Some Chemistry Of 5-Diazouracil And Its Derivatives

A thesis presented to The City University
in part fulfilment of the requirements for the
degree of Doctor of Philosophy

by

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Dedicated to my parents

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L.K.J. Aw

Abstract

The preparation of 6-alkoxy- and 6-aryloxy-5-diazo-1,6--dihydrouracils, from 5-diazouracil, is presented. A synthetic route to the parent compound, 5-diazo-1,6-dihydrouracil, is described and the EI-MS and FAB-MS of these novel compounds are given. The modified heterocyclic bases of nucleic acids often show biological activity, in particular, bases modified at the C-5 position of uracil. It is hoped that these compounds will show antibiotic and antitumour activity.

The reaction of diazouracils with dmso led to the formation of S,S-dimethyl-1,3,2-dioxathiolo[3,4-d]pyrimidines and the reaction with olefins resulted in the isolation of furano[2,3-d]pyrimidines. A 1,3-dipolar mechanism for the above reactions is presented.

The thermal decomposition of 5-diazouracil and 5-diazo-6--methoxy-1,6-dihydrouracil with a variety of reagents was investigated. Reaction with alcohols afforded 5-alkoxy- and 5,6-dialkoxy- uracils. 5-Methoxyuracil was obtained in the reactions of 5-diazo-6-methoxy-1,6-dihydrouracil. This suggested an intramolecular rearrangement of the intermediate obtained from the loss of the diazo group, and this was explored by isotopic labelling.

Additions of amines to the diazouracil gave a variety of 3-substituted 5(1-triazeno)uracils. An attempt to form an azaxanthine by the cyclisation of one of the isolated products, 5(3-propyl-1-triazeno)uracil, was undertaken.

The formation of 3,3a,5a,6,7,8-hexahydro-4H,8H-pyrazolo--[3,2:7,7a]furano[2,3-d]pyrimidin-7,9-dione via the addition of allyl alcohol to 5-diazouracil is reported.

Two approaches to the synthesis of 5-diazo-6H-uridine were attempted. One of the methods involving diazo transfer was found to be more promising resulting in the impure, desired product.

Abbreviations

A	=	adenine
Ac	=	acetyl
AMP	=	adenosine monophosphate
ADP	=	adenosine diphosphate
ATP	=	adenosine triphosphate
b.p.	=	boiling point
Bz	=	benzoyl
C	=	cytosine
c.	=	concentrated
cAMP	=	cyclic AMP
DBN	=	1,5-diazabicyclo[4.3.0]non-5-ene
DMF	=	N,N-dimethylformamide
dmso	=	dimethylsulphoxide
DNA	=	deoxyribonucleic acid
f.	=	fuming
G	=	guanine
h.p.l.c.	=	high performance liquid chromatography
i.r.	=	infrared
IMP	=	inosine monophosphate
Me	=	methyl
mmol	=	millimoles
m.p.	=	melting point
mRNA	=	messenger RNA
n.m.r.	=	nuclear magnetic resonance
Pi	=	inorganic phospate
PP	=	pyrophosphate
ppm	=	parts per million
pyr	=	pyridine
RNA	=	ribonucleic acid
r.t.	=	room temperature
T	=	thymine
t.l.c.	=	thin layer chromatography
TMS	=	tetramethylsilane
U	=	uracil
Ura	=	uracil
u.v.	=	ultraviolet
nm	=	nan ometres
$\triangle$	=	heat

Nomenclature

In this work, the naming and abbreviations of syntheic heterocyclic bases and nucleosides conforms to the IUPAC-IUB commision on Biochemical Nomenclature (1970)[1]. Other compounds have been named so that they conform to IUPAC 1957 Rules[2]. For the sake of simplicity, many of the accepted trivial names for the parent compound have been retained, *e.g.* in the naming of many of the uracil derivatives.

The terms used to describe the stereochemistry of the compounds conforms to the recommendations of IUPAC[3] and of the IUPAC-IUB joint commision on biochemical Nomenclature[4].

1 IUPAC-IUB Commision on Biochemical Nomenclature (CBN), *Eur. J. Biochem.* 1970, 15, 203.

2 Commision of Nomenclature and International Union of Pure and applied Chemistry, Paris 1957, "Definitive Rules for Nomenclature of Organic Chemistry", Butterworths Scientific Publications. (See also, J. Am. Chem Soc. 1960, 82,).

3 IUPAC, *J. Org. Chem.* 1970, 35, 2849.

4 IUPAC-IUB, *Eur. J. Biochem.* 1983, 131, 9.

PART A. Literature reviews.

Chapter 1. Structural aspects of nucleic acids and their components.

1.1 Early isolation.

1.1.1 Nucleic acids.

Nucleic acids are very important biological molecules, first isolated by Fredrich Miescher from pus cells in discarded surgical bandages. This rich source of nuclear material was digested with pepsin and hydrochloric acid and extracted with ether to give a residue termed "nuclein". Nuclein was found to be acidic in nature and contained a high proportion of phosphorus. The only other biological material then known to contain phosphorus was lecithin. As a consequence, the discovery was only reported [1] after the work had been checked [2-5]. The material isolated from pus cells is now thought to be a nucleoprotein rather than the pure nucleic acid.

Work with Rhine salmon sperm led to the isolation of a basic material which, being less complex than the known proteins, was termed "protamine". The analytical figure of 9.59% for phosphorus has led to the acceptance that this sample of material was a sample of nucleic acid [6].

Protein-free "nuclein" from yeast and animal tissue was obtained by a method described by Altmann [7], who also coined the term "nucleic acid". Subsequently, this method was improved [8-9].

1.1.2 Purine and pyrimidine bases.

Purine bases were discovered in nucleic acids [10-18]. Guanine was first found in the excreta of birds [19-21] and was identified as a component of nucleic acids [11,15]. The term "purine" was derived from the words "purum" and "uricum" [22-24].

Thymine was first discovered [6] and identified from the hydrolysate of nucleic acids [25]. Its structure was determined by degradation and synthesis [26,27] and shown to be a pyrimidine. Cytosine also was identified as a pyrimidine [28,29] as was uracil, which was isolated from yeast nucleic acid [30]. The structure of uracil was confirmed in 1901 by synthesis [31]. The term

"pyrimidine" had been coined from the names pyridine and amidine [32,33].

1.1.3 Sugar component of nucleic acid.

The partial acid hydrolysis of the protein-free material gave compounds which were termed "nucleotides" [34]. The hydrolysis of yeast nucleic acid gave adenine, guanine, cytosine, uracil, phosphoric acid and sugar. The sugar was recognised as a pentose [35] and specifically as ribose [36]. The confirmation came with the synthesis of amorphous ribose [37] and L-ribose [38]. D-Ribose was found to be the only sort of sugar in yeast RNA [39,42].

The hydrolysis of the protein-free nucleic acid from thymus "nuclein" gave adenine, guanine, cytosine, thymine, phosphoric acid and sugar. The sugar was shown to be a deoxypentose [43-45] and identified as D-2-deoxyribose by comparison with synthetic L-2-deoxyribose and D-2-deoxyribose [46-50]. The sugar moiety was demonstrated to exist in the furanose form [51,52] and to be linked to the N-1 position in pyrimidine ring [53].

1.1.4 DNA and RNA.

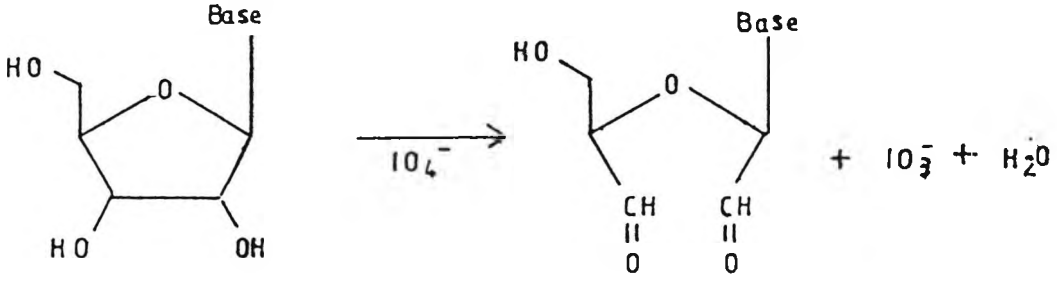
Nucleic acids were grouped into two types - ribonucleic acids (RNA) containing ribose and deoxyribonucleic acids (DNA) containing deoxyribose. An erroneous concept that DNA, "zoonucleic acid", was exclusive to animals and RNA, "phytonucleic acid", was exclusive to plant life developed [36,54]. Later, both RNA and DNA were shown to be present in all cells [55-69]. Further evidence came by means of a histochemical ribonuclease test, which was able to demonstrate RNA in animal tissue [70-73], and with UV spectroscopy, which detected the presence of pentose nucleotide and pentose polynucleotide in the cytoplasm of rapidly proliferating cells [74-78].

1.1.5 Glycosidic linkage.

The sugar component in RNA was shown to be in the furanose form using periodate [79-82] (Scheme 1.1). The failure of oxidation of deoxyribose with periodic acid indicated an absence of a *cis*-1,2-glycol system and hence, deoxyribose must be also in the furanose form [81]. Thus, the sugar moiety was linked to the purine or pyrimidine base via the C-1' anomeric carbon. The glycosidic link was shown to involve the N-1 position of the pyrimidine base [83-85]. This was confirmed by X-ray studies [86,87].

The ease with which purine bases may be removed from nucleosides indicated that the glycosidic linkage was via a C-N bond. Positions N-1 and N-3 were excluded as the site for the glycosidic link [86,87]. Position N-9 was chosen on the basis of comparisons of the absorption spectra [88-91] and confirmed in X-ray studies [92].

A β-configuration was proposed for the glycosidic linkage [93,94], which was confirmed by X-ray crystallography [86,95-97].



Scheme 1.1 Periodate oxidation of 1,2-diols

1.1.6 Internucleotide bond.

The nucleotides are phosphoric esters of the nucleosides. It was demonstrated that the phosphate ester resided on ribose and was located on the 3'-position [98-100]. The alkaline hydrolysis of RNA gave two isomers which were shown to be the 2'- and 3'-phosphate esters [101,102]. The interconversion of 2'- and 3'-phosphate isomers in dilute acidic solutions [103,104], but not alkaline conditions [105,106], was to complicate matters.

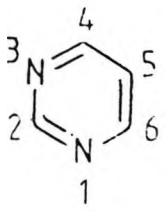
The nature of the internucleotide bond was first examined by electrometric titration of the acid and base groups [107-109] and on the basis of these results it was proposed that the internucleotide bond was a phosphate bridge between the sugar components with a linear polymer arrangement [110]. In RNA, the internucleotide was demonstrated to be a 5'-3' linkage between adjacent sugars [111-117]. Very accurate back titrations for the dissociation curve of highly polymeric DNA [118,119] led to the selection of the 5'-3' bridge as the internucleotide link [120-129].

1.2 Structure and terminology of nucleic acid components.

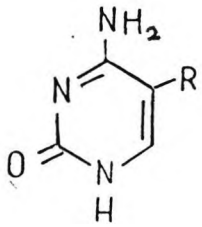
The atom numbering schemes and abbreviations for these components are given in a set of recommendations [130-133]. A set of standard definitions for terminology involved were proposed [134].

1.2.1 Heterocyclic bases.

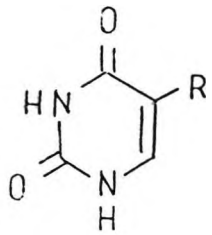
Of the pyrimidine series, the most commonly found naturally occurring heterocyclic bases in the nucleic acids are cytosine (1.2), uracil (1.4) and thymine (1.5). The most common of the purine class are guanine (1.7), xanthine (1.8), adenine (1.9) and hypoxanthine (1.10). The numbering of the heterocycles treats the pyrimidines as 1,3-diazines in which the atoms in the carbon-carbon double bond are numbered 5 and 6. In older literature, the previous system was to number them 5 and 4 respectively. Purine, being an accepted trivial name, has retained its old numbering instead of the typical systematic numbering for imido[4,5-d]pyrimidine.



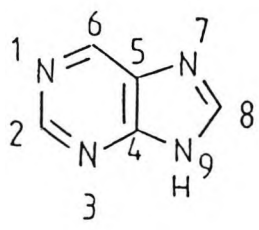
Pyrimidine (1.1)



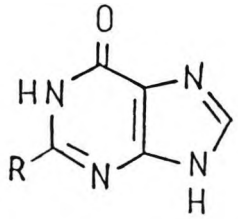
R=H, Cytosine (1.2)
R=Me, 5-Methylcytosine (1.3)



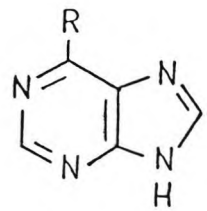
R=H, Uracil (1.4)
R=Me, Thymine (1.5)



Purine (1.6)



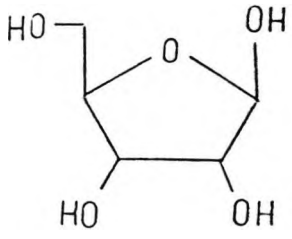
R=NH_2, Guanine (1.7)
R=OH, xanthine (1.8)



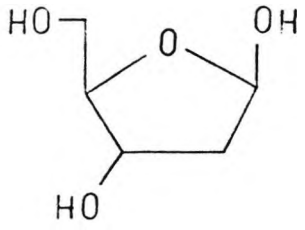
R=NH_2, Adenine (1.9)
R=OH, Hypoxanthine (1.10)

1.2.2 Sugar ring.

The two sugars found in nucleic acids are D-ribose (1.11) and D-2-deoxyribose (1.12). Both sugars are in the *B*-D-furanose form.



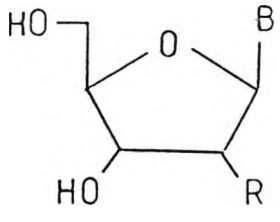
(1.11)



(1.12)

1.2.3 Nucleosides.

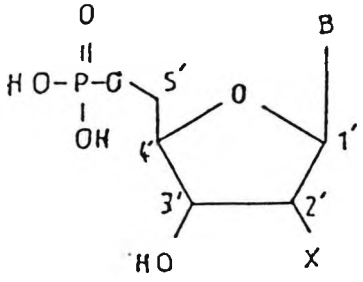
The term nucleoside is used to describe any molecule which has either a purine or pyrimidine base attached to a sugar. In nucleic acids, the configuration is β about the C-1 of the furanosyl form of the sugar (1.13-1.20).



Base, B	Nucleoside	
	ribonucleoside (R=OH)	deoxyribonucleoside (R=H)
adenine	adenosine (1.13)	deoxyadenosine (1.17)
guanine	guanosine (1.14)	deoxyguanosine (1.18)
cytosine	cytidine (1.15)	deoxycytidine (1.19)
uracil	uridine (1.16)	-
Thymine	-	deoxythymidine (1.20)

1.2.4 Nucleotides.

A nucleotide is a nucleoside phosphorylated at one of the sugar hydroxyls. In monophosphates (1.21), the isomers are distinguished by the position of the phosphate ester which is numbered with respect to the sugar ring. The monophosphates are also found as cyclic nucleotides and the position of the phosphate diester bonds numbered with respect to the sugar. Higher phosphates are also found and these di-, tri- and oligophosphates have a wide variety of roles.



Ribonucleoside-5'-monophosphate

X=OH

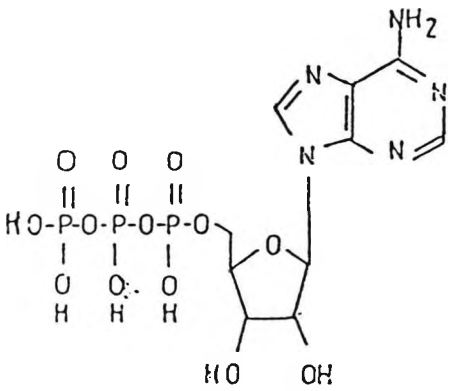
B=Adenine, Guanine, Cytosine, Uracil

Deoxyribonucleoside-5'-monophosphate

X=H

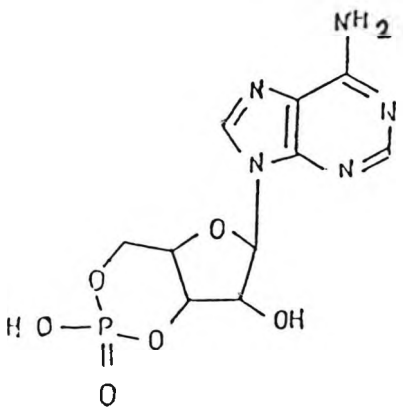
B=Adenine, Guanine, Cytosine, Thymine

(1.21)



Adenine-5'-triphosphate

(1.22)

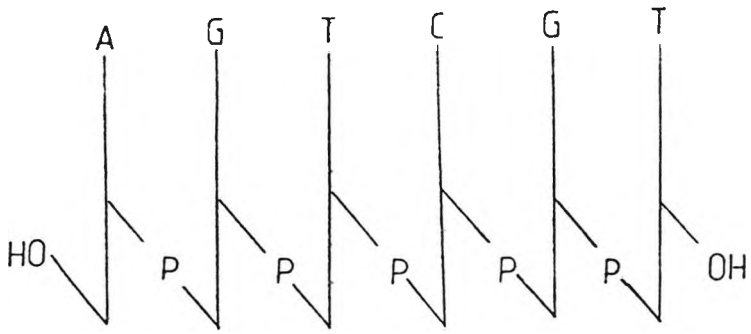


3,5'-Cyclicadenosine monophosphate

(1.23)

1.2.5 Primary structure of DNA.

DNA and RNA consist of a repeating motif of nucleotides linked by a phosphate diester bridge between the 3'- and 5'-positions of adjacent sugar rings. The sequence of the heterocyclic bases in such a structure is referred to as the primary structure (Scheme 1.2). Any strand of nucleic acid will have a linear direction, at one end the 5'-hydroxyl will be free, *i.e.*, not involved in the phosphate diester backbone, and at the other end the sugar ring will have a free 3'-hydroxyl and a 5'-phosphate bridge. Thus, the primary structure may be read from either end so long as the direction of reading is stated. A commonly used convention is that the sequence read in the 5'-3' direction is regarded as positive and the sequence read in the 3'-5' direction is negative.



Scheme 1.2. Representation of the primary structure +AGTCGT

1.2.6 Secondary structure of DNA.

Most DNA exists as two complementary strands of nucleic acid. The structure postulated for this double stranded DNA is that of a right handed double helix. The features of the double helix are that a) the chains run in opposite directions, b) the purine and pyrimidine bases are towards the centre of the helix and the deoxyribose and phosphate groups are towards the outside c) the diameter of the helix is approximately 20 Å with adjacent bases being 3.4 Å along the helix axis and related by a rotation of 36 degrees, d) the two chains are strongly hydrogen bonded via the complementary nature of the bases e) the two strands may only be separated by unwinding.

1.3 Conformation of nucleosides, nucleotides and nucleic acids.

1.3.1 Introduction.

In order to understand the biological activity of nucleosides, nucleotides and nucleic acids, it is important to know their detailed structural features. A detailed picture of their 3-D structures, including conformations, is now especially important, given the recent advances in drug design and computer modelling of receptor sites and substrate/drug interaction.

The structural features of nucleotides can be studied in several ways, of which the most powerful is X-ray crystallography. Recently, progress has been made in the identification of the primary structure of large biological polymers by mass spectroscopy. With the advent of high field n.m.r., studies on conformation of nucleosides, nucleotides and small fragments of nucleic acids in solution have been possible.

1.3.2 Definitions.

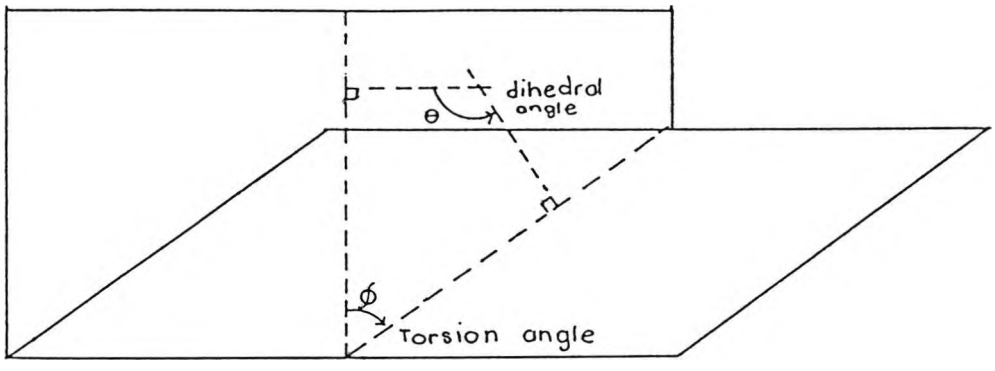
In order to understand conformation it is important to clarify some of the commonly used terms. Apart from the common terms, such as nucleoside and nucleotide, other names often appear. The prefix mono-, di-, tri-, tetra-, oligo- and poly- are usual in indicating the number of nucleotides in a chain. The prefix oligo- is normally used for chains containing 5-14 nucleotides and the prefix poly- for chains containing a greater number. The direction of the chain along the polynucleotide backbone is given by the atomic numbering sequence of P-O_5·-C_5·-C_4·-C_3·-O_3·-P. The three dimensional structure of the molecule is characterized by bond lengths, bond angles, and rotations of groups of atoms about bonds. A measure of such rotation is given by the torsion angle, ϕ (phi). Unfortunately, the complementary angle, the dihedral angle θ (theta), is often quoted for the torsion angle, whereas in a strict sense the two angles are different.

1.3.3 Torsion angle.

In a four atom sequence A-B-C-D, the torsion angle, ϕ, is defined as the angle between projected bonds, i.e., A-B and C-D, when looking along the central bond in the direction B-C. The angle

is defined as 0° if the bonds are eclipsed and is positive if the far bond is rotated clockwise with respect to the near bond. Thus, values may be reported between 0-360° or -180° to +180°.

Another definition of the torsion angle is the angle between the two planes, the first plane containing atoms ABC, the second plane containing the atoms BCD. This definition is useful when using the complementary "dihedral angle". The dihedral angle, θ, is given as the angle subtended by normals to the plane making up the torsional angle (Scheme 1.3) [134a].



Scheme 1.3

1.3.4 Preferential conformations.

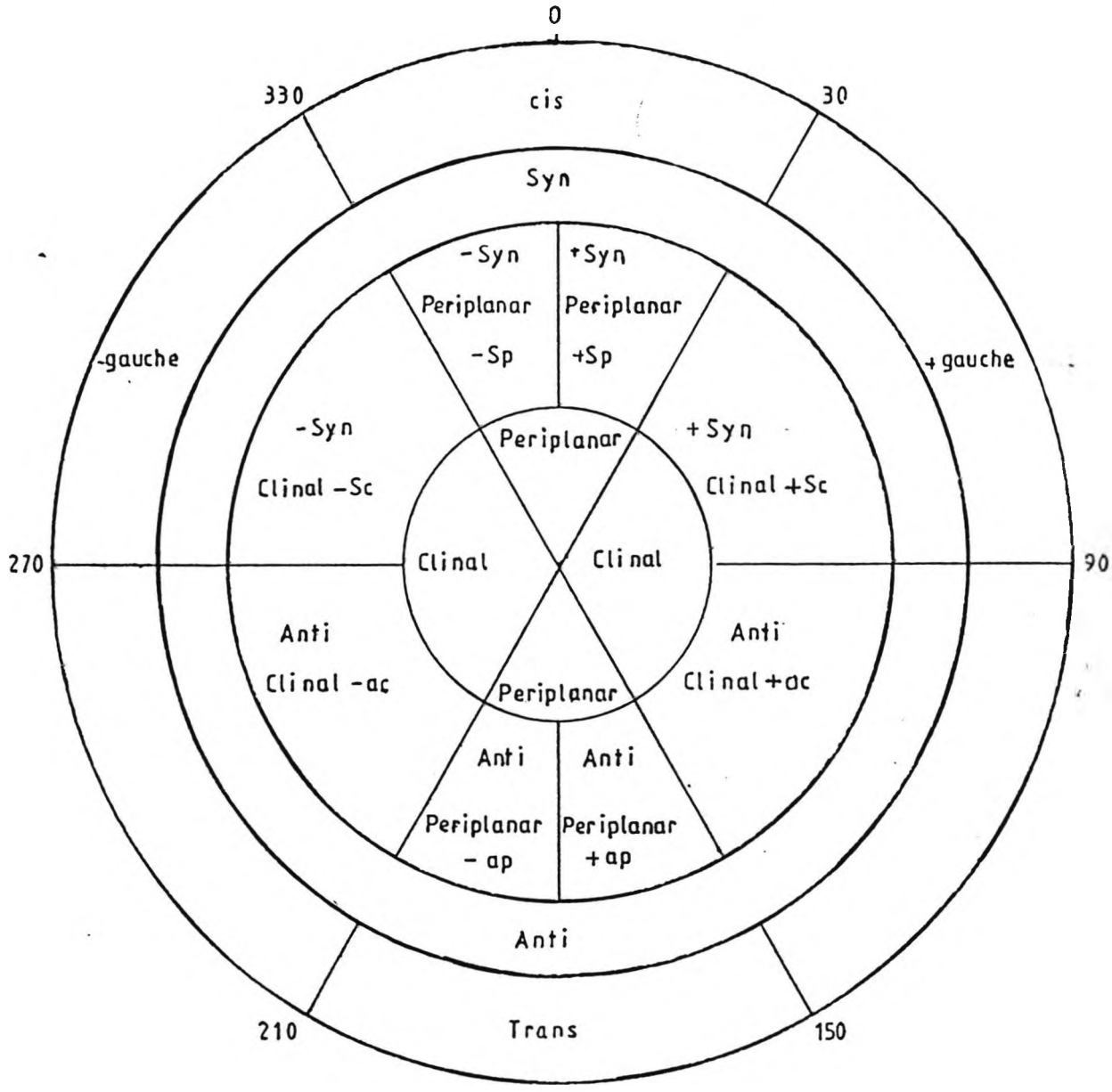
In molecules with rotational freedom about single bonds, not all the torsion angles are assumed for equal lengths of time. A series of preferred positions are often the norm. Terms commonly used to associate the angle of the torsion planes were proposed by Klyne and Prelog [134b] (Table 1.1 and Scheme 1.4).

Prelog term	angle	other common terms
Syn	~0°	*cis*
Anti	~180°	*trans*
+/-synclinal	+/-60°	*gauche*
+/-anticlinal	+/-120°	

Table 1.1 Torsion angle and terminology

1.3.5 Torsion angles in nucleotides.

A set of standard angles have been assigned to the various torsion angles in nucleotides (Table 1.2).


0
30
90
150
210
270
330
cis
Syn
-Syn
Periplanar
-Sp
+Syn
Periplanar
+Sp
-gauche
+gauche
-Syn
Clinal -Sc
Periplanar
+Syn
Clinal +Sc
Clinal
Clinal
Anti
Clinal -ac
Anti
Clinal +ac
Periplanar
Anti
Periplanar
-ap
Anti
Periplanar
+ap
Anti
Trans

Scheme 1·4

Torsion angle	Atom number sequence.
alpha	$_{(n-1)}O_3'$-P-O_5'-C_5'
beta	P-O_5'-C_5'-C_4'
gamma	O_5'-C_5'-C_4'-C_3'
delta	C_5'-C_4'-C_3'-O_3'
epsilon	C_4'-C_3'-O_3'-P
zeta	C_3'-O_3'-P-O_5'-$_{(n+1)}$
nu0	C_4'-O_4'-C_1'-C_2'
nu1	O_4'-C_1'-C_2'-C_3'
nu2	C_1'-C_2'-C_3'-C_4'
nu3	C_2'-C_3'-C_4'-O_4'
nu4	C_3'-C_4'-O_4'-C_1'
Chi	O_4'-C_1'-N_1-C_2 (pyrimidine)
	O_4'-C_1'-N_9-C_4 (purine).

Table 1.2 Nucleotide torsion angles

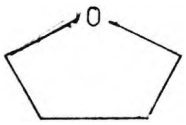
1.3.6 Sugar puckering modes.

Like all 5-membered rings the furanose form of the sugar ring is nonplanar. The two common terms used to describe the nature of the conformation are the envelope (E) form and the twist (T) form.

The envelope form is one in which four of the five ring atoms lie in one plane and the fifth atom being out of plane by 0.5 Å.

The twist form has three adjacent ring atoms in plane with the remaining two ring atoms being out of plane, one above and one below the plane.

Ring atoms which lie out of plane on the same side as the C-5' are referred to as *endo*, whilst those on the opposite side of the plane to the C-5' are referred to as *exo*. These sugar ring puckering modes are in a state of flux with transition between the various modes being facile. The term major puckering is used where there is the largest deviation from planarity and the term minor puckering is used where the deviation is lesser.

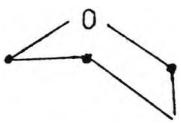


Planar 5-membered ring

A situation never observed

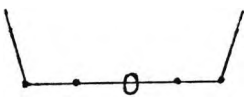
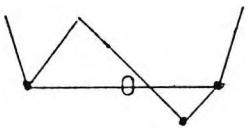
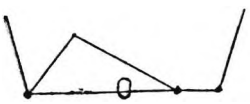
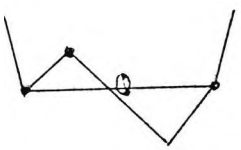
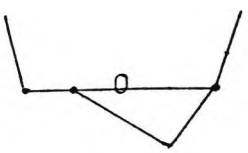
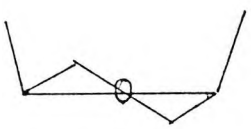
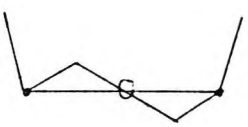
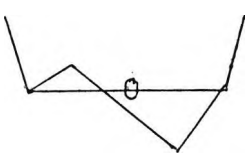
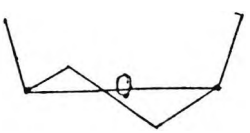
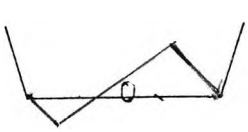
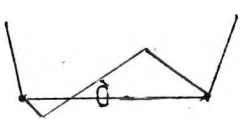
Envelope form

4 planar atoms



Twist form

3 planar atoms

	Description		Notation
	planar 5-membered ring (not observable)		
	envelope C_3· most out of plane		3 E (C_3· *endo*)
	envelope C_2· most out of plane		E (C_2· *exo*) 2
	Twist (symmetrical) half chair		3 T (C_2· *exo*) 2 (C_3· *endo*)
	Twist (*exo*) C_2· further out of plane than C_3·		3 T (C_2· *exo*) 2 (C_3· *endo*)
	Twist (*endo*) C_2· further out of plane than C_3·		2 T (C_2· *endo*) 3 (C_3· *exo*)

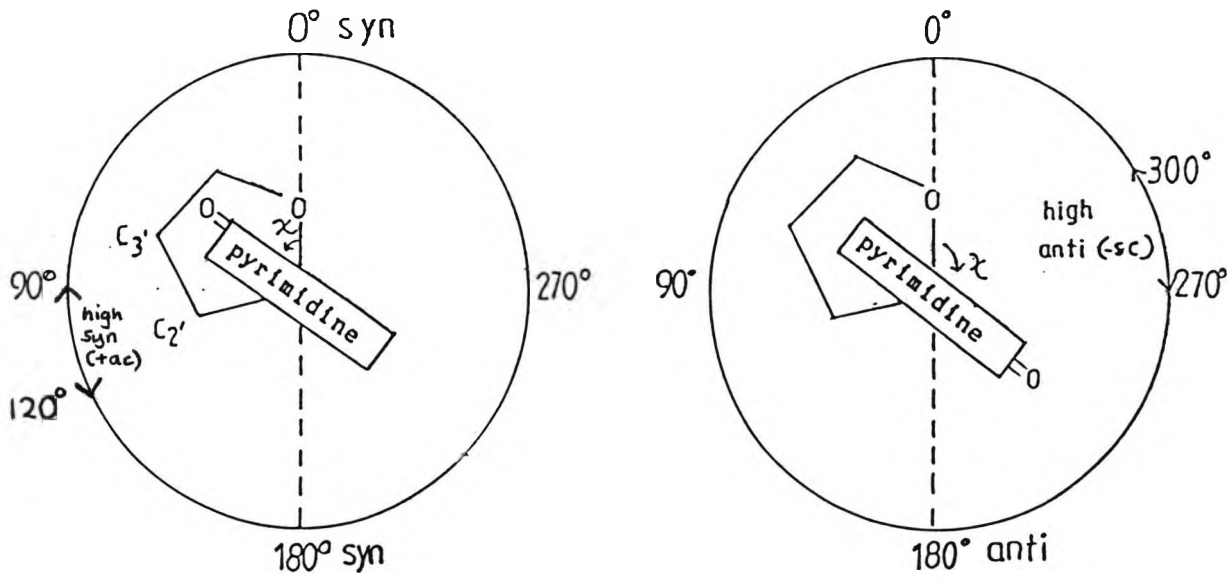
Scheme 1.5 Sugar pucker modes and their description.

An abbreviated notation exists to describe such ring modes. If the out of plane atom is endo, it is superscripted and if the out of plane atom is exo it is subscripted. The form of the ring is recorded as either E or T. If the subscript or superscript precedes the letter describing the form then the form is in the major puckering mode. If the superscript or the subscript follows the letter, then the ring is in the minor puckering form.

The preceding method for describing sugar puckering is an approximation and further treatment has produced the concept of pseudorotation [135-137]. Essentially, puckering is described by means of the maximum torsion angle and the pseudorotation phase angle P. For cyclopentane, changes in the ring conformation proceed virtually without potential energy barriers. However, when the ring is unsymmetrically substituted, potential energy thresholds are created which lead to preferred puckering modes. It is the formation of such conformers which is of interest in nucleoside chemistry.

X (approx)	Description
-89° to +90° +271° to +90°	*syn*
+91° to -90° +91° to +270°	*anti*
+90° to +120°	*high syn.*
+270° to +300°	*high anti.*

Table 1.3 Nucleoside orientation

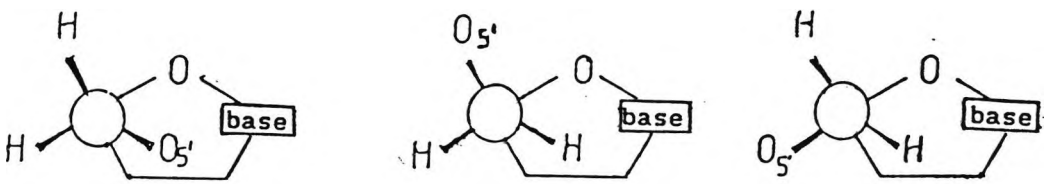


Scheme 1.6 "High syn" and "high anti" conformations

The heterocyclic base can adopt two major orientations about the glycosyl C_1·-N link. These are the *syn* and *anti* conformations, which are defined by the torsion angle X (O_4··-C_1··-N_9-C_4) or (O_4··-C_1··-N_9-C_4). The common terms and the associated torsional angles are given (Table 1.4). Two other terms often used are the *'high anti'* and the *'high syn'* (Scheme 1.6).

1.3.7 Orientation about the C_4··-C_5· bond.

The rotation about the C_4··-C_5· bond produces three major positions for the O_5· atom. These conformers are usually described by either the torsional angle ϕ_{oo} (O_5··-C_5··-C_4··-O_4··) or by the torsional angle ϕ_{oc} (O_5··-C_5··-C_4··-C_3··) [138]. The conformations may also be described by use of angular ranges or by the gauche/trans convention. The recommended method is to use the torsion angle ϕ_{oc} (Scheme 1.7).



ϕ_{oo}	300°	60°	220°
Angular convention	+SC	ap	-SC
gauche/trans convention	gauche/gauche	gauche/trans	trans/gauche

Scheme 1.7 Torsion angle and other conventions

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Chapter 2. Basis for biological activity of analogues of nucleic acid components.

In this chapter a brief summary of the biochemistry of nucleic acids and their components is given with the intent of illustrating targets for chemotherapy.

2.1 Importance of nucleic acids and their components.

The importance of nucleic acids and nucleotides are best illustrated by examining their roles in organisms [1-4]. In general, their involvement can be summarised as follows:-

a) Nucleic acids are the means by which genetic information is stored. The method of storage readily lends itself to use as a template for transcription, the product providing another template for the translation of the genetic code, by means of an assembly line process, for the formation of those vital macromolecules, the proteins.

b) The nucleotides are also used as regulators of biosynthetic pathways. They also occur as activated intermediates in biosynthesis.

c) In addition, nucleotides perform a role as secondary messengers in the general management of cell function.

d) The nucleotides play an integral part in the control of energy conversion within the organism.

e) They are also found as components in many of the coenzymes.

2.2 Biosynthesis of purine nucleosides. [5]

The purine heterocyclic bases are synthesized from a variety of precursors (Table 2.1)

Atom	Precursor
N-1	from aspartate
C-2	active derivatives of tetrahydrofolate
N-3	amide group of the side chain of glutamine
C-4	from glycine
C-5	from glycine
C-6	from CO_2
N-7	from glycine
C-8	activated derivatives of tetrahydrofolate
N-9	amide group of the side chain of glutamine

Table 2.1. Summary of the origin of purine atoms.

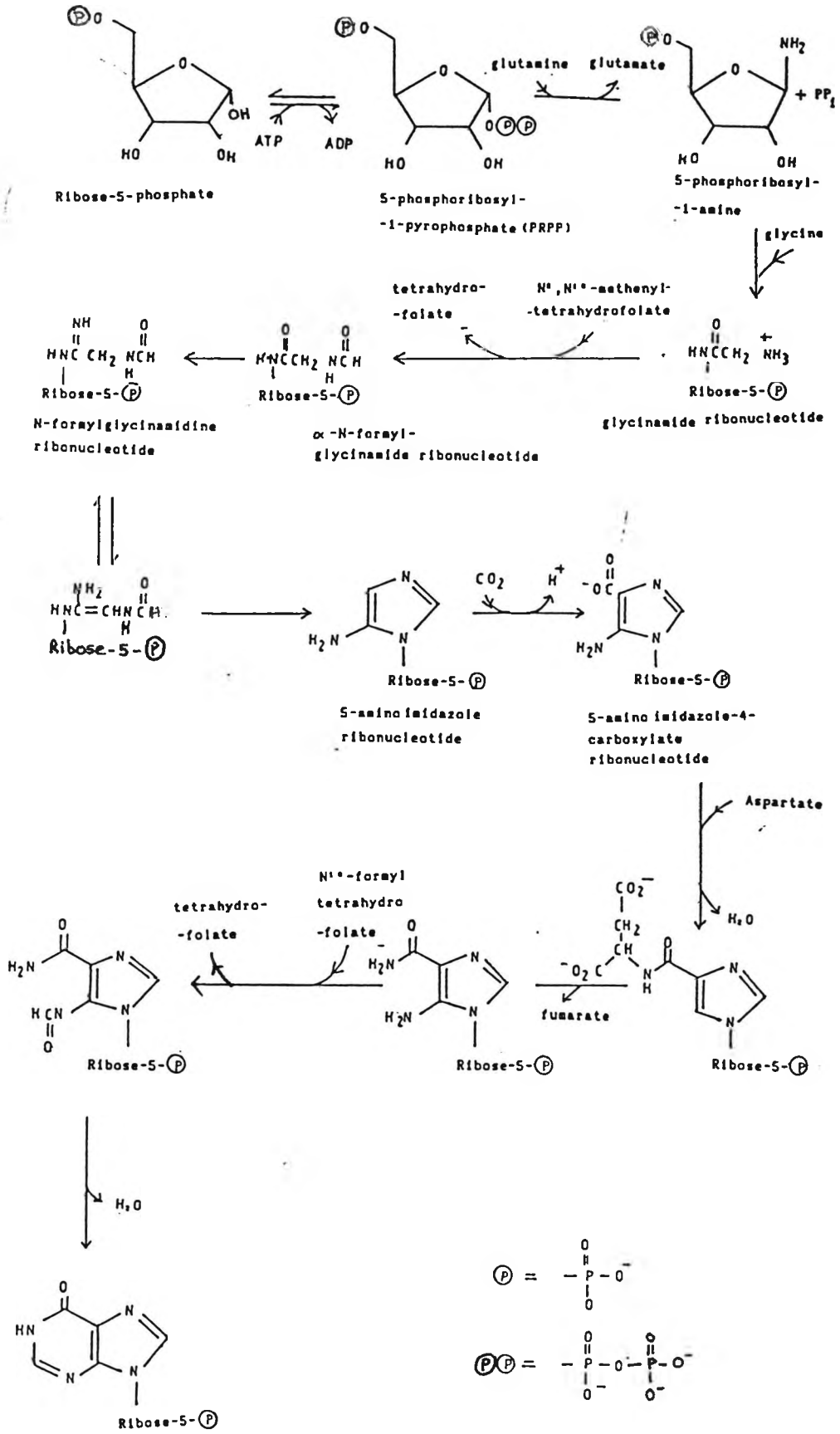
In the *de novo* synthesis of the purine ring, the initial step is the formation of 5-phosphoribosyl-1-amine from 5-phospho--ribosyl-1-pyrophosphate (PRPP) by the transfer of an amino group from glutamine [6-7]. It is the 5-phosphoribosyl moiety which serves as a support for the building of the purine heterocycle. The next stage is the reaction with glycine to give glycinamide ribonucleotide [8-9], which is then formylated at the free amine [10]. The amide at the ribose end of the molecule is converted to the amidine group (imino group) which obtains the amine moiety from another molecule of glutamine [11]. The resulting formylglycin--amidine ribonucleotide then undergoes ring closure to give 5-aminoimidazole ribonucleotide [12] which forms the 5-membered ring portion of the purine base. The 5-aminoimidazole ribonucleotide is carboxylated at the C-4 position [13-14], and then condensed with the amino group of aspartic acid [15]. The fumarate portion of the 5-amino-4-N-succinocarboxamide ribonucleotide is removed [16]. The 5-amino group is then formylated by N^{10}-formyltetrahydrofolate [17] and the resulting 5-formamidoimidazole-4-carboxamide ribonucleotide undergoes an intramolecular condensation to give inosine monophosphate (IMP) which contains the purine base hypoxanthine [18] (Scheme 2.1).

The remaining purine heterocycles are formed by postsynthetic modification of IMP (Scheme 2.2). Adenosine monophosphate is formed by the replacement of the carbonyl group by an amino group at C-6 using aspartate as the donor [19-21]. In this case the provision of the high energy phosphate for the formation of adenylosuccinate is from GTP.

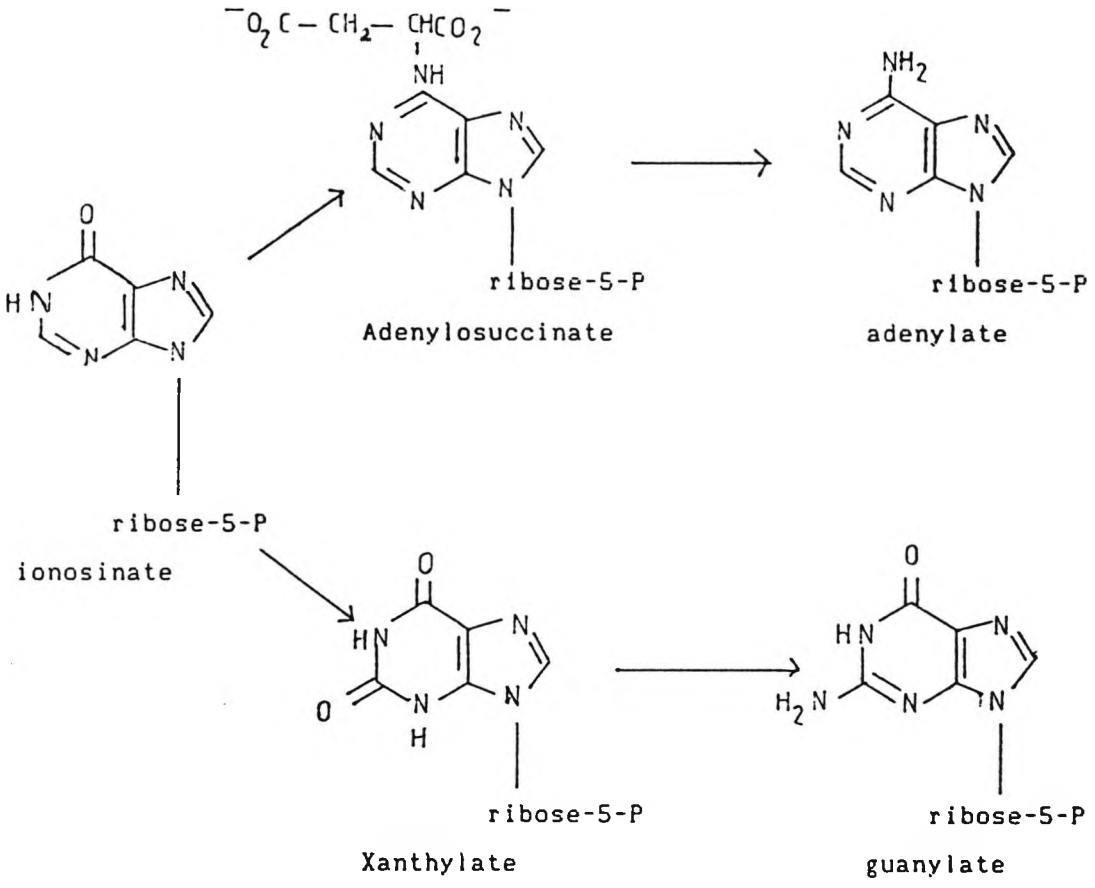
Guanosine monophosphate (GMP) is synthesized by the oxidation of IMP to xanthosine monophosphate [22-25], followed by the replacement of the C-2 carbonyl in the purine ring with an amino group obtained from the side chain of glutamine [26-27]. In this instance, the modification involves cleavage of ATP to AMP and pyrophosphate and the subsequent hydrolysis of the pyrophosphate.

It should be noted that, in the process, AMP and GMP act as feedback inhibitors for purine nucleotide biosynthesis (Scheme 2.3)[28].

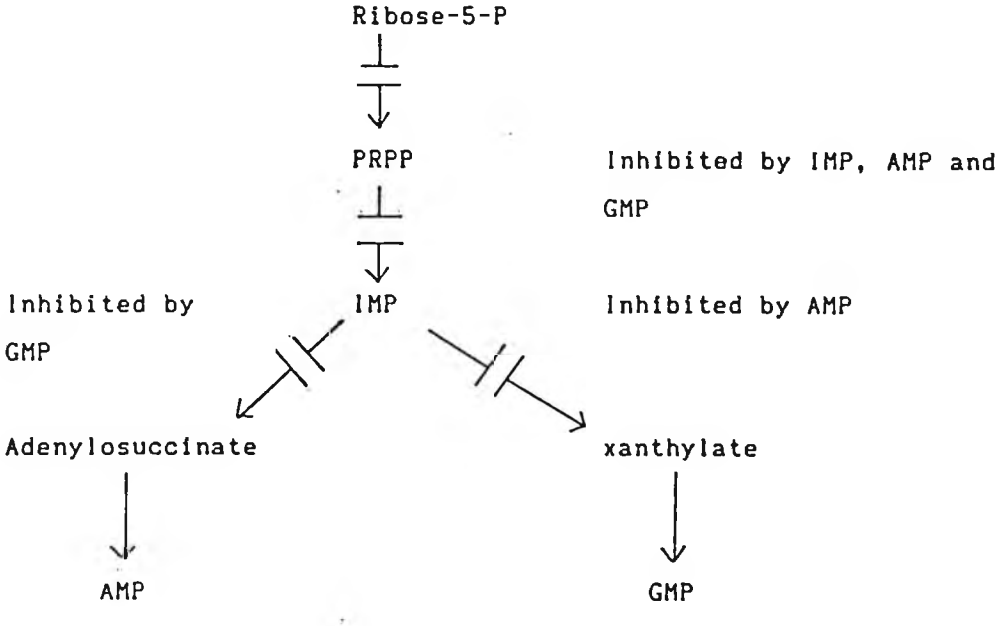
There also exists a mechanism for the reclamation of purine bases from the degradation of nucleic acids and nucleotides. This involves the use of enzymes to link the purine base to PRPP [29].


Ribose-5-phosphate
ATP
ADP
5-phosphoribosyl-
-1-pyrophosphate (PRPP)
glutamine
glutamate
+ PPi
5-phosphoribosyl-
-1-amine
glycine
tetrahydro-
-folate
N5,N10-methenyl-
-tetrahydrofolate
glycinamide ribonucleotide
α-N-formyl-
glycinamide ribonucleotide
N-formylglycinamidine
ribonucleotide
5-amino imidazole
ribonucleotide
5-amino imidazole-4-
carboxylate
ribonucleotide
Aspartate
fumarate
N10-formyl
tetrahydro
-folate
tetrahydro-
-folate
H2O
H2O

Scheme 2.1



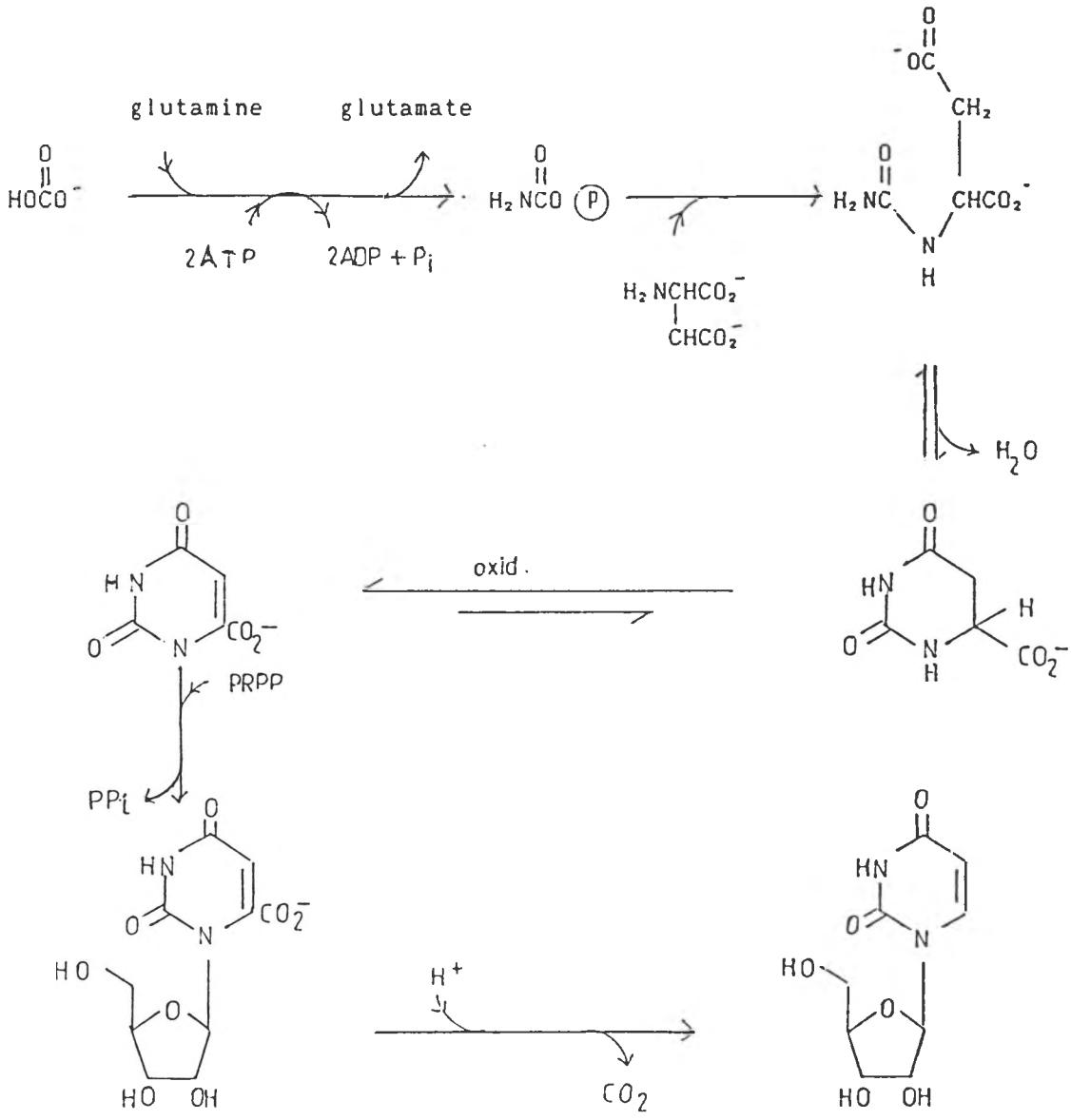
Scheme 2.2 Postsynthetic modification of Inosinate



Scheme 2.3 Feedback control of AMP and GMP biosynthesis

2.3 Pyrimidine biosynthesis.

The biosynthesis of pyrimidine bases begins with the formation of carbamoyl phosphate from glutamine, bicarbonate and two molecules of ATP [29-33]. The reaction of the resulting carbamoyl phosphate with aspartate, is catalysed by aspartate transcarbamoylase [34-35]. The molecule then undergoes an intramolecular cyclisation [36] to give dihydroorotate, which is oxidised using NAD $^{\bullet}$ to give orotate [37-41]. The formation of the nucleotide orotidine monophosphate (OMP) involves glycosidation with PRPP [42-43]. Uridine monophosphate (UMP) is formed by the subsequent decarboxylation of OMP (Scheme 2.4) [44].



Scheme 2.4 Biosynthesis of uridine

The formation of cytosine occurs by post-synthetic modification of UTP, in which the C-4 carbonyl is replaced by an amino group donated from glutamine [45-46].

In many of the post-synthetic conversions, the level of phosphorylation is often varied. The nucleoside may be converted to the nucleotide [47] and for the nucleotide monophosphates, diphosphates and triphosphates can be interconverted [48-50] (Scheme 2.5).

UMP + ATP ⇌ UDP + ADP ⇌ UTP + AMP

UDP + ATP ⇌ UTP + ADP

UDP ⇌ UMP + Pi

UTP + UMP ⇌ 2 UDP

UDP + GTP ⇌ UTP + GDP

UDP + ITP ⇌ UTP + IDP

Scheme 2.5 Interconversion of nucleotides

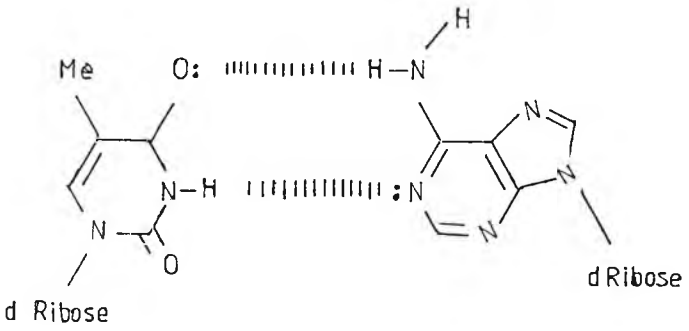
2.4 Biosynthesis of deoxyribonucleotides.

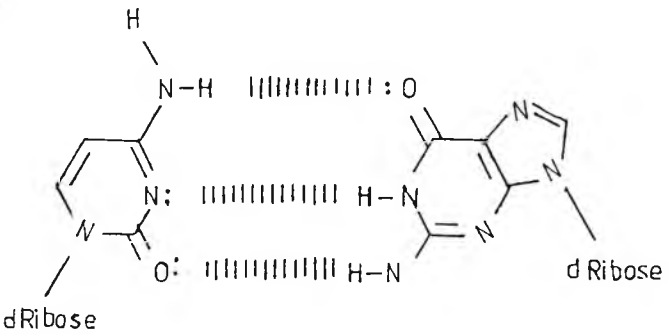
The formation of deoxythymidine monophosphate (dTMP) occurs via the methylation at the C-5 position of deoxyuridine monophosphate (dUMP). The methylating agent is N^5,N^{10}-methylene--tetrahydrofolate, which also serves as an electron donor in the reaction and results in dihydrofolate as the by-product (Scheme 2.6). The other deoxyribonucleotides are formed by reduction of ribonucleoside diphosphates. The reduction is catalysed by an enzyme system of four proteins which were identified as thioredoxin, thioredoxin reductase, enzyme B_1 and enzyme B_2. The reduction of ribonucleoside diphosphates is controlled allosterically via effectors such as ATP and dGTP. The system is also hindered by feedback inhibitors of dATP and dTTP [51].

2.5 Formation of DNA. [52]

Genetic information is conserved by duplication of the two complementary strands in double stranded DNA. After the unwinding of the two strands, each will act as a template in the duplication of the other via complementary base-pairing, where adenine pairs

with thymine and cytosine with guanosine (Scheme 2.6). Important in this process are two groups of enzymes. The first group is the polymerases which are responsible for duplicating the complementary strand. The other group includes the nucleases, lygases and postsynthetic modifiers which are responsible for DNA repair and for checking that duplication, for the most part, has been error free. The recognition of three types of polymerase and their associated functions has shown that the grouping of the enzymes into synthesizing and repair functions is a grouping of convenience rather than a rigid classification.





Scheme 2.6 Thymine-adenine and cytosine-guanine base-pairing

2.6 Formation of RNA [53]

DNA will also act as the template to form RNA. This process is referred to as transcription and is enzyme-controlled. In cell replication it is found that RNA, messenger RNA (mRNA), will serve as a template for protein synthesis and never for DNA synthesis. However, there exists one important exception, that of viral infection and it is this difference which may prove helpful in treatment with drugs. RNA is normally single stranded, having been synthesized by RNA polymerase in the direction of 5'-3' from only

one of the DNA strands. Of the two hybrids, DNA-DNA and RNA-DNA, it is found that the DNA-DNA hybrid is the thermodynamically more stable, Thus, the release of the DNA strand from the complementary RNA and the subsequent recombination with complementary DNA is overall an energetically favourable process.

RNA polymerase is a very large complex enzyme composed of four types of subunits. It is responsible for all aspects of RNA synthesis from DNA complexing through elongation to chain termination. The enzyme is also responsible for synthesizing the other two types of RNA, ribosomal (rRNA) and transfer RNA (tRNA), from the DNA template.

After the *de novo* synthesis of the RNA chain, the chain is post-transcriptionally modified which is referred to as "maturation" or "processing".

2.7. Biological basis for drug action.

2.7.1 Introduction.

Drug activity, in general, results from an adverse interaction between the foreign drug molecule and one or more of the macromolecules within the cell. In many cases, the drug molecule shows a marked resemblance to a molecule normally found within the organism or cell. In some cases the resemblance only occurs in some of the conformations that the drug may assume.

The complex of macromolecule and drug may be of several types, such as non-competitive inhibitors and competitive inhibitors. The interaction may be reversible or irreversible, which also referred to as un-competitive inhibition. The major difference between the non-competitive inhibitor and the competitive inhibitor is a distinction in site of action. In the case of the competitive inhibitor the site is usually the site associated with function, which in enzymes is referred to as the active site. In non-competitive inhibition the drug acts at another region of the macromolecule causing a conformational change which indirectly affects the active site. This secondary site of action is referred to as the allosteric site. In general, the action of the drug is to interfere with the normal working of the macromolecule, which may be achieved by physically blocking the path of the usual reactant. The other common mode of action is to alter the conformation of the

macromolecule so that it can no longer perform its function owing to the change in geometry of functional groups.

2.7.2 Pyrimidine analogues. [54-55]

Substitution at the C-5 position in many pyrimidine and pyrimidin-2,4-dione compounds has been a subject of great interest. Many of these compounds have shown biological activity as inhibitors of enzymes and of bacterial growth [70]. The positioning of substituents on the C-5 position has often been related to the recognition of thymine which can be regarded as a methyl-substituted uracil. The presence of enzymes which recognise this natural molecule also means that C-5 substituted analogues may interact with these enzymes by partially mimicking thymine with respect to substrate recognition. 6-Azauracil inhibits the rate at which pyrimidines are incorporated into the nucleic acid of Trypanosoma cruzi [57].

Substituent	Activity
F	Inhibition of DNA replication [61]
Cl	Insertion into E.coli DNA [62]
Br	Inhibits rate of incorporation of uracil in Trypanosoma cruzi [57]
I	
NH_2	Antimalarial activity [63]
	Inhibition of growth in E.Coli [64]
$N(CH_2CH_2Cl)_2$	activity against various carcinoma [59]
NO_2	Inhibition of bacterial growth [65]
	Effect as thymine antagonist in DNA biosyn.[66]
N_2	Inhibition of cell division in E.coli.[67]
	Cytotoxic effect. reduction of tumour size [68]
OH	Inhibits enzymatic degradation of uracil [56]
	Inhibits rate of incorporation of uracil in Trypanosoma cruzi [57]
	Antimalarial activity against Plasmodium berghei [58]
CH_2OH	-
CF_3	Incorporation into DNA with effective inhibition of DNA synthesis [60]

Table 2.2 C-5 Substituted uracils

2.7.3 Nucleoside analogues.

Nucleoside analogues have been a major source of antiviral and antitumour agents. Their effect probably comes from the disrupting the *de novo* synthesis of nucleotides and nucleic acids in the situation of rapid proliferation, which is symptomatic of viral infection or tumour growth. The manner in which these compounds exert their influence is not completely understood. Inhibition of the enzymes involved is a logical suggestion for a mode of action. Amongst the targeted enzymes are the specific kinases, especially thymidine kinase (TK), which convert nucleosides into nucleotides. Other enzymes which would form suitable targets are the 'post synthetic' modifiers of UMP and IMP.

The uses of these analogues have been hindered by problems of selectivity and resistance. The strategy concerning selectivity has relied on the changes in metabolism associated with the disorders. The increased rate of the enzymes concerned with synthesis, replication and duplication engenders an increased likelihood of those enzymes being affected by the drug. This sort of strategy is common in treatment of cancer.

The scope for selectivity in certain types of viral infection is larger. In addition to general targeting on *de novo* synthesis, fundamental differences between animal cells and viruses provide extra choices for selective action. The difference stems from the viral genetic code being stored, for the most part, in RNA. Viruses of this class include the common cold virus, influenza virus and the tumour viruses, rousviruses and leukoviruses. These viruses cause infection by transcription of their genetic code into DNA. The process of "reverse" transcription is mediated by an RNA-directed DNA polymerase, which is often referred to as "reverse transcriptase". This enzyme must be synthesized directly from translation of the original RNA and thus provides a possible target for drug action. RNA replicase (also referred to as RNA synthetase) is the major enzyme involved in RNA self-replication. This enzyme would also provides a selective target for antiviral agents. Thus, the use of RNA analogues to interfere with RNA synthesis would be more promising in treating such viruses. As there does not appear to be an RNA repair system in cells, this would tend to indicate that preserving the genetic code in RNA is

not so important as preserving it in the original cell's DNA and that if ribonucleoside analogues were to interfere in the cells own RNA, it would not be so critical as interfering with the viral RNA.

2.7.4 C-5 Substituted Nucleic Acid Components.

2.7.4.1 Substituted 2-Deoxyuridines.

There are numerous compounds in this category (Table 1.7). Most of them have shown antitumour (X= -CHO, -C.CH, -CN, $-NO_2$, -SCN, -F) or antiviral activity (X= -CH=CHBr, -CH=CHI) [1-2]. Like the C-5 substituted pyrimidin-2,4-diones, their effect is likely to result from mimicking deoxythymidine and inhibiting either thymidine kinase or thymidine synthetase.

2.7.4.2 Substituted uridines.

These compounds have shown mainly antiviral activity or inhibition of protein biosynthesis via disruption during translation. Substituents at the C-5 position probably act by interfering with systems which are keyed to cytosine. A list of some of the substituents are given in Table 2.3.

C-5 substituted deoxyuridines.	C-5 substituted uridines	
	$CH_2N(Et)_2$	[74]
CH_2OH	CH_2OH	[36]
CH_2OCH_3		
CH_2SH		
CH_2SCH_3		
CHO		
C.N		
CF_3		
Et		
Pr		
$CH_2CH=CH_2$		
$CH=CH_2$		
CH=CHBr		
CH=CHI		
C.CH		
	NH_2	[73]
NHR		
	N_2	[81]
NO_2	NO_2	[73]
$OCH_2C.CH$		
SH		
SC.N		
F	F	[72]
	Cl	[72]
Br	Br	[72-78]
I	I	[72]

Table 2.3 Base-modified deoxyuridines and uridines.

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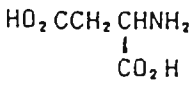
Chapter 3. Chemistry of diazo compounds.

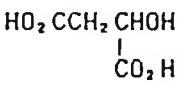
3.1 History and preparation.

3.1.1 Introduction.

The earliest reported reaction which is known to proceed via diazotization and subsequent reaction of the intermediate was the conversion of aspartic acid (3.1) to malic acid (3.2) [1,2]. A corresponding reaction was also found for aromatic amines with the conversion of aniline (3.3) to phenol (3.4) [3].



(3.1)

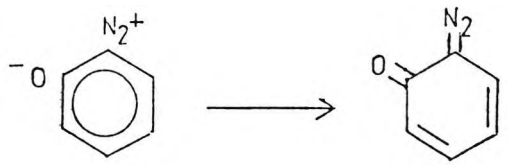


(3.2)

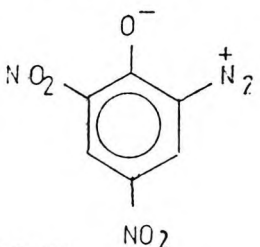
(3.3)

(3.4)

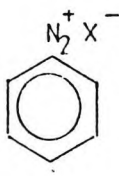
It was inferred that the intermediates in these reactions were members of a new class of compounds. This hypothesis was supported by the finding that the deamination of n-propylamine gave a variety of products [4,5]. Examples of this new class of compounds were furnished by the isolation of a neutral diazo oxide (Scheme 3.1), a diazotized picramic acid (3.5), and subsequently by the isolation of true diazonium salts (3.6) [6,7].



Scheme 3.1 Resonance forms of a diazo oxide



(3.5)



(3.6)

3.1.2 Sodium nitrite and mineral acids.

The most common method of diazotization has been the treatment of the relevant amine with hydrochloric acid and sodium nitrite. A simple working hypothesis is to regard the species involved to be nitrous acid, which is formed *in situ*. In practice, the species and the kinetics are rather dependent on the conditions of this reaction. This topic has been the subject of much work and has been well reviewed [8].

In acidic conditions with 2.5 mol mineral acid per mol of amine at temperatures between 0-10°C, the nitrosonium ion is the species which is involved in nitrosating the small quantity of free amine in equilibrium with the protonated amine, the generated nitrous acid being unstable in strongly acidic conditions (Scheme 3.2) [9].

$NaNO_2 + HCl \longrightarrow NaCl + HONO$

$HONO + H^+ \longrightarrow H_2O + {}^+NO$

Scheme 3.2 Formation of the nitrosonium cation

3.1.3 The nitrous anhydride method.

Under conditions of very dilute mineral acids (0.1 M hydrochloric acids), it was reported that the kinetics of diazotization of aromatic amines was overall second order [10] and could be expressed by the following equation.

$$rate = k[Ar.NH_3^+][HNO_2] \qquad \text{Eqn. 3.1}$$

When using methylamine the reaction was found to have third order kinetics and could be represented by a different equation [11] (eqn. 3.2).

$$rate = k[R.NH_3^+][HNO_2]_2 \qquad \text{Eqn. 3.2}$$

This apparent contradiction in the rates of reaction led to further work in which support for the first equation [12-14] has lessened in favour of the second equation [15-17].

Acceptance of the third order kinetics of deamination via nitrosation has led to the proposal of two mechanisms. The first mechanism [18] has two steps in which nitrous acid is involved.

$$ArNH_2 + H_2NO_2^{\bullet} \longrightarrow ArNH_2NO^{\bullet} + H_2O$$

$$Ar.NH_2NO^{\bullet} + NO_2^{-} \longrightarrow ArNH.NO + HNO_2$$

$$ArNHNO \longrightarrow ArN_2^{\bullet}$$

Scheme 3.3 nitrous acid diazotisation - mechanism 1

This first scheme (Scheme 3.3) has been rejected because of the failure to observe catalysis by an alternative base, such as acetate ions. The alternative scheme involves the formation of the nitrous anhydride species [19].

$$2HNO_2 \underset{}{\overset{\text{fast}}{\rightleftharpoons}} N_2O_3 + H_2O$$

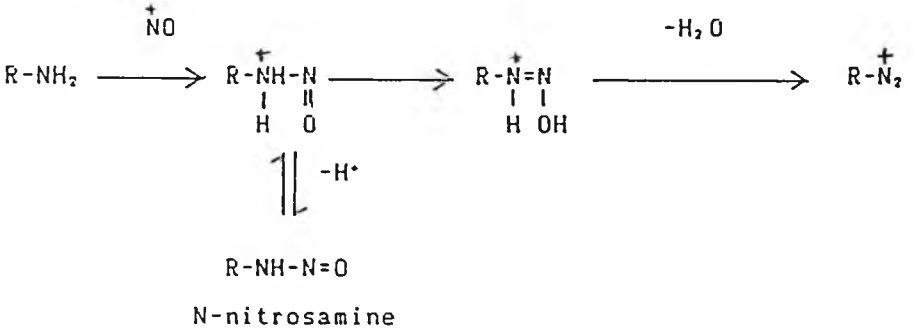
$$ArNH_2 + N_2O_3 \xrightarrow{\text{slow}} ArNH_2NO^{\bullet} + NO_2^{-}$$

$$ArNH_2NO \xrightarrow{\text{fast}} ArN_2^{\bullet} + H_2O$$

Scheme 3.4 Nitrous acid diazotisation - mechanism 2

Support for the second scheme (Scheme 3.4) has come from more detailed kinetic studies [20,21]. Isotopic exchange of ^{18}O between nitrous acid and water [22] also lend support to the formation of nitrous anhydride.

The method of diazotization can be summarised as follows:-



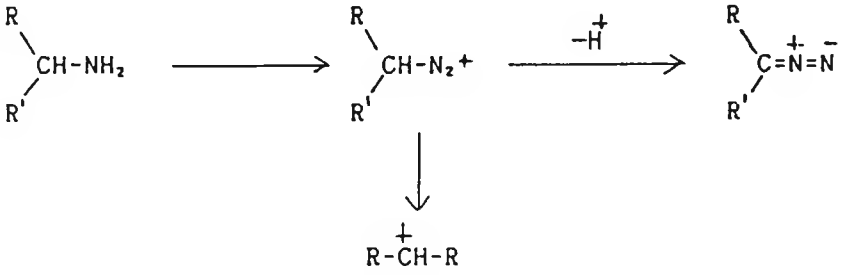
Scheme 3.5 Diazotization of an amine

3.1.4 Other nitrosating agents.

Recently, the intermediate primary n-nitrosamine was isolated [23] lending further support to the mechanism. There are a wide variety of donors for the nitroso cation. The relative strength under most conditions is as follows:-

$NO^{\bullet} > H_2N_2O > NCS\text{-}NO > Br\text{-}NO > Cl\text{-}NO > ON\text{-}NO_2$

The preparation of diazoalkanes by treatment of primary alkyl amines is dependent on conditions which facilitate the removal of a proton from the diazonium intermediate. With the exception of diazomethane, the predominant reaction is the loss of molecular nitrogen from the diazonium intermediate which results in diverse products being observed (Scheme 3.6)[4].

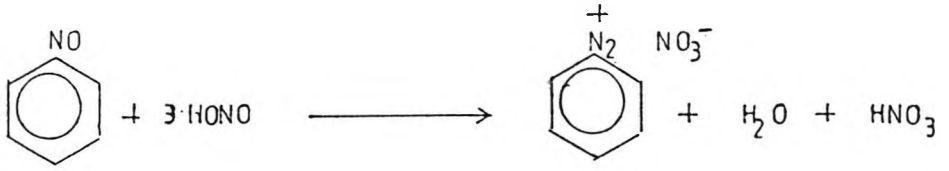


Scheme 3.6 Diazotisation of alkylamines

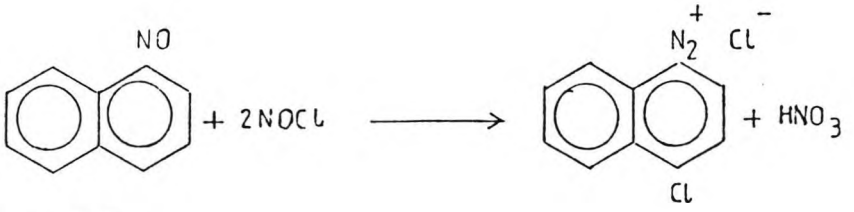
Thus, methylamine is readily diazotized by nitrosyl chloride at a temperature of -80°C. The intermediate nitrosamine can be detected by uv spectroscopy and trapped with potassium ethanoate to yield potassium methyldiazotate. Alkaline hydrolysis of either the nitrosamine or the methyldiazotate salt readily affords diazomethane [24-27].

An alternative to nitrosyl chloride is isopentyl nitrite, which is the commonly used nitrosating agent, under conditions where solubility in organic solvents is desirable [28].

In addition to diazotization by nitrosation of amines, it is also possible to form diazo compounds by nitrosation of nitroso compounds [29,30] (Scheme 3.7 and 3.8).

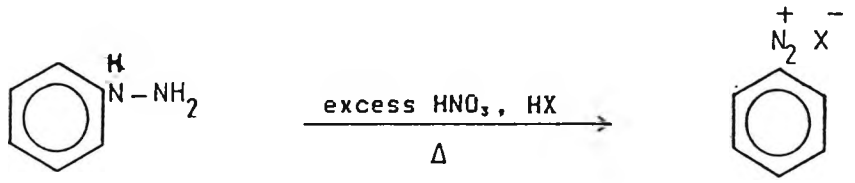


Scheme 3.7



Scheme 3.8

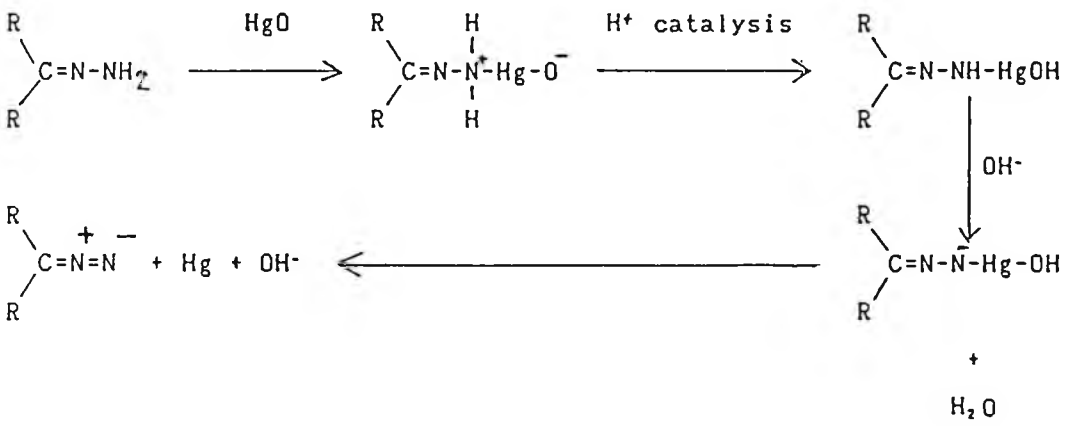
In excess nitrous acid aromatic hydrazines can be converted to diazonium salts [31] (Scheme 3.9). Normally, azides would be the expected product of treating hydrazines with nitrous acid but with gentle heating and excess nitrous acid the nitrosohydrazine decomposes to give aniline and nitrous oxide.



Scheme 3.9 Diazotization of phenylhydrazine

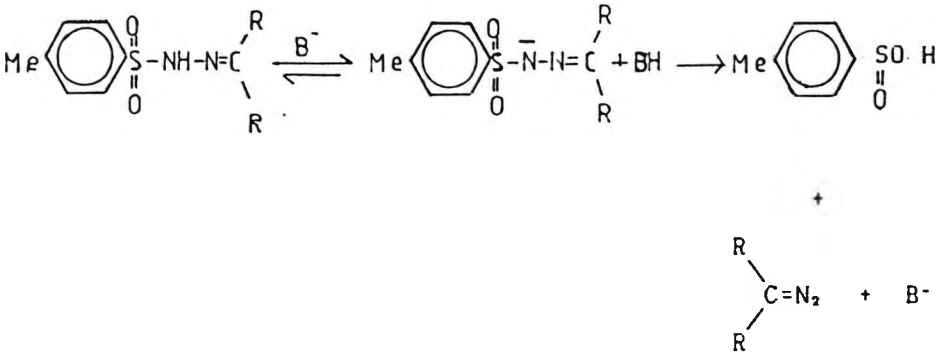
3.1.5. Metal oxides.

Diazo compounds may be prepared by the dehydrogenation of hydrazones by metal oxides such as mercuric oxide [32-38], silver oxide [39-41] and manganese dioxide [42-44]. A general mechanism has been postulated for the conversion of hydrazones to diazo compounds [45,46] (Scheme 3.10).



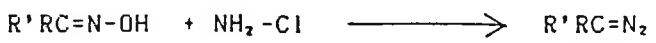
Scheme 3.10 Diazotization of hydrazones

In the case where substituents on the carbon carrying the diazo moiety are powerful proton activators, such as acyl groups, it has been proposed that a likely intermediate is the formation of mercury-bis diazoalkanes [47]. Other reagents include silver carbonate [48], lead tetraacetate [49] and iodine [50]. In addition, tosylhydrazones may also be converted into the corresponding diazo compound by the action of a base, this reaction is referred to as the Bamford-Stevens reaction [51](Scheme 3.11).



Scheme 3.11 Diazotization of Tosylhydrazones

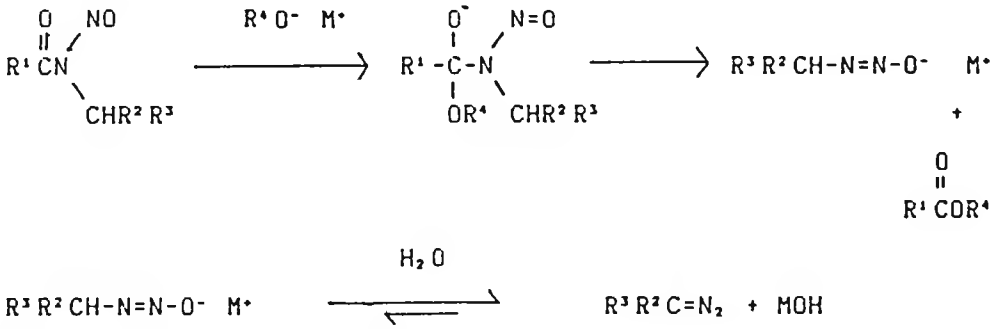
Oximes have been shown to diazotize with chloramine [52,53]. The mechanism advocated for this reaction remains a matter of conjecture.



Scheme 3.12 Diazotization of Oximes

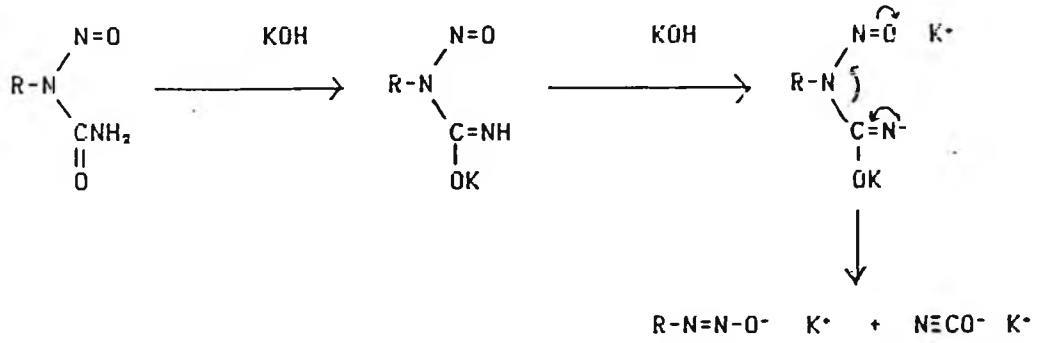
3.1.6 Acyl cleavage of N-alkyl-nitrosamides.

The alkaline cleavage of N-alkyl-N-nitrosoacyl compounds is perhaps the most common method for generating diazomethane. The earliest example of the preparation of diazo compounds from this class of compounds was the synthesis of diazomethane from N-methyl-N-nitrosourethane [54]. The use of diazourethane was superseded by the use of N-methyl-N-nitrosourea [55,56]. The mechanism of diazotization has been shown to involve a diazotate intermediate [57]. From the isolation of the by-products of the reaction a likely mechanism, which involves a nucleophilic attack on the carbonyl, was proposed for the diazotization of N-acyl-N-alkyl--N-nitrosoamines [58-61] (Scheme 3.13).



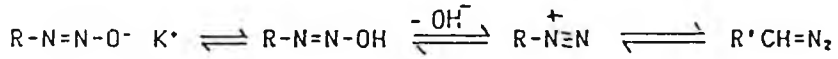
Scheme 3.13 Diazotization of N-Nitrosamines

With N-alkyl-N-nitrosourea, the reaction differs in that the alkali acts as a base abstracting a proton from the free amine to give the diazotate [61-64] (Scheme 3.14).



Scheme 3.14 Diazotization of N-nitrosoureas

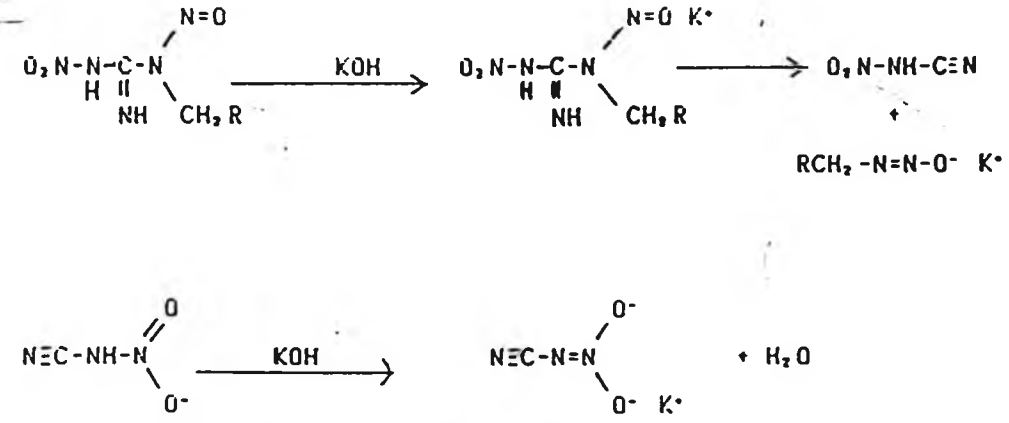
There exists a complex equilibrium involving the diazotate, the diazohydroxide, the diazonium ion and the free diazo compound. Although the equilibria concerning the alkyl diazonium ion have not been well investigated, owing to its tendency to decompose, its presence can be inferred from the products of reaction [65]. However, alkyldiazonium ions can be considered as being in equilibrium with the free diazo compound. The general equilibrium can be expressed by the following scheme:-



Scheme 3.15

The equilibrium between the diazotate and the diazonium ion is best demonstrated by arenediazotates [66] in which the diazonium ion is stabilized by interaction with the pi-electrons of the ring. In addition, increased stability exists because of the increased strength of a N-sp^2 hybridized bond over a N-sp^3 hybridized bond. An example of this complex equilibrium can be demonstrated for the p-nitrobenzenediazonium ion [67].

N-alkyl-N-nitro-N-nitrosoguanidines are cleaved by alkali in the manner similar to that of N-nitrosoureas. Their advantage over the nitrosoureas is their increased thermal stability. The isolation of the potassium salt of nitrocyanamide in some reactions suggests the following mechanism [68] (Scheme 3.16).

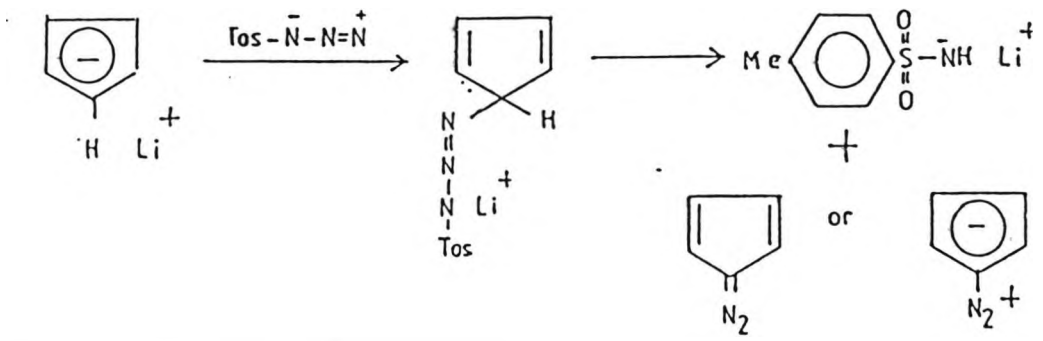


Scheme 3.16 Diazotization of N-nitrosoguanidines

Another group of reagents which belong to this class of N-acyl-N-nitrosoamines is the N-nitroso-N-*p*-toluene sulphonamides. Diazomethane can be rapidly generated from N-methyl-N-nitroso-*p*-toluene sulphonamide (diazald) at a temperature at which cleavage of the toluene sulphonic acid occurs (i.e. 50-75°C) [69]. The diazomethane/ether vapour is trapped out with a cold finger condenser, giving a relatively dry solution of diazomethane.

3.1.7 Diazo group transfer.

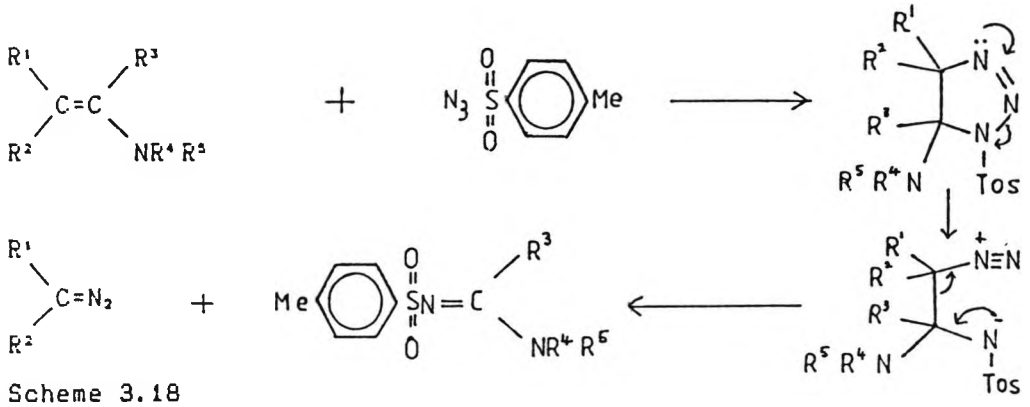
The transfer of the complete N_2 group from a donor such as azides to an acceptor compound is referred as diazo group transfer. An example of this reaction is the diazotization of cyclopentadienes using tosyl azide (Scheme 3.17). The reaction involves lithiation to produce cyclopentadienyllithium which reacts with tosyl azide. The probable intermediate is a triazene compound [70].



Scheme 3.17 diazo transfer to cyclopentadiene

Support for this mechanism comes from work on 1,4-diphenylcyclo--pentadiene in which isomeric products are obtained. This is consistent with the concept of stabilisation of a cyclopentadienyl carbanion by mesomeric structures [71].

Enamines are suitable acceptors for diazo transfer reagents. The reaction with tosylazide is associated with the cleavage of the enamine C=C and is believed to proceed via the formation of triazolines (Scheme 3.18) [72,73].

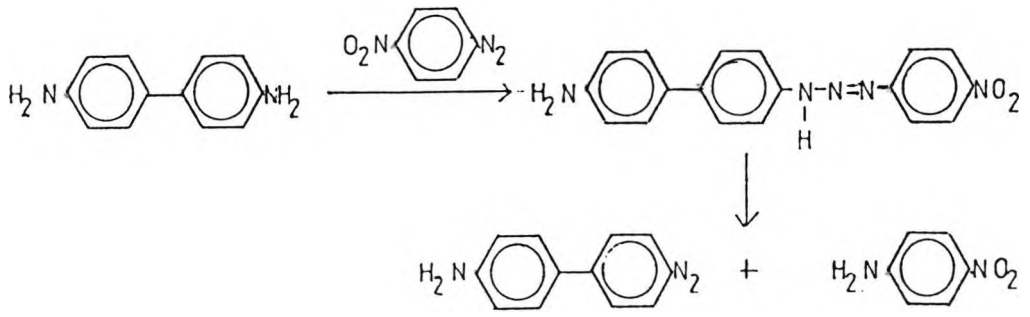


Scheme 3.18

As with enamines, a corresponding reaction has been reported to occur with alkoxyethylene and phosphoryl azide [74]. Similar reactions can be found for acetylenic compounds and are believed to proceed via triazoline type intermediate when the reagent used is cyanogen azide [75] or via 3H-pyrazole when using diazopropane [76].

3.1.8 Transdiazotization.

One special case of diazotization by diazo group transfer has been the transdiazotization in which the net result has been the transfer of the diazo function from one diazo compound to the target compound whilst retaining the functional groups within the system [77,78] (Scheme 3.19).

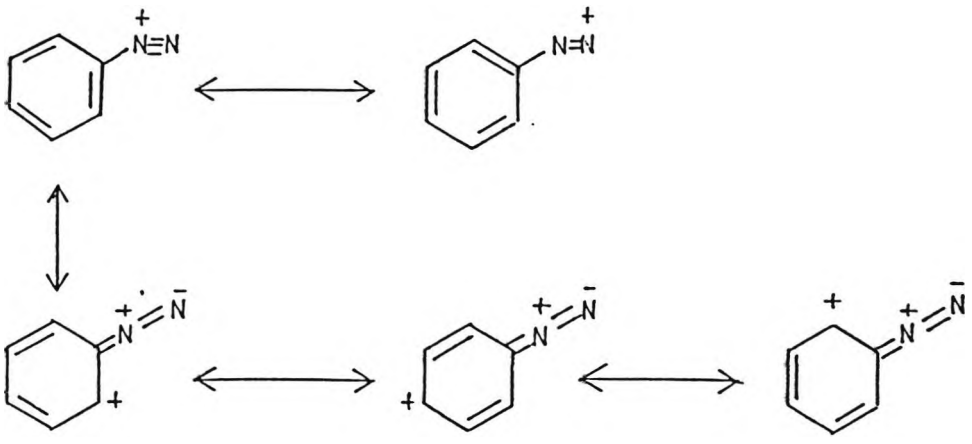


Scheme 3.19 transdiazotization

3.2 Structure.

3.2.1 Arene diazonium salts.

These are cationic diazonium compounds associated with an anionic counter ion [79-80]. The stability of the diazonium cation was attributed to its existence as a resonance hybrid of several canonical forms. In aromatic diazo compounds, these canonical forms also explained the strong electron withdrawing nature of the $-N_2^+$ group and its *ortho* and *para* directing effect for nucleophilic substitution [81-85]. Electrophilic substitution does not readily occur in aromatic diazo compounds. The nitration of the benzene--diazonium ion only occurs with difficulty and accordingly is *meta* directed [86].



Scheme 3.20 Canonical forms for the benzenediazonium ion

Arene diazonium salts have been examined by X-ray crystallography and the data for benzene diazonium chloride (Table 3.1) [87] shows the diazonium group to be linear to the $C_{(1)}$ atom of the benzene ring. The $N_{(1)}$-$C_{(1)}$ bond is 0.085 Å shorter than the aliphatic N-C. It was found that the benzene ring internal angles show a greater perturbation from that in benzene than the corresponding perturbation found in benzonitrile [88]. This increased perturbation along with the decrease in the aromatic bond lengths compared to benzene (1.397 Å) is attributed to the strong electron-withdrawing affect of the diazonium group. The closest distance of a chlorine to either of the diazo nitrogens is 3.22 Å which indicates a separation distance consistent with ionic bonding (3.50 Å) (cf. 1.77 Å for C-Cl in carbon tetrachloride).

Bond	length (A)
$N_{(1)}$-$N_{(2)}$	1.097
$N_{(1)}$-$C_{(1)}$	1.385
$C_{(1)}$-$C_{(2)}$	1.374
$C_{(2)}$-$C_{(3)}$	1.383
$C_{(3)}$-$C_{(4)}$	1.376

atoms	Bond angle (°)
$N_{(2)}$-$N_{(1)}$-$C_{(1)}$	180
$C_{(6)}$-$C_{(1)}$-$C_{(2)}$	124.8
$C_{(1)}$-$C_{(2)}$-$C_{(3)}$	117.6
$C_{(2)}$-$C_{(3)}$-$C_{(4)}$	119.8
$C_{(3)}$-$C_{(4)}$-$C_{(5)}$	121.7

Table 3.1 Bond lengths and bond angles for the benzenediazonium chloride

Similar results were obtained for benzene diazonium tribromide [89]. In this case, the separation of the nearest bromine to either of the nitrogens is 3.31 A, which implies an ionic interaction between the diazonium group and the anion. Further, the bond length between $N_{(1)}$-$N_{(2)}$ is of a value which matches the N-N bond in molecular nitrogen (1.097 A) [90]. Further support for the N-N triple bond has come from a combination of data from Raman spectroscopy, which gives a stretching vibration band of 2290 cm^{-1} [91-93], and from various theoretical calculations [94-95]. As a consequence of the triple bond, the ground state can be considered to be predominantly that of a phenyl group attached to the positive nitrogen of the diazonium group [96]. Thus, in the literature, the relative contribution of the extreme resonance forms has been related to considerations of bond length and bond order.

A point of caution that ought to be mentioned is that when two resonance forms of different bond order are contributing, the resultant hybrid has a bond length smaller than the interpolated value [97]. Another qualification is that this deduction of bond order from bond length is based on the averaging of bond order of covalent bonds and does not fully take into account the ionic character of covalent bonds. The idea of this covalent/ionic resonance may be considerable and be 0.1Å less than the interpolated value.

The stabilization of diazonium compounds can also be via intramolecular salts. Examples of these zwitterionic species have included diazo oxides, which can be represented either by a structure of diazonium phenolate or by the covalent quinone diazo compound [6,98]. Another example is the case of diazonium aryl-

-sulphonates, of which 2-diazonium-4-phenolsulphonate monohydrate and *p*-benzenediazonium sulphonate have been examined by X-ray crystallography [99,100]. In the diazonium arylsulphonates, the zwitterionic form is believed to predominate over the quinoid structure, whilst for the *p*-diazo oxides, as typified by *p*-diazo phenoxide ($OC_6H_4N_2$), it appears that the quinoid structure predominates.

3.2.2 Diazotates.

These compounds are the result of additions of a strong base to an aqueous solution of diazonium salt. The reaction can be summarised by the following scheme.

$ArN_2^+ + OH^- \xrightleftharpoons{\text{slow}} syn\text{-}ArN_2OH$

$syn\text{-}ArN_2OH + OH^- \xrightleftharpoons{\text{fast}} syn\text{-}ArN_2O^- + H_2O$

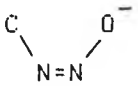
$syn\text{-}ArN_2O^- \xrightleftharpoons{\text{slow}} anti\text{-}ArN_2O^-$

$ArN_2^+ + OH^- \rightleftharpoons anti\text{-}ArN_2OH$

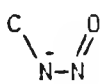
$anti\text{-}ArN_2OH \rightleftharpoons anti\text{-}ArN_2O^- + H^+$

Scheme 3.21 Diazotate equilibria

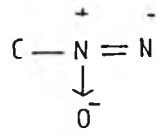
Direct confirmation of kinetic and spectral studies that show *syn*- and *anti*- diazotates to be structural isomers comes from X-ray structural analysis of potassium-syn-methyl- -diazotate [96]. The bond lengths for $N_{(1)}$-$N_{(2)}$ and $N_{(2)}$-O are consistent with a $N_{(1)}$-$N_{(2)}$ double bond and a $N_{(2)}$-O single bond rather than a $N_{(1)}$-$N_{(2)}$ single bond paired with a $N_{(2)}$-O double bond. This suggests that the extreme resonance form predominates (3.7). The distance $N_{(1)}$-O is much greater than $N_{(2)}$-O and thus the alternative structure (3.9) can be discounted.



(3.7)



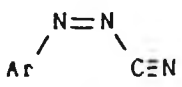
(3.8)



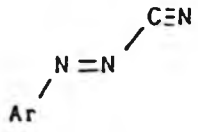
(3.9)

Scheme 3.22 Resonance forms for a diazotate

Similar to the diazotates are the aromatic diazocyanides. These compounds also show a *cis* (*syn*) (3.10) and *trans* (*anti*) geometry (3.11). Unfortunately, only the stable *anti* isomers have been studied by X-ray crystallography [97-100].

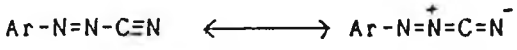


(3.10)



(3.11)

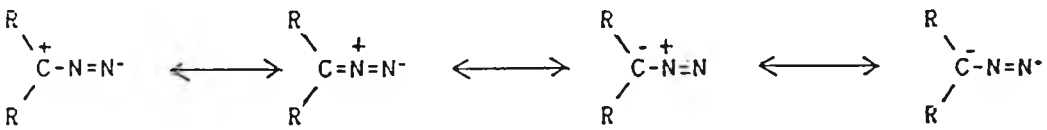
Suggestions that the nitrile group may also significantly contribute another resonance form (3.13) do not seem to be borne out by the X-ray crystallographic data. The C-N triple bond length is equal to or smaller than that in most nitriles.



(3.12) (3.13)

3.2.2 Diazoalkanes.

These compounds can be represented by several canonical forms. The simplest of this class is diazomethane. the carbon and two nitrogen atoms in diazomethane were shown to be linear and the molecule to be planar [101,102]. The stability of diazomethane can be attributed to it being a resonance hybrid of several forms (Scheme 3.23).



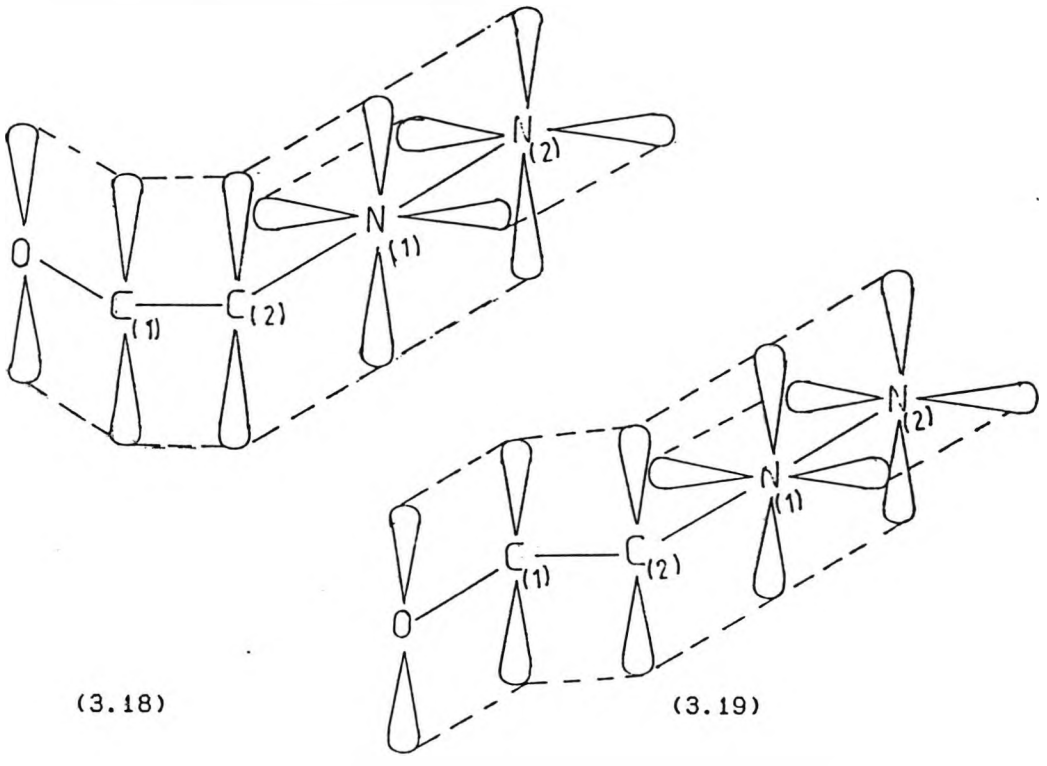
(3.14) (3.15) (3.16) (3.17)

Scheme 3.23 Resonance forms for a diazoalkane

From the diazoalkanes examined to date the C-N bond length (1.28-1.32 A) reflects a bond which can be represented as a hybrid between a C-N single bond (1.47 Å) and a C-N double bond (1.23-1.26 A). The N-N separation (1.12-1.13 Å) is greater than that found in dinitrogen and significantly greater than that found for the diazonium group in benzenediazonium chloride (1.09 Å). Thus it can be suggested that the $=CN_2$ group is a resonance hybrid between the forms (3.15) and (3.16) (Scheme 3.23).

3.2.3 Diazoketones.

From the resonance structures, it might be expected that ∝-substituents which provide further means of resonance stabilization should afford more stable diazo compounds. This hypothesis would seem to be particularly relevant with regard to ∝-diazoketones. From the study of 1,4-bisdiazo-2,3-butanedione [103], the $C_{(2)}$-$N_{(1)}$-$N_{(2)}$ bond angle and $C_{(1)}$-$C_{(2)}$-$N_{(1)}$ bond angle, respectively 176.8° and 116.5°, show that the diazo group is linear and that the hybridization of the $C_{(2)}$ carbon is essentially sp^2. Thus, a system where there is delocalization across the five atoms of the N_2CCO group can be envisaged. As a consequence of this delocalization, the $C_{(1)}$-$C_{(2)}$ bond may be regarded as having double bond character with hindered rotation and hence giving rise to rotational isomers (3.18 and 3.19).



(3.18) (3.19)

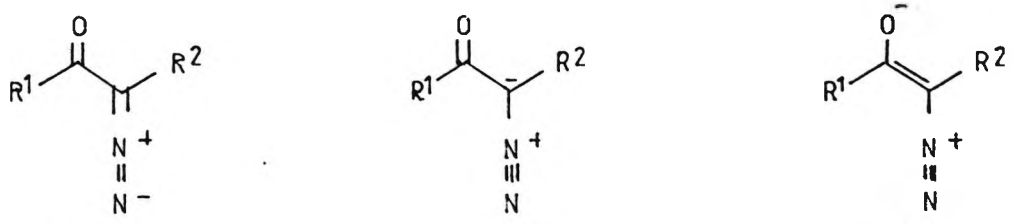
It has been observed using uv spectroscopy that the $-COCN_2$ group has an intense band at 245-250 nm which becomes weaker with increasing solvent polarity. At same time another band at 270-290 nm increases in intensity. The presence of an isobestic point is linked to the availability of the carbonyl lone pair of electrons. This suggests the existence of two species in equilibrium, that is, the result of transposable hydrogens within keto-enol tautomerism or alternatively within a diazo-isodiazo equilibrium. However, the observation of this isobestic point in diazoketones which do not

contain such transposable hydrogens [104,105] suggest that the two species are two states, one state being a condition where the carbonyl exhibits no hydrogen bonding and the other state is where there is hydrogen bonding [104]. A similar argument can be applied to favour this suggestion over the hypothesis that the isobestic point is the result of the conformational equilibrium between *cis* and *trans* forms [106,107].

In the IR, the carbonyl of simple diazoketones can be found around 1660 cm^{-1}. However, when hydrogen bonding increases, e.g. by using an increasing phenol/tetrachloroethene ratio, there is a decrease in the intensity of this band. At the same time there occurs an increasing of intensity for the peaks 1643 cm^{-1} and 1633 cm^{-1} [105]. In theory, hydrogen bonding may occur to both the carbonyl oxygen and also to the terminal nitrogen of the diazo group. Work with diphenyldiazomethane suggests that hydrogen bonding involves the carbonyl group in α-diazoketones [108].

The existence of the predicted rotamers in diazoketones was demonstrated by examination of the N-N stretching frequency in their infrared spectra. In apolar solvents, where the rotamers would be expected to be in equilibrium, the observation of the splitting of the diazo band has been taken to represent the two rotamers. The foundation for this proposal lies in the expectation that in certain diazo ketones the rotamers are stabilized by a sufficiently high energy barrier to prevent rapid interconversion [109,110]. Hence, 1-chloroacetyldiazomethane, 1-dichloroacetyl-diazomethane, 3-diazo--1,1,5,5-tetramethyl-2-hexanone, diazoacetophenone and 2-diazo-1--phenyl-1-propanone all show a single diazo stretching band whilst diazoacetone, 1-acetyl-1-diazoethane and benzoyldiazophenylmethane all show a splitting of the diazo band.

The frequencies for the carbonyl and diazo stretch are around 1650 cm^{-1} and 2080 cm^{-1} respectively [109-114]. Conjugation between these two groups shifts both their stretching frequencies from those observed when they are isolated, the carbonyl band moving to lower frequency and the diazo stretch to higher frequency. The conclusion is that the carbonyl group has less double-bond character than it does in ketones whilst the order of the N-N bond is correspondingly higher than in diazohydrocarbons. This shifting of frequencies can be assumed to demonstrate the resonance form (3.21) predominating over the form (3.22)(Scheme 3.24).



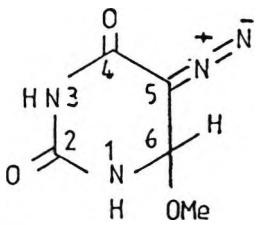
(3.20) (3.21) (3.22)

Scheme 3.24 Diazoketone resonance forms

The greater contribution of form 3.22 to the resonance hybrid is also consistent with the C-C bond of the $-COCN_2$ group displaying partial double bond character. Indeed, the greater the contribution of the enolate form, the higher the frequency of the diazo stretch band and the lower its intensity.

The existence of the rotamers at low temperatures was demonstrated in diazomethyl ketones using ^1H n.m.r. [115-117]. In the experiment, the singlet observed for the methine proton was demonstrated at low temperatures to split into two peaks.

X-ray crystallographic data on diazoketones has been on atypical compounds such as 1,4-*bis*diazo-2,3-butanedione [103]. Fortuitously, the other studies on atypical diazoketones studies included "5-diazo-6-methoxy-6-hydrouracil" (3.23) and 2'-deoxy-5--diazo-$O^{4'.5'}$-cyclouridine (3.24) which are of particular interest to the this work. Unfortunately, only the uncorrected bond lengths were reported [118].



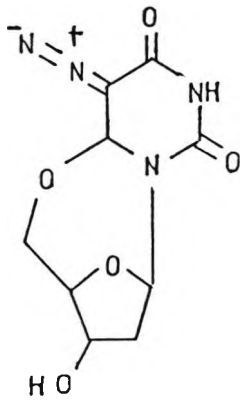
(3.23)

Bond	length (A)	Typical bond Lengths
$N_{(1)}-C_{(2)}$	1.337	1.47/C-N
$C_{(2)}-N_{(3)}$	1.370	
$N_{(3)}-C_{(4)}$	1.381	
$C_{(4)}-C_{(5)}$	1.436	1.54/c-c 1.34 C=C
$C_{(5)}-C_{(6)}$	1.472	
$C_{(6)}-N_{(1)}$	1.440	
$C_{(2)}-O_{(2)}$	1.241	1.16 or 1.22
$C_{(4)}-O_{(4)}$	1.218	
$C_{(5)}-N_{(5)}$	1.332	1.28-1.32
$N_{(5)}-N_{(7)}$	1.113	1.09 diazonium
$C_{(6)}-O_{(6)}$	1.421	
$O_{(6)}-C_{(8)}$	1.436	

Table 3.2 X-ray crystallographic data for 5-diazo-6-methoxy--1,6-dihydrouracil.

The molecules of ∝-diazoketones for which X-ray crystal structures have been reported are not suitable for making general conclusions about ∝-diazoketones and thus, there has been only a superficial attempt to interpret the observed data (Table 3.2 and Table 3.3) by simple comparison with other compounds, such as uracil, uridine, diazomethane and aromatic diazonium compounds.

Bond	length (Å)
$N_{(1)}$-$C_{(2)}$	1.349
$C_{(2)}$-$N_{(3)}$	1.381
$N_{(3)}$-$C_{(4)}$	1.361
$C_{(4)}$-$C_{(5)}$	1.431
$C_{(5)}$-$C_{(6)}$	1.484
$C_{(6)}$-$N_{(1)}$	1.457
$C_{(2)}$-$O_{(2)}$	1.219
$C_{(4)}$-$O_{(4)}$	1.234
$N_{(5)}$-$N_{(7)}$	1.120
$C_{(4)}$-$O_{(5')}$	1.415
$N_{(1)}$-$C_{(1')}$	1.486
$C_{(1')}$-$C_{(2')}$	1.529
$C_{(2')}$-$C_{(3')}$	1.519
$C_{(3')}$-$C_{(4')}$	1.522
$C_{(4')}$-$O_{(1')}$	1.449
$O_{(1')}$-$C_{(1')}$	1.404
$C_{(3')}$-$O_{(3')}$	1.431
$C_{(4')}$-$C_{(5')}$	1.519
$C_{(5')}$-$O_{(5')}$	1.433



(3.24)

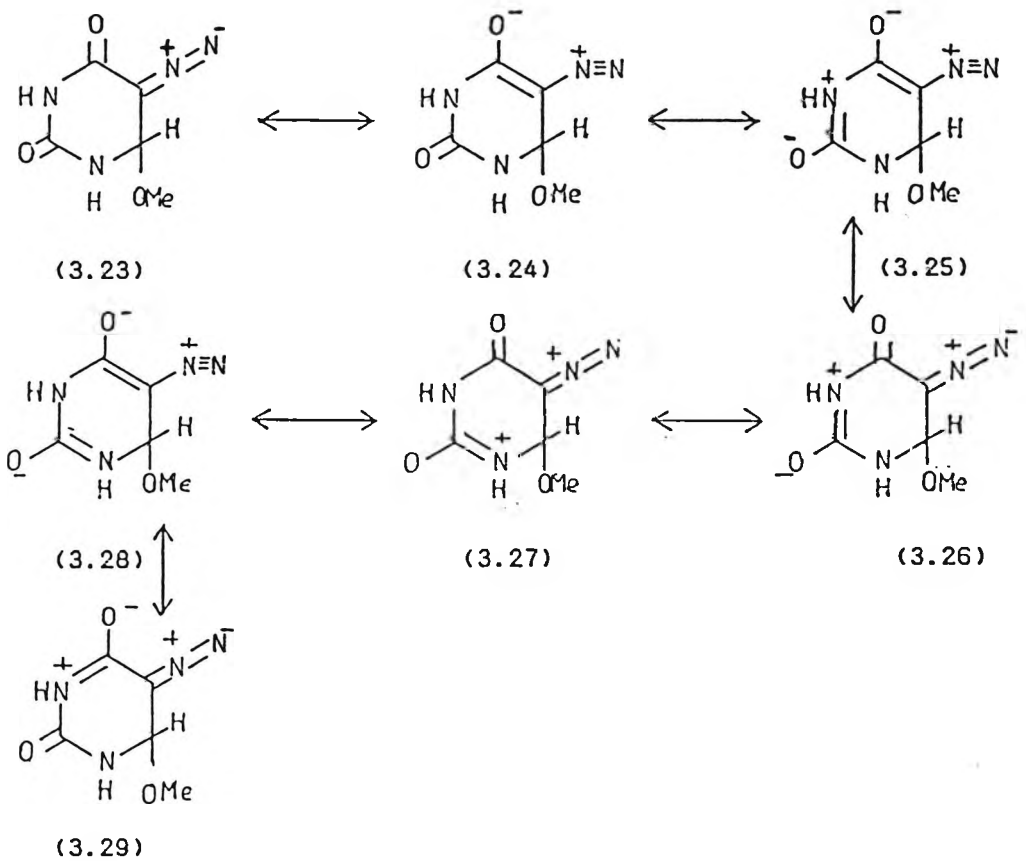
Table 3.3 X-ray crystallographic data for 2'-deoxy-5-diazo--1,6-dihydro-$O^{4,5'}$-cyclouridine

3.3 Conclusion.

The suggested structure for 5-diazo-6-methoxy-1,6-dihydrouracil (3.23) represents the uracil ring in its keto form. By extrapolation of the studies on diazoketones, several features might be proposed for the compound. It might be predicted that X-ray crystallography would demonstrate a linear diazo group and consequently, the N-N bond length of the diazo might be expected to be greater than that of the bond length for a diazonium group but less than that for an azo group. Similarly, the ∝-carbonyl bond length would be expected to be greater than a typical carbonyl in a ketone but less than a single C-O bond. It would be expected that the bond length for the C-2 carbonyl should be shorter than that of the C-4 carbonyl, owing to delocalization reducing the C-4 carbonyl bond order.

From the data, the expected finding for the N-N separation of the diazo group is consistent with the working hypothesis, that is indicating a delocalization. This observation is supported further by the finding of double bond character of the $C_{(4)}$-$C_{(5)}$ bond, as

demonstrated by its intermediate bond length. Inspection of the carbonyl bond lengths reveals an unexpected result. It shows that the bond length for the C-4 carbonyl is 1.218 A which compares favourably with the value for ketones and aldehydes (1.20 A) but longer than the C=O bond in carbon dioxide (1.16 A). The value compares favourably with the carbonyl bond length (1.22 A) in 1,4-bisdiazo-2,3-butadiene. The finding that the C-2 carbonyl has a longer bond length than the C-4 carbonyl is difficult to reconcile with the concept that the C-O bond is a simple carbonyl. From the bond length, suggestions as to the enol form can be dismissed since the C-O distance is very much smaller than that for the average C-O single bond (1.43 A). A clue to this anomaly can be obtained from the $C^{(2)}$-$N^{(3)}$ (1.37 A) and the $N^{(3)}$-$C^{(4)}$ (1.381 A) bonds. Both these bonds are shorter than the average C-N bond (1.47 A) and longer than the C=N bond (1.32 A) which prompts the conclusion that there is a resonance hybrid which totally agrees with the theory on imides. Thus, in this thesis it is now proposed that 5-diazo-6--methoxy-6-hydrouracil can be expressed as a hybrid of the following likely resonance forms (Scheme 3.25).



Scheme 3.25 Resonance forms of 5-diazo-6-methoxy-1,6-dihydrouracil

It can be predicted that for the most part 2'-deoxy-5-diazo--1,6-dihydro-$O^{6,5}$-cyclouridine will show the same characteristics as the simpler base. 5-diazo-6-methoxy-1,6-dihydrouracil. The N-N separation of the diazo group falls within the predicted range suggesting a resonance hybrid for the N-N bond. This is supported by the bond length for the $C_{(4)}$-$C_{(5)}$, which implies partial double bond character. Unlike the examined diazouracil, the nucleoside clearly shows a longer bond for the C-4 carbonyl which is consistent with the prediction of reduced double bond character owing to delocalization with the diazo nitrogens. The $C_{(2)}$-$N_{(3)}$, and $N_{(3)}$-$C_{(4)}$, bonds indicate that these bonds are hybridized. The bonds for the sugar portion of the nucleoside show a good correspondence with expected bond lengths as does the bond in the ether bridge between C-6 and C-5'.

3.4 References.

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(See J.H.Ridd, *J. Chem. Soc. Q. Rev.* 1961, 15, 418).

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(See J.H.Ridd, *J. Chem. Soc. Q. Rev.* 1961, 15, 418).

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Chapter 4 Spectroscopic analysis of nucleic acid Components.

In the field of nucleoside chemistry, one of the main problems has been the identification of the products. This chapter will review some of the spectroscopic techniques that have been used to determine structure.

4.1 Ultraviolet Spectroscopy.

The interaction of ultraviolet radiation with the valence electrons in organic molecules has provided a means to examine structures and environments of chromophores. In order to understand the u.v. spectra of pyridine-related nucleic acid components, it is appropriate to first consider the u.v. characteristics of the parent pyrimidine chromophore itself.

4.1.1 Pyrimidine.

Above the vacuum ultraviolet range, the u.v. spectra of heteroaromatic molecules show two bands. These bands are roughly grouped into short wavelength and long wavelength, termed B and L respectively [1]. Pyrimidine in gas phase spectra shows a complicated spectrum which is characteristic of aromatic compounds in general [2,3]. It has maxima at 191, 233, 238, 243, 248, 297, 305, 309, 312, 315, 316, and 322nm. An insight into the u.v. spectra of pyrimidine can be obtained by a simple comparison to benzene, since pyrimidine can be regarded as a diaza analogue of benzene. The maximum at 191nm corresponds to the 202-204nm absorption band in benzene, whilst the absorptions at 233, 238, and 248 correspond to the 255nm band in benzene. Thus, the broad bands at 191nm and around 240nm could be attributed to the 'forbidden' *pi-pi** transitions. The other peaks (297, 305, 309, 312, 316, and 322nm) can be attributed to differences in structure between benzene and pyrimidine. The most obvious difference is the presence of lone pair electrons on the azamethine nitrogens. It has been suggested that this longer wavelength grouping is due to n-pi* transitions. Support comes from the observation of the bands at wavelengths slightly higher than 300nm in other aza-analogues of benzene, for example, s-tetrazine [4].

Predictably, the group of absorption peaks around 300nm is particularly sensitive to the solvent environment. The influence of solvents can be explained in terms of two factors. One factor is the availability of the lone pairs for this transition. As might be expected, this band disappears in strongly acidic conditions. The other factor is the stabilization of either the ground or the excited state by solvent polarity, since a change in the dipole moment of the molecule occurs with the transition. It is observed that in apolar solvents the absorption of the n-*pi** transition occurs around 300nm, whilst in polar solvents this transition occurs at about 280nm. This implies that polar solvents stabilise the more polar ground state. As a corollary, the interaction of the nitrogen lone pair electrons with the pi-system results in the lowering of polarity in pyrimidine. This condition is better stabilised by apolar solvents resulting in the bathochromic effect of these solvents.

As with benzene, ring substitution causes the main absorption peak to exhibit a bathochromic effect. This is often accompanied with a hyperchromic effect [5]. The substituent effects for pyrimidine are more complex than in benzene, where there is a centre of symmetry. It appears that the bathochromic effect is determined by the nature of the substituent and its position on the ring. This is analogous to the effect of a further substituent in mono--substituted benzenes.

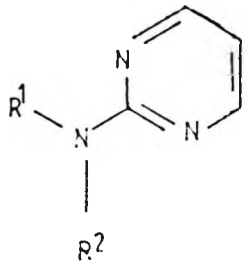
Electron-withdrawing substituents ($-CO_2H$, -Cl, -SOMe, and $-SO_2Me$) produce a small bathochromic shift when present on C-2 or C-5. When these substituents are on C-4, there is a larger bathochromic shift which is accompanied by a hyperchromic shift.

The electron-releasing substituents produce greater bathochromic and hyperchromic effects when on C-2 and C-5 than on C-4.

In polysubstituted pyrimidines containing one strongly conjugated substituent, the effects of the individual substituents are additive. This is analogous to substituent effects on the absorption in benzoyl derivatives.

4.1.2 Aminopyrimidines.

The u.v. spectra of 2-aminopyrimidine and its derivatives have been used in determining the structural features of these compounds (4.1-4.3).

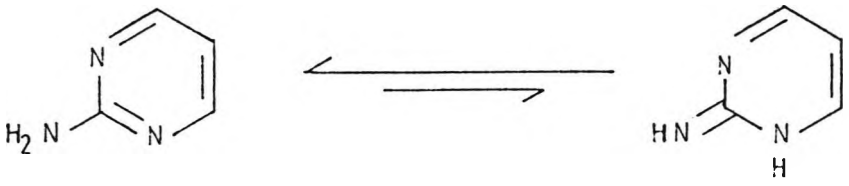


4.1) $R^1 = R^2 = H$,

4.2) $R^1 = H$, $R^2 = Me$,

4.3) $R^1 = R^2 = Me$.

2-Aminopyrimidine (4.1), 2-methylaminopyrimidine (4.2) and 2-dimethylaminopyrimidine (4.3) gave very similar u.v. spectra, *i.e.* they have two maxima with absorptions around 240nm and 300nm. The relative insensitivity of the band around 300nm to changes in solvent indicates that this band is not the n-*pi** transition found in pyrimidine but rather a splitting of the *pi*-*pi** transition band by a conjugating substituent. A similar observation was reported [5,6] for 2-phenylpyrimidine. Based on the similarity in spectrum, a similarity in chromophore structure can be deduced. The only consistent structure for 2-aminopyridine was to conclude that it existed in the amino tautomeric form (4.4) rather than the imino form (4.5).



(4.4) (4.5)

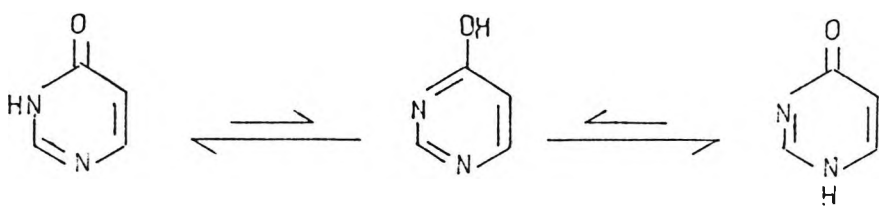
Examination of the cationic forms, *i.e.*, the conjugate acids, show the same spectral features [7]. This implies that protonation does not disturb or alter the conjugation of the substituent with the *pi*-system. Thus, protonation occurs at an azamethine nitrogen in the ring. The observations for the 2-aminopyrimidines are mirrored in the spectra for 4-aminopyrimidines [7].

Predictably, the u.v. spectrum for 2-imino-1-methyl-1,2--dihydropyrimidine is different to that of 2-aminopyrimidine. This difference is pronounced between the spectrum of 4-imino-1-methyl--1,4-dihydropyrimidine and 4-aminopyrimidine [8,9].

4.1.3 Hydroxy- and mercapto- pyrimidines.

Like the amines, hydroxy- and mercapto- pyrimidines can exist in two forms. For hydroxypyrimidines, the keto-enol equilibrium is dependent on the position of the hydroxy group on the ring. Ultraviolet spectral evidence has shown that whilst 2-methoxy--pyrimidine and 4-methoxypyrimidine show spectra similar to substituted pyrimidine rings, 2-hydroxy- and 4-hydroxy- pyrimidine have spectra which are different. The u.v. spectra of the latter two resemble their N-methylated derivatives. Thus, it can be concluded that 2-hydroxy- and 4-hydroxy- pyrimidines exist in the lactam form [8,9]. The method of comparing spectra of N-alkylated and O-alkylated derivatives provides a means of probing the lactam-lactim equilibrium. This method can be applied to the mercaptan-thio equilibrium by correspondingly comparing the sample with suitable S-alkylated and N-alkylated compounds. Similar arguments were applied to the observed spectra of pyrimidinthiones and show that the mercaptan-thio equilibrium lies towards the side of a pyridinethione [10]. 4-Pyrimidone can exist as two structural isomers, 1H-4-pyrimidone and 3H-4-pyrimidone (Scheme 4.1). The ratio of these isomers in solution has been estimated to be 5:2. However, 4-pyrimidinethione seems to exist solely as the 3H-4--pyrmidinethione. 5-Hydroxypyrimidine exists mainly as the phenolic form.

By comparing the u.v. spectrum of uracil with spectra of its O-alkylated and N-alkylated derivatives, it has been shown to exist totally in the ketonic form (2,4-pyrimidinedione) [11,12].



Scheme 4.1 Tautomeric forms of 4-pyrimidone

4.1.4 Purine.

In 9-methyl-9H-purine, a low intensity peak at 310nm corresponds to an n-pi* transition [13]. Some of the characteristics of the u.v. spectra of purines can be explained by treating them as derivatives of 5,6-diaminopyrimidine [14,15]. Most substituted

purines exhibit two broad bands, located near the 263nm and 188nm bands of an unsubstituted purine [13]. The peak at 263nm was reported to be a *pi-pi** transition comparable to the 255nm band in benzene [16]. The slight inflexion around 240nm was considered to be analogous to the benzene peak around 204nm.

In general, the effect of substituents on the absorption band is to shift it to longer wavelengths. The magnitude of the bathochromic effect would appear for the most part to be related to the position on the purine molecule. It was found that the bathochromic shift is greatest at C-2, then C-8 and finally C-6. This effect in purines has not proved to be additive and no empirical rules have been offered.

Purines substituted with hydroxy or amino groups are capable of existing in different forms. As with the pyrimidines, u.v. spectroscopy has provided evidence that amino substituents exist as the amine rather than the imine tautomer and that hydroxy groups in the lactim-lactam equilibrium favour the lactam form [4,9,13,17-18]. The use of the correct tautomer was important in the discovery of the structure of DNA [19].

The u.v. spectra of the pyrimidine nucleosides are very similar to those of the corresponding N-alkylpyrimidines. Only in very basic conditions (pH 11) does the spectrum begin to show different characteristics. This change in the spectrum has been associated with the ionization of the hydroxyls on the sugar ring and as such allows a distinction to be made between ribonucleosides and deoxyribonucleosides [20].

The u.v. spectra of nucleic acids and polynucleotides show a well defined maximum at 259nm associated with chromophoric pyrimidine and purine residues. The intensity of polynucleotides shows a hypochromic effect compared to the constituent nucleosides and hyperchromic changes are attributed to the degree of denaturation [21,22].

The 294nm wavelength is the usual monochromatic frequency which is chosen for measuring the concentration of polynucleotides.

4.1.5 Diazo compounds.

The u.v. spectra of simple diazoalkanes have been recorded in the gas phase [25] and in hexane [26]. The diazoalkanes are

characterised by a diffuse band around 450nm of very low intensity (ε, 3 - 10). There is a slight bathochromic shift as the homologues become larger. Aryl substitution causes a large hypsochromic shift. The u.v. spectrum shows two main regions of absorption, around 300nm and in the 135-200nm range. The u.v. spectra of several diazo compounds have been tabulated [27-30]. The u.v. spectra of diazoaldehydes show two absorption bands around 250nm and 280nm in addition to a band at long wavelength 350-400nm. In diazoketones, the conjugation of the diazo and the carbonyl groups shifts the long wavelength band hypsochromically when compared with the corresponding band for diazoalkanes. This hypsochromic shift is attributed to an n-*pi** transition [31]. The u.v. absorption bands are shifted bathochromically to the corresponding ketones. A quantum-mechanical treatment of the u.v. spectra of diazoketones has satisfactorily explained the observed features in terms of the existence of a *cis/trans* geometry [32]. A similar distinction between *syn*- and *anti*- diazotates can be determined in aqueous alkaline solution. The *anti*- isomers have absorption maxima between 270 and 280nm whereas the *syn*- isomers show a broad band inflexion in that region [33].

4.2 Infrared Spectroscopy.

4.2.1 Pyrimidines and Purines.

4.2.1.1 C=O absorptions.

The solid hydroxy derivatives of pyrimidines and purines show no strong absorptions in the ranges 3650-3590 or 3600-3200cm^{-1}. This implies an absence of free or H-bonded hydroxyl groups. This is consistent with the finding that the keto- tautomer predominates in both 2-hydroxy- and 4-hydroxy- pyrimidines. Similar observations were reported for uracil implying that it contains two carbonyls [34,35].

The normal carbonyl stretch around 1720cm^{-1} is shifted to lower frequency to 1700-1620cm^{-1} because of the α-nitrogen in compounds such as uracil. The intrinsic frequencies of the C=N and the C=C bonds are considered to be around 1600cm^{-1}. Therefore, the base residues often have very complicated vibrations in that region. The protonation of 2'-deoxycytidine produces two strong bands, the

one at 1720cm^{-1} being attributed to a "normal" carbonyl group by removing the conjugating effect of the N-3 atom. The other band at 1680cm^{-1} is attributed to the C=N vibration [36].

4.2.1.2 N-H absorptions.

As a consequence of having a cyclic amide/imide structure, these hydroxypyrimidines exhibit broad band absorptions around 3100cm^{-1}. These bands are associated with the N-H stretching vibrations. Often, the carbonyl absorptions are complicated by the presence of in-plane deformations of the N-H group around 1750-1550cm^{-1}. Pyrimidine shows a strong band around 1570cm^{-1}. Uracil is characterised by strong absorption bands around 1741 and 1719cm^{-1}. The absence of the band at 1741cm^{-1} in N-deuterated and 1-methyluracil leads to the uracil 1741cm^{-1} band being attributed to the H-1 in-plane deformation. The N-H and NH_2 out-of-plane deformation vibrations occur in the range 950-550cm^{-1}. The H-3 out-of-plane deformation in 1-methyluracil occurs at 870cm^{-1} and at 882cm^{-1} for 1-methylthymine.

Purine shows two bands at 1610 and 1570cm^{-1}. A weak band often observed with the two bands is the 1590cm^{-1} band which is assigned to the H-9 in-plane deformation. The out-of-plane deformation for N_9-H in purine is at 865cm^{-1} whilst those of adenine, hypoxanthine and guanine occur around 900cm^{-1}. The NH_2 waging band (out-of--plane deformation) is at 650cm^{-1}. The amino group in guanine has the same vibration a the lower frequency of 580cm^{-1}. The same vibration can be observed in 9-methylguanine at 690cm^{-1} [37].

Aminopyrimidines are characterised by the observation of strong absorption bands in the 3400-3100cm^{-1} range. In cytidine the band at 1670cm^{-1} is ascribed to the NH_2 scissoring vibration.

4.2.1.3 Skeletal absorptions.

The C-H stretches for hydroxy- derivatives of pyrimidine are not easily observed as they are normally overlapped by the stronger N-H absorption bands. One band which has been identified in pyrimidine is at 3047cm^{-1}. The C-H stretches of purines have been identified by comparing their spectra with the spectra of the corresponding deuterated compounds [38]. The absorptions for H-8, H-6 and H-2 in purine occur at 3098, 3060, and 3023cm^{-1} respectively.

In protonated adenine, the strong band at 1712cm^{-1} is attributed to the C=N$_1$ stretch, owing to protonation at N-1 which localises the *pi*-electrons within the bond [38,39]. The carbonyl absorption bands for the two carbonyls in hypoxanthine occur around 1680cm^{-1}. From comparisons between the un-deuterated and the deuterated spectra, absorptions observed at 1100, 804, and 790cm^{-1} have been attributed to the C$_4$-H deformations.

The standard practice of comparing deuterated spectra with the undeuterated ones allows skeletal vibrations to be assigned. After having taken into account absorption bands connected with the functional groups and with in-plane and out-of-plane deformations, the remaining absorptions can be attributed to vibrations between the ring atoms. These absorptions occur at 570, 450, and 410cm^{-1} for purine. Adenine has absorptions at 800, 720, 540, and 330cm^{-1}. Hypoxanthine has the frequencies at 800, 570, 370 and 350cm^{-1}, whilst for guanine they occur at 780, 705, 690, 515, 500, and 350cm^{-1}. The main absorptions are given below (Table 4.1 and 4.2).

wavenumber	assignment
3400-3100	NH_2 group in aminopyrimidines
~ 3100	N-H amide
~3050	C-H in hydroxypyrimidines
2190-2120	diazo str. in diazouracils
~1740	$N_{(1)}$-H in-plane deformations
~1720	$N_{(3)}$-H in-plane deformations carbonyls in N-protonated pyridones
1700-1620	carbonyl in uracils
~1680	C=N$^+$ vibration in protonated pyrimidines
~1670	NH_2 scissoring vibration in amino groups.
~1600	C=C, C=N
~1570	pyrimidine vibration
950-550	NH and NH_2 out-of-plane deformation
885-870	$N_{(3)}$-H out of plane deformation in 1-methyluracil and 1-methylthymine.

Table 4.1 I.r. absorptions in pyrimidine and its derivatives

wavenumber	assignment
~3100	$C_{(8)}$-H
~3060	$C_{(6)}$-H
~3020	$C_{(2)}$-H
~1710	C=N$_1$$^+$
~1680	carbonyl str. in hypoxanthine
1610-1570	$N_{(9)}$-H vibrations
~1100	$C_{(6)}$-H deformations
~865	$N_{(9)}$-H out-of-plane deformations
~805	$C_{(6)}$-H deformations
800-780	skeletal vibrations
~790	$C_{(6)}$-H deformations
720-700	skeletal vibrations
690-580	NH_2 wagging
570-500	skeletal vibrations
450-410	skeletal vibrations
370-330	skeletal vibrations

Table 4.2 I.r. absorptions in purine and its derivatives.

4.2.2 Nucleosides and nucleotides.

The i.r. spectra of nucleosides shows slight changes in the position of the absorptions of the usual base residues. The major difference is the appearance of a broad absorption band around 3400cm^{-1} connected with the hydroxyl groups in the sugar. In addition, the band is accompanied by several strong absorption peaks in the range 1100-1000cm^{-1}, which are connected with the C-O stretching vibrations. I.r. spectroscopy does not provide a reliable means of distinguishing a ribose sugar from a deoxyribose sugar. The phosphate moiety in nucleotides is characterized by two bands about 1200 and 1090cm^{-1} when dissociated. When the phosphate is doubly dissociated, the phosphate group is characterized by strong absorption peaks at 1100 and 980cm^{-1} [40].

4.2.3 Diazo compounds.

The i.r. spectra of diazomethane, deuterodiazomethane and dideuterodiazomethane have been recorded for the gas phase [41,42]. The diazo stretching vibration occurred at 2102, 2097, and 2096cm^{-1}, respectively. The i.r. spectra of monoalkyl-, alkylaryl-, and diaryl- diazomethanes in dichloromethane have been reported to have NN bond vibrations around the range 2070-2010cm^{-1} [43].

Bis(trifluoromethyl)diazomethane and bis(perfluoroethyl)diazo--methane show NN bands at 2137 and 2119cm^{-1}, respectively [44]. Dicyanodiazomethane displays an NN absorption band at 2140cm^{-1} [45].

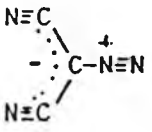
Arene diazonium salts in Nujol mulls have an absorption band for the NN vibration around 2295-2110cm^{-1} [46,47]. Similarly the NN vibration for ethylenediazonium salts were observed to occur in the range 2260-2110cm^{-1} [48].

It would appear that the increasing wavenumber for the NN band represents increasing triple-bond character, with the NN bond in neutral diazo compounds being represented by a doubly charged bond (Scheme 4.2)

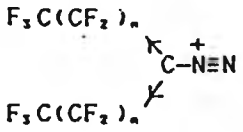
Increasing NN vibration frequency with increasing triple bond character

Scheme 4.2

It follows that any groups which were able to polarise the CN bond of a diazomethane would correspondingly show a NN vibration of a frequency higher than a diazoalkane, e.g. dicyanodiazomethane and bis(perfluoroalkyl)diazomethanes could be represented by resonance forms, which are zwitterionic in nature (4.6 and 4.7)

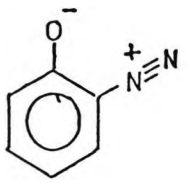


(4.6)



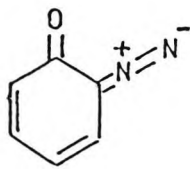
(4.7)

Even when considering the diazonium and diazo groups as resonance hybrid structures, the linking of the absorption frequency to structure could be explained in terms of the relative contribution of the triple-bond/double bond forms. This sort of consideration has led to the alternative zwitterionic forms for diazocyclopentadiene [49] and the diazophenols (4.8 and 4.9), which have a NN vibration in the range 2175-2015cm^{-1} and "carbonyl" vibration in the range 1640-1560cm^{-1} [50].



(4.8)

"Diazonium phenolate"



(4.9)

"Diazophenol"

This may prove too simple an interpretation. The structures proposed for diazouracils by application of this sort of hypothesis [51] have been recently challenged (cf. Chp. 3.2.3 and Chp.5.5). Even when the structure of the diazouracil is treated as a resonance hybrid, the frequencies of both the carbonyl and diazo stretch should not be used to advocate that the largest contribution comes from the zwitterionic diazoketone form. It is likely that the major contribution is from the form that corresponds more to the diazophenol than to the diazonium phenolate.

A large number of i.r. spectra of the diazoketones were tabulated [43,51-54]. It was observed that the carbonyl frequency (1645-1605cm^{-1}) was lower than expected in simple ketones. The diazo

NN band in ketones of the type $RCOCHN_2$ occurred around 2100-2080cm^{-1} and ketones of the type $RCOCRN_2$ around 2075-2050cm^{-1}. The diazoketones were also observed to exhibit an intense band around 1390-1380cm^{-1}. Absorption around 4250-4090cm^{-1} have been attributed to an asymmetric diazo stretching vibration [55]. The i.r. spectra of 2-diazo-1,3-diketones, α,β-unsaturated diazoketones and α-diazo--β-oxophosphonyl compounds have been reported [56-58].

4.3 ^1H N.M.R. Spectroscopy.

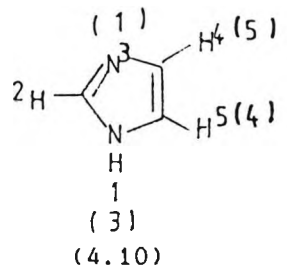
The use of nuclear magnetic resonance has become an increasingly prominent technique in structure elucidation since the first experiment in 1947 [59,60]. N.m.r. is the interaction of radio frequency electromagnetic radiation with nuclei capable of exhibiting spin quantization in an applied magnetic field. A clear and simplified account of the general aspects of n.m.r. theory can be found in several sources [61-63].

The structural information available from n.m.r. comes from three primary sources. The first source is the chemical shift and relates roughly to the chemical environment. The second is the coupling pattern which can be considered to provide information about neighbouring groups. The third source is relaxation effects which normally can be linked to spatial relationships.

4.3.1 Nitrogen-containing Heterocyclic Bases.

4.3.1.1 Imidazole.

The ^1H n.m.r. of imidazole (4.10) in dmso-d_6 is characterised by three peaks. The proton on the ring nitrogen appears as a broad peak because of the quadrupolar relaxation of the nitrogen nucleus. The chemical shift and assignments of the protons are given in the Table 4.3.



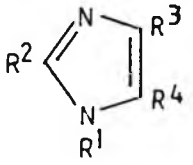
(4.10)

Imidazole

10.70	H-1
7.77	H-2
7.12	H-4, H-5

Table 4.3 ^1H n.m.r. chemical shifts for imidazole (δ)

Listed below are the chemical shift of some substituted imidazoles (4.11a-j) (Table 4.4).



(4.11)

	R^1	R^2	R^3	R^4
a)	H	Br	Br	Br
b)	H	Br	Br	H
c)	H	H	Br	Br
d)	H	H	Cl	Cl
e)	H	Cl	H	H
f)	H	H	H	I
g)	H	H	I	H
h)	H	$(Me)_2NH_2$	H	H
i)	H	NO_2	H	H
j)	sugar	H	$CONH_2$	NH_2

Cpd	N-1	C-2	N-3	C-4	C-5	
4.10	~10.7	7.7	-	7.17	7.17	
4.11a	~12.5	-(Br)	-	-(Br)	-(Br)	
4.11b	~12.5	-(Br)	-	-(Br)	7.38	
4.11c	~12.5	7.81	-	-(Br)	-(Br)	
4.11d	~12.5	7.73	-	-(Cl)	-(Cl)	
4.11e	~12.7	-(Cl)	-	7.07	7.07	[64]
4.11f	~	-(I)	-	7.0	7.0	[65]
4.11g	-	7.7	-	-(I)	7.1	
4.11h	~	$(CH_2)_2NH_2$	-	7.42	7.42	[66]
4.11j	12-13	NO_2	-	7.48	7.48	
4.11k	sugar	7.41	-	$CONH_2$ (6.83)	NH_2 (6.0)	

Table 4.4 Chemical shifts of some substituted imidazoles(δ)

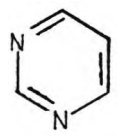
4.3.1.2 Deuterium-proton exchange in imidazoles.

The addition of deuterium oxide causes a disappearance of the N-H signal as the proton on the ring nitrogen is exchanged by a deuterium nucleus. The deuterium exchange of any nitrogen -containing heterocycle may give rise to unusual effects. For example, if deuterium oxide is added to a solution of imidazole and the ^1H n.m.r. run, it can be seen that the signals for H-4 and H-5 decrease. This is attributed to the exchange of the protons at these sites with the deuterium nucleus. However, if the addition of deuterium oxide is accompanied with a small quantity of sodium hydroxide-d, then it is the H-2 proton which is replaced with negligible exchange taking place at C-4 and C-5 [67].

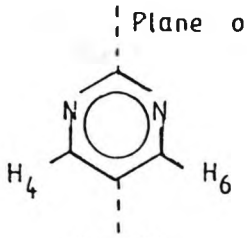
4.3.1.3 Pyrimidine.

Pyrimidine is often represented as a "diazacyclohexatriene" (4.12). In unsubstituted pyrimidine, a better way of presenting the ring is to show the double bonds as being delocalized and using the corresponding convention for representing benzene. By doing so, the magnetic equivalence of the H-4 and H-6 protons can be readily

visualised (4.13) and thus it would be expected that the ^1H n.m.r. of pyrimidine would give three types of signal (Table 4.5) [61,68]. Further support for the magnetic equivalence of H-4 and H-6 is provided by the ^1H n.m.r. of C-2 and C-5 substituted pyrimidines.



(4.12)



(4.13)

chemical shift(δ)	observed multiplicity	Assignment
9.26(9.2)	broad singlet	H-2
8.78(8.6)	doublet	H-4, H-6
7.36(7.1)	multiplet	H-5

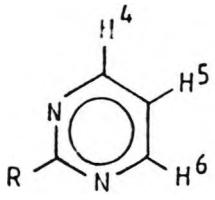
() values quoted in [61]

Table 4.5 ^1H n.m.r. of pyrimidine.

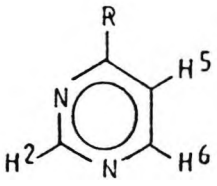
In the spectra of aminopyrimidines, attaching the amino group onto C-2 or C-5 of the ring demonstrates the magnetic equivalence of H-4 and H-6. The signal for the amino protons often mask the signal for H-5 at 6.7δ. The addition of D_2O removes the protons on the amino group and displays the H-5 triplet. The formation of a new peak at 4.03δ is attributed to the formation of HDO.

Mono-substituted pyrimidines do not show a simple quantitative additivity relationship. If the pyrimidine is mono-substituted then additivity does not follow a simple *ortho-*, *meta-* and *para-* relationship as in mono-substituted benzenes. However, as a general rule, the order of effects of substituents is the much the same with regards to the change in chemical shift induced when compared with benzene.

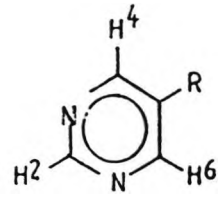
As can be seen from the data (Table 4.6), it was deduced that no general rule could be applied to monosubstituted pyrimidines with regards to calculating additivities. A simple approach based on treating protons as *ortho-*, *meta-* or *para-* to the substituent was inadequate. However, the concept of additivities allows a more subtle approach to be made without the need for complex calculation. Therefore, the question of whether changes in the chemical shift induced by the substituents are additive in polysubstituted pyrimidines is of interest.



(4.14)



(4.15)



(4.16)

Pyrimidine

Cpd	C-2	C-4	C-5	C-6
4.13	9.26	8.78*	7.36	8.78*

C-2 substituted pyrimidine

Cpd	C-2	C-4	C-5	C-6
4.14a	Me	9.04[+0.26](-0.09)	7.86[+0.50](+0.30)	9.04
4.14b	COOMe	8.99[+0.21](+0.08)	7.69[+0.33](+0.20)	8.99
4.14c	SCN	8.84[+0.06]	7.69[+0.33]	8.99
4.14d	Cl	8.78[+0.00](-0.06)	7.55[+0.19](-0.08)	8.78
4.14e	Br	8.72[-0.06](-0.12)	7.57[+0.14](-0.05)	8.72
4.14f	Me	8.68[-0.10](-0.09)	7.23[-0.13](-0.17)	8.68
4.14g	SMe	8.61[-0.17]	7.14[-0.22]	8.61
4.14h	OMe	8.60[-0.28](-0.1)	7.07[-0.29](-0.41)	8.60
4.14i	I	8.58[-0.30](-0.25)	7.57[-0.21](-0.02)	8.58
4.14j	SH	8.25[-0.58]	6.84[-0.52]	8.25
4.14k	NH_2 (6.7)	8.33[-0.45](-0.25)	6.70[-0.66](-0.63)	8.33*
4.14k'	NH_2	8.27[-0.51]	6.56[-0.80]	8.27
4.14l	$N(Me)_2$	8.31[-0.47](-0.1)	6.48[-0.88](-0.62)	8.31
4.14m	NHMe	8.28[-0.50](-0.3)	6.52[-0.84](-0.6)	4.82
4.14n	OH	8.23[-0.55](-0.13)	6.40[-0.96](-0.2)	8.33

C-4/C-6 substituted pyrimidine

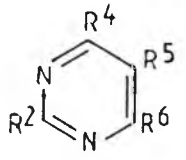
	C-2	C-4	C-5	C-6
4.15a	8.92[-0.34]	SMe	7.40[+0.04]	8.43[-0.35]
4.15b	9.22[-0.04](+0.08)	Me	7.23[-0.13](-0.16)	8.60[-0.18](-0.09)(Me=2.56)
4.15c	8.90[-0.36](+0.08)	Me	6.98[-0.38](-0.16)	8.41[-0.37]
4.15d	8.83[-0.43]	NHCOMe	8.02[+0.66]	8.61[-0.17]
4.15e	8.83[-0.43](-0.10)	OMe	6.97[-0.39](-0.46)	8.55[-0.23](-0.10)
4.15f	8.39[-0.86](-0.25)	NH_2	6.44[-0.92](-0.76)	8.04[-0.74](-0.25)
4.15g	8.26[-1.00]	SH	7.13[-0.13]	7.80[-0.98]
4.15h	8.17[-1.09](-0.13)	OH	6.29[-1.07](-0.49)	7.86[-0.92](-0.13)

C-5 substituted pyrimidine

	C-2	C-4	C-5	C-6
4.16a	9.44[+0.18](+0.30)	9.26[+0.48](+0.26)	CN	9.26
4.16b	9.28[+0.02](+0.25)	9.00[+0.22](+0.80)	COOH	9.00
4.16c	9.15[-.011](-0.05)	8.96[+0.18](+0.19)	Br	8.96
4.16d	8.99[-0.27](-0.17)	8.65[-0.13](-0.16)	Me	8.65
4.16e	8.78[-0.48](-0.10)	8.50[-0.28](-0.46)	OMe	8.50
4.16f	8.69[-0.57](-0.20)	8.39[-0.39](-0.49)	OH	8.39

[] difference between monosubstituted chemical shift and unsubstituted chemical shift.
() substituent additivity for benzene

Table 4.6 ^1H n.m.r. chemical shifts, δ, for pyrimidine and mono-substituted pyrimidines [69-71].



(4.17)

	C-2	C-4	C-5	C-6
4.17a	NH_2 (6.57)	NH_2 (6.13)	5.77	7.71
4.17b	7.83	NH_2 (6.35)	NH_2 (4.73)	7.58
4.17c	7.84	NH_2	5.42	NH_2
4.17d	Cl	NH_2 (6.87)	NH_2 (4.87)	7.57
4.17e	7.7	NH_2 (6.70)	NH_2 (4.92)	Cl
4.17f	Cl	Cl	7.45	8.62
4.17g	8.8	Cl	7.56	Cl
4.17h	Cl	Cl	7.46	Cl
4.17i	9.22	Me(2.56)	7.23	8.6
4.17j	Me(2.5)	9.0	7.09	Me(2.5)
4.17k	SMe	8.30	6.78	Me

Table 4.7. Di- and tri- substituted pyrimidines.

The effectiveness of applying additivities of substituents can be demonstrated by the following examples, for which experimental data is available for comparison (Table 4.7).

Applying additivities for 2,4-diaminopyrimidine

C-2	C-4	C-5	C-6
NH_2	-	-0.66	-0.45
-	NH_2	-0.92	-0.74
-	-	7.36	8.78
predicted chemical shift(δ)		5.78	7.59
found		5.77	7.71

Applying additivities for 2-chloro-4,5-diaminopyrimidine

C-2	C-4	C-5	C-6
7.83	NH_2	NH_2	7.58
Cl	-	-	+0.00
predicted chemical shift(δ)			7.58
found			7.57

Applying additivities for 2-thiomethyl-6-methylpyrmidine

C-2	C-4	C-5	C-6
SMe	-0.17	-0.22	-
-	-0.36	-0.38	Me
-	8.78	7.36	-
predicted(δ)	8.25	6.76	-
found	8.30	6.78	

The result of these calculations gives rise to optimism as to the general suitability of using the tabulated data for predicting the chemical shift of pyrimidine ring protons. It would appear that further positional considerations need not be applied, as these considerations are inherently included in the data for monosubstituted compounds. In addition, the application of additivity to 4,6-diaminopyrimidine gives the theoretical values for H-2 and H-5 as 7.78 and 5.51δ respectively. The values found are correspondingly 7.84 and 5.42δ and show a reasonable degree of agreement. Thus, there is a strong case for treating the Table 4.6 as an aid to predicting the chemical shift. Part of the difficulty in applying the data has been the wide variation in solubility of the substituted pyrimidine compounds and also the widely varying solvent effects which can occur [76].

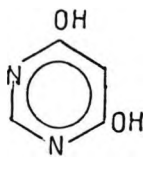
4.3.1.4 Uracil and its derivatives.

The ^{1}H n.m.r. of uracil shows it to be a dioxo compound rather than the enolic tautomer (2,4-dihydroxypyrimidine) [81]. The dioxo tautomer was characterised by the presence of a broad singlet at 10.8δ integrating for two protons, whilst the doublet at 5.5δ and the doublet at 7.4δ were assigned as H-5 and H-6, respectively (Table 4.8).

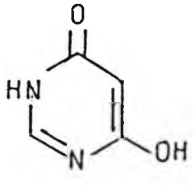
Chemical shift(δ)	Multiplicity	Assignment
10.8	Broad singlet	H-1; H-3
7.4	doublet J=8.0	H-6
5.5	doublet J=8.0	H-5

Table 4.8 ^{1}H n.m.r. of uracil in dmso-d_6

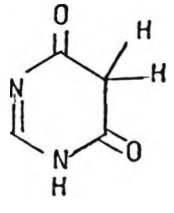
The isomeric 4,6-pyrimidinediol has several possible tautomers (4.18 - 4.22). The i.r. and u.v. evidence has shown that 4-hydroxypyrimidine tends to exist as the keto tautomer. The ^1H n.m.r. spectrum of 4,6-pyrimidinediol is characterised by three peaks, two sharp singlets at 5.32δ and 8.1δ of equal height and one broad peak around 9.45δ [81]. The spectrum best fits the structure in which the protons are attached to sp^2 hybridized carbons. The chemical shift of the peak around 5.32δ is comparable to the H-5 in uracil, which is consistent with the 4-oxo-tautomer. The chemical shift for the other peak at 8.1 is similar to H-2 for pyrimidines in which there is an electron-releasing group in a *meta*-position. In barbituric acid the methylene group between the two keto groups has a chemical shift of about 3.55δ. This would imply that the compound exists as the hydroxy-oxo- form (4.19). The broad signal at 9.45δ is in the typical range for phenol hydroxyls and amide protons.



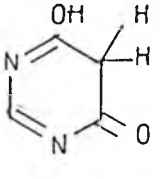
(4.18)



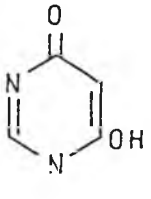
(4.19)



(4.20)



(4.21)



(4.22)

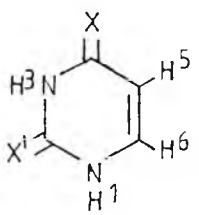
The ^1H n.m.r. spectra of several uracil derivatives have been recorded and are listed in Table 4.9. Broad NH peaks occur over a wide range. The ring proton signals will show which isomer is present in that the chemical shift for the ring proton for the C-6 isomer and the C-5 isomer differ considerably. It should be noted that in 5-fluorouracil the C-6 proton occurs as a doublet owing to the coupling with fluorine but otherwise the information from ring proton signals is extremely limited. It would be difficult to distinguish 5-bromouracil from 5-iodouracil on the basis of the ^1H n.m.r. Other spectra have been run in D_2O with sodium hydroxide-d

using tribasic sodium phosphate as the reference peak in place of TMS.

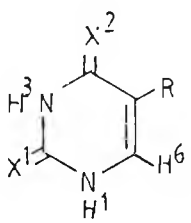
cpd.	N-1	C-2	N-3	C-4	C-5	C-6	
4.23a	10.8	=O	10.8	=O	5.5d	7.4d	
4.23b	14-11	=S	14-11	=S	6.55d	7.32d	
4.23c	-	=S	-	=O	5.95d	7.66d	D_2O
4.24a	11.3	=O	11.3	=O	I	7.87	
4.24b	11.2*	=O	11.5*	=O	Br	7.85	
4.24c	8-5	=O	8-5	=O	F	7.73d	
4.24d	-	=O	-	=O	Me(1.9)	7.73	D_2O
4.25	10.8	=O	10.8	=O	5.3	Me(2.03)	
4.26a	14-11	SMe(2.48)	14-11	=O	5.26	-OH	
4.26b	-	Me(2.24)	-	=O	5.2	-OH	D_2O
4.27	11.2	=O	11.2	=O	3.55	=O	

d=doublet

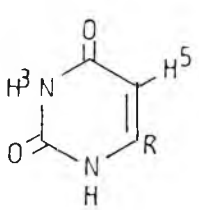
Table 4.9 The ^1H n.m.r. of some Uracil and related compounds(δ)



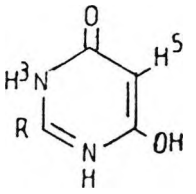
	X^1	X^2	
4.23 a)	=O	=O	uracil
4.23 b)	=S	=S	2,4-dithiauracil
4.23 c)	=S	=O	2-thiauracil



	X^1	X^2	R	
4.24 a)	=O	=O	I	5-iodouracil
4.24 b)	=O	=O	Br	5-Bromouracil
4.24 c)	=O	=O	F	5-Fluorouracil
4.24 d)	=O	=O	Me	thymidine



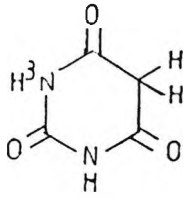
	R	
4.25	Me	6-methyluracil



R

4.26 a) -SMe 6-hydroxy-2-methylthio-,

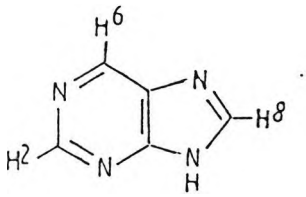
4.26 b) -Me 6-hydroxy-2-methyl-,



4.27 Barbituric acid
(2,4,6(1H,3H,5H)-pyrimidinetrione)

4.3.1.5 Purines.

The chemical shift of non-exchangeable protons in the heterocyclic base components of nucleic acids have been reported [77]. In keeping with the other data, the data has been recalibrated with respect to the tms standard, taking the aromatic signal of toluene, which was used as the external reference, to be 7.17δ.



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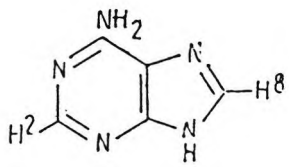
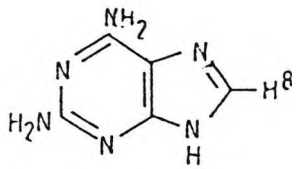


(4.29)



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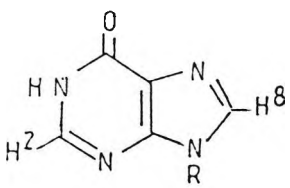

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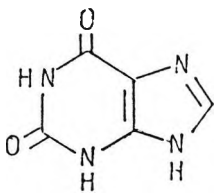

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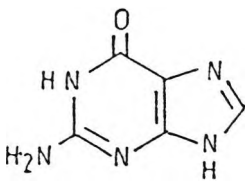
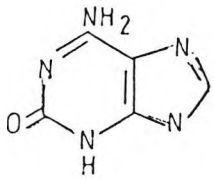
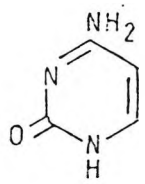


(4.33)



(4.34)



(4.35)

	(δ)				
	H-2	H-5	H-6	H-8	solv.
Purine	9.48	-	9.00	9.29	5.74
Adenine	8.79	-	-	8.79	5.76
2,6-Diaminopurine	-	-	-	8.47	5.76
Hypoxanthine	8.78	-	-	8.58	5.76
Xanthine	-	-	-	8.34	5.75
Guanine	-	-	-	8.31	5.73
Isoguanine	-	-	-	8.44	5.76
Cytosine	-	6.63	8.54	-	5.80
Uracil	-	6.46	8.4	-	5.80
Adenosine	8.7	-	-	8.95	5.80
Inosine	8.92	-	-	8.92	5.79
Xanthosine	-	-	-	8.60	5.76
Guanosine	-	-	-	8.59	5.80
Cytidine	-	6.81	8.54	-	5.73
Uridine	-	6.61	8.41	-	5.80

solvent 0.75 M of Na_2O_2 in D_2O

Table 4.10 Non-exchangeable proton chemical shifts [77].

4.3.2 Nucleosides.

Obtaining the ^1H n.m.r. of nucleosides involves the detection of protons on the sugar moiety in addition to those of the base. The protons on the sugar moiety which do not readily exchange with deuterium oxide are not always readily assignable due to the absence of fine coupling and the overlapping of signals. The easiest proton to assign is the anomeric or C-1' proton. This often appears as a singlet or a doublet reasonably downfield. With the exception of the protons on the heterocyclic base, the anomeric proton tends to be the most downfield signal as it is deshielded by the α-attachment of oxygen and nitrogen, both with a negative inductive effect.

The ^1H n.m.r. of nucleosides is dominated by the ^1H n.m.r. of the sugar moiety. The equivalence of the C-4 and the C-5 position in imidazole has been demonstrated by ^1H n.m.r., with the two protons H-4 and H-5 having the same chemical shift. However, the addition of the sugar at one of the ring nitrogens permanently changes the ring geometry. This permanent change in geometry

results in the H-4 and H-5 having different electronic environments and this is made manifest by the non-equivalence of their chemical shifts. For example, H-4 and H-5 in 2-nitroimidazole are represented by a single peak with a chemical shift around 7.44δ. The N-glycosidation with ribofuranose results in two singlets at 8.25δ (H-5) and at 7.30δ (H-4). An unusual feature is that the coupling constant between these different protons is zero. The ribofuranose anomeric proton has been assigned to the singlet at 6.36 [82].

The structures of cytidine and deoxycytidine have been explored by ^{1}H n.m.r. [77,83-85], the spectra being characterised by a doublet at 8.17δ (H-6). The corresponding doublet for H-5 was found to overlap with the anomeric proton (H-1') at 6.03δ. The observation of exchangeable protons at 7.50δ which integrate for 2H demonstrated that cytidine exists as the amino form. The claim that deoxycytidine existed as the imino form [83] was shown to be erroneous [84].

Uridine shows the expected ^{1}H n.m.r. signals for the uracil and ribofuranose moiety. The imide proton (H-3) occurs around 11.6δ.

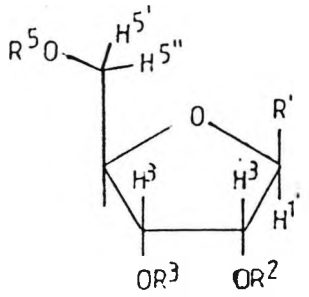
In general, the nucleosides show a wide degree of variation in the chemical shift of the non-exchangeable sugar ring protons. Unambiguous assignment of these protons is not always possible by inspection of low field n.m.r. (60-100 MHz) spectra. The chemical shifts are very dependent on the substituents on the ring as well as the position of substitution.

4.3.3 Nucleotides.

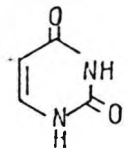
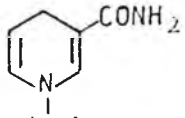
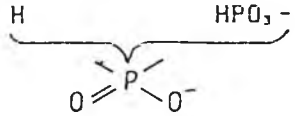
The ^{1}H n.m.r. spectra of nucleotides are much like those of the nucleosides. The chemical shifts of the protons on the ribofuranosyl ring are shifted upfield, the spectra normally being run in D_2O (Table 4.9). The cyclic monophosphates show only very slight upfield shifts compared to the unsubstituted nucleoside whereas the 5'-phosphate esters are characterized by very large upfield shifts.

4.3.4 General reasons for peak broadening.

In addition to peak broadening by quadrupolar relaxation, the C-H peaks may become broadened by the presence of paramagnetic species such as ferric and cupric ions. Molecular oxygen dissolved in solution may also cause line broadening. The spectra are



(4.36)

	R¹	R²	R³	R⁵
4.36 a)	uracil	H	H	H
4.36 b)		H	H	H
4.36 c)	F	Ph.CO-	Ph.CO-	Ph.CO-
4.36 d)	Ph.CO-	$MeSO_2$-	Ph.CO-	Ph.CO-
4.36 e)	F	Ac-	Ph.CO-	Ph.CO-
4.36 f)	Ac-	Ac-	Ph.CO-	Ph.CO-
4.36 g)	uracil	$(F_3C)_2C(OH)$-	$(F_3C)_2C(OH)$-	$(F_3C)_2C(OH)$-
4.36 h)	Adenine	H	H	HPO_3-
4.36 i)		H	H	HPO_3-
4.36 j)	Adenine	H		

Cpd	H-1'	H-2'	H-3'	H-4'	H-5'	H-5''	
4.36a	5.90	4.35	4.25	4.15	(3.82)		[72]
4.36b	4.99	4.36	4.33	4.00	3.88	3.71	[73,74]
4.36c	6.30	5.51	5.86	4.93	4.76	4.61	[75]
4.36d	6.58	5.47	5.82	4.87	(4.68)		[78]
4.36e	5.80	5.55	5.74	4.74	4.68	4.42	[75]
4.36f	6.30	5.58	5.82	4.63	(4.63)		[78]
4.36g	6.02	5.01	4.87	4.35	4.00	4.10	[72]
4.36h	2.91	1.60	1.33	1.18	0.85	0.83	[79]
4.36i	1.70	1.15	1.06	0.90	(0.68)		[79]
4.36j	6.09	4.71	4.75	4.37	4.60	4.39	[80]

adenosine	6.55	4.91*	4.60
inosine	6.58	4.91*	4.56
xanthine	6.43	4.88*	4.54
guanosine	6.46	4.92*	4.57
cytidine	5.73	4.82*	4.61

*quoted range for H-2', H-3' and H-4'

Table 4.11. Chemical shift of ribofuranose ring protons in some derivatives [72-80].

sensitive to the presence of small impurities which are themselves not paramagnetic but nevertheless generate magnetic field inhomogeneities in sections of the molecule.

In the present work, the sparingly soluble nature of uracil and its derivatives often necessitated the running of the spectrum in neat dmso-d_6. The resulting spectra were often of poor quality. The chemical shifts were prone to a limited amount of variability owing to concentration and solvent effects.

The free nucleosides produced spectra of a similar nature. However, nucleosides which had "protecting" groups on the sugar hydroxyls gave better spectra with increasing solubility in solvents such as chloroform. When the protecting groups were bulky, fine structure of the sugar moiety could be determined by the multiple couplings. The probable reason for the improved spectra is the reduction in the degrees of vibrational freedom and hence a reduction in signal broadening due to the "time average" effect. The free nucleosides also suffered from hydroxy proton-transfer and $^3J_{HCOH}$ couplings which have been shown to result in poor spectra owing to signal broadening [86].

4.3.5 Coupling constants.

In general, coupling constants reveal information about interacting neighbours. For magnetically equivalent protons the number of peaks is given by the n+1 rule, where n represents the number of those protons. The correlation between structure and interproton spin-spin coupling constants has been well covered [63,87].

The heterocyclic base components in nucleic acid do not show much coupling. In uracil, two doublets for H-6 and H-5 are observed. The coupling constant for these neighbouring protons is 7.7Hz [88] and falls within the typical range for cis vinyl protons. The example of uracil serves to illustrate the relationship between coupling constants and structure. Not all neighbouring protons show a coupling, for example, it can be seen that there does not appear to be any coupling between the H-1 and H-6 protons. There was no coupling observed in non equivalent neighbouring protons in the imidazole nucleosides.

The coupling constant is of limited use in determining the structure in heterocyclic bases. In conjunction with observations

on chemical shifts, the multiplicity of coupling can best be used to differentiate between structural isomers, for example, the distinction of thymidine from 6-methyluracil or from N-methyluracil.

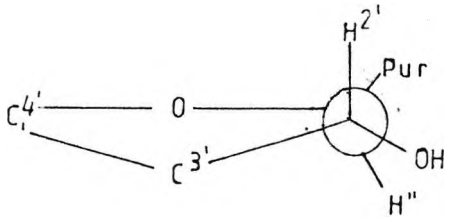
Assignment of the sugar ring protons can sometimes be made via the relationship of the vicinal protons. The ideal case is rarely observed at lowfield (4.37 and 4.38), nevertheless the assignment of the anomeric proton and the H-2' proton can often be made based on the magnitude of the coupling between them.

4.3.5.1 Using coupling constants and chemical shift to explore nucleoside conformation.

Coupling constants can be used to examine conformation. In the work of Karplus, dihedral angles between vicinal protons were found to be related to the magnitude of the coupling constant [89-91]. Early exploration of sugar ring conformation relied on examination of the magnitude of the coupling constant between H-1' and H-2' [82]. Thus, it was proposed that the magnitude of the coupling constant $J_{H-1',H-2'}$ could provide information on the orientation of these two protons with respect to each other and hence give information on the ring structure (Table 4.12). It was further concluded from examination of d-ribose nucleosides that the sugar ring had different conformations depending on whether the heterocyclic base was a pyrimidine or a purine [92,93]. The results suggested that purine nucleosides exhibited a preferred $C_{(3')}$-exo conformation or $C_{(2')}$-endo conformation (4.37). On the other hand, pyrimidine nucleosides possessed a $C_{(2')}$-exo conformation or a $C_{(3')}$-endo conformation (4.38).

Description of conformation	Dihedral angle	orientation H-1'	H-2'	predicted coupling
$C_{(2')}$-endo	150	axial	axial	6.9
$C_{(3')}$-exo	140	axial	axial	5.4
$C_{(3')}$-endo	115	equatorial	equatorial	1.7
$C_{(2')}$-exo	105	equatorial	equatorial	0.05

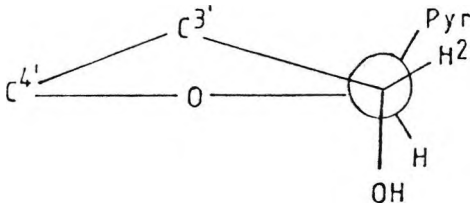
Table 4.12 Sugar pucker modes and $J_{H-1',H-2'}$.



C_2·-*endo*/C_3·-*exo*

H_1·-C_1·-C_2·-H_2· > 120°

(4.37)



C_2·-*exo*/C_3·-*endo*

H_1·-C_1·-C_2·-H_2· < 120°

(4.38)

Support for the C_2·-*exo*/C_3·-*endo* conformation for pyrimidine nucleosides has come from X-ray crystallographic studies [94,95]. The difference in the two types of preferred conformations for pyrimidine and purine nucleosides has been proposed as an explanation for the observation of specific rotations being positive for pyrimidine nucleosides but negative for purine nucleosides.

4.3.6 New techniques.

Three important developments which have aided the study of nucleic acid components has been the development of highfield n.m.r. [97-99], the use of probes other than the proton in n.m.r. studies, particularly phosphorus [99,100-102] and the utilization of computer simulations [103-105].

4.3.7 The conformation about the glycosidic bond.

A wide variety of techniques have been used to determine the orientation of the heterocyclic base to the sugar. The orientation of the base with respect ot the sugar ring is described by *syn*- and *anti*- conformations (chp.1). Attempts have been made to determine the position of the heterocyclic base in the *syn-anti* equilibrium by examination of the chemical shift of base ring protons. The underlying principle is the through space field effect of C-5' substituents on the electronic environment of the base protons [92,106-109]. As a method of analysing the conformation of the heterocyclic base relative to the sugar ring, it suffers from both solvation and base stacking effects [110]. Other attempts to derive the glycosidic bond conformation have used the magnetic anisotropy of the bases [111]

or rely on measurements between the proton-carbon couplings $^{3}J_{C-2,H-1'}$, which show a Karplus-type relationship [112,113].

One very powerful technique that has recently been applied increasingly to problems of conformation has been the use of nuclear Overhauser enhancement spectroscopy. This through space effect can be used to show intramolecular spatial relationships between protons [114-119]. The work on ^{1}H n.m.r. and conformation has been well reviewed [120] and the recent developments and refinement of ^{1}H n.m.r. techniques has also been well covered [121].

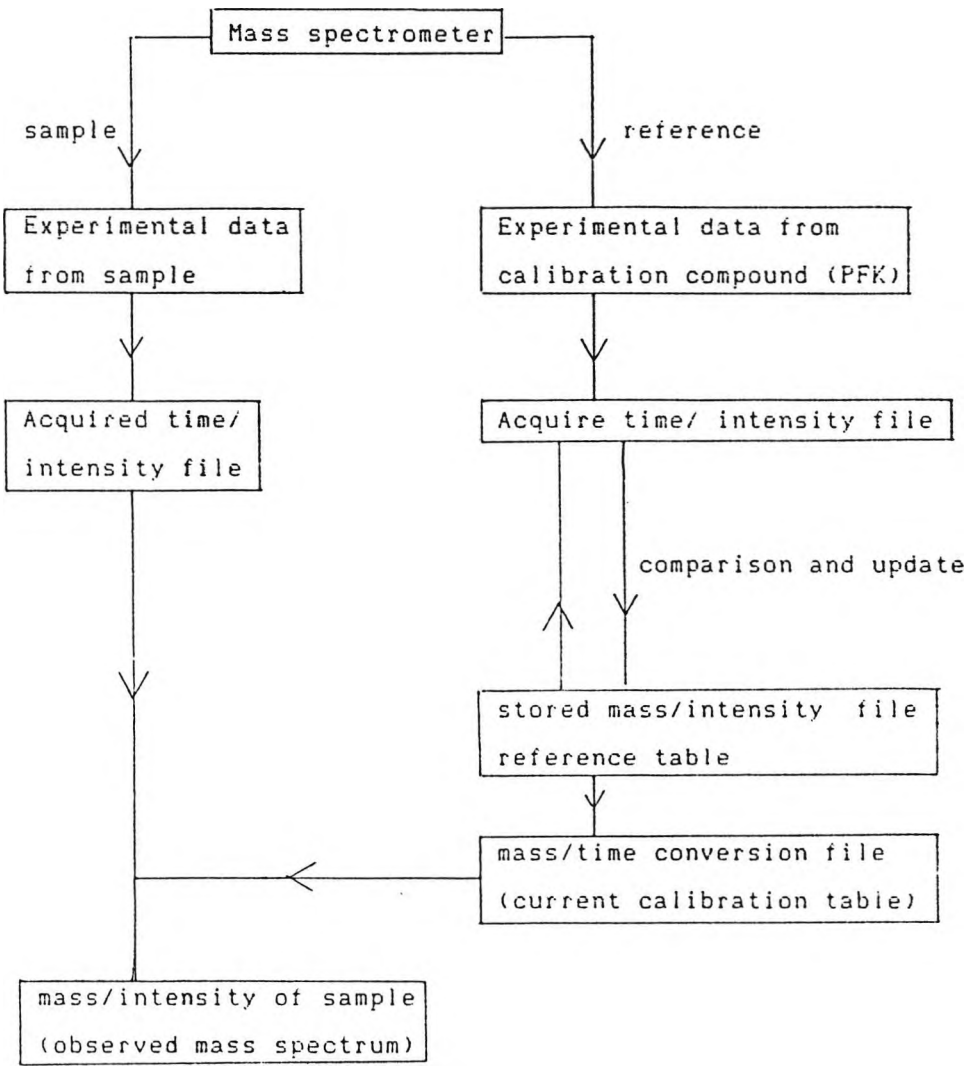
4.4 Mass spectrometry.

The primary requirement for electron impact mass spectrometry is that samples be thermally stable at temperatures required to volatilize the sample. If the sample is insufficiently volatile, its volatility often can be improved by derivatisation [122]. The conventional form of ionisation is electron bombardment of the vaporised sampled. This technique is referred to as "Electron Impact" or EI. As it is questionable whether the bombarding electrons actually collide with the sample's electrons, the abbreviation EI has more recently been used to stand for "Electron Ionisation".

4.4.1 Accurate mass measurement.

The term "accurate" is often applied to the m/z obtained from mass spectrometer which are given to four or more decimal places. More correctly, the observed values are referred to as "high resolution" masses. The use of computer matching programs to correlate either flight time or applied focussing field strength with mass are the basis of the modern high resolution mass spectrometer. In addition to this simple correlation, most spectrometers rely on a reference peak matching program to further improve the degree of accuracy at which masses may be quoted. Nevertheless, it is still often necessary to manually correct the data. An explanation of deviation of the "actual" mass from the computed measured mass can be seen from the flow chart of mass calibration (Scheme 4.3) [123]. Just as the internal energy of the molecules can not always be expected to be the same at any one particular instant, the peak intensity and fragmentation processes are not always the same. Thus, to be of any value the computer uses

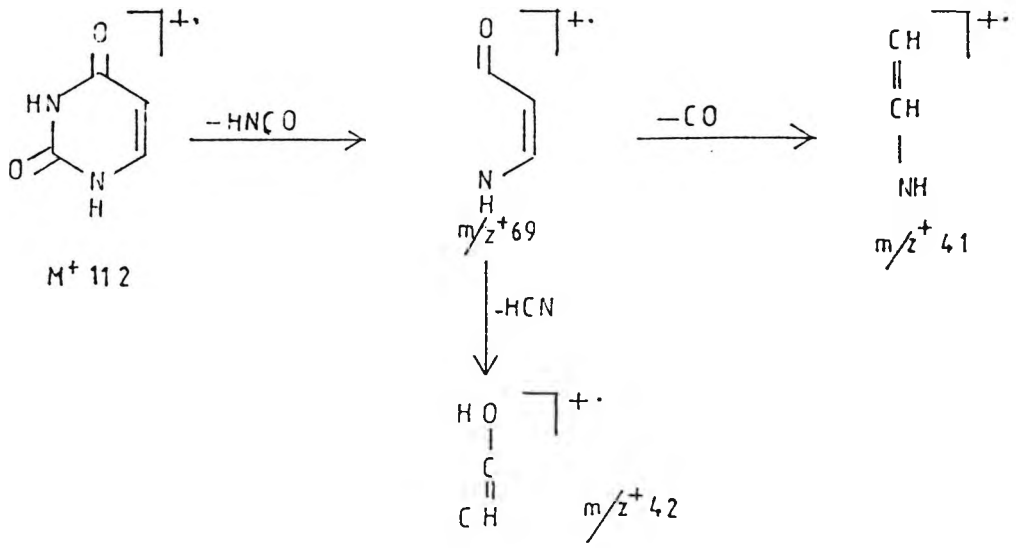
an idealised reference library and combines it with observed values for that time to create a "current and updated" reference file. Thus, this new file is more applicable for calibrating samples run around the same time. For practical purposes the current calibration file is updated or recreated every so often. During the intervals between updating, slight errors in the mechanics of producing the spectrum might occur and will be interpreted by the file and produce masses slightly off from the theoretical value. Often this is recognised by linear deviations in the difference between measured mass and theoretical mass. A common fault is a slight drifting in the focusing field. An obvious indication is the observed difference in measured mass and theoretical mass for PFK peaks in the sample. These peaks along with other suitable peaks, such as solvent or molecular gas peaks, can be used to determine the necessary correction.



Scheme 4.3 Mass spectrometer operation.

4.4.2 Pyrimidines.

Purines and pyrimidines are sufficiently volatile at temperatures between 130-200° [124]. Of particular interest is the mass spectrum of uracil and its derivatives. In the EI mass spectrum of uracil [125], apart from the molecular ion the main fragments were at 69, 42, or 41. These fragments were attributed to the loss of HNCO and then either HCN or CO (Scheme 4.4).



Scheme 4.4 Fragmentation of uracil

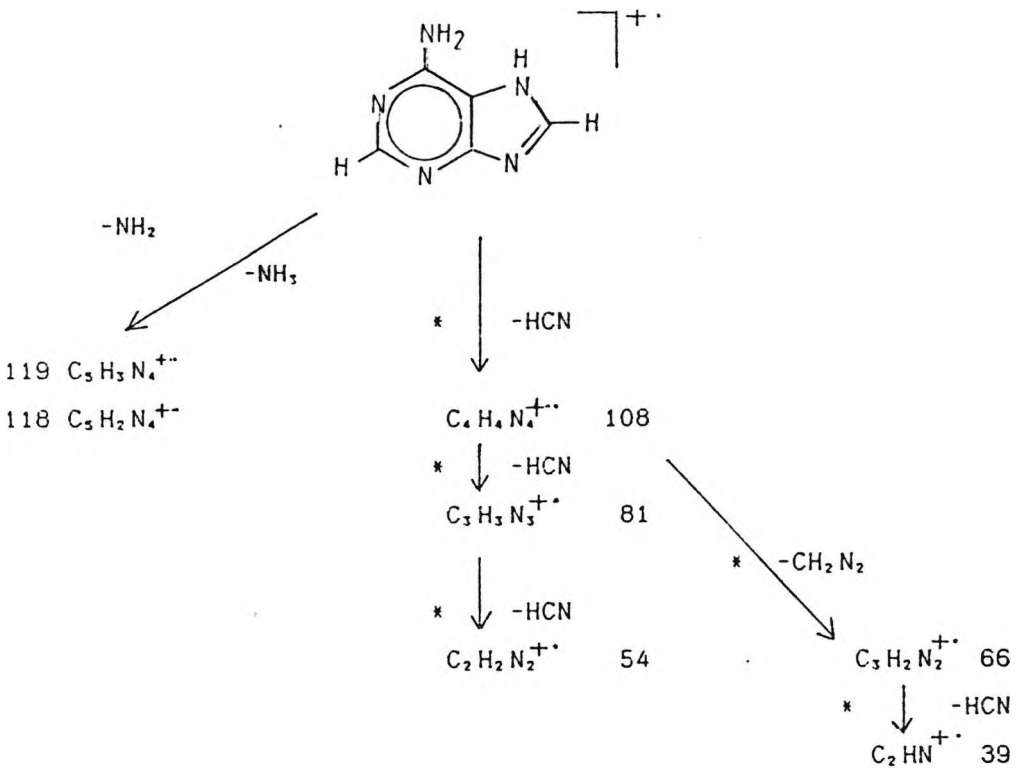
The loss of HNCO from pyrimidones is a common feature also seen in the EI spectra of 6-methyluracil [124], thymine (5-methyl--uracil) and cytosine. Other common features are the loss of HCN from pyrimidine type compounds.

One interesting feature has been the difference in spectra obtained at 70 eV and at 20 eV spectra. The use of 70eV during ionisation produces spectra of lower molecular ion intensity with a corresponding increase in the fragmentation process.

A number of simple pyrimidines have been examined by mass spectrometry [126,127].

4.4.3 Purines.

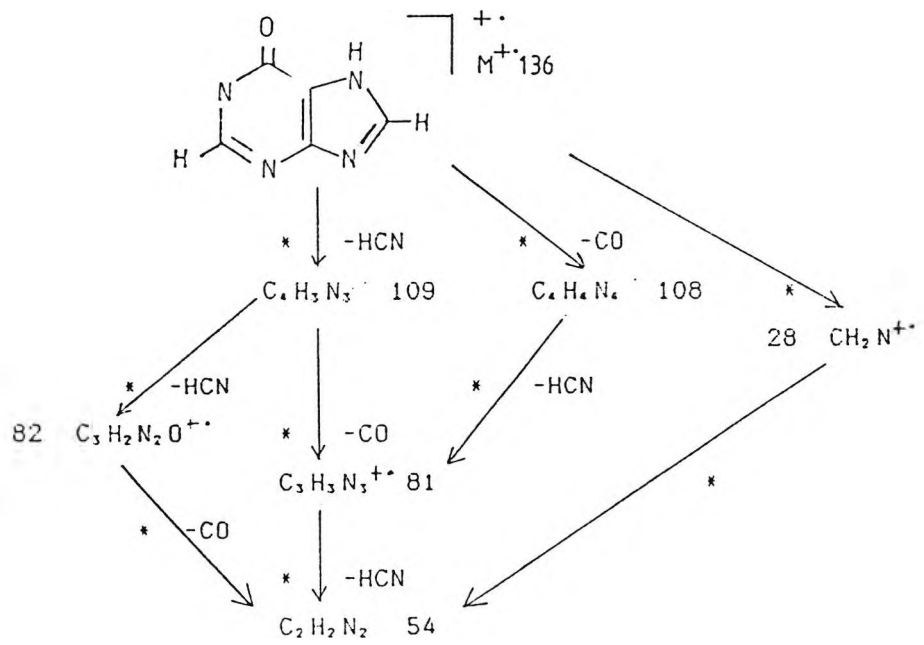
The EI spectrum of purine is characterized both by the molecular ion and by the fragments indicating the loss of HCN [129]. Studies using deuterium labels indicate that the loss of HCN can occur by several fragmentation pathways [130]. The same observations were made for adenine [124] (Scheme 4.5).



* metastable peak detected

Scheme 4.5 Fragmentation of Adenine

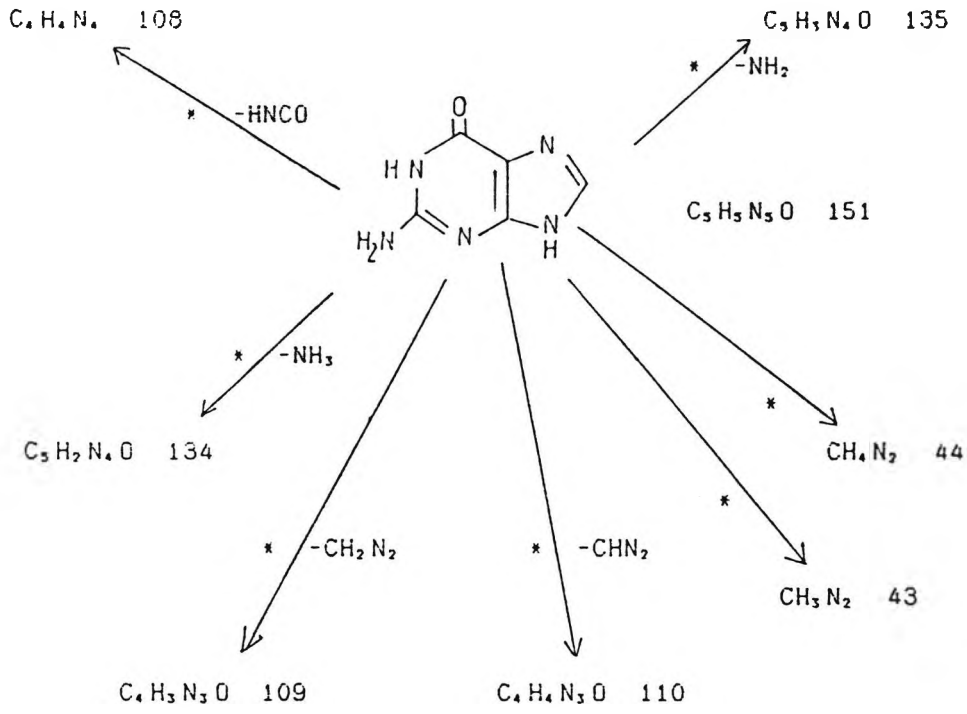
The spectrum of Hypoxanthine is characterised by the loss of HCN followed by the loss of CO and then HCN (Scheme 4.6).



* metastable peak detected

Scheme 4.6 Fragmentation Hypoxanthine

The spectrum of guanine is characterized by fragments which correspond to the loss of the various structural features (Scheme 4.7) [124]. Surprisingly, the loss of HCN is not a major feature.



Scheme 4.7 Fragmentation of guanine

4.4.4 Nucleosides.

Some nucleosides are sufficiently volatile to be ionised as the free nucleoside. A common feature of the EI spectra is the cleavage of the glycosidic bond. The heterocyclic base fragment is often identified by the observation of B+1 and B+2 peaks, which correspond to the mass for the base fragment plus either one or two hydrogens [131,132].

The D-ribose unit is characterised by a peak at M/Z 133, whilst deoxyribose is characterised by a peak at 117. One fragment which can be used to characterise the glycosidic link in free nucleosides is the base+30 peak. Labelling experiments show this to be composed of the base component, the anomeric carbon and its hydrogen and the furanoside oxygen with any abstracted proton [131]. A similar fragment containing the base and the C-1' and C-2' carbons might be observed at the base+44.

The nucleosides can be characterized by masses M-30 and M-89, which correspond to the fragmentation of the sugar moiety.

The high resolution spectra of adenosine [133] and thymidine [125] have been reported. Both of these compounds show spectra which follow the general pattern for nucleoside fragmentation rather than that for adenine or thymine respectively. Guanosine and cytidine are usually derivatised in order that they become sufficiently volatile for a spectrum to be obtained. Common peaks found in the spectra of trimethylsilylated compounds are the masses at 73 and 147. These correspond to the fragments $(CH_3)_3Si^+$, $((CH_3)_3Si)_2O^+$ [134,135]. A disadvantage of derivatisation of the nucleic acid component is that the fragmentation pattern is considerable more complex and consequently more difficult to interpret.

4.4.5 Alternative methods of ionisation.

A wide variety of techniques have been developed in order to ionise the sample. The appearance of the spectrum is very dependent on the method employed to ionise the sample. Most of the techniques now employed have been developed primarily to improve the observation of the molecular ion. These techniques are often referred to as "soft" ionisations as the molecular ion or quasi-molecular ion formed have less excess internal energy than for the corresponding molecular ion in EI.

Chemical ionisation is particularly suited for the mass spectra of nucleosides and has the added advantage that the technique is compatible with GC/MS and LC/MS. Field desorption is suitable for the examination of a wide variety of compounds, particularly nucleotides. The use of FAB has become increasingly popular because of the simplicity of the method, which conveys many of the aspects of FI and FD. It is suitable for use with compounds of low volatility such as sugars, peptides and nucleotides.

Although these newer methods are increasingly being used to tackle the spectra of nucleic acid components, much of the mass spectrometry data to date is from the more common technique of ionisation, namely EI. Many of the fragmentation processes in various classes of organic compounds have been reported [142-145] thus facilitating structural analysis of compounds from spectra

obtained with EI. A typical problem encountered with the EI spectra of uracil-type compounds is the lack of a molecular ion in the EI spectrum, consequently there is a greater reliance on information provided by the fragments. Thus, a more detailed examination of fragmentation is provided further on.

4.4.5.1 Photoionisation. [123,136-137]

This method involves bombardment of the molecules by photons of a wavelength around 100 nm. Its advantage lies in the absence of molecular distortion through the absence of excessive thermal energy. It also ensures that fewer energy states are involved owing to spin conservation.

4.4.5.2 Chemical ionisation. [123,138]

Instead of the high vacuum of 10^{-6} torr for electron ionisation, chemical ionisation works at a relatively higher pressure of 1 torr. In this method a carrier gas is ionised after mixing with the sample. In practice, the actual sample-ionising reagents are usually secondary species formed by interaction of the EI carrier gas cation radical with neutral gas molecules to form a simple cationic species. It is these secondary species containing only thermal and translational energy which ionise the sample during collision in the gas phase. During the collision, the secondary ion may behave like a strong Lewis acid abstracting a hydride ion, or like a strong Bronsted acid donating a proton to produce the molecular ion or the quasi molecular ion $(M+H)^+$ respectively. Another reaction which is often produced corresponds to the ionic complexation of the sample molecule with the secondary (Lewis acid) ion.

4.4.5.3 Field Ionization (FI) and Field Desorption (FD) [123,139-141].

These methods use high potential voltages to ionise the molecules. To achieve such high voltages, the anodes are effectively microneedles consisting of whiskers on a conducting wire. When the molecule approaches close to such an anode, the influence of the high potential field causes tunnelling of one of the sample's valence electrons to the anode. This tunnelling does not produce a high excess energy in the ion radical and hence, it reduces the

chance of fragmentation. In field ionisation the sample is vaporised, as in EI, and absorbs onto the emitter wire from the gas phase.

Field desorption uses much the same technique as FI, and differs in that the sample is applied as a solution to the unactivated anode. The solvent is allowed to evaporate from the sample thus leaving a film of the sample on the anode. The coated anode is then inserted into the spectrometer and the sample ionised by applying a high positive potential. The radical ions formed are repulsed from the anode and contain translational energy but with little excess energy for fragmentation. The method confers the added advantage of avoiding thermal volatilisation. Sometimes mild heating is applied to samples of low vapour pressure to aid desorption. In either case, the thermal energy of the molecule is much lower than in either EI or FI. Thus, this method reduces the chance of thermal decomposition of the sample. Ion-molecule collisions occurring during the desorption often produce $(M + nX)^+$ pseudomolecular ions, where X is a proton or a metal cation such as Na^+ or K^+.

4.4.5.4 Fast Atom Bombardment (FAB). [123]

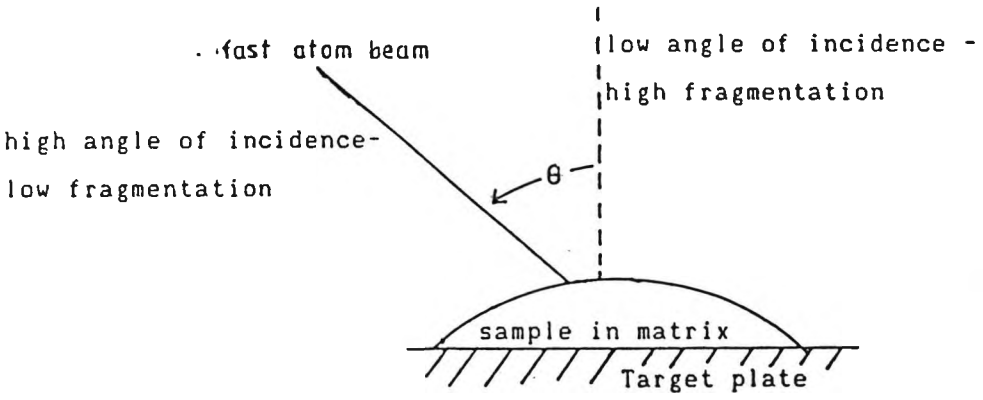
The method involves the desorption and ionising of a solid sample suspended in a matrix, using a bombarding beam of fast atoms. Initially, atoms of a gas such as argon are ionised by the removal of an electron to produce argon cations. These ions are then accelerated with an energy of 2 to 10 keV, usually via an electrostatic field, and focused into a collision chamber [143]. Within the collision chamber is stored an inert gas typically argon. In this chamber, ion-atom collisions takes place and the result is that the fast ions produce fast travelling atoms. Any of the resulting ions or unchanged fast ions are deflected away from the target plate, on which the sample in a medium such as glycerol has been deposited. The stream of fast moving atoms provide the required energy to both ionise and volatilise the sample. The role of the matrix material appears to be important in promoting the ionization of the sample and is under investigation [144-150].

This method is becoming increasingly popular as it can be controlled to provide atoms with energy sufficient not only to ionise and volatilise the sample, but also to allow some

fragmentation. The energy available for fragmentation is controlled by the angle of incidence of the atom beam (Scheme 4.8). If the beam is incident at near 90° then the beam only "grazes" the sample and the majority of the energy is limited to the surface layers of the matrix and thus, promotes the desorption of molecular-containing species [151]. If however, the angle of incidence is near 0° then there occurs localised and uneven energy deposition with the formation of "hot spots" and therefore the analyte is more prone to degradation [152], the optimum angle of incidence being between 60° to 70° [153].

The technique also allows the formation of quasi-molecular ions of either positive or negative charge. Ion-molecule collisions producing $(M + nX)^+$ species are again a feature of this "soft" ionization technique. In addition to the ions of the sample, ions of the bombarding atoms and ions of the matrix are also formed. One common problem is that the matrix material often dissolves salts which exist as preformed ions and these are readily desorped and appear in the spectrum [154].

The presence of these salts often complicate the FAB spectrum as the pseudomolecular ions formed occur as adduct ions in which the molecular-species has associated itself with an ion such as H^+, Na^+ or K^+. The spectrum may also be complicated by the tendency for the pseudomolecular ion to lose water. This multiplicity of peaks has often complicated the determination of the molecular weight of the sample. More recently, $AgNO_3$ has been deliberately introduced to the matrix [155] so that the resulting silver adduct ions are readily recognisable. Recognition of the silver ion adducts relies on the isotope abundance of silver, which exists as two nearly equally abundant isotopes (Ag^{107}/51.82% and Ag^{109}/48.18%).

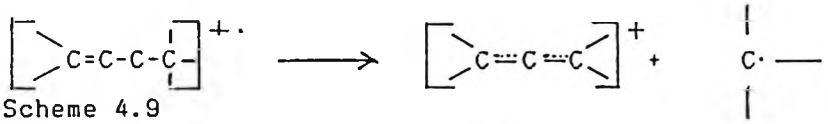


Diag. 4.8 Angle of incidence and fragmentation in FAB

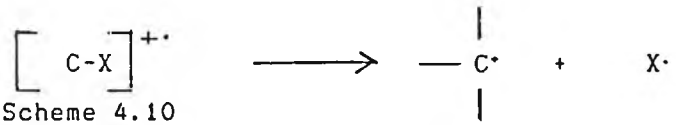
4.4.6 Fragmentation.

By itself, the molecular ion provides very little information about the molecular structure. Much more information can be obtained from looking at the fragments caused by bond fission owing to an excess internal energy during ionisation.

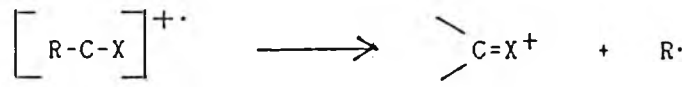
Currently, there are two qualitative theories which deal with fragmentation and seek to establish some of the empirical rules. One theory relies on fragmentation being determined by the relative stability of the fragments. After an initial ionisation, the molecular ion is considered to be a species of high energy. This energy is uniformly spread throughout the ion and manifested by bond fission. The bond fission is determined by the relative activation energy of the various fragmentation pathways. The transition state of each endothermic pathway can be represented by the energy of the resulting products. The fragmentation with the highest probability of occurring will be that which leads to the most stable fragments. On this basis, several fragmentation schemes have been proposed [132].



Scheme 4.9



Scheme 4.10



Scheme 4.11

Thus, products which were stabilised by factors such as the inductive and mesomeric effects are the more likely ions to be formed. Equally where the activation energy of fission might be offset by a large endothermic release, *e.g.* by the formation of carbon dioxide or other stable products, this would also be a favoured process.

The alternative theory is based on charge localization [158]. It is supposed that ionization leads to a cationic radical in which the charge can be localised in some portion of the molecular ion. This localization of charge acts as a trigger for fragmentation. The fragmentation proceeds by either one- or two- electron shifts,

which causes bond fission. The radical site has been advocated primarily as the driving force [142]. Any stabilization of the charge or radical can be considered as a bonus making the reaction more favourable. This theory readily lends itself to easy visualisation of the fragment structure and the observation of particular ions. By convention, a one-electron shift is represented by a single headed arrow, or "fish hook", the usual double-headed arrow being used to represent a double electron shift.

Neither theory completely explains all the features of fragmentation, because of their simplicity. Unusual fragments and rearrangements can not satisfactorily be explained if the product stability argument does not hold, as a tenet, the concept of charge localization. The charge localization theory whilst being ideal for predicting what is possible is somewhat weaker at predicting which fragmentation pathway is more favourable. It has been proposed that the theories are complementary and that the best approach to predicting fragments is to use the charge localization theory to predict the possible fragmentations and use the product stability argument to help select the more likely fragments to be observed [123]. Quantitative theories have been advocated to explain rates of fragmentation [159,160]. These theories involve complex calculations of the distribution of excess internal energy caused by ionisation to explain selective bond fission.

A problem common to all theories is the frequent absence of any definitive evidence of ion structure. Isotopic studies, particularly isotopic scrambling, suggest that the structure of the ion may not always be the obvious molecular structure. It is not always safe to assume that these ionic species behave rather like the ions found in solution.

The EI mass spectra cited in the present experimental studies were obtained by a double focusing spectrometer at 70eV. The analysis of fragmentation is based on the application of the charge localised theory using the high resolution masses to confirm elemental composition. Therefore, a brief summary of some of the determining factors is in order.

a) The ionisation of the molecule leads to a radical cation having a similar structure to the molecule.

b) The most favoured positions for localising charge are atoms bearing electrons with the lowest ionisation potential. As an

approximate guide to the probability of charge localization [142]:-

non-bonded > *pi*-conjugated > *pi* bonds > *sigma*

c) One-electrons shifts occur towards centres containing a radical, whilst two-electron shifts occur towards centres having a positive charge.

d) The bond is considered as having been split when it contains one unpaired electron or when it no longer has any electron.

e) electron movements are treated primarily in a step-wise manner to predict the possible fragments observed. Concerted reactions, such as a retro Diels-Alder reaction, are used to explain certain ions.

f) The decomposition of fragments follows the even-electron rule [161-163], which states that odd electron ions decompose by loss of a radical (odd electron fragment) or by the loss of an even electron fragment. Even electron ions almost always decompose by loss of even electron fragments.

g) For the most part, the positive charge should reside on the fragment with the lowest ionisation potential [164].

Some of the empirical fragmentation processes used are also included in this review.

a) **The *sigma* cleavage.**

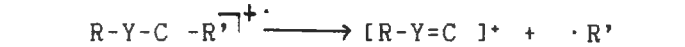
This refers to the ionisation of the single bond, in which a single electron has been removed from the bond. Thus, by rule d) the fission of this bond is envisaged to result in a radical and a cation.

b) **Charge site initiation.**

In this case the initiation of cleavage occurs via the positive charge which involves the attraction of an electron pair. This is similar to the *sigma* cleavage and results in the formation of R+ from RY+·. This sort of fission parallels the inductive effects of the heteroatom Y. Thus, halogens > oxygen or sulphur > nitrogen or carbon in displaying this sort of fragmentation.

c) **The *alpha* cleavage.**

The formation of a radical cause a bond fission between the alpha carbon and its neighbouring groups by a one-electron shift.



Scheme 4.12

The same process can also be initiated by a positive charge involving a two-electron shift. The ring opening of six membered rings by a retro Diels-alder can placed in this classification.

d) Rearrangement/cleavage at other sites.

In these sorts of reactions, either a radical or a positive ion initiates a migration of a substituent on an atom positioned $C_{(3)}$ or $C_{(4)}$ or further from the localised initiator. Typical to these migrations is a representation of the transition state as a cyclic structure involving several electron shifts. A typical example is the McClafferty rearrangement of ketones [164].

One source of confusion has been in using the term "*gamma*". The alpha cleavage has been defined as the cleavage of the $C_{(1)}$-X bond in a structure X-$C_{(1)}$-Y$^{+\cdot}$, where X is a substituent on the *alpha* carbon to Y. In order to be consistent, a *gamma* cleavage in ketones involves the cleavage of $C_{(4)}$-X bond. In this instance the carbonyl moiety is considered as part of Y, whilst the $C_{(2)}$ carbon is the *alpha* carbon.

e) Charge Migration.

As the heading suggests, one process that might occur is that of charge migration prior to fragmentation. Thus, the original structure is altered to give new initiator sites which then can be simply used to predict fragmentation. Often the migration of charge is effected by the migration of hydrogen radicals or hydride ions.

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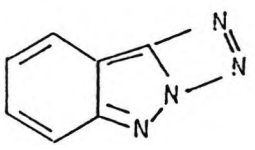
Chapter 5. Preparation of diazo heteroaromatic compounds.

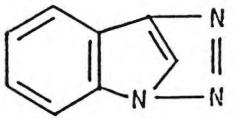
5.1 5-Membered rings containing nitrogen.

One of the earliest compounds to be diazotized was indazole. A structure containing a diazo group as part of a four-membered ring was proposed for the product (5.1) [1,2].

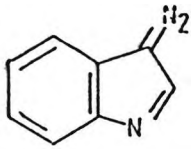
Similar work was undertaken with indole [3] and two structures proposed (5.2 and 5.3). One of them seems implausible given the geometrical constraints of bridgehead carbons.



(5.1)

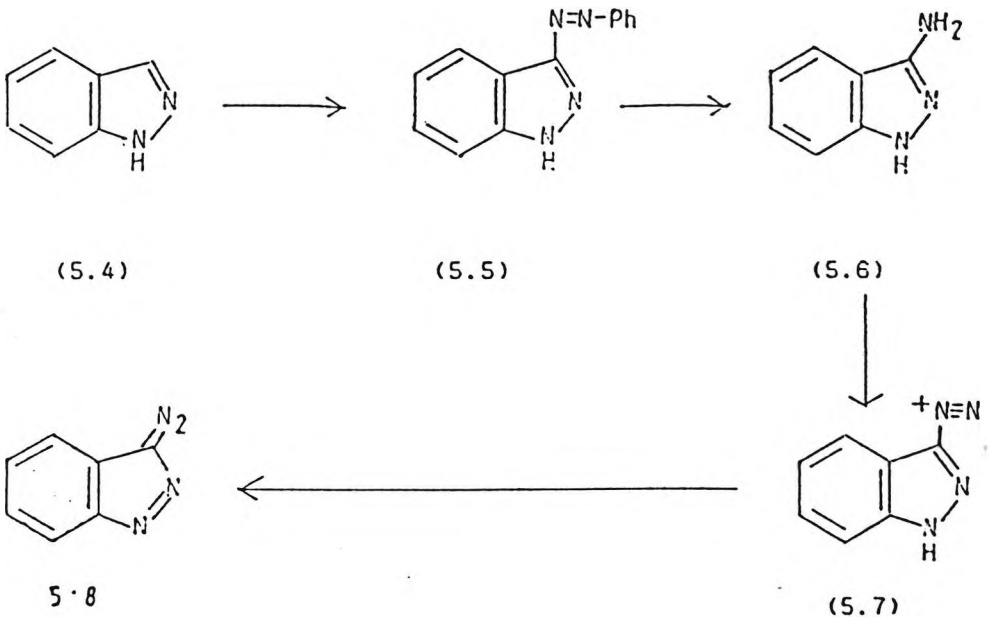


(5.2)



(5.3)

Diazoindazole was prepared from indazole (5.4) by coupling it with a benzene diazonium ion at the C-3 site through an electrophilic substitution. The resulting azo compound (5.5) was converted to 3-aminoindazole. The 3-aminoindazole was diazotized in dilute acid and afforded an indazole diazonium salt which on treatment with base yielded 3-diazoindazole (5.8) (Scheme 5.1). This method appears to be a general method from other studies [4,5].



(5.4) (5.5) (5.6)

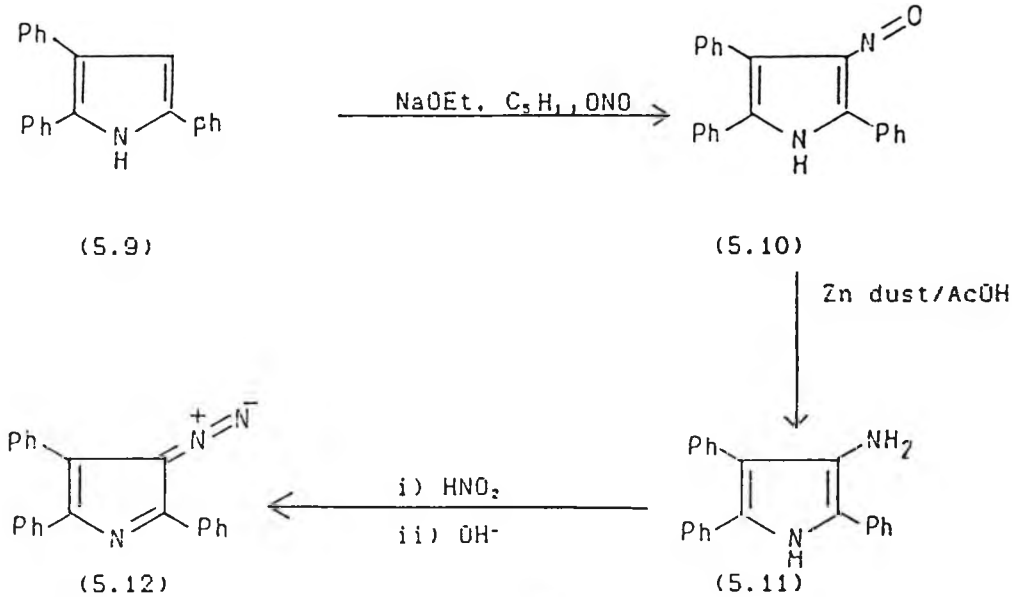
(5.7)

5·8

Scheme 5.1 Preparation of 5-diazoindazole

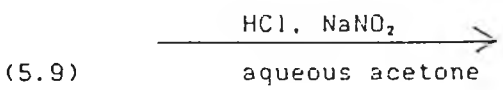
In principle, the diazotization of heterocyclic compounds should be no different from the usual preparative methods, outlined previously (Chp.3). A common feature of the diazotization of heterocyclic amines is the formation of the heteroaromatic diazonium salt which in the presence of a weak base readily forms the diazo heterocycle with the loss of a proton.

The diazotization of pyrrole proceeds by this general pathway. The pyrrole diazonium salts are sufficiently acidic to lose a proton in weakly acidic or neutral solutions. Often the only difficulty in the diazotization of the heterocycle is the preparation of the amine precursor. One method of circumventing this problem in both indoles and pyrroles has been to form the amine from the nitroso derivative rather than the usual nitro compound (Scheme 5.2) [6].



Scheme 5.2 Preparation of 3-diazo-2,4,5-triphenylpyrrole

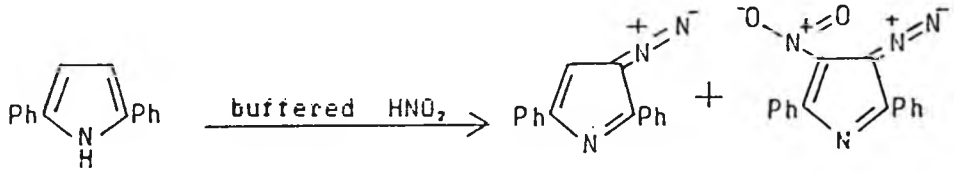
A one step introduction of the diazo group has been carried out at the C-3 position in pyrroles using an aqueous acetone solution of nitrous acid [7] (Scheme 5.3).



(5.9) (5.12)

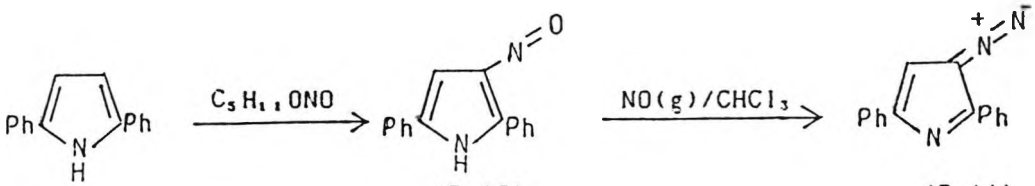
Scheme 5.3 One-step diazotization of 2,4,5-triphenylpyrrole

This direct insertion of the diazo group could be applied to pyrroles with vacant C-2 positions [8]. Generally, the 2-diazo--pyrroles are less stable than the 3-isomers. An unwanted side--reaction has been additional nitration, *e.g.* in the diazotization of 2,5-diphenylpyrrole (5.13) (Scheme 5.4). This can be avoided by nitrosation using pentylnitrite and sodium ethoxide to give the nitrosodiphenylpyrrole (5.16), which can then be converted to the diazo compound (5.14) by treatment with gaseous nitric oxide (Scheme 5.5).



(5.13) (5.14) (5.15)

Scheme 5.4

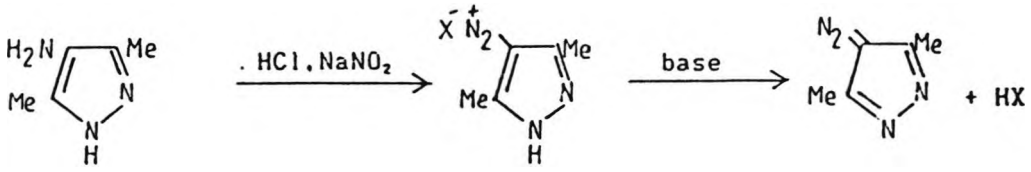


(5.16) (5.14)

Scheme 5.5

The preparation of 3-diazo-3H-pyrazole proceeds in a similar manner to that for pyrrole. The resulting diazonium salt is only weakly acidic and hence it was possible to isolate the diazonium salt [9]. The formation of 3-diazopyrazoles takes place in concentrated acids, otherwise the reaction of the product with the starting amine to give a triazene readily occurs [10]. The diazonium salts obtained readily underwent the normal coupling reactions [11-13]. Treatment with base resulted in deprotonation at the N-1 position leaving the diazo compound [14].

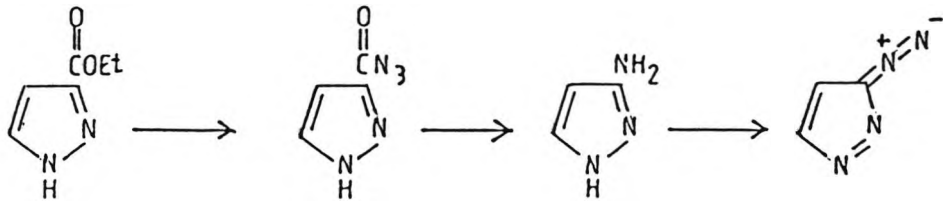
4-Amino-3,5-dimethylpyrazole (5.17) was diazotized to give a stable but highly reactive diazonium salt (5.18) [15-17]. The diazonium salt was readily converted to the diazo compound (5.19) by treatment with base [14,18] (Scheme 5.6).



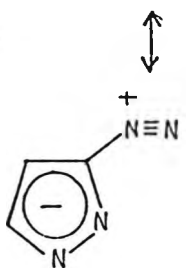
(5.17) (5.18) (5.19)

Scheme 5.6

Another route used in the pyrazole series goes via a Curtius rearrangement [9] (Scheme 5.7). It should be noted that the neutral diazo compound (5.23) is one possible structure. An equally valid structure is the representation of the diazo heteroaromatic compound as a zwitterion (5.24). In all probability, the best representation is a hybrid between the two forms as suggested by work with diazocyclopentadiene [19].



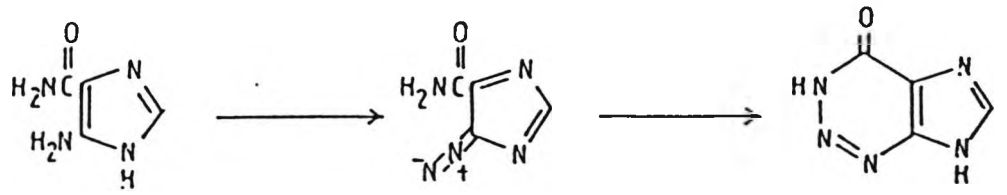
(5.20) (5.21) (5.22) (5.23)



(5.24)

Scheme 5.7 Preparation of 3-diazopyrrole

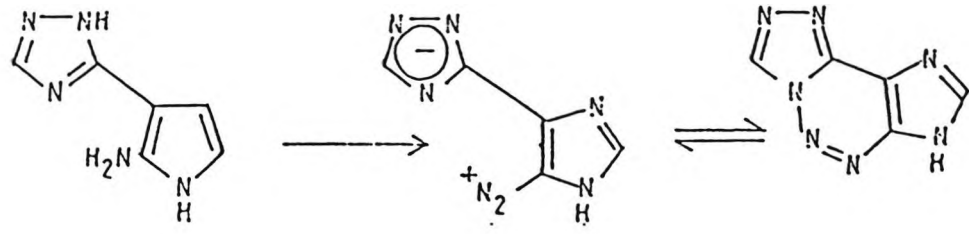
Several imidazoles have been diazotized, most of the methods are similar to the method for pyrrole [20-23]. 5-Aminoimidazole-4--carboxamide (5.25) has been diazotized with nitrous acid to yield the corresponding 5-diazoimidazole-4-carboximide (5.26). The diazo compound can react via an intramolecular cyclisation to give 2-azahypoxanthine (5.27) [24-27] (Scheme 5.8).



(5.25) (5.26) (5.27)

Scheme 5.8

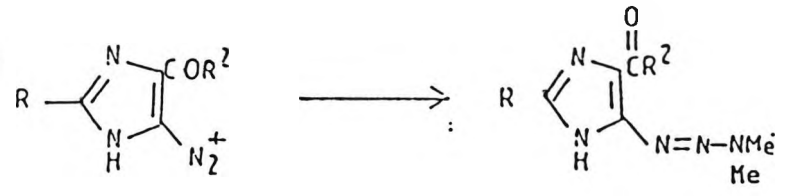
5-Amino-4-(1,2,4-triazol-5-yl)imidazole (5.28) when treated with sodium nitrite in hydrochloric acid gives an equilibrium mixture of the diazo compound (5.29) and the tricyclic compound, 6(H)-imidazo--[4,5-e]triazolo[3,4-c]-v-triazine (5.30) (Scheme 5.9) [28].



(5.28) (5.29) (5.30)

Scheme 5.9

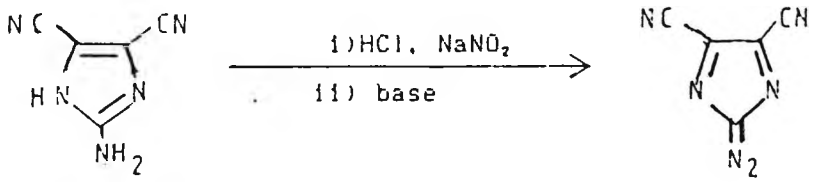
4-Amino-5-acylimidazoles substituted at the C-2 have been diazotized and coupled with dimethylamine to give triazene compounds (5.32) (Scheme 5.10) [29].



(5.31) (5.32)

Scheme 5.10

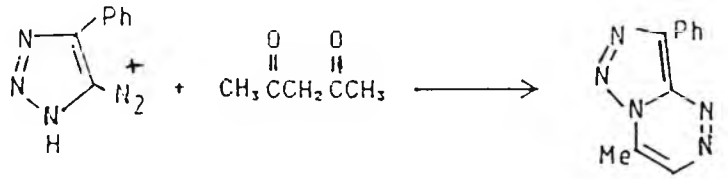
Diazotization of aminoimidazoles containing electron--withdrawing groups proceeds directly to the diazo compound. Thus, 2-amino-4,5-dicyanoimidazole (5.33) affords the diazo compound (5.34) when diazotized by the usual methods [30].



(5.33) (5.34)

Scheme 5.11

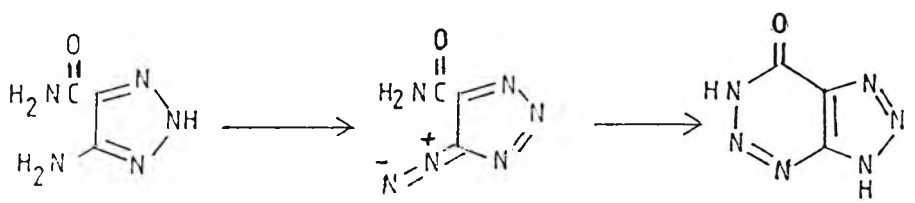
1,2,3-triazoles [21,31], and 1,2,4-triazoles have also been prepared in the same way as diazopyrroles. The diazonium salt of 5-amino-4-phenyl-1,2,3-triazole has been prepared and coupled with acetylacetone to form triazolotriazines [32].



(5.35) (5.36)

Scheme 5.12

The diazotization with pentyl nitrite in aqueous acetic acid of 5-amino-1,2,3-triazole-4-carboxamide gives a diazo compound that readily rearranges to afford 2,8-diazahypoxanthine [24].



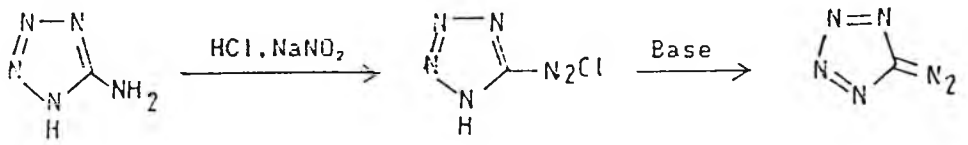
(5.37) (5.38) (5.39)

Scheme 5.13

5-amino-1,2,4-triazoles substituted with aryl groups at the C-3 position can be converted into the corresponding diazonium salts [33,34]. The reaction also proceeds when the C-3 substituent is an alkyl group [35,36]. In addition to aryl and alkyl substituents, a wide range of 5-amino-1,2,4-triazoles has been diazotized [37-40].

Although the N-H bond is extremely acidic, the isolation of the free diazo compound was not reported.

5-Aminotetrazole (5.40) on diazotization gives the diazonium salt which is capable of undergoing the usual coupling reactions [41-44]. Treatment with base readily gives the diazotetrazole (5.42) [45,46] (Scheme 5.14). Caution should be taken in ensuring that the diazotetrazole solution is extremely dilute. The concentrated solution is capable of spontaneous explosive decomposition [47].

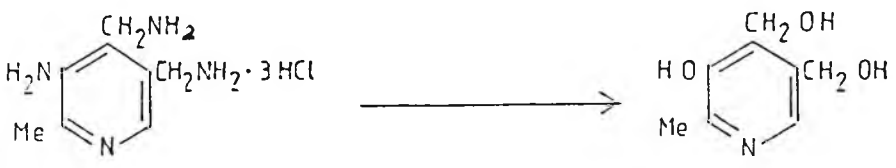


(5.40) (5.41) (5.42)

Scheme 5.14

5.2 Diazopyridines.

The preparations of some halopyridines via diazotization of the aminopyridine in the presence of halide have been reported [48-50]. Diazotization of 3-amino-4,5-bis-amino(aminomethyl)-2-methylpyridine trihydrochloride (5.43) gives pyridoxin(e), more commonly known as vitamin B6 (5.44)[51] (Scheme 5.15).

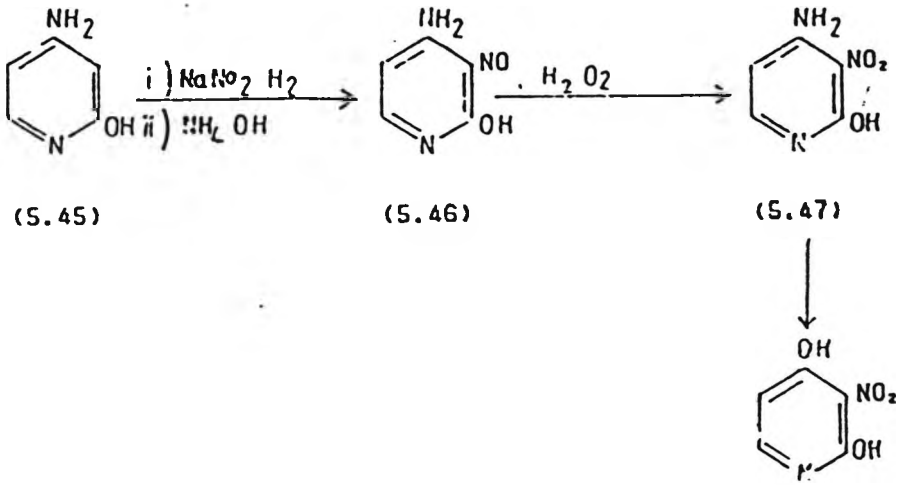


(5.43) (5.44)

Scheme 5.15

Diazotization of 3-amino-2-methylpyridine resulted in the formation of the 3-hydroxy-2-methylpyridine [52]. However, diazotization of 2-amino-4-methoxy-3-methylpyridine gave 7-methoxy-pyrazolo[3,4-b]pyridine. 4-Amino-2-hydroxypyridine (5.45) gave 4-amino-2-hydroxy-3-nitrosopyridine (5.46) which was oxidised by hydrogen peroxide to give the 3-nitro- compound (5.47). This

compound in turn could be converted into the corresponding 2,4-dihydroxy-3-nitropyridine (5.48) (Scheme 5.16) [53].

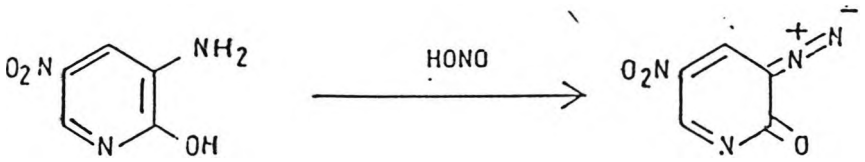


(5.45) (5.46) (5.47)

(5.48)

Scheme 5.16

Although the diazotization of aminopyrimidines leads to products which appear to be the result of the breakdown of a diazo intermediate, only the diazotization of 3-amino-2-hydroxy-5-nitro--pyridine (5.49) led to an isolatable diazo compound, 3-diazo-5--nitro-2-pyrimidone (5.50) (Scheme 4.21) [54].



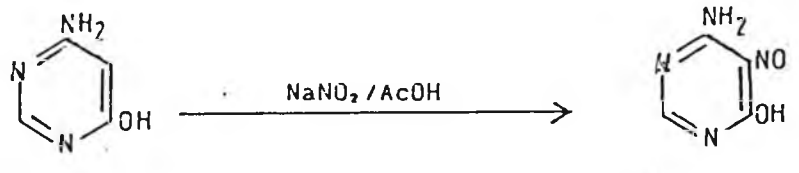
(5.49) (5.50)

Scheme 5.17

5.3 Diazopyrimidines.

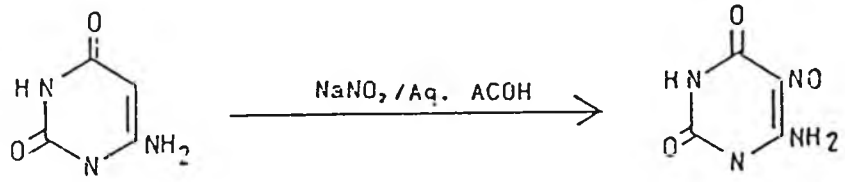
In the diazotization of aminopyrimidines it was observed that amino groups positioned both α- and γ- to the sp^2 nitrogens of the ring were resistant to diazotization. It has been reported that 2- and 4-aminopyridine when diazotized gave solutions which reacted with alkaline β-naphthol [55]. However, the products of the reaction were not elaborated upon and it must be concluded unless further evidence to the contrary is obtained that the preferential reaction remains the nitrosation of the C-5 position or at more reactive

sites on side chains [56-60] (Scheme 5.18-5.21).



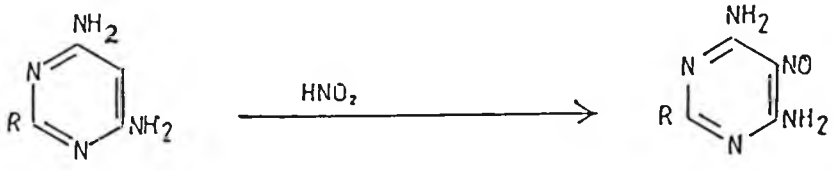
(5.51) (5.52)

Scheme 5.18



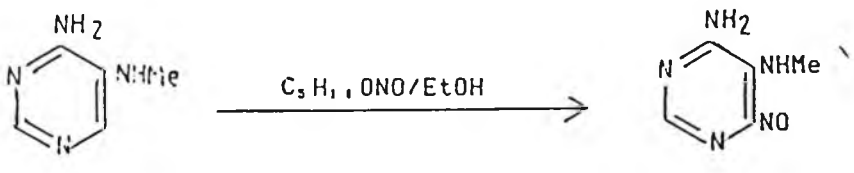
(5.53) (5.54)

Scheme 5.19



(5.55) (5.56)

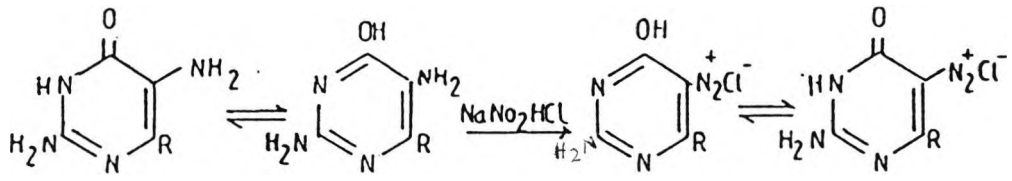
Scheme 5.20



(5.57) (5.58)

Scheme 5.21

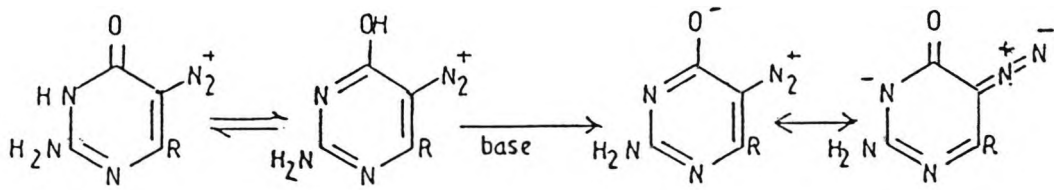
It has been reported that 5-aminopyrimidine does not produce the expected diazonium salt [62]. However, its derivatives are readily diazotized [61a]. The relative reactivities of C-2 and C-5 amino groups to diazotization has been reported for 2,5-diamino-4--hydroxy-6-methylpyrimidine (5.59) and the product was identified as the 5-diazonium salt (5.62) (Scheme 5.22), which underwent further reactions whereby the diazonium group was replaced [63].



(5.59) (5.60) (5.61) (5.62)

Scheme 5.22 Diazotization of 2,5-diaminopyrimidine

Support for the the relative reactivity of the C-5 amino over the C-2 amino group has been demonstrated with a different C-6 substituted diaminopyrimidine. The resulting diazonium salt (5.62) was converted to the 5-diazo compound (5.64) by treatment with base (Scheme 5.23) [64].



(5.62) (5.63) (5.64) (5.65)

Scheme 5.23

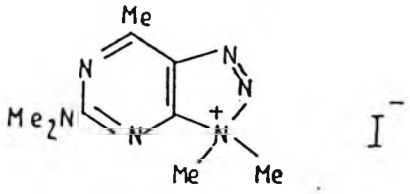
The C-5 aminopyrimidines when diazotized are capable of reacting with active substituents at C-4 and at C-6 to give fused pyrimidine ring systems (5.67). Hydroxyl, thiol and amino groups at these positions will react either with a diazo or a diazonium group to give the respective 1,2,3-oxadiazolo-, 1,2,3-thiadiazolo-, and 1,2,3-triazolo- pyrimidines [56,65-67] (Scheme 5.24).

X = O, S, NH, CH_2

(5.66) (5.67)

Scheme 5.24

2,6-Bis(dimethylamino)-4-methylpyrimidine diazonium iodide is thought to be particularly stable owing to the suspected formation of the pyrimidotriazolium iodide (5.68)[66].

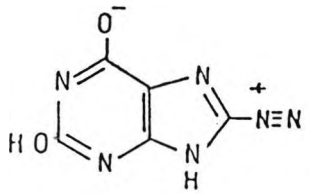


(5.68)

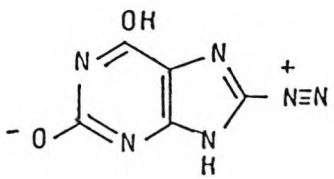
Within the scope of diazopyrimidines is the group of compounds referred to as the diazouracils. These compounds will be examined further in a latter part (Chp 6.0) because of our interest in them.

5.4 Diazopurines.

It has been reported that 8-aminopurines can be diazotized to yield the corresponding, stable diazopurines. For example, the synthesis of 8-diazoxanthine and 8-diazotheophylline [60]. The initial structures proposed were erroneous. The compounds were reinvestigated and two possible diazooxide structures for diazoxanthine (5.70 and 5.71) were thought to be unlikely [21, 68].

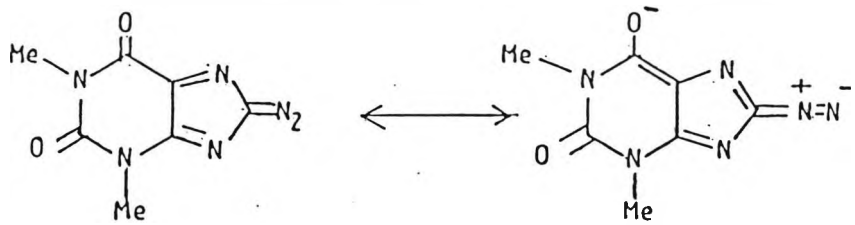


(5.69)



(5.70)

It was argued that the inability to synthesize a stable diazo compound from 8-amino-9-methylxanthine [21] suggested that the formation of a diazo oxide was not a major means of stabilization. It was suggested that the zwitterionic diazooxide was involved in as a resonance form and that this was responsible for a major part of the stabilization, for example 8-diazotheophylline (5.71 and 5.72).



(5.71) (5.72)

It was argued that the zwitterionic diazooxide was the major contributor to the hybrid structure since the i.r. absorption of diazo group in diazoxanthine(2250-2240cm^{-1}) and in diazotheophylline (2225cm^{-1}) were indicative of the "diazonium form" predominating.

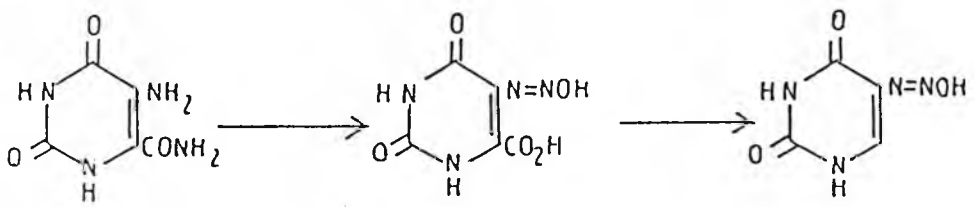
It would appear that the dominant factor in forming a stable diazopurine is the loss of the N-9 proton. The observation that synthesis of "diazocaffeine" from 8-aminocaffeine gave a very unstable "diazo" compound [69] lends further support to this view.

5.5 Diazouracil and some related compounds.

Within the scope of diazopyrimidines is the group referred to as the diazouracils. These compounds are characterised by the presence of hydroxyl groups at C-2 and C-4, *i.e.*, they are diazo derivatives of 2,4-dihydroxypyrimidine. It should be noted that the evidence to date indicates that the equilibrium position lies well over to the keto form (Chp. 4). Thus, a more accurate representation of this class of compounds is represent them as diazo derivatives of 1H,3H-pyrimidin-2,4-diones.[60]

The particular stability of the diazouracils stems from mesomeric stabilization conferred on the molecule by the carbonyls (Chp. 3). It is this stability which makes them ideal candidates for further study especially in view of their role as biologically active compounds.

It was reported that as early as 1890, diazotization of 5-aminouracil-6-carboxylamide (5.73) gave 5-diazouracil-6-carboxylic acid "monohydrate" (5.74), which in turn was decarboxylated to give diazouracil "monohydrate" (5.75) (Scheme 5.25)[70].

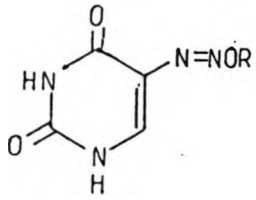


(5.73) (5.74) (5.75)

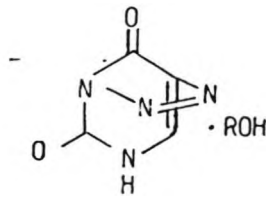
Scheme 5.25 Preparation of "5-diazouracil hydrate"

The rationale for considering the diazo compound as a "monohydrate" (5.75 or 5.76) was based on the observation that if the decarboxylation reaction was performed in ethanol, the product

had an empirical formula consistent with "anhydrous" diazouracil which had co-crystallized with one molecule of solvent. Two structures were proposed for this diazo compound (5.78 and 5.79).

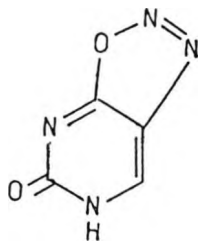


5.75) R = H
5.77) R = Et

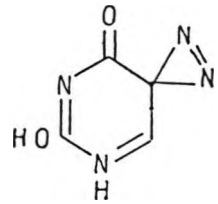


5.76) R = H
5.78) R = Et

A red "diazouracil hydrate" was formed from the diazotization of 5-aminouracil [71] and was assigned corresponding structures (5.75 and 5.76). Diazouracil was also prepared [72] and two possible structures (5.79 and 5.80) were assigned for the compound.

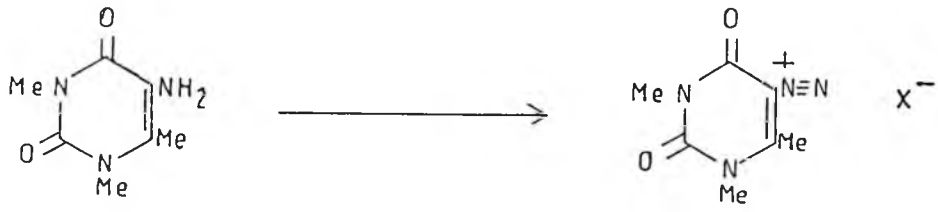


(5.79)



(5.80)

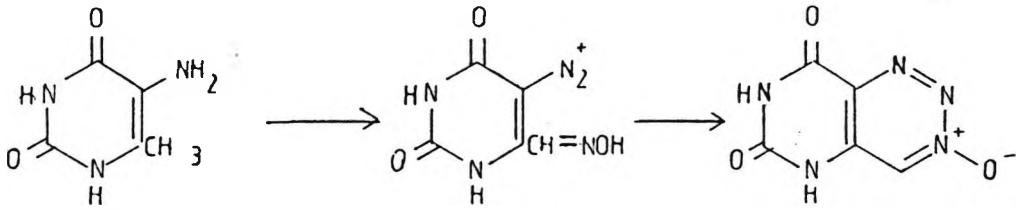
5-amino-1,3,6-trimethyluracil (5.81) when diazotized yielded the corresponding diazonium salt (5.82) (Scheme 5.26) [73].



(5.81) (5.82)

Scheme 5.26

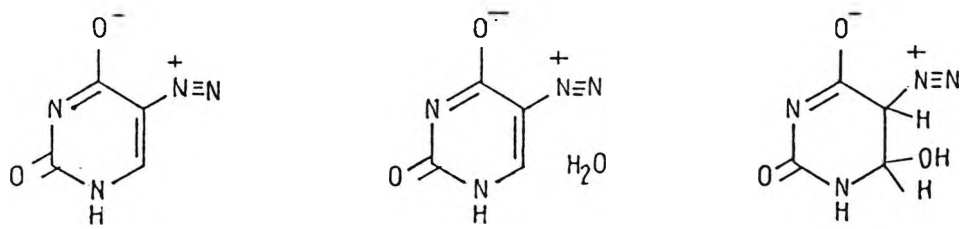
5-amino-6-methyluracil (5.83) with excess nitrous acid in acetic acid gave pyrimido-v-triazine (5.85) [65,73-74] (Scheme 5.27).



(5.83) (5.84) (5.85)

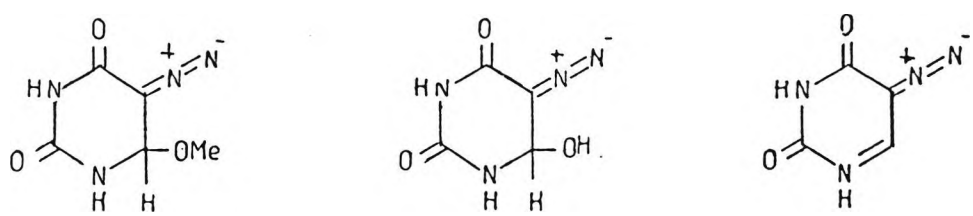
Scheme 5.27

More recently, the structure of diazouracil and its hydrate was subject to further investigation [75]. On the basis of the i.r. spectroscopy, an alternative structure for diazouracil was proposed (5.86). The hydrate derivative was considered to be one molecule of diazouracil co-crystallized with one molecule of water (5.87). An alternative structure which had the water molecule covalently attached was also suggested (5.88).



(5.86) (5.87) (5.88)

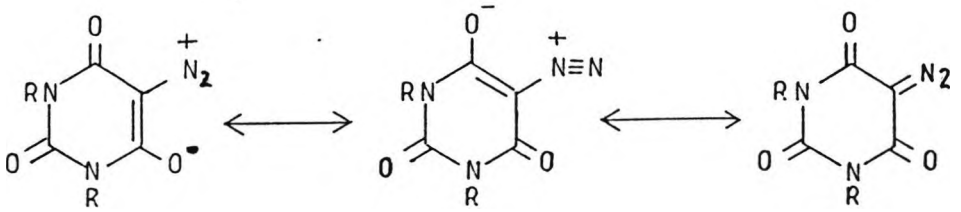
The structure of diazouracil and some relevant derivatives were examined by ^1H n.m.r. spectroscopy and structures assigned on the basis of the data for the methanol adduct (5.89) the water adduct (5.90) and anhydrous diazouracil (5.91) [76].



(5.89) (5.90) (5.91)

Other diazopyrimidines referred to in the literature include 5-diazobarbituric acid (5.92) and 5-diazo-1,3-dimethylbarbituric acid (5.95). The structures of both these compounds were assigned

on the basis of i.r. carbonyl and diazo stretch frequencies and the proposed correlation with structural features [77].



5.92) R=H 5.93) R=H 5.94) R=H

5.95) R=Me 5.96) R=Me 5.97) R=Me

The observation that the diazo group readily couples with sulphite ions to give a diazosulphonate, would also lend support to the concept of a diazonium species being present, a simple analogy being found with arene diazonium salts [78-79].

The same i.r. spectroscopic arguments were applied in proposing a structure for diazouracil and its methanol adduct. In view of the n.m.r. study, it would be reasonable to assume that the structure of diazobarbituric acid may well be as a diazoketone in keeping with the structure found for diazouracil and its methanol adduct. An alternative is to consider the diazobarbituric acid as a resonance hybrid of several canonical forms and with a major contributor being the diazo ketone form (5.94).

5.6 Diazonucleosides.

Both 5-aminouridine [80,81] and 5-amino-2'-deoxyuridine have been diazotized to afford the corresponding diazo compound [80-84]. The diazonucleotides will be dealt in more detail in a latter chapter (Chp.9).

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Part B: Results and Discussion.

Chapter 6. Synthesis and spectroscopic studies of 5-diazouracil derivatives.

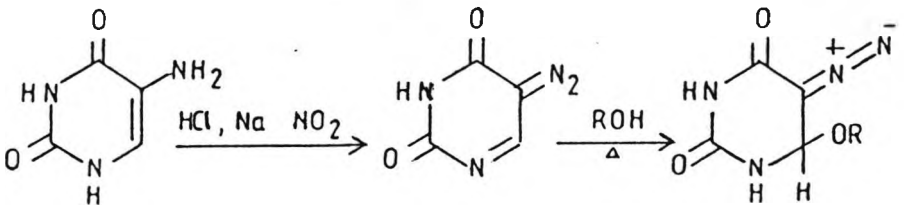
6.1 Introduction

A substantial amount of published work on diazouracil has been concerned with examining its biological activity. Its cytotoxic [1-3], antiviral [4,5], antibacterial [3,6-8] and antitumour activity [9-11] and its action as an antimetabolite [11-13] have been reported. Although most biological studies involved a compound which was referred to as 5-diazouracil, it is highly likely that the actual active agent was the hydrated derivative (6.3) [14].

It is surprising that, in view of the considerable medicinal potential indicated by the biological properties of this class of compounds, little has been published concerning their chemistry. Thus, apart from the parent 5-diazouracil only the 6-hydroxy-, 6-methoxy- and 6-ethoxy- dihydro-5-diazouracils are known and there have been no systematic investigations reported of their chemistry.

In the light of this background, the objectives of the present work have been to synthesize a range of derivatives of the 1,6-dihydro-5-diazouracil and to explore the chemistry of the diazo group. This chapter is concerned with the synthesis of a number of compounds, including the hitherto unknown parent dihydro compound without a 6-substituent, and their detailed examination by spectroscopic methods. The reactivity of the diazo function is described in the subsequent chapters.

There are very few ways in which 5-diazouracil (6.2) can be derivatised whilst retaining the structural integrity of the diazo group. The earlier published work on 5-diazouracil showed that the 1,6-double bond is particularly susceptible to addition. The formation of 5-diazo-6-hydroxy-1,6-dihydrouracil (6.3), 5-diazo-6--methoxy-1,6-dihydrouracil (6.4) and 5-diazo-6-ethoxy-1,6-dihydro--uracil (6.5) can be represented as the addition of ROH to the double bond (Scheme 6.1). Thus, the reaction scheme suggests a general method by which a variety of new diazo compounds may be produced.



(6.1) (6.2) 6.3) R=H

6.4) R=Me

6.5) R=Et

Scheme 6.1 Addition to 1,6-double bond of 5-diazouracil

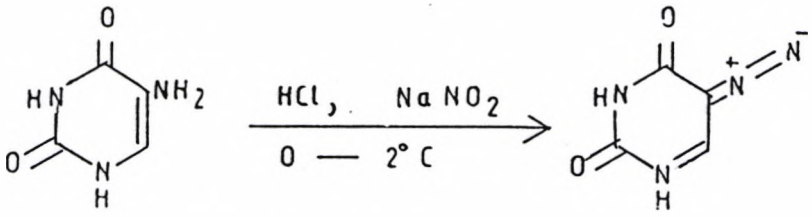
In the present work, a wide selection of alcohols was chosen for reaction with "anhydrous" diazouracil (5-diazouracil) (6.2), which was prepared from commercially available 5-aminouracil (6.1) [14]. The selection included a secondary alcohol, 2-propanol, and a tertiary alcohol, 2-methyl-2-propanol. Phenol and 4-methoxyphenol were chosen to represent the phenolic type alcohols.

In general, the poor solubility of uracil compounds in organic solvents has often been a hindrance in their identification and in studying aspects of their structure. Although uracil can be clearly identified with ^1H n.m.r. from its two vinylic doublets, any subsequent substitution of either of these protons removes the characteristic coupling. Thus, identification and structural information can be very ambiguous. For example, 5-diazouracil has a simple spectrum, being composed of two singlets and like many substituted uracils it presents a problem, especially when one of the singlets, the H-3 signal, is prone to changes in broadness and in chemical shift depending on the sample conditions. The derivatisation of diazouracil at the C-6 position should provide a readily identifiable label when examining subsequent derivatives by ^1H n.m.r. Two spectroscopic methods in which problems of solubility can be circumvented for the purpose of structural investigation are the techniques of infrared spectroscopy and mass spectrometry. There appears to be a dearth of literature on mass spectra of diazo compounds and more specifically on the mass spectra of diazouracils. Thus, a study of the mass spectrometry of this class of compounds would be of particular interest.

6.2 5-Diazouracil.

6.2.1 Preparation.

5-Diazouracil was prepared from commercially available 5-aminouracil. The 5-aminouracil was dissolved in 1M hydrochloric acid and cooled to a temperature between 0-3°C. A 6.9% aqueous solution of sodium nitrite solution was added in a dropwise manner and the mixture constantly stirred. The yellow precipitate formed was filtered from the acid solution and washed with cold ethanol at (-15°C). The diazo compound was obtained with a crude yield of 95% and was dried in vacuo at room temperature (Scheme 6.2).



Scheme 6.2

The yellow product was characterised in its ^1H n.m.r. by three singlets, a 1H broad singlet at 10.9δ (H-3), a sharper singlet at 9.1δ (H-6) also integrating for one proton and a singlet around 3.3σ (H_2O) integrating for slightly more than one proton. The spectrum was consistent with the literature but showed traces of residual water. Further drying in vacuo at 50°C, did not entirely remove all the water as indicated by a smaller signal around 3.0δ. The melting point was of the doubly dried material gave fine droplets at around 130°C with the bulk of the crystals melting between 198-205°C (Lit. 210°C).

The compound was characterised in the i.r. by a strong diazo absorption peak around 2130cm^{-1} (diazo), which was approximately equal in intensity to the peaks in the carbonyl region around 1650cm^{-1} and 1710cm^{-1}.

Attempts to prepare 5-diazouracil using a cold water wash to remove traces of acid gave a mixture, which was identified by ^1H n.m.r. to contain 5-diazouracil and 5-diazo-6-hydroxy-1,6-dihydro--uracil.

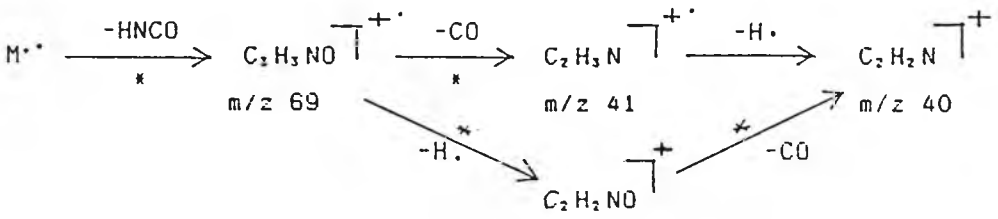
The molecular formula of 5-diazouracil was confirmed in the mass spectrum by finding peaks at mass 138 and 139. These two masses

were shown in the high resolution spectrum to correspond to the molecular ion [M$^{+\cdot}$] and the isotopic ion [M+1]. Thus, the molecular formula $C_4H_2N_4O_2$ can be deduced for the product of the diazotization of 5-aminouracil.

6.2.2 EI Mass spectrum.

6.2.2.1 Uracil.

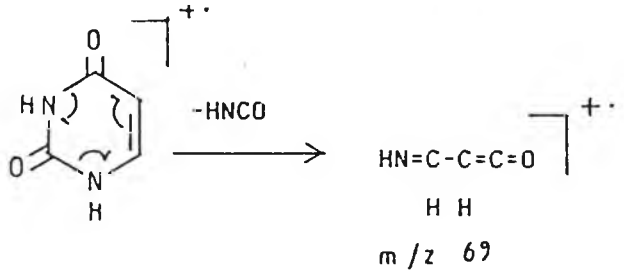
In order to facilitate the analysis of the mass spectrum of 5-diazouracil, a review of the EI spectrum of uracil would be helpful. Therefore, a sample of uracil was analysed by mass spectrometry using EI at 70eV. The spectrum was characterised by the peaks at m/z 113, 112, 69, 68, 42, 41 and 40. The peaks at m/z 113 and 112 correspond to a [M+1] ion and the molecular ion [M]. The percentage relative intensity of these two peaks of (8:100) indicated that the peak representing the fragment [M+1] includes both the fragment with the isotopic carbon and a molecular ion with an extra hydrogen, or so called pseudo-molecular ion [M+H]. In addition to the characteristic peaks at m/z 69, 42, and 41, fragments at m/z 68 and 40 were obtained. These fragments were shown by high resolution mass spectrometry to result from either the loss of hydrogen from the the daughter ion at m/z 69 followed by the loss of CO or the result of two separate fragmentations in which atomic hydrogen is ejected (Scheme 6.3).



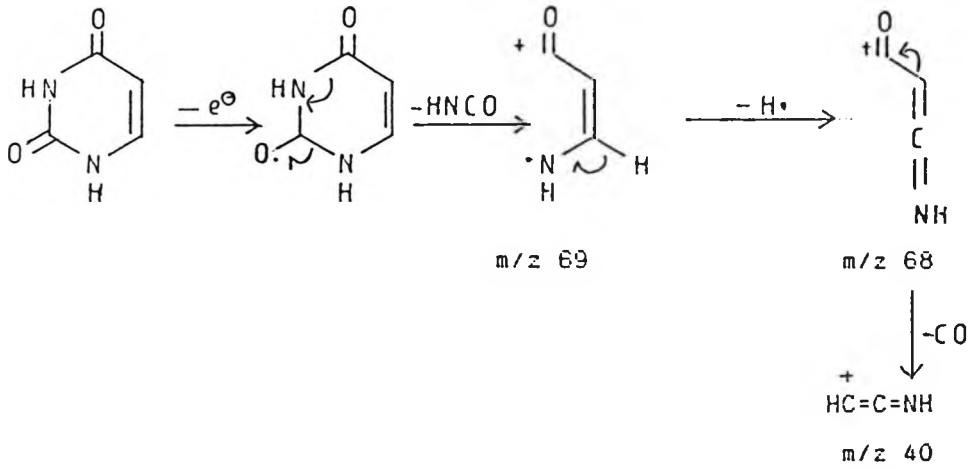
* metastable peak found
Scheme 6.3 EI-MS fragmentation of Uracil

The fragmentation can be explained by theoretical paths worked out using a charge localization theory. It might also be predicted that alternative fragmentation pathways might occur and these would lead to different masses being observed. Such theoretical considerations are often in good agreement with the observed results. The loss of CHNO can be explained either as a concerted retro-Diels Alder (Scheme 6.4) or by a sequential

fragmentation (Scheme 6.5). The fragments [M-43] and [M-71] are characteristic of pyrimidin-2,4-diones.

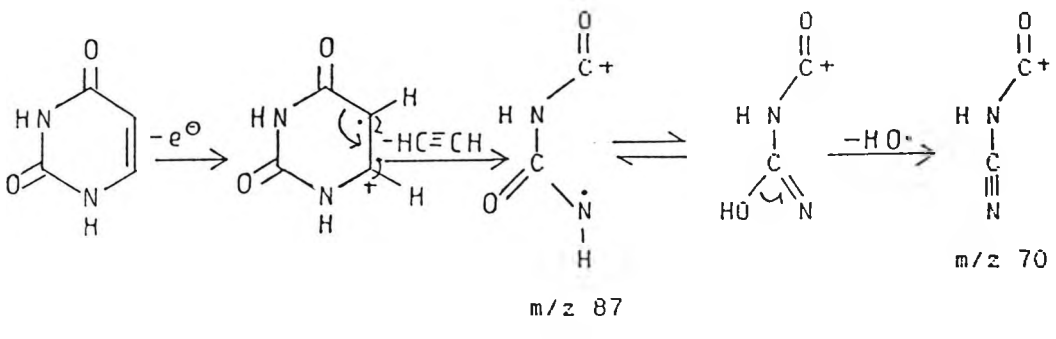


Scheme 6.4 Retro Diels-Alder fragmentation



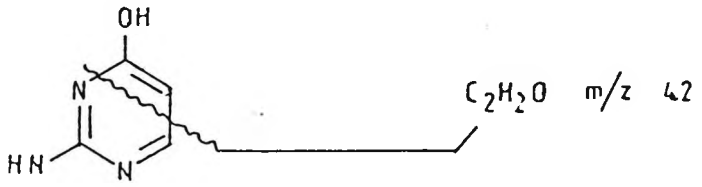
Scheme 6.5

Two peaks found in the spectrum at m/z 70 and 42 have been shown to correlate to the respective formulae C_2HN_2O and C_2H_2O. It is not immediately obvious as to how these fragments arise. However, if charge localization fragmentation is applied to the alternative tautomeric form, a reasonable explanation may be put forward (Scheme 6.6 and 6.11).



(6.6) (6.7) (6.8) (6.9) (6.10)

Scheme 6.6



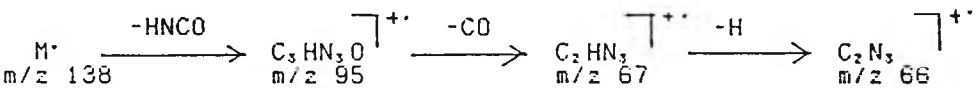
(6.11)

Evidence to support the proposed pathway was obtained in a second high resolution spectrum which contained the intermediate fragment at m/z 87 which agreed with the formula required for (6.8).

6.2.2.2 5-Diazouracil.

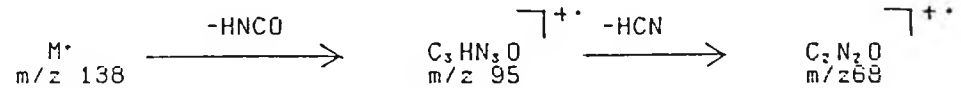
The high resolution EI spectrum of 5-diazouracil showed peaks at m/z 139 and 138. The observed ratio of relative intensities for the peaks 139:138 was 4:51. This suggests that in addition to the [M+1] ion of isotopic carbon (expected at a ratio of 2:51), there exists another species, which corresponds to the pseudomolecular ion [M+H].

Like uracil, fragments are observed which represent the sequential loss of HNCO [M-43], carbon monoxide [M-71], and hydrogen [M-72] (Scheme 6.7).



Scheme 6.7

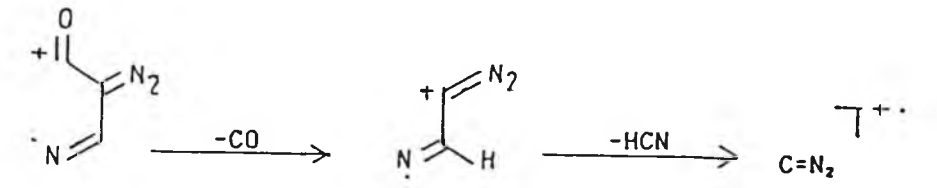
The observation of a peak at m/z 68 appears to follow another commonly observed fragmentation pathway of uracil, *i.e.* the loss of HNCO followed by the loss of HCN (Scheme 6.8).



Scheme 6.8

For convenience, a charge localised intermediate (6.12) may be postulated. It can be seen that this intermediate could lose carbon monoxide or hydrogen cyanide (Scheme 6.9). In addition, it can be

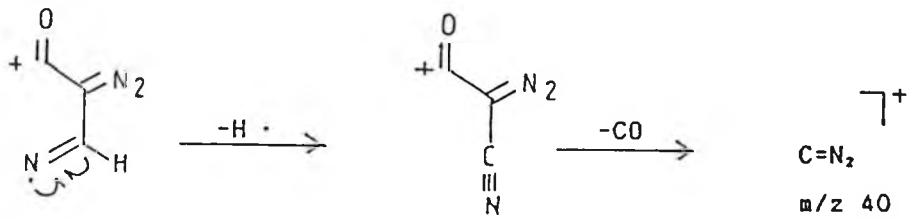
predicted that the loss of atomic hydrogen might also be a plausible fragmentation (Scheme 6.10). The postulated fragment from such a process was identified in a second high resolution mass spectrum. Whilst the loss of CO and HCN were always observed (Scheme 6.11), the loss of atomic hydrogen did not always show up, which suggests that the loss of the neutral species CO and HCN are more favoured than the ejection of a hydrogen radical.



m/z 95 m/z 67

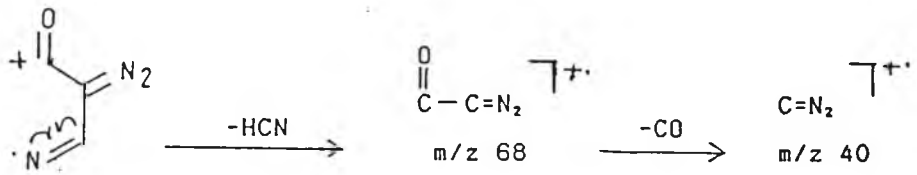
(6.12) (6.13) (6.14)

Scheme 6.9



m/z 95 m/z 94

Scheme 6.10



m/z 95

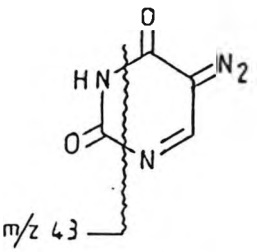
Scheme 6.11

The observation of the peak at m/z 112 implies a rather unusual fragmentation. High resolution spectrometry shows that this represents a loss of CN from the molecular ion. This loss of the CN fragment implies the presence of a nitrile group or a imine moiety. Ejection of the cyanide radical would have to involve a hydrogen rearrangement. An alternative suggestion is that the peak at m/z 112

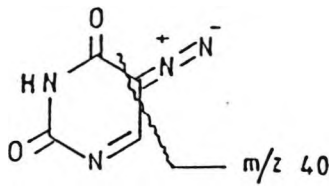
is not the daughter ion of the molecular ion but rather the result of the pseudomolecular ion expelling the neutral species HCN.

The peak at m/z 69 appears to correspond with the loss of a cyanide radical from a fragment m/z 95. However, this could be from an undetected daughter ion, m/z 96, of the pseudomolecular ion or from other fragmentations. Without the ability to detect metastable ions, the actual source(s) of the fragment cannot be traced.

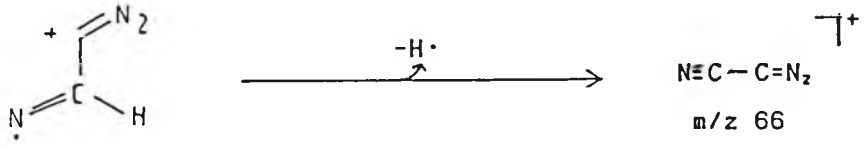
Other small fragments can be detected at masses 66 (C_2N_3), 43 (CHNO), 41 (CNO), and 40 (CN_2) and can be related to various structural features of 5-diazouracil (6.15 and 6.16, and Scheme 6.12).



(6.15)



(6.16)



m/z 67

Scheme 6.12

6.3 5-Diazo-6-methoxy-1,6-dihydrouracil.

6.3.1 Preparation.

This diazo compound was prepared from 5-aminouracil by the usual diazotization in 1M hydrochloric acid with the addition of sodium nitrite solution. The bright yellow 5-diazouracil which precipitated out was filtered from the acidic solution and washed with cold water until the washings gave a negative chloride ion result with silver nitrate solution.

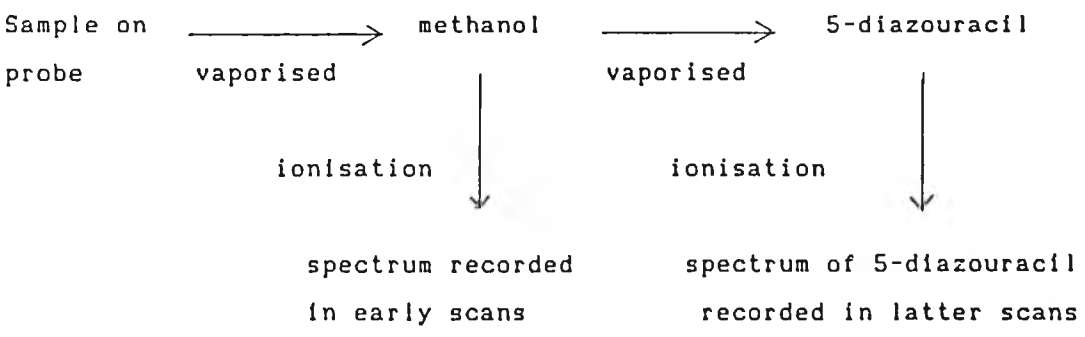
The product was dissolved in boiling methanol and activated charcoal was added to remove coloured impurities. The solution was filtered hot and the filtrate allowed cool to room temperature. On standing, the solution afforded bright yellow crystals in 90% yield.

The compound was characterised in the ^1H n.m.r. by four signals. A broad singlet around 10.3σ, which was exchangeable with D_2O and integrating for one proton, was assigned to the H-3 proton. A doublet at 8.6δ with a coupling constant J=4.0Hz, exchangeable with D_2O, and also integrating for one proton was assigned to the H-1 proton. The other doublet, J=4.0Hz, at 5.75δ which also integrated for one proton was assigned to the H-6 proton. In D_2O, this doublet collapsed to a singlet. The remaining 3H singlet at 3.35δ was assigned to the methyl protons of the methoxy group. The spectrum was consistent with the literature.

6.3.2 EI mass spectrum.

The EI spectrum of 5-diazo-6-methoxy-1,6-dihydrouracil is of interest as a representative of the dihydrouracils. At 70 eV the fragmentation of the molecule is too extensive and neither the molecular ion nor a pseudomolecular ion were detected. Peaks at m/z 139, 138, 95, 67 and 66, characteristic of 5-diazouracil, were observed. High resolution mass spectrometry confirmed that the these peaks had formulae which were consistent with fragments of 5-diazouracil. The 5-diazouracil species may have been generated by either one of two means or as a combination of the two. It was thought likely that the 5-diazouracil species could arise as an artefact of the operating method, *i.e.*, as a result of pyrolysis of the original 6-methoxy- derivative during the vaporising the sample on the probe. The second way in which 5-diazouracil may be derived is as a stable intermediate in a logical fragmentation process.

If the first hypothesis is correct then as a consequence it might be expected that the earlier scans would predominantly be of methanol whilst the latter scans give spectra identical for the most part with that of 5-diazouracil (Scheme 6.13).

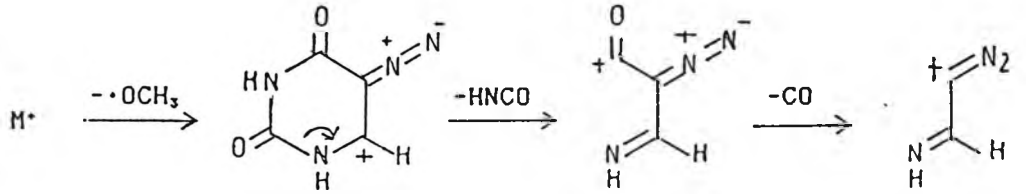


Scheme 6.13

If the second hypothesis is correct then it might be expected that, in addition to the 5-diazouracil spectrum, other fragments also will be detected, unless the fragmentation to 5-diazouracil is the most favoured to the exclusion of all other pathways.

Examination of the spectrum shows that the relative intensity of the peaks at m/z 139 and 138 for the [M+1] to [M] peaks are 8:100 for 5-diazouracil. Similarly, for uracil, the ratio of the molecular ion (m/z 112) to the pseudomolecular ion (m/z 113) is again in the order of 8:100. However, for the 6-methoxy diazo compound, the ratio of the peaks at m/z 139 and 138 is 9.4:11 which is equivalent to 85:100. This ten-fold increase of the relative intensity of the peak at m/z 139 is so large as to indicate that likely explanation is that this peak is formed as a fragment from a larger molecule rather than as a pseudomolecular ion.

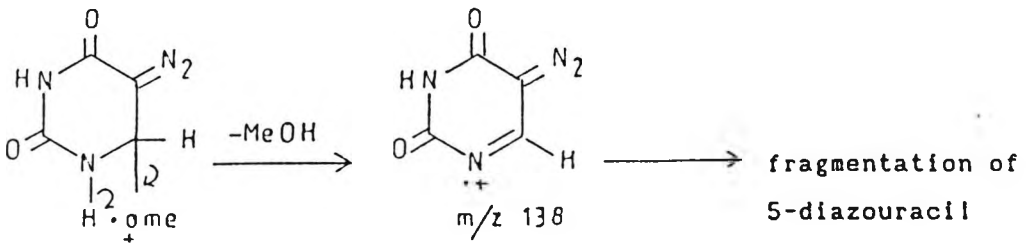
The observation of peaks at m/z 139, 96 and 68 in the high resolution spectrum suggest the formation of a daughter ion [M-31] (6.17) which can be attributed to the loss of a methoxyl radical. The fragments at m/z 96 (6.18) and 68 (6.19) are consistent with the loss of CHNO and CO from it [M-MeO] (Scheme 6.14).



m/z 139 (6.17) m/z 96 (6.18) m/z 68 (6.19)

Scheme 6.14

In the same way, a fragmentation can be envisaged in which ionisation lead to the ejection of the neutral alcohol molecule resulting in the 5-diazouracil species which then undergoes the usual fragmentations (Scheme 6.15).

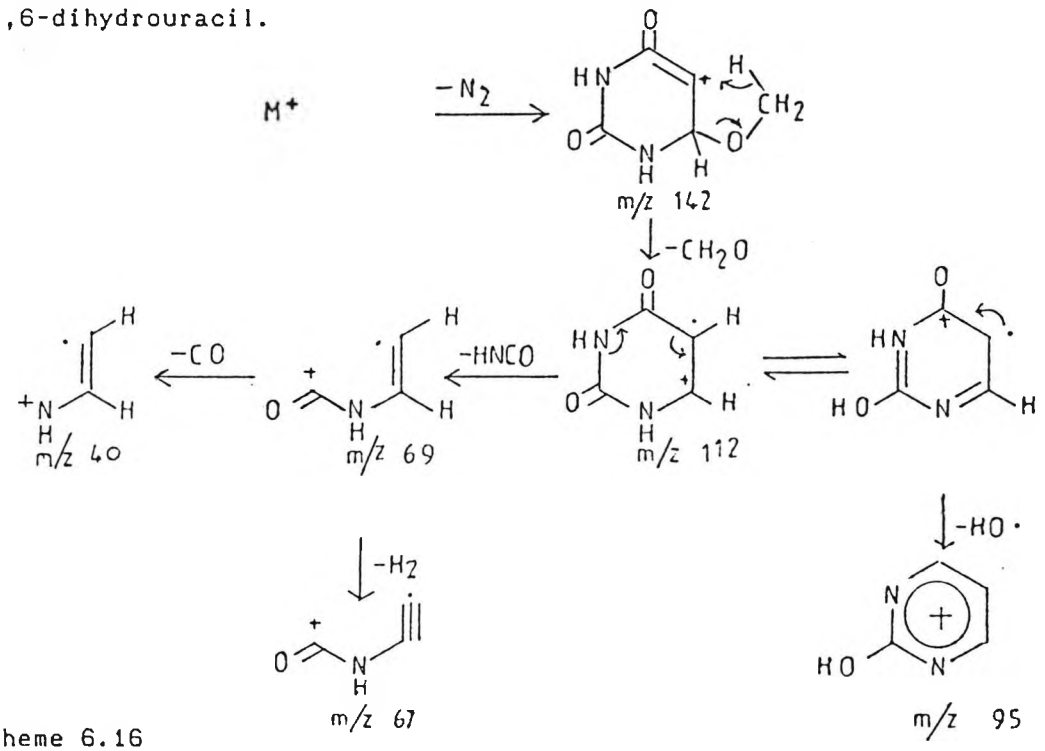


(6.20) (6.21)

Scheme 6.15

The detection of other peaks at masses higher than m/z 139 would also support the second hypothesis. Although the molecular ion was not detected, peaks at m/z 142 and 140 were observed. The high resolution mass indicated that the peak at 142 can be represented by only two possible formulae. These fragments correspond to [M-N_2] and/or [M-CO]. Neither type of fragmentation was observed for 5-diazouracil nor uracil. Surprisingly, the usual fragmentation pathway associated with the sequential loss of HNCO moiety from the molecular ion followed by the loss of CO or a hydrogen radical was not observed. The intensity of the peak at 142 was too low for detection of the isotopic ion, m/z 143, which would have been able to distinguish if the fragment had contained 4 or 5 carbons depending on whether carbon monoxide was lost or nitrogen was lost. Examination of the accurate mass does indicate that a better agreement for the mass is obtained for the formula (M-N_2), which represents the ejection of the two nitrogens, presumably the diazo nitrogens.

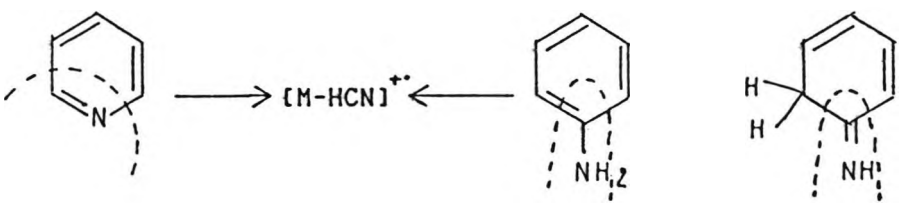
The fragments found at m/z 142 (6.22), 112 (6.23), 69 (6.24), and 40 (6.25) can be expressed as a sequential fragmentation pathway (Scheme 6.16). The formulae of the fragments along this pathway indicate that these fragments contain only two nitrogens. Thus, this pathway would lend support for the formation [M-N2] (6.22), a loss of nitrogen from a putative molecular ion for 5-diazo-6-methoxy--1,6-dihydrouracil.



Scheme 6.16

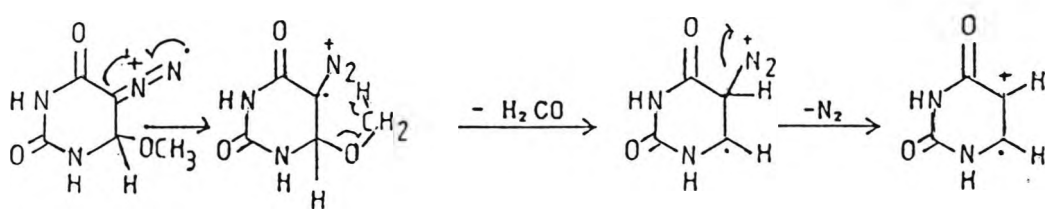
Although the peak at m/z 95 can be explained as a fragment of the fragmentation of 5-diazouracil, this peak (6.28) may also arise from the fragmentation of 5-diazo-6-methoxy-1,6-dihydrouracil. The only way of resolving whether either or both fragmentations occur would be to look for the corresponding metastable peaks.

As in the previous studies, a charge localization approach did require the use of an alternative tautomeric form to explain the observation of certain fragments although it is possible that these fragments could be obtained by other less obvious processes. The use of tautomeric structures enables a simple fragmentation to be visualised but it is not possible to demonstrate the existence or absence of such tautomers in mass spectrometry. For example, the loss of HCN occurs both from pyridine (6.29) and from aniline (6.30) [103]. The loss from aniline could equally be expressed using the imine tautomer (6.31).



(6.29) (6.30) (6.31)

The loss of formaldehyde appears to come from the methoxyl group in which there is a hydride migration to a positive centre or a hydrogen migration to an atom carrying an unpaired electron (Scheme 6.17). The peak at m/z 140 (6.34) is attributed to such a fragmentation.

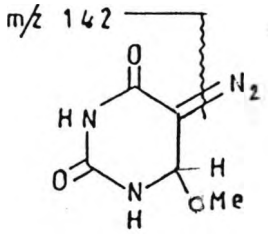


M+ 170 m/z 140 m/z 112

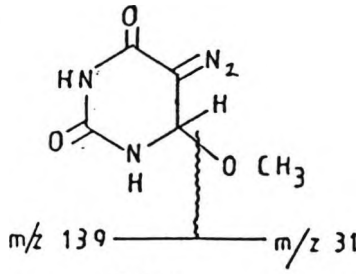
(6.32) (6.33) (6.34) (6.35)

Scheme 6.17

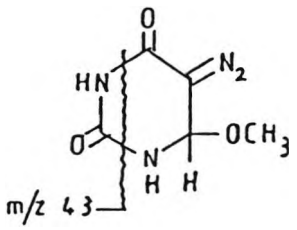
Other fragments can be simply related to structural features of the molecule (6.36, 6.37 and 6.38).



6.36



6.37



6.38

In conclusion, it can be seen that the spectrum of 5-diazo-6-methoxy-1,6-dihydrouracil is different to the spectrum of 5-diazouracil. The surprising aspect of the spectrum is the observation that a fragment relating to the loss of HNCO, m/z 127, could not be detected, nor could a fragment showing the loss of HNCO followed by a loss of CO be detected at m/z 99. It would also appear that saturation of the 1,6-bond would lead to the preferential loss of molecular nitrogen from the diazo compound.

6.4. 5-Diazo-6-hydroxy-1,6-dihydrouracil.

6.4.1 Preparation.

5-Diazouracil was prepared by the usual method. The filtered yellow solid was washed with distilled water until the washings showed an absence of chloride ions using the silver nitrate test. The compound was collected and dissolved in boiling water. Decolourising charcoal was added and the solution filtered whilst boiling hot. The solution was allowed to stand at ambient temperature over the weekend. The crystals formed were filtered from the solution and dried in an oven at 80°C overnight to afford 1.3g of a bright yellow glistening solid.

This compound was characterised in the i.r. by strong peaks at 1720 and 1650cm^{-1} which were attributed to the carbonyls of the uracil ring. The presence of the diazo group was inferred from the absorption in the i.r. at 2150cm^{-1}. The peaks around 3300cm^{-1} could be taken as evidence of the hydroxyl group, however this is not definitive because the stretch of the amide and imide ring hydrogens also give absorption in that region.

The melting point of this compound was not sharp and it discoloured to produce fine droplets at a temperature between 120-140°C, however with the bulk of the material remaining solid up to a temperature of > 190°C.

Evidence for the structure of the isolated compound was obtained from the ^{1}H n.m.r. in dimethyl sulphoxide. The broad signal at 10.08δ integrating for one proton was assigned to the H-3 proton. The broad signal at 8.44δ was assigned to the H-1 proton. The doublet of doublets (J=3.0Hz, J=7.5Hz), integrating for one proton, at 5.86δ was assigned to the H-6 proton. The observed doublet at 6.72δ (J=7.5Hz) was assigned to the proton in the hydroxyl group. The coupling, J=7.5Hz was attributed to the coupling between the hydroxyl proton and the H-6 ring proton. Although the coupling between the H-6 proton and the H-1 amide proton could not be seen at the 8.38δ, the size of its coupling could be inferred from the other coupling at 5.86δ. The ^{1}H n.m.r. data was in good agreement with printed spectrum in the literature, which did not cite coupling constants or chemical shifts and did not make any assignments. The addition of deuterium oxide to the n.m.r. sample resulted in the disappearance of the peaks at 10.30δ, 8.38δ and 6.72δ would supports the assignments made. A new sharp singlet at 5.94δ was attributed to the H-6 ring proton which no longer shows any proton-proton coupling. This confirmed the structure of the 5-diazouracil "hydrate" to be 5-diazo-6-hydroxy-1,6-dihydrouracil (6.3). The chemical shifts of the exchangeable protons, H-3, H-1 and OH, are often observed to vary and it is likely that differences in the sample conditions are responsible for the changes in chemical shift.

6.4.2 EI mass spectrum.

A molecular ion, $M^{\cdot}$, at m/z 156 was observed in the spectrum, albeit at an extremely low intensity (0.03%). High resolution mass measurement gave the formula for the species to be $C_4H_4N_4O_3$, which was in agreement with the formula obtained from microanalysis. The peak at m/z 138 was identified as 5-diazouracil. Thus, it can be deduced that the appearance of 5-diazouracil in spectrum is an artefact of the EI-MS process. The observed ions were predominantly those ions associated with the fragmentation of 5-diazouracil, *i.e.*, m/z 138, 112, 96, 68, 67, and 43, which suggests that the loss of

water from the product to afford 5-diazouracil is a major feature. The low intensity of the sample peaks can be related to poor volatility of these substituted diazouracils.

6.5 5-Diazo-6-ethoxy-1,6-dihydrouracil.

6.5.1. Preparation.

The structure of 5-diazouracil which was "recrystallized" from methanol has shown the solvent methanol as being covalently bonded. It was therefore desirable to investigate previous claims that "recrystallization" of 5-diazouracil in ethanol" results in it being co-crystallized with one equivalent of ethanol".

The compound was prepared by the diazotization of 5-amino--uracil. Ethanol at -10°C was added to the mixture and the 5-diazo--uracil filtered from the solution and washed with absolute ethanol at -10°C. The solid material was heated in absolute ethanol with reflux for 24h until the compound had dissolved. On standing at room temperature for 3-4 days, a bright yellow precipitate was obtained. This was filtered from the mother liquor and dried in vacuo at room temperature overnight. The compound was obtained in an overall yield of 82%.

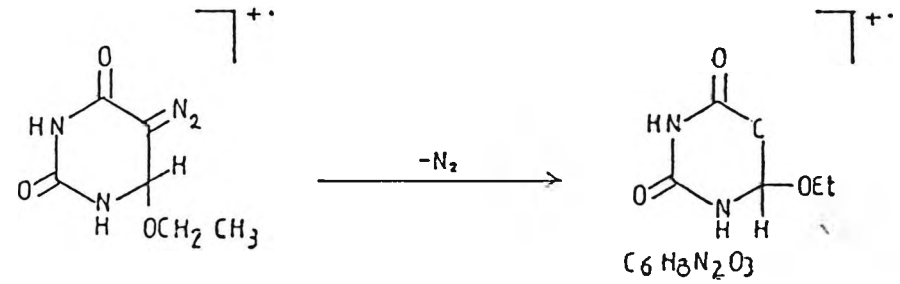
The compound was characterised in the i.r. by a diazo stretch around 2100cm^{-1} with an intensity roughly equal to the peaks at 1760 and 1660cm^{-1}, which were attributed to the ring carbonyls. In its ^1H n.m.r. spectrum, the compound showed a broad singlet at 10.0δ integrating for one proton, which was assigned to the H-3 proton. The observed doublet (J=3.0Hz) at 8.89δ was assigned to the H-1 proton on the basis of the coupling with the H-6 proton. Correspondingly, the doublet at 6.68δ (J=3.0Hz), integrating for one proton, was assigned to the H-6 proton. The quartet at 3.45δ (J=6.5Hz), integrating for two protons, was assigned to the methylene moiety in the ethoxyl group. Correspondingly, the triplet (J=6.5Hz) at 1.10δ which integrated for three protons was assigned to the methyl moiety in the ethoxyl group, thus confirming the structure of the ethanol adduct of 5-diazouracil to be 5-diazo--6-ethoxy-1,6-dihydrouracil (6.5).

6.5.2 EI mass spectrum.

Like 5-diazo-6-methoxy-1,6-dihydrouracil, the spectrum of the 5-diazo-6-ethoxy-1,6-dihydrouracil did not show a molecular ion nor

a pseudomolecular ion. The mass spectrum was found to contain peaks at m/z 139, 138, 95, 67, and 66 which are characteristic of a 5-diazouracil species. The peaks at m/z 139 and 138 gave an intensity ratio of 100:40, respectively. This ratio is in keeping with the observation for the methoxy compound (6.4), showing higher than expected intensity for the peak at m/z 139.

As might have been predicted from the mass spectrum of the methoxy compound, this spectrum contains a peak at m/z 140 which high resolution shows to correspond to a fragment with an empirical formula $C_4H_4N_4O_2$. Correspondingly, a fragment [M-28] which represents the probable loss of nitrogen or carbon dioxide from a putative molecular ion should be detectable. The peak at m/z 156 was attributed to [M-28] and at high resolution, its formula was shown to be $C_6H_8N_2O_3$ which indicates the loss of N_2 and confirms the presence of a 6-ethoxyl group. A corresponding structural assignment (6.40) could then be made. Further support for the ethoxyl moiety can be found at lower masses by the detection of the mass for ethanol at m/z 46 and for an ethoxyl radical cation at m/z 45.

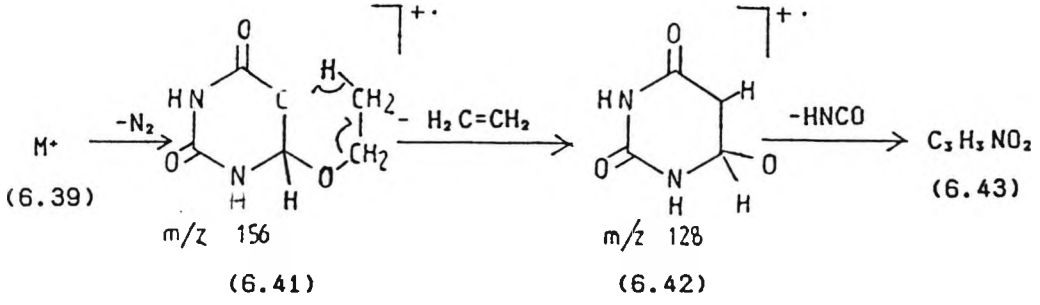


$M^{+\cdot}$, $C_4H_6N_4O_2$
m/z 184
(6.39)

$C_6H_8N_2O_3$
m/z 156
(6.40)

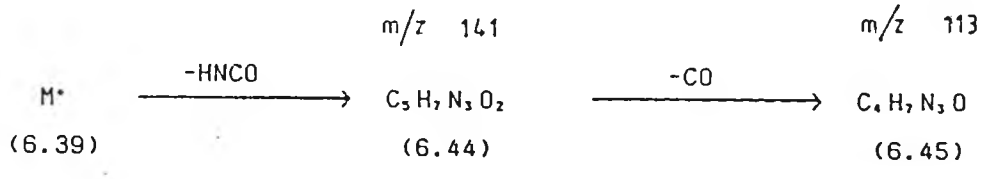
In the spectrum for 5-diazo-6-methoxy-1,6-dihydrouracil, a fragmentation pathway was put forward to explain the observed peaks at mass 140, 142, and 112 (Schemes 6.16 and 6.17). The major feature of this fragmentation was the migration of hydrogen from the alkoxyl group to a radical or cationic centre via a 5-membered transition state. It was postulated that if such a mechanism existed, the 5-diazo-6-ethoxy- compound would show the same mechanism to afford the same fragment, *i.e.*, a peak at mass 140 . A peak at m/z 140 was observed and high resolution mass measurement confirmed the formula ($C_4H_4N_4O_2$). A possibility that hydrogen migration might occur via a

six-membered transition state can be envisaged for the ethoxy-compound. The consequence would be the loss of a fragment of ethylene, mass 28. High resolution mass spectrometry showed that fragments corresponding to [M-N_2] (6.41) and [(M-N_2)-28] (6.42) could be detected (Scheme 6.18).



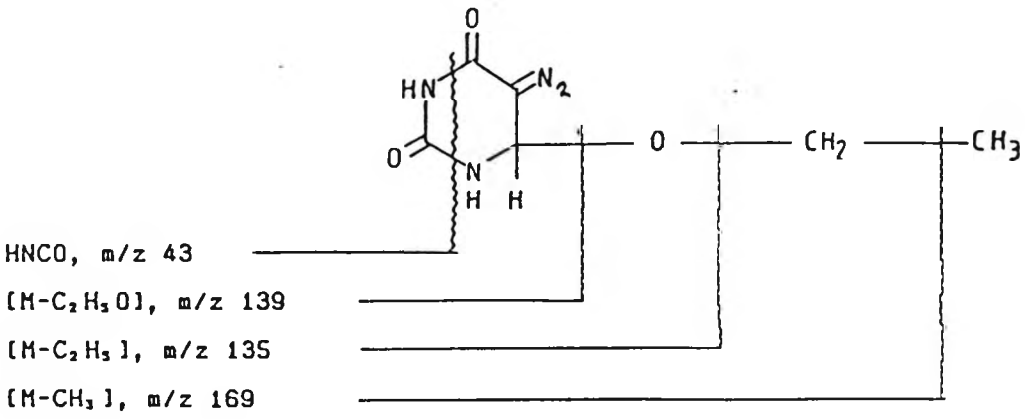
Scheme 6.18

A fragment which corresponds to [M-HNCO] could be detected. The formula assigned to this fragment was found to be at the limit of accuracy, +/-20mmu from the theoretical value, for high resolution mass spectrometry. Thus, there is a low confidence in the structural assignment of this fragment. The peak at m/z 113 ($C_4H_7N_3$) (6.45), which corresponds to the expected loss of CO from the putative daughter ion [M-HNCO] (6.44), was also found to lie outside the accepted limits for the proposed formula. Hence, it is doubtful whether a fragmentation occurs in which there is a loss of HNCO followed by the loss of CO from the molecular ion (Scheme 6.19), a result which was also observed for 5-methoxy-1,6-dihydro--uracil.



Scheme 6.19

Another fragment which could be identified was [M-CH_3] at m/z 169. The fragment at m/z 43 was attributed to the species $HNCO^+$. The fragment at m/z 155 was attributed to the species [M-C_2H_3]. These fragments can be related to various structural features of the molecule (Scheme 6.20).



Scheme 6.20

6.6 5-Diazo-6-isopropoxy-1,6-dihydrouracil.

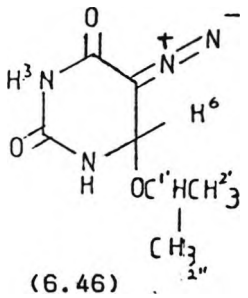
6.6.1 Preparation.

This compound was prepared by the diazotization of 5-amino--uracil with 1M hydrochloric acid and sodium nitrite. The resulting 5-diazouracil was washed free of acid with distilled water. The solid was then triturated with absolute ethanol and filtered to remove residual water. The suspension was filtered. The residue was dissolved in boiling 2-propanol and decolourised with charcoal. The yellow solution was filtered whilst hot and the filtrate reheated to dissolve any of the material which had come out of the solution during the filtration. The solution was left standing at ambient temperature for 1 week. The bright yellow crystalline material was filtered from the mother liquor and dried in a vacuum desiccator.

The compound was characterised by i.r. and showed the presence of the diazo group with an absorption band around 2120cm^{-1}. The presence of the amide and imide carbonyls were indicated by absorptions around 1720 and 1660cm^{-1}. The ether linkage of the isopropoxyl group was indicated by an absorption around 1060cm^{-1}.

The ^{1}H n.m.r of the compound indicated a pattern consistent with a covalently bound isopropoxyl group. The broad singlet at 10.10δ was assigned to the H-3 imide proton. The broad singlet at 8.64δ was assigned to the H-1 amide proton on the basis that it showed similar chemical shifts to those in the methoxy- and ethoxy-compounds although it did not show the expected doublet. The

coupling (J=4.5Hz) between the amide proton and the neighbouring H-6 proton could be measured from the doublet observed at 5.80δ, which integrated for one proton, also demonstrates the covalent nature of the 2-propanol adduct. The multiplet at 3.86δ (J=6.0Hz) was assigned to the H-1' methine proton on the propoxyl group. The corresponding doublet (J=6.0Hz) integrating for six protons was assigned to the two methyls C-2' and C-1' (6.46).



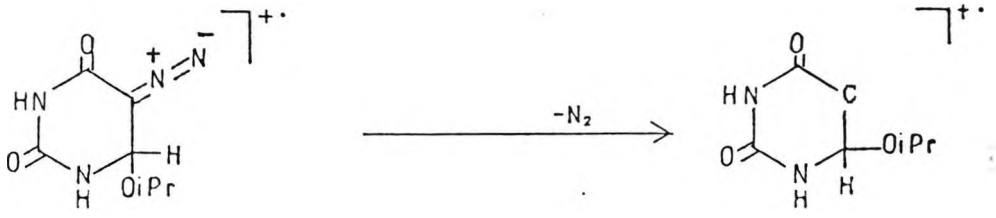
(6.46)

6.6.2 EI mass spectrum.

The mass spectrum of this compound followed a similar pattern to those of the ethoxy- and methoxy- compounds. Although a molecular ion was not observed, a pseudomolecular ion species $[M+H]^+$ was present at m/z 199. High resolution measurement showed that the mass of this peak was consistent with the proposed formula of $C_7H_{11}N_4O_3$.

The peaks at m/z 139 and 138 were each unusually broad and this suggested that there are probably two or more peaks of similar mass for each of the masses. The ratio of the intensities of the two peaks m/z 139 and 138 showed a preponderance for m/z 139 (100:40). The detection of the peak at m/z 140 confirms that the product is an adduct of 5-diazouracil.

The peak at mass 170 was attributed to a species $[M-N_2]$ (6.48) which also supports the finding that loss of molecular nitrogen from the diazo-dihydrouracils is a common fragmentation process for these compounds (Scheme 6.21). This peak shows that the compound is a derivative of 5-diazouracil and contains a C_3H_8O moiety.

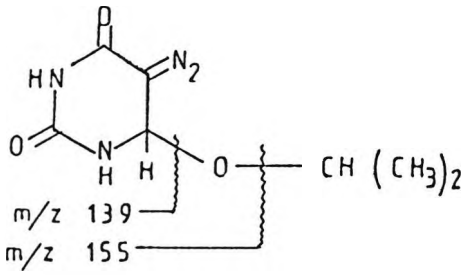


M^+, $C_4H_{10}N_4O_3$, m/z 198 (6.47)

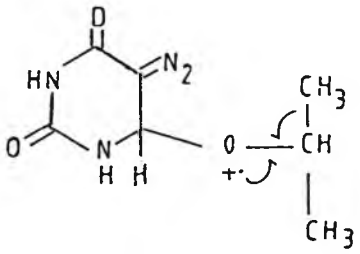
$C_6H_{10}N_2O_3$, m/z 170 (6.48)

Scheme 6.21

The peaks at m/z 155 ($C_4H_3N_4O_3$), 139 ($C_4H_3N_4O_2$), and 138 ($C_4H_2N_4O_2$) further support the conclusion that the derivative differs from 5-diazouracil by a C_3H_7O moiety and that the N-1 position has a hydrogen (6.49). The peak at m/z 155 also implies that this group is linked to diazouracil via an ether linkage. Thus, it can be deduced that the substituent is a propoxy group.



(6.49)



(6.50)

The observation of a species [M-CH_3] at m/z 183 appears to indicate that there is an α-methyl on the *ipso*-carbon to the ether linkage and this would imply the presence of a 2-propoxyl group (6.50).

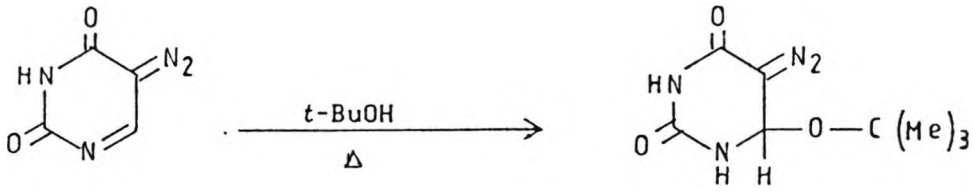
6.7 Attempted preparation of 6-butoxy-5-diazo-1,6--dihydrouracil.

The reaction of freshly prepared 5-diazouracil with n-butanol did not give the anticipated 6-butoxy-5-diazo-1,6-dihydrouracil. Instead, a product was obtained in which the diazo group had been eliminated. This reaction is covered in the following chapter (chp. 7).

6.8 5-Diazo-6-*tert*-butoxy-1,6-dihydrouracil

6.8.1 Preparation.

This compound was prepared by suspending 5-diazouracil in hot *tert*-butanol (Scheme 6.22). The mixture was heated with reflux for 24h and the undissolved material filtered off. The filtrate was allowed to stand for a considerable period of time to afford the desired product, 5-diazo-6-*tert*-butoxy-1,6-dihydrouracil (6.51) in <1% yield. The poor yield was attributed to the problem of dissolving the starting material in *tert*-butanol.



(6.51)

Scheme 6.22 Preparation of 5-Diazo-6-*tert*-butoxy-1,6-dihydrouracil

The compound was characterised in its i.r. by a strong absorption at 2130cm^{-1} which indicated the presence of the diazo nitrogens. The amide and imide groups were identified from the absorptions at 3340, 1710 and 1660cm^{-1}, which correspond to the NH and carbonyl functional groups. The presence of the *tert*-butoxyl group could not distinguished from the typical absorptions for nujol, which was used as the mulling agent.

It's ^{1}H n.m.r. spectrum showed two broad singlets at 10.0δ and at 8.4δ, which were assigned to the H-3 and H-1 protons, respectively. The presence of the *tert*-butoxy group was given by the doublet at 5.6δ which demonstrated the saturated nature of the 1,6-double bond. The singlet at 3.3δ was assigned to the *tert*-butoxy group.

Microanalysis showed the amount of carbon (37.96%) and hydrogen (3.97%) to be lower than required, whilst the nitrogen (31.06%) content was higher than required. It is likely that the presence of 5-diazouracil is responsible for these values. 5-Diazouracil was either co-crystallized with the product or it was formed by the loss of *tert*-butanol when drying the product in vacuo. The formula could not be confirmed by high resolution EI-MS in the absence of a molecular ion or a pseudomolecular species. The FAB spectrum gave a pseudomolecular ion [M+H] but high resolution mass measurement of this ion was not provided.

6.8.2 EI mass spectrum.

Three high resolution spectra were obtained for this compound; two low temperature spectra and a high temperature spectrum. In neither spectrum were the molecular ion [m+] nor a pseudomolecular ion [P] observed.

The low temperature spectra (100°C) were dominated by the peak at m/z 139 (100%), a characteristic feature of the 6-alkoxy 5-diazouracils. A species at m/z 185.0791 was assigned to a species [P-N_2] ($C_8H_{12}N_2O_3$+H, requires 185.09261). Although *tert*-butanol was not observed in the spectrum, the *tert*-butyl group was observed a m/z 57. The most prominent peaks in the spectrum were those associated with 5-diazouracil, m/z 96, 95, 68, 67, 66, and 43.

The high temperature spectrum (250°C) was characterised by peaks associated with the fragmentation of 5-diazouracil, m/z 138, 112, 96, 67 and 43. In this spectrum, a peak at m/z 138 was observed in place of the peak at m/z 139. From the earlier work, only for 5-diazouracil (Chp. 6.2.2.2) is the ratio of m/z 138 to m/z 139 very large. This suggested that the alkoxyl group had been eliminated, presumably as the alcohol, to leave 5-diazouracil on the probe prior to ionisation.

6.9 5-Diazo-6-phenoxy-1,6-dihydrouracil.

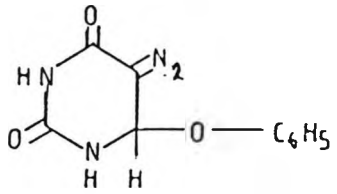
6.9.1 Preparation.

5-Diazouracil was suspended in hot benzene and phenol, dissolved in benzene, was added to the suspension. The mixture was heated with reflux overnight and allowed to stand at ambient temperature for 3 days. The orange precipitate was filtered from mother liquor and washed with dry benzene. The solid was triturated with dry ether to remove any unreacted phenol and refiltered. The residue was dried in a vacuum desiccator at ambient temperatures.

This compound was characterised by an absorption in the i.r. at 2140cm^{-1} which was attributed to the diazo stretch. The absorption bands at 1725, and 1660cm^{-1} were assigned to the two carbonyls in uracil. The absorption band at 1510cm^{-1} was assigned to the aromatic vibrations of the phenoxyl group. The absorption band at 1020cm^{-1} was assigned to the C-O stretch of an ether.

The compound was also characterised by ^{1}H n.m.r. The 1H broad singlet at 10.0δ was assigned to the H-3 proton and the 1H broad singlet at 8.2δ was assigned to the H-1. The phenoxy group was identified by the aromatic portion of the spectrum integrating for five protons. The typical pattern for the phenoxy group was observed with a 2H multiplet at 7.15δ, assigned to the *meta*- protons, and a 3H multiplet at 6.8-6.6δ assigned to the *ortho*- and *para*- protons.

The aromatic portion of the spectrum did not show a first order coupling pattern with the *ortho*- and *para*- protons having similar chemical shifts. The 1H doublet around 5.7δ (J=4.5 Hz) was assigned to H-6 and couples with H-1 (6.52).



(6.52)

6.9.2 EI mass spectrum.

The EI mass spectra for this compound was obtained at high and low temperature. The high temperature spectrum of the compound was characterised by the observation of peaks at m/z 139 and 138. This compound also showed a high ratio of intensity for the peak at m/z 139 to the peak at m/z 138, *viz.*, 32.5:22.9 which gives a relative ratio of 142:100. Both the molecular ion and the pseudomolecular ion were not observed. A peak at m/z 140 ($C_4H_4N_4O_2$) was probably the result of a hydrogen migration from the phenyl moiety. Confirmation that the molecule contained an ether linkage was given by the peaks at m/z 156 ($C_4H_4N_4O_3$) and at m/z 128 ($C_4H_4N_2O_3$). Evidence of the phenoxy group was obtained by observing a low intensity peak for phenol at m/z 94. Other peaks which also indicated the presence of the phenoxy group were found at m/z 95 (C_5H_5NO), 52 (C_4H_4), and 51 (C_4H_3). The main finding was that the spectrum of the compound was dominated by fragments from the 5-diazouracil moiety. In this first spectrum, the anticipated [M-N_2] fragment was not detected.

In the low temperature EI spectrum, an accurate mass measurement showed a molecular ion at m/z 232.0613, which confirmed the formula as $C_{10}H_8N_4O_3$. The very low intensity for the molecular ion suggests that the compound is prone to extensive fragmentation. A peak at m/z 204 was identified as the [M-N_2] species; the loss of molecular nitrogen being a common feature of the diazouracils. The peak at m/z 202 was assigned to the [(M-N_2)-H_2] species. In this spectrum, phenol was detected at m/z 94. The peak at m/z 112 was

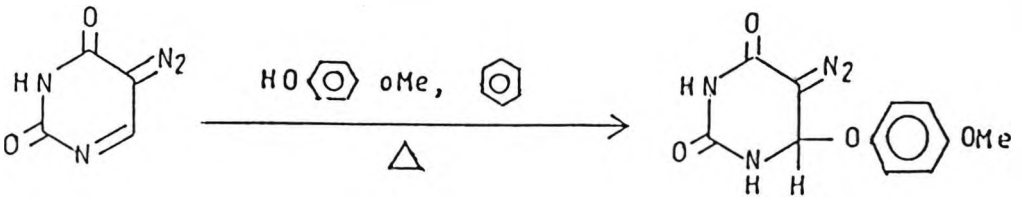
assigned to a dihydrouracil species. In common with the first spectrum, peaks at m/z 69, 45, 44 and 44 were observed.

6.10 5-Diazo-6(4-methoxyphenoxy)-1,6-dihydrouracil

6.10.1 Preparation

Further investigation of 6-aryloxy- diazouracils was undertaken with the selection of 4-methoxyphenol as the alcohol to be added across the 1,6-double bond of 5-diazouracil. An advantage of choosing the methoxyphenoxy group was that the methoxyl group would provide a signal which could be readily identified in the ^1H n.m.r.

The compound was prepared by suspending 5-diazouracil in benzene and adding a large excess of *p*-methoxyphenol with stirring (Scheme 6.23). The mixture was heated with reflux overnight and then left standing at ambient temperature for a further 24h. The resulting orange precipitate was filtered from the solution and triturated in cold dry benzene before refiltering. The product was found to be readily soluble in cold methanol or THF, which suggested a far less polar compound than the original 5-diazouracil. The residue was purified by preparative t.l.c. using dichloromethane/--methanol (85:15) as the mobile phase. The major band on silica (R_f=0.43) was removed and eluted with the mobile phase. The eluent was evaporated to dryness using a rotary evaporator to afford a yellow/orange solid in 49% yield.



Scheme 6.23

5-Diazo-6(4-methoxy)phenoxyuracil was characterised in its i.r. spectrum by a strong absorption band at 2130cm^{-1}, showing the presence of the diazo nitrogens. Absorption bands around 1720 and 1650cm^{-1} were attributed, respectively, to imide and amide carbonyls in the uracil ring. The absorption at 1610cm^{-1} represented aromatic vibrations of the phenol group. The absorption at 810cm^{-1} was observed, as might be expected of a *para*-substituted benzene ring.

Absorptions around 3340 and 3240cm^{-1} were attributed to the N-H stretching of strongly hydrogen bonded amides in the solid state.

The compound was characterised in its ^{1}H n.m.r. by a broad singlet at 10.6δ, assigned to the H-3 proton. This proton could be distinguished from the H-1 proton because of the absence of a coupling. The H-1 proton appeared as a 1H doublet (J=3.0Hz) at 8.7δ. As expected, the *para*-disubstituted phenyl protons appeared as a large 4H singlet. This observation was in keeping with similar observations for other *para*-dialkoxy- benzenes and is represented by an aa'bb' coupling pattern, which at 100MHz gives a singlet. The pattern for the aromatic protons also confirmed the ether linkage between the uracil and phenoxy moieties. The coupling now observed for the H-6 doublet (J=3.0Hz) at 6.1δ, demonstrates the saturated nature of the 1,6-bond. A sharp singlet at 4.0δ was assigned to the methoxyl group in the *para*-methoxyphenoxyl moiety.

6.10.2 EI mass spectrum.

The mass spectrum of this compound was characterised by a molecular ion at m/z 262 ($C_{11}H_{10}N_4O_4$) and pseudomolecular ion at m/z 263. In addition, the usual fragment at m/z 139 ($C_4H_3N_4O_2$) was observed. There was no fragment at m/z 138, indicating an absence of phenol elimination from the molecular ion in this compound. The peaks at m/z 124 and 123 were indicative of methoxyphenol and methoxyphenoxy radical. No fragment corresponding to [M-N_2] was observed. Unlike the previous 6-substituted uracils, the intensity for the molecular ion was comparatively large, which suggested that the addition of the methoxyphenoxyl radical to 5-diazouracil leads to less fragmentation, either by making the compound more volatile or by providing a region where ionisation results in a stable, localised charge.

As in the case of the phenol adduct, the majority of the peaks were associated with the fragmentation of the protonated 5-diazouracil moiety and fragments associated with the methoxyphenoxyl group were not observed. However, the peaks at m/z 81 (C_5H_5O) and 56 (C_3H_4O) could be assigned to fragments from the aromatic portion of the compound.

The compound was mixed with D_2O and the EI mass spectrum then rerun under the same conditions. In addition to the molecular ion

and pseudomolecular ion, peaks were observed at m/z 264, 265, and 266. These could be ascribed to various deuterated compounds (Table 6.1).

Formula			Mass
$C_{11}H_{10}N_4O_4$			262
$C_{11}H_9DN_4O_4$	$C_{11}H_{10}N_4O_4+H$		263
$C_{11}H_8D_2N_4O_4$	$C_{11}H_9DN_4O_4+H$	$C_{11}H_{10}N_4O_4+D$	264
	$C_{11}H_8D_2N_4O_4+H$	$C_{11}H_9DN_4O_4+D$	265
	$C_{11}H_8D_2N_4O_4+D$		266

Table 6.1

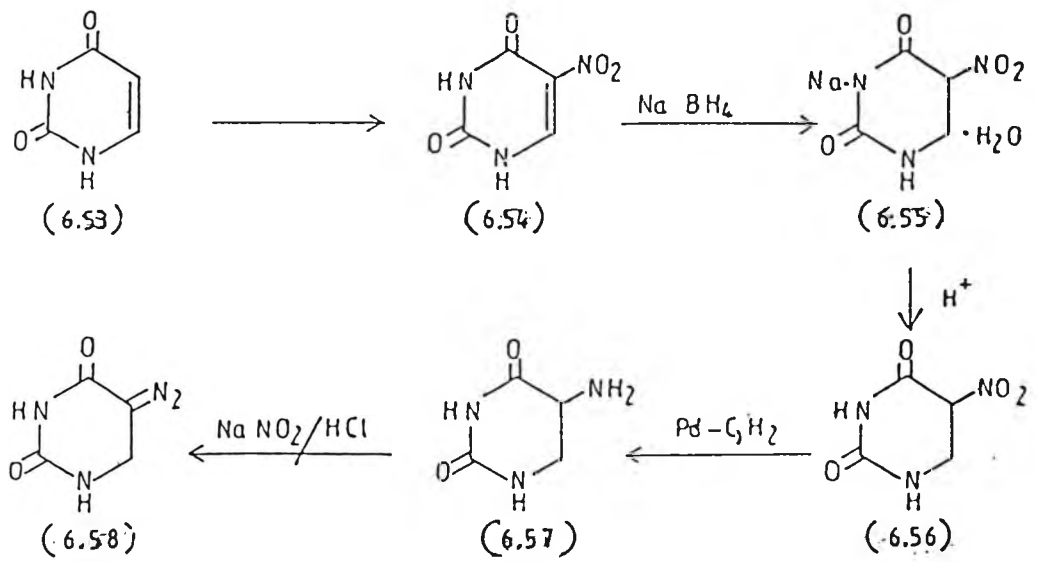
It can be seen that all the masses from 262 to 266 can be explained in terms of the molecular ion, pseudomolecular ion and deutero- derivatives of these species. Although the mass at 264 could be conceivably explained by an M+2H species, it isn't necessary to refer to such a species. These fragments suggest that the likely exchangeable protons are the acidic protons of the amide and the imide of the uracil moiety. Examination of the peak intensities suggests that a large proportion of the sample exists as the deuterated compound with only a small quantity being free of deuteration. Surprisingly, the spectrum of the compound after treatment with deuterium oxide gave many of the expected fragments and their corresponding deutero derivatives which were not detected in the initial undeuterated spectrum. There are several possible explanations; the first is that natural statistical variance or choice of scans happens to select a spectrum with more fragmentation peaks for the deuterated compound then for the undeuterated precursor; another possibility is that deuterated fragments are slightly more stable and consequently many of the daughter ions which would have been detected due to further fragmentation are now observed; yet another possibility is that the addition of water to the sample in some way affect volatilization and subsequent fragmentation of the compound.

The detection of the $[M-N^2]$ was observed at m/z 234. The formula was confirmed by high resolution mass measurement and by the mono- and di- deuterated species at m/z 235 and 236. The presence of the *p*-methoxy-phenoxy group was indicated by the peaks at m/z 123, 124, and 125.

6.11 5-Diazo-1,6-dihydropyrimidin-2,4(3H)-dione.

To date there has been no reported synthesis of the parent compound of the 1,6-dihydro- derivatives. Interest in this compound stems from studies on 5-diazouracil. One approach to the synthesis of 5-diazo-1,6-dihydrouracil would be to add hydrogen across the 1,6-double bond of 5-diazouracil. The use of metal catalysts and dissolved gaseous hydrogen was considered unfavourable owing to the poor solubility of 5-diazouracil in cold solvents. In addition, the possibility exists that the metal catalyst may cause decomposition of the diazo compound. Moreover, it is known that 5-diazouracil will explode in contact with finely divided platinum (II) solids and with platinum black [15]. The reduction with Raney nickel was considered unsuitable because of the high pressure and elevated temperature required in the hydrogenation.

An alternative method for reducing the imine (1,6-double bond) would be to use a metal hydride reagent such as lithium aluminium hydride or sodium borohydride. It was decided that both reagents would lead to complications in obtaining 5-diazo-1,6-dihydrouracil. Lithium aluminium hydride, although able to reduce the imine, has been known to reduce the carbonyls of amides to give the cyclic amine. The problem of solubility was again considered to be an adverse factor. The question of the stability of the diazo group's to such reagents during the reduction process was also considered. Therefore, an alternative scheme (Scheme 6.24) was chosen, whereby the last stage is the formation of the diazo group via a diazotization of a suitable precursor.

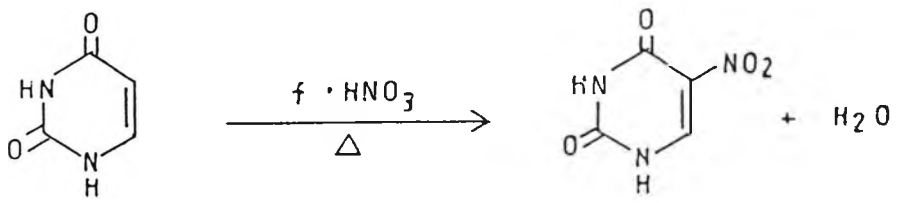


Scheme 6.24

The obvious precursor would be 5-amino-5,6-dihydrouracil (6.57), which could be potentially prepared either by the reduction of 5-aminouracil, or from the direct reduction of 5-nitrouracil (5.54), or in a two step procedure from 5-nitrouracil via the 5-nitro-5,6-dihydrouracil (6.56). 5-Nitrouracil (6.54) could be obtained by the nitration of uracil (6.53).

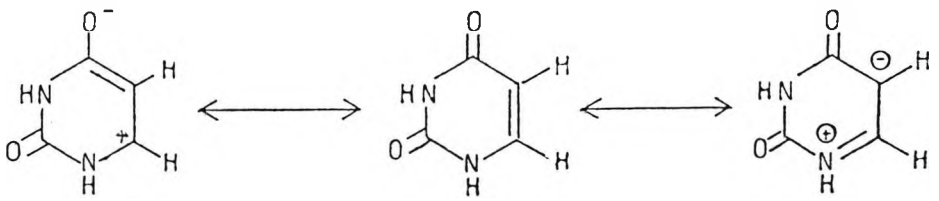
6.11.1 5-Nitrouracil.

The treatment of uracil with boiling fuming nitric acid leads to nitration at the 5-position of the molecule (Scheme 6.25)[17-18].



Scheme 6.25 Nitration of uracil

Unlike halogenation, the nitration of the molecule results in exclusively mono-substitution. The reaction is an electrophilic substitution and presumably corresponds to the nitration of aromatic compounds. The olefin may be considered as a conjugated system as it seems likely the lone pair of electrons on the N-1 nitrogen will feed in to the olefin thus activating the 5-position relative to the C-6 position, which is deactivated by conjugation with the C-4 carbonyl (Scheme 6.26). The active agent in the nitration with fuming nitric acid is the nitronium ion.

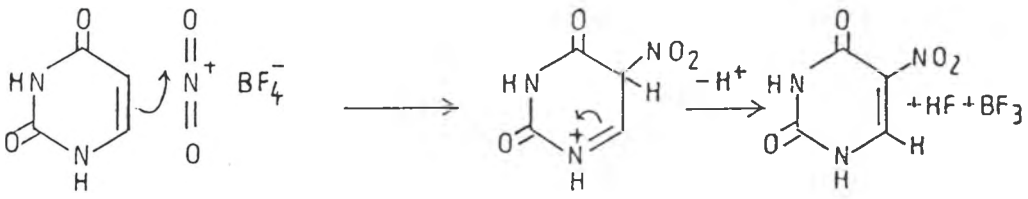


Scheme 6.26 C-5 activation and C-6 deactivation of uracil

An alternative reagent to fuming nitric acid is to use nitronium salts [19-20], which provide a mild but highly effective means of nitrating pyrimidine bases with a typical yield between 80-90%.

6.11.1.1 Preparation.

Uracil was nitrated using nitronium tetrafluoroborate in tetramethylene sulphone at 20°C for 20h (Scheme 6.27) [21]. The reaction was quenched by the addition of chloroform and this caused the product to precipitate out of solution. The fine yellow solid was filtered and recrystallized from hot methanol. The product was obtained in 60% after further purification by preparative t.l.c. using methanol/chloroform (3:7) as the mobile phase.



Scheme 6.27

The product was characterised in its i.r. spectrum by absorption bands at 1735 and 1690cm^{-1} which correspond to the carbonyls of the uracil ring. The observed absorption at at 1530cm^{-1} was attributed to the presence of a nitro group. The nitro group normally has an absorption of 1560 cm^{-1} (asymmetric stretch), but with conjugation this band can be lowered by 30cm^{-1}. The observed value indicated that the nitro group was attached to an unsaturated atom. A second nitro band around 1325cm^{-1} was also observed (symmetrical stretch). The presence of the olefinic band was indicated by the absorption at 1630cm^{-1}. The i.r. spectrum is dominated by the absorption bands associated with the amide/imide groups of the ring, absorptions around 3400 cm^{-1} being assigned to the N-H vibration and the absorption bands at 1690 and 1720cm^{-1} being associated with the amide imide carbonyls. Other peaks in the latter region were also observed for out of plane vibrations of the N-H groups.

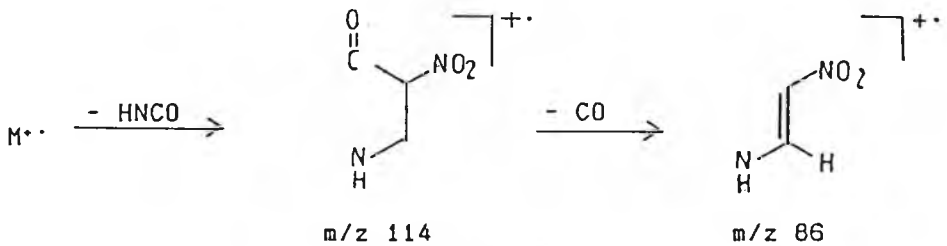
The compound was characterised in its ^{1}H n.m.r by a sharp, non-exchangeable singlet at 8.48δ which was assigned to the C-6 proton. The N-H protons could not be observed in dmso-d$_6$ between the range 0-11δ and probably suggest extremely upfield protons and/or extensive signal broadening resulting in the signals being lost in the noise.

6.11.1.2 EI Mass spectrum.

The ^1H n.m.r. of 5-nitrouracil typifies some of the difficulties of identifying uracil-type compounds. The sharp singlet at 8.48δ provides little information as to the compound. The i.r. spectrum whilst indicating the functional groups, provides very little information on the configuration of the molecule or the multiplicity of the groups.

The mass spectrum of 5-nitrouracil is characterised by extensive fragmentation. Fortunately, the compound is sufficiently volatile and stable to provide the molecular ion at m/z 157. Like uracil and diazouracil, 5-nitrouracil also shows the formation of an M+H species at 158.

The peaks at m/z 114 and 86 were shown by high resolution spectrometry to correspond to [M-HNCO] and [(M-HNCO)-CO] (Scheme 6.28). These fragments correspond with species obtained in the fragmentation of uracil. The dominant peak in the spectrum occurs at m/z 46. This peak represents the nitro cation. Unlike uracil, the absence of a peak at m/z 130 would appear to indicate that the loss of HCN from the molecular ion is an unfavourable process.



m/z 114 m/z 86

Scheme 6.28

6.11.2 Attempted one-step double reduction of 5-nitrouracil.

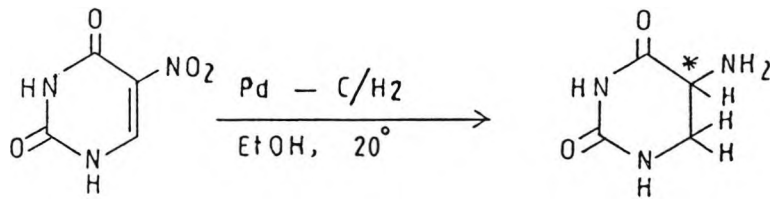
An obvious route to the desired amine would be to reduce the 5,6-double bond and to reduce the nitro group to the amine. In principle, it should be possible to reduce both the nitro group and the double bond in a one-pot reaction. The majority of metal catalysts used for hydrogenation are non-selective and therefore, suitable for reducing both the nitro group and the double bond.

In the literature [17], 5-nitrouracil was reduced to 5-amino--uracil using 10% chloroplatinic acid at 2atm and at 75°C. The attempt to further reduce the 5-aminouracil to give 5-amino-5,6--dihydrouracil did not produce the expected 5-amino-5,6-dihydro--uracil and an impure compound of unknown composition was obtained.

It was suggested that the reduction product had lost ammonia. No spectroscopic evidence was provided for either 5-aminouracil, or 5-amino-5,6-dihydrouracil or the resultant hydrolysis product, 5-hydroxyuracil. The failure to obtain the aminodihydro- product confirms the literature finding [19] that it is the reduction of the 5,6-double bond to give "hydrouracil" (5,6-dihydrouracil) which is the difficult part of the reduction.

It was also reported that 5-amino-5,6-dihydrouracil hydrochloride was prepared by catalytic reduction of 5-aminouracil hydrochloride using 5% rhodium on alumina and hydrogen at 3.1atm and recrystallized from ethanol-water [20]. No attempt was made to generate the free amine from the salt.

Our first attempt to produce 5-amino-5,6-dihydrouracil from 5-nitrouracil by reduction at ambient conditions in a one-pot reaction, using palladium on carbon as the catalyst, gave only a poor yield, confirming the literature findings [17] (Scheme 6.29).



Scheme 6.29

5-Nitrouracil was suspended in absolute ethanol and 10% palladium on carbon was added. The suspension was hydrogenated under ambient conditions with only a slight positive pressure. When the uptake of hydrogen had slowed to a very low rate, the mixture was removed from the hydrogenator, filtered through celite (Hi-flow filter aid) to remove the catalyst and the filtrate was evaporated to dryness on a rotary evaporator to leave an orange-brown solid.

The compound was characterised by a strong broad absorption in the infrared spectrum around 3400-3200cm^{-1}. The region of the spectrum between 1800-1650 cm^{-1} was dominated by a large broad peak centred about 1710 cm^{-1}. It was not clear whether the nitro group, previously indicated by absorptions at 1530 and 1325cm^{-1}, was retained. Although no strong absorptions were observed at those values, a strong absorption at 1290cm^{-1} might have masked the lower of two characterising bands. A peak of medium absorption around

1570cm^{-1} was observed, which might indicate the presence of an aliphatic nitro group. The absence of any strong stretches between 2500 and 1800cm^{-1} excluded the possibility that the azouracil had been formed.

The ^{1}H n.m.r. indicated a large broad singlet around 3.5δ which was taken as evidence of reduction of the nitro group to the amine. Two sets of multiplets were also observed around 4.0δ and 3.3δ. The spectrum also contained solvent ethanol peaks as well as the two sets of multiplets at 2.1δ and 2.9δ associated with methylene sulphone. The high field portion of the spectrum showed two singlets at 8.8δ and 8.1δ which were attributed to the N-H protons of the ring.

The low resolution mass spectrum of the crude material indicated a fragment at m/z 129, which would suggest the formation of 5-amino-5,6-dihydrouracil.

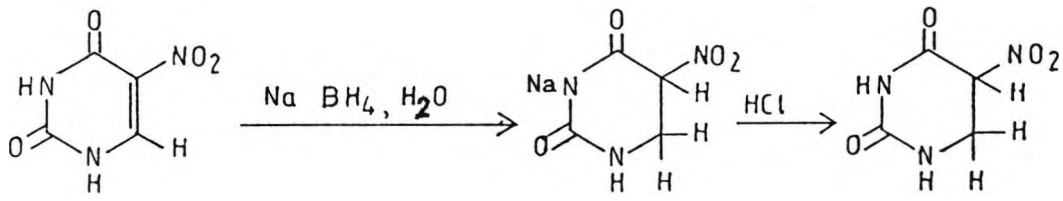
Only 58% of the crude product, contaminated with methylene sulphone, was recovered after filtration and it is assumed that the filtration through celite removed much of the unreacted 5-nitrouracil. The failure of the hydrogenation to afford the desired product in a high yield was attributed to minute traces of methylene sulphone possibly poisoning the catalyst and primarily to the poor solubility of 5-nitrouracil in ethanol.

6.11.3 5-Nitro-5,6-dihydrouracil.

The failure to produce 5-nitro-5,6-dihydrouracil via catalytic hydrogenation resulted in the adoption of a different approach based on a reduction using a metal hydride salt. Such agents present an opportunity to work at ambient temperatures and under mild conditions. Thus, a strategy was proposed in which the double bond was first reduced to generate a nitroalkane. The relatively easy reduction of the nitro group could then be employed to generate the desired amine.

Although lithium aluminium hydride was considered unsuitable, sodium borohydride will readily reduce a majority of strongly polarised double bonds without reacting with the nitro group [24-26] or amide groups. The conjugation of the nitro group and the C-4 carbonyl serves to polarise the 5,6-double bond in 5-nitrouracil sufficiently for Michael addition of hydride to occur. The reduction

would result in the initial formation of a sodium salt of uracil with the retention of the nitro group. The resulting salt, which could then be converted into the neutral nitrodihydrouracil by treatment with a mineral acid (Scheme 6.30). One advantage conferred by sodium borohydride is its slow rate of decomposition in water, which allows the reduction of 5-nitrouracil to be carried out in an aqueous medium [24]. With a slight modification of the literature method [27], an improvement on the yield was obtained.



Scheme 6.30

6.11.3.1 5-Nitro-5,6-dihydrouracil monosodium monohydrate.

5-Nitrouracil was suspended in an aqueous ethanolic solution. To this, sodium borohydride in an aqueous ethanol solution, was added in a dropwise manner and the mixture heated with reflux for 10 minutes. The literature method was modified by the addition of sodium bicarbonate to the sodium borohydride solution and by using ethanol in place of methanol. The addition of bicarbonate conferred the twin advantages of aiding the solubility of 5-nitrouracil and of preventing the acid-catalysed decomposition of the reducing agent. The solution was left stirring at ambient temperature overnight and a further quantity of sodium borohydride added. Finally, a small quantity of 2M sodium hydroxide solution was added in order to maximise the yield of the product. A white solid which had precipitated out of solution was filtered and recrystallized from an ethanol and water. The mother liquor was left standing and afforded 5-nitro-5,6-dihydrouracil monosodium monohydrate. The apparent yield of the product can vary between 95-120%. Yields higher than 100% are probably the result of the formation of disodium monohydrate salts.

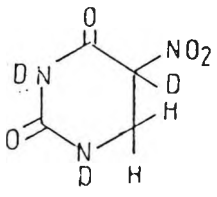
6.11.3.2 5-Nitro-5,6-dihydrouracil.

6.11.3.2.1 Preparation.

The sodium salt was dissolved in a minimum of boiling water and acidified with dilute hydrochloric acid until the solution was pH 2.5. The solution left standing at ambient temperature until the formation of white crystals. The crystals were filtered from the mother liquor and dried in an oven overnight to afford 5-nitro-5,6--dihydrouracil (yield 75.5%) obtained in an overall yield of 44.3% (lit. 39.9%). When 5-nitro-5,6-dihydrouracil monosodium monohydrate was used directly after being filtered without recrystallization, then, 5-nitro-5,6-dihydrouracil was obtained in an overall yield of 80%.

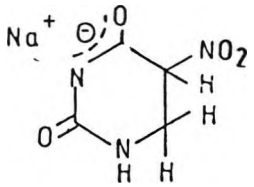
The product was characterised in the infrared by peaks at 1720 and 1670cm^{-1} which were assigned to carbonyl groups. The spectrum was also characterised by a strong absorption at 1560 and a medium intensity absorption at 1350cm^{-1} which are attributed to the presence of an aliphatic nitro group.

The ^{1}H n.m.r. spectrum showed two broad singlets at 10.40δ and 7.60δ, each integrating for one proton, which were respectively assigned to H-3 and H-1 protons. The observation of these signals in the ^{1}H n.m.r. spectrum confirms the conversion from the salt back to the neutral molecule. A 1H triplet at 5.5δ (J=6.0Hz) was assigned to the proton at C-5. A 2H multiplet centred around 3.70δ was assigned to the protons at C-6. Expansion of the multiplet suggested coupling to both H-5 and H-1 (J=0.5Hz). Further confirmation of assignment was provided by a study of the deuterium exchange in which deuterium oxide was added to ^{1}H n.m.r. sample and the reaction was followed by recording the spectrum of the exchanging mixture at different times. The signal for the H-5 proton at 5.5δ was removed at a faster rate than either the H-3 proton at 10.4δ or the H-1 proton at 7.6δ. Thus, the disappearance of the triplet for H-5 was followed by the disappearance of the broad singlet for the H-3 and finally the removal of the signal for the H-1 proton to give the tri-deutero-5-nitro-5,6-dihydrouracil (6.59).

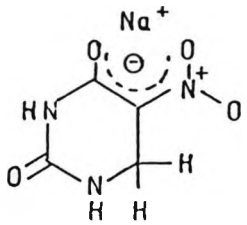


(6.59)

The deuterium study allows speculation about the structure of the sodium salt. It was suggested that anion to the sodium involved delocalization of the negative charge about the N-3 position (6.60) but there was no data other than the melting point [24]. The deuterium study indicated that the kinetically favourable anion is probably a resonance hybrid with the negative charge delocalized over the C-4 carbonyl, the C-5 atom and the nitro group (6.61) and hence, it is possible that the salt occurs as the 5-sodium salt or as a mixture of both the 3-sodium and 5-sodium salts.



(6.60)



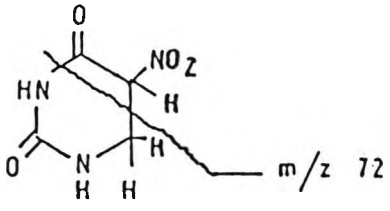
+ BH

(6.61)

It was not possible to examine the sodium salt using proton spectroscopy owing to the poor solubility of the sodium salt in deuterated organic solvents. It is suggested that structure of this salt might be resolved by the use of solid state n.m.r. or by X-ray crystallography.

6.11.3.2.2 <u>EI mass spectrum.</u>

The EI mass spectrum did not afford a molecular ion at m/z 159. However, pseudomolecular species at m/z 160 and 158 were observed, which accurate mass measurement confirmed to corresponded to [M+H] and [M-H], thus confirming the formula $C_4H_5N_3O_4$. Typical fragments include [M-H_2] at m/z 157, [M-NO_2] at m/z 114, [M-HNO_2] at m/z 112 and [M-(C_2HNO_3) at m/z 72 (6.62). Unlike many of the uracil derivatives a peak for [M-HNCO] was not observed, however, a peak at m/z 114, which corresponds to [(M-H_2)-HNCO], was observed. Unlike the diazodihydrouracils, [M-28] or [M-30] was not observed which might have been expected if the loss of CO was a common fragmentation pathway.



(6.62)

6.11.4 5-Amino-5,6-dihydrouracil.

The synthesis of the amine can be achieved by further reduction of 5-nitro-5,6-dihydrouracil using a heterogeneous catalyst, which permits a wide choice of solvents. Palladium was selected as it is one of the stronger catalysts.

6.11.4.1 Preparation.

5-Nitro-5,6-dihydrouracil was dissolved in warm distilled water. The catalyst was added and the mixture placed on the hydrogenator. In order to maintain the solubility of the starting material, the reaction mixture was kept at a steady 40°C using a water bath. The reaction was allowed to proceed until the rate of reaction, as given by the rate of uptake of hydrogen gas, had slowed to zero. The end of the reaction was reached after the uptake of approximately three equivalents of hydrogen gas. The solution was filtered through a sintered glass funnel containing a large quantity of filter aid in order to remove the suspended catalyst. The colourless filtrate was evaporated to dryness on a rotary evaporator over a water bath at 40°C. The product was a creamy white solid and was obtained in a quantitative yield. The compound was stored at a temperature below 0°C with the exclusion of light. The compound remained stable under those conditions for several weeks. Impurities were found to drastically reduce the compound's stability and the compound was thought to decompose with the release of ammonia, which could be detected by smell and confirmed by the formation of ammonium hydrochloride near fuming hydrochloric acid. The failure to fully reduce all of the nitro compound to the amine also drastically reduced the stability of the product.

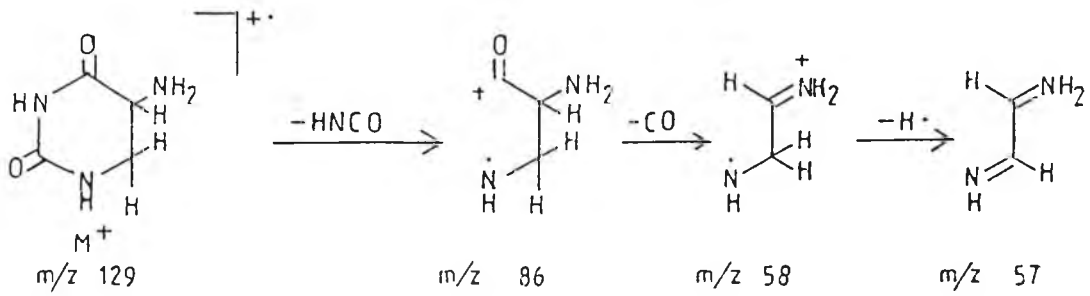
The infrared of the product was characterised by peaks at 1730 and 1680cm^{-1} which were assigned to the two carbonyls. The broad absorption between 3400 and 2900cm^{-1} were attributed to the N-H vibrations of the amino, amide and imide protons. The absence of absorptions at 1560 and 1350cm^{-1} corresponds to the absence of an aliphatic nitro group.

Its ^1H n.m.r. spectrum in dmso-d_6 showed two broad singlets at 10.4δ and 7.70δ, which were assigned to the H-3 and H-1 protons. The 5-amino group appeared as a broad peak centred about 4.7δ. The remaining signals were covered by a complex multiplet between 3.8δ and 3.1δ which was assigned to the C-5 methine and the C-6

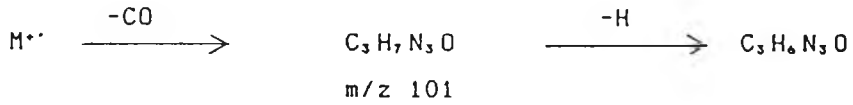
methylene. The coupling of these protons generates a complex, second order spectrum. As in the case of 5-nitro-5,6-dihydrouracil, the two protons of the C-6 methylene are not magnetically equivalent, owing to the chiral centre at C-5. Further complexity is introduced by likely coupling between the N-1 to one or both of the C-6 methylene protons. In addition, the C-5 proton may also couple to the protons on the amino group, a coupling which is absent in the nitro precursor.

6.11.4.2 EI mass spectrum.

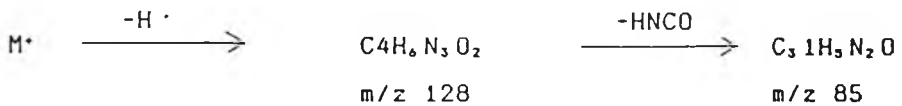
The mass spectrum of the compound contained the molecular ion, $C_4H_7N_3O_2$, at m/z 129. Pseudomolecular species [M+H] and [M-H] were also observed. The fragmentation was typical of uracils in that the observed fragments were consistent with species which lost HNCO, then lost CO, and finally lost H (Scheme 6.31). These fragments match the species resulting from the fragmentation observed for uracil. In addition to the loss of HNCO, species which correspond to the loss of carbon monoxide, or the amino group or atomic hydrogen from the molecular ion can be identified by peaks at m/z 101, 113 and 128 respectively (Scheme 6.32-6.35).



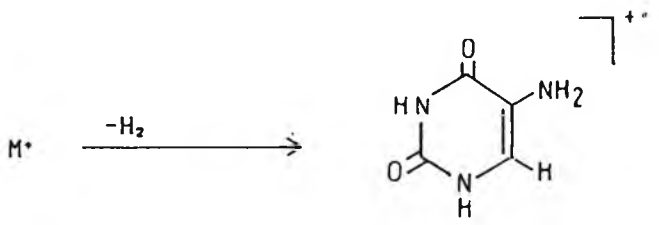
Scheme 6.31



Scheme 6.32

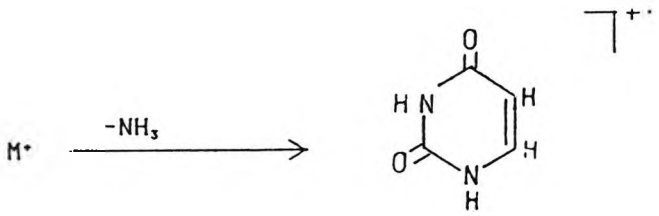


Scheme 6.33



m/z 127

Scheme 6.34



m/z 112

Scheme 6.35

A common process for some dihydrouracils is the loss of HX from the molecular ion, where X represents a substituent positioned at C-6 or C-5. As in the case of precursor, 5-nitro-5,6-dihydro--uracil, it can be deduced that species at m/z 127 [M-2] (Scheme 6.34), arose as a result of molecular hydrogen being lost from the molecular ion. A likely driving force is the greater stability gained by the fragment with the introduction of conjugation. If this hypothesis is correct, similar losses of other groups might be expected. Thus, the peak observed at m/z 112 can be attributed to the loss of ammonia (Scheme 6.35).

6.11.5 5-Diazo-1,6-dihydrouracil.

The intention was to prepare this novel compound by the diazotization of 5-amino-5,6-dihydrouracil. As a corollary, its synthesis also confirms the reduction of the starting material, since diazotization has long been an accepted method for the determination of amino groups.

6.11.5.1 Preparation.

A 1M solution of hydrochloric acid was cooled to 0°C in an ice/salt bath. 5-Amino-5,6-dihydrouracil was dissolved in the cold solution. Sodium nitrite as a 6.9% w/v solution was added in a dropwise manner over a period of 40min and the temperature was

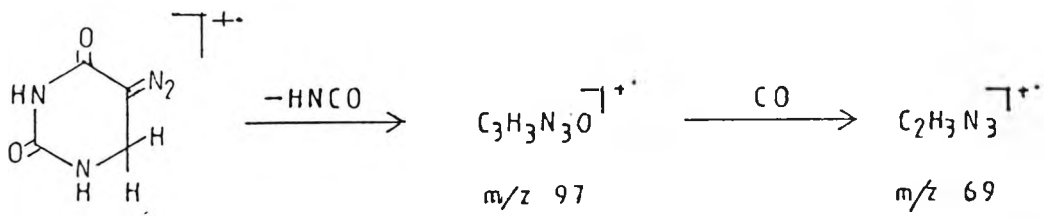
allowed to rise to 2°C. The bright yellow precipitate which formed was filtered from the solution. This residue was washed with cold distilled water until the washings gave a negative chloride test with silver nitrate solution. The compound was allowed to dry in a vacuum desiccator to afford the diazo compound in 90% yield.

The compound was characterised in its infrared spectrum by a strong absorption band at 2130cm^{-1} for the diazo group. Absorptions around 1710 and 1660cm^{-1} were attributed to the carbonyls of the dihydrouracil ring.

The ^{1}H n.m.r. of the compound was noticeable in its simplicity in comparison with the spectrum of 5-amino-5,6-dihydrouracil. The spectrum of the diazo compound showed a 1H broad singlet at 10.72δ. Another broad singlet at 7.8δ, which also integrated for one proton, was assigned to the N-1 proton. A finely split 2H doublet at 4.24δ, which may appear as a singlet, was assigned to the C-6 methylene protons. The coupling constant between H-1 and the protons on C-6 was determined from the C-6 doublet and gave a value $J_{1,6}$=1-2Hz.

6.11.5.2 <u>EI mass spectrum.</u>

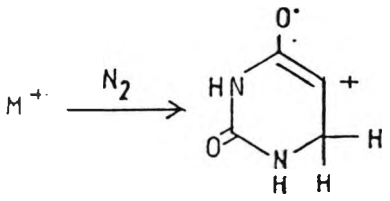
The mass spectrum of 5-diazo-1,6-dihydrouracil included peaks at m/z 140 and 141. These peaks were shown by high resolution mass spectrometry to be consistent with the expected molecular ion, $C_4H_4N_4O_2$, and the pseudomolecular ion, M+H. The peaks at m/z 97 and 69 were assumed to represent fragments formed by the loss of HNCO and subsequent loss of carbon monoxide from the molecular ion in a fragmentation pathway similar to that found in uracil (Scheme 6.36).



Scheme 6.36

The peaks at m/z 98 and 70 were correspondingly assigned to the loss of HNCO, followed by the loss of CO from the pseudomolecular species. The peak at m/z 112 was shown to have a formula consistent with $C_4H_4N_2O_2$, which represents the loss of molecular nitrogen from

the molecular ion (Scheme 6.37), which is a common feature to all the 5-diazo-1,6-dihydrouracils examined so far.



m/z 112

Scheme 6.37

Unlike the 6-alkoxy derivatives of 5-diazouracil a peak at m/z 139 was not observed. The absence of this peak might have been predicted. It has been previously suggested that the observation of the peak at m/z 139 in the EI spectra of the 6-alkoxy derivatives was the result of the loss of the alkoxyl radical from the molecular radical cation. In absence of alkoxyl group in 5-diazo-1,6-dihydro--uracil, a peak at m/z 139 could only arise by the loss of hydrogen and this is a far less favourable process. A peak at m/z 139 might be observed if the formation of the pseudomolecular ion is formed in abundance, then the peak would be the result of the loss of molecular hydrogen from the pseudomolecular species. The low intensity of the pseudomolecular ion suggests that a species at m/z 139 arising from it might have been at too low an intensity to been observed.

Thus, the synthesis of 5-diazo-1,6-dihydrouracil was achieved for the first time, via 5-amino-5,6-dihydrouracil, and this diazo compound should be a useful precursor to a variety of new 5-substituted dihydrouracils.

6.12 Examination of 5-diazo-6-methoxy-1,6-dihydro--uracil by h.p.l.c.

6.12.1 Introduction.

In the early investigation of nucleic acids and their components, chromatography played a major role. Nucleotides were first separated by the use of ion-exchange columns [28,29] from the acid-soluble fraction of the plant and animal tissues [30-32]. High performance liquid chromatography (h.p.l.c.), originally introduced in 1969, was soon applied to difficult-to-separate biological molecules, such as the nucleotides [33-37]. Both anion

and cation ion-exchangers were used to separate purines, pyrimidines and nucleosides. Such separations were found to be very dependent on careful choice of pH [38]. Despite the use of microparticle chemically bonded ion-exchange packings, a poor retention of pyrimidines was usually obtained and often there was a failure to separate complex mixtures of nucleosides and bases [39-42].

The development of the reversed-phase mode with microparticle chemically bonded packings has enabled the separation of complex mixtures of bases and nucleosides [43]. The reversed-phase mode proved ideal for nucleosides and bases and it has become the method of choice for the separation of nucleosides and/or bases. It was adapted for all types of analyses, from a single nucleoside such as adenosine [44] to complete assays of all the nucleosides and bases in a physiological fluid such as serum or plasma [45]. Purine and pyrimidine bases and their nucleosides are now analysed routinely by reversed-phase liquid chromatography [44-46]. The use of reversed-phase liquid chromatography has been extended to the separation of some nucleotides [47] and some oligonucleotides [48].

6.12.2 Operating system.

The equipment consisted of a Walters pump, Cecil variable wavelength detector and Rheodyne injector. A wide variety of column packings were examined to establish whether they would be suitable for the analysis and purification of diazouracils.

6.12.2.1 5Um ODS-Hypersil column.

A study on the h.p.l.c. of 5-diazo-6-methoxy-1,6-dihydro--uracil was initiated, using a 5um ODS-HYpersil column, 25.0cm x 0.45 i.d., which contains an octadecyl hydrocarbon chain bonded to silica. The column was high pressure slurry packed in the laboratory and was tested using a prepared mixture of benzamide, acetophenone, benzophenone, and biphenyl. Typical retention times for these compounds were measured (Table 6.2) using methanol/water (70:30) at 2ml/min as the mobile phase. The peaks were detected on a Cecil detector set at a wavelength of 254nm.

	t_R [min]	k'
Benzamide	1.65	0.179
acetophenone	2.2	0.571
benzophenone	4.3	2.071
biphenyl	9.0	5.428
t_o	1.4	

Table 6.2 Retention times on a 5um ODS-Hypersil Column

A sample of 5-diazo-6-methoxy-1,6-dihydrouracil was run on the same column. Using a similar mobile phase, methanol/water (70:30) at 2ml/min, a retention time of 1.4min was observed. This is the same value noted for the hold up volume t_o.

In an effort to promote retention, the methanol content was reduced in favour of the more polar component, water. This has been observed to lead to an increase in the retention time in nucleosides and their purine and pyrimidine bases [49]. When the mobile phase was changed to methanol/water (40:60), the retention was increased to 1.8min. On further reduction of the methanol content, methanol/--water (20:80), two poorly resolved peaks were obtained. The smaller peak had a retention time of 1.9min and larger peak, approximately twice the height, had a retention time of 2.3min. Attempts to further separate these peaks by increasing the water content resulted in a single broad peak.

One of the problems associated with the chromatography of purine and pyrimidine bases is their sensitivity to pH. In the uracil derivatives, the amide and imide nitrogens are capable of acting both as an acid and also as a proton acceptor. The two carbonyl oxygens are rich in electron lone pairs and readily associate with protons in solution providing these compounds with an amphoteric nature. In addition, the lactam/lactim tautomerism means that several species may exist in solution. In order to reduce the number of potential species, the water in the mobile phase was replaced with a phosphate buffer solution of known pH since it would be expected that many of these potential species are pH dependent.

The results from the studies with the ODS-Hypersil column suggested that 5-diazo-6-methoxy-1,6-dihydrouracil was too polar for a C-18 reversed-phase column. It was decided that a different type of column might prove more promising.

6.12.2.2 5Um Phenyl-Hypersil column.

A column (25.0cm x0.45cm i.d.) was packed with Phenyl--Hypersil, which consists of a phenylpropyl group bonded to silica and is a medium polarity, reversed-phase packing [53]. The test mixture, containing benzamide, acetophenone, benzophenone, and biphenyl, was used to check the column. A mobile phase of methanol/--water (70:30) did not successfully resolve all four components of the test mixture. However, when a mixture of methanol/water (55:45) was used, a satisfactory resolution of all four components was achieved (Table 6.3).

	t_R [min]	k'
Benzamide	2.2	1.75
acetophenone	3.15	2.94
benzophenone	7.1	7.88
biphenyl	8.15	9.19
t_o	1.6	

Table 6.3 Retention Times on a Phenyl-Hypersil column

A sample of 5-diazo-6-methoxy-1,6-dihydrouracil was also run on the column with the same mobile phase and gave a retention time of 1.8min.

6.12.2.3 Cyanopropyl(CPS)-Hypersil column.

The short retention time observed for the diazo on the Phenyl-Hypersil and ODS-Hypersil columns prompted the search for an alternative packing material. The diazo compound was then tested on a CPS-Hypersil column (25.0cm x 0.45cm i.d.), which contains a cyanopropyl group bonded to silica support via a silyloxy bond. This column slightly increased the retention time to 2.2min (t_o 1.0min) was obtained for 5-diazo-6-methoxy-1,6-dihydrouracil. It was concluded that to increase the retention time yet further, a more polar column was required.

6.12.2.3 Nitro-Nucleosil column.

The Nitro-Nucleosil column (25.0cm x 0.45cm i.d.) contains packing material in which a *p*-nitrophenylpropyl group is bonded to silica. The packing material represents an intermediate between true reversed-phase materials and normal phase materials and has

been classified as a "polar reversed-phase" [54].

A sample of 5-diazo-6-methoxy-1,6-dihydrouracil was run on the column using acetonitrile as the mobile phase. Two peaks were observed; a small peak with a retention time 2.2min (t_o), and a large peak with a retention time of 8.4min. Under the same conditions, 5-diazo-6-isopropoxy-1,6-dihydrouracil had a retention time of 8.5min and 5-diazo-6-*tert*butoxy-1,6-dihdyrouracil had a retention time of 10.1min.

Thus, it appears that the Nitro-Nucleosil column would be a suitable starting point for future h.p.l.c. studies. The results indicated a reasonable retention time for the 5-diazo-6-alkoxy--1,6-dihydrouracils tested. A variety of mobile phase conditions should be explored to optimise separation.

6.13 FAB spectra of some of the diazo compounds.

6.13.1 Introduction.

The difficulty in obtaining a molecular ion in the EI spectra of many of the diazouracils synthesized was attributed to their poor volatility and intrinsic instability of the molecular ions

Many biological compounds are poorly volatile and are prone to extensive fragmentation in EI spectrometry and recently, FAB mass spectrometry has been used to examine them e.g. lipids [55,56], penicilloic acids [57], carbohydrates [58-64], peptides [65-69], proteins [66,70], leukotrienes [71], nucleotides and nucleosides [72], and oligodeoxyribonucleotides [73].

The diazouracils present the typical problem which a soft method of ionization (see Chp 4.4.5) might be able to solve. The technique of fast atom bombardment (FAB) mass spectrometry was applied to some of the synthesized diazouracils in an effort to observe their molecular ions.

6.13.2 5-Diazo-6-methoxy-1,6-dihydrouracil.

The FAB spectrum of the diazo compound, suspended in 3-nitrobenzyl alcohol (matrix material), was characterised by an intense peak at m/z 171 (100%), which corresponds to the expected formation of a pseudomolecular species, denoted by P and represents [M+H]. The absence of a molecular ion at m/z 170 is not significant as it is a common feature of FAB spectra that they rarely show the

molecular ion. Alternative pseudomolecular ions to [P], were also detected. These alternative species included [M+Na] (m/z 193) and [M-H] (m/z 169) (Table 6.4).

m/z	intensity	Assignment*
511	0.4	3M+H
493	0.6	2M+mat+H
477	1.5	P+2mat
341	3.5	2M+H
324	19.9	P+mat
193	3.1	M+Na
171	100.0	P
169	3.8	M-H
143	8.4	$P-N_2$, P-CO
142	4.3	$M-N_2$, M-CO
139	40.6	P-MeOH
128	2.9	P-HNCO
115	1.1	$P-N_2$-CO
114	1.1	$M-N_2$-CO
111	1.2	P-MeOH-N_2
100	2.9	P-CO-HNCO, $P-N_2$-HNCO
85	1.8	P-2HNCO
84	1.2	M-2HNCO

* P=M+H

Table 6.4 Masses and their assignment in FAB of 5-Diazo-6-methoxy-1,6-dihydrouracil.

In common with the EI spectrum (see chp. 6.3.2), where the fragmentation of the molecule results in the formation of an [M-CO] or [$M-N_2$] species (m/z 140), a species [$P-N_2$] or [P-CO] at m/z 141 was observed. The peak at m/z 139 was attributed to the species formed by the loss of methanol from the pseudomolecular ion. The loss of the neutral methanol in the FAB spectra corresponds to the ready loss of the methoxyl radical in the EI mass spectra. Whereas the loss of HNCO, a characteristic of uracil derivatives, was not observed in the EI spectrum, this fragmentation was confirmed in the FAB spectrum by a peak at m/z 128 [P-HNCO].

Higher mass species than the molecular ion were also detected at m/z 341, 363 and 511, which respectively represent [2M+H], [2M+Na] and [3M+H] species. In addition to the dimers and trimers found, complex mixtures of the molecular ion and the matrix material were also observed. A particularly intense peak which might have been mistaken for the pseudomolecular ion was the peak at m/z 324, which actually represents an adduct of the matrix material (mat), nitrobenzyl alcohol, and the pseudomolecular ion. Other species which were detected include a complex of the pseudomolecular ion with a matrix material dimer [P+2mat]; a complex of the dimer and the pseudomolecular ion of the matrix material [2M+H+mat].

The results of the FAB spectrum complement the EI study and confirm the synthesis of 5-diazo-6-methoxy-1,6-dihydrouracil. Confirmation that the molecular ion had the formula $C_5H_6N_4O_3$ was obtained by high resolution mass measurement of the pseudomolecular ion. A measured mass of 171.05154 was obtained which agrees with the formula for the pseudomolecular ion [$C_5H_6N_4O_3$+H].

6.13.3 5-Diazo-6-hydroxy-1,6-dihydrouracil.

The FAB spectrum of 5-diazo-6-hydroxy-1,6-dihydrouracil was obtained. The spectrum was characterised by a peak at m/z 156 (1.7%, M+) but an absence of a peak at m/z 157 [P]. Theabsence of a pseudomolecular ion was unexpected. However, a typical feature of FAB spectra of hydroxyl-containing compounds is the ready loss of water, and consequently a peak at m/z 139 [P-H_2O] was observed. Examination of the FAB spectrum showed many peaks which could be assigned to daughter ions formed by the fragmentation of 5-diazouracil (Table 6.5).

The generation of 5-diazouracil was indicated by the more complex species formed, with m/z values greater than the pseudomolecular ion, at m/z 437, 415, 385, 277, 247 and 246.

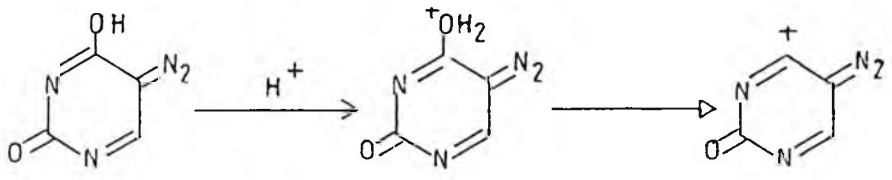
The presence of the hydroxy derivative was indicated by the [M+] peak and by complexes with a higher value m/z. Of particular importance are the complexes of the molecular species with common cations, such as sodium or potassium, which are frequently observed in FAB spectra. The peaks at m/z 179 and m/z 195 can be assigned to complexes of 5-diazo-6-hydroxy-1,6-dihydrouracil with sodium and potassium cations respectively. The species M-H which was observed

for the methoxy derivative (see Table 6.4) was also similarly observed for the putative hydroxy derivative.

		assignments for the Uracil derivatives	
m/z	intensity	5-diazo-6-hydroxy-	5-diazo-
469	0.6	3M+H	
437	2.5		3M2+Na
415	0.5		3M2+H
385	5.1		2M2+H+mat
287	1.1	M+mat+Na	
277	3.4		2M2+H
265	1.6	P1+mat	
264	5.5	M1+mat	
247			P2+mat
246	1.4		M2+mat
195	2.2	M1+K	
179	3.7	M1+Na	
177	2.3		M2+K
161	2.3		M2+Na
157	-	P1 or [M1+H]	
156	1.7	M1	
155	2.2	M1-H	
139	99.8	P1-H_2O	P2
138	1.6	M1-H_2O	M2
129	4.3	P1-N_2, P1-CO	
128	3.3	M1-N_2, M1-CO	
121	2.3		P2-H_2O
120	1.1		M2-H_2O
113	71.1		P2-CN
112	4.5		M2-CN
86	2.3	P1-HNCO-N_2, P1-HNCO-CO	
85	1.4	M1-HNCO-N_2, M1-HNCO-CO	P2-N_2-CN, P2-N_2-CO
84	2.2		M2-N_2-CN, P2-N_2-CO
82	1.2		M2-N_2-CO
71	1.0	P1-2HNCO	
70	6.7	M1-2HNCO	
68	1.2		P2-HNCO-CO

Table 6.5 FAB of 5-diazo-6-hydroxy-1,6-dihydrouracil

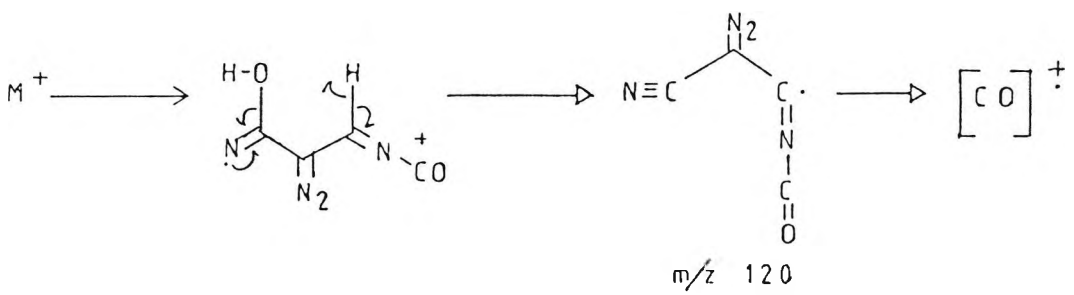
The high mass complexes point to the existence of the hydroxyl derivative. It would appear that 5-diazouracil is also present and it would be reasonable to suggest that if it is derived from the hydroxyl derivative by the loss of water, then its formation occurs primarily in the matrix material. One particular fragment at m/z 121 suggests the loss of water from the 5-diazouracil pseudomolecular ion, a reaction not observed in the methoxy- derivative or from the hydroxy- derivative. Although water is lost from the hydroxyl derivative, the water loss from 5-diazouracil must involve the lactim form (6.62), a form not associated with the dihydrouracils. The lactim form may be preferred or readily achieved because of the increased conjugation, especially since 5-diazouracil is unsaturated in the 1,6-position (Scheme 6.38).



(6.62)

Scheme 6.38

The fragment at m/z 120 can be explained by a species [M2-H_2O]. However, such a simplistic assignment remains awkward since it would necessitate the loss of the C-6 proton with the lactim hydroxyl. Apart from an alternative assignment, a ring-opened molecular ion radical could be envisaged for its occurrence and driven by the formation of nitrile (Scheme 6.39).



Scheme 6.39

The existence of 5-diazo-6-hydroxy-1,6-dihydrouracil can also be shown through some of the fragments. The loss of HNCO and N_2

and/or CO were observed in the FAB spectrum of 5-diazo-6-methoxy--1,6-dihydrouracil. Similar fragmentations might be expected from the hydroxyl derivative, given their structural similarity. The observation of species, resulting from such fragmentations, can be taken as evidence supporting the presence of the hydroxy-derivative.

6.13.4 5-Diazo-6-ethoxy-1,6-dihydrouracil

The FAB spectrum of this compound (Table 6.6) was similar to the spectrum of the methoxy- derivative.

m/z	intensity	Assignment
553	0.4	3M+H
522	0.4	P+M+mat
491	1.0	P+2mat
369	2.2	2M+H
338	14.6	P+mat
337	1.1	M+mat
223	1.1	M+K
205	4.7	M+Na
185	100.0	P
184	1.6	M
183	5.1	M-H
169	2.3	$M-CH_3$
167	1.9	$P-H_2O$
166	2.5	$M-H_2O$
163	1.1	$P-N_2$, P-CO
156	3.6	$M-C_2H_4$
142	2.6	P-HNCO
141	4.4	M-HNCO
139	48.3	P-EtOH
114	2.3	P-HNCO-N_2, P-HNCO-CO
113	12.4	M-HNCO-N_2, M-HNCO-CO
99	1.9	P-2HNCO
98	1.2	M-2HNCO

Table 6.6 Assignment of FAB peaks for 5-diazo-6-ethoxy-1,6--dihydrouracil

The spectrum contained both a molecular ion at m/z 184 of low intensity and by a pseudomolecular ion [P] of high intensity at m/z

185. Similar to the hydroxy- and methoxy- derivative, the spectrum was also characterised by a [M-H] species. Complexes with the common cations, sodium and potassium, were also observed at m/z 207 and m/z 223. A surprising feature of the spectrum was the presence of peaks at m/z 166 and m/z 167. These can be tentatively assigned to [M-H_2O] and [P-H_2O], which is at odds with the spectrum of the methoxy- derivative.

Typical fragments observed were the loss of the alcohol, and the loss of the diazo nitrogens or carbon monoxide and the loss of HNCO. As might be expected of the uracil ring, the loss of two HNCO-groups is observed.

The formula for 5-diazo-6-ethoxy-1,6-dihydrouracil was confirmed by the high resolution mass measurement of the high intensity pseudomolecular ion. A mass of 185.06698 was measured and confirmed $C_6H_8N_4O_3$+H (requires m/z 185.06745) as the formula for the psuedomolecular ion.

6.13.5 5-Diazo-6-isopropoxy-1,6-dihydrouracil.

The FAB spectrum of this diazo compound was very similar to those of the other 5-diazo-6-alkoxy-1,6-dihydrouracils. The diazo compound produced a strong pseudomolecular ion [P] at m/z 199 and a molecular ion at m/z 198, which demonstrates that simple ionisation of the sample also occurs. Therefore, the finding of species related to the fragmentation of the molecular ion is not unreasonable. A peak at m/z 197 which represents the [M-H] species was also observed. Although it is possible to regard such a species as [M-H], it is equally possible to express such a fragment as [P-H_2].

The expected fragments were also observed; these include [P-N_2] and/or [P-CO] at m/z 171 and also [M-N_2] and/or [M-CO] at m/z 170 (Table 6.7). Although the species [P-HNCO] and [M-HNCO] were not observed, the species [P-2HNCO] and [M-2HNCO] were detected. A peak at m/z 128 was observed and this could be assigned to the fragments [P-HNCO-CO] or [P-HNCO-N_2] or [(P-N_2)-HNCO] or [(P-CO)-HNCO]. Thus, the failure to detect the [P-HNCO] species can result from a subsequent and rapid, further fragmentation or a preference for fragmentation via an alternative process. A corresponding peak at m/z 127 was also observed, in which the same fragmentations of the molecular ion could be assigned. The loss of

isopropanol from the pseudomolecular ion to give a peak at m/z 139 was also observed. As in the spectra of the other diazo compounds, this peak was the second largest peak in the spectrum.

m/z	intensity	Assignment
617	1.4	3M+Na
595	0.3	3M+H
505	0.3	P+2mat
435	0.5	2M+K
419	5.5	2M+Na
397	2.7	2M+H or P+M
352	4.2	P+mat
237	1.0	M+K
221	18.3	M+Na
199	100.0	P
198	1.0	M
197	2.9	M-H
183	1.4	$M-CH_3$ or $P-CH_4$
171	4.9	$P-N_2$, P-CO
170	3.2	$M-C_2H_4$
139	83.8	P-*i*PrOH
128	4.3	$P-HNCO-N_2$, P-HNCO-CO
127	1.5	$M-HNCO-N_2$, M-HNCO-CO
113	8.1	P-2HNCO
112	3.6	M-2HNCO
84	1.1	*i*PrOH+Na
61	2.1	*i*PrOH+H

Table 6.7 Assignment of FAB peaks for 5-diazo-6-isopropoxy-1,6--dihydrouracil

The peak at m/z 183 also demonstrated the loss of the methyl group from the alkyl portion of the molecule and this can be ascribed to the loss of a methyl radical from the molecular ion radical or the loss of methane from the pseudomolecular ion. A similar fragment was detected in the spectrum of 5-diazo-6-ethoxy--1,6-dihydrouracil (see Table 6.6).

Complexes with a mass above 199 were also formed, including a complex with the matrix material, nitrobenzyl alcohol, at m/z 352 and the dimer and trimer at m/z 397 and 595 respectively. Complexes with sodium and potassium cations were also identified.

Confirmation of the formula was obtained by a high resolution mass measurement for the pseudomolecular ion. A value of 199.08321 was obtained, which fits the expected formula ($C_7H_{11}N_4O_3$+H requires 199.0831).

6.13.5 5-Diazo-6-*tert*-butoxy-1,6-dihydrouracil.

5-Diazo-6-*tert*-butoxy-1,6-dihydrouracil was examined by FAB mass spectrometry. Two samples of this compound were run using a different matrix material in each case. In the first spectrum (column A, Table 6.8), the matrix material used was 3-nitrobenzyl alcohol and in the second spectrum (column B, Table 6.7), the matrix material was thioglycerol.

The compound gave a weak signal for the pseudomolecular ion at m/z 213 in both spectra. At such a weak intensity, it was unlikely that either the molecular ion or the [M-H] species would be observed and this was found to be the case. Further support for the molecule came from the observation of sodium and potassium complexes in both spectra.

The fragmentation pattern provided the typical fragments even though the intensity of some of the peaks was different to the previous pattern shown by the other diazo compounds. An extremely intense peak was observed at m/z 139, this corresponds to the loss of *tert*-butanol from the pseudomolecular ion. This would suggest that the loss of *tert*-butanol is a highly favoured process. The loss of butanol would appear to occur primarily to the pseudomolecular ion, since the intensity of the sodium complexes of 5-diazouracil is low especially when compared with the spectrum of 5-diazo-6-hydroxy--1,6-dihydrouracil (m/z 161 has an intensity of 1.1 in spectrum A and an intensity of 1.0 in spectrum B). This result is also reflected in the potassium complex of 5-diazouracil (m/z 177), which was found only in spectrum B with an intensity of 1.0%.

Typical fragmentations include the loss of HNCO from both the molecular ion and the pseudomolecular ion. This is also accompanied by a species representing the loss of two HNCO species, which is consistent of the amide-imide portion of the uracil ring. The loss

of N_2 and/or CO was observed and would also be a typical characteristic of these compounds. The loss of CO cannot be readily distinguished from loss of nitrogen on the basis of the spectra obtained as both result in the loss of 28amu. The loss of either or both of these species is a favourable process and also occurs to the [M-HNCO] daughter ion to produce the [M-HNCO-CO] or [M-HNCO-N_2] species.

m/z	intensity (A)	intensity (B)	Assignment
463	0.4	-	2M+K
447	0.4	-	2M+Na
425	-	-	2M+H or P+M
366	N/A*	-	P+mat
321	-	N/A	P+mat
251	1.9	2.8	M+K
235	3.4	2.6	M+Na
213	3.0	2.3	P**
197	-	1.1	M-CH_3 or P-CH_4
185	50.9	35.6	P-N_2, P-CO
184	1.1	1.4	M-N_2, M-CO
170	-	2.0	P-HNCO
169	2.1	2.8	M-HNCO
155	4.0	-	P-(*t*-BuH)
154	2.3	-	M-(*t*-BuH)
142	2.1	4.7	P-HNCO-N_2, P-HNCO-CO
141	6.4	16.8	M-HNCO-N_2, M-HNCO-CO
139	100.0	100.0	P-(*t*-BuOH)
127	2.1	3.9	P-2HNCO
126	1.1	2.2	M-2HNCO
113	18.1	9.7	*t*-BuOH+K
97	4.0	2.1	*t*-BuOH+Na
75	-	1.2	*t*-BuOH+H
74	2.1	3.4	*t*-BuOH

*N/A = Not applicable,

**P = M+H

Table 6.8 Assignment of FAB peaks for 5-diazo-6-*tert*-butoxy--1,6-dihydrouracil

A high resolution mass measurement of the pseudomolecular ion was not made because of the low intensity of the ion. It should be noted that the nature of matrix material did not particularly affect the general shape of the spectrum, especially in view of the number of common species seen in both spectra. There were some minor differences in the observed peaks, but these peaks were usually of a low intensity.

6.13.6 5-Diazo-1,6-dihydrouracil.

The FAB spectrum of the compound contained a peak at m/z 141, of low intensity, which was assigned to the pseudomolecular species [M+H] (Table 6.9). In accordance with expected behaviour, a peak at m/z 139 was observed. This peak can be expressed as a [M-H] species, but it seems much more likely that the peak is in reality a [P-H_2] species. Further evidence for 5-diazo-1,6-dihydrouracil is provided by the peak at m/z 179, which represents a complex of the molecular species and potassium. Although the complex with sodium (M+Na, m/z 163) was not observed in this spectrum, its presence was observed in a second spectrum.

Higher complexes of the diazo compound were also detected, for example, the [2M] species at m/z 280 and the [(P+M)-H_2] species at m/z 279. Other complexes involve the matrix material, thioglycerol, such as the [M+Na+mat] species and m/z 271.

The loss of 28amu from the pseudomolecular ion, P, was observed and attributed to the loss of the diazo nitrogens (P-N_2) or the loss of carbon monoxide (P-CO). A peak for the loss of HNCO was observed but the peak for the expected loss of two HNCO was not detected and this was at odds with the other compounds in the series. The species [(M-H)-HNCO] was observed, which is better expressed as the loss of molecular hydrogen from the pseudomolecular species, [(P-H_2)-HNCO]. This has the advantage in that the loss of the neutral, stable, molecular hydrogen is a much more energetically favourable process.

The base peak was at m/z 187, which was assigned to the species [P+EtOH]. The ethanol originated from the work-up procedure. The ethanol seems to be quite strongly associated with the diazo product as many of the peaks in the spectrum can be directly related to a product-ethanol complex, for example, [P+EtOH], [(M+EtOH)+Na] and [(M+EtOH)+K].

m/z	intensity	Assignment
465	0.4	P+3mat
463	0.5	(P+3mat)-H_2
423	0.3	(M+Na)+2mat+EtOH
421	0.5	P+2M
419	0.5	(P+2M)-H_2
403	0.7	(P+EtOH)+2mat
317	2.3	(M+Na)+mat+EtOH
301	2.7	(P-H_2)+M+Na
295	6.2	(P+EtOH)+mat
294	1.3	(M+EtOH)+mat
293	15.1	(P+EtOH)+mat-H_2
280	3.4	2M
279	18.0	(P+M)-H_2, 2M-H, P+3EtOH
278	1.1	M+3EtOH
271	1.7	(M+Na)+mat
263	2.6	2mat+EtOH+H
262	1.7	2mat+EtOH
247	1.3	P-H_2+mat
233	2.7	P+2EtOH
232	8.7	M+2EtOH
225	1.2	(M+K)+EtOH+K
209	11.7	(M+Na)+EtOH
200	1.0	mat+2EtOH
187	100.0	P+EtOH
185	3.9	(P+EtOH)-H_2]
179	6.7	M+K
159	24.2	(P+EtOH)-N_2, (P+EtOH)-CO
158	16.3	(M+EtOH)-N_2, (M+EtOH)-CO
155	2.1	mat+EtOH+H
154	4.4	mat+EtOH
144	18.3	(P+EtOH)-HNCO]
143	1.6	(M+EtOH)-HNCO]
141	2.2	P
140	1.3	M
139	2.6	P-H_2 or M-H
115	1.3	(P+EtOH)-HNCO-N_2, (P+EtOH)-HNCO-CO
113	4.4	P-CO, P-N_2
101	9.2	(P+EtOH)-2HNCO
98	1.5	P-HNCO
96	17.1	M-HNCO-H, P-H_2-HNCO

Table 6.9 Assignment of FAB peaks for 5-diazo-1,6-dihydrouracil

The large number of peaks and the intensity of those peaks in the spectrum, which contain ethanol, suggest that the ethanol is closely associated with the product. However, it is likely that the ethanol is not a covalently bonded moiety. The observation of complexes with multiples of ethanol would be in keeping that the ethanol is not covalently bonded but a free species, rather in the manner in which the multiples of the matrix material are often observed in complexes. Further evidence to suggest that the ethanol is a separate species comes from the observation of the peaks at m/z 263, 262, 155, and 154, which all represent complexes of ethanol and matrix material only.

6.14 ^{13}C-n.m.r. of some of the studied compounds.

6.14.1 Introduction.

A search of the literature has failed to reveal any ^{13}C-n.m.r. data for any of the compounds studied in this chapter. In this section the spectra of some of the compounds are discussed and tentative assignments made for this class of compounds.

The spectra were run on a Jeol FX60 spectrometer. The spectral width was 4000Hz unless otherwise stated. Proton decoupling was carried out at 59.75MHz and the observation frequency was fixed at 15.0MHz.

6.14.2 5-Diazo-6-methoxy-1,6-dihydrouracil.

The ^{1}H-decoupled spectrum of the compound contained five peaks in addition to the solvent peaks (dmso-d_6) and the reference peak (TMS). From ^{13}C-chemical shift tables, the various peaks could be assigned to the different carbons in the molecule (Table 6.10).

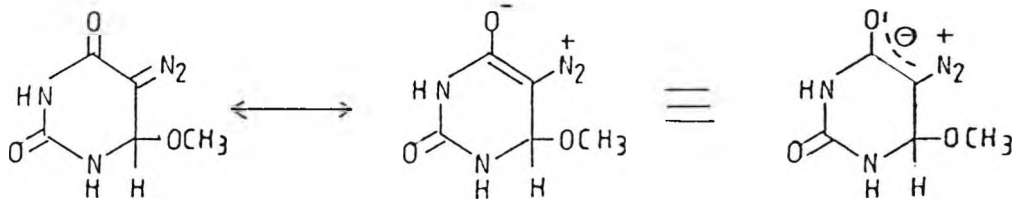
chemical shift	intensity	assignment
162.56	1416	C-2
151.65	1591	C-4
77.12	1539	C-6
57.24	739	C-5
51.59	1430	C-1' (MeO)

Table 6.10 ^{1}H decoupled spectrum of 5-Diazo-6-methoxy-1,6--dihdyrouracil

The assignment of the C-6 and C-5 atoms is based on the intensity of each signal. It can be seen that the large of the two signals is the signal at 77.1δ. The absence of any hydrogen nuclei at C-5 means that the C-5 carbon will relax at a much slower rate than the C-6 carbon.

A poor ^{13}C-coupled spectrum provided sufficient data to confirm the assignments. The spectrum was complicated by the observation that the compound had undergone decomposition in the following coupled spectrum. Presumably, the heat generated over the several hours required to accumulate the spectrum was responsible for the decay of part of the sample material.

In the coupled spectrum, a singlet at 162.564δ was observed for one of the carbonyls and a doublet observed for the other. The doublet consisted of signals at 152.039δ and 151.00δ (centred about 151.52δ), giving a coupling constant J_{HC}=15.6Hz. It is the observation of this coupling which suggests that the upfield signal at 151.6δ represents the C-4 carbonyl. It might have been anticipated that the C-4 carbonyl would have been the most downfield signal, by simple comparison to uracil. One possible explanation is the influence of the the diazo group, which might be providing paramagnetic shielding, or alternatively a different hybridized state may be contributing to the overall increase in sp^3 character (Scheme 6.40).



Scheme 6.40

The signal at 77.1δ had split into a doublet (71.081δ and at 82.646δ, centred at 76.864δ), thus, confirming the attachment of a proton to that carbon. This would agree with the assignment of that signal to the C-6 carbon. The signal for the C-5 atom was not sufficiently resolved from the noise, as were two of the quartet for the methoxyl carbon. Only two peaks of the methoxyl quartet were above the baseline noise; these signals appeared at 56.657δ and 47.041δ (centred at 51.849δ).

6.14.2 5-Diazo-6-ethoxy-1,6-dihydrouracil.

The ^{1}H-decoupled spectrum of this compound contained six peaks. The observed chemical shifts of the ring carbons were similar to those found in the methoxy- derivative and this allowed the assignment of the signals (Table 6.10).

As expected, the C-5 signal at 57.81δ was substantially smaller than either the putative C-6 or C-1' signals. Further support for the correct assignment of the C-5 position was obtained in the enhanced quarternary spectrum (EQS). In this spectrum a low

decoupling power is applied, which reduces the peak heights of signals for carbons to which hydrogen is attached. Qua ternary carbons, which have no hydrogens, retain their signal height because of the absence of the C-H interaction.

chemical shift	intensity	assignment
163.02	1538	C-2
152.02	2102	C-4
76.24	2656	C-6,
60.22	2763	C-1', OCH_2
57.81	1064	C-5
15.0	2454	C-2', Me

Table 6.11 ^{1}H-decoupled spectrum of 5-Diazo-6-ethoxy-1,6-dihdyro--uracil

The EQ spectrum, was characterised by two strong downfield signals at 163.02δ and 152.015δ, which agree with the assignment of carbonyls to those signals. In upfield region, the spectrum showed two peaks at 60.16δ (reduced EQS intensity) and 57.81δ (partially reduced EQS intensity). This confirmed the their assignments as $-OCH_2$ and C-5 respectively. The reduced intensity of peaks at 76.2δ and at 15.0δ confirmed them to be hydrogen-carrying carbons, C-6 and $-CH_3$ of ethoxy, respectively. The methyl group was also identified from the coupled spectrum, which split the signal at 15.0δ into a quartet. The C-6 carbon appeared as a doublet in the coupled spectrum (J_{HC}=168.9Hz).

6.14.3 5-Diazo-6-isopropoxy-1,6-dihydrouracil.

This compound showed a similar spectrum to the methoxy- and ethoxy- derivatives. There were two downfield peaks which could be assigned to the carbonyls as before (Table 6.12).

The coupled spectrum showed that only one of the carbonyls was coupled. The C-4 signal split into a doublet ($^3J_{HC}$=5.87Hz). A doublet ($^1J_{HC}$=167Hz) centred about 75.16δ (80.73δ and 69.596δ) was observed in the coupled spectrum. This fits with an assignment to the C-6 carbon. A doublet was also observed for C-1 centred at 70.31δ (75.98δ and 64.85δ), which had a coupling constant $^1J_{HC}$= 169.9Hz.

chemical shift	intensity	assignment
163.15	1633	C-2
151.95	2524	C-4
75.00	5773	C-6,
67.58	5384	C-1', OCH
58.59	1630	C-5
23.31	5256	C-2'a, Me
22.27	5460	C-2'b, Me

Table 6.12 Decoupled spectrum of 5-Diazo-6-isopropoxy-1,6--dihydrouracil

6.15 Conclusions.

6.15.1 Synthesis.

The preparation of 5-diazouracil from the commercially available 5-aminouracil represents a convenient starting point for the preparations of many 6-alkoxy- and 6-aryloxy- compounds. The failure to prepare 5-diazo-6-butoxy-1,6-dihydrouracil was surprising in view of the success in the preparation of the *tert*-butoxy-derivative. Inspection of the boiling points (Table 6.13), shows that *n*-butanol has a higher boiling point than either *tert*-butanol or the benzene. Thus, the failure to form the desired product is probably the result of decomposition of the diazo compound at the elevated temperature.

Solvent	B.p.
methanol	64.6
Ethanol	78.0
2-propanol	82.4
n-butanol	117.7
2-methylpropanol	108.0
benzene	80.0

Table 6.13 Boiling points of solvents and reagents in the preparation of 6-alkoxy- diazouracils

One possible route to the preparation of the desired butoxy-derivative would be to use a solvent mixture, for example, heating the diazo with reflux in a mixture of *n*-butanol and benzene over a lengthy period of time. One problem encountered in the preparation

of the *tert*-butoxy- derivative has been the decreasing solubility of the diazo compound in the higher alkyl chain alcohols. One possible alternative to benzene would be to use THF as solvent for both the 5-diazouracil and the alcohol.

In the synthesis of 5-diazo-1,6-dihydrouracil, the main difficulty encountered was the preparation of the precursor 5-amino--5,6-dihydrouracil. It was particularly important to the stability of the precursor compound that all the catalyst was totally removed from the solution and that the solvent water was removed from the filtrate under mild conditions. This diazo compound should provide a ready route to 1,6-dihydro-N-substituted- compounds. Unlike the 6-alkoxy- derivatives, there is no chance that the removal of the N-1 proton will result in the elimination of the 6-alkoxy- or 6-aryloxy- substituent. Thus, this compound should also prove an interesting starting material for glycosidation and lead to various diazonucleosides.

6.15.2 Purification and H.p.l.c.

The methoxy-, ethoxy- and isopropoxy- derivatives all readily recrystallized out of the excess solvent/reagent alcohol. The other diazo compounds were obtained by precipitation from the alcohol. The h.p.l.c. study on the alkoxy- derivatives indicated that non-polar reversed phase columns were unsuitable as a means of separating these compounds. However, the most promising of columns tested was the nitro-phenyl- column. For the future, the development of a system capable of resolving and identifying these diazo compounds will serve to complement any biological studies.

6.15.3 Spectroscopy.

6.15.3.1 Infrared.

In general, the i.r. spectra of the diazouracil derivatives were characterised by a strong intense absorption around 2120cm^{-1}, which represents the diazo functional group. The region between 1750cm^{-1} and 1600cm^{-1} was much more complex than expected. Instead of two sharp and strongly absorbing regions for the carbonyls, the peaks were often broad and contained more than one peak because of the presence of in-plane and out of plane deformations for the amide

and imide N-H bonds. Infrared spectroscopy was not suited for distinguishing between the various substituent groups.

6.15.3.2 N.m.r. spectroscopy.

The ^1H n.m.r. of the various derivatives showed several common features. The most important feature was the detection of the N-1 proton. The observation of this signal in the spectrum of a diazouracil establishes the saturation of the 1,6-position of the ring and consequently the covalent nature of the alcohol addition to 5-diazouracil. Even in 5-diazo-1,6-dihydrouracil, the saturated position can be deduced from the presence of the N-1 proton. The coupling between the N-1 proton and the C-6 proton is best established in most of derivatives by measuring the C-6 doublet. The coupling, $J_{1,6}$, was not the same magnitude in all of the diazo derivatives and covered a range between 0.0 to 8.0 Hz. The four carbons of the diazouracil ring was clearly indicated by ^{13}C n.m.r.

6.15.4 Mass spectrometry.

6.15.4.1 EI spectrometry.

The alkoxy- diazouracils showed similar characteristics in their EI spectra. The spectra all contained an intense peak at m/z 139 and a weaker peak at m/z 138. Several common fragmentation pathways were observed. The ready loss of the HNCO and N_2 fragments from the molecular ion was a regular feature. A typical fragmentation process was the loss of the alkoxyl radical. In spite of the absence of the molecular ion, the fragments allow the structure of the diazo to be determined because of the fragments $M-N_2$ and M-HNCO.

6.15.4.2 FAB spectrometry.

The FAB spectra of these compounds were characterised by the pseudomolecular species. Complexes with sodium and potassium ions are also a common feature as are complexes with the matrix material. A peak at m/z 139 is a common feature but unlike the EI spectrum, this peak is probably the result of the loss of the alcohol from the pseudomolecular species. Higher multiples of the molecular ion are

often detected, and these are often associated with sodium, potassium or hydrogen. Typical fragments resulted from the loss of CO and/or N_2 and the loss of HNCO or 2(HNCO). Overall, FAB spectrometry is a vast improvement over EI spectrometry, where the low volatility of compounds has resulted in excessive fragmentation. However, the one advantage of EI is the ease with which high resolution spectra may be obtained, leading to a more reliable determination of the formula of fragments.

6.15.5 Suggestions for future work.

5-Diazouracils are extremely polar. The polarity could be offset if the amide and imide could be alkylated to give the secondary imide and/or tertiary amide, *viz.*, 3-methyl-, 1-methyl- and/or 1,3-dimethyl- 5-diazouracil. The N-methylation of uracil compounds was carried out by using diazomethane in methanol [74]. It is suggested that in order to avoid the possible exchange of the alkoxy group with a methoxy group in methanol that an alternative solvent such as THF is used. The alternative reagent for N-methylation would be to use dimethyl sulphate with a base [75].

The N-alkylated diazouracils would represent a novel series of compounds. It is anticipated that they will be easier to handle and characterise than the un-alkylated diazouracils. Alkylated bases have been of interest because of their mutagenic and antitumour properties [76] and there is the possibility that the alkylated diazouracils could be biologically active.

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Chapter 7 Reactions resulting in the loss of the diazo nitrogens.

7.1 Introduction.

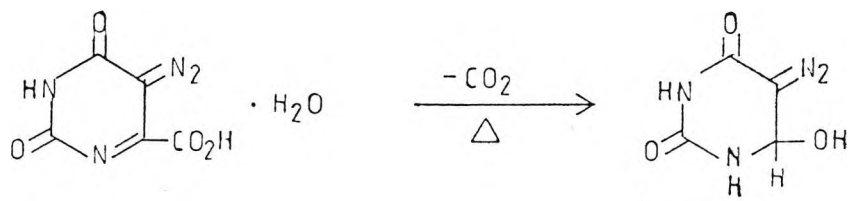
7.1.1 Reactions of diazopyrimidines.

Reactions of diazo compounds can be divided into three classes, correspondingly designated Class I, II and III. Class I reactions are those in which the diazo group is unaffected; reactions in class II are those in which the diazo nitrogen atoms are involved and are retained in the product molecule. Finally class III reactions are ones in which there occurs a resultant loss of the nitrogen atoms from the molecule.

For convenience, the previously reported reactions involving diazopyrimidines have been sorted into these groups and will also serve as examples for these general categories.

7.1.1.1 Class I reactions.

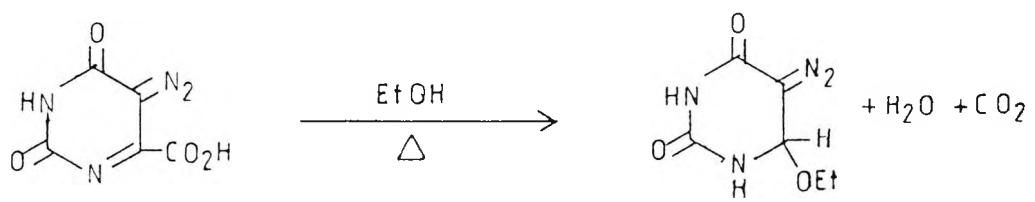
The reaction in which 5-diazouracil-6-carboxylic acid "monohydrate" (7.1) was decarboxylated to give the corresponding diazouracil "monohydrate" (Scheme 7.1) belongs in this class [1].



(7.1) (7.2)

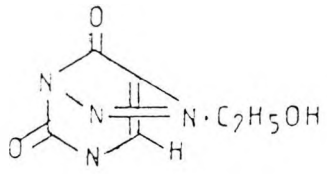
Scheme 7.1

When the reaction was similarly carried out in hot ethanol, the product was thought to have co-crystallized with one molecule of ethanol. It is now clear that the product of decarboxylation in ethanol is 5-diazo-6-ethoxy-1,6-dihydrouracil (Scheme 7.2) and not as 2,3a,4,5-tetraza-3,6-dioxo-bicylo[3.2.1]octa-$\Delta^{4,5}$-$\Delta^{5a,1}$-diene (7.5) nor as 5-(N'-ethoxy)-5-diazouracil (7.6) as proposed in the original work [1].

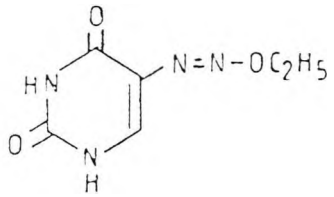


(7.3) (7.4)

Scheme 7.2

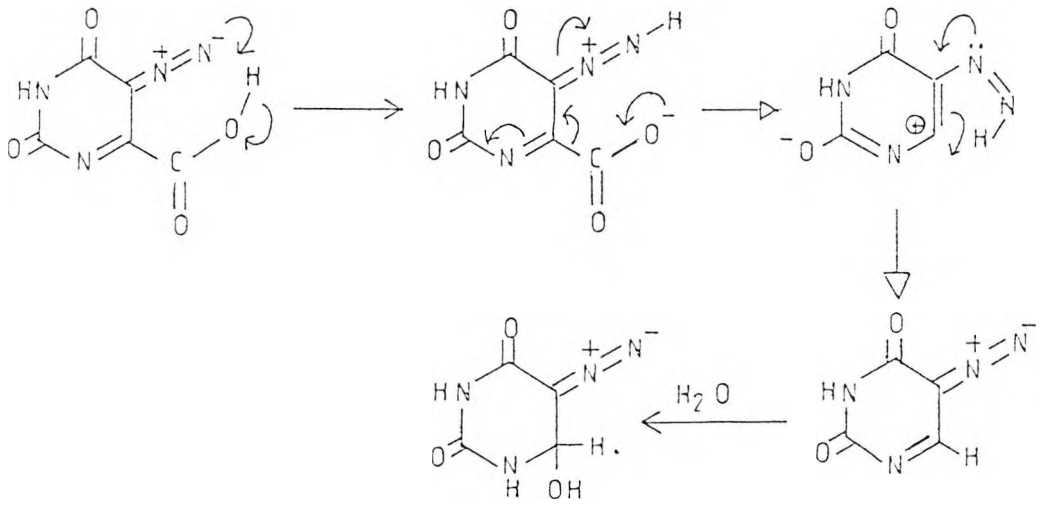


(7.5)



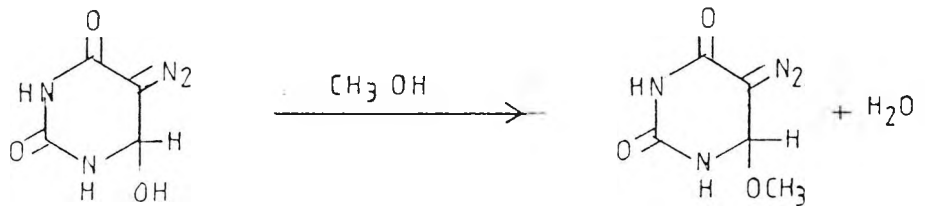
(7.6)

The decarboxylation also occurs at a temperature above 10°C when the compound is left standing in air [1]. The ease with which the decarboxylation occurs is noteworthy. Decarboxylation is often observed in β-carboxy acids and β-keto acids [1a], however, the carbonyls in 5-diazouracil-6-carboxylic acid are positioned *gamma* to the carboxylic group. It is known that uracil-6-carboxylic acid is stable to decarboxylation. Thus, the decarboxylation must be promoted by another factor. Evidently, loss of the carboxylic group is promoted by the adjacent group. One explanation is that the diazo group as a proton acceptor assisting the decarboxylation (Scheme 7.3).

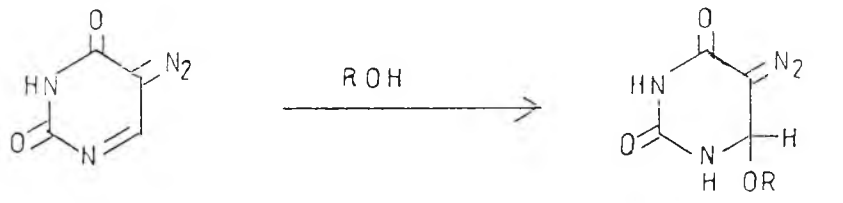


Scheme 7.3

The conversion of 5-diazo-6-hydroxy-1,6-dihydrouracil into the 6-methoxy derivative (Scheme 7.4) [2] and the addition of water or an alcohol across the 1,6-double bond of 5-diazouracil (Scheme 7.5) are class I reactions.



Scheme 7.4



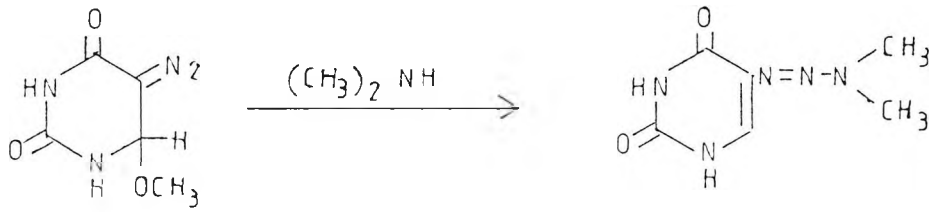
R=H alkyl, aryl

Scheme 7.5

7.1.1.2 Class II Reactions.

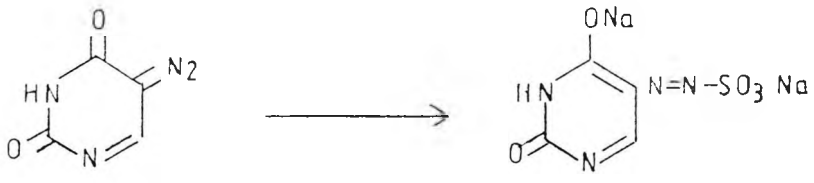
This class of reactions is characterized by the involvement of the diazo group whilst retaining the diazo nitrogens. The earliest example of this reaction was the characterization of the diazo group in 5-diazouracil-6-carboxylic acid "monohydrate" by reduction to the corresponding hydrazine using tin chloride and concentrated hydrochloric acid for 5-diazouracil-6-carboxylic acid and 5-diazo--6-ethoxy-1,6-dihydrouracil.

It was reported that 5-diazo-6-methoxy-1,6-dihydrouracil reacts with dimethylamine to afford 5-(3,3-dimethyl-1-triazeno)--uracil with the concomitant loss of the 6-methoxy group (Scheme 7.6) [2]. It was proposed that, in an alkaline solution, aromatic amines and phenols coupled very slowly to give unidentified products [3]. A more detailed study of the diazo group with amines is dealt with later on.

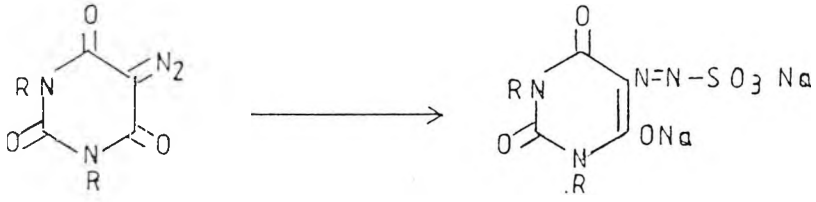


Scheme 7.6

It has been reported that diazouracil, diazobarbituric acid and its N,N'-dimethyl derivative will react with sodium hydrogen sulphate to give the corresponding sodium salt of the diazo sulphonate [4,5] (Scheme 7.7 and 7.8).

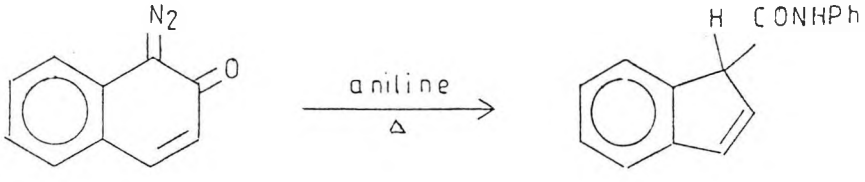


Scheme 7.7

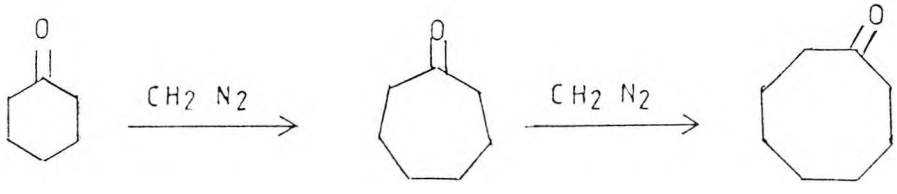


Scheme 7.8

It is known that diazo compounds will undergo ring expansion and ring contraction, for example the ring contraction of 1-diazo-2-tetralone [6](Scheme 7.9). An example of a ring expansion is the preparation of octanone via heptanone from hexanone by successive additions of diazomethane [7](Scheme 7.10). These changes in ring size involve the loss of the diazo nitrogens to generate a highly reactive intermediate capable of breaking old bonds and forming new ones.

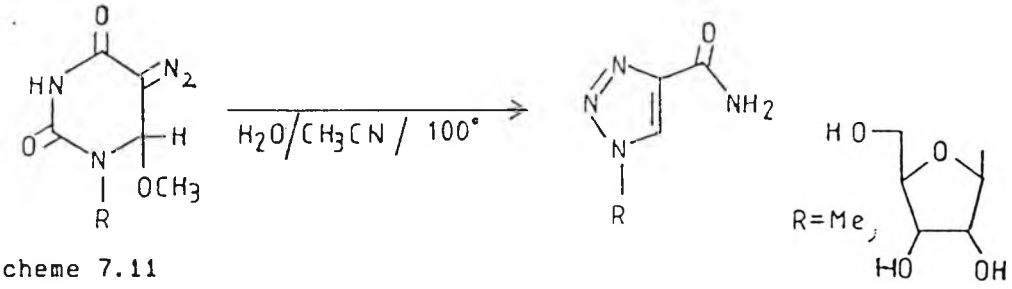


Scheme 7.9



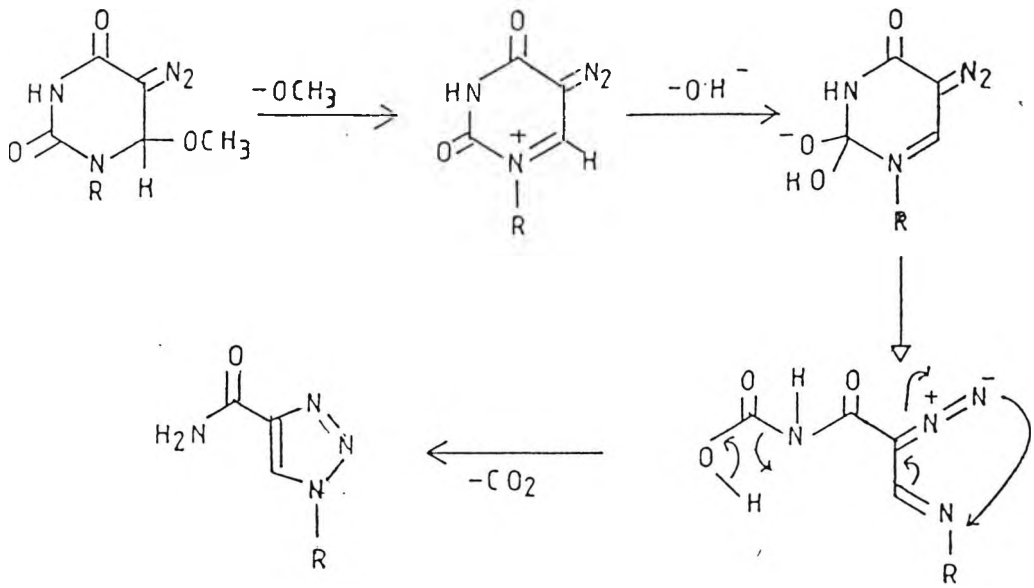
Scheme 7.10

It has been reported [8,9] that 5-diazouracils will undergo ring contraction. This reaction is unusual because the diazo nitrogens are not lost but are retained and form part of the new ring in the resulting triazole carboxamides (Scheme 7.11).



Scheme 7.11

It was also demonstrated that these compound will retain the N-alkyl groups on the precursor N-alkylated 5-diazouracil. Thus, this reaction possibly provides an effective route to *seco*-carboximide nucleoside analogues. It is likely that the mechanism involves the hydrolysis of the imide bond followed by ring closure (Scheme 7.12).



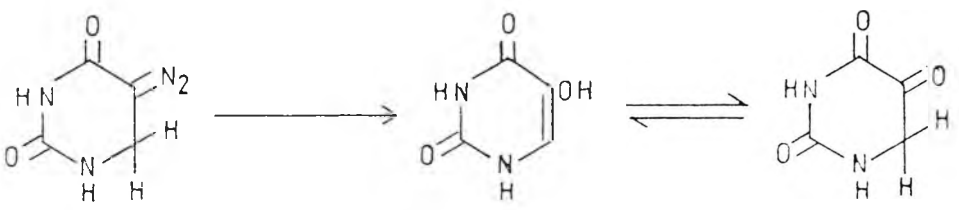
Scheme 7.12

7.1.1.3 Class III reactions.

This class represents the more familiar type of reaction in which the diazo nitrogens are lost to afford a reactive intermediate which readily couples with nucleophiles.

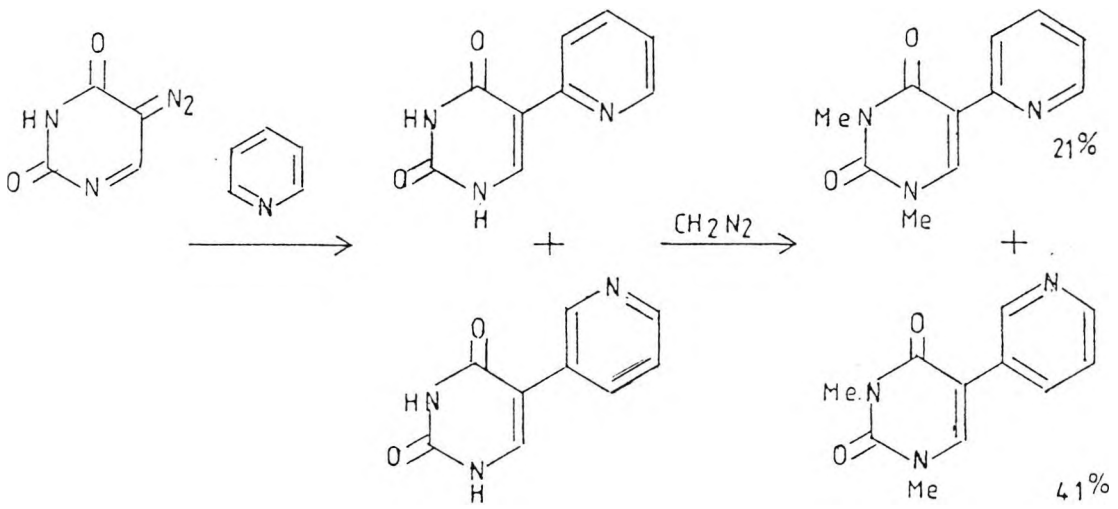
One early example was the reported action of aqueous dilute hydrochloric acid on 5-diazo-6-methoxy-1,6-dihydrouracil to give

5-hydroxyuracil [1,10] (Scheme 7.13).



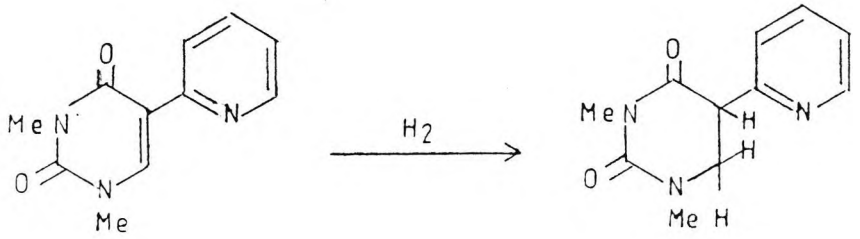
Scheme 7.13

The thermal decomposition of these diazouracils in pyridine has afforded a mixture of isomers in which the diazo group has been substituted with pyridine yielding isomeric 5-pyridyluracils [11]. When either diazouracil, or its hydrated product or its methanol adduct are heated with reflux in pyridine, 5(2-pyridyl)uracil and 5(3-pyridyl)uracil were obtained in a ratio 1:2. These products could not be separated, even by h.p.l.c. However, N-methylation with diazomethane afforded the 1,3-dimethyl derivatives which were amenable to separation by chromatography (Scheme 7.14).

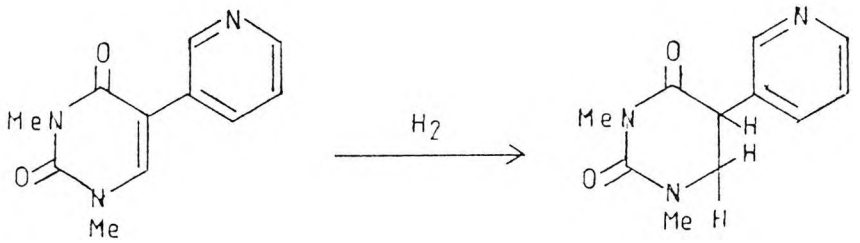


Scheme 7.14

The attachment to the C-5 position of the pyrimidine ring was determined via reduction to the dihydro- derivative using palladium on charcoal under hydrogen at 50 psi in a Paar apparatus and by subsequent isotopic substitution (Scheme 7.15).

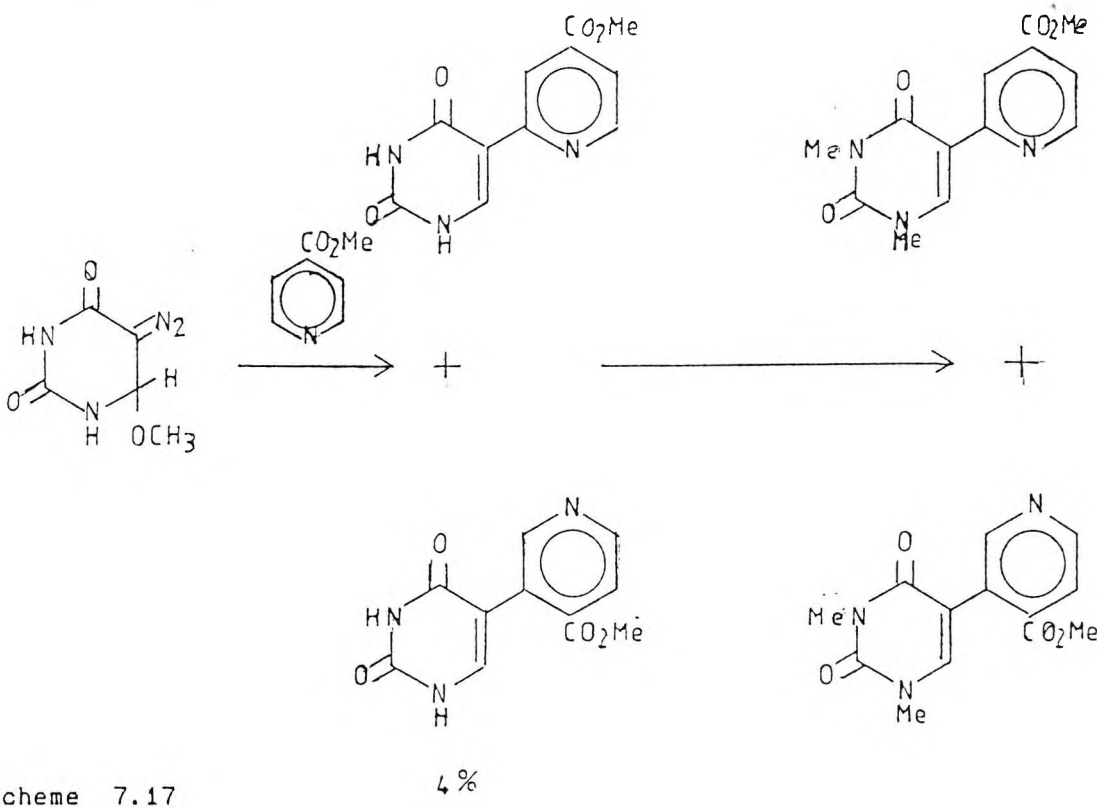


Scheme 7.15



Scheme 7.16

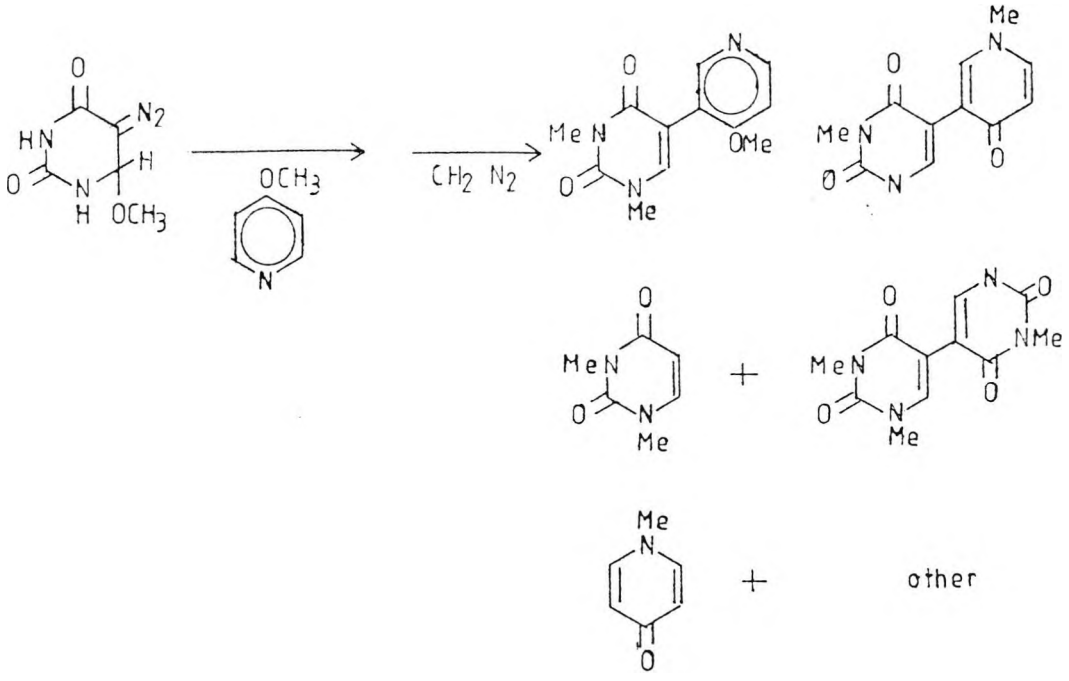
The reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with 4-carboxymethylpyridine (methyl isonicotinate) afforded two isomeric products which correspond to the substituted 2- and 3-pyridyl compounds (Scheme 7.17).



Scheme 7.17

Reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with 4-methoxypyridine afforded a wide variety of products (Scheme 7.18)

but only one containing the substituted pyridyl group was obtained, name 5(3-(4-methoxypyridyl))uracil.



Scheme 7.18 Reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with 4-methoxypyridine

The catalytic decomposition of these diazo compounds has been unexplored since the original work in which these compounds were found to explode with the addition of platinum black. The products of this reaction were unidentified [1].

Despite the early synthesis of diazopyrimidine compounds, the number of reactions involving these compounds has been extremely limited. This is surprising on account of the large number of publications dealing in the biological activity of diazouracil. It is also noteworthy in that well over half a century elapsed from its initial synthesis to the elucidation of the structure of diazouracil and of its methanol adduct and its hydrate [2].

7.2 Solubility study.

Since the intermediate formed when the diazo group is cleaved from the precursor is extremely reactive, it was decided that controlling certain aspects of the reaction conditions would facilitate such a study. The safety of such reactions would be

enhanced if the release of nitrogen was slow and not explosive. Explosive release could be avoided by the use of a limiting initiator, such as a small quantity of catalyst. This condition could be further enhanced by dilution of the starting material in a large quantity of solvent. It was assumed that reactions might be facilitated by the use of a suitable solvent. High dilution would also help prevent reaction of the carbenoid intermediate with itself or its precursor.

Thus, it was considered necessary to make a preliminary study of the solubility of 5-diazo-6-methoxy-1,6-dihydrouracil. The criteria for solvent suitability were the ability to dissolve the diazo compound, the organic reagent and the proposed catalyst to be used for decomposition. In addition, the solvent should be inert to the diazo compound and the reactive intermediate generated during the reaction.

7.2.1 Results and discussion.

The solubility tests on the compound were carried out on a small scale (1-2 mg) in 1.5 ml of the selected solvent in a sample tube, the solubility being evaluated by qualitative inspection. If the solvent became yellow, then the material was deemed to be partly soluble. If the solution was strongly coloured and the crystalline diazo compound was no longer visible then the selected solvent was considered as effective. The solubility of the diazo compound was examined in three temperature ranges. The results are summarised below (Table 7.1).

	Temperature		
Solvent	10-15°C	25-30°C	50-55°C or b.p.
Acetone	insol.	sl. sol.	-
Acetic acid	sol.	-	-
Pyridine	sol.	-	-
dmso	sol.		
MeCN	insol.	sl. sol.	sl. sol.
DMF	sol.	-	-
dichloromethane	insol.	insol.	insol.
chloroform	insol.	insol.	insol.
ether	insol.	insol.	insol.
THF	insol.	sol.	-
MeOH	sl. sol.	sol.	-
EtOH	insol.	sol.	-
triethylamine	sl. sol.	discolours	-

sol. = soluble, sl. sol. = slightly soluble, insol. = insoluble

Table 7.1 Solubilities of 5-diazo-6-methoxy-1,6-dihydrouracil

A similar study was carried out for 5-diazo-1,6-dihydrouracil (Table 7.2).

Solvent	Temperature		
	10-15°C	25-30°C	50-55°C or b.p.
Acetone	insol.	insol.	insol.
Acetic acid	insol.	sl. sol.	-
Pyridine	sol.	-	-
dmso	sol.	-	-
MeCN	insol.	insol.	insol.
DMF	sol.	-	-
dichloromethane	insol.	insol.	insol.
chloroform	insol.	insol.	insol.
ether	insol.	insol.	insol.
THF	insol.	sl. sol.	sol.
MeOH	insol.	sl. sol.	sl. sol.
EtOH	insol.	insol.	sl. sol.
water	insol.	insol.	insol.
NaOH	sol.	decolours	-

sol. = soluble, sl. sol. = slightly soluble, insol. = insoluble

Table 7.2 Solubilities of 5-diazo-1,6-dihydrouracil

From the results, both THF and dimethyl sulphoxide appear to be likely candidates for further testing as they seem to fit the criteria of dissolving the diazo compound and the catalyst and should dissolve or be miscible with a wide range of organic compounds. However, their lack of interference with the reactants or the intermediate formed from the catalytic decomposition of the diazo compound needed to be established.

The possible interaction of the solvent with the diazo compound was studied under three different conditions. The first test was to follow the stability of the diazo compound under ambient conditions with respect to time. The second test was to raise the temperature to 50°C and observe the integrity of the diazo group. Finally, it would be necessary to see if the catalyst would produce any reaction between the two components. The purpose of the first experiment was to demonstrate if dmso has any autocatalytic activity. The second part was aimed at seeking out a safe operating temperature. The third part of the experiment was to explore if the two components are stable in the presence of the catalyst at ambient temperature or at a higher temperature.

7.3 Stability in dimethyl sulphoxide.

5-Diazo-6-methoxy-1,6-dihydrouracil was dissolved in dimethyl sulphoxide. After 1h, a sample of the mixture was removed and examined by analytical t.l.c. using a solvent mixture of chloroform and methanol in a ratio 7:3. The reaction mixture was heated to 50°C. The reaction mixture was allowed to cool to ambient temperature and examined by analytical t.l.c. Rhodium acetate catalyst was added and the mixture heated once more to 50°C. After 1h, the mixture was allowed to cool to ambient temperatures and once again analysed by analytical t.l.c. (Table 7.3).

description of expt (temperature, °C)	R_f 0.08	0.51	0.69	0.77
dmso	-	-	-	x
cpd & dmso (rt)	-	-	x	x
cpd & dmso (50)	-	-	x	x
cpd, dmso & cat (rt)	x	x	x	x
cpd, dmso & cat (50)	x	x	-	x

x = position of material

solvent: Chloroform/Methanol (7:3)

Table 7.3 T.l.c. results.

The observation of only two spots, at R_f=0.69 and R_f=0.77 in the analytical t.l.c., showed that at room temperature (15°C) and with heating (50°C) that the starting diazo compound was unaffected by dimethyl sulphoxide (dmso). However, the addition of the catalyst at room temperature resulted in the appearance of two new spots which indicated two new products. The formation of new compounds was confirmed by the t.l.c. of the catalysed reaction at 50°C which showed that the starting material was no longer present.

The findings of the analytical t.l.c. were supported by the results of the infrared spectroscopy of the reaction mixture. The infrared could be used to follow the presence of the starting material by examination for the characteristically strong diazo

stretch around 2100cm^{-1} (Table 7.4).

experiment	diazo stretch
diazo compound & dmso at r.t. (15°C)	present
diazo compound & dmso at 50°C	present
diazo compound, dmso & cat. at 15°C	present
diazo compound, dmso & cat at 50°C	absent

Table 7.4. Infrared results.

7.3.1 Results and discussion.

The t.l.c. results of the stability testing showed that the diazo compound was stable in dmso at ambient and at elevated temperature. The presence of catalyst was sufficient to produce by-products. The products were derived by the breakdown of the diazo starting material because of the absence of the diazo starting material in t.l.c. of the sample containing the catalyst at 50°C. I.r. gave a spectrum free of the diazo absorption peak around 2100cm^{-1}.

7.4 The reaction of diazouracils with dmso.

7.4.1 5-Diazo-6-hydroxy-1,6-dihydrouracil.

5-Diazo-6-hydroxy-1,6-dihydrouracil was dissolved in dimethyl sulphoxide to give a bright yellow solution. Rhodium acetate catalyst was added and the mixture heated to 50°C with stirring for 1h, after which t.l.c. showed the absence of starting material.

The crude reaction product (approximately 2g) was purified by column chromatography, using a column packed with silica gel H (100g). A mobile phase of dichloromethane/methanol (280:20) was used to elute the product fractions. Each fraction was tested by analytical t.l.c., using chloroform/methanol (7:3) as the mobile phase. The relevant fractions were then combined.

The fraction R_f=0.2 (chloroform/methanol system) was then evaporated to dryness to afford a yellow solid. The i.r. spectrum of the compound was characterised by broad band absorption around

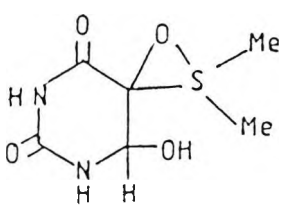
3600-3000cm^{-1} which was attributed to N-H and O-H vibrations. A strong absorption at 1720cm^{-1} was assigned to the imide carbonyl of the uracil moiety. The i.r. spectrum confirmed the absence of the strong absorption around 2120cm^{-1} of the diazo group. The solid nature of the product and the t.l.c. confirmed that the isolated product was free of dimethyl sulphoxide. The absence of free dimethyl sulphoxide was also provided in the i.r. spectrum, which was devoid of the strong sharp absorptions at 1400, 1030, 940, and 870cm^{-1} and by the absence of a triplet of peaks between 2300 and 2200cm^{-1}, which are characteristic of dimethyl sulphoxide.

The product was examined by high resolution EI mass spectrometry at 70eV. The absence of peaks at m/z 140, 139 and 138, which have been shown to be associated with 5-diazouracils, supports the i.r. and t.l.c. findings that the original diazo compound was no longer present. Despite the absence of a peak at m/z 206 ($C_6H_{10}N_2O_4S$), the product was believed to correspond to the addition of dimethyl sulphoxide with a putative carbenoid intermediate. The EI spectrum revealed numerous fragments which are consistent with this.

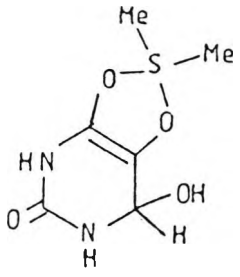
The spectrum contained peaks at m/z 78, 63, and 48, which were consistent with the species dmso, dmso-Me and S=O. The presence of of these fragments support the hypothesis that the product is an adduct with dimethyl sulphoxide.

The presence of the uracil moiety was indicated by the peaks at m/z 113 and 112, which correspond to the fragments $C_4H_5N_2O_2$ and $C_4H_4N_2O_2$. Further support of the presence of the uracil moiety was provided by the peaks m/z 94 ($C_4H_2N_2O$) and 43 (CHNO).

At this point, two possibilities were considered for the structure of the adduct (7.7 and 7.8).

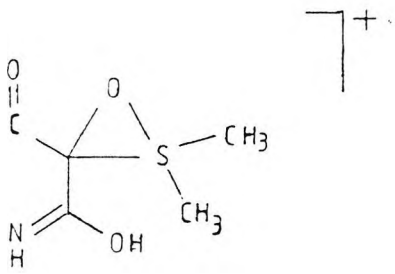


(7.7)



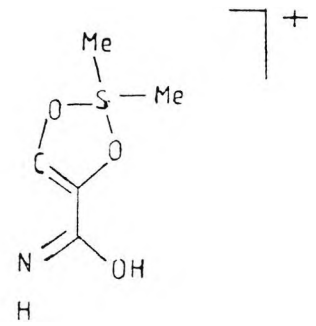
(7.8)

A peak at m/z 162 was assigned a formula $C_5H_6NO_3S$ and can be envisaged as a fragment (7.9 or 7.10) formed by the loss of HNCO and H from the putative molecular ion.



m/z

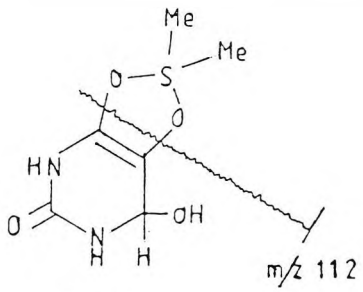
(7.9)



(7.10)

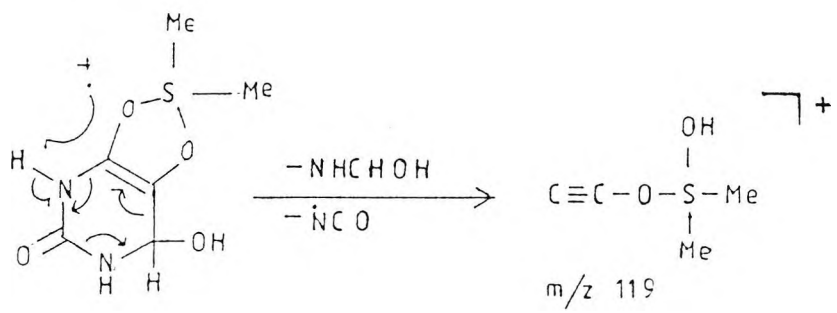
In the spectrum, peaks at m/z 94 and m/z 78.9 were found to correspond to fragments with the formulae $C_2H_6O_2S$ and CH_3O_2S, evidently related to dimethyl sulphone.

The formation of the fragment isomeric to uracil would appear to be supported by the fragment at m/z 119 which corresponds to $C_4H_7O_2S$ (Scheme 7.19). This structure (7.8) will also explain the formation of the ion at m/z 112 (7.11) and the fragments at m/z 119, which corresponds to $C_4H_7O_2S$ (Scheme 7.19).



m/z 112

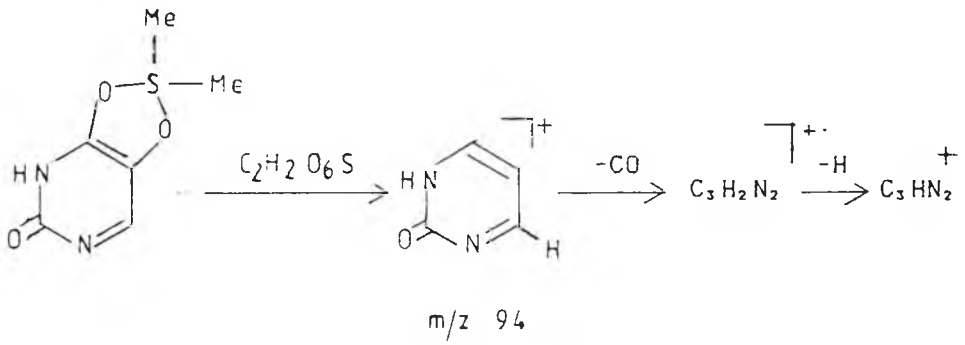
(7.11)



Scheme 7.19

It was possible that in addition to (7.8), the dehydro-derivative (7.12) was also present. The peaks at m/z 94, 66 and 65 can be assigned to the loss of dimethyl sulphone, CO and H from the

dehydro- derivative (Scheme 7.20). Although there was no molecular ion, many of the anticipated fragments were observed, for example, m/z 119, 94, 79, 66, 64, 63, 62, 61, 43 and 39, with many of the peaks common for both products (7.8 and 7.12).



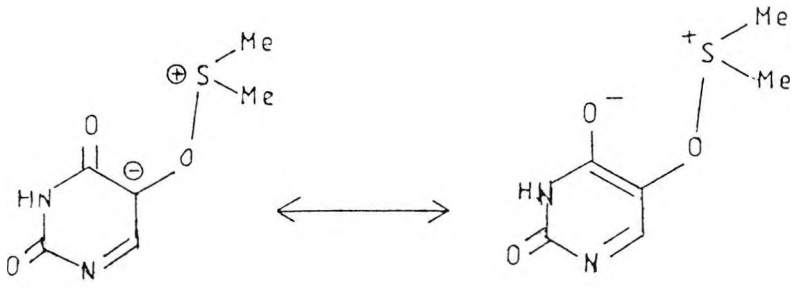
m/z 94

(7.12)

Scheme 7.20

The formation of (7.14) could have arisen from the loss of water from (7.8), possibly as an artefact of the EI-MS process, however, it was found to be a separate product in reaction of dmso with 5-diazo-6-methoxy-1,6-dihydrouracil. The sample proved too weak to produce a ^1H n.m.r. spectrum, which would confirmed if it was present in the isolated material or if it was produced as an artefact of the EI-MS process.

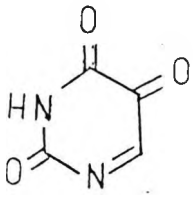
The product (7.8) is presumably formed via the reaction of the carbenoid intermediate with the solvent, via a zwitterion (7.13 and 7.14)



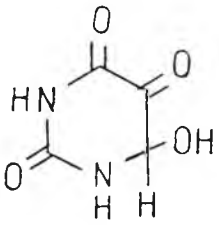
(7.13) (7.14)

This type of zwitterion is similar to the intermediate formed during Moffat oxidation reactions [12,13]. A thorough examination of

the reaction failed to reveal any evidence for the formation of ketones (7.15) or (7.16).



(7.15)



(7.16)

7.4.2 EI Mass spectrum of dimethyl sulphoxide.

One possibility was that dimethyl sulphone, in the spectrum of the adduct (7.4.1), was observed because it was a contaminant of the dimethyl sulphoxide used in the reaction. The most common commercial process to produce dimethyl sulphoxide does involves the oxidation of dimethyl sulphide [14-17]. Consequently the process often results in a small quantity of dimethyl sulphide being further oxidised to the sulphone [18-20]. Despite purification by column chromatography, it was conceivable that either the sulphoxide or the sulphone might have been carried through as a closely associated complex.

A high resolution mass spectrum of the dmso was obtained in order to determine the extent of dimethyl sulphone contamination (Table 7.5).

m/z	% relative intensity	Assignment
94.01	0.38	dimethyl sulphone
79.01	1.45	(M+H)
78.02	5.39	($M^{\bullet}$)
62.99	9.30	(M-Me)
47.98	2.05	(S=O)
46.99	14.48	(CH_3S)

Table 7.5 Mass spectrum of dimethyl sulphoxide

From the results, it can be seen that the detection of a peak at m/z 94 in the product (7.8) is not in itself sufficient to confirm its formation from the ion (7.7). Apart from the peak at

m/z 94, the spectrum of dimethyl sulphoxide did not contain any of the expected fragments for dimethyl sulphone: noticeably absent were peaks for the loss of one or both of the methyls from the sulphone. The low intensity of the peak at m/z 94, the absence of a pseudomolecular ion for the putative sulphone and the absence of any of the expected fragments for dimethyl sulphone, all suggests that the peak at m/z 94 might not be attributable to dimethyl sulphone present in dimethyl sulphoxide.

7.4.3 EI Mass spectrum of perdeuterodimethyl sulphoxide.

In order to aid the identification of the product, it was decided that perdeuterodimethyl sulphoxide might prove a suitable reference material (Table 7.6) as dmso-d_6 is usually produced by deuterium exchange from dmso [16,18].

m/z	%relative intensity	Assignment
86.06	4.10	M+D
85.06	2.26	M+H
84.06	82.61	dmso-d_6, M^+
82.04	1.42	M-H
66.01	100.00	M-CD_3
50.01	28.85	CD_3S
47.97	6.95	S=O

Table 7.6 Mass spectrum of dmso-d_6

The mass spectrum of perdeuterodimethyl sulphoxide possessed both a molecular ion and pseudomolecular ions formed by the addition of deuterium or by the addition of hydrogen. The spectrum for the perdeuterodimethyl sulphoxide gave no indication of a sulphone or the perdeuterodimethyl sulphone.

Having established perdeuterodimethyl sulphoxide was free of the perdeutero- sulphone, it is an obvious choice as a reagent for probing the reaction of the diazouracil with dmso.

7.4.4 5-diazo-6-hydroxy-1,6-dihydrouracil and dmso-d_6

5-Diazo-6-hydroxy-1,6-dihydrouracil was dissolved in dmso-d_6 and rhodium acetate catalyst added. The mixture was heated to 50°C with stirring for 1h, during which nitrogen was evolved. After the

effervescence had ceased, the reaction was left stirring at ambient temperature for 24h. The crude product was examined by mass spectrometry without purification.

The mass spectrum was devoid of peaks at m/z 156, 140, 139, and 138 which would be formed from the parent diazo compound. The spectrum contained peaks at m/z 128 ($C_4H_5N_2O_3$), 113 ($C_4H_5N_2O_2$), 112 ($C_4H_4N_2O_2$), and 43 (CHNO) which were indicative of a substituted uracil moiety. As expected, a peak at m/z 100 was detected and high resolution confirmed this to be perdeuterodimethyl sulphone. The presence of the sulphone was confirmed by the peak at m/z 82 which high resolution mass measurement indicates it to be the [sulphone-CD_3] species. The strongest peaks were associated with the spectrum of dmso-d_6. The peaks at m/z 85 (C_2D_6OS+H), 84(C_2D_6OS), 66(CD_3OS), and 47.9(SO) were all observed.

It is noteworthy that the peaks associated with the uracil portion of the adduct remain undeuterated as might have been anticipated.

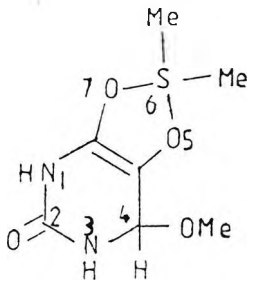
7.4.5 5-Diazo-6-methoxy-1,6-dihydrouracil and dmso.

5-Diazo-6-methoxy-1,6-dihydrouracil was dissolved in the reactant dmso and rhodium acetate catalyst was added. The mixture was left stirring at ambient temperature until both the catalyst and the diazo compound had dissolved. The solution was observed to effervesce on gently warming and this was accompanied by a colour change from bright yellow to dark orange. The product was purified by preparative t.l.c. using dichloromethane/methanol (85:15) as the mobile phase, a polar component, R_f=0.25 being recovered as a yellow solid.

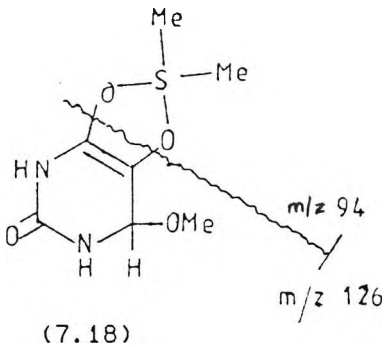
The compound was characterised in its i.r. spectrum by the absence of a diazo absorption band. Strong absorption bands around 1720 and 1670cm^{-1} were attributed to the C-2 imide carbonyl and the out of plane N-H vibration.

The product was examined by EI mass spectrometry. Although the molecular ion was not detected, a pseudomolecular ion [M+H] was observed in very low intensity. The accurate mass measurement of the species gave a mass of 221.0572, which is consistent with the formula $C_7H_{12}N_2O_4S+H$ (requires 221.05960). The uracil moiety was identified from the fragments at m/z 143 ($C_5H_7N_2O_3$), 126 ($C_5H_4N_2O_2$), 86 ($C_3H_4NO_2$), 83 (C_3HNO), 67 (C_3HNO), 59 (C_2H_5NO), and 43 (CHNO).

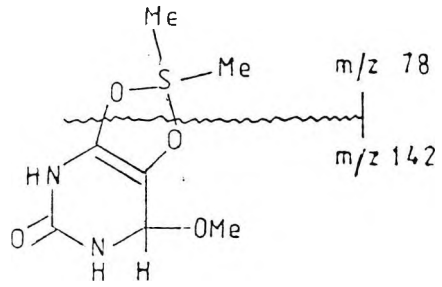
The evidence for the sulphone portion of the molecule was provided by the fragments at m/z 79 (CH_3O_2S), 78 (C_2H_6OS), 63 (CH_3OS), 62 (C_2H_6S), and 48 (S=O). Although a peak at m/z 94 was detected, its value differed too much from the value for dimethyl sulphone (C_2H_6OS). The dimethyl sulphone fragment (7.18) was subsequently detected in a second spectrum at 94.0145 (1.41%), confirming $C_2H_6O_2S$ (requires 94.0089) as the formula. The second spectrum showed that a peak at 142.0514 could be assigned to $C_5H_6N_2O_3$ (requires 142.0378) (7.19). In addition to the pseudo--molecular species, the fragments at m/z 176 ($C_6H_{10}NO_3S$), 158 ($C_5H_6N_2O_4$) (7.20), 146 ($C_5H_8NO_2S$) and 119 ($C4H_6O_2S$) demonstrate that attachment of the dimethyl sulphone to the uracil moiety.



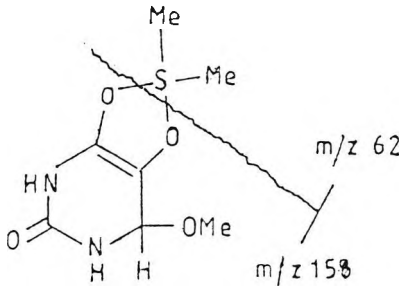
(7.17)



(7.18)



(7.19)



(7.20)

The spectrum also contained peaks which could be related to the presence of the dehydro- product (7.12) at m/z 146, 144, 133, 119, 105, 104, 103, 101, 79, 78, 66, 65, 66, 62, 61, 53 and 43. Many of the peaks were common to both products (7.17 and 7.12). The peaks at m/z 33 (MeOH+H), 32 (MeOH) and 31 (CH_3O) were associated with free methanol which suggests that the dehydro- compound may, in part, arise as an artefact of the EI-MS process.

The presence of the two species in the isolated material was obtained from the ^1H n.m.r. The spectrum in dmso-d_6 was of a very poor quality. It was found that the addition of a small quantity of D_2O to sample gave a large improvement in the quality of the spectrum, which suggests that the sample may be soluble in water.

The methoxy- compound (7.17) was characterised by two singlets of nearly equal height at 2.5δ and 2.55δ. A sharp singlet at 3.65δ was assigned to the methoxyl group (at C-4) and a broad singlet at 5.3δ was assigned to the H-4 proton of S,S-dimethyl-4-methoxy--3,4-dihydro-1H-1,3,2-dioxathiolo[4,3-d]pyrimidin-2-one (7.17).

The dehydro- compound was characterised by a sharp singlet at 8.0δ for the H-4 and a sharp singlet at 2.65δ for the two methyl groups.

The nature of the S-methyl groups is consistent with the dioxathiolopyrimidine structure. In the dehydro- compound the two S-methyl groups were likely to have the same chemical shift since there exists a plane of symmetry for the molecule, hence they would experience the same magnetic environment. For the methoxy- compound, the methoxyl group will break this symmetry so that one side of the plane has a methoxyl group whilst the other side has a hydrogen. Thus, the two S-methyl groups, which similarly occur on either side of the ring-plane, experience different environments.

7.4.5.1 FAB spectrum.

A sample of the putative adduct (7.17) was analysed by FAB spectrometry (Table 7.7). The spectrum gave peaks which were consistent with the presence of (7.17) and (7.12).

The dioxathiolo- adduct was characterised by the pseudomolecular species [P1] at m/z 221. A complex with potassium was observed at m/z 259, but no corresponding sodium complex was observed at m/z 243. The commonly expected complexes with the matrix material were visible in the spectrum but have been left out of the table because their low intensities (<5%). A higher complex containing sodium and dmso was observed at m/z 321. Further evidence of the adduct was provided by the fragments at m/z 177, 161, 159, 158, 103 and 231, which represents a complex of the matrix material and the fragment at m/z 123.

	Assignment	
	Methoxy- product	Dehydro- product
m/z		
375		M2+P-H_2
367		M2+Na+2dmso
321	M1+Na+dmso	
296		P2-H_2+mat
289		M2+Na+dmso
267		P2+dmso
259	M1+K	
239	2mat+Na	
231	m/z123+mat	
227		M2+K
221	P1•	
211		M2+Na
209	mat+Na+dmso	
189		P2••
188		M2
187		M2-H, P2-H_2
179	2dmso+Na	
177	M1-HNCO	
161	M1-HN=CHOMe	M2-HCN
159	P1-Me_2S	
158	M1-Me_2S	
149		M2+Na-Me_2S
141	mat+MeOH+H	
133		M2-HCN-CO
131	mat+Na	
123	$C_4H_6O_2S$+H	
119		P2-HCN-CO
109	mat+H	
103	P-$C_4H_4O_2S$	
101	dmso+Na	
78	dmso	
63	dmso-Me	

• P1=M1+H

•• P2=M2+H

Table 7.7 Assignment of FAB peaks for the diazo-dmso adduct

The evidence for the dehydro- product was provided by the molecular ion [M1], pseudomolecular ion [P1], and the [P1-H_2] species. In addition, the expected higher complexes with the common cations, sodium and potassium, were also identified. Complexes with dmso for the pseudomolecular ion (m/z 267) and for the sodium complex was observed (m/z 367).

Various fragments were identified and the loss of dimethyl sulphide seems to be common to the methoxy- and dehydro- products. A peak at m/z 161 (M1-HNC) is consistent with the 1,6-position in the pyrimidone portion being unsaturated. The peak at m/z 119 was assigned to a species formed by the loss of HNC and CO. The peak at m/z 109 is better explained as the pseudomolecular ion of the matrix material rather than by the species [P1-H_2O-Me_2S].

The base peak at m/z 131 (100%) was assigned to the sodium complex of the matrix material. The peaks at m/z 78 and 63 were associated with dmso.

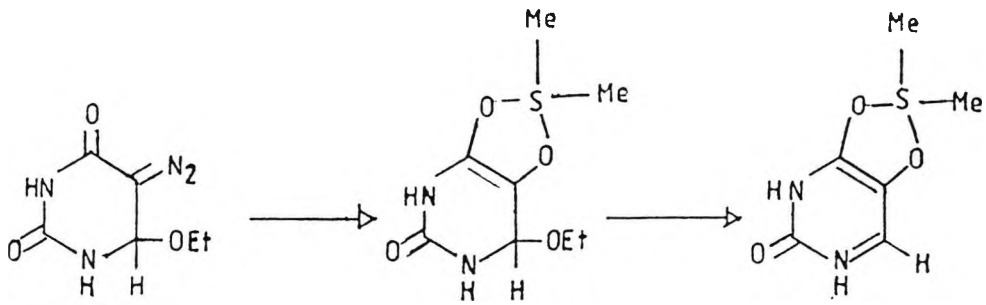
7.4.6 5-Diazo-6-ethoxy-1,6-dihydrouracil with dmso.

5-Diazo-6-ethoxy-1,6-dihydrouracil was dissolved in warm dmso/benzene (2:18) to give a yellow solution. A small quantity of rhodium acetate was added to the stirring solution and the solution heated with reflux for 48h. The crude material was purified by preparative t.l.c. using dichloromethane/methanol (90:10) as the mobile phase, the major band having R_f=0.48.

The ^{1}H n.m.r. of the solid in methanol-d_4 gave three signals; a ^{1}H singlet at 7.6δ, which was assigned to the C-6 proton in the dehydro- product (7.12), a singlet at 4.8δ (OH), and a 6H singlet at 2.7δ, which was assigned to the two methyl groups. The upfield N-3 proton was not visible having been exchanged with deuterium in the solvent. The magnetic equivalence of the two S-methyls is consistent with the high degree of symmetry in S,S-dimethyl-1,3,2--dioxathiolopyrimidine (7.12). The spectrum also agrees with the earlier identification in the isolated material from the reaction of the methoxy diazouracil (6.4) with dmso.

The EI mass spectrum did not give a molecular ion for either the dehydro- product (7.12) or the expected ethoxy- compound (7.21). The mass spectrum gave peaks that were consistent with fragments of the dehydro- product. In keeping with members of the dioxathiolo--pyrimidines, a peak for dimethyl sulphone at m/z 94 was observed.

Other peaks associated with the dehydro- compound were observed at m/z 81, 79, 78, 77, 67, 63, 62, 61.98, 61, 54, 43, 40, 39. The absence of peaks at 47 (EtoH+H), 46 (EtOH) and 45 (C_2H_5O) were consistent with the absence of the ethoxy- product (7.21). The loss of ethanol might have resulted from heating the compound in benzene at reflux temperature.



(6.5) (7.21) (7.12)

Scheme 7.21

7.5 Reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with THF.

7.5.1 In the presence of ethanol.

From the solubility studies, a possible alternative solvent was found to be tetrahydrofuran. The 6-methoxy- derivative is reasonably soluble in the THF and therefore two experiments were carried out in this solvent.

The first reaction was carried out with 5-diazo-6-methoxy-1,6--dihydrouracil (6.4) and ethanol in THF. The product was isolated by removing the excess solvent on a rotary evaporator, giving a waxy compound which was characterised in the infrared by the absence of the diazo stretch. The sample was examined by ^1H n.m.r. and was found to have a very complicated spectrum, with two sets of methylenes around 4.25δ and another methylene envelope between 3.20-3.90δ. Further downfield, another three groups of multiplets were observed around 2.30, 1.85 and 1.15δ.

From the spectrum, it was realised that there were a high number of deshielded methylenes of a chemical shift similar to the methylenes of diethyl ether and ethanol. Comparison with THF showed that the compound isolated did not contain THF as an impurity. It is likely that a reaction with the solvent THF took place.

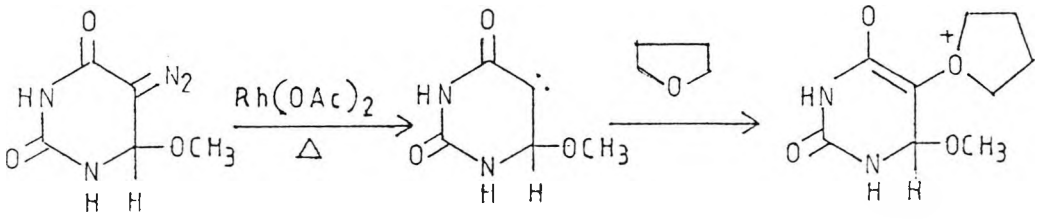
7.5.2 In the presence of 2,3-dihydrofuran.

The second reaction involved the reaction of the same diazo compound (6.4) dissolved in THF with 2,3-dihydrofuran present and rhodium acetate catalyst added.

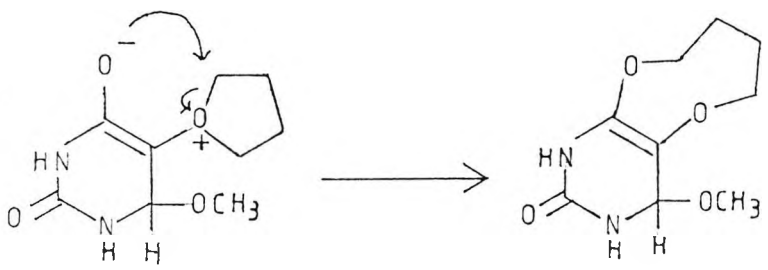
The ^1H n.m.r. of the isolated waxy product was similar to the product observed for the above reaction. The spectrum was characterised by a sharp singlet at 3.76δ rising above a widely extended methylene envelope at around 3.64-4.32δ. The singlet was assumed to be the methoxyl group at C-6.

Thus, both attempts to trap the rhodium-carbenoid with well known trapping agents (alcohol or olefin), which should have led to the 5-alkoxy or 5-spirocyclopropyl adducts, yielded similar, complex product mixtures. In both cases, the n.m.r. spectrum indicated extensive presence of the $-CH_2-O-$ function, consistent with the addition reaction of the starting material with the THF solvent.

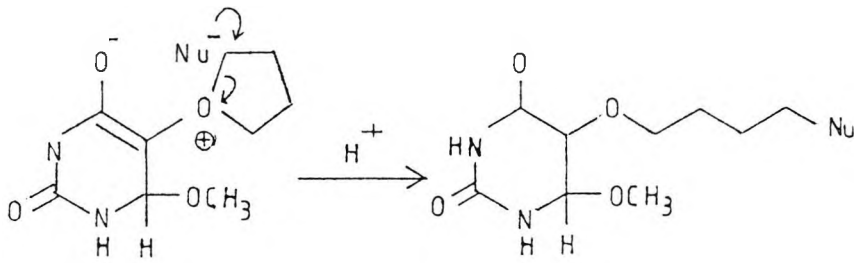
A possibility is that the putative carbene intermediate reacted with an oxygen lone pair to form an oxonium ylide (Scheme 7.22), a reaction analogous to the formation of sulphur ylides has been previously reported [21]. The ylide then undergoes several possible reactions in which there is a ring opening of the tetrahydrofuran ring by a nucleophile (Scheme 7.23 and Scheme 7.24).



Scheme 7.22



Scheme 7.23



Scheme 7.24

It can be concluded that THF, like dmso, is unsuitable as a solvent in which to carry out the reactions of the diazouracils.

7.6 Reaction with ammonium hydroxide.

5-Diazo-6-methoxy-1,6-dihydrouracil was dissolved in methanol. To the stirring solution, ammonia solution was added dropwise. The reaction was left stirring at ambient temperature for 48h and solvent was removed on a rotary evaporator to afford a bright yellow solid.

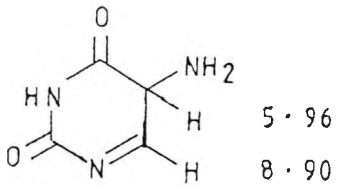
The compound was characterised in its i.r. spectrum by the absence of any absorption between 2500 and 1800cm^{-1}. This indicated the absence of a diazo group. A strong absorption at 1710cm^{-1} was assigned to the uracil carbonyl group.

The ^{1}H n.m.r. spectrum showed two doublets at 5.96δ and 8.90δ, both with a coupling constant of 9.0Hz. One very broad N-H peak was detected at 12.0δ, integrating for one proton . A peak integrating for two protons was observed at 5.02δ and was attributed to an amino group. A broad singlet at 3.40δ was attributed to water, which had not been removed or was a contaminant in the deuterated solvent (dmso-d_6). The doublet at 8.9δ was assigned to the H-6 proton. The absence of a sharp singlet around 3.8δ for the methoxyl group and the absence of one of the N-H protons led to the deduction that both N-1 and C-6 were unsaturated. The absence of the methoxy signal suggested that the product was a C_4-compound.

The i.r. spectrum suggested that a carbonyl is positioned between the two nitrogens giving an imide since the expected value for the conjugated carbonyl would be around 1685cm^{-1} whilst that for

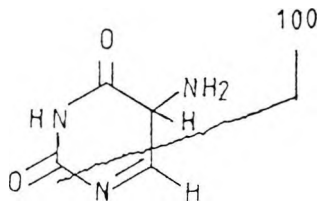
the imide lies at 1710cm^{-1}. Thus, if a carbonyl exists at C-2, then the position of the unsaturated bond must in the 1,6-position.

The mass spectrum identified a C_4-species at m/z 127. The formula $C_4H_5N_3O_2$ was confirmed by high resolution mass spectrometry. A pseudomolecular ion [M+H] at m/z 128 was also observed. With C-6 containing only one hydrogen, the other hydrogen had to be positioned at C-5 to observe such a large coupling constant. The only positions available for the amino group and the remaining oxygen would be the C-5 and C-4 positions respectively. Thus, the proposed product is 5-amino-3H,5H-pyrimidine-2,4-dione (7.22).

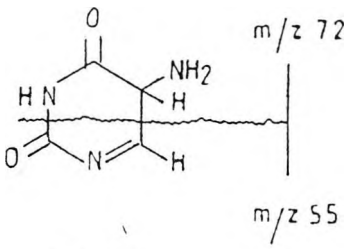


(7.22)

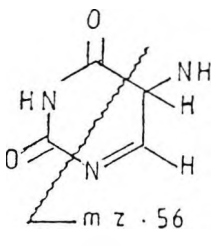
Support for the structure can be found in the high resolution EI spectrum. A peak at m/z 100 was identified with the loss of HCN (7.23) and a peak at m/z 84 suggests a loss of HNCO, typical of many uracil compounds. The loss of CO from the molecular ion, another common feature of uracils, to give a peak at m/z 99 was also observed.



(7.23)



(7.24)



(7.25)

The fragments $C_2H_4N_2O$ (m/z 72) (7.24), $C_2H_4N_2$ (m/z 56) (7.25), C_2HNO (m/z 55.06), and $C_2H_3N_2$ (m/z 55.03) can all be related to the proposed structure.

The formation of the product can be readily explained by a simple insertion of a carbenoid intermediate into the N-H bond of ammonia.

7.7 Reactions with carbonyl containing compounds.

7.7.1 Acetone.

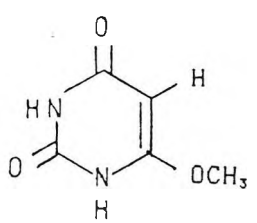
7.7.1.1 Preparation.

The 5-diazo-6-methoxy-1,6-dihydrouracil was stirred in a large excess of acetone. The mixture was heated with reflux and rhodium acetate catalyst added. The mixture was left stirring for 48h and allowed to cool to ambient temperature. The glistening cream coloured crystals were filtered from the solution.

The melting point of the product was 212°C. The compound was characterised in its i.r. spectrum by the absence of the diazo stretch around 2130cm^{-1}. The absorption bands around at 1760 and 1720cm^{-1} respectively were assigned to the imide and the amide carbonyls. The strong absorption at 1670cm^{-1} was consistent with an enol double bond (1690-1650cm^{-1}).

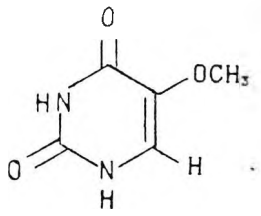
The ^1H spectrum was quite simple, being composed only of singlet peaks and devoid of any signals in the region 0-3.0δ. The possibility that the product was a acetone adduct was discounted because of the absence of any C-methyls signals. Although no N-H peaks were observed in methanol-d_4, these peaks were observed when the solvent was changed to dmso-d_6 and were located at 10.64δ (bs, 1H, H-3) and 9.5δ (bs, 1H, H-4). A 3H singlet at 3.66δ was assigned to the methoxyl group and the 1H singlet at 6.36 δ assigned to the olefinic proton.

In the starting material, the methoxyl group was positioned at C-6 and consequently, 6-methoxyuracil (7.26) was initially advocated as the product. However, applying the Tobey-Simon rules for olefinic proton chemical shifts to 5-methoxyuracil (7.27) and 6-methoxy--uracil, the n.m.r. spectrum suggests that the material isolated is the 5-methoxyuracil.



(7.26)

	gem CONH	*trans* NHCO	*cis* OR	
δ_{α} = 5.25	+ 1.37	+ -0.72	+ -1.07	= 4.83



(7.27)

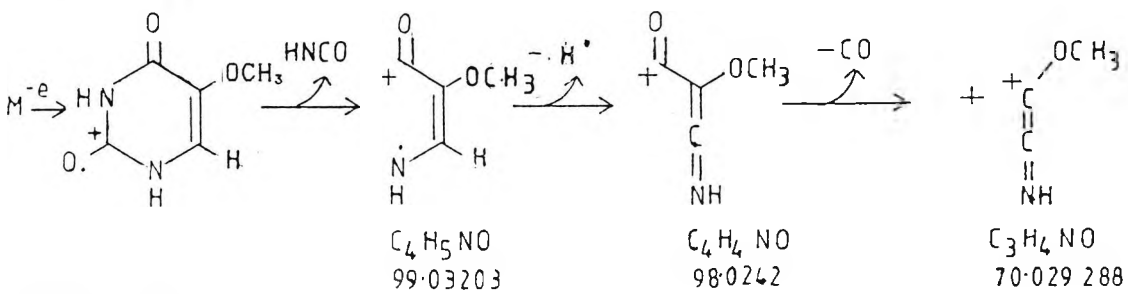
	gem NHCO		*trans* CONH		*cis* OR		
δ_H = 5.25	+ 2.08	+	0.46	+	-1.07	=	6.72

7.7.1.2 EI spectrum.

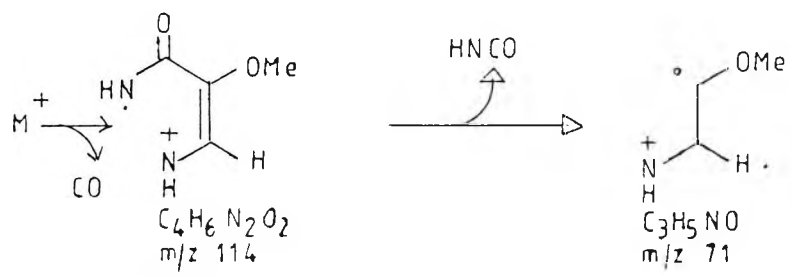
The spectrum of 5-methoxyuracil was characterised both by the molecular ion (m/z 142) and the pseudomolecular ion (P, m/z143), which had an intensity which was 8% of the molecular ion. A weak intensity peak representing the species [M-H] or [P-H_2] was observed at m/z 141.

The fragmentation pattern was very similar to most uracils in that species representing the loss of HNCO or CO from the molecular ion were readily observed. The absence of the starting material was indicated by the absence of peaks at m/z 140, 139, and 138, and confirmed the lack of a diazo group, as indicated by the i.r. spectrum.

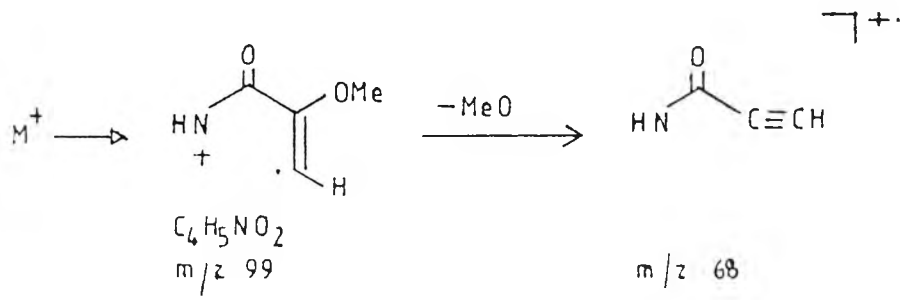
Some simple fragmentation pathways can be proposed (Schemes 7.25-7.27).



Scheme 7.25



Scheme 7.26



Scheme 7.27

The unavailability of metastable ion observations on the mass spectrometer used means that the proposed pathways remain speculative.

7.7.1.3 ^{13}C n.m.r.

The spectrum of the compound showed five peaks, excluding the solvent and TMS peaks, which was consistent with the product having five carbon atoms. The two most downfield peaks were assigned to the carbonyls of the uracil ring and the most upfield signal was assigned to the methoxyl group (Table 7.8). The remaining two signals were assigned to the olefinic carbons of uracil.

decoupled spectrum	EQ Spectrum	assignment	Literature [21]
162.5	162.8	C-4	160.8
153.9	154.0	C-2	150.7
139.8	-	C-6	122.6
114.7	114.8	C-5	135.9
61.8	-	MeO	MeO*
			$J_{C-6,H}$=180Hz**

* Chemical shift for the methoxy group not quoted

**Coupled spectrum not given

Table 7.8 13C n.m.r. for 5-methoxyuracil

7.7.1.4 Discussion.

The chemical shift of H-5 in 6-methoxyuracil in dmso-d_6 was reported to be 4.94δ [22], which is in good agreement with the value calculated (4.83δ) by the Tobey-Simon rules. In TFA, the

reported chemical shift for H-5 was further downfield at 5.63δ [23]. By contrast the observed chemical shift for the olefinic proton in the product occurred further downfield at 6.52δ. The predicted chemical shift of H-6 (6.72δ) in 5-methoxyuracil was in close agreement. Thus, the ^{1}H n.m.r. data suggests that the product is not 6-methoxyuracil but 5-methoxyuracil.

The proton decoupled ^{13}C n.m.r. spectrum gave chemical shifts sufficiently close to those given in the literature [22] to suggest that the product is 5-methoxyuracil. However, the assignment of C-5 and C-6 remains unresolved. In literature, the ^{1}H n.m.r. and ^{13}C coupled spectrum were not provided and consequently their assignments cannot be verified. In our work, the assignment of C-5 and C-6 was obtained from the EQ spectrum.

In the literature of methoxyuracils [22-28], direct comparisons of those results cannot be made because of the absence of complete data for either 5-methoxy- or 6-methoxy- uracil.

7.7.2. Benzophenone.

7.7.2.1 Benzene as the solvent.

The diazo compound was suspended in hot benzene with stirring. To the stirring solution, benzophenone dissolved in benzene was added. Rhodium acetate catalyst was added and the mixture heated with reflux for 1h. The solution was allowed to cool to ambient temperature and left stirring for a further 72h. The solid was filtered from the solution and analysed by i.r. spectroscopy, which showed two peaks at 2120 and 2160cm^{-1} associated with the presence of two diazo compounds. The strong absorptions at 1760, 1720 and 1670cm^{-1} indicated the presence of the uracil moiety.

It was deduced that no significant reaction had taken place. The result of the hot benzene was to remove methanol from part of the methoxy diazouracil to leave two species, *viz.*, 5-diazouracil and the original diazo starting material. The failure of the reaction was attributed to the lack of solubility of the diazo compound in a benzene/benzophenone solution.

7.7.2.2 DMF as the solvent.

A further attempt was made to react benzophenone with 5-Diazo-6-methoxy-1,6-dihydrouracil In order to improve the solubility of the components, DMF was used in place of benzene. DMF was chosen because THF and dmso have shown that they are liable to react with the diazo when heated in the presence of the catalyst.

Both the diazo starting material and benzophenone were dissolved in DMF and the stirred solution heated with reflux. Rhodium acetate catalyst was added to the hot solution and the mixture left stirring for 1h. The solution was then allowed to cool to ambient temperature and left stirring for a further 72h. The solution was condensed on a rotary evaporator and the resultant oil/gum left to stand for 24h, whereupon a crystalline material was observed in the oil. The oil was triturated in dry ether, which further precipitated out a white solid.

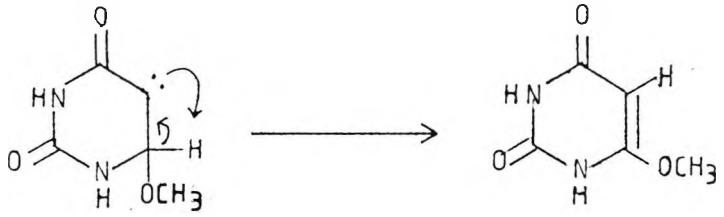
The i.r. spectrum indicated that the product was not a diazouracil because of the absence of the diazo absorption. The presence of the carbonyl peaks pointed to the product being a uracil of some description. Analytical t.l.c. confirmed this product to be identical to 5-methoxyuracil obtained in the reaction with acetone. The EI mass spectrum was sufficient to identify the compound as 5-methoxyuracil, with the observation of both the molecular ion (m/z 142) and the pseudomolecular ion (P, m/z 143). The same fragments were also observed, i.e. m/z 114 (M-CO), 99 (M-HNCO), 71((M-HNCO)-CO), 68 (C_3H_2NO), 56 (M-2HNCO).

The failure of benzophenone, in benzene, to produce the desired dioxalopyrimidine or even the spirooxiranouracil serves to demonstrate the problems of poor solubility. With poor solubility, the product is formed through intramolecular rearrangement of the intermediate species rather than through intermolecular reaction.

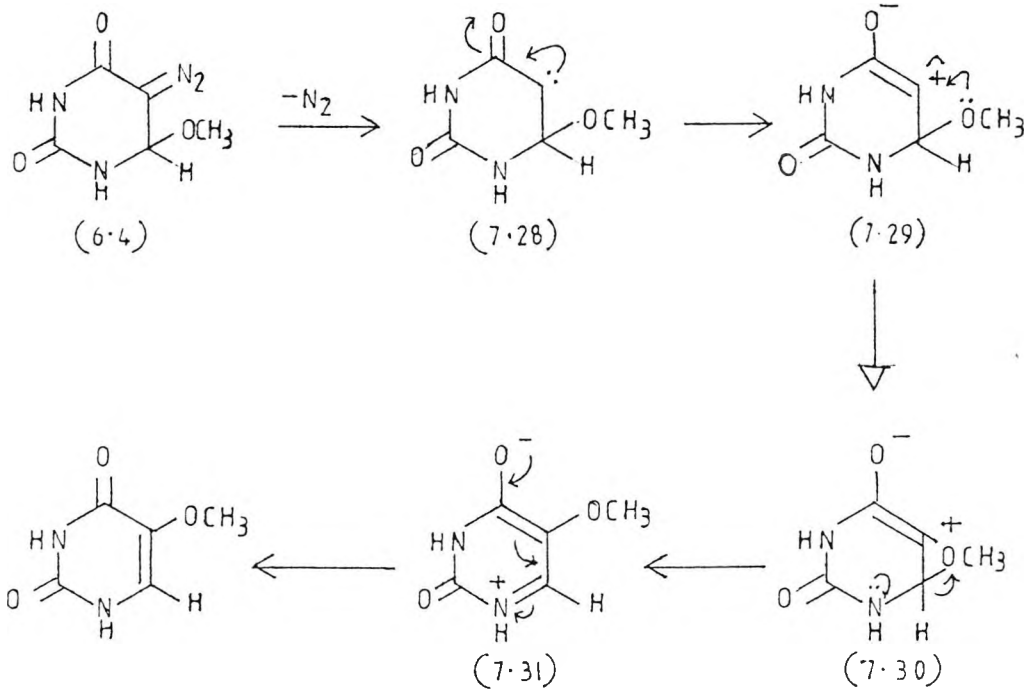
7.7.3 Discussion.

One reported reaction of ketocarbenes was the 1,2-shift of an α-hydrogen [29,30]; if this reaction had occurred, then resulting product would have been 6-methoxyuracil (Scheme 7.28). However, the formation of 5-methoxyuracil in the previous reactions indicates that there was a migration of the methoxy group. A mechanism for the rearrangement of the intermediate (7.29) can be proposed, in

which an oxirano intermediate (7.30) is formed. The involvement of similar species, *i.e.* an oxiren intermediate, in some ketocarbene reactions has been demonstrated [29,30].



Scheme 7.28



Scheme 7.29 Formation of 5-methoxyuracil

7.7.4 Suggestions for further work.

Although acetone did not succeed in producing the dioxalo--pyrimidine, the potential for 1,3-dipolar addition reactions should be explored further. An alternative source of carbonyls would be to react the diazo compound with aldehydes. Preliminary studies with acetaldehyde seem promising. The i.r. spectrum of the crude material shows that the diazo starting material will react in acetaldehyde. The ^{1}H spectrum indicated the crude material to contain several products none of which being 5-methoxyuracil. Signals in the n.m.r.

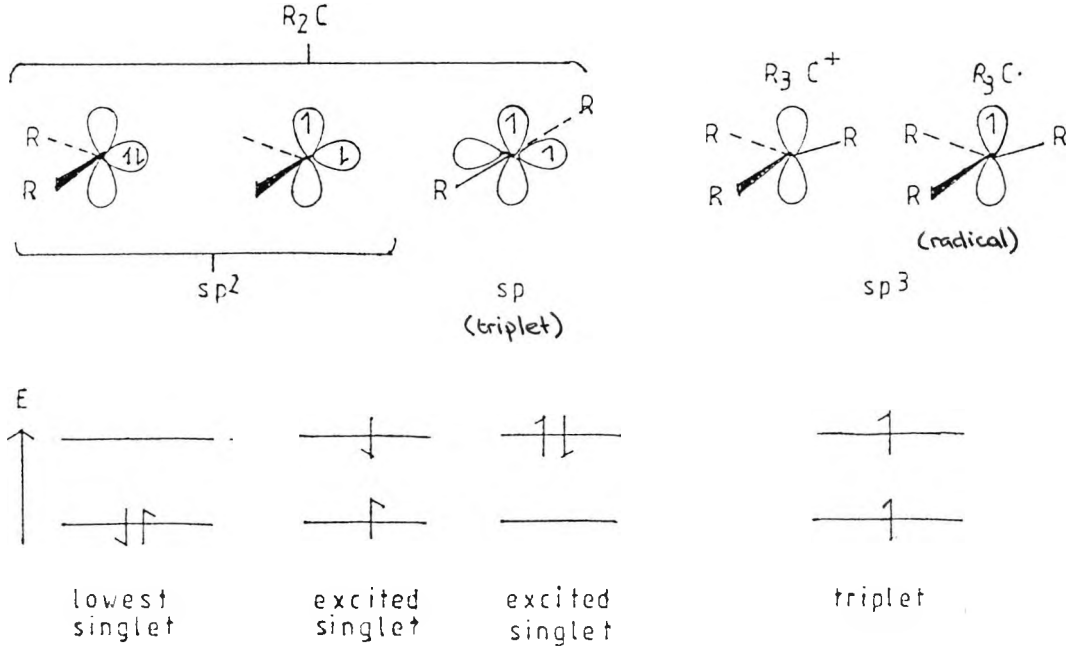
of the products included multiplets between 6.0 and 1.0δ. The mass spectrum of the crude material also indicated that 5-methoxyuracil formation is not significant, with an absence of a peak at m/z 142. At present the problem of purification of the crude product is being tackled.

7.8 Reactions with olefins.

7.8.1 Introduction.

7.8.1.1 Carbene structure.

Much of the chemistry of diazo compounds may be explained by supposing the reacting species to be a carbene. A carbene is a neutral bivalent species, which has two covalent bonds to other groups and possesses two non-bonding orbitals containing two electrons. These may be paired or unpaired with respect to their quantum spin number. Carbenes can be described with reference to the distribution of two non-bonding electrons (Scheme 7.30). If the electrons are paired, *i.e.* having antiparallel spins, the carbene is a singlet. The carbene is referred to as a triplet when the electrons are unpaired. Having less than the usual tetravalency, the carbene behaves like a very strong electrophile and its reactivity means that it exists only as a transient intermediate.



Scheme 7.30

As a general rule, carbenes formed by photolysis tend to give indiscriminate reactions. By comparison, the decomposition of diazo compounds by thermal means leads to a less energetic carbene species which shows more selectivity in its reactions. The disadvantage of thermal decomposition is that in going from the more stable diazoalkane to the reactive carbene other side reactions involving the diazoalkane rather the carbene might take place [31]. Consequently, an obvious rationale would be to lower the activation energy for formation of the active species thus reducing the possibility of the diazoalkane participating in side reactions. This reduction of activation energy might be achieved by catalysis. A common process is the addition of a suitable metallic catalyst. It should be noted that when using a catalyst the reactive species may not be the simple carbene but rather a metal-carbenoid complex [32].

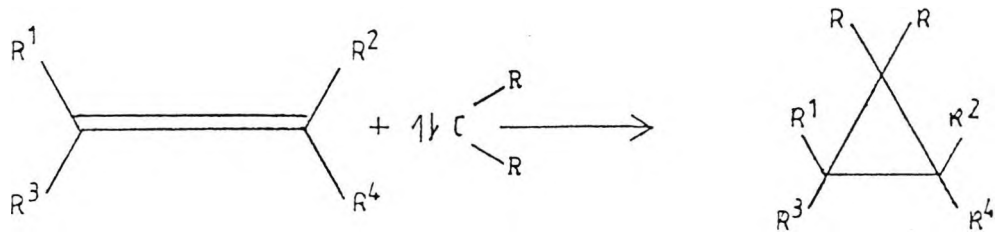
7.8.1.2 Spectra of carbenes.

Despite their short lifetimes, it has been possible to obtain spectral evidence of the presumed carbene intermediates formed from the decomposition of diazoalkanes. The putative carbene has usually been generated by flash photolysis and its spectrum taken. The visible and u.v. spectra of photolysed diazomethane have been recorded [33] and the microwave spectrum of difluorocarbene has been reported [34]. Most of the techniques involve the generation of the carbene in an inert matrix at low temperatures in order to isolate the highly reactive intermediate. The i.r. spectrum of fluorocarbene in such a matrix has also been reported [35]. The unpaired spin found in triplet state carbenes, which have been generated in a crystalline matrix or in a rigid glass has been explored with e.s.r. [36,37]. The identification by various types of spectroscopy of a large number of carbenes has been reviewed in depth [38].

7.8.1.3 Cycloadditions.

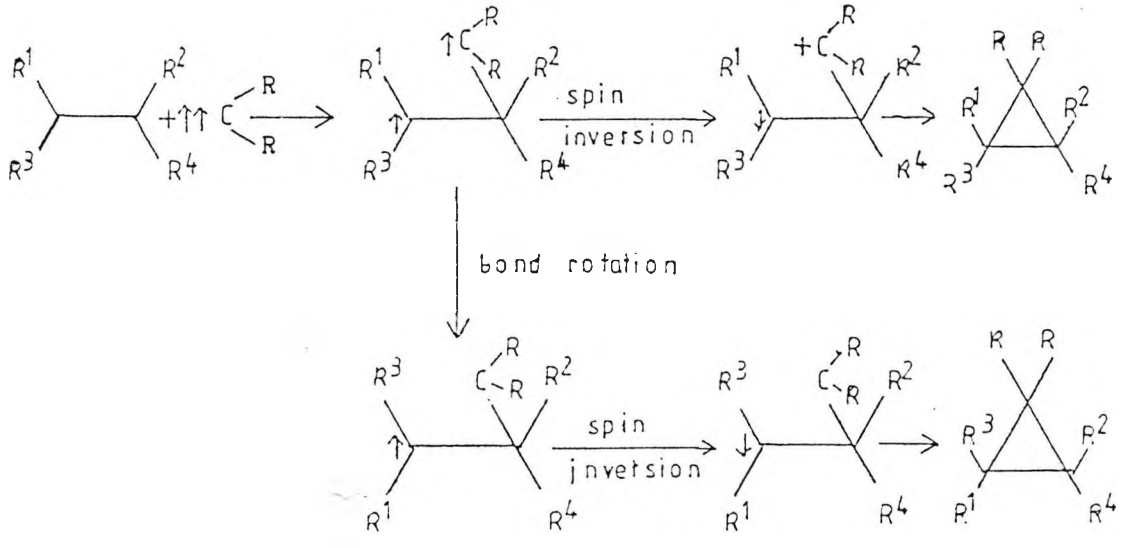
Carbenes will readily react with electron rich molecules such as olefins to yield cyclopropanes. The mechanism of the cyclo-addition has been suggested to be dependent on the nature of the intermediate carbene. A guide to the numerous examples in literature concerning the preparation of cyclopropanes from carbenes or

carbenoids has been given elsewhere [39]. Provided that the products of the cycloaddition were themselves not prone to rearrangement, then a totally stereospecific addition was regarded as the result of a concerted reaction. It was rationalised that such concerted reactions involved the singlet carbene (Scheme 7.31).



Scheme 7.31

An alternative species was proposed for the observation of cycloadditions in which stereospecificity was not observed. It was suggested that the reactions which proceeded with partial inversion of configuration were the result of reaction with the triplet carbene [40-42]. It was concluded that an intermediate biradical was formed and that rotation occurred before spin inversion allowed the completion of the cycloaddition (Scheme 7.32). A more sophisticated treatment based on orbital symmetry has been offered [43-45].

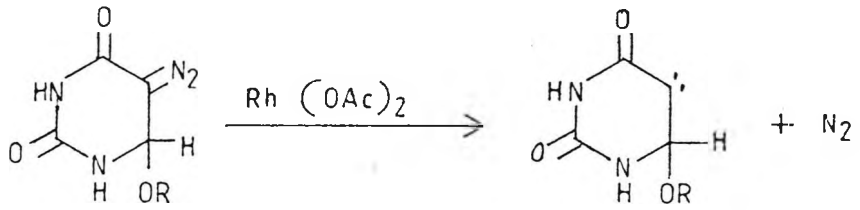


Scheme 7.32

It had been demonstrated that with copper salts or finely divided copper, the carbenoid species formed behaves very much like the putative carbene formed by other means [46-52]. Rhodium acetate

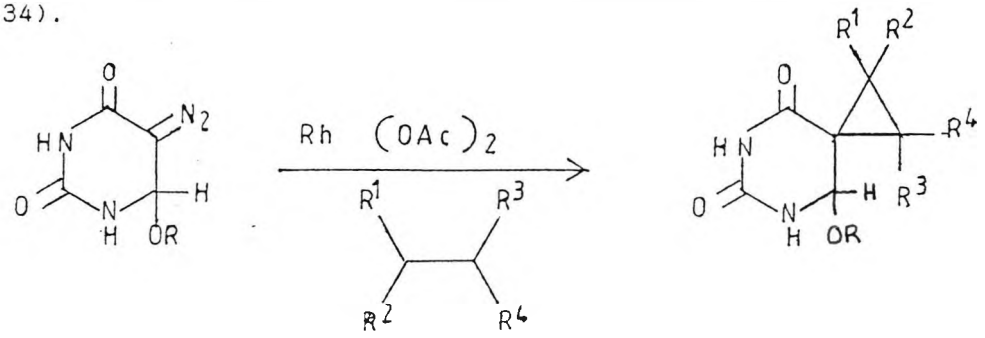
also appears to behaves in a similar manner. Thus, the catalyst would act on diazo compounds with the formation of a carbenoid and the action of this carbenoid could be explained by treating the reactive intermediate as a simple carbene.

By analogy, it might be expected that the treatment of the diazouracil with rhodium acetate would produce such a reactive intermediate, which for the purpose of simplicity might be represented by a carbene structure (Scheme 7.33).



Scheme 7.33

The putative intermediate might be expected to react with a double bond and lead to the formation of spirocyclopropanes (Scheme 7.34).



Scheme 7.34

In principle, the reaction with olefins could be extended to other electron rich systems. For example, compounds containing carbonyls, thiocarbonyls, imino groups, azo groups or nitriles [53].

In order to examine the nature of the intermediate formed by the decomposition of the diazouracils, work was undertaken using 5-diazo-6-methoxy-1,6-dihydrouracil as this compound was easy to prepare and readily purified producing a stable crystalline material which was easy to handle. In addition, the methoxyl group on the molecule would provide an identifying label in the ^1H n.m.r. spectra to demonstrate the presence of the uracil moiety.

Rhodium acetate was chosen as the catalyst because it is known to be a superior catalyst to copper. This was demonstrated by when an attempt to react copper cyanide with 5-diazo-6-methoxy-1,6--dihydrouracil showed, in the i.r. spectrum, that the diazo moiety had not been significantly altered. Addition of rhodium acetate resulted in the removal of the NN stretch in the i.r. spectrum of the reaction product.

7.8.2 Results and discussion.

7.8.2.1 Reaction with selected olefins.

The difficulties of finding an inert solvent in which to carry out the reactions limited the choice of olefins for reaction. It was proposed that suitable candidates for reactions would be olefins which existed either as a liquid at room temperature or as a low melting point solid.

7.8.2.2 Styrene.

One of the readily available olefins which satisfy the criterion of being a liquid at ambient temperatures is styrene with its melting point -31°. Thus, it was decided that reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with styrene would be a good starting point.

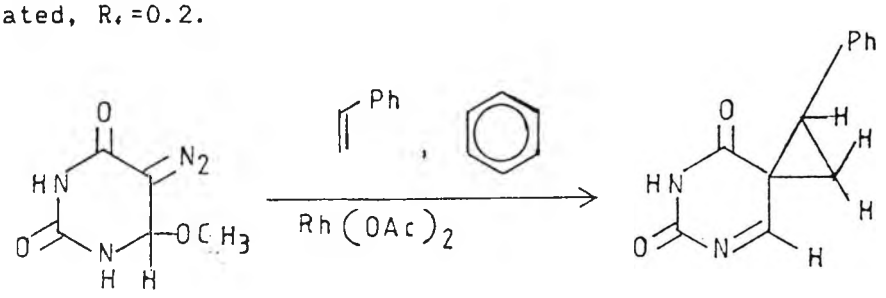
7.8.2.2.1 Neat styrene.

5-diazo-6-methoxy-1,6-dihydrouracil was warmed in styrene at 30°C with constant stirring. A small quantity of catalyst was added and the mixture left stirring overnight. After 14h, the solution had completely solidified to give a green plastic solid. This initial attempt failed owing to the ease with which the styrene polymerised.

7.8.2.2.2 Styrene in benzene.

It was decided that the problem of polymerisation might be avoided if the styrene in the reaction was present in high dilution. Thus, styrene was mixed with the relatively more inert benzene. The reaction mixture was warmed to 30°C and rhodium acetate catalyst

added (Scheme 7.35). The reaction was left stirring at ambient temperature overnight. The solvent benzene and the unreacted excess styrene was removed on a rotary evaporator. An attempt to purify the compound with preparative t.l.c. using silica plates and dichloromethane/methanol (82:15) resulted in a broad band being isolated, R_f=0.2.



Scheme 7.35

The i.r. spectrum of the product, a yellow solid, contained no diazo absorption peak at 2120cm^{-1}. The spectrum also showed strong absorptions at 890, 790, 770 and 750cm^{-1} which were attributed to the material possessing an aromatic group. The i.r. showed two strong absorptions at 1720 and 1680cm^{-1}, which were indicative of the uracil portion in the molecule.

The compound was identified by its ^{1}H n.m.r. as 5-methoxyuracil. A sharp 3H singlet at 3.88δ (methoxyl group); a 1H singlet at 6.56δ (C-5 proton), and the N-3 and N-1 protons both giving broad 1H singlets at 10.6δ and 9.4δ. The finding was confirmed by the EI spectrum which was characterised by the peaks at m/z 143, 142 and 141, representing the pseudomolecular species, the molecular ion and the [M-H] species of 5-methoxyuracil.

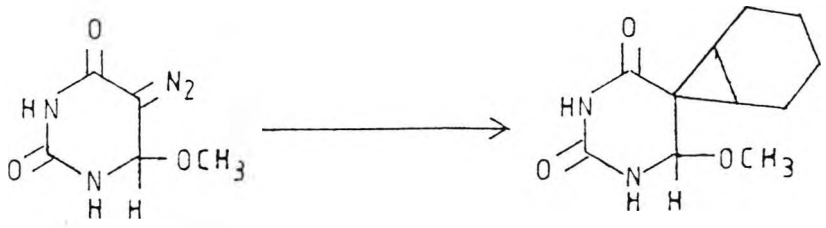
7.8.2.3 Cyclohexene.

7.8.2.3.1 Preparation.

Reaction with cyclohexene might be expected to produce the 5-spirobicyclo[4.1.0]heptyluracil (Scheme 7.36).

5-Diazo-6-methoxy-1,6-dihydrouracil was suspended in hot cyclohexene and rhodium acetate catalyst added. The mixture was left under reflux for 48h. The formation of a white precipitate was observed and on standing for a further 24h at ambient temperature, the precipitate was observed to have redissolved. The excess

cyclohexene was removed on a rotary evaporator to afford a bright yellow oil. A sample of the material was purified by preparative t.l.c. using dichloromethane/methanol (95:5) as the mobile phase. The major band, R_f=0.73, was isolated as a yellow oil.



Scheme 7.36

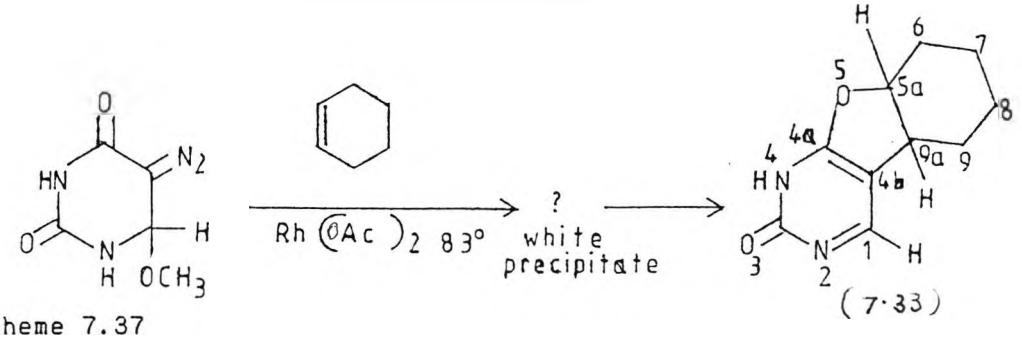
The i.r. spectrum of the product was characterised by the loss of the strong absorption at 2120cm^{-1}. The carbonyl region of the spectrum was surprisingly sharper and less spread out than in other diazo compounds. The spectrum was characterised by a strong absorption only at 1680 and 1660cm^{-1} (c.f. the more typical 1720, 1680cm^{-1}). The peak at 1680cm^{-1} assigned to a carbonyl absorption and the peak at 1660cm^{-1} suggested the presence of a enol type double bond. The carbonyl absorption was more typical of a urea or an amide than imide.

The fingerprint region of the spectrum was dissimilar to the fingerprint region in cyclohexene as to confirmed that this oily material was a derivative of the uracil rather than material suspended or dissolved in cyclohexene.

The ^{1}H n.m.r. confirmed the absence of cyclohexene as the three characteristic sharp broad singlets at 5.56δ, 1.94δ and 1.56δ were absent. The absence of a signal for the methoxy group indicated the product contained an unsaturated uracil moiety. The spectrum was characterised by a broad 1H singlet at 5.8 8δ (H-1) two sets of multiplets (doublet of triplets) at 7.2δ (J=10.6Hz, J=4.5Hz, H-5a) and 6.2δ (J=10.6Hz, J=2.5Hz, H-9a), each integrating for 1H. A 1H broad singlet was observed at 5.92δ. A complex 8H multiplet about 2.4-1.8δ was the most prominent feature and was assigned to the methylene protons of the cyclohexane portion. The high value for the coupling between these two sets of signals suggests that the dihedral angle between them is close to 0° or that the angle is 180°. The chemical shifts of the observed multiplets are

exceptionally high and suggest that there must be some secondary deshielding effect present.

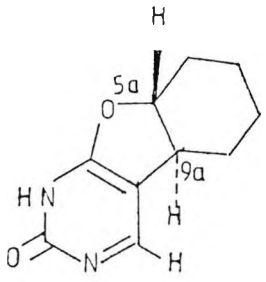
The observed ^1H n.m.r. spectrum is more consistent with a structure in which the cyclohexene has reacted with the oxygen of the C-4 carbonyl and with the C-5 position (Scheme 7.37). A finding which would agree with the observation in the i.r. spectrum of a loss of one of the imide characteristic.



Scheme 7.37

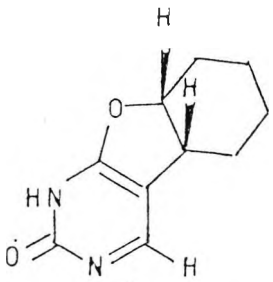
The proposed alternative structure to the spirocyclopropane would also be capable of showing diamagnetic deshielding. Instead of a carbonyl being responsible, the deshielding could equally be attributed to a secondary magnetic field generated by a ring current in the double bond of the enol in the furano portion of the compound.

In principle there are two possible isomers, depending on whether the two protons C-5a and C-9a are on the same side of the fused ring plane or on opposite faces. The *trans*-isomer (7.34) has an absolute configuration of R about the C-5a atom and a configuration S about the C-9a atom. The *cis*-isomer corresponding has an absolute configuration of 5aR,9aR (7.35). Each of these geometric isomers will also have an enantiomer.



'trans'
5aR 9aS

(7.34)

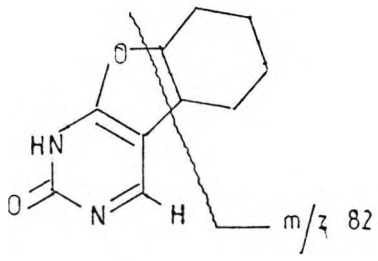


'cis'
5aR 9aR

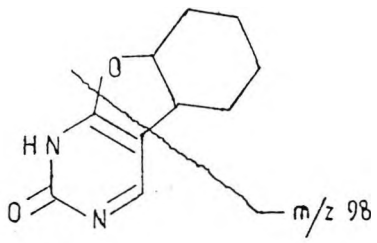
(7.35)

7.8.2.3.2 EI spectrum.

The high resolution mass spectrum of the product was recorded at 70eV. The peaks at m/z 193, 192 and 191 were assigned to the pseudomolecular, the molecular ion ($C_{10}H_{12}N_2O_2$) and to a [M-H] species. A peak at m/z 82 for the ion, C_4H_{10} (7.36), and a more intense ion at m/z 83 for C_4H_{11}. The peak at m/z 98 was assigned to the fragment ($C_4H_{10}O$)(7.37), which mirrors the formation of dimethyl sulphone in dioxathiolopyrimidine (7.11).

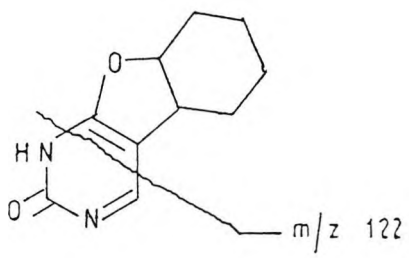


(7.36)

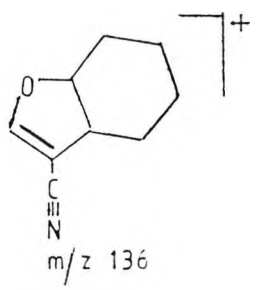


(7.37)

The fragment at m/z 122 (7.38) was demonstrated with high resolution to represent the fragment $C_8H_{10}O$. The observation of fragments (7.38 and 7.39) is consistent with the reactant adding across the proposed 1,3-dipole ($O_{(4)}$-$C_{(4)}$-$C_{(5)}$) in the intermediate (7.29).

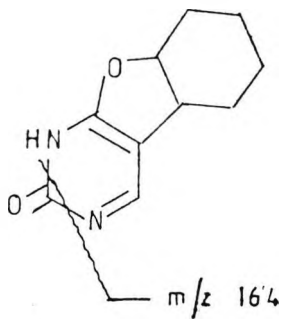


(7.38)

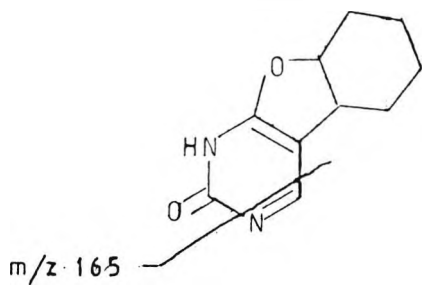


(7.39)

Although a fragment [M-HNCO] was not observed, a fragment [M-CO] was found at m/z 164 (7.40). Like many uracil derivatives which are 1,6-unsaturated, the loss of HCN is observed (7.41).



(7.40)

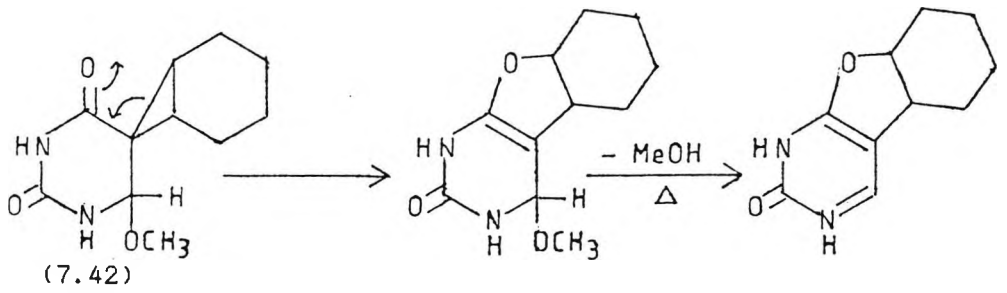


(7.41)

The fragment at m/z 136 was assigned to $C_8H_{10}NO$, which probably originated from the loss of HNCO from the molecular ion, followed by the loss of H, *i.e.* (M-HNCO-H) (7.41).

7.8.2.3.3 Discussion.

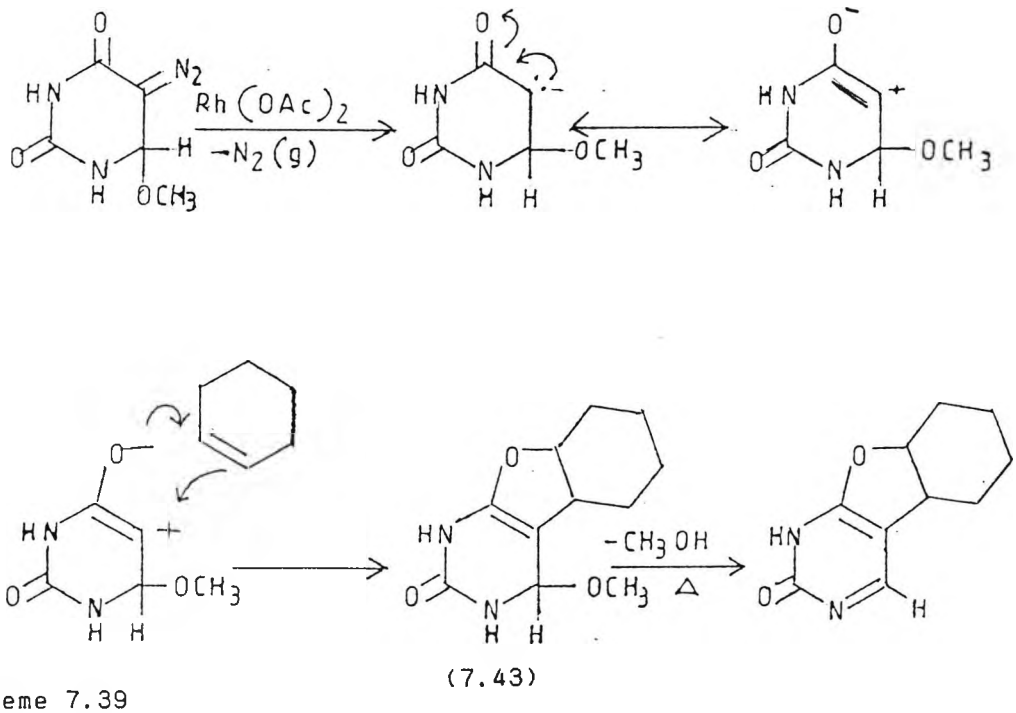
One possible route to the tricyclo- compound (7.33) is via the formation of the cyclopropane derivative (7.42), which subsequently rearranges to provide the observed product (Scheme 7.38).



(7.42)

Scheme 7.38

An alternative mechanism would be to consider that the product arose from 1,3-dipolar reaction in which the "keto carbene" exists as the tautomeric 1,3-dipole (7.44) (Scheme 7.39).



(7.43)

Scheme 7.39

The failure to detect cyclopropane intermediate (7.42) tends to suggest that Scheme 7.39 is the more likely.

Evidence that the methoxy- derivative (7.43) was present was provided in the mass spectrum of the crude material. In this spectrum peaks at m/z 224 and 225 were observed, which represent the molecular ion and pseudomolecular ion of this compound (7.43). The peaks were shown by high resolution to have a formula $C_{11}H_{16}N_2O_3$ and $C_{11}H_{16}N_2O_3+H$.

The product (7.33) appears to be a single geometric isomer from the ^1H spectrum. The formation of the *cis*- or *trans*- isomer would fit with the observed coupling of J=10.6Hz, which according to the Karplus equation gives a dihedral angle between H-5a and H-9a of around 0° or 180°. However, it is conceivable that the *cis*- and *trans*- forms possess the same chemical shifts and coupling constants and thus, given a common spectrum. The methoxy derivative (7.43) was detected in the MS of the crude material but not isolated. It is likely that the reaction conditions are responsible for the loss of methanol from either the starting diazo compound or from (7.43) to give the major product, *viz.* the dehydro- product (7.33). It is noticeable that the addition of large cyclohexane moiety allows the product to dissolve in chloroform.

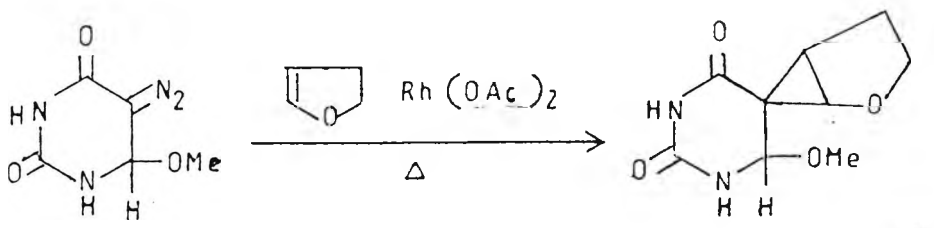
7.8.2.3.4 Suggestions for further work.

The geometry of the product might be determined by examining the spatial relationship of the atoms. A suitable technique would be highfield ^1H n.m.r. spectroscopy, using n.O.e.'s to determine the proximity of various protons to H-1 and thus, determine the exo/endo geometry.

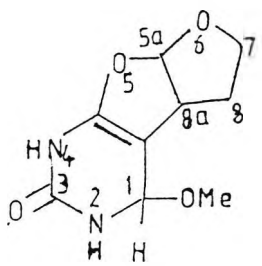
7.8.2.4 2,3-Dihydrofuran.

7.8.2.4.1 Introduction.

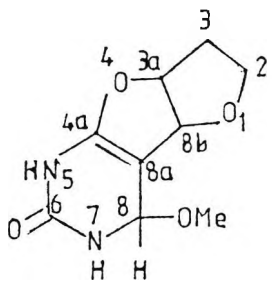
To avoid any complications with competing reactions, it was proposed that the diazo compound should be decomposed in neat dry 2,3-dihydrofuran. Three possible products were predicted; a spiro-product formed by cyclopropanation (Scheme 7.40) and two isomeric furano- compounds (7.46 and 7.47).



Scheme 7.40



(7.46)



(7.47)

7.8.2.4.2 Preparation.

The diazo compound was stirred in a large excess of dry dihydrofuran and the mixture warmed to 40°C. Water contamination was kept to a minimum by drying the dihydrofuran over sodium lead alloy for several days before use. Rhodium acetate catalyst was added to the solution and an effervescence was observed. The solution was left stirring at 30°C in a sealed vessel for 1h. The reaction mixture was then allowed to cool to ambient temperature and left stirring for a further 48h. The reaction mixture was evaporated to remove the excess dihydrofuran and afforded a viscous oil.

The product was analysed without purification as it was observed to fume in air and it was feared that purification would result in the material deteriorating. The i.r. spectrum of the product was free of an absorption band around 2120cm^{-1}, and confirmed that the starting material had reacted with the loss of the diazo group. The uracil moiety was identified by an strong absorption at 1680cm^{-1}, which was assigned to an amide-type carbonyl.

5-Methoxyuracil was identified as one of the products (Table 7.9).

δ	integral		assignment
	5-methoxyuracil		
10.56	2	(1)	N-3
9.4	2	(1)	N-1
6.6	2	(1)	H-6
3.36	6	(3)	OMe
1-methoxy-hexahydrofurano[2',3':6,7]furanopyrimidine-3-one			
9.0	bs	1H	H-4
8.4	bs	1H	H-2
6.4	d	1H	H-1 (J=2.0Hz)
5.2	d	1H	H-5a (J=3.0Hz)
4.24	t	2H	H-7 (J=9Hz)
3.36	s		MeO
2.54	dt	1H	H-8a (J=9Hz, J=3.0Hz)
2.2-1.7	m	2H	H-8,

Table 7.9 Observed chemical shifts in dmso-d_6

Apart from the signals for 5-methoxyuracil, other signals were also detected (Table 7.9). An examination of these signals showed that they were consistent with the formation of furanopyrimidine. The presence of N-H and H-5a signals were taken clear evidence of the uracil moiety. From the distribution of the signals, the data would appear to fit best the furano[2',3':6:7]furanopyrimidine structure (7.46).

Model studies show that only when H-5a and H-8a are *cis*-configuration can there be two fused 5-membered rings. Examination of the model suggests that the dihedral angle between H-5a and H-8a can lie in the region 0-60°. The model also shows that when H-5a and H-8a are eclipsed (dihedral angle is 0°), then the H-8a/H-8', O-5/C-8b, C-5a/C-7 and C-8b/H-8' are also eclipsed. Correspondingly, when H-5a and H-8a are gauche, then previous pairs have gauche relationship to each other. The observed 3.0Hz coupling suggest a dihedral angle of around 45-50°, which suggests that the compound is in the more stable conformation, *i.e.* where the bonds about C-5a, C-8a and C-8 are gauche.

The ^{13}C n.m.r. of the compound showed 9 peaks in addition to the solvent dmso-d_6, 5-methoxyuracil, and TMS peak in the ^{1}H decoupled spectrum (Table 7.10).

δ	%intensity	Assignment	Typical functional Groups**
5-methoxyuracil*			
162.692	2719	>C=O	
153.968	3121	=C-O-	
140.036	4324	CH(OMe)N	
114.843	3271	=C	
61.914	4229	OMe	
1-methoxy-hexahydrofurano[2',3':6,7]furanopyrimidine-3-one			
146.093	1362	C-3,	amide C=O
139.932	385	C-4a	=C-O-
102.083	447	C-5a	O-CH-O
99.671	1421	C-4a	C=c-O
74.343	571	C-1	
68.944	1643	C-7	$O-CH_2-R$
66.406	1619	MeO	MeO
32.030	1385	C-8a	CH
28.645	1500	C-8	CH_2

* Compared with 5-methoxyuracil (see 7.7.2.2)

** relevant functional groups that fit the given value [54]

Table 7.10 Observed ^{13}C chemical shifts.

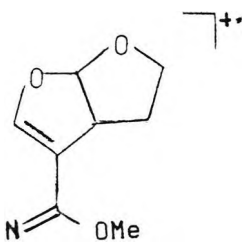
The ^{13}C n.m.r. data confirmed the observations in the ^{1}H spectrum. The major component was 5-methoxyuracil. However, the other peaks in spectrum were consistent with the adduct (7.48). The peaks were assigned from ^{13}C chemical shift tables [54,55] and are only tentative assignments. The C-1 carbon is an sp^3 hybridized carbon attached to nitrogen and oxygen, which is not a common combination. Fortunately, the previous studies on ^{13}C n.m.r of some 5-diazo-6-alkoxy-1,6-dihydrouracils (see 6.14) gives the chemical

shift of the C-6 carbon at 75.00δ (see 6.14.3). Thus, the observed value of 74.0δ and the proposed assignment correlates very well. The observation of the two signals of similar chemical shift, 28.6δ and 32.0δ, are in more typical of branched alkyl carbons than an ether methine and an alkyl methylene (*c.f.* 7.47, C-3 and C-3a). This observation is consistent with a furano[2',3':6,7]furanopyrimidine product (7.46). The absence of any other spectral peaks also agrees with the hypothesis that the adduct formation results in one isomer being formed.

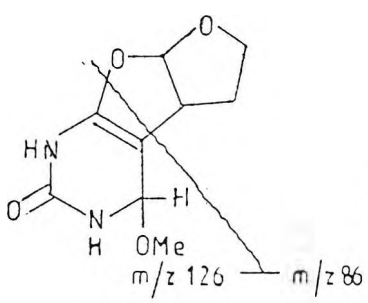
7.8.2.4.3 <u>EI Mass spectrum at 70eV.</u>

The EI spectrum showed that the crude mixture contained 5-methoxyuracil. The peaks at m/z 142 and 143 indicated the presence of the molecular and pseudomolecular ions. Many of the fragments commonly associated with 5-methoxyuracil (see Chp. 7.7.1) were also observed; the spectrum contained peaks at m/z 141, 114, 113, 99, 98, 70, 68 and 56.

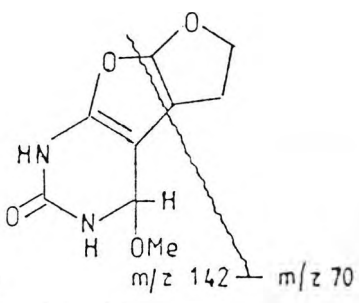
The furano[2',3':6,7]furanopyrimidone (7.46) was also detected in the EI spectrum with peaks at m/z 212 and 213, *i.e.* the molecular and pseudomolecular ions. High resolution mass measurement confirmed the formula to be $C_9H_{12}N_2O_4$. The peaks at m/z 169 and m/z 168 (7.48) were attributed to [M-HNCO] and [M-HNCO-H], which is consistent with uracil derivatives (*c.f.* 7.10).



(7.48)



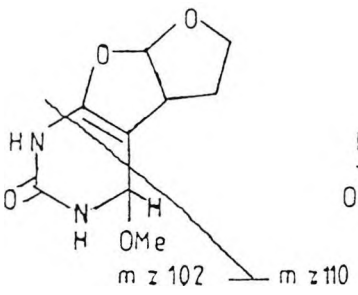
(7.49)



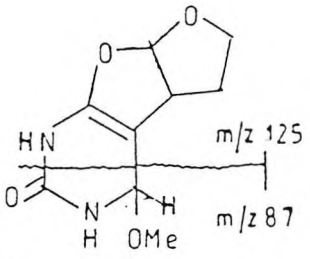
(7.50)

Many of the observed fragments could be related to the structural features of the adduct, *e.g.* the uracil moiety was demonstrated by the fragments at m/z 126 (7.49), 142 (7.50) and 102 (7.51). The peaks at m/z 110 (7.51) and 125 (7.52) are consistent with the

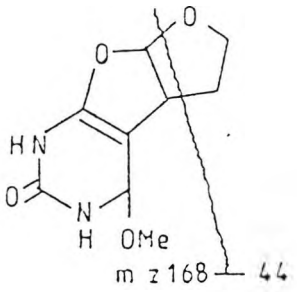
presence of the fused furan rings. The furanopyrimidine portion was given by the peak at m/z 168 (7.53).



(7.51)

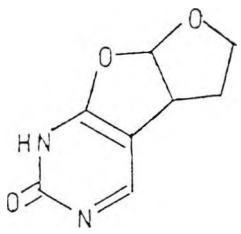


(7.52)

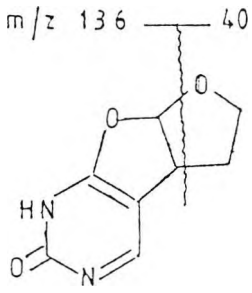


(7.53)

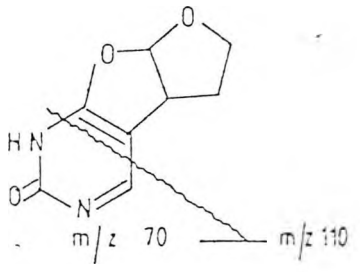
The mass spectrum also provided evidence of the dehydro-product (7.54) at m/z 181 ($C_8H_8N_2O_3$+H), 136 (7.55), 110 (7.56), 70 and 40. The n.m.r. spectra have shown the dehydro- derivative to be absent in the crude mixture, therefore, its detection in the EI spectrum suggests that it is formed as an artefact of the EI-MS process.



(7.54)



(7.55)



(7.56)

7.8.2.4.4 Discussion.

The EI spectrum suggested that, as well as forming 5-methoxy--uracil, the diazo compound also reacted with the activated alkene to produce an adduct (7.46). The reaction would appear to be regiospecific. The fusion of two five-membered rings would in any case lead to one stereoisomer and a conclusion about whether the reaction proceeded by a concerted mechanism can not be inferred.

7.8.3 Discussion of reactions with carbon-carbon double bonds.

7.8.3.1 Introduction.

The experiment with cyclohexene and with 2,3-dihydro- -furan have shown that diazouracils react with carbon-carbon double bonds to form 5-membered cyclic compounds with the loss of the diazo nitrogens. For reference, a review of cycloadditions of 1,3-dipoles is presented.

7.8.3.1.1 5-Membered ring cycloadditions.

The concept of 1,3-dipolar reactions was developed as the result of studies of the addition of diazoalkanes to strained double bonds [56,57]. Such 1,3-additions had been previously reported for diazoalkanes, azides and ozone [58-60].

Cycloadditions could be classified according to either the number of new bonds formed or according to ring size of the product. A typical reaction is the formation of two *sigma*-bonds at the expense of two *pi*-bonds. The formation of five-membered rings can be produced by 3+2 cycloaddition. The only means by which such a reaction can lead to a uncharged five-membered ring is to consider a three centre molecule which contains a formal separation of a negative and positive charge. Thus, a dipole over the three centres is created. This dipole will react with unsaturated systems having double or triple bonds.

7.8.3.1.2 Classification of dipoles.

The 1,3-dipole, *a-b-c*, can be described so that atom *a* possess a sextet, *i.e.*, being inherently less stable with an incomplete octet. Such an atom can be represented by having a positive charge. The atom *c* contains an unshared electron pair, which formally carries a negative charge. The 1,3-dipole and the unsaturated system, referred to as the dipolarophile, coalesce by means of a cyclic electron displacement with extinction of the formal charges to produce a 5-membered ring. It can be concluded that if the electron sextet were located on an oxygen, nitrogen, or sulphur atom it would be unstable. Some 1,3-dipoles can be stabilized if there is a non-bonding pair of electrons on atom *b* which can stabilise the positive charge through mesomeric resonance.

The 1,3-dipoles have been classified according to the nature of the valency of the three atoms of the dipole [56]. Of the various classifications advocated, the most relevant to the diazouracils are as follows. The first group are the 1,3-dipoles with a double bond and internal octet stabilization. Within this class, the important subgroup is that of the diazonium betaines (Table 7.11).

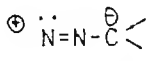
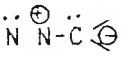
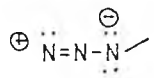
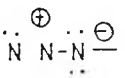
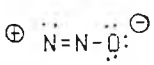
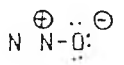
	1,3-dipolar structure	alternative resonance form
Diazoalkane		
Azides		
Nitrous oxide		

Table 7.11 Diazonium betaines. 1,3-Dipoles with a double bond and internal octet stabilization.

The other relevant class is that of unsaturated carbenes and azenes as 1,3-dipoles (Table 7.12).

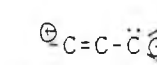
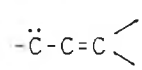
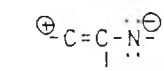
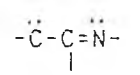
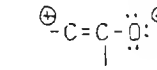
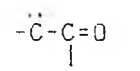
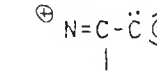
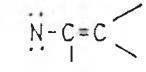
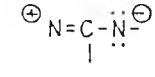
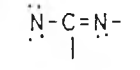
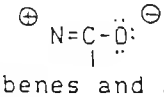
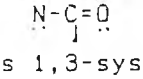
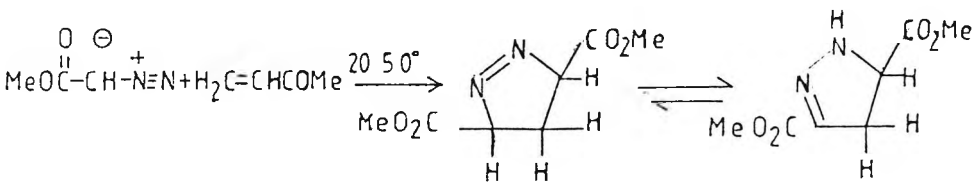
Type of group	1,3-dipolar structure	alternative resonance form
Vinyl carbene		
Imino carbene		
Keto carbene		
Vinyl azenes		
Imino azene		
Keto azene		

Table 7.12 Unsaturated carbenes and azenes as 1,3-systems

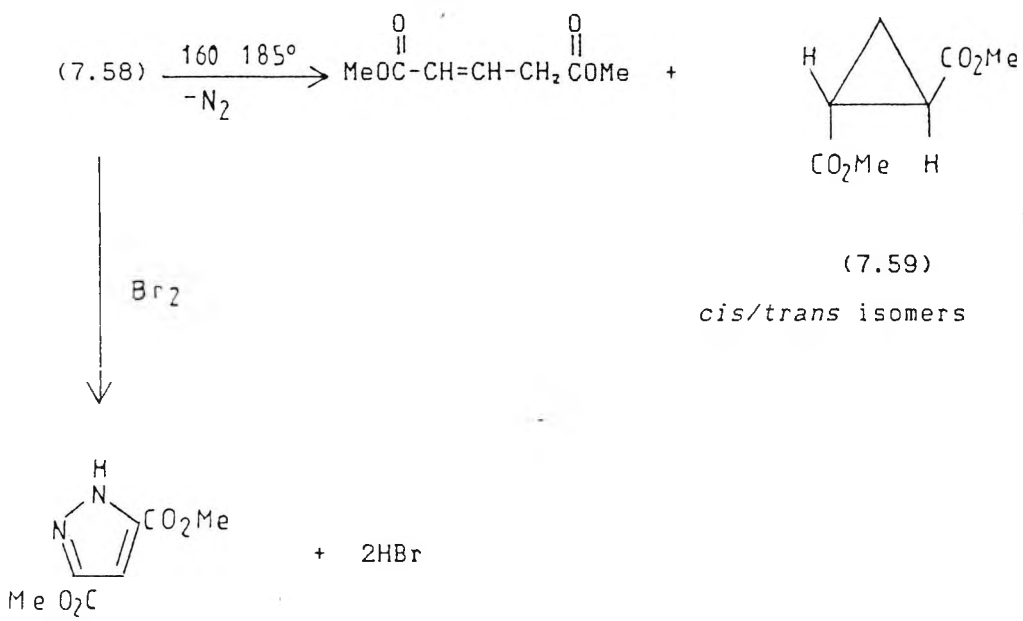
7.8.3.1.3 Addition of 1,3-dipoles with internal octet stabilization.

Diazoalkanes have been shown to be capable of reacting with olefins to produce pyrazoline intermediates. For example, diazo--acetic acid ester was shown to couple with methyl acrylate to form 1,2-pyrazoline-3,5-dicarboxylate (7.57). The initial product of addition was rapidly isomerised to produce the 2,3-pyrazoline (7.58), which then underwent further reaction (Scheme 7.42). The ring opening of the pyrazoline was thought to go via an open chain zwitterionic species [61].



(7.57) (7.58)

Scheme 7.41

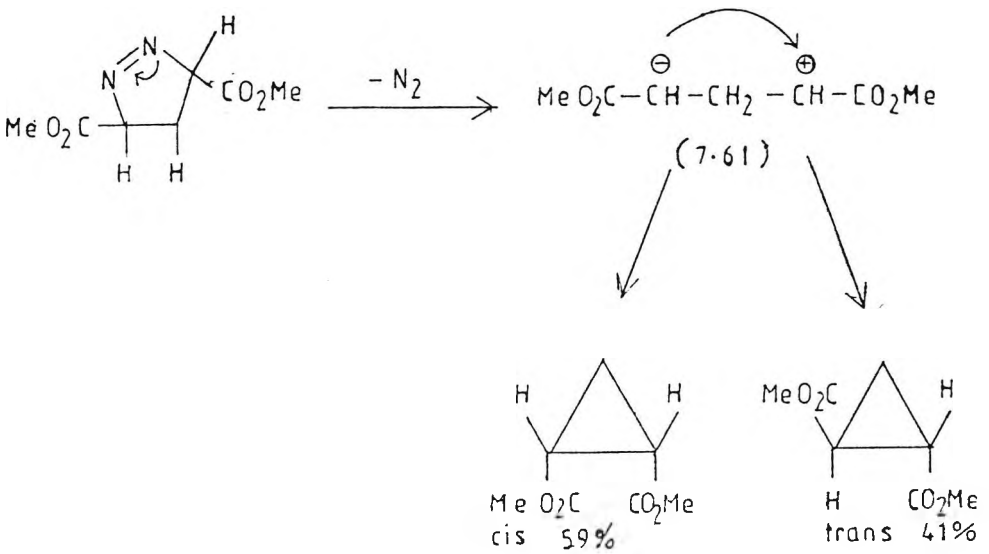


(7.60)

(7.59)
cis/trans isomers

Scheme 7.42

The conversion of pyrazoline intermediate to a cyclopropane with the loss of the diazo nitrogens as molecular nitrogen suggests that this reaction proceeds by a stepwise mechanism involving the generation of a zwitterionic (7.61) or biradical form, which was deduced from the *cis/trans* ratio of 1:0.7. Hence, the loss of stereospecificity occurred as the result of bond rotation in an open-chain intermediate before cyclisation (Scheme 7.43) [62,63].



Scheme 7.43

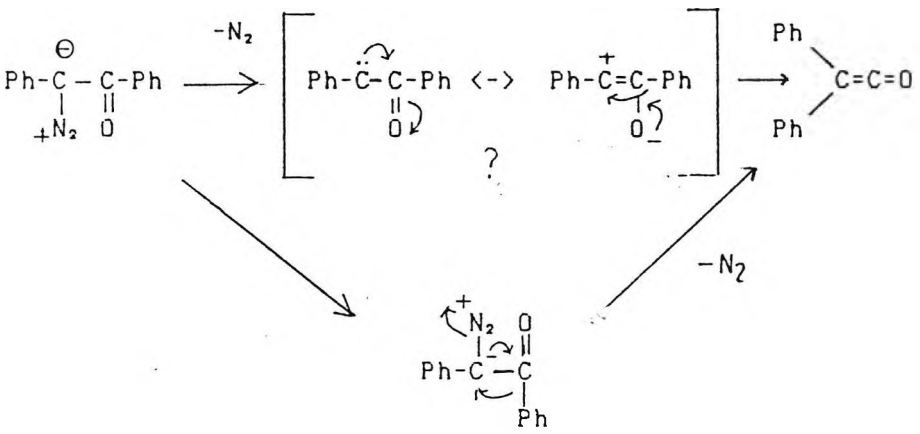
The order of ease of addition of the diazo compounds was diazomethane > diphenyldiazomethane > diazoacetic ester > diazo--ketones > α-diazo- β-dicarbonyl compounds.

7.8.3.1.4 Additions of 1,3-dipoles without octet stabilization.

The chemistry of 1,3-dipolar systems which are not octet--stabilized is a relatively newly explored field of 1,3-cyclo--addition chemistry [56]. As mentioned earlier, systems in which the sextet is located on a carbon, nitrogen or oxygen atom in the three-atom system is unstable and thus, these reactive species are generated and used in situ.

A classic example of 1,3-dipoles without octet stabilisation is the formation of a ketocarbene by thermolysis of a diazoketone.

The ketocarbene can be described by neutral or zwitterionic resonance structures and is capable of exhibiting Wolff rearrangement to produce a ketene [64] (Scheme 7.44). The failure to trap the ketocarbene during thermal Wolff rearrangement suggests that it might not exist as a discrete intermediate [65], or be too short lived for intermolecular trapping.



Scheme 7.44

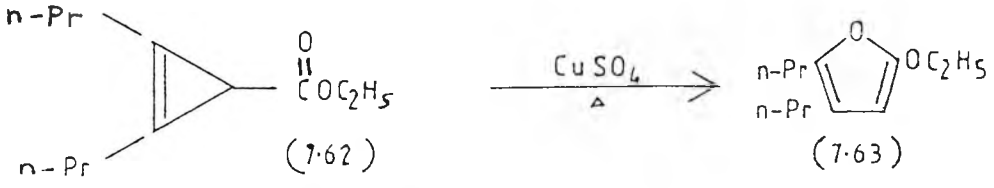
The formation of 2,5-diphenyloxazole by the decomposition of diazoacetophenone in benzonitrile was taken as support for the keto-carbene intermediate having a 1,3-dipolar nature. It was proposed that the copper induced decomposition of diazoketones both promotes the liberation of the diazo nitrogens and stabilises the resulting carbene. A consequence of this is the suppression of the Wolff rearrangement with the favourable formation of acylcyclopropanes [66].

In the case of cyclic ketones, the Wolff rearrangement results in ring contraction [67]. The chemistry of photolytically and thermally generated α-ketocarbenes has been summarised as involving the processes of cyclopropanation, insertion, rearrangement and dimerization [68-71] .

The behaviour of the alpha-ketocarbenes has been shown to produce a wide variety of ring compounds. The α-ketocarbenes are capable of forming cyclopropanes and heterocyclic rings such as oxazoles [72-74], dioxoles [75], lactones [76,77] and furans [78-80]. The formation of the five membered ring has been ascribed to one of two possible mechanisms, a 1,3-dipolar action [56] or via a rearrangement. Support for the 1,3-dipolar mechanism has come

from work carried out with α-diazoketones, α-diazides and α--diazoesters. These compounds were reacted with a wide variety of ketones, olefins, alkynes, nitriles and ketones. The failure to detect cyclopropane and cyclopropene products from reactions which lead to furans and dihydrofurans implies that five-membered rings are not formed by rearrangement of cyclopropane intermediates [81,72].

Evidence has also been advanced against the hypothesis of a 1,3-dipolar cycloaddition. The results which were initially used in support of a 1,3-dipolar mechanism for the formation of furan from alpha-diazoesters [78] were later re-interpreted in favour of a rearrangement from a cyclopropene ester. it was argued that furan formation was dependent on the reaction temperature and the type of copper catalyst used in generating the reactive intermediate [79]. An example of this reaction was the conversion of 1,2-di-*n*-propyl-3--(ethoxycarbonyl)cyclopropene (7.62) to 2,3-di-*n*-propyl-5-ethoxy--furan (7.63) by copper sulphate (Scheme 7.52). Further evidence to support this alternative hypothesis was the observation that the furans only appeared after a substantial build up of the cyclopropene [83]. Thermal isomerizations of cyclopropenyl ketones and esters have been reported [84] lending further support to the rearrangement hypothesis. These rearrangements are similar to the formation of cyclopentane from vinylcyclopropane [85,86]. Vinyloxirane has also been reported to rearrange to dihydrofuran [87-90].



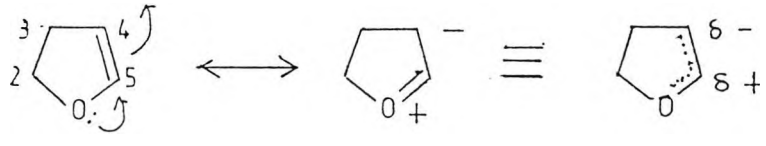
Scheme 7.45

7.8.3.2 Experimental Observations.

In the present work, the reaction of the putative carbenoid with cyclohexene resulted in the formation of a tricyclic product via the fusion of two rings to give a third ring in a 3+2

cycloaddition. The absence of any evidence to support cyclopropan--ation gave rise to the hypothesis that the thermally generated ketocarbene in the cycloaddition existed as a non-octet stabilised 1,3-dipole.

The use of 2,3-dihydrofuran resulted in a regiospecific cycloaddition. The furano[2',3':6:7]furanopyrimidone (7.46) is consistent with the expected product of the reaction between a 1,3-dipole and an "activated" olefin. If the electronic structure of 2,3-dihydrofuran is examined, it is expected that the enolic double bond should be polarised such that the C-4 position is slightly negative and the C-5 position slightly positive (Scheme 7.46). The regiospecificity of the adduct (7.46) agrees with literature examples of 1,3-dipolar addition using polarised, nucleophilic olefins [91,92].



Scheme 7.46

The adducts (7.33 and 7.46) of the cycloaddition reactions were stereospecific, with only one stereoisomer obtained. Both adducts were thought to possess a *cis*- geometry, which would be in keeping with the literature [92].

7.8.3.3 Suggestions for further work.

Ideally more types of dipolarophile should be explored, potential candidates include vinyl ethers and 3,4-dihydro-2H-pyran. All the reactants to be tried should be as dry as possible and if possible used neat to avoid solvent reactions becoming significant. Reactions with triple bond systems would be of interest as the expected products would be the unsaturated cycloadduct, for example, an alkyne might be expected to lead to the fused furan system. It is suggested that the less polar N-methylated diazouracils might prove more soluble in organic solvents and be better for exploring the reactions of these *o* -diazoimides.

7.9 Reactions with alcohols.

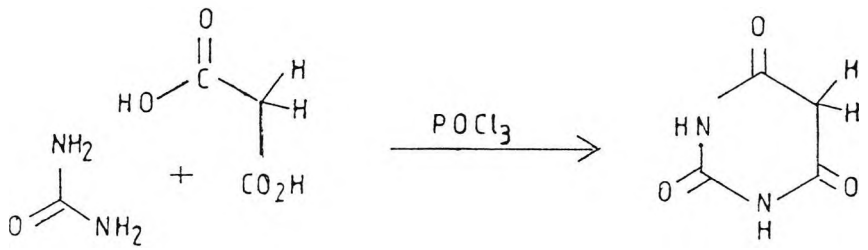
7.9.1 Introduction.

7.9.1.1 Synthesis of pyrimidines derivatives.

The parent compound of this series is pyrimidine [93,94] or m-diazine. It can be regarded as a cyclic amidine with positions C-2, C-4 and C-6 being similar with regards to reactivity [95]. Position C-5, however, is characterised by a different behaviour being more aromatic in nature.

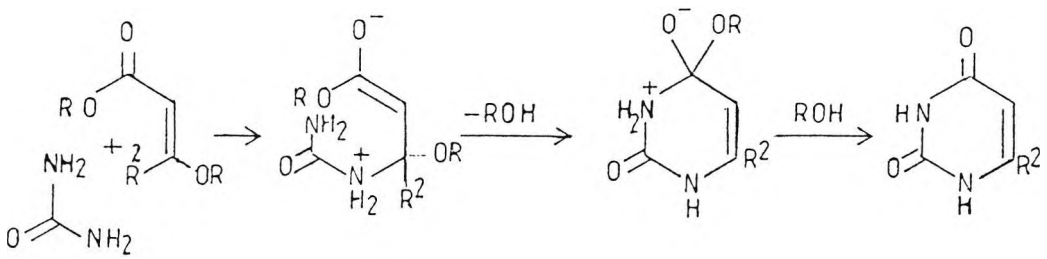
7.9.1.1.1 Uracil.

The commonest type of pyrimidine ring synthesis is by a double condensation of two aliphatic portions. For example, the double condensation of urea with malonic acid, effected by phosphorus oxychloride, affords barbituric acid (Scheme 7.47).



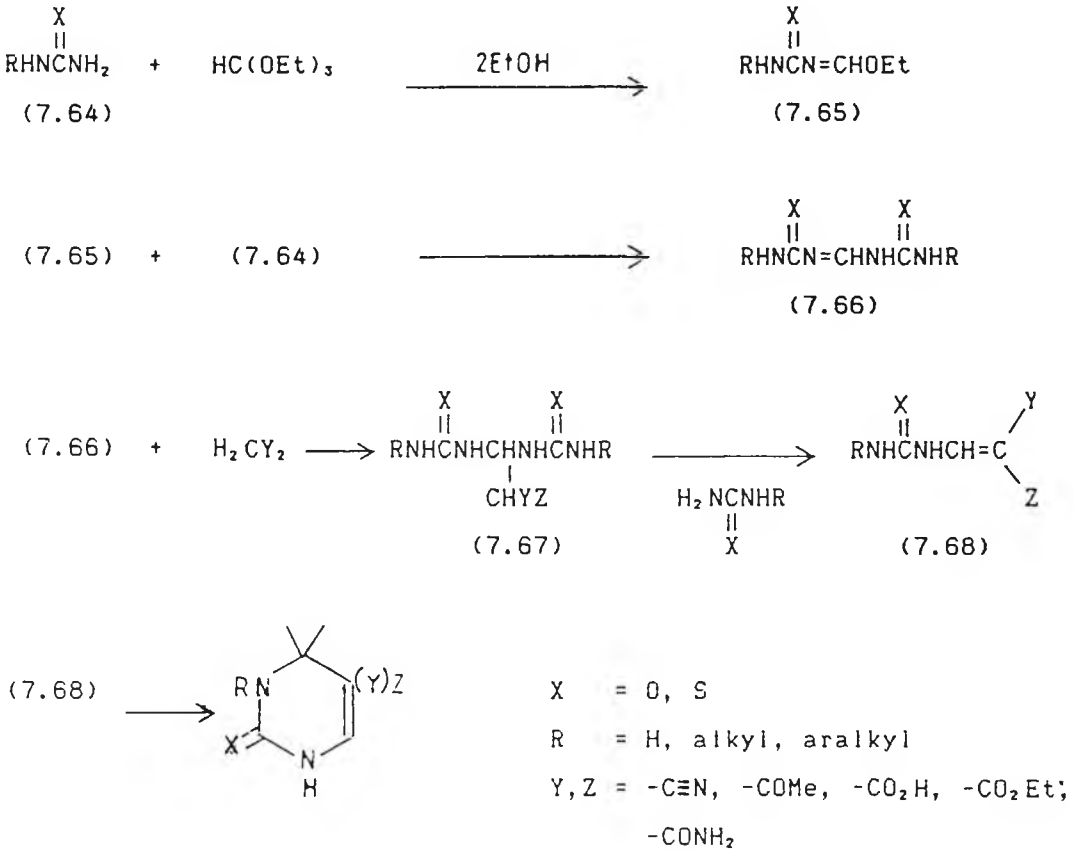
Scheme 7.47

A related reaction is the double condensation of urea with *B*-keto esters to produce C-6 alkyl substituted uracils [96]. This reaction is a general one and works with the enol ethers of these compounds (3-alkoxypropenoic acid), where alkyl substituents on the C-3 position of the propenoic acid are carried through to the C-6 position of the resulting uracil (Scheme 7.48). Urea has also been condensed with nitriles [11+86-89].



Scheme 7.48

A general synthetic procedure for the preparation of a large number of pyrimidines has been outlined [101] (Scheme 7.49).



Scheme 7.49

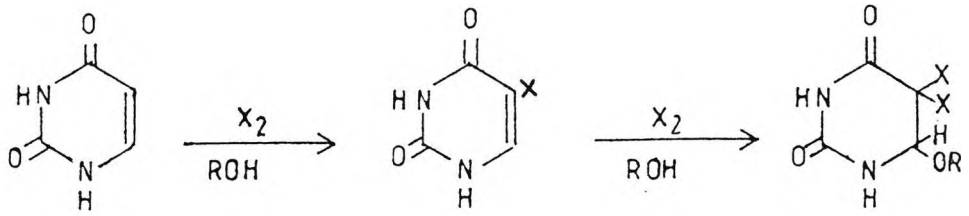
The essential reaction of all the methods for cyclisation to pyrimidines is the nucleophilic attack of nitrogen onto an electro--philic carbon centre. Thus, the basicity of the nitrogens is an important factor in deciding the ease of reaction.

As an alternative to using urea or thiourea, a wide variety of pyrimidines can be prepared by the condensation of guanidine with a wide variety of carbonyl [102-106], enol ether [107] and nitrile [108,109] containing compounds.

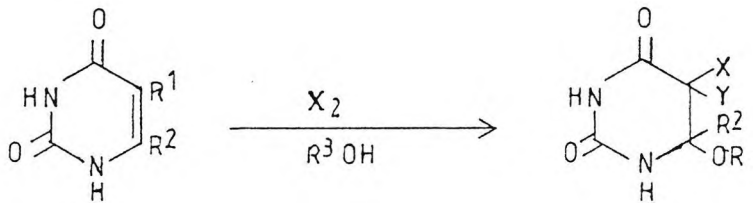
7.9.1.1.2 5-Substituted pyrimidines.

The aromatic character of the C-5 position of the pyrimidine ring is exemplified by the tendency of this carbon to participate in electrophilic substitution reactions. The halogenation of uracil in methanol is a particularly facile reaction and occurs at the C-5 position. The reaction of uracils with halogenating agents has been

extensively investigated [110-112]. There is a tendency for addition to follow substitution (Scheme 7.50).



scheme 7.50



7.69) R^1=I, R^2=H

7.70) X=Y=Cl, R^2=H, R^3=H

7.71) R^1=CO_2H, R^2=H

7.73) X=Cl, Y=NO_2, R^2=H, R^3=H

7.72) R^1=NO_2, R^2=H

7.75) X=Br, Y=Me, R^2=H, R^3=H

7.74) R^1=Me, R^2=H

7.77) X=Y=Cl, R^2=$PhCH_2$, R^3=Me

7.76) R^1=H, R^2=$PhCH_2$-

5-Iodouracil (7.69) and uracil-5-carboxylic acid (7.71) both giving 5,5-dichloro-6-hydroxy-5,6-dihydrouracil (7.70) [111]. In other cases, addition occurs with retention of the original C-5 substituent, for example, 5-nitrouracil (7.72) will yield 5-chloro--6-hydroxy-5-nitro-5,6-dihydrouracil (7.73) [111]. Thymine (7.74) reacts with hypobromous acid to afford 5-bromo-6-hydroxy-5-methyl--5,6-dihydrouracil (7.75) [113].

Few examples of C-6 substituted uracils have been studied; 6-benzyluracil (7.76) undergoes addition to give 6-benzyl-5,5--dichloro-6-methoxy-5,6-dihydrouracil (7.77) [110]. The halogenation of 1-methyluracil, 1,3-dimethyluracil and 1,3-dimethylcytosine occurs in manner expected for uracil [114]. However, Uracil-6--carboxylic acid affords 5-chlorouracil-6-carboxylic acid [111].

Fluorination of 2,4,6-trifluoropyrimidine at the C-5 position has been achieved using silver fluoride [115]. Direct C-5 iodination has been achieved in pyrimidines using N-iodosuccinimide [116,117].

The nitration of pyrimidines goes exclusively on the C-5 position when using the standard nitrating agents. The addition is less complex than halogenation, with little evidence of olefinic addition. Although pyrimidine is stable to nitration by concentrated nitric acid or its mixtures with sulphuric acid, the presence of any electron-releasing group, *e.g.* a hydroxyl or amino group will facilitate nitration. Thus, nitration of 2-amino- or 2-hydroxy--pyrimidine with concentrated nitric acid gives, in both cases, 2-hydroxy-5-nitropyrimidine [118,119]. The nitropyrimidines are a convenient route to the corresponding aminopyrimidines. This reduction may be achieved by stoichiometric or catalytic means. It should be noted however, that chemical reduction of nitrouracils has a tendency to remove the nitro group and replace it with a hydroxy group, *e.g.* 1-methyl-5-nitrouracil yields 1-methylisobarbituric acid [120].

Nitrosation, like nitration is directed to the C-5 position. In the presence of other substituents such as the amino group or the hydroxyl group positioned at C-2, C-4 or C-6, the reaction still leads to the 5-nitroso derivative [121]

Formaldehyde reacts with uracil derivatives, substituting at C-5. For example, 6-methyluracil in acid or in alkaline solution affords 5-hydroxymethyl-6-methyluracil [122]. A similar reaction for 1,6-dimethyluracil gives 5-chloromethyl-1,6-dimethyluracil.

2-Aminopyrimidine can be converted to 2-amino-5-sulpho--pyrimidine using chlorosulphonic acid [123]. When carried out with 6-amino-1,3-dimethyluracil, the reaction affords 6-amino-5-chloro--1,3-dimethyluracil [124].

Pyrimidines have been shown to couple to diazonium salts. In a reaction analogous to nitrosation, the electron-releasing groups promote coupling, especially if these groups are located at C-2 and C-4 or at C-4 and C-6. In addition, the diazonium salt must be particularly reactive. Thus, diazotized aniline does not couple, whilst diazotized *p*-nitroaniline successfully coupled with uracil type compounds [121,126] giving the C-5 arylazo derivative.

7.9.1.1.3 5-Alkyl- and 5-Aryl- pyrimidines.

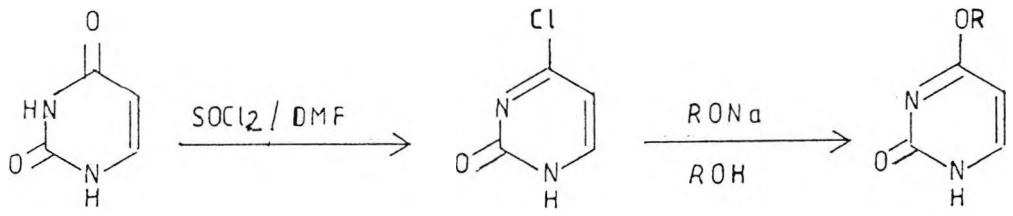
Most commonly, C-5 alkyl and aryl pyrimidines are prepared by ring synthesis from appropriately substituted precursors. Exceptions include the formation of the methyl group from the hydroxymethyl or chloromethyl pyrimidine via chemical reduction [127] and the formation of 5,5-diarylated or 5,5-dialkylated barbiturates.

7.9.1.1.4 5-Hydroxypyrimidines.

The existence of 5-hydroxypyrimidines has never been well authenticated in the literature [128]. The only other pyrimidine containing a 5-hydroxy group which has been reported is "isouracil" (2,5-dihydroxypyrimidine) [129]. The presence of "hydroxy" groups on the pyrimidine ring often results in tautomerism. Thus, 2,4,6-tri--hydroxypyrimidine (barbituric acid) exists as the 2,4,6-pyrimidine--trione tautomer. It is reported that isobarbituric acid exists as the 5-hydroxyuracil whilst dialuric acid and isodialuric acid exist as the hydroxypyrimidinetriones [128]. Interest in these 5-hydroxy compounds has centered on their potential biological activity [130].

7.9.1.1.5 Alkoxypyrimidines.

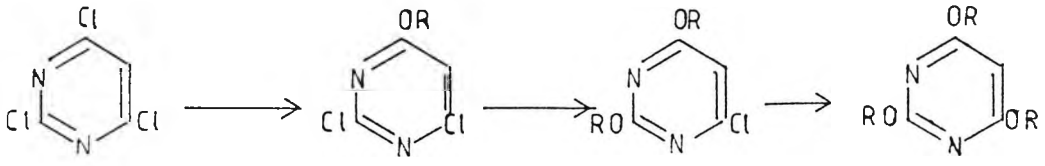
There have been two main methods for forming the alkoxy-pyrimidines. One method involves the nucleophilic attack of an alkoxide on the corresponding chloropyrimidine. Uracil can be converted to 4-chloro-1H-pyrimidin-2-one using thionyl chloride in DMF. This compound readily reacts with alkoxides to form 4-alkoxy-2(1H)-pyrimidinone (Scheme 7.51).



Scheme 7.51

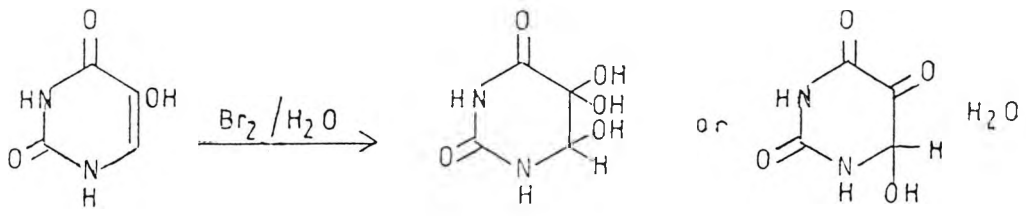
The reaction of 2,4,6-trichloropyrimidine with one equivalent of alkoxide in the corresponding alcohol at 0°C readily forms 4-alkoxy-2,6-dichloropyrimidine. It should be noted that this

compound is exactly the same as 6-alkoxy-2,4-dichloropyrimidine. If the reaction was carried out at 25°C with two equivalents of the alkoxide, the resulting product was 2,4-dialkoxy-6-chloropyrimidine or 2,6-dialkoxypyrimidine. If the reaction was carried out at 70-100°C all the chlorines were replaced by alkoxyl groups (Scheme 7.52) [131-133].



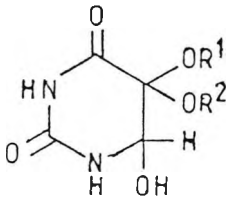
Scheme 7.52

The alternative method is the *de novo* synthesis of the alkoxy--pyrimidine by ring formation from two acyclic portions, for example, the synthesis of 5-alkoxypyrimidines via a cyclisation involving guanidine [134,135]. However, such ring forming methods do not readily afford the alkoxy-5,6-dihydrouracils. Thus, there exists only very limited literature on these compounds. It has been reported [136] that isodialuric acid monohydrate (5,5,6-trihydroxy--5,6-dihydrouracil monohydrate) or an isomeric alternative was formed by the action of bromine water on isobarbituric acid (5-hydroxyuracil) (Scheme 7.53). The isomer was alleged to be 6-hydroxy-2,4,5(1H,3H,6H)pyrimidinetrione dihydrate. The treatment of the intermediate pyrimidinetrione/trihydroxydihydropyrimidine with concentrated hydrochloric acid and methanol readily gave 5,6-dihydroxy-5-methoxy-5,6-dihydrouracil monohydrate [137].



Scheme 7.53

The reaction of bromine- or chlorine- water with isobarbituric acid in methanol or ethanol resulted in a dialkoxy derivative. This suggested that the earlier product obtained was the trihydroxy- derivative (Scheme 7.53). Treatment of the dimethoxy product with diazomethane resulted in the N.N'-dimethyl dimethoxy- derivative. It was claimed that the failure to lose water meant that of the two possible isomers for the double alkoxy- derivative, the compound was better represented by a 5,5-dialkoxy-6-hydroxy-5,6-dihdyrouracil (7.78).



(7.78)

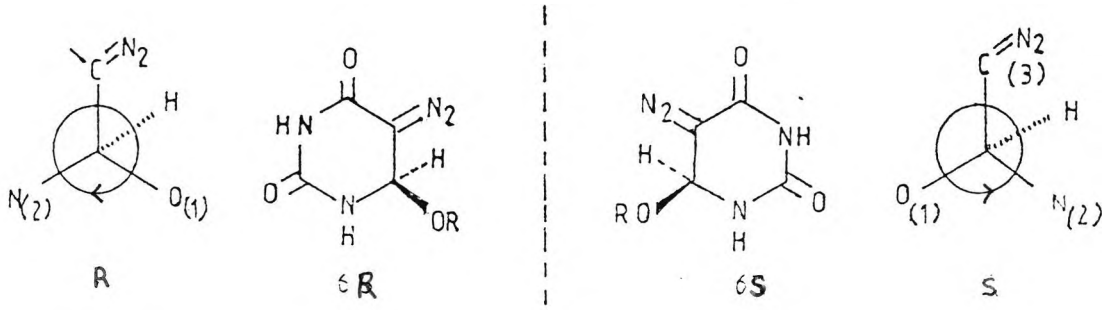
A similar mechanism was also advanced for the reaction of 5-methoxy-1,3-dimethyluracil with chlorine water and methanol. It was assumed that 6-chloro-5,5-dimethoxy-5,6-dihydrouracil was obtained. When treated with sodium methoxide this compound was reported to produce 5,5,6-trimethoxy-5,6-dihydrouracil. The reported reactions have never been verified by any spectroscopic evidence.

The preparation of saturated uracils, collectively referred as the dihydrouracils, usually involves a double condensation to form the ring in which the substituents have been previously placed on the acyclic precursors. Compounds formed by this method have included the 5,5-disubstituted- and 5,6-disubstituted- 5,6-dihydro--uracils. The substituents in these compounds have been non-labile alkyl, aryl or alkenyl substituents. Ultraviolet radiation can initiate the addition of water across the 5,6-double bond in 1,3-dimethyluracil to give exclusively 6-hydroxy-1,3-dimethyl-5,6--dihydrouracil [138].

The preparation of the 5-diazo-6-alkoxy-1,6-dihydrouracils has provided a means to obtain the 5,6-dialkoxy-5,6-dihydrouracils. These compounds form a novel group of compounds. The difficulty in preparing this type of compound has meant that their biological activity and significance has remained largely unexplored.

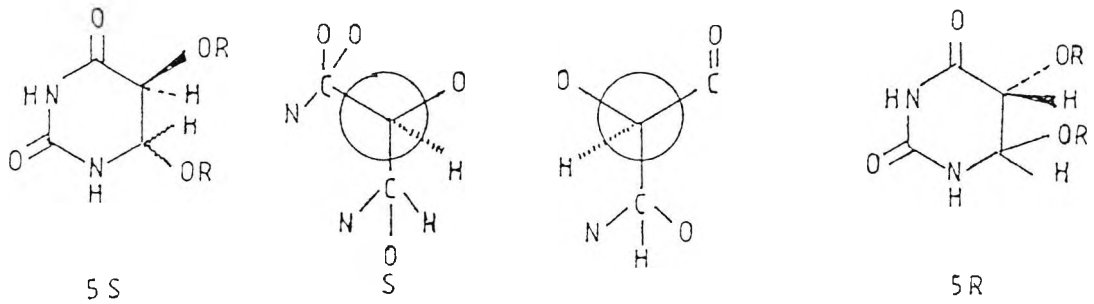
7.9.1.2 The stereochemistry of 5,6-dialkoxy-5,6-dihydro--uracils.

The precursor 6-alkoxy-5-diazo-1,6-dihydrouracil has one chiral centre at C-6 and therefore occurs as a pair enantiomers (Scheme 7.54). The configuration of the compound can be expressed by the R/S convention developed by Cahn, Ingold and Prelog [132].



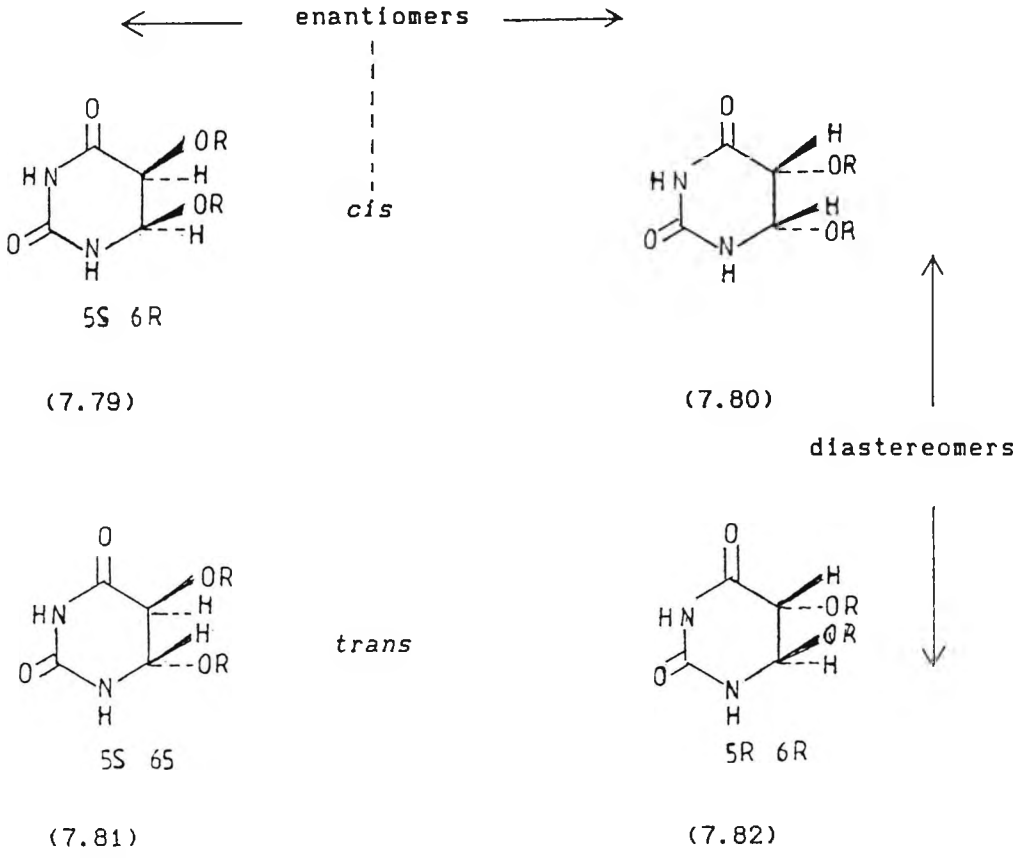
Scheme 7.54

In 5,6-dialkoxy-5,6-dihydrouracils, there exist two chiral centres located at C-5 and C-6 and hence, there are four possible stereoisomers. Examination of the absolute configuration about the C-5 atom similarly gives two stereoisomers with either an "R" or an "S" configuration, where according to the rules for establishing priority, the carbon atom of the carbonyl is assigned a higher order than the C-6 carbon (Scheme 7.55).



Scheme 7.55

Thus, the four stereoisomers consisting of two sets of diastereoisomeric pairs of enantiomers (Scheme 7.55). The diastereomeric relationship between the pairs of enantiomers (7.79 and 7.80 with 7.81 and 7.82) is alternatively denoted as a relationship between geometric (*cis*- and *trans*-) isomers.



7.9.2 Results and discussion.

7.9.2.1 5-Butoxyuracil.

7.9.2.1.1 Introduction.

The reaction of 5-diazouracil and *n*-butanol, in the absence of rhodium acetate catalyst, did not afford the expected 5-diazo-6--butoxy-1,6-dihydrouracil. Instead, a product without the diazo group was obtained and was identified as 5-butoxyuracil.

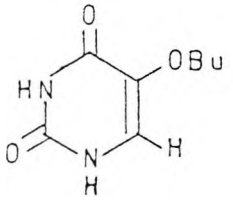
7.9.2.1.2 Preparation.

Freshly prepared 5-diazouracil was dissolved in boiling *n*-butanol for 5h to give a dark orange solution. The hot solution was left to stand overnight at ambient temperature, giving a small amount of a fine orange precipitate. The solution was evaporated to dryness using a rotary evaporator to afford a brown/yellow waxy material. An attempt was made to purify the product by preparative t.l.c. using dichloromethane/methanol (90:10) as the mobile phase. A white product with an R_f=0.6 was isolated from the the silica.

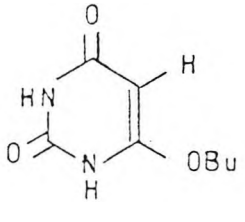
The i.r. of the white solid showed an absence of a diazo stretch around 2100cm^{-1}, which indicated that the diazo moiety was no longer present. The spectrum also showed an absorption band at 1770cm^{-1} of medium intensity which was assigned to out-of-plane vibrations for N-H bonds. The two bands at 1730, and 1710cm^{-1} were attributed to conjugated amide carbonyls (1740-1710cm^{-1}) and an imide carbonyl (six-membered ring imides, 1700-1720cm^{-1}). An absorption at 1660cm^{-1} was attributed to the presence of a double bond conjugated with a carbonyl group.

The ^{1}H n.m.r. of the compound was characterised by a sharp singlet at 6.84δ, which integrated for one proton, a broad singlet at 4.92δ integrating for three protons, a triplet at 4.12δ (J=8Hz) integrating for two protons, a multiplet between 1.2-1.8δ which integrated for four protons, and a triplet at 0.98δ (J=10Hz) which integrates for three protons.

It was concluded from the n.m.r. data that a *n*-butoxyl group had been introduced onto a uracil ring with the removal of the diazo nitrogens. Thus, the likely product is either 5-butoxyuracil (7.83) or 6-butoxyuracil (7.84)



(7.83)



(7.84)

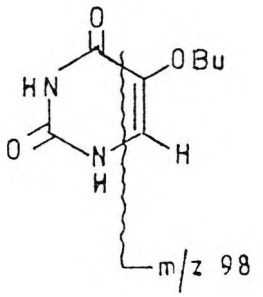
The chemical shift of the sharp singlet at 6.84δ, attributed to the olefinic proton, suggests that the product is the 5-isomer (c.f. 5-methoxyuracil, Chp. 7.7.1). The integral for the N-H protons seems too large by one proton, a likely cause being differences in relaxation of these protons which can give rise to an erroneous integral for them.

7.9.2.1.3 <u>EI mass spectrum.</u>

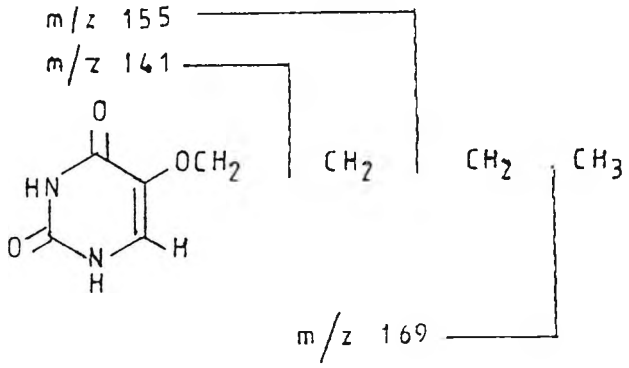
The mass spectrum gave peaks at m/z 185, 184 and 183. which correspond to the pseudomolecular ion, molecular ion and M-H species. High resolution mass measurement of the peak at m/z 184

showed the ion to be consistent with the formula $C_8H_{12}N_2O_3$.

The expected fragments [M-HNCO] and [M-CO] were not detected. The [M-2HNCO] species and the [$C_4H_{10}O$] species were observed (7.85). The fragmentation of the molecular ion was dominated by the alkyl portion, with fragments [M-CH_3], [M-C_2H_5] and [M-C_3H_7] at m/z 169, 155 and 141 (7.86).



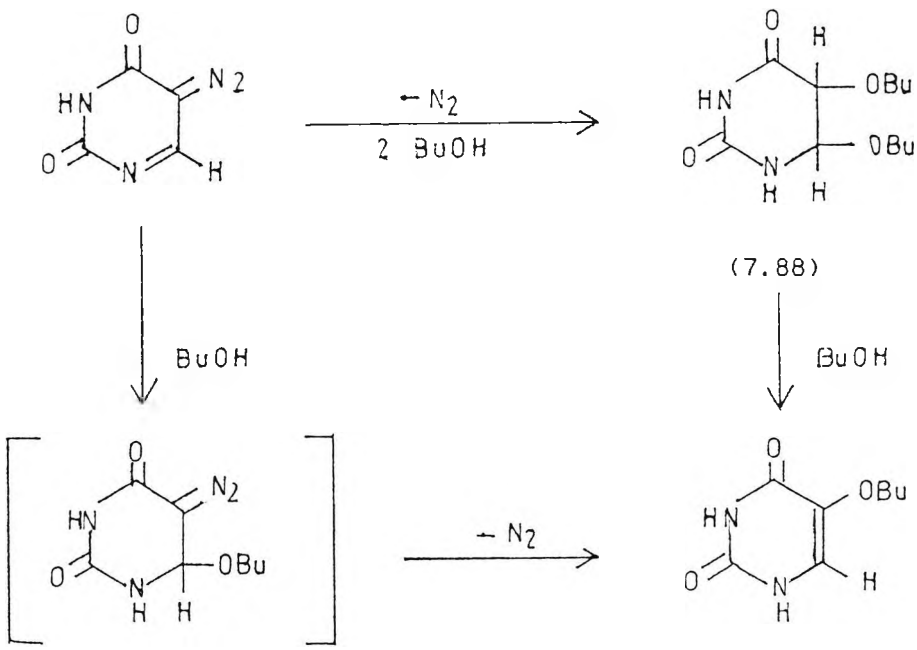
(7.85)



(7.86)

7.9.2.1.3 Discussion.

The formation of 5-butoxyuracil may have occurred by two possible routes. The first route, 5-diazouracil reacts with butanol to form 6-butoxy-5-diazo-1,6-dihydrouracil (7.87) which then loses nitrogen and undergoes a migration of the butoxyl group in a manner similar to formation of 5-methoxyuracil (see also Chp. 7.7.3). The second route involves the formation of the 5,6-dibutoxy-5,6-dihydro--uracil (7.88) as an intermediate (Scheme 7.56).



(7.87) (7.88) (7.83)

Scheme 7.56.

Evidence for the formation of the dialkoxy- derivative (7.88) was obtained in an EI mass spectrum of the crude mixture. The spectrum gave peaks at m/z 258.1611 and 257.1580, which were shown by high resolution mass measurement to be consistent with the molecular ion ($C_{12}H_{22}N_2O_4$ requires 257.1579) and the [M-H] species. This species was also identified by fragment peaks at m/z 185 (M-BuO). This species can be distinguished from butoxyuracil because of the absence of the peak at m/z 184. The spectrum was also characterised by a strong peak for C_4H_9O at m/z 73. The evidence for the uracil portion was given by the peak at m/z 112 ($C_4H_4N_2O_2$).

7.9.2.2 5,6-Dimethoxy-5,6-dihydrouracil.

7.9.2.2.1 Introduction.

The reactions of 5-diazo-6-methoxy-1,6-dihydrouracil (6.4), in a series of rhodium acetate catalysed reactions, were explored. The first of these reactions was reaction with methanol.

7.9.2.2.2 Preparation.

The 5-diazo-6-methoxy-1,6-dihydrouracil was stirred in hot methanol until the compound had dissolved to give a yellow solution. Rhodium acetate catalyst was added and the effervescing solution was left stirring at room temperature overnight. The solvent methanol was removed on a rotary evaporator to give a white crystalline material. The crude material was purified by preparative t.l.c. using dichloromethane/methanol (90:10) as the mobile phase. Two major products were isolated, one (58% yield) with an R_f=0.58 and the other (26% yield) with an R_f=0.37.

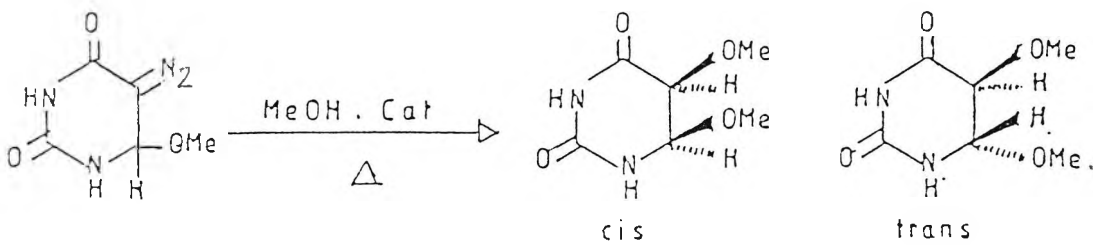
The product with an R_f=0.58 was characterised by i.r. and showed the absence of a diazo stretch around 2100cm^{-1}, thus, confirming that the material isolated was not the starting diazo compound.

The ^1H n.m.r. of the less polar component was found to contain four groups of signals. The two 3H sharp singlets at 3.72δ and

3.58δ were assigned to methoxyl groups. The broad singlets at 4.86δ and 4.64δ were attributed to N-H protons.

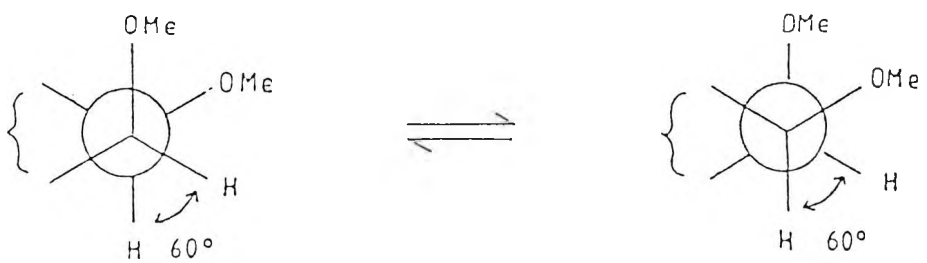
The signal around 3.40δ was complicated by the multiplet obtained from the residual proton found in incompletely deuterated methanol. However, an expansion of this region revealed two doublets which were at 3.38δ and 3.42δ. A coupling constant of J=1.5Hz was measured for these two doublets.

The more polar component, R_f=0.37, also was isolated and was similarly characterised by ^{1}H n.m.r. The spectrum was found to be exactly the same as the spectrum for the less polar component and thus, the two products are isomeric compounds (Scheme 7.57). This was quite surprising as it was thought that the methoxyls and the C-5 and C-6 protons might have different chemical shifts owing to different environments owing to a *cis* and *trans* geometry. The conformation of the isomers was explored with the use of models.



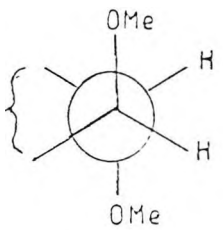
Scheme 7.57

If the models of the two isomers are examined, despite of the near planarity of the nitrogens and carbonyls in the uracil ring, the C-5 and C-6 atoms still permit a gauche conformation when viewed along the C-5/C-6 bond. For the *cis*-isomer, a gauche conformation results in a dihedral angle of approximately 60° (7.89 and 7.90), which according to the Karplus equation would result in a coupling of approximately 1.8Hz. Thus, the model is in agreement with the observed value.

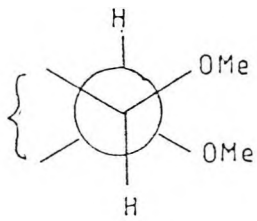


(7.89) (7.90)

Examination of the model for *trans*-isomer reveals that the two gauche conformers result in different dihedral angles between H-5 and H-6 (7.91 and 7.92). When the methoxyl groups are equatorial, the dihedral angle between the hydrogens is approximately 180°. This would lead to a predicted coupling of J=9.2Hz. However, if the methoxyl groups are axial than the dihedral angle between the hydrogens is 60°, which results in the same coupling as the *cis*-isomer. Thus, the models show that the two isomers may have the same coupling. Although and equatorial arrangement for the methoxy groups is sterically more favoured, the preference for an axial arrangement for the methoxyl groups may be explained by dipole--dipole repulsion. Intuitively, the models suggest that the two isomers will have different polarities and this may explain the different solubilities of the two isomers.



(7.91)



(7.92)

The product (R_f=0.58) could not be sufficiently purified for microanalysis to confirm the formula. The obtained values were low in carbon (37.2%), hydrogen (4.32%) and nitrogen (14.9%) ($C_6H_{10}N_2O_4$ requires C-41.4%, H-5.8%, N-16.0%). The values were also lower than those required for methoxyuracil. One possible explanation is that the difficulty in drying the sample without the loss of methanol from the adduct led to the observed differences.

7.9.2.2.3 EI mass spectrum.

The spectrum of this compound (R_f=0.58) did not provide either a molecular ion at m/z 174 nor a pseudomolecular ion. The spectrum was similar to spectrum for 5-methoxyuracil. The spectra differed in one major respect, whilst the spectrum for 5-methoxyuracil possesses an intense peak at m/z 142 and a weaker peak at 143 for the molecular and pseudomolecular ions, the spectrum for the dimethoxy- derivative tends to show the opposite effect, *viz.* an

intense peak at m/z 143 and a weaker intensity for the peak at 142, which often is absent. The observation can be explained by the ready loss of a methoxy group to afford an ion at m/z 143. This ready loss of one of the alkoxyl groups was also observed for the dibutoxy-derivative. The dialkoxy derivative was also characterised by peaks at m/z 115 [M-MeO-CO], 100 [M-MeO-HNCO], 87 [$C_3H_5NO_2$] and 72 [m/z 115-HNCO].

7.9.2.2.4 FAB spectrum.

The FAB spectrum of the product was obtained. The spectrum showed peaks at 175 and 173, which were assigned to the pseudomolecular ion [P] and [P-H_2] species. However, accurate mass measurement was not available and hence, the formula could not be confirmed. Higher complexes were observed at m/z 283 (P+mat), 281 (P-H_2+mat), 213 (M+K), 207 (P+MeOH), 205 (P-H_2+MeOH) and 197 (M+Na). The more intense peaks were the fragment peaks at m/z 165 (M+Na-MeOH), 143 (P-MeOH), 100 (P-MeOH-HNCO) and 57 (P-MeOH-2HNCO).

7.9.2.3 5-Methoxyuracil.

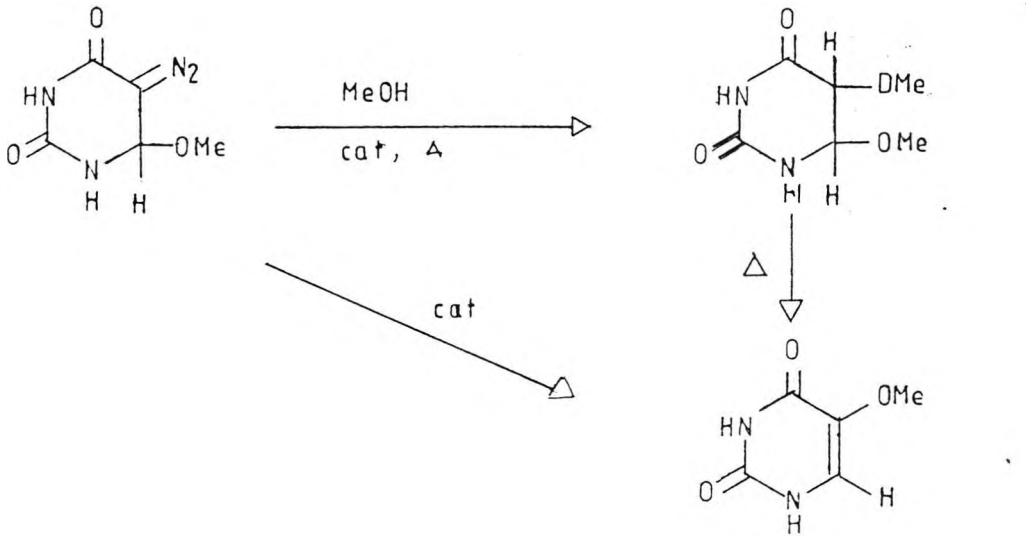
7.9.2.3.1 Preparation.

5-Diazouracil was dissolved in hot methanol and rhodium acetate was added to the solution and the mixture left stirring overnight. The solution was evaporated to dryness to afford a yellow green solid. The sample was triturated in a minimum of acetonitrile to remove the catalyst and the solid filtered. The resulting 5,6-dimethoxy-5,6-dihydrouracil was re-dissolved in boiling methanol and allowed to cool whereupon, a precipitate was obtained, which was filtered from solution. The solid residue was identified as 5-methoxyuracil from its ^{1}H n.m.r., which contained two broad singlets at 10.4δ and 9.24δ, a 1H sharp singlet at 6.36δ and a 3H singlet at 3.66δ. The methoxy uracil was also identified by the EI mass spectrum which contained the characteristic peaks at m/z 143, 142, 141, 114, 99, 71 and 56.

7.9.2.3.2 Discussion.

The reaction showed that by heating the dimethoxy- derivative vigo rously, it could be converted to give the monoalkoxy- product. The spectroscopic data suggested that this product was similar to

the product isolated previously (see Chp. 7.7.1). This suggests that the same product can be derived from two possible routes; thermal decomposition of the dialkoxy- derivative and by rearrangement of the putative 1,3-dipolar intermediate (7.29). The observation that the product tends to precipitates out of methanol suggests that it is being formed from the more soluble dimethoxy- derivative. This would also suggests that the dimethoxy- derivative is not formed by an initial formation of 5-methoxyuracil followed by methanol addition across the 1,6-double bond (Scheme 7.58)



Scheme 7.58

The reaction with methanol behaves in much the same way as the reaction with *n*-butanol, in which the monoalkoxy- derivative was thought to have been produced from the dialkoxy-derivative.

If the starting diazouracil is reacted with methanol using rhodium acetate as the catalyst at ambient temperature, then a precipitate is obtained. The precipitate was identified as 5-methoxyuracil from its ^{1}H n.m.r. and its formula confirmed by microanalysis. The reaction at ambient temperature confirms that 5-methoxyuracil is also formed directly from the decomposition of the starting diazo compound (Scheme 7.58).

7.9.2.4 5,6-Diethoxy-1,6-dihydrouracil.

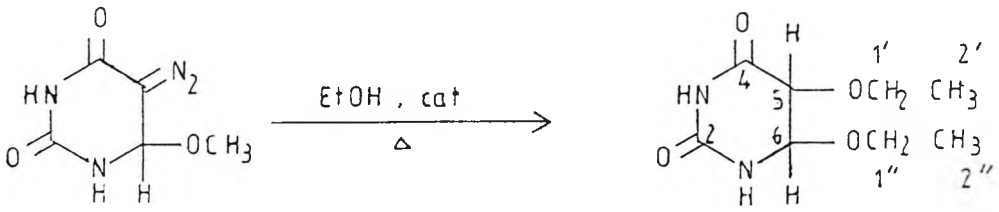
7.9.2.4.1 Preparation.

The reaction of 5-diazo-6-methoxy-1,6-dihydrouracil (6.4) with ethanol (Scheme 7.59) in the presence of rhodium acetate catalyst

afforded a white solid. This crude material was purified by preparative t.l.c. using a mobile phase of dichloromethane/methanol (85:15). The major component, R_f=0.55, was removed from the silica by eluting with ethanol.

The i.r. spectrum of the product was devoid of an absorption peak around 2100cm^{-1} which indicated that the material isolated was not starting material.

The ^1H n.m.r. was characterised by a 6H multiplet at 1.1δ which was assigned to the methyl groups (C-2' and C-2") in the two ethoxy groups. Expansion of this region showed the multiplet to contain two triplets each with a coupling of approximately J=6.0Hz. The methylenes (C-1' and C-1") in the two ethoxy group were represented by a 4H multiplet centred at 3.92δ, J=6.0Hz. The ring protons H-5 and H-6 also appeared as a 2H multiplet at 3.50δ. Expansion of this multiplet showed it to be two doublets at 3.46 and 3.48δ, each having a coupling of approximately J=1.5Hz. The amide and imide N-H protons were not directly visible because of exchange with deuterium in the solvent, methanol-d_4.



(7.93)

Scheme 7.59

The crude material also indicated that a wide variety of products were formed. In addition to diethoxy- derivative (7.93), 5-methoxyuracil was also detected in the spectrum. There also appears to be some 6-ethoxy-5-methoxy-5,6-dihydrouracil present in the crude mixture, which was identified in the n.m.r. spectrum without being isolated.

The formula of the diethoxy- derivative could not be confirmed by microanalysis. The sample was low in carbon (43.63%) and hydrogen (4.85%) but high on nitrogen (16.92%) ($C_8H_{14}N_2O_4$ requires C-47.5%, H-6.98%, N-13.85%).

7.9.2.4.2 <u>EI mass spectrum of ethoxyuracil.</u>

As in the case of the dimethoxy- product, the EI mass spectrum did not afford either the molecular ion or a pseudomolecular ion. Examination of the scan profile shows intermittent sample evaporation, commonly associated with solvent evaporation. This suggests that with increasing temperature, the diethoxy- product loses ethanol to leave an ethoxyuracil. A molecular ion for ethoxyuracil was observed at m/z 156.0452 (M^+, $C_6H_8N_2O_3$ requires 156.05348). The peaks in the spectrum can be related to the fragmentation of this species. The peak at m/z 128 (M-CO), 113 (M-HNCO), 85 ($C_4H_5O_2$), 78 ($C_3H_7NO_2$), 70 (M-2HNCO).

7.9.2.4.3 <u>Discussion.</u>

The main feature of the reaction was the replacement of the methoxyl group with an ethoxyl group. It is not clear at which stage the substitution of the methoxyl group takes place, *viz.*, either the exchange occurs during the reaction or whilst eluting the compound from the silica or in evaporating the eluent to dryness.

It appears that only one isomer is present, however, the work with 5,6-dimethoxy-5,6-dihydrouracil (see Chp. 7.9.2.2) suggests that both *cis-* and *trans-* isomers may have the same n.m.r. spectrum and thus, the observation of one coupling constant for H-5 and H-6 does not indicate the presence of only one isomer.

7.9.2.4.4 <u>5,6-Diethoxy-5,6-dihydrouracil from 5-diazo--6-ethoxy-1,6-dihydrouracil.</u>

5-Diazo-6-ethoxy-1,6-dihydrouracil was dissolved in hot ethanol to afford a bright yellow solution, which was allowed to cool before the rhodium acetate catalyst was added. Effervescence was observed and the reaction left stirring overnight at ambient temperature. The excess reactant/solvent ethanol was evaporated to afford a waxy residue, which was purified using dichloromethane/--methanol (85:15) as the mobile phase. The main band of silica,

R_f=0.35, was removed and eluted with the mobile phase. The eluent was evaporated to dryness to afford a white, crystalline material.

The i.r. spectrum showed an absence of a strong absorption around 2100cm^{-1}, indicating that the diazo compund had reacted by the loss of the nitrogens. The compound was identified by its ^{1}H n.m.r. and was identical to the previous spectrum (see Chp. 7.9.2.4.1).

7.9.2.5 Reaction with water/THF.

7.9.2.5.1 Preparation.

5-Diazo-6-methoxy-1,6-dihydrouracil was dissolved in THF/water (15:5). Rhodium acetate catalyst was added and the solution gently warmed. At 21°C, effervescence was observed from the surface of the catalyst. The solution left stirring overnight and then evaporated to dryness. The residue was purified by preparative t.l.c. using dichloromethane/methanol as the mobile phase (85:15) and the largest band of silica, R_f=0.38, removed and eluted with methanol. The eluent was evaporated to dryness to afford a yellow solid.

The compound was identified by its ^{1}H n.m.r. as identical to methoxyuracil isolated from other reactions, *i.e.* having an olefinic proton at 6.4δ.

7.9.2.5.2 Discussion.

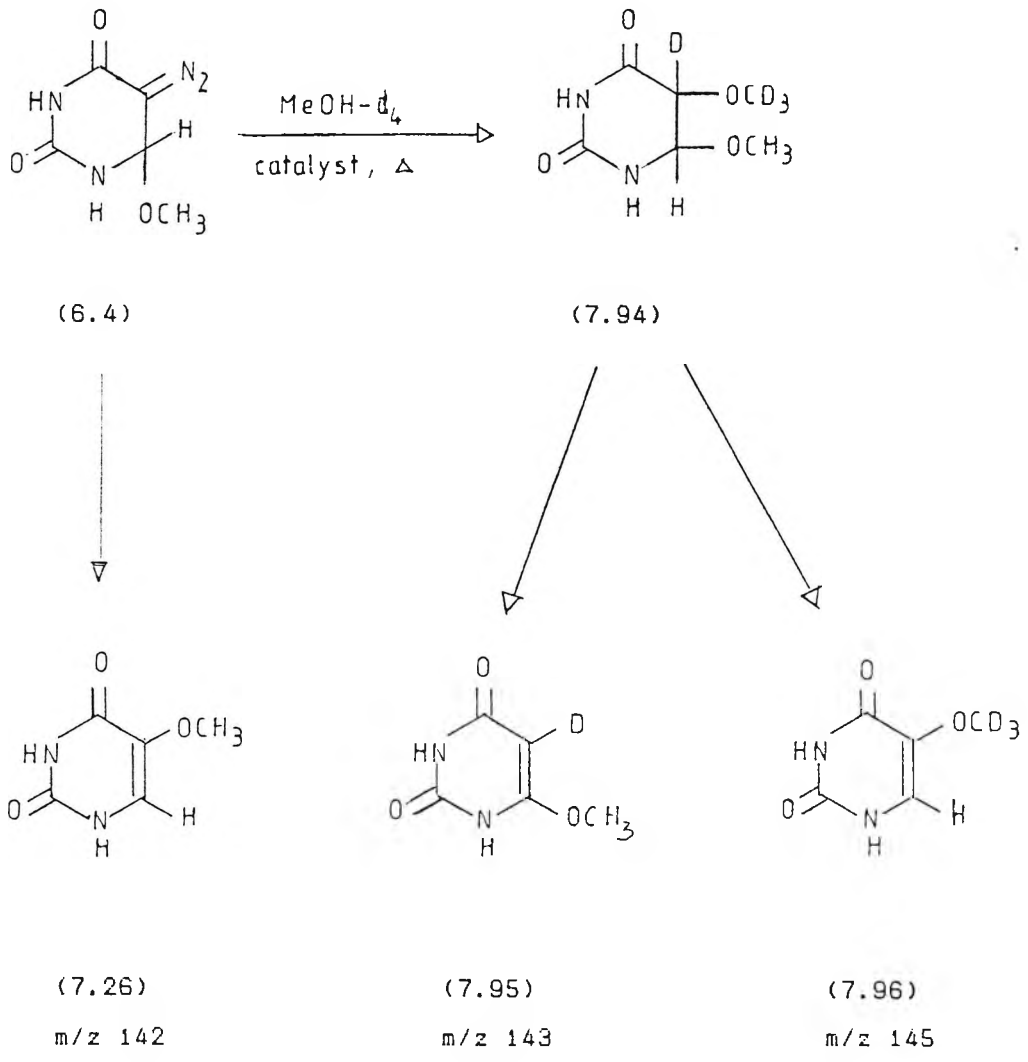
Although the diazo starting material was dissolved in THF and water, the reaction product indicates the strong preference of the intramolecular reaction to occur. It is noticeable that in many of the reactions, the formation of the methoxyuracil is favoured by a low temperature for the catalytic decomposition of the starting diazo material. This probably reflects the lower energy required to move the methoxy group into a positon to react than to move an external molecule into position.

7.9.2.6 5-Trideuteromethoxyuracil.

7.9.2.6.1 Introduction.

In many of the reactions, a methoxyuracil was obtained. Based on the literature (see Chp. 7.7.1), it was concluded that the product was the 5-isomer. An experimental method was used to try to confirm this assignment. From the work with dimethoxydihydrouracil,

it was observed that the same isomeric methoxyuracil could be obtained. Thus, if the two methoxy groups could be distinguished, a direct means of determining the isomer is available. By the use of isotopic labelling, it is possible to distinguish the two methoxy groups. A ready source for isotopically labelled methoxy groups is found in methanol-d_4. The aim was to introduce a trideuteromethoxy-group to give a dimethoxydihydrouracil by reacting the starting material (6.4) with methanol-d_4 (Scheme 7.60). This attaches the isotopically labelled methoxy group to the C-5 position. The resulting material (7.94) could then be converted to methoxyuracil (7.95 or 7.96) and thus, the retention or absence of the trideuteromethoxy- group will show which isomer was formed.



(6.4) (7.94)

(7.26) m/z 142 (7.95) m/z 143 (7.96) m/z 145

Scheme 7.60

7.9.2.6.2 Preparation.

5-Diazo-6-methoxy-1,6-dihydrouracil (6.4) was dissolved in hot methanol-d_4. The solution was allowed to cool to 25°C before rhodium acetate catalyst was added and the mixture left stirring for 12h. The solution was filtered to afford a yellow solid (A) and the filtrate evaporated to dryness. The residue (B) was then dissolved in hot benzene/THF and heated with reflux overnight. This solution was evaporated to dryness and the residue(C1) purified by preparative t.l.c. using dichloromethane/methanol (85:15) as the mobile phase. To afford a pale yellow crystalline material (C2).

The precipitate (A) was examined by EI-MS and 1H n.m.r. The n.m.r. spectrum showed two 1H broad singlets at 10.5 and 9.3δ, which were assigned to the N-H protons. A sharp 1H singlet at 6.4δ was assigned to an olefinic proton and the 3H singlet at 3.8δ was assigned to the methoxy group. This indicated that the methoxyuracil associated with intramolecular rearrangement was present. The compound was confirmed as the methoxyuracil by the EI spectrum (Spectrum 1) by a molecular ion at m/z 142 (61.4%) and an pseudomolecular ion at m/z 143 (4.0%) (expected percentage of C-13 isotope is 3.1%).

The crude product (B) was examined by EI-MS (spectrum 2) a molecular ion for 5-trideuteromethoxy-6-methoxy-5D,6H-dihydrouracil was observed at m/z 178.1004 ($C_6H_6D_4N_2O_4$ requires 178.08943). The molecular ion was present in very low intensity (0.18%) and confirms that the reaction of the diazo compound (6.4) with methanol leads to the dimethoxy product (see Chp. 7.9.2.2). The mass spectrum also showed peaks at m/z 142 (61.0%) for methoxyuracil and m/z 145 (10.0%) for trideuteromethoxyuracil. A peak at m/z 143 (4.16%) was also observed. The expected intensity for methoxyuracil (7.26) owing to ^{13}C isotopic abundance is 3.05%. Thus, the peak at m/z 143 need not necessarily be 5-deutero-6-methoxyuracil.

The crude solid (C1) was examined by EI-MS and found to contain peaks at m/z 145 (2.3%), 143 (0.3%) and 142 (4.7%). The purified solid (C2) gave a very weak n.m.r. spectrum. A singlet was observed at 6.5δ, which was assigned to the olefinic proton. A singlet at 3.5δ was observed and assigned to the methoxy group in methoxyuracil (7.26) carried over in the purification. The EI spectrum of the material was also obtained and contained the anticipated peaks at m/z 145 (5.5%), 143 (1.5%) and 142 (15.5%).

7.9.2.6.3 Discussion.

The results can be interpreted by assuming that two reactions take place (Scheme 7.60). The first reaction is direct formation of methoxyuracil (see Scheme 7.29). The other reaction is the formation of the dimethoxyuracil. In first stage of the experiment, much of the less soluble methoxyuracil precipitates out of solution to give the solid (A). Trideuteromethoxyuracil (m/z 145) was also detected in the EI spectrum. This can be explained by assuming that some of the dimethoxy- derivative (7.94) had co-precipitated out with the methoxyuracil, which leads to a fragment at m/z 145 being detected in the EI spectrum, or by assuming that a small amount of trideuteromethoxyuracil (7.96) was generated during the reaction, which then precipitates out with 5-methoxyuracil.

The EI spectrum (Spectrum 2) of residue (B), which should be the dimethoxy- compound (7.94), had a molecular ion for the compound. This compound serves to illustrate the problems of assessing the proportion of a compound present from its intensity. The intensity of the dimethoxy- compound was far less than the quantity of the methoxyuracil, but it is thought that this is because of the greater tendency for the dimethoxy- compound to fragment. However, in the comparison of m/z 145 to m/z 143 or to m/z 142, the intensities refer to essentially the same compound, *i.e.* methoxyuracil, and consequently a good guide as to proportion of each can be obtained from the examination of the intensity of the molecular ions. In this spectrum, it would be anticipated that the ratio of m/z 145:142 should be larger than in the previous spectrum (Table 7.13). A ratio of m/z 145:142 of 0.16 was observed (Spectrum 2) compared to a ratio of 0.08 (Spectrum 1).

The third residue (C1) should be the methoxyuracil (7.95 or 7.96), which previously had been shown to be the same as the one obtained by rearrangement of 1,3-dipolar intermediate. If the compound was the 6-isomer, then the peak at m/z 145, representing the 5-trideuteromethoxyuracil, would be small or absent and the peak at m/z 143 should be present in a high intensity. The spectrum of the crude material (Spectrum 3) shows that the ratio of the peaks m/z 142:143 is much the same as in the isolated methoxyuracil (A) and in the residue (B). This suggests that the peak at m/z 143 consists of a mixture of the C-13 isotopic isomer and the pseudo-

-molecular ion of methoxyuracil rather than the molecular ion of 6-methoxyuracil (7.95). In the purified product (C2), where a single band was obtained, it suggests that either m/z 145 or 143 would be predominate. In both spectra of (C1) and (C2), the largest intensity is for the peak at m/z 145 which is evidence that the dimethoxy--compound is converted to 5-methoxyuracil. The presence of methoxy--uracil (7.26) formed by rearrangement was provided by the peak at m/z 142. As anticipated the ratio of peak m/z 145:142 has increased from 0.16 to 0.35. The significance of finding a large intensity for m/z 142 (spectrum 4) is that it confirms the single band of silica to be the 5-methoxyuracil/5-trideuteromethoxyuracil band, which agrees with the earlier conclusion that the product obtained by rearrangement is the same as the product formed by the loss of methanol form the dimethoxydihydrouracil.

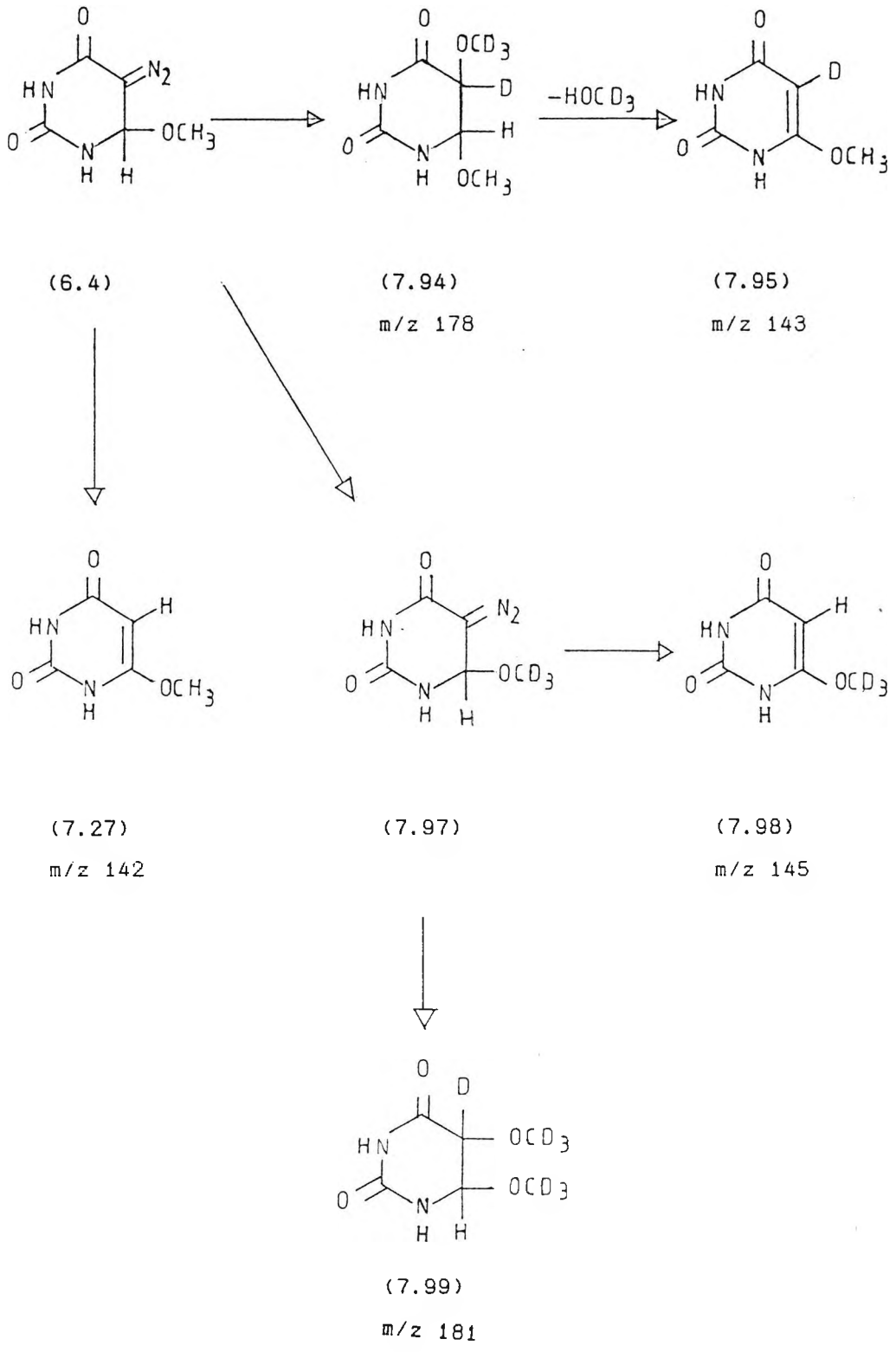
	Relative intensities(%)				ratio (1)	ratio (2)	ratio (3)
Spectrum	178	145	142	143			
1	-	5.5	61.4	4.0	(1:11.2)	(1.4:1)	(15.4:1)
2	0.2	10.0	61.0	4.2	(1:6.1)	(2.4:1)	(14.5:1)
3	-	2.3	4.7	0.3	(1:2.0)	(7.7:1)	(15.7:1)
4	-	5.5	15.5	1.5	(1:2.8)	(3.7:1)	(10.1:1)

ratio (1) intensity of m/z 145: intensity of m/z 142
ratio (2) intensity of m/z 145: intensity of m/z 143
ratio (3) intensity of m/z 142: intensity of m/z 143

Table 7.13 relative intensities of methoxyuracil and trideutero--methoxyuracil.

The examination of the ratios of the intensities is important as an alternative explanation for the results can be offered. The results can be explained in terms of several participating reactions (scheme 7.61). In the scheme, the two reactions are the 1,2-shift of hydrogen in the 1,3-dipolar intermediate (7.29) to give the 6-methoxyuracil (7.27) and the exchange of the methoxyl group in the

starting material to give 5-diazo-6-trideuteromethoxy-1,6-dihydro--uracil (7.97), which then undergoes decomposition and rearrangement to give the 6-trideuteromethoxy isomer (7.98).



Scheme 7.61

In this scheme, a peak at m/z 145 is also formed and consequently the peak masses can not by themselves provide sufficient information to distinguish between the schemes. In this scheme (Scheme 7.61), the 6-trideuteromethoxy- isomer (7.98) must be formed from the trideuteromethoxy- intermediate (7.97). Although a peak at m/z 146 was detected, it was shown that this peak can be explained in most cases by the ^{13}C isotopic abundance for the peak at m/z 145 rather than by the diazo intermediate (7.97)(fragment P-N_2). In addition, the absence of the peaks at m/z 139, 138 and 103, representing the commonly associated fragments [M-CD_3O], [M-CD_3OD] and [M-HNCO] (see Chp. 6), confirms that peak at m/z 146 was not from the intermediate (7.97). The spectra did not provide any evidence for the expected formation of the *bis*-dideuteromethoxy- compound (7.99). It may be argued that this peak is unlikely to be observed because of excessive fragmentation and the low percentage of the precursor (7.97) available to undergo addition with methanol-d_4.

In the second scheme (Scheme 7.61), the way the intensities of the various peaks alter at each stage can be used to distinguish it from the first scheme (Scheme 7.60). In the first spectrum (Spectrum 1), the intensity of the peaks at m/z 145 and 142 should be high, since they represent the precipitation of the 6-methoxyuracils (7.27 and 7.98). The residue (B) should then contain a high quantity of the dimethoxy- derivative (7.94), which when heated leads to the 6-methoxyuracil (7.95). Thus, the residue (C1) should now contain a high quantity of the newly formed 5-deutero-6-methoxyuracil (7.95) and the intensity of the peak at m/z 143 should increase. Correspondingly, the ratio of 142:143 and the ratio of 145:143 should be smaller than in the previous spectra (Spectrum 1 or 2). In addition, with no further formation of 6-methoxyuracil (7.27) and 6-trideuteromethoxyuracil (7.98) after the first step, the ratio of m/z 145:142 will remain constant in the remaining spectra (Spectrum 2, 3 and 4). Since neither the ratios m/z 145:143 nor 142:143 is smaller and the ratio of 145:142 does not remain constant in spectra 2 and 3, it can be concluded that the data best fits the first scheme (Scheme 7.60). Thus, the experiment with methanol-d_4 also supports the previous finding that the isolated methoxyuracil is the 5-isomer.

Surprising features of the results are the differences between the last spectra (Spectrum 3 and Spectrum 4). Unexpectedly,

the ratio 145:143 has decreased, however, this is not evidence for second scheme (Scheme 4.61) since a decrease in the ratio 145:142 is also observed and thus, the increase in the relative amount of m/z 143 can be explained as the resulting increase of the pseudo--molecular ion of methoxyuracil (7.26). The difference in the ratio 145:143 can be attributed to differences in sample composition and statistical distributions of ion population and energy levels. The higher amounts of m/z 143 can be explained in part by the operating conditions leading to more pseudomolecular formation (Table 7.14).

	Relative intensities(%)					
Spectrum	146	145	142	143	145:146	142:143
1	0.2	5.5	61.4	4.0	27.5:1	15.4:1
2	0.5	10.0	61.0	4.2	20.0:1	14.5:1
3	0.2	2.3	4.7	0.3	11.5:1	15.6:1
4	0.6	5.5	15.5	1.5	9.2:1	11.1:1

Table 7.14 relative intensities of methoxyuracil and trideutero- -methoxyuracil.

7.9.2.6.4 Suggestions for further work.

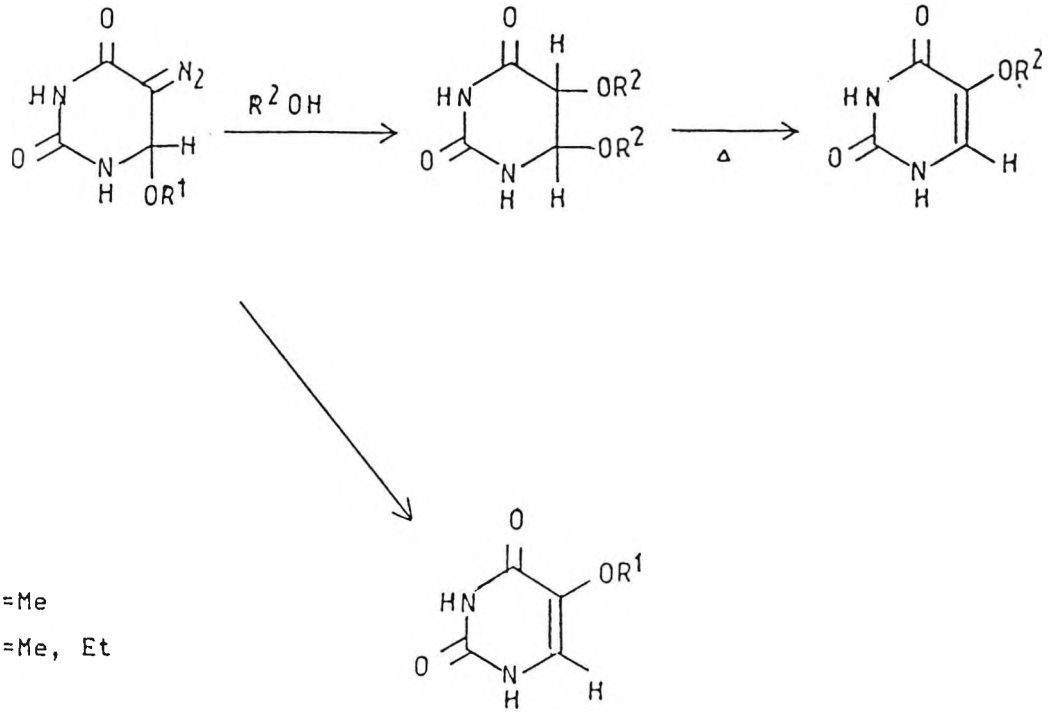
One approach would be to examine if the trideuteromethoxy--uracil (7.97) species could be isolated. This would involve stopping the reaction at around 50% completion and examining the products. In this approach, quenching the reaction may prove a problem. A suggested method would be to add dichloromethane in an attempt to precipitate out the polar uracils. The reaction mixture may then be analysed by EI-MS without further purification. The purification by t.l.c. to remove the catalyst should be avoided if possible as there is a likelihood that silica might be responsible for the exchange of alkoxyl groups in these type of compounds (see Chp. 7.9.2.4.3). Another approach to examining the migration of the methoxyl group would be to use ^{13}C enriched methanol to prepare the starting diazo (6.4). This should allow a coupling constant between C-6 and the carbon in the methoxyl to be measured. The reaction of the diazo compound can then be carried out in acetone

and the ^{13}C spectrum run. It should then be possible to determine which of the two ring carbons is coupled in the product.

The exchange of the alkoxy groups should be studied by exploring the formation of mixed dialkoxy- dihydrouracils. The different 6-alkoxy- and 6-aryloxy- 5-diazo-1,6-dihydrouracils which have been prepared (Chp. 6) could be used to react with different alcohols. A study to see whether the 5-alkoxy- uracils are capable of undergoing exchange with different alkoxyl groups would also be of interest.

7.9.3 General Discussion.

The reaction of the 5-diazo-6-methoxy-1,6-dihydrouracil with ethanol and methanol readily produce the expected 5,6-dialkoxy-5,6--dihydrouracils (Scheme 7.62).

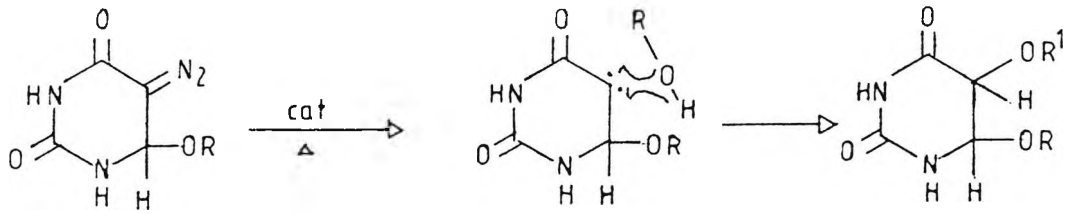


R^1 =Me

R^2 =Me, Et

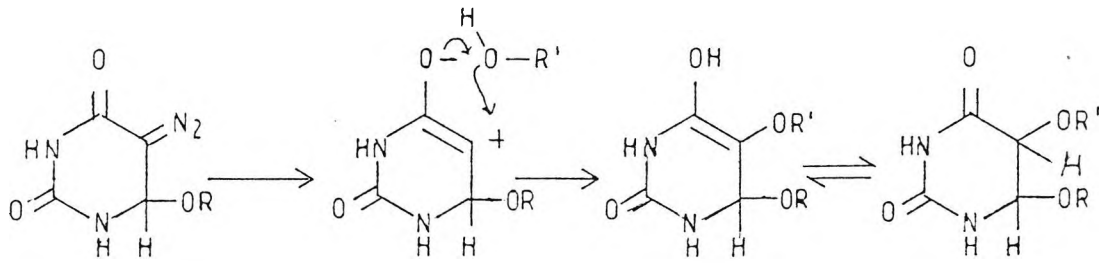
Scheme 7.62

The formation of the dialkoxydihydrouracil can be explained by assuming the formation of a keto-carbenoid species, which then reacts by inserting into the O-H bond to form the 5-alkoxyl-5H-species (Scheme 7.63).



Scheme 7.63

However, the formation of the dialkoxy species does not necessary mean that the the putative keto-carbenoid has a structure similar to a carbene. An equally feasible mechanism to the dialkoxyl adducts involving a 1,3-dipolar structure for the keto carbenoid can be proposed (Scheme 7.64)



Scheme 7.64.

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Chapter 8 Reactions in which the diazo group is retained.

8.1 Introduction.

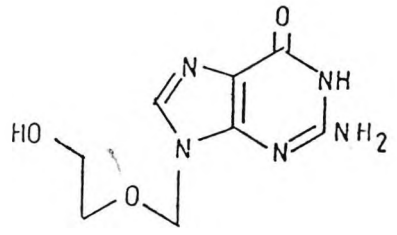
8.1.1 Biological activity of nucleoside analogues: Acyclovir-type drugs.

In general, the action of drugs can be attributed to the interaction of the drug molecule with specific receptors possessed by the organism. The efficacy of the drug depends on factors which influence the binding of the drug and its target receptor and the the extent of the response elicited by the binding. The importance of "binding" is related to its major role in selective activity of drugs. The mechanism of enzyme-ligand recognition was proposed to be analogous to the workings of a "lock and key" mechanism [1]. In this analogy it was proposed that the enzyme binding site and the substrate had a structural complementarity [1]. This theory can be extended to cover the wide spectrum of receptor-ligand interactions.

In principle, there must exist a structural similarity between the drug compound and the natural ligand, which allows the specific recognition of the drug molecule. Based on the "lock and key" analogy, the natural ligand was considered to be the best fit in terms of structural recognition. It can be inferred that the greater the similarity in structure between the drug and the natural ligand the better the recognition. However, evidence from binding studies of putative transition state analogues showed that in terms of structure recognition, as given by the stronger binding, the natural ligand was not the best fit and that better binding was observed with transition state analogues [2-3]. This observation led to the induced-fit theory [4-6] in which the enzyme arranges itself to fit the ligand. The energy required to fit the enzyme, although considerable, was offset by the energy released in forming the complex. An alternative to expending this energy and generating strain, in altering the conformation of the macromolecule to fit the ligand, would be to allow the drug to have or be able to attain the transition state. This may be achieved by giving the ligand a greater flexibility. Thus, the ligand would be able to arrange itself to match the active site and the energy for binding would be much lower. In the case where the substrates are cyclic, a greater degree of freedom may be achieved in related drugs by the use of the conformationally more flexible open chain structures, *i.e.* in acyclic analogues.

In nucleosides, modified acyclic analogues may be achieved by either replacing the cyclic sugar moiety with a corresponding acyclic portion or by replacing the heterocyclic base with its acyclic equivalent. It is known that the replacement of the sugar moiety with an acyclic residue has led to analogues with extremely potent antiviral activity [7]. Important compounds of these type include guanosine analogues, *e.g.* 9-(2-hydroxyethoxymethyl)guanine, which is under clinical use as acyclovir (8.1) [8], 9-[(2-hydroxy-1--(hydroxymethyl)ethoxy)methylguanine [DHPG] (8.2) [9] and (S)-9--(2,3-dihydroxypropyl)adenine [DHPA], an adenosine analogue (8.3) [10-12].

Recently, the C-nucleoside antibiotics formycin and pyrazofurin have been examined and have demonstrated both antiviral and antitumour activity [13-15]. Unfortunately, these compounds proved too toxic for antiviral use. However, pyrazofurin is still being evaluated as an anti-tumour agent [16].



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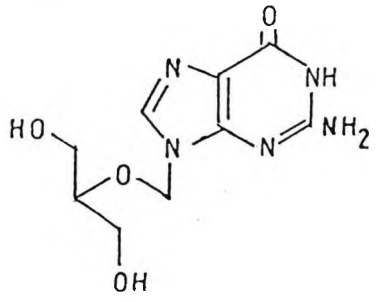

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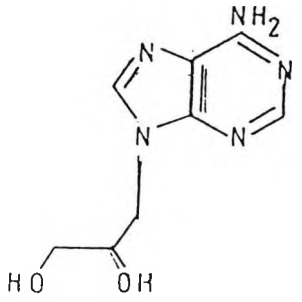

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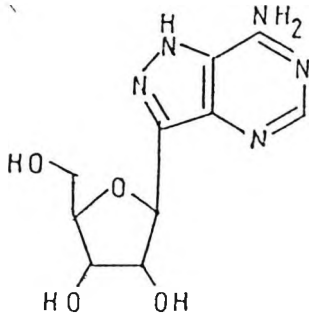

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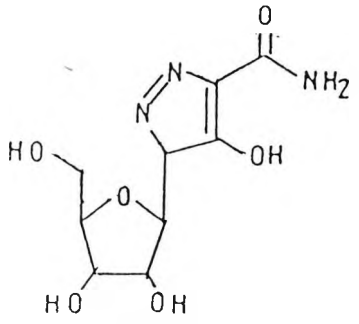

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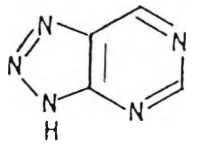
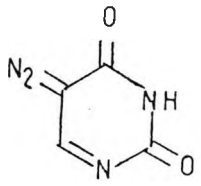
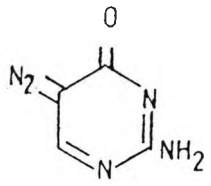


(8.6)



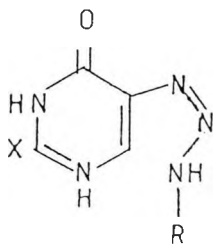
(8.7)



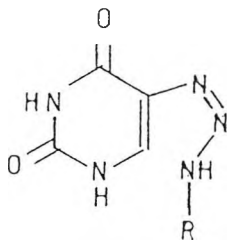
(8.8)

A logical extension of the work on acyclovir, DHPG, formycin (8.4) and pyrazofurin (8.5) would be the synthesis of acyclic analogues of 8-azapurines (8.6). A good starting point would be the synthesis of an acyclic analogue of one of the purines which would be based on the 5(3-alkyl-1-triazeno)pyrimidine. One method by which the triazine side chain might be synthesised would be by the reaction of a suitable diazo compound with an amine [17-20]. A suitable choice for the diazopyrimidine would be either to use 5-diazouracil (8.7) or 2-amino-5-diazopyrimidin-4(1H,3H)-one (8.8).

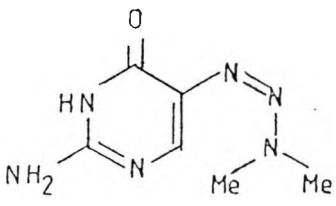
When reacted with an amine, the diazo compounds would lead to the formation of 5(3-alkyltriazeno)-4-pyrimidone (8.9) which could be considered as acyclic analogues of azapurine. If diazouracil is used with an amine, then the expected product would be an acyclic azaxanthine analogue (8.10). This compound is of interest because of the central role of xanthine triphosphate in the biosynthesis of nucleic acids. The reaction of 2-amino-5-diazopyrimidin-4(3H)-one with an amine would lead to acyclic azaguanosine analogues (8.11).



(8.9)



(8.10)



(8.11)

Whilst examples of azaguanosine analogues could be found in the literature, there did not appear any reports of azaxanthine analogues. The formation of 5-(3,3-dimethyl-1-triazeno)uracil from

5-diazouracil and the secondary amine, diethylamine, had been reported previously [21]. It was therefore decided to explore the nature of the triazine reaction with a tertiary amine, an aromatic amine and primary amines.

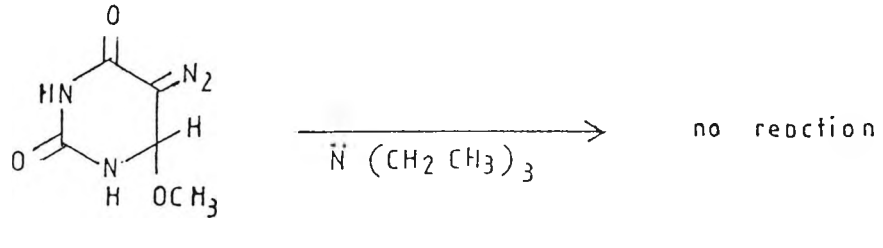
The tertiary amine decided upon was triethylamine, which was chosen because the simpler tertiary amine, trimethylamine, was only commercially available as an aqueous solution or as a hydrochloride salt. Aniline was chosen as the aromatic amine. Several primary amines were also selected as it was hoped that, by altering the alkyl group, a wide variety of analogues could be generated and that these experiments would make accessible analogues with moieties having similarities to a sugar ring.

8.2 Results and Discussion.

8.2.1 Attempted reaction of 5-Diazo-6-methoxy-1,6--dihydro-uracil with triethylamine.

5-Diazo-6-methoxy-1,6-dihydro-uracil was dissolved in triethylamine and stirred. After 10 minutes, a precipitate was formed. The excess solvent was evaporated to afford an orange solid.

The precipitate displayed an infrared spectrum which was characterised by a strong absorption at 2110 cm-1 which showed that the diazo stretch had remained unaffected. A comparison of infrared spectra, confirmed the precipitate to be starting material. It can be concluded that there is no reaction (Scheme 8.1).



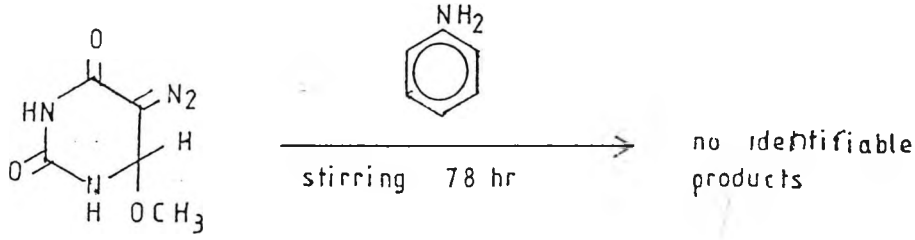
Scheme 8.1

8.2.2 Reaction of 5-Diazo-6-methoxy-1,6-dihydrouracil with aniline.

5-Diazo-6-methoxy-1,6-dihydrouracil was dissolved in aniline and heated with reflux for 48h. The material was allowed to cool to room temperature and left standing. After 1 week, there was no

visible change from the dark red/brown solution. The solution was added to acidified ice/water and partitioned with ether. The red ether layer was separated from the orange aqueous phase and washed with distilled water and dried before being condensed to afford a brown oil.

Attempts to purify the oil by column chromatography or by preparative t.l.c. failed to achieve a separation into components (Scheme 8.2).

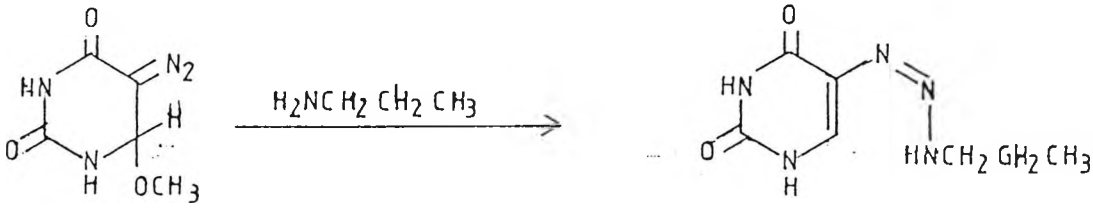


Scheme 8.2

8.2.3 5(3-Propyl-1-triazeno)uracil.

8.2.3.1 Preparation.

The 5-diazo-6-methoxy-1,6-dihydrouracil was stirred in *n*-propylamine until it had dissolved. After 5min., a yellow/white precipitate was obtained. The precipitate was filtered from the red solution, washed with dry ether and dried in a vacuum desiccator (Scheme 8.3).



Scheme 8.3

The i.r. of the compound was characterised by the absence of the diazo stretch associated with the starting material at 2110cm^{-1}. The broad absorption at 1680cm^{-1} was assigned to the carbonyl functional groups in the uracil ring. Strong sharp absorptions around 3360 and 3310cm^{-1} were associated with the N-H stretching vibrations in amines, amides.

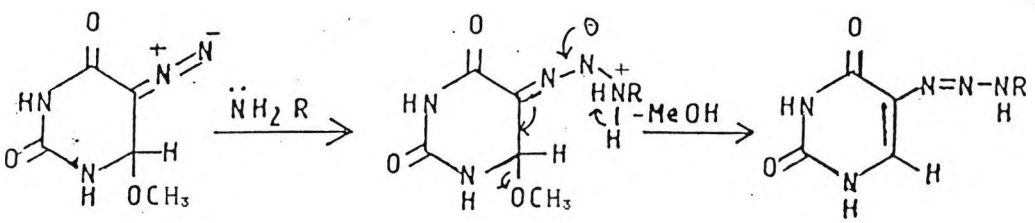
The ^{1}H n.m.r. of the compound was consistent with the proposed structure (8.3a). The *n*-propyl group is readily recognized by its clearly defined multiplets and well separated signals (Table 8.1).

chemical shift, δ	Assignment
10.18 s 1H	H-3' exchangeable in D_2O
9.18 s 1H	H-6
8.00 bs 1H	H-3 exchangeable in D_2O
7.66 bs 1H	H-1 exchangeable in D_2O
4.58 t 2H J=6.75Hz	H-1"
1.92 m 2H J=6.75Hz	H-2"
0.98 t 3H J=6.75Hz	H-3"

Table 8.1. ^{1}H n.m.r. 5(3-propyl-1-triazeno)uracil (dmso-d_6)

There are several features of the spectrum which support the proposed structure. One feature, obtained from the examination of the chemical shifts of the protons in the alkyl group, clearly indicates that the amine is attached to a strongly electron withdrawing group. This feature is made manifest by the downfield chemical shifts of the methylenes in the propyl group when compared with their shifts in the unreacted *n*-propylamine. The C-1" methylene was shifted downfield by 1.82δ. The C-2" methylene was shifted downfield by 0.7δ. The methyl C-3" retained the same chemical shift.

The loss of the methoxyl group was indicated by the absence of a sharp 3H singlet around 3.4δ. Instead, a sharp 1H singlet at 9.14δ was observed. The loss of the methoxyl group but the retention of both the H-1 and H-6 protons suggests a likely mechanism which leads to an anionic C-5 intermediate (Scheme 8.4).



Scheme 8.4

8.2.3.2 EI mass spectrum.

The retention of the diazo nitrogens was confirmed by the observation of a peak at m/z 197.0882 for the molecular ion ($C_7H_{10}N_5O_2$ requires 197.09126). An [M+1] species was also observed owing to ^{13}C isotopic abundance. The peaks at m/z 181, 169, 168 were assigned to [M-CH_4], [M-C_2H_4] and [M-C_2H_5]. The diazouracil portion of the molecule was identified from the $C_4H_5N_4O_2$ fragment at m/z 139. The propylamine portion was also present at m/z 58. A peak at 154.0658 was observed and possibly resulted from the unresolved peaks for [M-HNCO] (m/z 154.091316) and [M-C_3H_7] (m/z 154.042351).

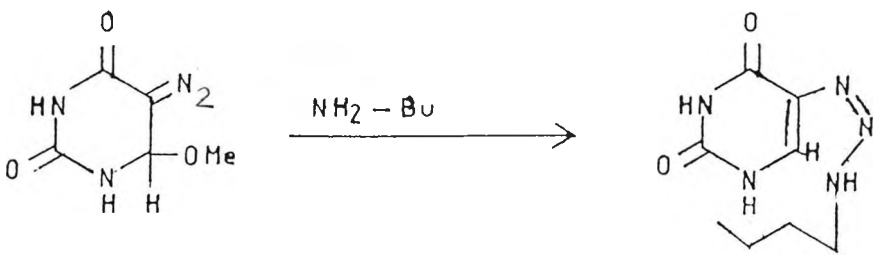
8.2.4 5(3-Butyl-1-triazeno)uracil.

8.2.4 Preparation.

5-Diazouracil was mixed with freshly distilled *n*-butylamine and the bright orange solution was left stirring overnight, whereupon a colour change to bright yellow and the formation of a precipitate was observed. An off-white residue was filtered from the yellow solution and washed with ether before being left to dry in a vacuum desiccator.

The i.r. of the compound (8.13) did not contain the strong absorption band for the diazo group. The presence of the uracil group was inferred from the carbonyl absorption around 1680cm^{-1}.

The formula of the compound was confirmed by microanalysis; found C45.44%, H6.19% and N32.8% (5(3-*n*-butyl)triazenouracil, $C_8H_{13}N_5O_2\cdot$ requires C45.49%, H6.20%, N33.15%) and confirmed that the reaction had proceeded with retention of the diazo nitrogens (Scheme 8.5). The EI spectrum this compound did not show either a molecular ion nor a pseudomolecular ion. The compound underwent extensive fragmentation and resulted in fragments containing little structural information.



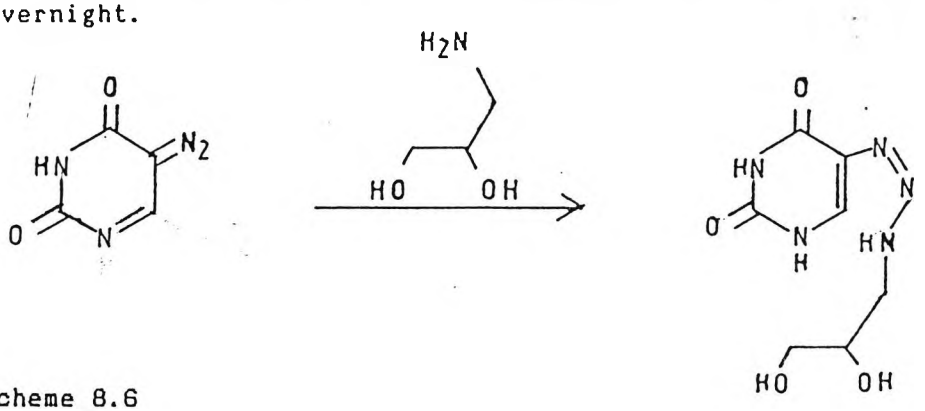
(8.13)

Scheme 8.5

8.2.5 5(3-(2,3-Dihydroxypropyl)-1-triazeno)uracil.

8.2.5.1 Preparation.

5-Diazouracil was added to a mixture of 3-amino-1,2-propane--diol and 1,4-dioxan (Scheme 8.6). The dioxan was added because it was thought that the high viscosity of 3-aminopropane-1,2-diol would hinder reaction. In order to aid the mixing of the diazo compound, ultrasonics was employed. The mixture turned orange and it was assumed that the compound was going into solution. The mixture was then left stirring overnight to afford a precipitate. The solvent was removed by decanting the liquid phase and its subsequent filtration. The residues were combined and dried under vacuum overnight.



Scheme 8.6

The i.r. spectrum of the compound was characterised by the absence of the starting diazo absorption band. The presence of the uracil moiety was inferred from the carbonyl absorptions at 1720 and 1680cm^{-1}.

The ^{1}H n.m.r. of the compound was characterised by the singlet at 9.14δ associated with the C-6 position of the uracil ring. As in the formation of the 5(3-propyl-1-triazeno)uracil, a chemical shift of 9.14δ can be related to the vinylic nature of the C-6 position. Although most of the propyl protons were masked by the broad envelope of the hydroxyl protons, the attachment of the amine to the diazo nitrogens was deduced from the relative downfield shifts. It was possible to observe that the C-1" methylene was present at 3.12δ, which represented a downfield shift of 0.52δ when compared to the starting amine. The C-2 proton on the propanediol was similarly shifted downfield but to a lesser amount, 0.4δ.

The addition of deuterium oxide simplified the spectrum by removal of the hydroxyl protons and allowed three signals, two doublets and a multiplet at 4.56δ (J=4.0Hz), 4.40δ (J=7.5Hz) and

4.12δ (J=4.0Hz and J=4.0Hz) respectively, to be observed. From these couplings, it is possible to assign the signals (Table 8.2).

Chemical shift, δ	Assignment
9.14 s 1H	H-6
7.20 bs 1H	H-3
6.84 bs 1H	H-1
4.20 d 1H (J=4.0Hz)	Ha-3"
4.06 d 1H (J=7.5Hz)	Hb-3"
3.86 m 1H (J=4.0Hz, J=7.5Hz)	H-2"
3.12 m 2H (J=4.0Hz)	H-1"

Table 8.2 ^{1}H n.m.r. of 5(3-propyl-1-triazeno)uracil

The microanalytical data showed the compound to be contaminated with dioxan, water and aminopropanediol. The difficulty in removing the viscous, highly polar aminopropanediol from the product was resulted in the low nitrogen but high carbon and hydrogen values obtained: carbon (37.04%), hydrogen (5.49%), nitrogen (25.24%) ($C_7H_{11}N_5O_4$ requires C36.68%, H4.84%, N30.56%). The observed values gave a higher carbon value than the starting diazo compound, which indicates the addition of the aminopropanediol. The value for nitrogen indicates that the product contained more nitrogen than aminopropanediol and this indicates the presence of the diazouracil moiety.

8.2.5.2 EI mass spectrum.

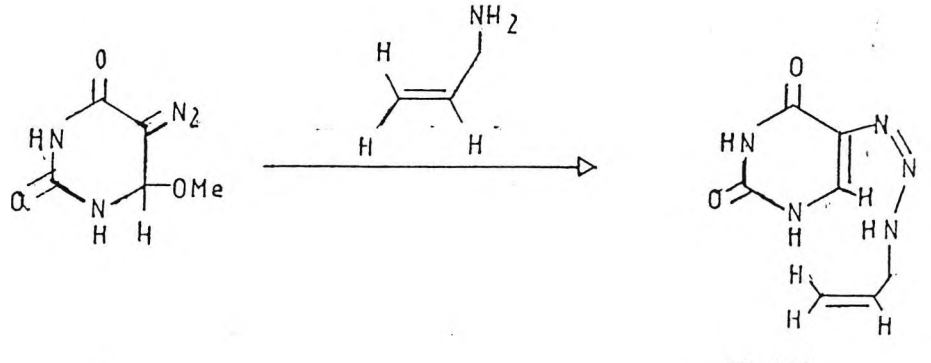
The EI spectrum of 5(3-(2,3-dihydroxypropyl)-1-triazeno)uracil was characterised by extensive fragmentation and neither the molecular ion nor the pseudomolecular ion was observed. Many of the fragments. Surprisingly, the anticipated peaks at m/z 139, 138 associated with the diazouracil moiety were also not observed. The fragments at m/z 89, 91 and 119 were identified as $C_3H_7NO_2$, $C_3H_9NO_2$ and $C_3H_8N_3O_2$. These fragments provide evidence of the aminopropane diol and the triazeno- dihydroxypropyl group. Evidence for the uracil portion was provided by the fragment $C_4H_4N_2O_2$ at m/z 112. The closest species to the molecular ion are the [M-CH_2O] and [M-CH_3O]

species at m/z 199.0898 ($M-CH_2O$ requires 199.07052) and m/z 198.0618 ($M-CH_3O$ requires 198.0627). The fragment $M-C_2H_5O_2$ was also observed at m/z 168. It appears that the fragmentation of the alkyl chain is the most prominent process.

8.2.6 5(3-(2-Propenyl)-1-triazeno)uracil

8.2.6.1 Preparation.

5-Diazo-6-methoxy-1,6-dihydrouracil was stirred in neat allylamine at 23°C. After 5min., a white precipitate was obtained. The solution was then left to stir at ambient temperature for 24h. The white precipitate was filtered from solution and washed with dry ether, before drying in a vacuum desiccator (Scheme 8.7)



(8.15)

Scheme 8.7

The i.r. of the product contained no diazo absorption around 2120cm^{-1} which indicated that the starting material had reacted. The carbonyl absorptions at 1760 and 1670cm^{-1} were present and indicated the uracil moiety.

The ^1H n.m.r. showed the characteristic sharp 1H singlet at 9.2δ, assigned to the H-6 proton. The broad 1H singlets at 10.24, 8.2 and 7.9δ were assigned to the N-3', N-3 and N-1 protons, respectively. The complex second order signals, integrating for 5H, between 6.7-5.3δ, provided evidence that the vinylic portion of the allyl chain had not reacted with the diazo. A change in the coupling pattern was observed and this was attributed to the triazeno portion of the molecule.

8.2.6.2 Mass Spectra.

The FAB spectrum of the product, in a glycerol matrix, was characterised by an intense pseudomolecular species [P] at m/z 196,

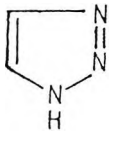
which was the base peak for the spectrum. At high resolution, it had a measured mass of m/z 196.08427 ($C_7H_9N_3O_2$+H requires 196.08343). Both the molecular ion [M+] and the [M-H] species gave very small peaks. A peak at m/z 218 was assigned to the complex with sodium and the peak at m/z 234 associated with the complex with potassium. the peak at m/z 287 was assigned to the species [P+mat], which represents a complex of the pseudomolecular species with the matrix material. Thus, all the expected complexes were observed. An [M+P] species was observed at m/z 191.

Typical fragments include both [M-HNCO] at m/z 152 and [P-HNCO] at m/z 153. As expected, peaks at m/z 140 and 139 and 138 all identified the starting material. The species [P-2HNCO] and [M-2HNCO] were observed at m/z 110 and 109, respectively. The loss of the carbon monoxide from the pseudomolecular ion and from the molecular ion were observed at m/z 168 and 167.

8.2.7 Cyclisation of triazenes.

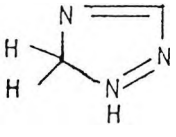
8.2.7.1 Introduction: Approaches to triazoline formation via the cyclisation of triazenes.

Any five-membered ring containing three nitrogens is a triazoline. Triazolines can be divided into two classes according to the arrangement of the nitrogens, *i.e.* 1,2,3-triazolines (8.16) and 1,2,4-triazolines (8.17).



2,4-1,2,3-triazine

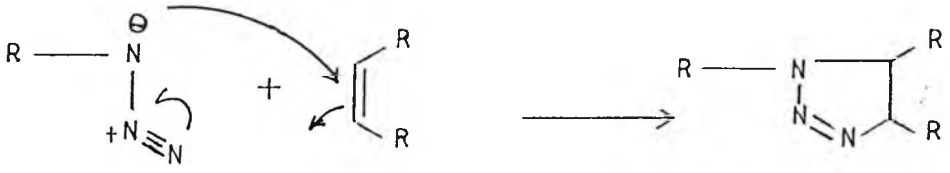
(8.16)



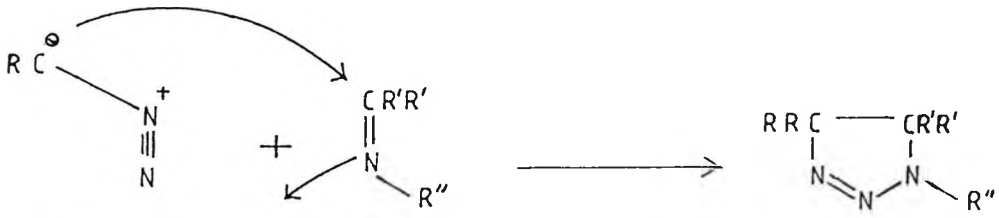
1,3-1,2,4-triazine

(8.17)

Triazolines have been of interest because of their similarity to imidazole and consequently, they might be expected to show biological activity. 1,2,3-Triazolines are readily prepared by the cycloaddition of an azide with an olefin (Scheme 8.8) or by the reaction of a diazo compound with an imine (Scheme 8.9).

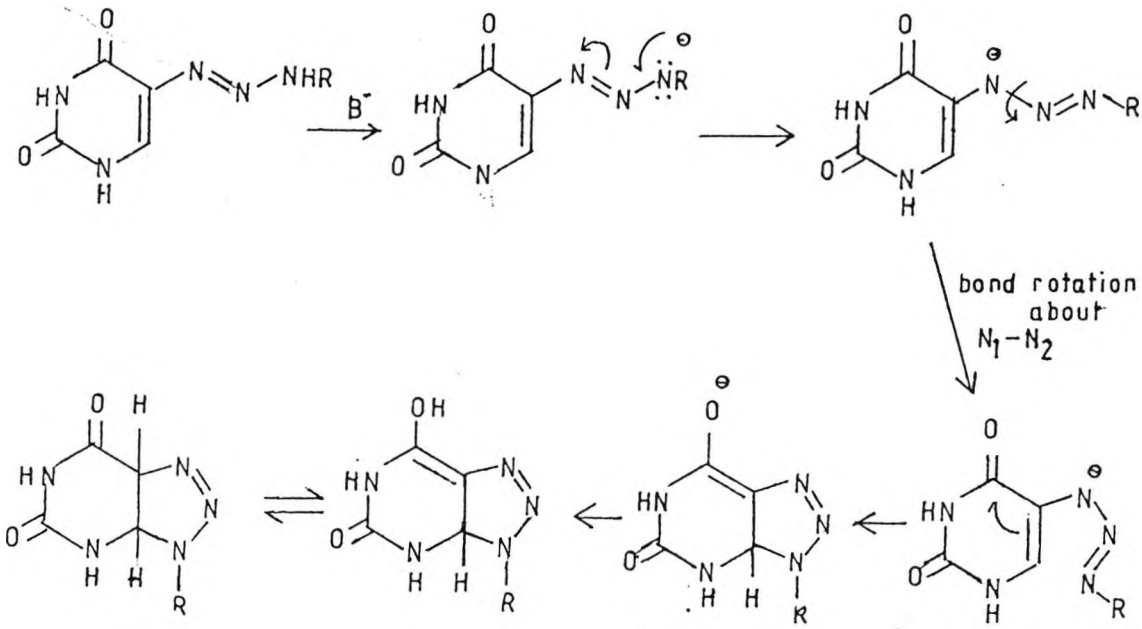


Scheme 8.8



Scheme 8.9

The reaction of diazouracil with simple amines gave the open-chain triazeno- compounds, which could be considered as acyclic analogues of azapurines. It would be of interest if the 8-azaxanthine also could be formed. Inspection of the ^1H n.m.r. of 5(3-propyl-1-triazeno)uracil suggested that the most acidic proton was the one located on the terminal triazeno nitrogen, rather than either the imide or amide protons. It was proposed that under the right conditions, a Michael-type addition of the 3'-nitrogen to form the triazoline group might occur. The C-6 carbon of the ring was considered to be a suitable site for addition, it being the *B*-position of an enone system and hence prone to nucleophilic substitution. To help in this reaction, it was decided that treatment with a base would provide an anion which would be better at attacking the C-6 atom. It was hoped that formation of the ion might help energetically in the inversion of the expected *trans* geometry of the triazeno- group (Scheme 8.10). The selection of the base was directed by considerations about a competing reaction: if the base was also a strong nucleophile, it could also attack the C-6 position of the uracil ring instead of abstracting the N-H proton. In order to avoid this reaction a reagent with strong basicity and poor nucleophilicity, 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (8.16) was used. The reaction was followed by n.m.r.

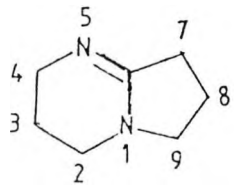


Scheme 8.10

8.2.7.2 Reaction of 5(3-propyl-1-triazeno)uracil with DBN.

8.2.7.2.1 ^{1}H n.m.r. studies.

The reaction was carried out in an n.m.r. sample tube which allowed the reaction to be followed directly. A small quantity of 5(3-propyl-1-triazeno)uracil was dissolved in dmso-d_6 and its spectrum run. For comparison, the spectrum of DBN was also run (Table 8.3). A small quantity of DBN was added to the sample and the ^{1}H n.m.r.spectrum run at suitable time intervals.



(8.16)

δ		Assignment
3.42 t	2H J=6 Hz	(H-4)
3.04 t	4H J=6 Hz	(H-2,9)
2.40 t	1H J=7.5	(H-7)
1.84-1.76 m	2H	(H-3,8).

Table 8.3 ^{1}H N.M.R. 1,5-Diazabicyclo[4.3.0]non-5-ene

δ	assignment
9.5 bs	N-H
8.76 s	C-6
7.62 bs,	
7.56 bs,	
4.36 t,	propyl
1.90 m,	Propyl
0.88 t.	propyl

Table 8.4 The n.m.r. of the propyltriazenouracil before the addition of DBN

δ	assignment
8.76 s,	Uracil C-6
8.46 s,	New peak
7.64 bs,	Uracil NH
7.4 bs,	Uracil NH
4.36 t	(H-1",propyl)
3.26-3.06 m,	DBN
3.30 t,	DBN
2.06-1.56 m,	DBN (H-2",propyl)
0.86 t	(H-3',propyl)

Table 8.5 N.m.r. of the reaction mixture, 5 minutes after the addition of DBN

Time	relative heights of signals 8.76δ : 8.46δ
5 minutes	62:18
20 minutes	69:47
40 minutes	32:52
1h 10 minutes	23:63
22h 00 minutes	10:71
41h 20 minutes	0:81

Table 8.7 Relative peak heights.

It was anticipated that the base would remove the most down field signal of triazenouracil. If no further reaction took place then it might be anticipated that the only observation would be a chemical shift of the first methylene in the propyl side chain. However, an indication that reaction was occurring at the the C-6

position would be the disappearance of the signal associated with the H-6, 8.76δ, signal in the diazouracil derivative. A new signal at 8.46δ was observed to have formed approximately at a rate at which the H-6 signal was being lost (Table 8.7). The retention of the signals associated with the N-3 and N-1 hydrogens suggest that any observed shift is not due to deprotonation at these positions.

8.2.7.2.2 ^{13}C n.m.r. studies.

The reaction was also investigated with ^{13}C n.m.r. The ^{1}H decoupled ^{13}C n.m.r. spectrum of 5(3-propyl-1-triazeno)uracil (Table 8.8) and ^{1}H decoupled ^{13}C n.m.r. of DBN were collected for reference (Table 8.9). DBN was mixed and with the triazenouracil and a spectrum collected. More starting material was added to the reaction mixture and the spectrum repeated after 48h (Table 8.10).

δ	intensity	assignment
160.226	342	C-4
153.079	352	C-6
140.666	286	C-2
127.868	292	C-5
51.394	386	C-1"
22.805	401	C-2"
10.525	360	C-3"

Table 8.8 ^{1}H decoupled ^{13}C spectrum of triazenouracil

δ	intensity	assignment
159.316	3616	
50.679	5840	
43.207	5838	
42.493	6430	
30.472	5697	
29.661	5323	
19.232	5659	

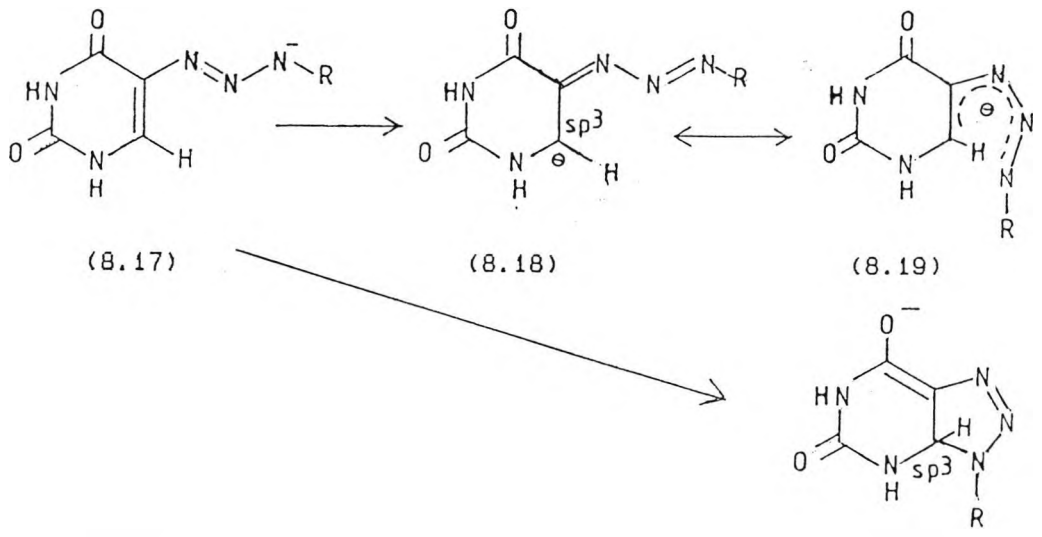
Table 8.9 ^{1}H decoupled ^{13}C spectrum of DBN

δ	intensity	assignment
161.655	509	C-6
160.548	1387	C-4
142.683	326	C-2
127.309	498	C-5
46.326	308	C-1" (propyl)
22.935	509	C-2" (propyl)
10.590	351	C-3" (propyl)

Table 8.10 ^{1}H decoupled ^{13}C spectrum of the reaction mixture

The chemical shifts were assigned for the starting material on the basis of the data on 1,2,3-triazoles [22]. The ^{13}C n.m.r. showed that the product differed from the starting material in several ways: all the propyl carbons were shifted upfield. The signals associated with the uracil portion were shifted downfield. One important observation was the disappearance of the signal at 153δ. A likely explanation for the absence of the C-6 signal is that it has been shifted to a position under one of the DBN peaks. The peak at 127δ has stayed much the same. The peaks at 160δ, and 140δ appear to have been shifted slightly downfield.

The ^{13}C data did not provide clear evidence for the cyclised product. Several possibilities were considered (Scheme 8.11): the first product (8.17), the charge remained on the N-3' nitrogen; the second product (8.18), the charge is localised at C-6 or delocalized about the atoms C-6, C-5 and the three nitrogens of the triazeno group (8.19); the third possibility was that the product had cyclised to the triazoline (8.20).



Scheme 8.11

If the charge remained localised at the N-3' position of the triazeno group, then the shielding effect of the anion would be confined largely to the propyl group and an upfield shift of the propyl carbons would be observed. It is anticipated that the effect of the anion on the uracil portion of the molecule would be small.

If the charge were localised at C-6, then an upfield shift might be expected for C-6 and smaller shift observed for C-5. Also, the chemical shift of the propyl carbons would remain much the same.

The case in which the charge was delocalized (8.19), it is not clear as to what the effect on the chemical shift this would have. It might be anticipated that changes in the chemical shift would be observed for C-6, C-5 and the methylenes in the propyl group.

In the case of the cyclised product, it would be anticipated that the C-6, C-5 and C-4 would all show changes in their chemical shift with the largest effect at C-6. A small significant change in the chemical shift of the methylenes of the propyl group might also be anticipated. Any large upfield shift for C-6 in going from an sp^2 to an sp^3 hybridized state might be will offset by a downfield shift because of the attachment of the triazeno group.

The results would suggest that the structure (8.17) is not likely to be the product since the chemical shift of the uracil portion of the carbon should be unaffected. Equally, the structure (8.18) would not explain the observed effect on the carbons of the propyl group.

Thus, two possibilities remain, *viz.* (8.19) and (8.20). As a consequence of the proposed structure (8.19), no change or a small upfield shift is anticipated for the chemical shift of C-5 and C-6. Similarly, it might be anticipated that the triazoline structure (8.20) would have the chemical shifts of C-2, C-4 and C-5 positions remained much the same, whilst the C-6 position signal would shift because of the attachment of the N-3' nitrogen. The formation of the triazoline is only one of the possibilities which can account for a downfield shift of C-6 and an upfield shift (via an inductive effect) of the methylene carbons in the propyl group. Without a precise knowledge as to how chemical shift is influenced in charged species, the structure proposed remains speculative.

The ^{13}C n.m.r. sample was added to distilled water in the hope that the product would precipitate out. The solvent water was removed after the failure to observe precipitation. Attempts to

purify the compound on silica proved to be unsuccessful as a wide variety of decomposition products were obtained in addition to the starting material. This was attributed to the reversible nature of cyclisation reactions to triazolines [23-31].

With a more powerful spectrometer, the question of the *in situ* cyclisation may be resolved by running a coupled spectrum in which it should be possible to observe a ^{3}J coupling between the H-7a and the C-1' methylene through the N-7 of the 7-propyl-triazolo--[5,4-d]pyrimidine. The coupled spectrum would also provide information on $J_{C(6)-H}$ which can be related to the hybridized state at C-6. if the reaction did not afford the triazoline product (8.20) then an sp^2 state would be envisaged and the one bond C-H coupling should be approximately 165Hz. if the reaction had occurred then C-6 would be sp^3 hybridized and the coupling constant would be approximately 125Hz. One recent technique which is now just becoming available is the use of heteroatom n.O.e. to determine the spatial relationship between atoms and should allow the determination of any proximity of H-7a and the C-1" atom. The standard homonuclear n.O.e.d.s. should similar show a interaction between the protons at the the two positions.

8.2.7.2.3 Discussion.

The reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with primary aliphatic amines leads readily to the formation of 5-(3-alkyl)triazenouracils. A principle feature observed in all reactions was the loss of the 6-methoxy- group and the generation of the 5,6-double bond. The loss of the methoxyl group probably results from the formation of anion intermediate and results in the more stable conjugated product.

The failure to isolate a product with aniline was possibly due to a lower electron availability on the amino group, due to -M mesomeric effects with the aromatic ring.

The attempted cyclisation of the triazene, whilst promising, did not lead to the isolation of 4,5-dihydro-8-azaxanthine.

8.3 Suggestions for further work.

The failure to isolate the phenyltriazenouracil suggests that the formation of aryl compounds should be explored further. Since the diazo group is quite stable in the absence of the catalyst, a

solvent system such as ether could used. Further, the use of substituted anilines, such as methoxy anilines might improve the reaction. The secondary amines have yet to be explored and are likely to afford the desired triazeno products.

The ease of preparing many of these triazenouracils should provide several samples for biological testing. A search of the literature, suggests that the biological activity of these compounds has yet to be explored.

8.4 Reaction of 5-diazouracil with allyl alcohol.

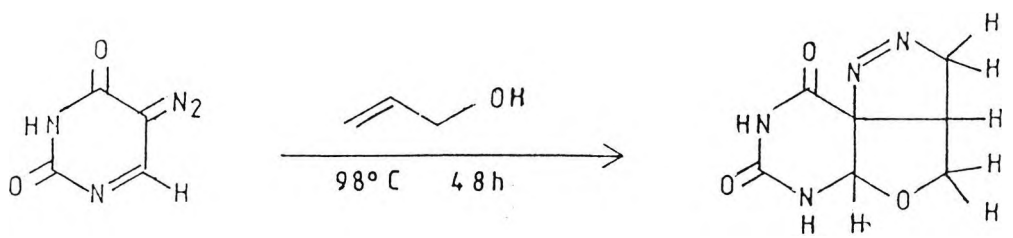
5-Diazouracil was suspended in allyl alcohol and the two components heated with reflux, 98°C, for 48h. A white precipitate was observed to have formed and was filtered from the solvent and dried in a vacuum pistol at 70°C.

The compound was characterised in its i.r. spectrum by an absorption at 3210cm^{-1} associated with C-H stretches of a saturated alkane. Two intense peaks at 1730 and 1690cm^{-1} were observed and from these peaks, it was deduced that the product was a derivative of the uracil.

The microanalysis data for the compound showed it to be 1:1 and that the compound was an adduct of 5-diazouracil with allyl alcohol, $C_7H_8N_4O_3$, in which the diazo nitrogens had been retained.

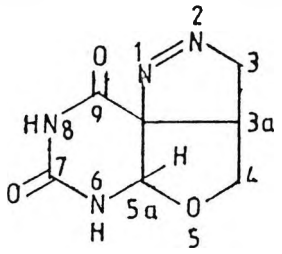
The compound fluoresces extremely strongly in daylight and especially in the u.v. region of the spectrum.

There are numerous reports of the reaction of diazo compounds with olefins resulting in the formation of pyrazolines. It was therefore proposed that the reaction had formed a pyrazolo- derivative (Scheme 8.12).



Scheme 8.12

The compound was named as a pyrazolofurano- derivative of pyrimidine, *i.e.*, 3,3a,5a,6,7,8-hexahydro-4H,8H-pyrazolo[3,2:7,7a]--furano[2,3-d]pyrimidin-7,9-dione (8.21).



(8.21)

The ^{1}H n.m.r. of the compound confirmed the loss of the allyl double bond. The spectrum was characterised by a broad singlet at 8.66δ assigned to the N-6 proton. A finely split doublet at 5.54δ (J=1.0Hz), integrating for 1H, was assigned to the 5a methine. A 2H doublet at 4.86δ (J=5.75Hz) was assigned to the C-3 methylene. The 1H multiplet at 4.14δ (J=5.75Hz, J=3.0Hz) was assigned to the C-3a methine. Finally, the two doublets at 4.78δ and 4.68δ, each with the same measured coupling (J=3.0Hz), were assigned to the two protons of the C-4 methylene. It is assumed that the N-8 proton lies above the scanned range, *i.e.*, above 10.0δ and hence, was not observed. It should be noted that the assignments for the C-4 and C-3 methylene might be the other way round. However, the broadness of the two doublets at 4.78δ and 4.68δ when contrasted with the sharp signal observed at 4.86δ suggest a small ^{4}J coupling with the 5a methine for each of the two protons in the C-4 methylene. It is also thought likely that the azo functional group would be more deshielding than a ether functional group.

Confirmation of the structural formula was provided by the a molecular ion at m/z 196 in a high resolution EI-MS spectrum.

8.4 Discussion.

This novel double reaction of allyl alcohol represents the simple addition of the alcohol across the 1,6-double bond of 5-diazouracil and a 3+2 cycloaddition of a diazo group with a double bond to produce a fused, five-membered ring. The reaction of the diazouracil with a double bond has great potential for the preparation of novel spiro pyrazoline uracils.

8.5 References.

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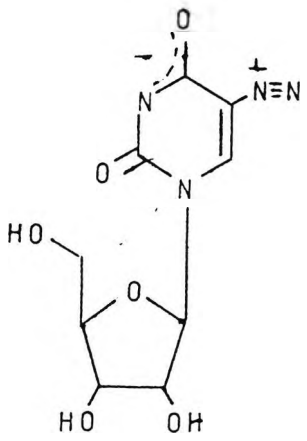
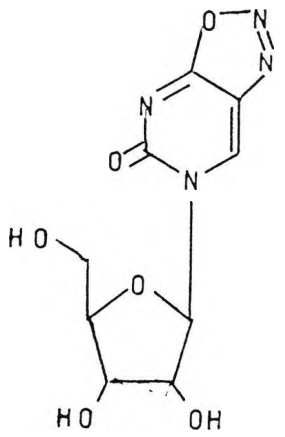
Chapter 9 Diazonucleosides.

9.1 Introduction.

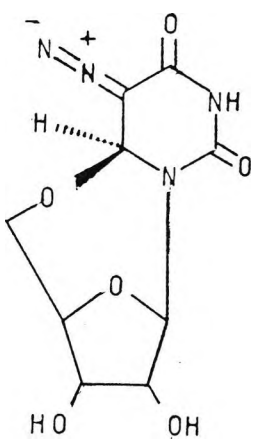
The treatment of 5-aminouridine with hydrochloric acid and sodium nitrite solution gave a product which was referred to as 5-diazouridine. Two tentative structures [1,2] were assigned to the compound (9.1 and 9.2). However, the structures proposed seemed at odds with the ^{1}H n.m.r. of the compound and a third structure was assigned to it (9.3).



(9.1)



(9.2)

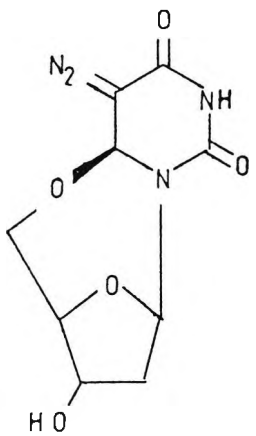


(9.3)

The ^{1}H n.m.r. indicated a broad signal 10.62δ assigned as the NH. The chemical shift of 6.15δ for the C-6 proton suggested that the C-6 carbon had a deshielding group attached to it. It was correctly deduced that the C-5' atom had become attached to the C-6 atom via O-5' oxygen. The configuration at the C-6 atom was

determined by examination of models. The chemical shift of the C-6 proton was further downfield than might have been expected for a methine attached to nitrogen and oxygen. The models showed that if the absolute configuration was 6S then the C-6 methine would lie over the sugar ring oxygen and as a consequence would also be subject to induced secondary field effects of the lone pairs on the ring oxygen. Consequently, this was used to explain the downfield shift of the C-6 proton to 6.15δ from the value of 5.72δ observed in 5-diazo-6-methoxy-1,6-dihydropyrimidin-2,4(1H,3H)-dione.

5-Diazo-2'-deoxyuridine was deduced from its ^{1}H n.m.r. spectrum to have similar structural features. The structure of the compound was represented by 1(2-deoxy-*B*-D-ribofuranosyl)-$O^{5'}$-6(*S*)--cyclo-5-diazo-1,6-dihydropyrimidin-2,4(3H,6H)-dione (9.4).



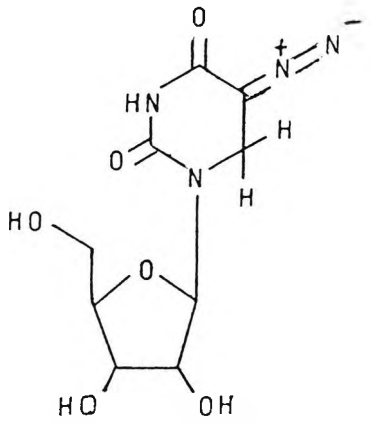
(9.4)

These cyclonucleosides are very restricted as to the number of conformations which they can adopt. The heterocyclic bases in these molecules are in "high *anti*" conformation. If this conformation is suitable for biological activity then this molecule will be an ideal example. However, if the alternative situation holds true, whereby the heterocyclic base with a *syn* conformation may be more active, then the restriction imposed by this particular diazo compound will reduce its potential. Further, large constraints on the degrees of conformational freedom are imposed on the sugar ring, as well as depriving such molecules of the ability to form phosphates esters on the C-5' position of the sugar. Thus, it would appear that potential

antiviral and antibacterial agents based on the 5-diazo moiety being present in the nucleoside or nucleotide analogue may well be excluded.

Cyclisation at the C-6 position atom could be avoided if the 1,6-dihydro- derivative of uridine was the precursor to diazotization. The proposed target in the present work was therefore the formation of 5-diazo-1-(B-D-ribofuranosyl)1,6-dihydropyrimidin--2,4(3H,6H)-dione.

Nomenclature of the diazo compounds has often been confusing because of the early literature and the retention of non-systematic names. There are two approaches to the nomenclature of these compounds. The first approach is to look at the difference in the unsaturation of the C-5 and C-6 atoms. The 5-diazo group retains the unsaturation of C-5 atom of uracil (pyrimidin-2,4(1H,3H)-dione) whilst sp3 C-6 atom is referred to by the use of the term "6H" to denote increased saturation. The other method is to start with 5-diazouracil as the parent compound and then to develop the degrees of saturation. Thus, a methylene at C-6 would denote hydrogenation of the 1,6-bond . Consequently, the prefix "1,6-dihydro-" would be applied and any other groups may be regarded as substituents and accordingly the derivative can named as substituted 5-diazo-1,6--dihydrouracil. Thus the proposed diazo compound (9.5) could be named as 5-diazo-6H-uridine or 5-diazo-1,6-dihydrouridine or as 5-diazo-1(1'-B-d-ribofuranosyl)-1,6-dihydrouracil.

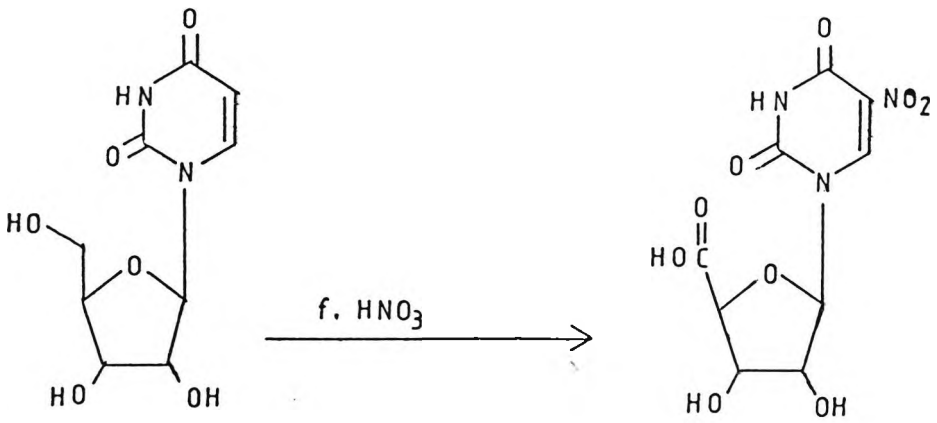


syn conformation

(9.5)

The required precursor to 5-diazo-1,6-dihydrouridine is the corresponding amino compound. One approach is to synthesize the desired 5-amino-5,6-dihydrouridine through a functional group interconversion (FGI) by the reduction of a nitro group. The precursor for the synthesis of 5-nitro-5,6-dihydrouridine might be the 5-nitrouridine which could be obtained by nitration of uridine. This approach parallels the method used to synthesis 5-diazo-1,6--dihydrouracil.

When nitrated with nitric acid [4], uridine gave the nitronucleoside. Subsequent work [5] elucidated the structure of the product and demonstrated it to be the 5-nitro-1-(B-D-ribosyluronic acid)-uracil, in which the 4'-hydroxymethyl group had been oxidised (Scheme 9.1).

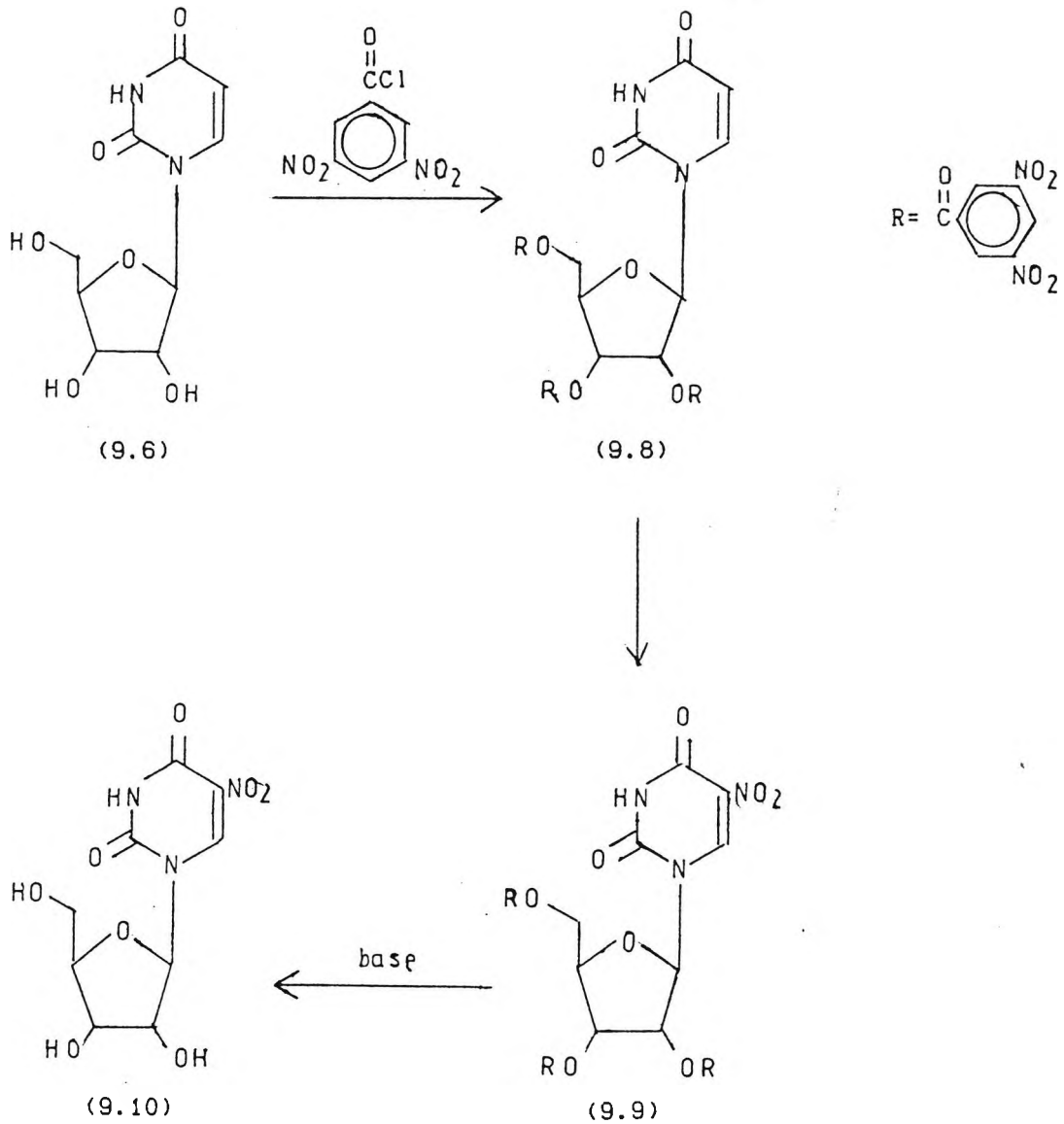


(9.6) (9.7)

Scheme 9.1

In order to prevent such an oxidation occurring when nitrating uridine, it was necessary to protect the free hydroxyls. The choice of protecting group must take into account the need for retention in the strongly acidic medium of the nitrating reagent and inertness to nitration by the reagent. It was found that if 3,5-dinitrobenzoyl chloride was used to protect all the free hydroxyls that the resulting protected nucleoside could withstand a very reactive nitrating system [5]. The compound can be deprotected to give the free nucleoside (Scheme 9.2). The nitrated nucleoside (9.10) obtained should lend itself to reduction to give the 5-amino-5,6--dihydrouridine which could then be diazotized to afford the

proposed diazonucleoside (9.5).



Scheme 9.2

Earlier work on the reduction of uracils and their derivatives [8,9] demonstrated that whilst uracil was easily reduced to dihydrouracil, 5-aminouracil gave an ambiguous mixture of compounds. Further, it was concluded that the use of colloidal platinum and hydrogen gas at twice atmospheric pressure was extremely susceptible to trace impurities. More recently, it has been shown that 5-amino--uracil may be reduced [10] by using powdered rhodium on alumina as a catalyst at 45psi. This reduction works only on the hydrochloride salt of aminouracil and results in the formation of the hydrochloride salt of the 5-amino-5,6-dihydrouracil.

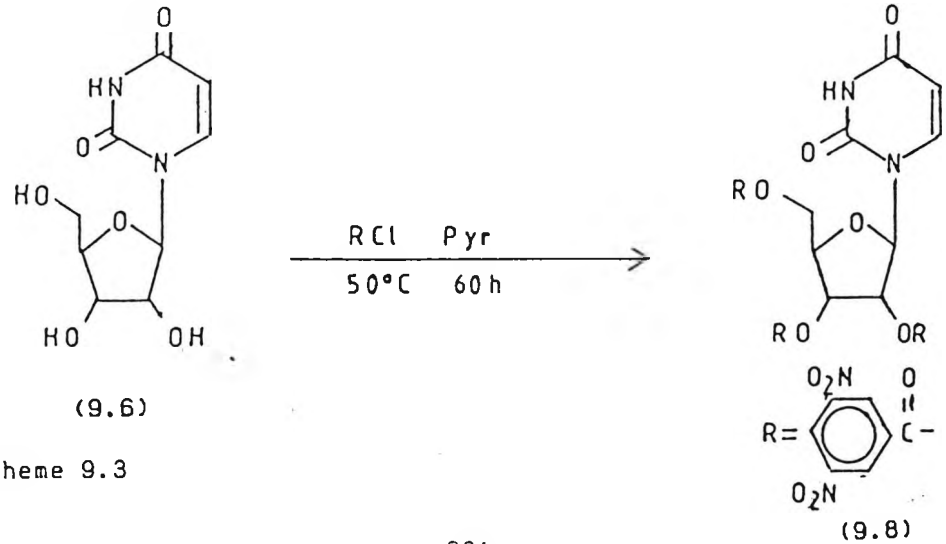
The C-6 position in the 5-nitrouracil moiety is especially enhanced for nucleophilic attack. Thus, it might be expected to lend itself to metal hydride reductions as was demonstrated in steroids [11]. This stratagem was employed for 5-nitrouracil, 5-acetyluracil, 5-nitro-1(4-nitrophenyl)uracil and 5-nitro-1-(*B*-D--ribofuranuronic acid ethyl ester)uracil [12]. A surprising omission was that of 5-nitrouridine. Thus, experiments were undertaken to see if either the 5-nitro-2',3',5'-tri-O-(3,5-dinitrobenzoyl)uridine or 5-nitrouridine would be reduced to the corresponding 5-nitro-5,6--dihydro- derivatives.

By combining the two themes, studies were undertaken to examine if this would produce a viable method for the synthesis of 5-diazo-6H-uridine with the added bonus of producing intermediates which would be likely candidates to have biological activity.

9.2 Results and Discussion.

9.2.1 Preparation of 2',3',5'-Tri-O-(3,5-dinitrobenzoyl)--uridine.

Uridine was dissolved in pyridine and slowly added to a 3,5-dinitrobenzoyl chloride. The mixture was left stirring at 50°C for 60h. The clear dark brown solution was then concentrated under vacuum to give a brown oil. The oil was added to ice-water with stirring to give an off-white precipitate. The solid was filtered from the aqueous phase and purified by soxhlet extraction using absolute ethanol until the condensate was colourless. The solid was filtered and dried in a vacuum oven to afford the protected uracil in 80% yield (lit. 93%) (Scheme 9.3).

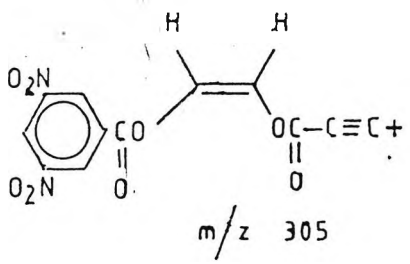


(9.6) (9.8)

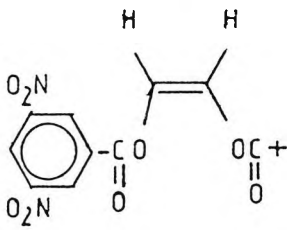
Scheme 9.3

The compound was characterised in the i.r. by absorption peaks at 1700 and 1690cm^{-1} which were associated with the carbonyls of the uracil ring. The presence of the protecting group was determined by the absorption peaks at 1600, 1590, 1350, 1740, 1730, 1700, 900, and 800cm^{-1}, which correspond to the vibrations for the aromatic rings (1600cm^{-1}), the nitro groups (1590 and 1350cm^{-1}), ester carbonyls (1740, 1730 and 1720cm^{-1}) and the aromatic hydrogen (920 and 810cm^{-1}).

The molecular ion was not observed in the mass spectrum. The compound underwent extensive fragmentation, which suggests that a softer ionization method would be more appropriate. The spectrum is dominated by fragments from the protecting groups. The peaks at m/z 212, 195, 167, and 121 can all be related to fragments from 3,5-dinitrobenzoic acid. The uracil moiety can be deduced from the fragment at m/z 140 which corresponds to a species N-methanaluracil ($C_5H_4N_2O_3$). The peaks at m/z 305 and 281 are in keeping with fragments of an O,O'-diacylated ethene (9.11 and 9.12). A peak at m/z 305 can be related to a fragment $C_{12}H_5N_2O_8$ which implies a species containing a C-2 or C-3 mono-acylated sugar component. Acylation at the C-5 position is provided by the peak at m/z 225 which corresponds to a species $C_8H_5N_2O_6$.



(9.11)



(9.12)

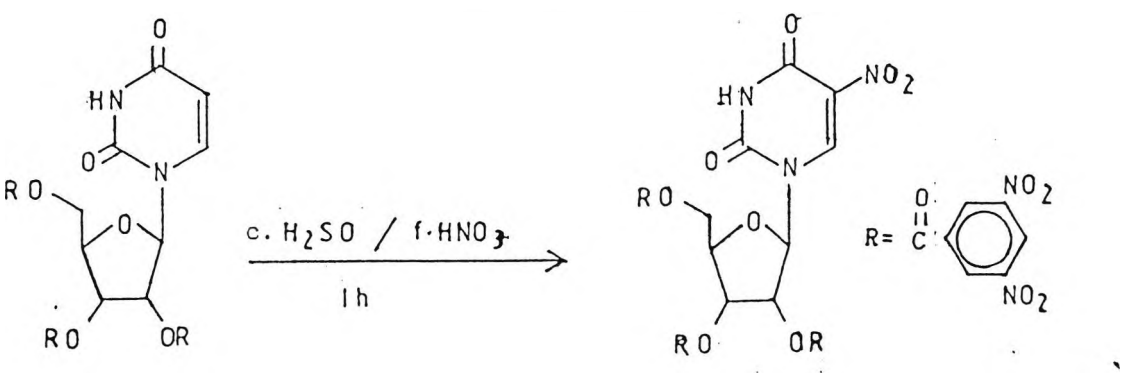
The ^{1}H n.m.r. spectrum of the protected nucleoside in dmso-d_6 was very poor in quality as might be expected [6] However, two main features of the spectrum are noteworthy; that the uracil moiety was still attached to the ribose unit and the extent to which the ribofuranose was protected.

The observation of doublets at 7.92δ and 5.88δ (J=7.5Hz) in its n.m.r spectrum were assigned to the H-5 and H-6 protons of the un-substituted uracil group. A signal for the anomeric proton,

H-1' at 6.12δ confirmed the presence of the sugar portion of the molecule. The presence of a complex aromatic portion implied that protection had occurred and that the sugar was protected at more than one site. The ratio of the integral for the aromatic signals to that of either the H-5 or H-6 proton was 9:1, which is evidence of the tri-*O*-(3,5-dinitrobenzoyl)ribofuranosyl moiety. Although water was present, the material was used without further drying in the next stage. Confirmation of tri-protection was also given by microanalysis which confirmed the formula of the product.

9.2.2 Preparation of 5-Nitro-1-B-D-(2',3',5'-tri-*O*--(3,5-dinitrobenzoyl))uridine.

The protected uridine (9.8) was added to a mixture of concentrated sulphuric acid and fuming nitric acid (55:66). The mixture was left stirring at room temperature for 1.5h. and formed a dark red/brown solution. The choice of nitration with a mixture of concentrated sulphuric acid and fuming nitric acid was determined by the failure of nitronium tetrafluoroborate, previously used to nitrate uracil, to nitrate uridine [13]. The reaction mixture was poured into an ice/water mixture and stirred. The formation of a creamy white precipitate was observed. The solution was allowed to stand until all the ice had melted and the solution was then filtered. The white residue was washed with ice-cold water until the washings were pH 6.5. The solid was dried under vacuo at 70°C to afford the nitro derivative (Scheme 9.4) in crude yield of 95% (Lit. 71%). Microanalysis showed the crude material to have a formula close to $C_{30}H_{17}N_9O_{23}.5H_2O$, giving a recalculated yield of 80%. A slightly low value for the analysis for carbon (found 39.13%, calculated 41.35%) and slightly low value for nitrogen (found 12.73%, calculated 14.46%) suggest that the product contained a small proportion of the diprotected species [5].



Scheme 9.4

The i.r. spectrum contained strong absorption peaks in the range 1750 to 1680cm^{-1}, which were assigned to the carbonyls of uracil and of the ester carbonyls of the protecting groups. Sharp absorptions at 1590 and 1350cm^{-1} were assigned to the nitro groups.

The mass spectrum demonstrated nitration on the uracil portion by the presence of a peak at m/z 185 which corresponds to the nitrated N-methanaluracil ($C_5H_4N_2O_3$), and the absence of the original peak at m/z 140 for an un-nitrated methanaluracil fragment. A fragment at m/z 182 was identified as a nitro N-ethenouracil component, which also suggests nitration of the uracil portion of the compound. The spectrum was again dominated by the protecting group and its related fragments. The baseline peak for 3,5-dinitro--benzoic acid confirmed the i.r. observation that the protecting groups had been attached at the hydroxyl positions of the ribofuranose moiety.

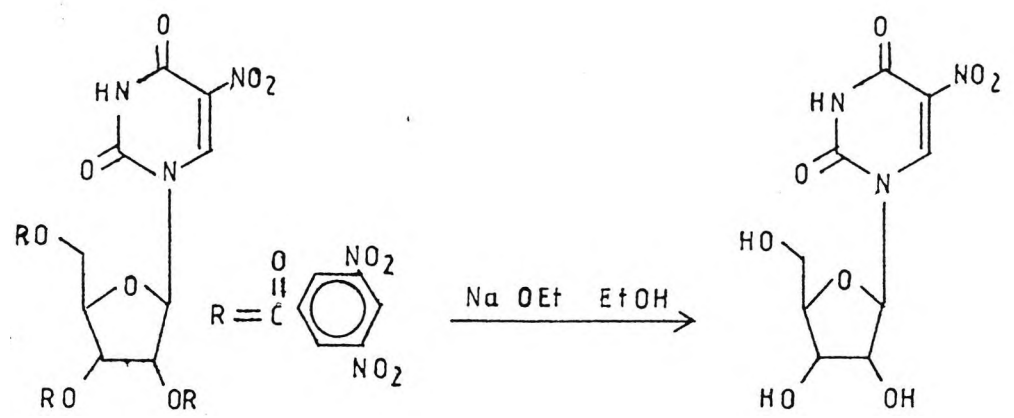
The clearest indication of uracil nitration was provided by the ^{1}H n.m.r. spectrum (in pyridine-d_5). The spectrum was of poor resolution, with the multiplicity of the signals being unresolved. The absence of the doublets associated with unsubstituted uracil indicated that the compound had been substituted. The position of nitration was confirmed to be the C-5 atom by the observation of a singlet at 8.96δ, which is very close to the observed value for 5-nitrouracil (8.48δ in dmso-d_6).

9.2.3 Preparation of 5-nitrouridine.

The nitrated protected uridine (9.9) was suspended in absolute ethanol. Sodium ethoxide was added to the stirring solution, which turned dark red. The solution was left stirring overnight and heated to reflux for 3h, then allowed to cool to ambient temperature and the precipitate filtered off. The residue was triturated with warm ethanol and refiltered until the filtrate became colourless. The residue was suspended in hot ethanol and acidified with concentrated sulphuric acid until the solution was pH 3.0. The solution was filtered and the filtrate evaporated to dryness to afford an pink/white solid (Scheme 9.5).

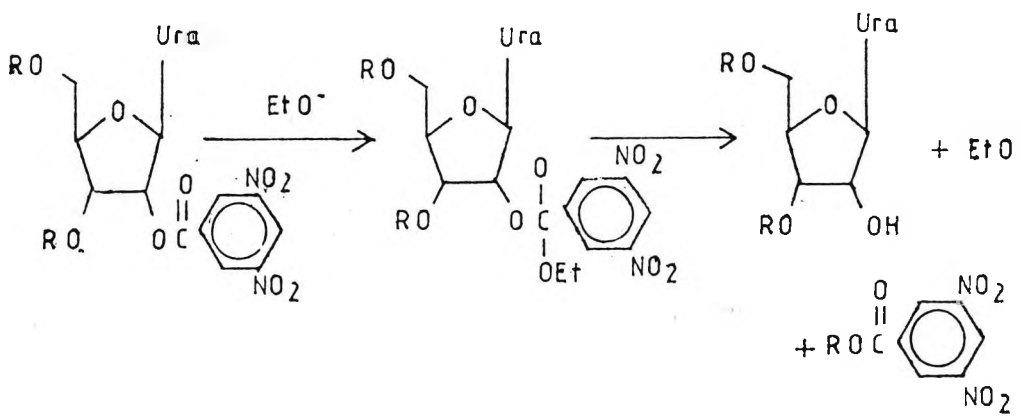
The i.r. spectrum of the compound was denoted by a strong absorption band around 1700 to 1680cm^{-1}, which was assigned to the carbonyls of the uracil ring. It should be noted that an absence of

strong absorptions covering the range just above 1700cm^{-1} suggest the removal of the ester carbonyls associated with the protecting groups.



Scheme 9.5

Although the individual ribose protons could not be unambiguously assigned, it was clear from the ^{1}H n.m.r. that deprotection had occurred, as evidenced by the absence of the signals associated with the dinitrophenyl group. The nitro group on the uracil ring is still present, as shown by the downfield signal at 9.40δ. The upfield shift observed for the ribose protons indicates the removal of the protecting groups. This large downfield shift, anything between 1.0 to 3.0δ from the expected values around 4.0 to 5.0δ and 6.0 to 7.0δ, shows that the deshielding of the ribose ring protons in the triaryl ester resulted from anisotropic effects of the aromatic ring current or the carbonyl groups in the protecting groups.



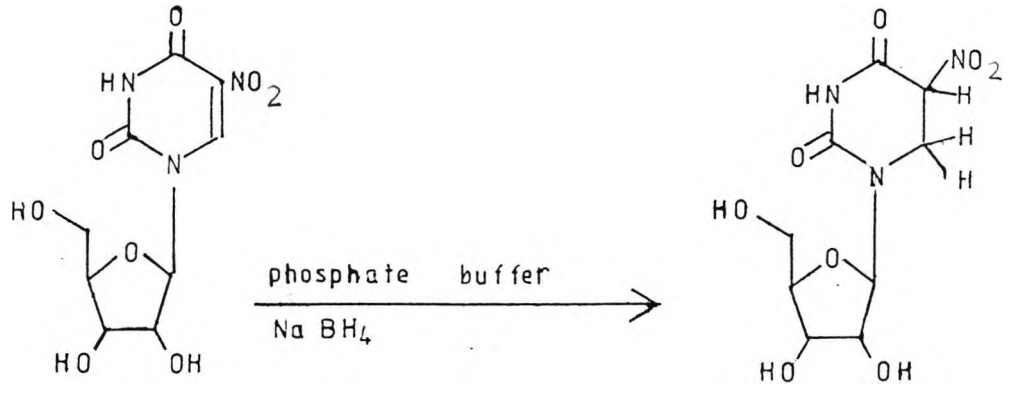
Scheme 9.6

It was also possible to isolate in high purity the removed protecting group. The ^1H n.m.r. spectrum of the by-product showed that the nucleoside ester had been deprotected by nucleophilic attack to afford ethyl 3,5-dinitrobenzoate (9.13) (Scheme 9.6).

9.2.4 Attempted preparation of 5-nitro-5,6-dihydrouridine and 5-nitro-5,6-dihydro(2',3',5'-tri-*O*-acetyl)--uridine.

The aim of this experiment was to carry out the reduction of the 5,6-double bond in the uracil portion of 5-nitrouridine by the method in which 5-nitrouracil was converted to 5-nitro-5,6-dihydro--uracil (Scheme 9.7).

5-Nitrouridine was dissolved in a buffered solution at pH 7.0. Sodium borohydride also dissolved in buffered solution was added to the nucleoside. The mixture was left stirring overnight. The aqueous reaction mixture was extracted with ethyl acetate and the organic phase dried over anhydrous sodium sulphate. The solution was filtered and the filtrate evaporated to dryness.



(9.10) (9.11)

Scheme 9.7

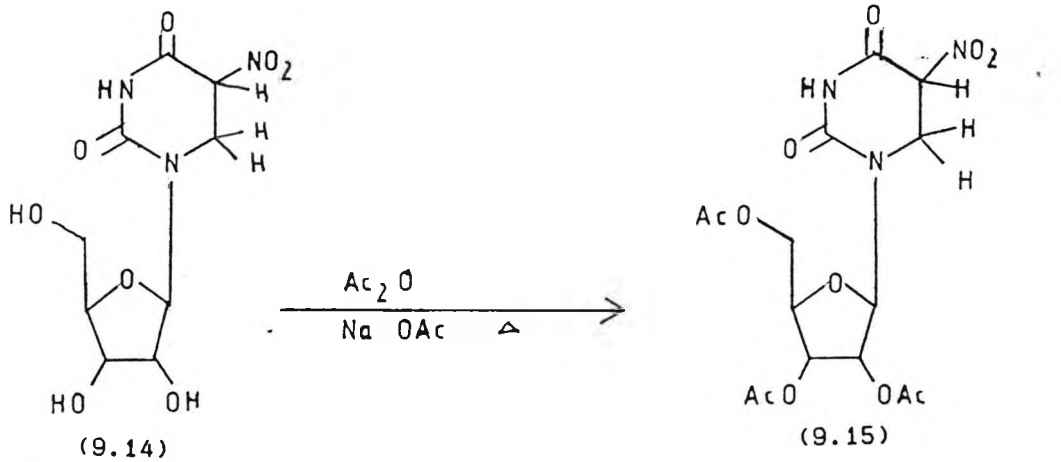
The compound was examined by ^1H n.m.r.. The spectrum was too poorly resolved to be of any value as most of the signals occurring in a large methylene envelope in the range 5.7 to 3.7δ.

The aqueous phase was frozen and the water removed by evaporation using a high-vacuum oil pump. The freeze-dried solid was a very hard material which was soluble in acetic acid. The solvent

acetic acid was removed to leave a bright yellow oil which on standing formed a yellow crystalline material.

The material was characterised by a broad absorption band between 1740 to 1630cm^{-1} which was assigned to the presence of carbonyl groups in the compound. The presence of a nitro group was inferred from the absorptions at 1560 and 1350cm^{-1}.

The ^{1}H n.m.r. spectrum contained several multiplets between 5.20δ and 3.80δ, which were associated with the ring protons of the ribofuranose. Strong hydrogen bonding and the presence of hydroxyl protons may serve to reduce resolution. Since the spectrum was poor it was proposed that if this compound were the desired nucleoside, it should be possible to derivatise it to the tri-*O*-acetyl-derivative (Scheme 9.8). The absence of a sharp singlet at 9.40δ suggested that there was no longer a strongly deshielded vinylic proton.



(9.14) (9.15)

Scheme 9.8

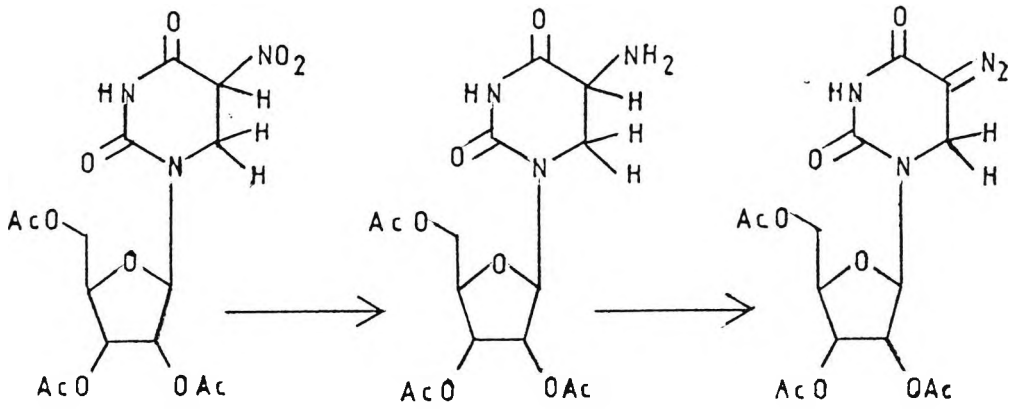
The putative 5-nitro-5,6-dihydrouridine (9.14) was mixed with sodium acetate and acetic anhydride and heated over a boiling water bath with reflux for 5h., as in the literature method [7]. The solution was left stirring overnight. The reaction mixture was taken up in ethyl acetate and washed with water. The organic layer was separated out, dried and purified by preparative t.l.c. using ethyl acetate as the mobile phase. A component, R_f=0.69, was isolated. The product was examined by ^{1}H n.m.r. (Table 9.1). The compound was poorly soluble in deuterochloroform.

δ		
7.32 s		(chloroform),
7.2 t?	1H	
5.96 d?	1H	
5.75 t?	1H	
5.50 bs	1H	
4.60-4.25 m	4H	
2.64 s	1.5H	(OH)
2.34 s	2H,	
2.20+2.16 2s	9H,	
3.82 q J=7.5 Hz, 1.32 t J=7.5 Hz - residual ethyl acetate		

Table. 9.1 ^1H n.m.r. ($CDCl_3$) observed signals for acetate derivative

The singlets at 2.20 and 2.16δ were assigned to the three acetyl methyls of a putative protected nucleoside. The absence of the downfield singlet around 9.0δ, supported the view that the nitro vinyl moiety of nitrouridine had been reduced. The numerous signals integrating for one or two protons were taken as an indication of the ribofuranose ring protons and include the dihydrouracil ring protons. A comparison with uridine suggests that the signals at 5.96, 5.75, 5.50δ and 4.6 to 4.25δ correspond to the ring protons of ribose. The expected chemical shift of the hydrogens in the 5-nitro-5,6-dihydrouracil moiety would have been expected to occur at 5.50δ and 3.70δ. No such signals were observed, but neither were the doublets associated with uracil. One explanation is that the chemical shift of these protons has been shifted either by diamagnetic shielding from a C-5' acetyl carbonyl or the substituted uridine has a high *anti* configuration which places the C-6 and C-5 positions near or over the lone pairs of the oxygen in the furanose ring.

The overall yield of the two steps was 15% for the crude material and about 5% for the purified compound. Thus, although it should be now possible to reduce the protected 5-nitro-5,6-dihydro--uridine to the protected 5-amino-5,6-dihydrouridine and then diazotize the proposed intermediate to the diazo compound (Scheme 9.9), a simpler route to the desired diazo compound was sought which would afford a higher overall yield.

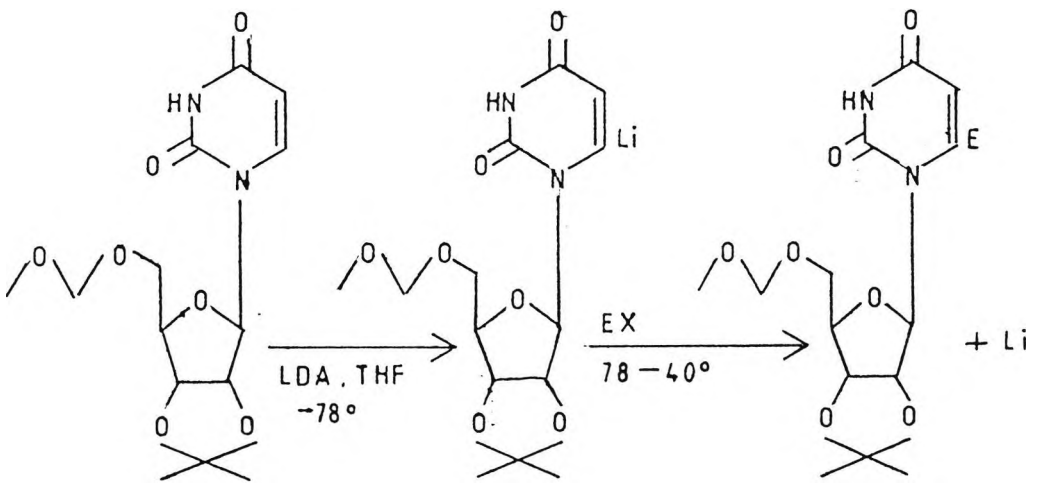


(9.15) (9.16) (9.17)

Scheme 9.9

9.2.5 Approaches to 5-diazo-6H-uridine via diazo transfer reagents.

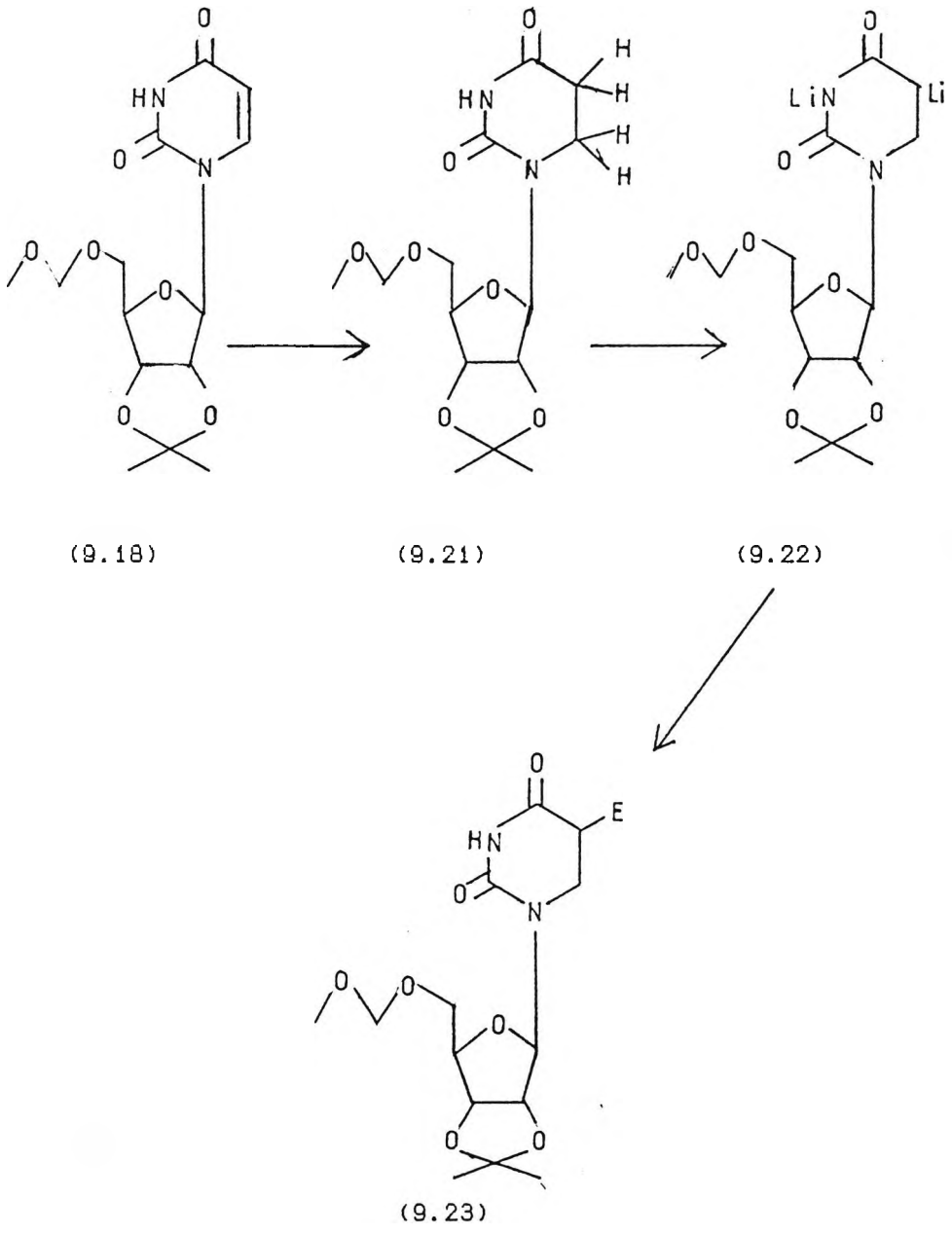
Recent work on uridines demonstrated that the use of a lithiating reagent would result in the regiospecific metallation of the C-6 position of the uracil ring. The carbanion generated in this position would be suitable for reaction with an electrophile (Scheme 9.10). This method was further expanded as a general route to the introduction of electrophiles in this position of the ring or to carry out a nucleophilic attack on other reagents [14-17].



(9.18) (9.19) (9.20)

Scheme 9.10

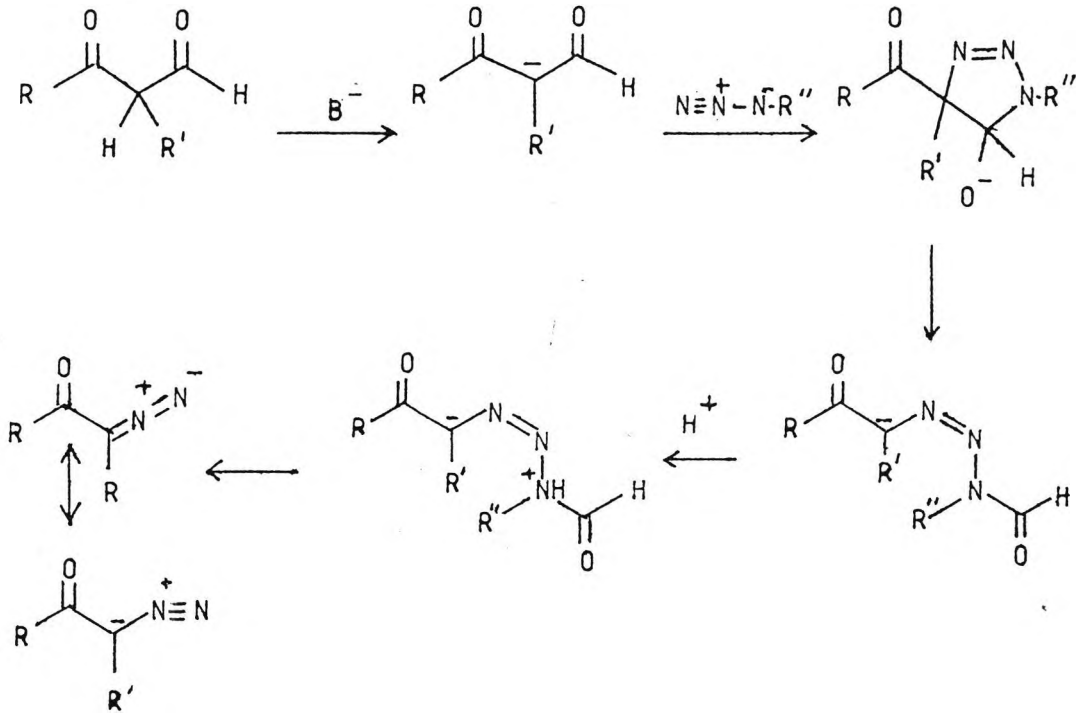
By contrast, in 5,6-dihydrouracils lithiation occurs at the C-5 position [18]. The emphasis of the reported work has been on methods of introducing a wide variety of electrophiles into the C-5 position (Scheme 9.11) and subsequently producing the 5-substituted uridine by regenerating the double bond via catalytic dehydrogenation.



Scheme 9.11

Interest in the procedure came from an examination of the some of the products of this method. It was noted that acyl groups could be introduced into the the C-5 position in high yields.

Previously, it had been shown that *B*-formyl keto- systems were able to undergo substitution of the formyl group by a diazo group using suitable diazo transfer reagents [19]. This suggested an approach via 5-formyl-5,6-dihydrouridine, which could then be reacted with a diazo transfer reagent to yield the desired product (Scheme 9.12).

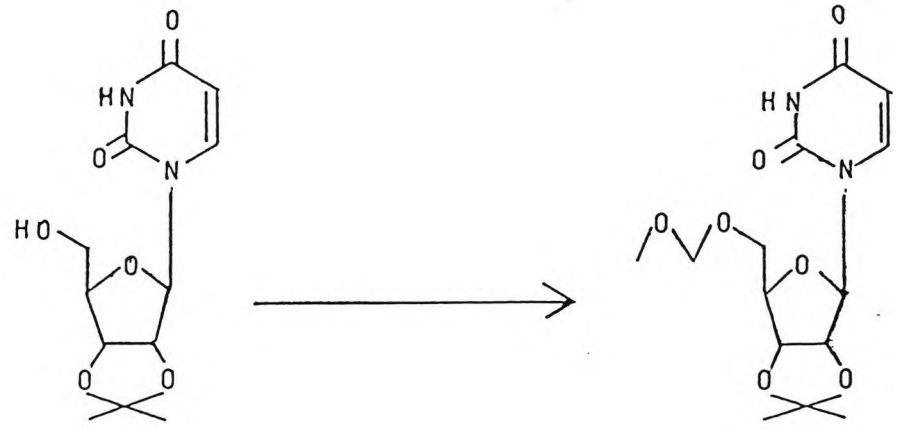


Scheme 9.12

9.2.6 Preparation of 2',3'-O-isopropylidene-5'-O--methoxymethyluridine.

The nucleoside was protected in the C-5' position by attaching an *O*-methoxymethyl blocking group. 2',3'-Isopropylidene uridine was suspended in dimethoxymethane and acetone. Methane sulphonic acid was added to the turbid mixture and was left stirring at room temperature. The solution rapidly became opaque with the formation of a white precipitate after 30min. The precipitate was dissolved by addition of 28% ammonium hydroxide solution and evaporated to dryness. The residue was partitioned between chloroform and a saturated solution of sodium chloride. The organic layer was evaporated to give a yellow oil and purified by column chromatography. After vacuum drying, the product was isolated as a

white, foamy solid in 97% yield (Scheme 9.13).



(9 24) (9 18)

Scheme 9.13

The compound was characterised by an absorption in the i.r. by strong bands between 1720 and 1680cm^{-1} which were assigned to the carbonyls of the uracil group and absorption bands between 1070 to 1130cm^{-1} which are associated with the ether C-O stretching of the protecting groups.

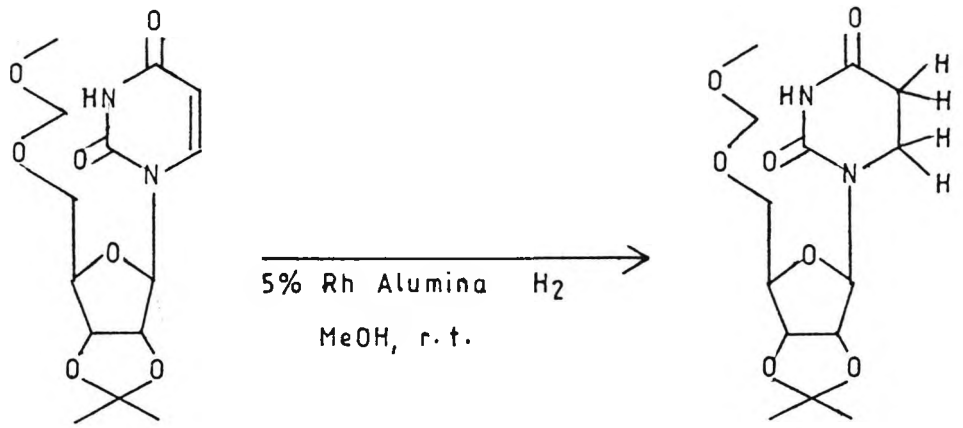
The compound was also examined by ^{1}H n.m.r. and was determined on the basis of chemical shift, coupling and decoupling studies (Table 9.2) [18].

δ	assignment
5.88 d 1H J=1.5 Hz	H-1'
4.88 m 2H J=1.5 Hz, J=3.0Hz	H-2', H-3'
4.72 s 2H	OCH_2O
4.36 m 1H J=3.0 Hz	H-4'
3.66 s 3H	OCH_3
2.68 t 2H	H-6
1.56 s 3H	C-Me
1.36 s 3H	C-Me

Table 9.2 Assignment of ^{1}H n.m.r of 2',3'-O-Isopropylidene--5'-O-methoxymethyluridine

9.2.7 Preparation of 2',3'-*O*-isopropylidene-5'-*O*-methoxymethyl-5,6-dihydrouridine.

Lithiation is directed to the C-5 position, when the uracil group is reduced. The protected uridine was dissolved in methanol and reduced using 5% rhodium on alumina catalyst under a hydrogen atmosphere (Scheme 9.14). Reduction was continued until the sample no longer displayed at the C-5 and C-6 doublets in its ^{1}H n.m.r. spectrum. The compound was purified by preparative t.l.c. using dichloromethane/methanol as the mobile phase to afford a white solid in a yield of 99%.



9·18 9·21

Scheme 9.14

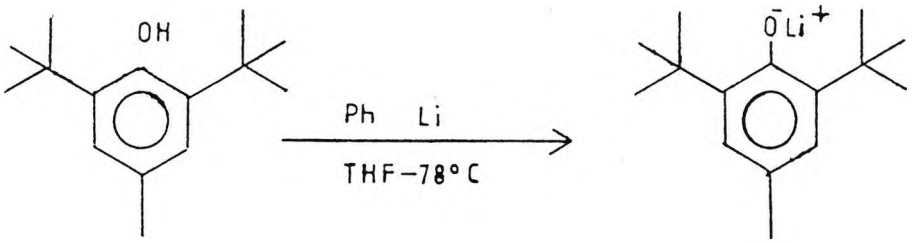
The ^{1}H n.m.r. spectrum was consistent with the hydrogenation of the 5,6-double bond (Table 9.3).

δ	assignment
5.70 d 1H J=3.0Hz	H-1'
4.71-4.87 m 2H J=3.0Hz	H-2', H-3'
4.66 s 2H	OCH_2O
4.14-4.36 m 1H J=3.0Hz	H-4'
3.69-3.74 m 2H	H-5'
3.52 t 2H	H-5
3.30 s 3H	OCH_3
2.68 t 2H J=6.5Hz	H-6
1.56 s 3H	C-Me
1.36 s 3H	C-Me

Table 9.3 Assignment of ^{1}H n.m.r. of 2',3'-*O*-isopropylidene-5'-*O*-methoxymethyl-5,6-dihydrouridine

9.2.8 Preparation of 5-formyl-2',3'-*O*-isopropylidene--5'-*O*-methoxy-methyl-5,6-dihydrouridine.

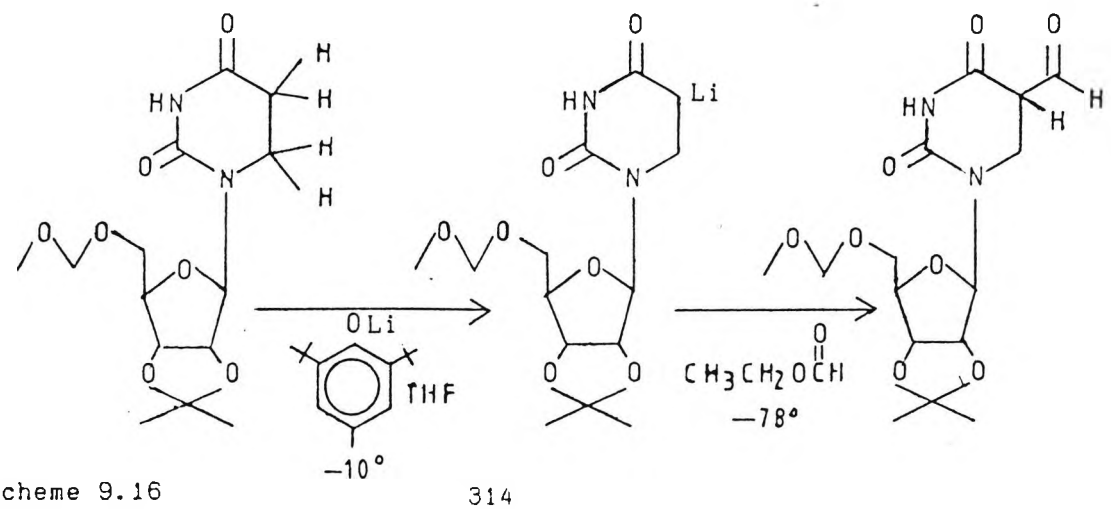
The preparation of the formyl derivative was based on the lithiation of the dihydrouracil portion of the nucleoside which was subsequently reacted with ethyl formate in order to introduce the formyl group in the C-5 position. The reagent, lithium 2,6-di-*tert*--butyl-4-methylphenoxide [20], was used in preference to lithium diisopropylamide (LDA) [21] because of the ease at which both the excess reagent and its by-product could be removed in purification. The lithiating reagent was prepared by reacting phenyllithium with 4-methyl-2,6-di-*tert*-butylphenol in THF (Scheme 9.15).



(9.25) (9.26)

Scheme 9.15

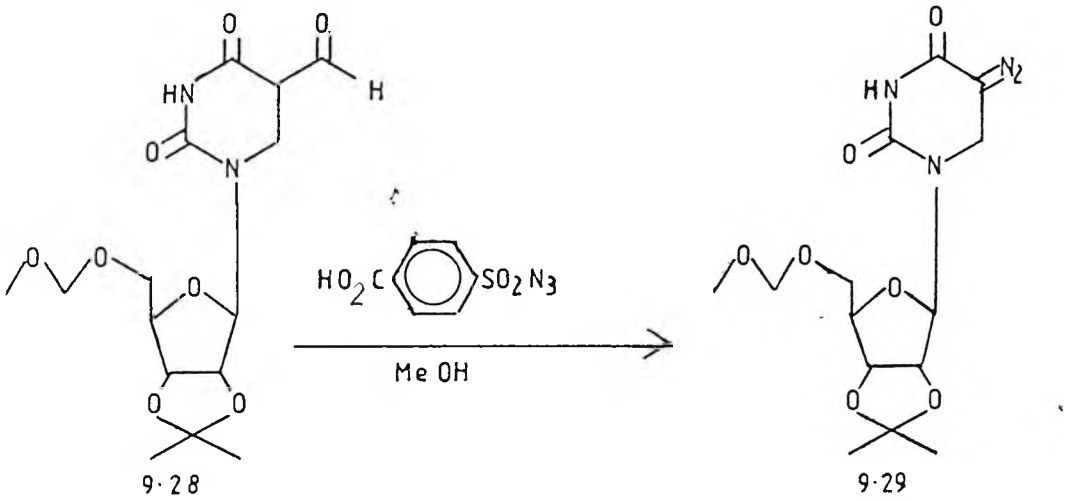
The protected dihydrouridine was dissolved in THF and added to the mixture. The reaction temperature was allowed to rise to -10°C to allow the formation of the lithiated nucleoside and then rapidly cooled to -78 °C before the addition of ethyl formate (Scheme 9.16). The product was obtained by partitioning the reaction mixture between chloroform and water. The chloroform phase was evaporated to dryness to afford yellow/orange solid in 30% yield, which was used for the next stage without further purification.



Scheme 9.16

9.2.9 Attempted synthesis of 5-diazo-1,6-dihydrouridine.

The putative formyl derivative was dissolved in methanol and added to a suspension of carboxybenznesulphonyl azide in methanol and triethylamine (Scheme 9.17). The mixture was left stirring over the weekend. The solution was evaporated to dryness. Three different methods were investigated in order to find a suitable method to isolate the product.



9·28 9·29

Scheme 9.17

Work up procedure A

A sample was triturated with dichloromethane. The solution was observed to become turbid with a bright white precipitate. The precipitate was removed by filtration and the filtrate condensed on a rotary evaporator to afford a bright yellow oil which on standing gave a yellow crystalline material.

The compound was characterised by a medium intensity absorption at 2120cm^{-1}. Comparison with the spectrum for carboxy benzene sulphonyl azide showed that the spectra were similar. Carboxybenzene sulphonyl azide is characterised by two peaks at 2220 and 2150cm^{-1} and in its finger print region by sharp distinct peaks at 1020, 870, 730, and 690cm^{-1}. The product spectrum differed in that no peak was observed at 2220cm^{-1} and the absorption associated with the diazo stretch occurred at 2120cm^{-1}. The spectrum of the product also differed in the fingerprint region with the sharp absorption at 870 cm^{-1} accompanied by absorptions at 760, 750 and 710cm^{-1}.

The i.r. suggests that a component containing a group similar to an azide but not an azide is present. This would agree with the formation of the diazo compound. The sharp peak at 870cm^{-1} and the similarity in the spectrum would suggest that the component isolated was not pure but contained the carboxybenzene sulphonic acid by-product.

Work up procedure B

A sample was triturated with dichloromethane, filtered and the filtrate evaporated. An attempt was made to purify the sample from contaminating carboxybenzene sulphonic acid by preparative t.l.c. using dichloromethane as the mobile phase. Each band was eluted with methanol and the eluent was evaporated to dryness.

The attempt to purify the material by preparative t.l.c. using silica plates proved unsuccessful. The ^{1}H n.m.r. of the isolated fractions contained none of the strong peaks associated with either the isopropylidene blocking group nor the methoxymethyl protecting group.

Work up procedure C

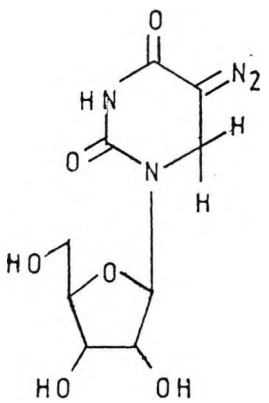
A sample was triturated with dichloromethane and filtered. The filtrate was washed with saturated sodium bicarbonate solution in an attempt to remove the *para*-carboxybenzene sulphonic acid. The two layers were separated and the organic layer was dried with anhydrous sodium sulphate, filtered and the filtrate evaporated to dryness to give a yellow oil which on standing rapidly afforded yellow rhombic needles in a yellow oil.

The ^{1}H n.m.r. of the material suggested that the material was a nucleoside with the observation of a singlet at 6.0δ, a triplet at 4.82δ, a triplet at 4.34δ, a multiplet at 3.88 δ, and a broad singlet at 3.50δ rising above a multiplet located at 3.48δ. These signals were attributed to the ribofuranosyl moiety. The ^{1}H n.m.r. spectrum also indicated a large multiplet centered at 7.70δ, which indicated the material contained carboxybenzene sulphonic acid. A sharp singlet at 10.40δ was also observed.

The i.r. spectrum indicated an extremely weak absorption at 2110cm^{-1}. The spectrum was characterised by a broad absorption between 3600 and 3000 cm^{-1} which was associated with the presence of hydroxyl groups. The carbonyl portion of the spectrum showed an

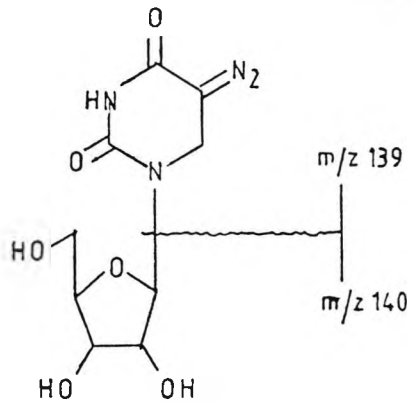
intense absorption at 1700cm^{-1} and weaker absorptions at 1720 and 1680cm^{-1}. These were attributed to the carboxyl group of the carboxy benzene sulphonic acid and to the carbonyls of the uracil moiety. A strong absorption at 870cm^{-1} was associated with *para*-disubstituted benzenes.

The absence of the protective groups in the ^{1}H n.m.r. suggested that the possible product was 5-diazo-6H-uridine (9.30), which was confirmed in the EI-MS spectrum. A pseudomolecular ion at m/z 313 was identified by high resolution mass measurement. Many of the observed fragments could be related to its structure. The peaks at m/z 285 and 270 were assigned to the species [M-N_2] and [M-HNCO]. The peaks at m/z 133 (9.31), 104, 90 and 74 were assigned to to sugar moiety and its related fragments. Confirmation of the 5-diazo-6H-uracil moiety was provided by the peaks at m/z 140, 139 (9.31), 138, 74, 69 and 54. The peaks at m/z 182 and 170 were assigned to fragments associated with both the uracil and sugar moiety (9.32 and 9.33).

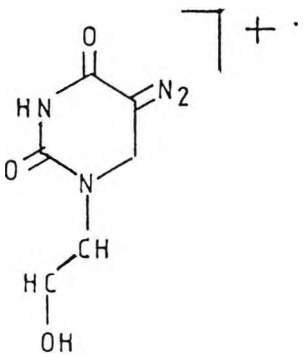


m/z 313

(9.30)

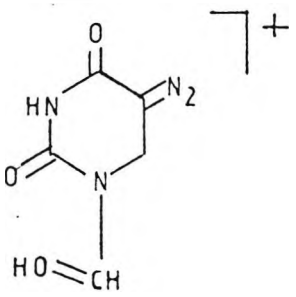


(9.31)



m/z 182

(9.32)



m/z 169

(9.33)

The spectrum also provided evidence for a small quantity of the protected nucleoside (9.29) with a peak at 3.29.1205 which represents the loss of nitrogen from a pseudomolecular species [P2-N_2]($C_{14}H_{20}N_4O_7-N_2$ requires 329.1348). Peaks for the protecting group were also observed at m/z 75 ($MeOCH_2OCH_2$), 61 ($MeOCH_2O$) and 58 (C_3H_6O). The fully protected sugar moiety was also detected at m/z 217.

Although neither the desired diazo nor the 5-formyl nucleoside were isolated in a high state of purity, the spectral evidence suggests that the lithiation, subsequent formylation and diazo transfer is a promising route to the 5-diazonucleoside. The success of this method will rely firstly on optimising the formation pf the 5-formyl derivative. It is suggested that possibly a better approach would be to react the 5-formyl nucleoside *in situ* with the diazo transfer reagent before an an attempt is made to isolate the product. Success will depend, secondly, on finding a suitable isolation and purification procedure for the diazo compound, *e.g.* preparative reverse phase h.p.l.c.

9.3 References.

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Chapter 10 Experimental

Melting points, which were determined on a Kofler hot-stage apparatus, are in degrees centigrade and are uncorrected. Infrared spectra were obtained using a Perkin-Elmer 159G spectrophotometer and were performed on nujol mulls unless otherwise stated. The frequencies of maximum absorptions are quoted in wavenumbers (cm^{-1}) using a polystyrene film as a reference. ^1H n.m.r. spectra were recorded on either a Jeol MH 100 spectrometer or a Jeol PMX 60 spectrometer. Tetramethylsilane (TMS) was used as internal reference and chemical shifts quoted in δ. Coupling constants are quoted in Hertz (Hz). The abbreviations: s=singlet, d=doublet, dd=double doublet, t=triplet, q=quartet, m=multiplet, bs=broad singlet, exc=exchangeable, *i.e.* disappears on the addition of D_2O, are used in the description of ^1H n.m.r. data. ^{13}C-n.m.r data were determined on a Jeol FX-60 spectrometer at 15MHz with TMS as an internal standard unless otherwise stated. Mass spectra were recorded on a AEI MS 30 instrument. FAB spectra were obtained through the SERC Mass Spectrometry service Centre, University College Swansea. Microanalysis were analysed in CARLO ERBA elemental analyser, model 1106. T.l.c. analytical separations were performed on 0.2mm or 1.0mm thick Merck Kieselgel GF254 silica plates and monitored by irradiation of the plates with UV light or by exposure to iodine. Large-scale column silica gel chromatography was performed using Merck Kieselgel HF.

10.1 5-Diazouracil [1]

A solution of hydrochloric acid (90ml, 0.996M) was chilled to 0°C using an ice/water/salt bath. To this solution, 5-aminouracil (4.1g, 32.2mmol) was added with stirring until the amine had dissolved to give a bright yellow solution. A 6.9% w/v solution of sodium nitrite (20ml, 1.08M) was slowly added in a dropwise manner to the cold acid/aminouracil solution. The temperature of the reaction mixture was not allowed to rise higher than 2°C. A bright yellow precipitate was obtained and the solution left stirring for another 10min. The precipitate was filtered from the solution and washed with ice-cold distilled water (4x150ml) until the washings showed the absence of chloride ions using silver nitrate solution. The residue was then washed with cold ethanol (3x200ml) at -18°C. and dried in a vacuo at ambient temperature for several days to

afford a bright 5-diazouracil(4.2g, 95%); *high resolution mass measurement* 138.0236 ($C_4H_2N_4O_2$, requires 138.01776); *m.p.* 198-205°C; *i.r.* (nujol, cm^{-1}) 3320(NH), 2130 (N_2), 1710 (CO, imide), 1650 (CO, amide), and 1510 (conj. C=N); δ_H (100MHz, dmso-d_6) 10.9 (1H, bs, H-3), 9.1 (1H, s, H-6), 3.3 (1H?, s, H_2O); *EI-MS* (Spectrum I) (m/z, % rel. intensity) 139 (4.0%, M+H), 138 (51.0%, M+), 112 (6.4%), 96 (2.6%), 95 (9.0%), 68 (12.2%), 67 (86.2%), 66 (1.3%), 43 (11.4%), 39 (100.0%); *EI-MS* (Spectrum II)(m/z, % rel. intensity) 138.0057 (16.3%, M+), 112 (85.2%), 96 (4.9%), 95 (5.8%), 94 (15.9%), 70 (14.3%), 69 (86.4%), 68 (29.7%), 67 (47.0%), 44 (95.6%), 43 (86.0%), 40.0 (52.3%).

10.2 5-Diazo-6-methoxy-1,6-dihydrouracil. [1]

5-Aminouracil (6.25g, 49.1mmol) was dissolved in hydrochloric acid (120ml, 0.8M) and cooled to 0°C. Sodium nitrite solution (32ml, 1.93M) was added to the dissolved amine in a dropwise manner and the temperature allowed to rise to 2°C. 5-Diazouracil precipitated out and after stirring for a further 5 min., absolute ethanol at -10°C (80ml) was added. The mixture was cooled to -10°C using an acetone/dry ice slush bath. The diazo compound was collected by filtration and washed with cold (-10°C) absolute ethanol (2x20ml). 5-Diazouracil was then dissolved in boiling methanol (500ml, hplc grade) and decolourising charcoal (0.5g) added. The hot solution was filtered and gave a clear yellow filtrate, which was allowed to stand at ambient temperature for several days. The clusters of glistening yellow needles were filtered from the mother liquor, which was then reduced to half its original volume on a rotary evaporator and on further standing yielded a second crop of of crystals (7.46g overall, 90%); *FAB high resolution mass measurement* 171.05154 ($C_5H_4N_4O_3$+H requires 171.05180); *m.p.* 198-205°C; *i.r.* (KBr, cm^{-1}) 3410 (N-H), 2820 (OMe), 2110 (N_2), 1710 (CO, imide), 1650 (CO, amide), and 1050 (C-O, ether); δ_H (100 MHz, dmso-d_6) 10.3 (1H, bs, H-3, exc.), 8.6 (1H, d, J=4.0Hz, H-1), 5.75 (1H, d, J=4.0 Hz, H-6), 3.35 (3H, s, Me); δ_C (15MHz, dmso-d_6, TMS) 162.22 (1416, C-2), 151.65 (1591, C-4), 77.12 (1539, C-6), 57.24 (7.39, C-5), 51.59 (1430, MeO); *EI-MS* (Spectrum I) (m/z, % rel. intensity) 142 (0.7%), 140 (0.6%), 139 (11.7%), 138 (11.0%), 112 (2.0%), 96 (0.8%), 95 (2.0%), 69 (2.6%), 68 (4.2%), 67 (22.2%), 58 (0.2%), 55 (4.7%), 53 (2.5%), 43 (3.8%), 40 (6.3%), 32 (26.1%), 31 (35.9%), 28

(100.0%); *EI-MS*(Spectrum II) (m/z, % rel. intensity) 142 (0.4%), 140 (1.9%), 139 (31.9%), 138 (11.7%), 112 (0.4%), 111(3.9%), 96 (0.7%), 95 (2.0%), 69 (4.1%), 68 (4.3%), 67 (19.5%), 55 (5.3%), 53 (1.8%), 43 (3.8%), 40 (6.1%), 32 (28.1%), 31 (36.7%), 28 (100.0%); *FAB-MS* (3-nitrobenzyl alcohol) (m/z, % rel. intensity) 511 (0.4%), 494 (0.6%), 477 (1.5%), 341 (3.5%), 324 (19.9%), 193 (3.1%), 171 (100%, M+H), 169 (3.8%), 143 (8.4%), 142 (4.3%), 141 (1.4%), 139 (40.6%), 137 (8.3%), 128 (2.9%), 114 (1.1%), 113 (8.7%), 111 (1.2%), 100 (2.9%), 85 (1.8%), 84 (1.4%), 70 (1.8%).

10.3 5-Diazo-6-hydroxy-1,6-dihydrouracil

Freshly prepared 5-diazouracil (2g, 14.46mmol) was dissolved in boiling water (120ml). Decolourising charcoal was added and the solution filtered whilst hot. An additional volume of distilled water (100ml) was added and the solution reheated until the material which had come out of solution during the filtration had redissolved. The solution was allowed to stand at ambient temperature over the weekend and the bright yellow solid filtered from the solution. Only one crop of 5-diazo-6-hydroxy-1,6-dihydro--uracil (1.3g, 58%) was afforded and further attempts to obtain a second crop were unsuccessful; *microanalysis*, found C30.96%, H2.37%, 35.79% ($C_4H_4N_4O_3$ requires C30.78%, H2.58%, N35.89%), *high resolution mass measurement*, 156.0307 ($C_4H_4N_4O_3$ requires 156.028325); *m.p.* 120-140°C (droplets), 208°C (decomp.); *i.r.* (nujol, cm^{-1}) 3320 (N-H), 3040 (OH), 2150 (N_2), 1720 (CO, imide), 1620 (CO, amide), 1330 (OH), 1460 (CH); δ_H (100MHz, dmso-d_6, TMS), 10.3 (1H, bs, H-3, exc.), 8.38 (1H, bs, H-1, exc.), 6.72 (1H, d, J=7.5 Hz, C6-OH), 5.83 (1H, dd, J=7.5 and J=3.0Hz, H-6), 3.38 (s, H_2O); *EI-MS* (m/z, % rel. intensity) 156 (0.03%, M+), 138 (4.54%), 119 (1.06%), 112 (1.04%), 96 (4.46%), 70 (1.82%), 69 (4.93%), 68 (2.06%), 67 (7.30%), 55 (1.92%), 54 (1.84%), 53 (1.57%), 47 (3.06%), 45 (2.03%), 43 (6.4%), *FAB-MS* (m/z, % rel. intensity) 437 (2.5%), 415 (0.5%), 385 (5.1%), 287 (1.1%), 277 (3.4%), 265 (1.6%), 264 (5.5%), 247 (55.6%), 246 (1.4%), 195 (2.2%), 179 (3.7%), 177 (2.3%), 161 (2.3%), 158 (2.9%), 156 (1.7%), 155 (2.2%), 139 (99.8%), 138 (1.6%), 129 (4.3%), 121 (2.3%), 120 (1.1%), 113 (71.1%), 112 (4.5%), 86 (2.3%), 85 (1.4%), 84 (2.2%), 82 (1.2%), 71 (1.0%), 70 (6.7%), 68 (1.2%).

10.4 5-Diazo-6-ethoxy-1,6-dihydrouracil.

The crude 5-diazouracil from 5-aminouracil (6.04g, 47.5mmol) was heated with reflux in absolute ethanol (150ml) for 24h. On standing a bright yellow precipitate was obtained. The precipitate was filtered from the mother liquor and dried in a vacuum desiccator at ambient temperature for 2h. to afford 5-diazo-6-ethoxy-1,6--dihydrouracil (7.2g, 82%); *microanalysis*, found C38.99%, H4.29%, N30.49% ($C_6H_8N_4O_3$ requires C39.13%, H4.38%, N30.43%); *high resolution mass measurement* (FAB), 185.06698 ($C_6H_8N_4O_3$+H, requires 185.06745); *m.p.* 199°C; *i.r.* (nujol, cm^{-1}) 3220 (N-H), 2120 (N_2), 1700 (CO, imide), 1660 (CO, amide), 1060 (C-O, ether); δ_H (100 MHz, dmso-d_6) 10.03 (1H, bs, H-3, exc.), 8.89 (1H, d, J=3.0Hz, H-1, exc.), 6.68 (1H, d, J=3.0 Hz, H-6), 3.45 (2H, q, J=6.5Hz, H-1'), 1.10 (3H, t, J=6.5Hz, H-2'); δ_C (15MHz, ^{1}H decoupled, dmso-d_6, TMS)(intensity), 163.02 (1538, C=O, C-4), 152.02 (2102, C=O, C-2), 76.24 (2656, OCH_2, C-1'), 60.221 (2763, $C{=}N_2$, C-5), 57.812 (1064, C-H, C-6), 14.973 (2454, CH_3, C-2'); δ_C (15MHz, EQ, dmso-d_6, TMS) 163.02 (2727), 152.02 (3231), 60.16 (1349), 57.81 (1570); δ_C (15MHz, coupled, dmso-d_6, TMS) 163.21 (724), 162.95 (656), 152.28 (583), 151.89 (789), 81.90 (346), 75.98 (388), 70.64 (375), 60.03 (389), 59.76 (398), 55.87 (218), 19.27 (380), 14.39 (390); *EI-MS* (m/z, % rel. intensity) 141 (0.8%), 140 (7.3%), 139 (100%), 138 (41.8%), 115 (0.5%), 113 (19.6%), 112 (4.7%), 96 (3.8%), 95 (7.6%), 86 (0.6%), 70 (23.4%), 69 (16.2%), 68 (17.2%), 67(67.0%), 66 (1.2%), 55 (27.5%), 53 (8.0%), 46 (24.6%), 43 (14.5%), 42 (23.2%), 40 (20.3%), 39 (72.3%), 32 (62.3%), 31 (87.32%); *FAB-MS* (m/z, % rel. intensity) 553 (0.4%), 522 (0.4%), 491 (1.0%), 369 (2.2%), 338 (14.6%), 337 (1.1%), 223 (1.1%), 205 (4.7%), 185 (100.0%), 184 (1.6%), 183 (5.1%), 169 (2.3%), 167 (1.9%), 166 (2.5%), 163 (1.1%), 156 (3.6%), 142 (2.6%), 141 (4.4%), 139 (48.3%), 114 (2.3%), 113 (12.4%), 99 (1.9%), 98 (1.2%), 70 (2.1%).

10.5 5-Diazo-6-isopropoxy-1,6-dihydrouracil.

Freshly prepared 5-diazouracil (3.0g, 21.7mmol) was triturated in absolute ethanol (30ml) and filtered. The residue was dissolved in boiling 2-propanol (300ml) and decolourised with charcoal. The solution was filtered whilst hot to remove the charcoal and coloured impurities. The clear, yellow filtrate was reheated to dissolve any

material which had come out of solution during the filtration and was left standing at ambient temperatures for several days. The fine crystalline material was filtered from the mother and dried in a vacuum desiccator at ambient temperatures overnight to afford 5-diazo-6-isopropoxy-1,6-dihydrouracil (1.47g, 35%); *high resolution mass measurement*, 199.08321 ($C_7H_{10}N_4O_3$+H requires 199.0831); *m.p.* >210°C; *i.r.* (nujol, cm^{-1}) 3240 (N-H), 2930 (CH_2), 2120 (N_2), 1710 (CO, imide), 1660 (CO, amide), 1470 (CH), 1060 (C-O, ether); δ_H *(100 MHz, dmso-d_6) 10.10 (1H, bs, H-3, exchangeable in D_2O), 8.64 (1H, bs, J=4.5Hz, H-1, exchangeable in D_2O), 5.80 (1H, d, J=4.5 Hz, H-6), 3.86 (1H, m, J=6.0Hz, H-2'), 1.10 (6H, d, J=6.0Hz, H-1',H-3')*; δ_C (60 MHz, dmso-d_6), 163.15 (1633, C=O, C-4), 151.95 (2524, C=O, C-2), 75.00 (5773, OCH_2, C-2'), 67.58 (5384, C-6), 58.59 (1630, $C=N_2$, C-5), 23.31 (5236, Me), 22.27 (5460, Me); *EI-MS* (m/z) 199 (0.8%, M+H), 183 (7.7%), 170 (2.0%), 156 (0.4%), 155(2.2%), 140 (12.2%), 139 (100%), 138 (39.9%), 128 (44.3%), 127 (1.1%), 126 (0.3%), 113 (1.3%), 112 (7.7%), 111 (2.6%), 97 (0.4%), 96 (5.4%), 95(8.0%), 86 (0.6%), 85 (10.1%), 71 (1.7%), 70 (8.5%), 69 (11.4%), 68 (19.8%), 60 (0.7%), 59 (7.4%), 58 (3.2%), 57 (40.6%), 54 (5.0%), 53 (11.2%), 43 (47.4%), 41 (6.4%), 40 (36.6%); *FAB-MS* (m/z, % rel. intensity) 617 (1.4%), 595 (0.3%), 505 (0.3%), 435 (0.5%), 419 (5.5%), 397 (2.7%), 352 (4.2%), 237 (1.%), 221 (18.3%), 199 (100.0), 198 (1.0%), 197 (2.9%), 183 (1.4%), 171 (4.9%), 170 (3.2%), 139 (83.9%), 128 (4.3%), 127 (1.5%), 113 (8.1%), 112 (3.6%), 84 (1.1%), 61 (2.1%).

10.6

5-Diazo-6-tert-butoxy-1,6-dihydrouracil

Freshly prepared 5-diazouracil (3g, 21.7mmol) was triturated in absolute ethanol and filtered. The residue was dried in vacuo and heated with reflux for 24h in *tert*-butanol and (300ml). The solution was filtered hot and allowed allowed to stand at ambient temperature for 1 week. The fine yellow needles formed were filtered from solution to afford 5-diazo-6-tert-butoxy-1,6-dihydrouracil (62mg, 2.92mmol, 0.1%); microanalysis, C37.96%, H3.97%, N31.06%, ($C_8H_{12}N_4O_3$, requires C45.28%, H5.70%, N26.40%); m.p. 210°C discolours; *i.r.* (nujol mull, cm^{-1}) 3340 (NH), 2950 (C-H), 2870 (C-H), 2130 (N_2), 1750 (NH), 1710 (CO, imide), 1660 (CO, amide),

1055 (C-O, ether); δ_H (100 MHz, dmso-d_6, TMS) 10.0 (1H, bs, H-3), 8.40 (1H, bs, H-1), 5.6 (1H, d, J=4.5Hz, H-6), 3.3 (9H, s, Me); *EI-MS* (spectrum I) (m/z, % rel. intensity) 185.0791 (0.2%, P-N_2, $C_8H_{12}N_2O_3$+H, requires 185.09261), 129 (0.2%), 141 (0.6%), 140 (5.7%), 139 (83.8%), 138 (47.2%), 112 (4.7%), 96 (3.7%), 95 (7.7%), 94 (0.4%), 70 (3.4%), 69 (6.4%), 68 (12.8%), 67 (64.2%), 55 (18.4%), 45 (68.4%), 44 (7.3), 43 (1.0%); (Spectrum II, 100°C) (m/z, % rel. intensity) 139 (100.0%), 95 (7.2%), 67 (80.7%), 57 (15.0%), 55 (53.9%), 43 (37.2%); (Spectrum III, 250°C) 138 (6.3%), 112 (9.2%), 97 (3.6%), 69 (17.6%), 67 (16.7%), 55 (14.7%), 44 (100.0%), 43 (62.0%); *FAB-MS* (Spectrum I) (m/z, % rel. intensity) 463 (0.4%), 447 (0.4%). 251 (1.9%), 235 (3.4%), 213 (3.0%), 185 (50.9%), 184 (1.1%), 169 (2.1%), 155 (4.0%), 154 (2.3%), 142 (2.1%), 141 (6.4%), 139 (100%), 127 (2.1%), 126 (1.1%), 113 (18.1%), 97 (4.0%), 74 (2.1%); *FAB-MS* (spectrum II, m/z) 251 (2.8%), 235 (2.6%), 213 (2.3%), 197 (1.1%), 185 (35.6%), 184 (1.4%), 170 (2.0%), 169 (2.8%), 142 (4.7%), 141 (16.8%), 139 (100%), 127 (3.9%), 126 (2.2%), 113 (9.7%), 97 (2.1%), 75 81.2%), 74 (3.4%).

10.7 5-Diazo-6-phenoxy-1,6-dihydrouracil.

The 5-diazouracil (1.51g, 10.94mmol) was stirred in benzene/THF (90:10) to give a fine suspension. Phenol (1.19g, 12.64mmol) was dissolved in benzene (20ml) and added to the suspension. The mixture was heated with reflux overnight and then left to stand at ambient temperatures. The orange precipitate was filtered from the solution and dried in vacuo to afford 5-diazo-6-phenoxy-1,6-dihydrouracil (1.27g, 50%); *high resolution mass measurement*, 232.0613 ($C_{10}H_8N_4O_3$ requires 232.059625); *i.r.* (nujol, cm^{-1}) 3340 (NH), 3060 (amide, NH), 2140 (N_2), 1725 (CO, imide), 1660 (CO, amide), 1650 (Ar), 1510 (Ar), 1020 (-O-Ar); δ_H (100MHz, dmso-d_6, TMS) 10.0 (1H, bs, H-3), 8.2 (1H, bs, H-1), 7.15 (2H, m, H-3'/5'), 6.8-6.6 (3H, m, H-2'/6' and H-4'), 5.7 (1H, d, J=4.5Hz, H-6); *EI-MS* (spectrum I, 250°C((m/z, % rel. intensity) 141 (0.3%), 140 (1.9%), 139 (32.5%), 138(22.9%), 112 (2.8%), 111 (0.8%), 110 (0.7%), 96 (1.7%), 95 (4.5%), 94 (0.9%), 70 (4.0%), 69 (4.5%), 68 (10.2%), 67 (54.0%), 66 (1.2%), 53 (8.0%), 52 (1.9%), 51 (1.0%), 46 (13.4%), 45 (32.8%), 44 (11.1%), 43 (19.2%), 42 (10.6%), 41 (7.1%), 40 (19.1%), 39 (76.5%); (spectrum II, 100°C) (m/z, % rel.

intensity) 232 (0.5%), 204 (2.4%), 202 (1.3%), 128 (4.5%), 112 (31.7%), 96 (22.2%), 94 (22.3%), 69 (17.9%), 57 (16.1%), 56 (44.8%), 55 (15.6%), 45 (16.5%), 44 (100.0%), 43 (85.5%), 42 (21.1%), 41 (53.8%), 40 (21.1%), 39 (22.8%).

10.8 5-Diazo-6(4-methoxy)phenoxy-1,6-dihydrouracil

Freshly prepared 5-diazouracil (697mg, 5.05mmol) was suspended in benzene (50ml) and *p*-methoxyphenol (3g, 24.17mmol) added. The mixture was heated with reflux overnight. The reaction mixture was allowed to stand for 24h. at ambient temperature. The orange solid was filtered from the solution and purified by preparative t.l.c. using dichloromethane/methanol (85:15) as the mobile phase. The band of silica, R_f=0.46 was isolated and eluted with methanol. The eluent evaporated to dryness to afford a pale yellow micro--crystalline solid, which was identified as the desired product (640mg, 49%); *high resolution mass measurement*, 262.0762 ($C_{11}H_{10}N_4O_4$ requires 262.07019); *i.r.* (KBr, cm^{-1}), 3340 (NH), 3240 (NH), 2130 (N_2), 1720 (CO, imide), 1700 (NH), 1650 (CO, amide), 1610 (Ar), 1440 (Me), 1050 (C-O, ether), 890 (Ar-H), 810 (Ar); δ_H (100MHz, dmso-d_6, TMS) 10.6 (1H, bs, H-3), 8.70 (1H, d, J=3.0Hz, H-1), 6.88 (4H, s, Ar-H), 6.1 (1H, d, J=3.0Hz, H-6), 4.0 (3H, s, MeO); *EI-MS* (m/z, % rel. intensity) 263 (1.8, M+H), 262 (11.5, M^+), 219 (0.9%), 191 (1.3%), 176 (1.8%), 148 (5.3%), 139 (4.0%), 124 (4.9%), 123 (9.7%), 113 (3.5%), 112 (50.0%), 110 (2.2%), 109 (7.5%), 96 (3.5%), 95 (4.9%), 86 (4.4%), 81 (5.3%), 69 (51.3%), 68 (23.9%), 66 (4.0%), 65 (7.5%), 56 (6.6%), 55 (23.0%), 53 (15.5%), 52 (14.2%), 51 (8.2%), 43 (96.0%), 41 (46.0%), 40 (39.8%), 38 (11.5%); (Spectrum II, sample in D_2O) (m/z, % rel. intensity) 266 (0.3%), 265 (1.7%), 264 (5.7%), 263 (6.6%), 262 (2.8%), 234 (0.3%), 125 (2.1%), 124 (7.6%), 123 (7.6%).

10.9 5-Nitrouracil [2]

Uracil (245mg, 2.19 mmol), nitronium tetrafluoroborate (700mg, 5.27 mmol) and tetramethylene sulphone (14.9g) were left stirring at 20°c for 18h. The reaction mixture was quenched by the addition of chloroform. The product was filtered from solution and recrystallized from hot methanol to afford 5-nitrouracil (286mg,

83%). A sample further purified, for spectroscopic purposes, by preparative t.l.c. using methanol/chloroform (3:7) as the mobile phase. The major band R_f=0.36 was removed and eluted with methanol. The eluent was evaporated to dryness to afford pale yellow solid, 5-nitrouracil; *high resolution mass measurement* 157.0099 ($C_4H_3N_3O_4$ requires 157.01235); *m.p.* >220°c; *i.r.* (nujol, cm^{-1}) 3400(NH), 1735 (CO, conj. imide), 1690 (CO, amide), 1630 (C=C, conj. with carbonyl), 1530 (NO_2), 1325 (NO_2), 840 (>C=CRH); λ_{max} (CH_3OH, nm) 212, 239, 264, 246; δ_H (dmso-d_6, TMS) 8.45 (1H, s, H-6); $_c$ (^{1}H-decoupled, dmso-d_6, TMS) 157.09 (286%), 156.05 (569%), 123.30 (195); o_c (enhanced quaternary, dmso-d_6, TMS) (intensity) 157.22 (327), 156.86 (231), 123.37 (200); δ_c (coupled, dmso-d_6, TMS) (intensity) 162.17 (283), 157.42 (134), 156.96 (231), 155.33 (105), 150.13 (288), 123.37 (200); *EI-MS* (m/z, % rel. intensity) 158 (0.9%), 157 (6.5%), 141 (1.2%), 114 (0.4%), 112 (0.4%), 86 (0.6%), 71 (6.3%), 49 (11.3%), 44 (85.5%), 43 (100%); *FAB-MS* (3-nitrobenzyl alcohol, m/z, intensity not provided) 464 (M+2mat), 315, 314 (2M), 180 (M+Na), 158 (M+H), 93 (M+Na-2HNCO).

10.10 Attempted hydrogenation of 5-nitrouracil

5-Nitrouracil (1g) was suspended in ethanol (50ml) and the catalyst (30mg), 5% palladium on carbon, added. The mixture was stirred under hydrogen. A measured quantity of hydrogen (70cm3) was taken up and reaction stopped. The catalyst was filtered from the solution through high flow filter aid and the solvent ethanol was removed using a rotary evaporator to afford an orange-brown solid (482mg); *i.r.* (nujol, cm^{-1}) 3400-3200 (NH), 1800-1650(COs), 1590(R-NO_2?), 1570, 1290; δ_H (100MHz, $CDCl_3$+DMSO-d_6, TMS) 8.8 (bs, H-3), 8.1 (bs, H-1), 6.5 (s), 3.2-3.8 (bs, NH2), 2.9 (m, methylene sulphone), 2.1 (m, methylene sulphone); *EI-MS* (m/z, % rel. intensity) 129 (0.3%), 111 (0.3%), 79 (100%), 78 (11.5%), 68 (9.7%), 52 (45.8%), 51 (24.7%), 50 (18.2%).

10.11 5-Nitro-5,6-dihydrouracil sodium salt monohydrate [3]

5-Nitrouracil (30.36, 193.3mmol) was dissolved in Ethanol/ distilled water (200:150). A solution of sodium borohydride (4.32g, 114.2mmol) and sodium bicarbonate (2 spatula, 40mg) in ethanol/water (10:10) was prepared and added in a small quantities to the 5-nitrouracil solution. The reaction mixture was left stirring at

ambient temperature overnight to afford a powdery white solid. A small quantity of sodium hydroxide solution (2M, 2ml) was added and the mixture stirred and filtered to afford the crude product (42.64g, 111%). The product was recrystallized from ethanol/water (300:300) to afford fine needle-shaped crystals. The crystals were filtered from the mother liquor and dried under vacuo at room temperature to afford 5-nitro-5,6-dihydrouracil monosodium monohydrate (22.6g, 58.7%); *microanalysis*, found C24.02%, H2.96%, N20.96%, ($C_4H_4N_3O_4.Na.H_2O$ requires C24.13%, H3.03%, N21.11%; m.p. 327°C discolours; *i.r.* (Nujol, cm^{-1}) 3460 (NH), 3200 (H-bonded OH), 1720 (CO, imide), 1670 (CO, amide), 1350 (NO_2), 1130, 1100.

10.12 5-Nitro-5,6-dihydrouracil [3]

5-Nitro-5,6-dihydrouracil monosodium monohydrate (15.16g, 76mmol) was dissolved in boiling water (250ml) and acidified to pH 2.5 using concentrated hydrochloric acid. The solution was left to stand at ambient temperature to afford a white crystalline material. The crystals were filtered from solution and dried under vacuo at ambient temperature overnight to afford 5-Nitro-5,6-dihydrouracil (9.15g, 75.5%); *microanalysis*, found C30.31%, H3.03%, N26.49% ($C_4H_3N_3O_4$ requires C30.20%, H3.17%, N26.41%); *high resolution mass measurement*, 160.0429 ($C_4H_3N_3O_4$+H requires 160.03582) *m.p.* 193°C; *i.r.* (nujol, cm^{-1}) 3460 (NH), 3200 (H-bonded OH), 1720 (CO, imide), 1670 (CO, amide), 1560(NO2), 1350(NO2), 1150, 1130, 1105; δ_H (100MHz, dmso-d_6, TMS) 10.4 (1H, bs, H-3), 7.56 (1H, bs, H-1), 5.52 (1H, t, J=5.7Hz, H-5), 3.7 (2H, m, H-6', H-6"); *EI-MS* (m/z, % rel. intensity) 160 (1.7%), 158 (0.8%), 157 (9.7%), 114 (2.2%), 113 (7.6%), 112 (100%), 72 (0.6%).

10.13 5-Amino-5,6-dihydrouracil

5-Nitro-5,6-dihydrouracil (1.06g, 6.66mmol) was suspended in warm distilled water (100ml). To the suspension, 5% palladium on carbon (257mg) was added. The mixture was left stirring under hydrogen until the volume taken up remained constant (540ml). The solution was filtered through celite filter aid in order to remove the catalyst. The colourless filtrate was evaporated to dryness to afford a white solid, 5-amino-5,6-dihydrouracil (0.836g, 97.2%); *high resolution mass measurement* 129.0512 ($C_4H_7N_3O_2$ requires 129.0469); *i.r.* (nujol, cm^{-1}) 3400-2900 (NH, amino, amide and

imide), 3230, 3080, 2920, 2850, 1730 (CO, imide), 1680 (CO, amide), 1500, 1460, 1380, 1250, 1100, 1010, 970, 800, 755, 720, 660; δ_H (100MHz, dmso-d_6, TMS) 10.4 (1H, bs, H-3), 7.70 (1H, bs, H-1), 4.7 (2H, bs, NH_2), 4.8-2.9 (3H, m, H-5, H-6a, H-6b); *EI-MS* (m/z, % rel. intensity), 130 (0.3%), 129 (5.3%, M+), 128 (0.3%), 127 (2.8%), 114 (1.5%), 112 (0.6%), 101 (3.7%), 100 (5.8%), 86 (1.7%), 85 (2.2%), 72 (0.6%), 58 (5.4%), 57 (18.6%), 56 (5.6%).

10.14 5-Diazo-5,6-dihydrouracil

A 36% Hydrochloric acid solution (5.05g) was diluted with distilled water (50ml) to give a 1N solution. The acid solution (30ml) was cooled to a temperature slightly lower than 0°C using and ice/salt slush bath. 5-Amino-5,6-dihydrouracil (716.6mg, 5.55mmol) was dissolved in the acid to give a yellow solution. Sodium nitrite (3.51g) was dissolved in distilled water (50ml, 7.0%solution). To the stirring mixture, the 7.0% sodium nitrite solution (10ml) was slowly added in two portions. The first portion (5ml) at 0°C over a period of 20min. The temperature of the reaction mixture was allowed to rise to 2-3°C and the second portion (5ml) was slowly added. The bright yellow precipitate formed was filtered from the solution. The yellow residue was washed with iced water (4x50ml) until the washing was shown to be free of chloride ions using the silver nitrate test. The residue was pressed into a yellow paste and dried under vacuo to afford 5-Diazo-1,6-dihydrouracil (699mg, 90%); *high resolution mass measurement*, 141.0502 ($C_4H_4N_4O_2$+H requires 141.041235); *i.r.* (KBr, cm^{-1}) 3590 (N-H), 3600-2500 (C-H), 2130 (N_2), 1720 (CO), 1680 (CO); δ_H (100MHz, dmso-d_6, TMS), 10.9 (1H, bs, H-3), 7.8 (1H, bs, H-1), 4.2 (2H, d, J=1Hz, H-6); *EI-MS* (m/z, % rel. intensity) 141 (0.5%, M+H), 140 (3.8%, M^+), 113 (0.2%), 112 (0.4%), 98 (1.6%), 97 (1.3%), 87 (0.2%), 86 (0.8%), 70 (5.3%), 69 (1.7%), 68 (3.8%), 67 (1.5%), 57 (1.0%), 55 (2.9%), 44 (12.5%); *FAB-MS* (Thioglycerol, m/z, % rel. intensity) 423 (0.3%), 403 (0.7%), 317 (2.3%), 295 (6.2%), 294 (1.3%), 293 (15.1%), 279 (18.0%), 278 (1.1%), 263 (2.6%), 262 (1.7%), 233 (2.7%), 232 (8.7%), 225 (1.2%), 209 (11.7%), 200 (1.0%), 187 (100.0%), 185 (3.9%), 159 (24.2), 158 (16.3%), 155 (2.1%), 154 (4.4), 145 (1.6), 144 (18.3), 143 (1.6%), 141 (2.2%), 139 (2.6%), 138 (1.8%), 131 (7.3%), 115 (1.3%), 113 (4.4), 109 (12.9%), 101 (9.2%), 98 (1.5%), 96 (17.1%).

10.15 Solubility study of 5-diazo-6-methoxy-1,6-dihydrouracil

5-Diazo-6-methoxy-1,6-dihydrouracil (1-2mg) was placed in a clear glass sample tube and the selected solvent (1.5ml) added. The temperature of the samples was controlled by standing in a water bath set at one of three temperatures; ambient (10-15°C), warm (25-30°C) and hot (50-54°C) or at the b.p. of the selected solvent. The samples were shaken periodically to aid mixing. The solubility evaluated by qualitative inspection: if the solvent became yellow, then the material was deemed to be partly soluble; if the solution was strongly coloured and the crystalline diazo compound was no longer visible then the compound was considered soluble. Results, see Table 7.1 p194).

10.16 Solubility study of 5-diazo-1,6-dihydrouracil

5-Diazo-1,6-dihydrouracil (1-2 mg) was placed in a clear glass sample tube and the selected solvent (1.5ml) added. The temperature of the samples was controlled by standing in a water bath set at one of three temperatures; ambient (10-15°C), warm (25-30°C) and hot (50-54°C) or at the b.p. of the selected solvent. The samples were shaken periodically to aid mixing. The solubility evaluated by qualitative inspection: if the solvent became yellow, then the material was deemed to be partly soluble; if the solution was strongly coloured and the crystalline diazo compound was no longer visible then the compound was considered soluble. Results, see Table 7.2 p195.

10.17 Stability of 5-diazo-6-methoxy-1,6-dihydrouracil in dmso

Procedure A

5-Diazo-6-methoxy-1,6-dihydrouracil was dissolved in dimethyl sulphoxide. After 1h, a sample of the mixture was removed and examined by analytical t.l.c. using chloroform/methanol (7:3) as the mobile phase. Dmso (R_f=0.77) and the starting diazo compound (R_f=0.69) were the compounds observed.

Procedure B

The reaction mixture (procedure A) was heated to 50°C. The reaction mixture was allowed to cool to ambient temperature and examined by analytical t.l.c. Dmso (R_f=0.77) and the starting diazo compound (R_f=0.69) were the compounds observed.

Procedure C

The reaction mixture (procedure B) was allowed to cool to ambient temperature and rhodium acetate catalyst was added. The new mixture was stirred at ambient temperature for 1h and then examined by analytical t.l.c. In addition to dmso (R_f=0.77) and the starting diazo compound (R_f=0.69), two new spots were observed at R_f=0.51 and R_f=0.08

Procedure D

The reaction mixture (Procedure C) was heated to 50°C with stirring. After 1h, the mixture was allowed to cool to ambient temperatures and once again analysed by analytical t.l.c. Three components were observed; dmso (R_f=0.77), and the two unidentified components at R_f=0.51 and R_f=0.08. The starting diazo compound was no longer observed.

10.18 Reaction 5-diazo-6-hydroxy-1,6-dihydrouracil with dmso

5-Diazo-6-hydroxy-1,6-dihydrouracil (30mg) was dissolved in dimethyl sulphoxide (2ml) to give a bright yellow solution. Rhodium acetate catalyst was added and the mixture heated to 50°C with stirring for 1h. A t.l.c. of the reaction mixture showed and absence of starting material. The reaction mixture (approximately 2g) was purified by column chromatography, using a column packed with silica gel H (100g). A mobile phase of dichloromethane/methanol (14:1) was used to elute out the products and the eluent was collected. Each fraction was tested by analytical t.l.c., using chloroform/methanol (7:3) as the mobile phase and the relevant fractions were then combined. The fraction R_f=0.2 (chloroform/methanol system) was then evaporated to dryness to afford a yellow solid (21.3mg); *i.r.* (nujol, cm^{-1}) 3600-3000 (N-H, O-H), 1720 (CO, imide); *EI-MS* (m/z, % rel. intensity) 162.0237 (0.1%, M-HNCO-H, $C_5H_6NO_3S$ requires 162.022489), 119 (0.3%), 113 (0.1%), 112 (0.2%), 94 (0.3%), 79 (0.9%), 78 (15.3%), 63 (21.3%), 62 (5.4%), 48 (2.0%), 43(2.2%); *FAB-MS* (thioglycerol, m/z, % rel. intensity) 415 (3.6%, P2+M1-H_2), 413 (36.5%, P2+M2), 411 (3.2%, P2+M2-H_2), 405 (5.3%, P1+2mat); 399 (5.8%, 2M1+Na), 393 (3.7%, P2+mat+dmso); 377 (P1+M1), 315 (M1+mat+H), 297 (M+mat+H), 229 (M1+Na), 209 (19.5%, P2+H_2), 207 (3.4%, $C_6H_{10}N_2O_4S$+H, P2); 205 (5.9%, P2-H_2), 189 (8.7%, $C_6H_8N_2O_3S$+H P1), 188 (5.3%, P1-H_2), 179 (11.6%, 2dmso+Na), 163 (3.8%, M1-HNCO),

147 (5.3%, mat+K), 131 (100%, mat+Na), 129 (3.5%, P2-dmso), 115 (5.6%, P1-dmso+H_2), 113 (12.0%, P1-dmso), 79 (48.4%, dmso+H) 77 (3.2%, dmso-H);

10.19 EI-MS of dimethyl sulphoxide

A sample of the dmso used for reaction (Chp. 10.18) was examined by high resolution mass spectrometry; *EI-MS* (m/z, % rel. intensity) 94.01 (0.38%), 79.01 (1.45%), 78.0200 (5.39%, M+, C_2H_6OS requires 78.01394), 62.99 (9.3%), 47.98 (2.05%), 46.99 (14.48%).

10.20 EI-MS of perdeuterodimethyl sulphoxide

A sample of the dmso-d_6 used for reaction (Chp. 10.21) was examined by high resolution mass spectrometry; *EI-MS* (m/z, % rel. intensity) 86.06 (4.10%), 85.06 (2.26%), 84.06 (82.61%, M+, C2D6OS requires), 82.04 (1.42%), 66.01 (100.0%), 50.01 (28.85%), 47.97 (6.95%).

10.21 5-Diazo-6-hydroxy-1,6-dihydrouracil and dmso-d_6

5-Diazo-6-hydroxy-1,6-dihydrouracil (141mg) was dissolved in dmso-d_6 (2ml) and rhodium acetate catalyst added. The mixture was heated to 50°C with stirring for 1h. and an effervescence was observed from the granular surface of the catalyst. After the effervescence had ceased, the reaction was left stirring at ambient temperature for 24h. The crude product was examined by mass spectrometry without purification; *EI-MS* (m/z, % relative intensity) 128 (0.2%), 113 (0.7%), 112 (0.3%), 100.0418 (0.8%, $C_2D_4O_2S$, requires 100.046924), 85 (2.7%), 84 (79.6%), 82 (1.9%), 66 (100%), 48 (9.3%), 43 (21.5%).

10.22 Reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with dmso

5-diazo-6-methoxy-1,6-dihydrouracil (100mg) was dissolved in the reactant dmso (0.5ml) and rhodium acetate catalyst (10mg) was added. The mixture was left stirring at ambient temperature until both the catalyst and the diazo compound had dissolved. On warming, the solution was observed to effervesce and this was accompanied by a colour change from bright yellow to dark orange. The product was purified by preparative t.l.c. using dichloromethane/methanol (85:15) as the mobile phase. The polar component, R_f=0.1 was

extracted from the silica. The solvent was removed on a rotary evaporator to afford a yellow solid (32mg); *high resolution mass measurement*, 221.05960 ($C_7H_{12}N_2O_4S$+H requires 221.05960); *i.r.* (nujol, cm^{-1}) 1720 (CO; imide), 1680 (O-C=C, enol) δ_H (MHz, dmso-d_6+D_2O, TMS) 5.30 (1H, bs, H-4), 3.65 (3H, s, OMe), 2.55 (3H, s, Me), 2.50 (3H, s, Me), also 8.00 (s), 2.65 (s); *EI-MS* (Spectrum I) (m/z, % rel. intensity) 221 (0.2%), 219 (0.3%), 176 (0.3%), 161(1.1%), 155 (0.5%), 146 (0.4%), 137 (1.1%), 126 (0.4%), 120 (0.9%), 119 (2.1%), 94 (2.5%), 86 (0.6%), 83 (12.5%), 79 (4.3%), 78 (9.8%), 67 (6.8%), 63 (0.3%), 62 (3.1%), 59 (2.5%), 48 (1.1%), 44 (100%), 43 (39.0%) (Spectrum II, D_2O treated sample) (m/z, % rel. intensity) 128 (0.3%), 127 (0.3%), 120 (0.4%), 119 (0.8%), 118 (0.2%), 111 (0.8%), 110 (0.5%), 105 (0.7%), 104 (0.6%), 103 (0.4%), 102 (0.5%), 101 (0.4%), 100 (0.3%), 99 (0.6%), 96 (0.9%), 95 (0.9%), 94 (94%), 93 (0.8%), 88 (0.5%), 87 (0.6%), 86 (0.7%), 85 (1.5%), 84 (2.5%), 83 (2.3%), 79 (1.6%), 78 (8.7%), 70 (3.0%), 69 (11.4%), 68 (1.2%), 67 (1.2%), 62 (3.9%), 61 (5.0%), 60 (4.1%), 59 (1.9%), 58 (3.7%), 44 (100%), 43 (27.8%). *FAB-MS* (thioglycerol, m/z, % rel. intensity) 375 (4.1%), 367 (4.2%), 321 (5.5%), 295 (10.0%), 289 (6.6%), 267 (7.6%), 259 (4.8%), 239 (27.3%), 231 (4.4%), 227 (4.7%), 221 (5.7%, P2), 211 (5.7%), 209 (60.6%), 189 (33.6%, P1), 188 (5.2%), 187 (69.2%), 179 (63.3%), 177 (6.2%), 161 (5.1%), 159 (40.3%), 158 (25.4%), 149 (20.1%), 141 (6.6%), 133 (6.9%), 131 (100%), 123 (11.5%), 119 (4.8%), 109 (4.7%), 103 89.5%), 101 (89.5%), 78 (7.7%), 63 (4.3%).

10.23 Reaction of 5-diazo-6-ethoxy-5,6-dihydrouracil with dmso

5-diazo-6-ethoxy-5,6-dihydrouracil (106mg) was dissolved in warm dmso/benzene (2:18ml) to give a yellow solution. A small quantity of rhodium acetate catalyst (10mg) added with stirring and the solution heated with reflux for 48h. The material was mixed with chloroform (150ml) to in order to precipitate out the product. The product failed to precipitate out and the excess solvent was removed on a rotary evaporator. The residue was dissolved in methanol and purified by preparative t.l.c. using dichloromethane/methanol (90:10) as the mobile phase. The major band of silica (R_f=0.17) was eluted with methanol and the eluent evaporated to dryness to afford a yellow solid (94.0mg); *i.r.* (nujol, cm^{-1}) 1720, 1660-1680 (C=C-O-, C=C); δ_H (100MHz, dmso-d_6, TMS) 7.6 (1H, s), 4.8

(s, OH), 2.7 (6H, s); *EI-MS* (m/z, % rel. intensity) 94 (2.3%), 81 (0.5%), 79 (7.7%), 78 (72.3%), 77 (0.8%), 67 (0.4%), 63 (82.5%), 62.01 (6.0%), 61.98 (5.0%), 61 (20.3%), 54 (0.6%), 43 (18.4%), 40 (2.3%), 39 (1.0%).

10.24 Attempted reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with ethanol in THF

5-Diazo-6-methoxy-1,6-dihydrouracil (150mg) was added to dry ethanol/THF (2:15) and heated to reflux to give a bright yellow solution. The solution was allowed to cool so that the solution was no longer at reflux temperature but still remained warm. Rhodium catalyst (5-10mg) was added. A rapid efferevescence was observed and the soluiton became rapidly turbid. On standing at ambient temperature the solution once again became clear. Examination of the product by analytical t.l.c. using dichloromethane methanol (85:15) showed one major component (R_f=0.5). The sample was evaporated to dryness to give an off-white waxy solid (168mg); *i.r.* (film, cm^{-1}) 3660-3240-3000 (N-H, C-H), 1730-1710 (CO), 1580, 1040 (ether), δ_H (60MHz, $CDCl_3$ + MeOH-d_4, TMS) 7.9s, 5.5 bd, 4.6m, 4.2m, 3.3-3.8m, 2.2-2.5m, 1.7-2.1, 1.1-1.4m.

10.25 Reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with ammonia solution

5-Diazo-6-methoxy-1,6-dihydrouracil (120.3mg) was dissolved in methanol (25ml) by stirring at room temperature for 0.5h. To the yellow methanol solution, ammonia solution (2ml, sp.gr. 0.88) was added dropwise. The solution was left stirring over 2 days at ambient conditions and the evaporated to dryness to give a bright yellow solid (80.4mg, 91%); *high resolution mass measurement*, 127.0332 ($C_4H_5N_3O_2$, requires 127.038165); *i.r.* (cm^{-1}), 1710 (CO), δ_H (100MHz, dmso-d_6 + $CDCl_3$, TMS) 12.0 (1H, bs, H-3, exc.), 8.9 (1H, d, J=9.0Hz, H-6), 5.96 (1H, d, J=9.0Hz, H-5), 5.08 (2H, bs, NH_2), 3.72 (H_2O, shifts to 4.3 on addition of D_2O); *EI-MS* 128 (0.4%), 127 (0.3%, M+) 100 (0.8%), 99(1.9%), 84 (3.4%), 72 (1.1%), 56 (6.1%), 55 (3.4%).

10.26 Reaction in acetone (5-methoxyuracil)

5-Diazo-6-methoxy-1,6-dihydrouracil (350mg, 2.06mmol) was dissolved in freshly distilled acetone (25ml) by warming to 35°C. Rhodium acetate (5mg) was added and effervescence was observed. After stirring for 2 minutes a white precipitate was obtained. The solution was left stirring at ambient temperature overnight and the precipitate was filtered from solution and dried in a vacuum pistol to afford 5-methoxyuracil (180mg); *microanalysis*, found C42.21%, H4.21%, N19.43% ($C_5H_6N_2O_3$ requires C42.26%, H4.26%, N19.71%); *high resolution mass measurement*, 142.0550 ($C_4H_6N_2O_3$, requires 142.037835); *i.r.* (KBr, cm^{-1}) 3000 (NH), 2860 (CH), 1760 (CO, imide), 1720 (CO, amide), 1670 (C=C), 1050 (ether); δ_H (100MHz, dmso-d_6, TMS) 10.4 (1H, bs, H-3), 9.26 (1H, bs, H-1), 6.36 (1H, bs, H-6), 3.66 (3H, S, MeO); *EI-MS* (m/z, % rel. intensity) 143 (4.0%), 142.0550 (61.4%, M+), 141 (0.7%), 114 (1.5%), 99 (8.8), 71 (80.7%), 56 (23.4%), 43 (14.7%).

10.27 Reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with benzophenone in benzene

5-Diazo-6-methoxy-1,6-dihydrouracil (105mg) was suspended in hot benzene (10ml) and benzophenone (1.078g), which was dissolved in benzene (5ml), added. The solution was heated to reflux for 1h. and rhodium acetatecatalyst (20mg) added at the onset of heating to give a clear green solution. The mixture was left stirring for 3 days at ambient temperature (10°C). The precipitate was filtered from the solution. The precipitate was washed with cold methanol to afford a pale yellow solid (66.4mg); *i.r.* (KBr, cm^{-1}) 3180 (NH), 2860 (CH), 2160, 2120 (N_2), 1760 (CO, imide), 1720 (CO, amide), 1670 (C=C), 1410, 1280, 1220, 1170, 1120, 1050 (ether), 890, 820, 760, 6500, 760, 650.

10.28 Reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with benzophenone in DMF

5-Diazo-6-methoxy-1,6-dihydrouracil (105mg, 0.61mmol) was dissolved in DMF (10ml) to afford a bright yellow solution. Benzophenone (1.144g) was dissolved in the DMF solution and the mixture heated to reflux. Rhodium acetate catalyst (20mg) was added to the hot solution and the green solution left stirring for 1h. The solution was allowed to cool and left stirring for 3 days. The

solution was condensed using a rotary evaporator and left to stand. After 2 days, a crystalline material was observed in the oil. The oil was triturated with dry ether to further precipitate an off-white solid (87mg, 98%); *high resolution mass measurement*, 142 ($C_5H_6N_2O_3$ requires 142.0000); *m.p.* 195°C droplets, 235°C decomp.; *i.r.* (nujol, cm^{-1}) 3180 (NH), 2860 (CH), 1760 (CO, imide), 1720 (CO, amide), 1670 (C=C), 1410, 1280, 1220, 1170, 1120, 1050 (ether), 890, 820, 760, 650; σ_H (dmso-d_6, TMS) 6.6 (1H, s, H-5), 3.8 (3H, s, MeO); *EI-MS* (m/z, % rel. intensity) 143 (3.1%), 142 (31.0%), 141 (0.4%), 114 (1.4%), 99 (5.9%), 71 (100%), 56 (30.7%).

10.29 Attempted reaction of 5-diazo-6-methoxy-5,6-dihydrouracil with styrene

5-Diazo-6-methoxy-5,6-dihydrouracil (103.7mg) was suspended in neat styrene (20ml) and the suspension heated to 30°C with stirring. Rhodium acetate catalyst (20mg) was added to the soluiton to afford a green solution and the mixture left stirring overnight at ambient temperature. The product obtained was a hard, solid, green material which proved insoluble in most organic solvents and was assumed to be polystyrene.

10.30 Reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with styrene in benzene.

5-Diazo-6-methoxy-5,6-dihydrouracil (157mg) was suspended in styrene/benzene (0.3:3) at 30°C. Rhodium acetate catalyst (10mg) added and the mixture left stirring for 24h. The unreacted styrene and solvent benzene were removed on a rotary evaporator to afford a yellow residue (118mg); *high resolution mass measurement*, 142.0529 ($C_5H_6N_2O_3$, requires 142.037985), *i.r.* 3140 (NH), 2850 (CH), 1780 (CO), 1720 (CO), 1680 (C=C), 1030 (ether); δ_H (100MHz, dmso-d_6 + $CDCl_3$), 10.6 (1H, bs, H-3), 9.4 (1H, bs, H-1), 6.54 (1H, s, H-5), 3.9 (MeO); *EI-MS* (m/z, % rel. intensity) 143 (1.7%), 142 (19.0%, M+), 141 (0.3%), 114 (1.5%), 71 (29.7%), 56 (23.4%)

10.31 5a,6,7,8,9,9a-Hexahydro-4H-benzo[1',2':6,7]furano[2,3-d]--pyrimidin-3-one

5-Diazo-6-methoxy-1,6-dihydrouracil (108.2mg, 0.64mmol) was suspended in hot cyclohexene (50ml) and rhodium acetate catalyst (10mg) added. The mixture was heated with reflux for 48h. and

afforded a white precipitate, which on further stirring, was observed to have gone into solution. The unreacted cyclohexene was removed by a rotary evaporator and the residue (250mg) purified by preparative t.l.c. using dichloromethane/mehanol (95:5) as the mobile phase. The band of silica (R_f=0.7) was removed and eluted with mobile phase and the solvent removed from the resultant filtrate to afford a yellow oil (62.3mg, 51%); *high resolution mass measurement*, 192.0864 ($C_{10}H_{12}N_2O_2$ requires 192.08987); *i.r.* (film, cm^{-1}) 3300 (NH), 2980 (CH), 1710 (CO, imide), 1670 (C=C-O-, enol), 1070 (ether); δ_H (100MHz, $CDCl_3$, TMS) 7.2 (2H, dt, J=10.6Hz, J=4.5Hz), 6.18 (1H, dt, J=10.6Hz, J=2.5Hz), 5.92 (1H, bs), 5.88 (1H, bs), 2.4-1.8 (8H, m) *EI-MS* (m/z, % rel. intensity) 193 (2.5%), 192 (2.4%, M+), 191 (2.6%), 175 (3.2%), 166 (0.7%), 165 (1.0%), 164 (1.1%), 149 (3.6%), 137 (4.5%), 122 (2.3%), 110 (3.6%), 98 (14.7%), 82 (12.6%), 81 (100%), 56 (6.5%), 55 (21.8%), 43 (24.5%); *EI-MS* (Spectrum II) (m/z, % rel. intensity) 224.1049 (0.13%, $C_{11}H_{16}N_2O_3$ requires 224.1161), 193 (3.78%), 192(3.78%, M+), 191 (2.34%), 165 (2.54%), 164 (2.68%), 149 (4.85%), 136 (5.1%), 123 (6.3%), 122 (3.12%), 110 (5.2%), 98 (18.7%), 97 (52.3%), 94 (8.5%).

10.32 1-Methoxy-1,2,5a,7,8,8a-Hexahydro-4H-furano[2',3':6,7]furano--[2,3-d]pyrimidin-3-one

5-Diazo-6-methoxy-1,6-dihydrouracil (812mg) was stirred in a large excess of dry 2,3-dihydrofuran (10ml). The mixture was warmed to 30°C and rhodium acetate catalyst (15mg) added, which resulted in an effervescence being observed, and stirred for 1h. in a sealed system. The mixture was allowed to cool to ambient temperature and left stirring for 48h. The excess 2,3-dihydrofuran removed on a rotary evaporator to afford a khaki coloured, viscous oil (763mg); *high resolution mass measurement*, 212.0669 ($C_9H_{12}N_2O_4$ requires 212.0795) *i.r.* (film, cm^{-1}) 3420 (NH), 2930 (CH), 1760 (CO), 1690(C=C), 1070 (ether); δ_H (100MHz, dmso-d_6, TMS) methoxyuracil, 10.56 (2H, bs, H-3), 9.4 (2H, bs, H-1), 6.6 (1H, s, H-5), 3.78 (6H, s, MeO-C6a) + adduct 10.2 (1H, bs, H-4), 8.4 (1H, bs, H-2), 6.4 (2H, d, J=3.0Hz, H-1) 5.2 (1H, m, H-5a), 4.24 (2H, m, H-7), 3.36 (3H, s, MeO), 2.5 (1H, m. H-8a), 1.88 (2H, m, H-8); δ_C (15.0MHz, dmso-d_6, TMS) (intensity) Methoxyuracil 162.692 (2719, C-2/C-4), 153.968 (3121, C-2/C-4), 140.036 (4324, C-5), 114.843 (3271, C-6) 61.914 (4229, OMe) + adduct 146.093 (1362, C-3), 139.932 (385, C-4a),

102.083 (447, C-5a), 99.671 (1421, C-8b), 74.343 (571, C-1), 68.944 (1643, C-7), 66.406 (1619, MeO), 32.030 (1385, C-8a), 28.645 (1500, C-8); *EI-MS* (m/z, % rel. intensity) 213 (0.7%), 212 (0.3%), 211 (1.1%), 181 (1.2%), 180 (0.3%), 169 (1.0%), 168 1.0%), 54 (2.3%), 152 (0.7%), 143 (2.7%), 142 (1.6%), 141 (4.3%), 136 (0.5%), 126 (2.1%) , 125 (4.1%), 110 (2.5%0, 109 (3.4%), 102 (0.6%), 87 (100%), 85 (11.7%), 70 (48.5%), 43 (17.0%), 40 (4.3%); *FAB-MS* (3-nitro--benzyl alcohol, m/z, intensity not provided) 423 (M+P-H_2), 388 (M+mat+Na), 366 (P+mat), 337 (M-CO+mat), 235 (M+Na), 213 (P+), 197 (P-CH_4), 181 (P-MeOH).

10.33 5,6-Dibutoxy-5,6-dihydrouracil

5-Diazouracil (285mg, 2.064mmol) was suspended in *n*-butanol (100ml). The mixture was heated with reflux overnight. The solution was evaporated to dryness to afford a waxy solid, 5,6-dibutoxy-5,6--dihydrouracil (369mg, 69.2%); *high resolution mass*, 258.1611 ($C_{12}H_{22}N_2O_4$ requires 258.15795); *m.p.* 180°C discol., >230°C; *i.r.* (film, cm^{-1}) 3600-2600 (NH, CH), 1720 (CO, 1680 (CO), 1060 (-O-R); δ_H (60MHz, dmso-d_6, TMS) 10.9 (1H, bs, H-3), 7.5 (1H, bs, H-1), 4.3-3.5 (10H, m, H-5, H-6, OCH_2CH_2), 0.9-1.7 (10H, m, $-CH_2CH_3$) *EI-MS* (m/z, % rel. intensity) 258 (0.07%, M+), 257 (0.09, M-H), 215 (0.22%), 185 (1.23%), 129 (16.1%), 112 (23.1%), 73 (32.9%), 57 (100%), 43 (40.4%).

(5-Butoxyuracil)

An attempt to purify the dibutoxy-dihydrouracil by preparative t.l.c. using methanol/dichloromethane (15:85) as the mobile phase resulted in 5-butoxyuracil being isolated; *high resolution mass measurement,* 184.0767 ($C_8H_{12}N_2O_3$ requires 184.08479); i.r. (KBr, cm^{-1}) 3000-3600 (C-H, N-H); 1730 (CO), 1710 (CO), 1660 (C=C); δ_H (MeOH-d_4, TMS) 6.84 (1H, s, H-6), 4.10 (2H, t, J=6.0Hz, H-1'), 1.3-1.9 (4H, m, J=6.0Hz, H-2'and H-3'), 0.98 (3H, t, J=6.0Hz, H-4'); *EI-MS* (m/z, % rel. intensity) 184 (0.5%, M+), 183 (1.6%), 169 (0.7%), 155 (0.2%), 141 (0.4%), 128 (0.5%), 112 (16.5%), 98 (0.2%), 97 (0.4%).

10.34 5,6-Dimethoxy-5,6-dihydrouracil

5-Diazo-6-methoxy-1,6-dihydrouracil was stirred in hot methanol until the compound had dissolved to give a yellow solution. Rhodium acetate catalyst (10mg) was added to the hot solution and

the effervescing solution was left stirring at ambient temperature overnight. The solvent methanol was removed on a rotary evaporator to give a white crystalline material. The crude material was purified by preparative t.l.c. using dichloromethane/methanol (90:10) as the mobile phase. Two major products were isolated, one (58% yield) with an R_f=0.58 and the other (26% yield) with an R_f=0.37.

5,6-Dimethoxy-5,6-dihydrouracil (R_f=0.58)

Microanalysis, found, C37%, H4.24%, N15.32% ($C_6H_{10}N_2O$ requires C41.38%, H5.79%, N16.08%); *i.r.* 3500-2700 (NH, CH); 1760 (CO), 1730 (CO), 1060 (ether) δ_H (100MHz, MeOH-d_4, TMS) 5.1 (OH) 3.72 (3H, s, MeO), 3.58 (3H, s, MeO), 3.38 (1H, d, J=1.5Hz) 3.42 (1H, d, J=1.5Hz); *EI-MS* (m/z, % rel. intensity) 144 (0.4%), 143 (0.3%), 115 (22.7%), 100 (0.2%), 87 (20.6%), 86 (11.5%), 72 (3.1%); *EI-MS* (Spectrum II) (m/z, % rel. intensity) 149 (0.11%), 143 (1.6%), 142 (25.9%), 87 (0.25%), 86 (0.94%); *FAB-MS* (thioglycerol, m/z) 305, 213, 197, 175 (M+H), 173, 143, 127, 113, 100, 91, 85, 73.

5,6-Dimethoxy-5,6-dihydrouracil (R_f=0.37)

δ_H (100MHz, MeOH-d_4, TMS) 5.1 (OH) 3.72 (3H, s, MeO), 3.58 (3H, s, MeO), 3.38 (1H, d, J=1.5Hz) 3.42 (1H, d, J=1.5Hz); 143 (2.5%), 115 (26.9%); 100 (0.6%), 87 (4.1%9, 86 (37.6%), 72 (0.9%), 43 (78.2%), 32 (12.3%), 31 (23.3%).

10.35 5-Methoxyuracil from 5,6-dimethoxy-5,6-dihydrouracil

5-Diazo-6-methoxy-1,6-dihydrouracil (180mg) was dissolved in hot methanol (25ml). The solution was allowed to cool and rhodium acetate catalyst (10mg) added. The effervescing mixture was left to stir for several hours. The excess methanol was removed on a rotary evaporator to afford a white solid tinted green. The green colour was removed by triturating the solid in acetonitrle and the resulting purple acetonitrile solution decanted to afford a off-white solid. The solid was dissolved in boiling methanol methanol (10ml) which resulted in the precipitation of a white solid. The solid was filtered from solution and dried in a vacuum pistol at ambient tempterature; high resolution mass measurement, 142.0346 ($C_5H_6N_2O_3$, requires 142.037985); *i.r.* (KBr, cm^{-1}) 3360 (NH), 3000 (C-H), 1760 (CO), 1710 (CO), 1670 (C=C), 1070 (C-O,

ether); δ_H (100MHz, dmso-d_6, TMS) 10.4 (1H, bs, H-3), 9.24 (1H, bs, H-1), 6.34 (1H, s, H-5/H-6), 3.68 (3H, s, MeO); *EI-MS* (m/z, % rel. intensity) 143 (0.7%), 142 (10.7%, M+), 141 (0.3%), 114 (0.3), 113 (5.1%), 99 (1.6%), 71 (16.8%), 56 (4.8%), 28 (100%).

10.36 5,6-Diethoxy-5,6-dihydrouracil

5-Diazo-6-methoxy-1,6-dihydrouracil (111mg, 0.65mmol) was dissolved in absolute ethanol (15ml) at 40°C. Rhodium acetate (10mg) was added to the stirring solution and effervecence was observed. The solution became turbid shortly afterwards and on further stirring the solution becomes clear. The solution was evaporated to dryness on a rotary evaporator to afford off-white solid. The solid was purified by preparative t.l.c. using dichloromethane/methanol (85:15) as the mobile phase. The major band (R_f=0.7) of silica was removed and eluted with ethanol. The eluent was evaporated to dryness to afford a white solid (125mg, 95%); *microanalysis*, found C43.63%, H4.85%, N16.92% ($C_8H_{14}N_2O_4$ requires C47.5%, H6.96%, N13.85%); *i.r.* (KBr, cm^{-1}) 3000-3300 (C-H, N-H), 1760 (CO), 1710 (CO), 1660, 1050 (O-C); δ_H (100MHz, MeOH-d_4, TMS) 3.85 (4H, m, OCH_2', OCH_2''), 3.5 (2H, m, J=1.5Hz, H-5, H-6), 2.0 (6H, m, J=6.0Hz, CH3', CH3"); *EI-MS* (m/z, % rel. intensity) 135 (0.2%), 115 (1.0%), 70 (2.1%), 43 (16.4%); *EI-MS* (Spectrum II, 5-ethoxyuracil) 156.0452 (31.75%, $C_6H_8N_2O_3$ requires 156.053485), 128 (79.7%), 113 (2.94%), 85 (28.0%), 8 (5.4%), 70 (4.7%).

10.37 Attempted preparation of 5-Hydroxy-6-methoxy-5,6-dihydrouracil

5-Diazo-6-methoxy-1,6-dihydrouracil (118mg) was dissolved in THF/water (15:5). The solution was heated to 20°C with stirring and rhodium acetate catalyst (10mg) added and effervescence was observed. The reaction mixture was evaporated to dryness to afford an green-white solid. The product was purified by preparative t.l.c. using dichloromethane/methanol (85:15) as the mobile phase. The largest band was removed and eluted with methanol to yield a bright yellow solid (5-methoxyuracil) (103mg); *i.r.* (nujol, cm^{-1}) 3160 (NH), 2920 (C-H), 1770 (CO), 1720 (CO), 1670 (C=C), 1050 (C-O, ether); δ_H (100MHz, MeOH-d_4, TMS); 6.37 (1H, s), 4.67 (HO), 3.73 (3H, s, MeO); o_c (1H decoupled) (intensity) 162.76 (515), 153.968 (618), 140.30 (1162), 114.78 (687), 61.98 (1066); o_c (enhanced quaternary) (intensity) 162.76 (563), 154.04 (800), 114.71 (755).

10.38 Preparation of 5-trideuteromethoxyuracil

5-Diazo-6-methoxy-1,6-dihydrouracil (180mg, 1.06mmol) was dissolved in hot methanol-d_4 (5ml), the solution was cooled to 25°C and rhodium acetate catalyst added. The solution was left stirring at ambient temperature for 12h. The opaque solution was filtered to afford a yellow solid (5-methoxyuracil, compound I). The filtrate was evaporated to dryness to afford an off-white solid (mainly 5-trideuteromethoxy-6-methoxy-5,6-dihydrouracil, compound II). The residue (compound II) was disolved in THF/benzene (10:90) and the mixture heated at reflux for a further 12h. The solution was then evaporated to dryness to afford an off-white solid (mixture III). This material was purified by preparative t.l.c. using dichloromethane/methanol (85:15) as the mobile phase. The band of silica (R_f=0.4) was isolated, eluted with methanol and the eluent evaporated to dryness to afford an off white solid (mixture IV).

5-Methoxyuracil (Compound I)

High resolution mass measurement, 142.0374 ($C_5H_6N_2O_3$ requires 142.037835); δ_H (60MHz, dmso-d_6, TMS) 10.5 (1H, bs, H-3), 9.4 (1H, bs, H-1), 6.37 (1H, s, H-6), 3.7 (3H, s, OMe); *EI-MS* (m/z, % rel. intensity) 143 (4.0%), 142 (61.4%, M+), 114 (1.9%), 112 (0.6%), 99 (9.1%), 83 (1.0%), 56 (32.3%).

Also observed:- 5-Trideuteromethoxyuracil (Compound III)

EI-MS (m/z, % rel. intensity) 145.0560 (5.5%, $C_5H_3D_3N_2O_3$ requires 145.05687)

5-Trideuteromethoxy-6-methoxy-5,6-dihydrouracil (Compound II)

High resolution mass measurement, 178.1004 ($C_6H_6N_2O_4D_4$ requires 178.08943) *i.r.* (nujol, cm^{-1}) 2900-3200 (C-H, N-H), 2420 (C-D), 1720 (CO), 1670 (CO), 1020 (O-C) δ_H (60MHz, dmso-d_6, TMS) 9.43 (1H, bs, H-1), 3.73 (3H, s, OCH_3), 3.2 (1H, bs, H-6) *EI-MS* (m/z, % rel. intensity) 178 (0.2%, M+), 146 (0.5%), 91 (10.7%), 76 (0.2%), 59 (0.4%).

Also observed:-

EI-MS (m/z, % rel. intensity) 146 (0.5%), 145.0537 (10.0%, $C_5H_3D_3N_2O_3$ requires 145.05687) *EI-MS* (m/z, % rel. intensity) 143 (4.2%), 142.0362 ($C_5H_6N_2O_3$ requires 142.037835)

Mixture III (Compound I + Compound III)

Compound I

EI-MS (m/z, % rel. intensity) 143 (0.5%), 142 (4.7%), 112 (4.2%), 99 (0.3%), 71 (36.9%), 56 (6.4%)

Compound III

EI-MS (m/z, % rel. intensity) 146 (0.2%), 145 (2.3%), 113 (2.8%), 102 (0.3%), 74 (1.9%), 59 (2.1%)

Mixture IV (Compound I + Compound III)

σ_H (60MHz, dmso-d_6, TMS) 6.4 (s, H-6), 3.7 (s, OMe)

Compound I

EI-MS (m/z, % rel. intensity) 143 (1.5%), 142 (15.5%), 112 (5.6%), 99 (3.9%), 71 (62.2%), 56 (14.5%)

Compound III

EI-MS (m/z, % rel. intensity) 146 (0.6%), 145 (5.5%), 113 (8.0%), 102 (1.4%), 74 (4.5%), 59 (2.5%)

10.39 Attempted reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with triethylamine.

5-Diazo-6-methoxy-1,6-dihydrouracil (109.7mg) was dissolved in triethylamine (15ml). The mixture was left stirring at ambient temperature. An orange precipitate was observed and the excess solvent/reactant triethylamine was removed. The precipitate was identified from its infrared spectrum as the starting diazo compound; *i.r.* (KBr, cm^{-1}) 3100-3200 (N-H), 2110 (N2), 1680-1710 (CO), 1050 (O-C).

10.40 Attempted reaction of 5-diazo-6-methoxy-1,6-dihydrouracil with aniline

5-Diazo-6-methoxy-1,6-dihydrouracil (107.4mg) was left stirring in aniline (15ml) with reflux for 48h. The solution goes brown. On standing for several days no change was observed in the solution. After several months a fine crystalline material was obtained. Attempts to separate this material from aniline by filtration proved unsuccessful. The attempt to obtain the product by adding aniline solution to ice/water and extracting with ether also proved unsuccessful. The addition of the aniline solution to hexane

did not precipitate out any product. A sample of the aniline solution was purified by preparative t.l.c. A single band was obtained, which when eluted and the eluent evaporated down, gave back the original brown oil.

10.41 5(3-Propyl-1-triazeno)uracil

5-Diazo-6-methoxy-1,6-dihydrouracil (114mg, 0.67mmol) was dissolved in freshly distilled *n*-propylamine (10ml) to produce a pale yellow solution. The solution was left stirring at ambient temperature to afford a white precipitate after 5min. The precipitate was filtered from the solution, washed with ether and dried in vacuo to afford the product (105mg,80%); microanalysis C42.51%, H5.65%, N35.25% ($C_7H_{11}N_5O_2$ requires C42.63%, H5.62%, N35.51%); *high resolution mass measurement*, 197.0882 ($C_7H_{11}N_5O_2$ requires 197.091255) *i.r.* (KBr, cm^{-1}) 3350-3100 (C-H, N-H), 1690 (CO), 1660, 1640; o_H (100MHz, dmso-d_6, TMS) 10.18 (1H, s, H-3', exc.), 9.18 (1H, s, H-6), 8.00 (1H, bs, H-3 exc.), 7.66 (1H, bs, H-1, exc.), 4.58 (2H, t, J=6.75Hz, H-1"), 1.92 (2H, m, J=6.75Hz, H-2"), 0.98 (3H, t, J=6.75Hz, H-3"); o_C (15MHz, dmso-d_6, TMS) (intensity) 160.226 (342, C-4), 153.079 (352, C-6), 140.666 (286, C-2), 127.868 (292, C-5), 51.394 (386, C-1"), 22.805 (401, C-2"), 10.525 (360, C-3"); *EI-MS* (m/z, % rel. intensity) 198 (0.3%), 197 (2.1%, M+), 181 (0.7%), 169 (1.1%), 168 (0.3%), 154 (1.4%), 139 (0.3%), 140 (20.4%), 58 (1.5%), 43.05 (96.0%), 43.00 (11.6%).

10.42 5(3-Butyl-1-triazeno)uracil

5-Diazouracil (103.5mg, 0.75mmol) was dissolved in freshly distilled *n*-butylamine (15ml) to give a bright orange solution. The solution was left stirring overnight and gave a yellow, turbid solution. The solution was filtered to afford an off-white residue, which was dried in vacuo (44.3mg, 30%); *microanalysis* C45.44%, H6.19%, N32.8% ($C_8H_{13}N_5O_2$ requires C45.49%, H6.20%, N32.15%); *i.r.* (KBr, cm^{-1}) 3440-3130 (C-H, N-H), 1680 (C=O), 1580, 1370, 1210; o_H (100MHz, dmso-d_6, TMS) 9.68 (1H, bs, H-3', exc.), 8.72 (1H, s, H-6), 6.68 (1H, bs, H-3, exc.), 6.3 (1H, bs, H-1, exc.), 4.42 (2H, t, J=6.8Hz, H-1"), 1.92 (2H, m, H-2"), 1.26 (2H, m, H-3"), 0.90 (3H, t, J=6.8Hz, H-4"); *EI-MS* 119 (0.2%), 115 (0.4%), 87 (0.6%), 83 (0.3), 66 (1.1%), 57 (0.9%).

10.43 5(3(2,3-Dihydroxy)propyl-1-triazeno)uracil

5-Diazouracil (102.5mg, 0.74mmol) was suspended in a solution of 3-aminopropan-1,2-diol (372mg) and 1,4-dioxan (10ml). The mixture was treated with ultrasonics which caused the suspension to turn orange. The mixture was left stirring overnight and the precipitate filtered from solution. the precipitate was dried in vacuo to afford the product (71.8mg, 32.7%); *microanalysis*, found C37.04%, H5.49%, N25.24% ($C_7H_{11}N_5O_4$ requires C36.66%, H4.84%, N30.56%); *i.r.* 3340-3130 (C-H, N-H), 3000-2500 (OH), 1720 (CO), 1710 (CO), 1680; δ_H (100MHz, dmso-d_6+$CDCl_3$, TMS) 9.14 (1H, s, H-6), 7.20 (1H, bs, exc., H-3), 6.84 (1H, bs, exc., H-1), 4.20 (1H, d, J=4.0Hz, Ha-3"), 4.06 (1H, d, J=7.5Hz, Hb-3"), 3.86 (1H, m, J=4.0Hz, J=7.5Hz, H-2"); 3.12 (2H, m, J=4.0Hz, H-1"); *EI-MS* 199.0898 (2.7%, M-CH_2O requires 199.07052), 168 (6.8%, M-$C_2H_5O_2$), 158.0706 (4.5%, (M-HNCO)-CO requires 158.08036), 118 (3.8%), 91 (8.9), 75 (3.2%).

10.44 5(3(2-Propenyl)-1-triazeno)uracil

5-Diazo-6-methoxyuracil (511mg, 3.0mmol) was stirred in neat allylamine (50ml) at 23°C for 24h. The white precipitate was filtered, washed with ether and dried in vacuo to afford the product (458.5mg, 78.2%); *microanalysis*, found C45.28%, H4.64%, N36.13% ($C_7H_9N_5O_2$ requires C43.0%, H4.64%, N35.88%); *FAB high resolution mass measurement*, 196.08427 ($C_7H_9N_5O_2$+H requires 196.08343); *i.r.* (film, cm^{-1}) 3140-3340 (C-H, N-H), 1760 (C=O), 1710-1660 (C=O, C=C); δ_H (100MHz, dmso-d_6, TMS) 10.24 (1H, bs, H-3'), 9.20 (1H, s, H-6), 8.2 (1H, bs, H-3), 7.9 (1H, bs, H-1), 6.7-5.3 (5H, m, allyl-H); *FAB-MS* (glycerol, m/z) (intensities not provided) 288 (14.8%), 234 (1.5%), 218 (2.7%), 197 (13.3%), 196 (100%), 195 (1.9%), 194 (1.9%), 193 (1.4%), 153 (9.2%), 152 (2.9%), 140 (3.3%), 139 (10.1%), 138 (25.3%), 110 (2.2%), 109 (4.6%), 168 (2.2%), 167 (1.5%).

10.45 Attempted cyclisation of 5(3-propyl-1-triazeno)uracil

^{1}H n.m.r. study

5(3-propyl-1-triazeno)uracil (20mg) was dissolved in dmso-d_6 (0.4ml) and its ^{1}H n.m.r. spectrum recorded. DBN (3 drops) was added to the sample and the reaction followed by ^{1}H n.m.r. over a period of 2 days. The reaction was quenched by the addition of water (0.5ml). For comparison the spectrum of DBN was also recorded. Results: see Chp. 8.2.7.2.1.

^{13}C n.m.r. study

5(3-Propyl-1-triazeno)uracil (100mg) was dissolved in dmso-d_6 (2.5ml) and its proton-decoupled ^{13}C n.m.r. spectrum recorded. The spectrum DBN was also recorded. A small quantity of DBN in dmso-d_6 was added to the sample and the sample left standing for several hours. The proton-decoupled spectrum recorded. The reaction was quenched by the addition of water. The reaction mixture was transferred to a preparative silica t.l.c. plate and run using dichloromethane/methanol (95:5) as the mobile phase. The band of silica R_f=0.55 was removed, eluted and the eluent evaporated to dryness to afford a white solid. A ^1H n.m.r. spectrum of the isolated material showed this to be a mixture of several compounds with the predominant component being the starting triazeno-compound. Results: see Chp. 8.2.7.2.2.

10.46 3,3a,5a,6,7,8-Hexahydro-4H,8H-pyrazolo[3,2:7,7a]furano--[2,3-d]pyrimidin-7,9-dione

5-Diazouracil (135.4mg, 0.98mmol) was dissolved in hot allyl alcohol (20ml) and left stirring for 48h. The off-white precipitate was filtered from solution and dried in vacuo to afford the product (96.3mg, 50.0%); *microanalysis*, found C43.30%, H4.12%, N28.33% ($C_7H_8N_4O_3$ requires C42.85%, H4.11%, N28.56%); *high resolution mass measurement*, 196.0821 ($C_7H_8N_4O_3$ requires 196.05963); *i.r.* (KBr, cm^{-1}) 3210 (C-H), 1730 (CO), 1690 (CO), 1470, 1270, 1240, 1100, 1080 (O-C); δ_H (100MHz, $CDCl_3$, TMS) 8.66 (1H, bs, H-6), 5.54 (1H, d, J=1.0Hz, H-5a), 4.86 (2H, d, J=5.75Hz, H-3), 4.78 (1H, d, J=3.0Hz, Ha-4), 4.68 (1H, d, J=3.0Hz, Hb-4); *EI-MS* (m/z, % rel. intensity) 197 (6.4%), 196 (2.5%, M+), 168 (32.4%, M-N_2), 166 (0.5%, M-CH_2O), 153 (4.7%, M-HNCO), 110 (6.1%, M-2HNCO); *FAB-MS* (nitrobenzyl alcohol, m/z, intensity not provided) 393 (P+M), 350 (P+mat), 232 (M+2H_2O), 219 (M+Na), 198 (P), 169 (P-N_2), 157 (P-C_2O), 109 (P-2HNCO-H_2), 140 (P-C_3H_5O).

10.47 2',3',5'-Tri-*O*-(3,5-dinitrobenzyl)uridine [4]

Uridine (10.3g, 41mmol) was dissolved in calcium hydride dried pyridine (400ml) and the solution slowly added to 3,5-dinitro--benzoyl chloride (39.6g). The mixture was stirred at 50°C for 60h to afford a clear, dark brown solution. The mixture was concentrated to afford a brown oil, which was added to ice-water

(2000ml) with stirring. An off-white precipitate was formed and was filtered from the aqueous solution. The solid material was by soxhlet extraction using absolute ethanol as the solvent. The material was washed for 72h. until the ethanol washings are no longer orange but remain colourless. The solid was filtered and dried (29.8g, 85%); *microanalysis*, C43.67%, H2.23%, N13.33% ($C_{30}H_{18}N_8O_{21}$ requires C43.60%, H2.20%, N=13.56%); *m.p.* 231-233°C; *i.r.* (KBr, cm^{-1}) 3370-3080 (C-H, N-H), 1750-1680 (CO), 1620 (C=C), 1600 (Ar-H), 1500 (Ar-H), 810 (Ar-H), 730 (C-H); δ_H (pyridine-d_5, TMS) 9.18-9.10 (9H, m, Ar-H), 8.58 (1H, s, H-3), 7.9 (1H, d, J=7.5Hz, H-6), 7.48 (1H, d, J=1.5Hz, H-1'), 7.1 (1H, s, H-4'), 6.72-6.64 (2H, m, H-2' and H-3'), 5.88 (1H, d, J=7.5Hz, H-5), 5.18 (2H, s, H-5'); *EI-MS* (m/z, % rel. intensity) 305 (0.3%), 293 (0.7%), 212 (57%), 195 (3.4%), 167 (2.4%), 166 (24.5%), 140 (0.3%), 121 (4.2%), 111 (2.1%), 96 (7.3%), 68 (2.6%); FAB-MS (thioglycerol, m/z, intensity not provided) 849 (M+Na), 827 (P), 826 (M+), 825 (P-H_2), 781 (P-NO_2), 715 (M-$C_4H_3N_2O_2$), 652 (P-$C_6H_3N_2O_4$).

10.48 5-Nitro-1-*β*-D-(2',3',5'-tri-*O*-(3,5-dinitrobenzoyl))uridine [4]

The protected uridine (26g, 31.5mmol) was added to a mixture of concentrated sulphuric acid/fuming nitric acid (55:66) and left stirring at room temperature for 1.5h. The red/brown liquid was poured into ice/water (500g/500ml) and stirred to afford a creamy white precipitate, which was filtered and washed with ice-cold water until the washings were pH 6.5. The solid was dried under vacuo (18.55g, 68%); *microanalysis*, found C39.13%, H2.44%, N12.73% ($C_{30}H_{17}N_9O_{23}$ requires C41.35%, H1.97%, N14.46%); *m.p.* 153°C; *i.r.* (cm^{-1}) 3090 (N-H), 1720-1740 (CO), 1630 (C=C), 1540 (Ar-H), 1350 (C-NO_2), 920 (Ar-H), 720 (C=C-H); δ_H (100MHz, $CDCl_3$, TMS) 9.18 (1H, s, H-3), 8.78-8.98 (9H, m, Ar-H), 7.28 (1H, s, H-6), 6.36 (1H, d, J=1.0Hz, H-1'), 6.16 (2H, m, H-5'), 4.68-5.0 (2H, m, H-2' and H-3'), 4.3 (1H, m, H-4'); δ_C (15MHz, pyridine-d_5, TMS) 143.6 (525%), 143.2 (647%), 136.1 (441%), 129.7 (2587%), 113.9 (669%), 112.8 (947%), 110.3 (2352%), 107.8 (582%), 74.5 (311%), 61.3 (295%), 56.8 (331%); *EI-MS* (m/z, % rel. intensity) 292 (3.5%), 212 (100%), 167 (3.8%), 113 (4.9%), 100 (13.1%), 97 (14.1%), 68 (14.7%); FAB-MS (3-nitro--benzyl alcohol, m/z, intensity not provided) 872 (P), 715 (P-B), 677 (P-R), 661 (P-OR), 196 ($C_7H_3N_2O_5$+H=R+H), 157 ($C_4H_2N_3O_4$+H=B+H).

10.49 5-Nitrouridine [4]

5-Nitro-(3,5-dinitrobenzoyl)uridine (11.84g, 1.36mmol) was suspended in absolute ethanol (500ml). Sodium metal (0.7g) was added to absolute ethanol (120ml) and stirred until all of the sodium metal had reacted and the ethanolic sodium ethoxide added to the protected uridine. The reaction mixture became red and the turbidity of the solution decreased. initially and then increased. On stirring, a white precipitate was obtained. The mixture was left stirring overnight and heated with reflux for 3h. The precipitate was filtered from the hot ethanol to afford 5-nitrouridine. The precipitate was suspended in hot ethanol (150ml) and acidified with concentrated sulphuric acid to pH 3.0. The solution was filtered and the yellow filtrate evaporated to dryness to afford an off-white solid (1.5g).

On standing the orange filtrate produced needle like crystals which were filtered from the solution and identified as ethyl 2,5-dinitrobenzoate.

5-Nitrouridine.

Microanalysis, C33.12%, H3.10%, N11.82% ($C_9H_{11}N_3O_8$ requires C37.37%, H3.83%, N14.52%); *i.r.* (nujol mull, cm^{-1}) 3600-3000 (OH), 1680 (CO), 1660 (CO), 1350 (NO_2); m.p. 175-179°C; δ_H (100MHz, dmso-d_6, TMS) 9.28 (1H, bs, H-3), 9.2 (1H, s, H-6), 5.82 (1H, d, H-1'*), 4.8-5.6 (bs, OHs), 4.32 (1H, bs, H-4'*), 3.98-4.16 (2H, m, H-2'*, H-3'*), 3.68 (2H, bs, H-5'), 3.5 (H2O); *EI-MS* (m/z, intensity) 169 (1.1%), 60 (0.6%), 46 (0.7%), 44 (100%), 43 (11.4%) (*tentative assignment).

Ethyl 2,5-dinitrobenzoate.

High resolution mass measurement, 240.0362 ($C_9H_8N_2O_6$ requires 240.03823); δ_H (100MHz, $CDCl_3$, TMS) 9.32 (2H, s, H-2, H-6), 7.4 (1H, s, H-4), 4.60 (2H, q, J=6.8Hz, H-1'), 1.52 (3H, t, J=6.8Hz, H-2'); *EI-MS* (m/z, intensity) 241 (0.9%), 240 (8.0%), 224 (0.6%), 196 (20.0%), 195 (40.8%), 168 (0.2%), 166 (11.0%), 75 (96.3%), 73 (4.8%), 45 (39.5%).

10.50 Attempted synthesis of 5-nitro-5,6-dihydrouridine

5-Nitrouridine (1.59g, 5.5mmol) was dissolved in 100ml of buffer solution (pH 7.0) to afford a yellow solution. Sodium borohydride (370mg) in buffer solution (10ml) was added and the

solution became red in colour. The solution was left stirring for 3h at ambient temperature. The mixture was filtered and the filtrate was extracted with ethylacetate (4x50ml). The organic phase was washed with distilled water (50ml) and the two phase separated. The aqueous phase was frozen and placed under vacuo to remove the water by freeze-drying. The yellow residue was extracted with acetic acid (200ml) and filtered. The solvent was removed from the filtrate and left standing to afford bright yellow crystals (1.2g, 75%); *microanalysis*, found C22.00%, H3.59%, N8.30% ($C_9H_{13}N_3O_8$ requires C37.11%, 4.49%, N14.42%); *i.r.* (nujol, cm^{-1}) 3600-3000 (OH), 1720-1680 (CO); δ_H (100MHz, acetic acid-d_4, TMS) 10.5 (1H, bs, H-3), 6.48 (1H, bs), 5.3 (2H, bs,), 4.76-4.60 (4H, m), 2.2 (2H, m); δ_C (acetic acid-d_4, TMS) (intensity), 162.76 (162), 153.84 (184), 92.19 (96), 85.94 (140), 82.10 (77), 75.33 (50), 70.0 (45), 62.89 (126), 42.64 (99); *EI-MS* (m/z, % rel. intensity) 260 (0.1%), 259 (1.2%), 158 (0.5%, $C_4H_4N_3O_4$), 115 (1.5%, $C_5H_7O_3$), 101 (0.5%), 73 (1.4%), 43 (100%).

10.51 Attempted preparation of 5-nitro-5,6-dihydro(2',3'5'--tri-*O*-acetyl)uridine

5-Nitro-5,6-dihydrouridine (640mg), sodium acetate (450mg) and acetic anhydride (20ml) were heated at reflux for 5h. The solution was left stirring at ambient temperature for 17h. The solid precipitate was filtered from the yellow solution and ethyl acetate (100ml) added. The ethyl acetate solution was washed with distilled water (2x50ml), dried with anhydrous sodium sulphate and filtered. methanol (50ml) was added to the filtrate at the mixture left stirring for 5min. at ambient temperature. The solution was evaporated to dryness and the gummy solid purified by preparative t.l.c. using ethyl acetate as the mobile phase. The largest band of silica, Rf=0.69 was isolated, eluted with ethyl acetate and eluent evaporated to dryness to afford a off-white gum (340mg); *microanalysis*, found C50.67%, H7.09%, N3.97% ($C_{15}H_{19}N_3O_{11}$ requires C43.17%, H4.58%, N10.06%) ($C_{15}H_{19}N_3O_{11}.7C_4H_8O_2$ requires C49.95%, H7.31%, N4.06%) δ_H (100MHz, $CDCl_3$, TMS) 10.4 (1H, bs, H-3), 5.96(1H, d), 5.76 (1H, d), 5.5 (1H, m), 4.5-4.36 (5H, m). 3.88 (1H, m), 2.26 (3H, s), 2.16 (6H, s), δ_C (15MHz, $CDCl_3$, TMS) (intensity) 171.01 (86), 170.75 (104), 170.42 (76), 169.84 (112), 169.22 (145), 80.57 (97), 78.10 (187), 73.62 (108), 72.97 (76), 71.02 (69), 70.63 (71),

20.73 (183), 20.531 (221); *EI-MS* (m/z, % rel. intensity) 181 (0.2%), 169 (0.3%), 80 (3.8%), 60 (1.3%), 59 (0.8), 47 (17.9%), 46 (11.6%).

10.52 2',3'-*O*-Isopropylidene-5'-*O*-methoxymethyluridine [5]

2',3'-Isopropylidene uridine (5.068g, 15.4mmol) was suspended in dimethoxymethane (150ml) and acetone (75ml). Methane sulphonic acid (4ml) was added and the slightly turbid mixture was left stirring at room temperature. After 30min. a white precipitate was obtained, which was dissolved by the addition of 28% ammonia solution (200ml) and this solution evaporated to dryness. The residue was partitioned between chloroform and saturated sodium chloride solution. The organic layer was dried with anhydrous sodium sulphate, filtered and the solvent removed from the filtrate to give a yellow oil. The product was purified by preparative t.l.c. using ethanol/chloroform (1:99) as the mobile phase. The largest band (R_f=0.5) was removed and eluted with ethanol and the eluent evaporated to dryness to afford the product, a pale yellow gum, which drying under high vacuum results in a clean white foamy solid (4.89g, 84.4%); *i.r.* (film, cm^{-1}) 3200-2900 (C-H), 1720-1690 (CO), 1380 (O-C); δ_H (100MHz, CD_3OD+$CDCl_3$, TMS) 7.74 (1H, d, J=7.5Hz, H-5), 5.90 (1H, d, J=1.5Hz, H-1'), 5.72 (1H, d, J=7.5Hz, H-6), 4.80-5.0 (2H, m, J=1.5Hz, H-2'and H-3'), 4.74 (2H, 2, OCH_2O), 4.38 (1H, m, J=3.0Hz, H-4'), 3.78 (2H, d, J=3.0Hz, H-5'), 3.36 (3H, s, OCH_3), 1.60 (3H, s, Me), 1.40 (3H, s, Me); δ_C (15MHz, proton--decoupled, CD_3OD+$CDCl_3$, TMS) (165.94 (4501, C-4), 151.91 (4776, C-2), 143.59 (4464, C-6), 115.00 (6901, OCH_2O), 102.66 (5554, C-5), 97.59 (6132, C-1'), 94.59 (5032, $OCMe_2O$), 86.68 (6164, C-2'), 85.64 (5744, C-3'), 82.39 (6010, C-4'), 68.74 (4920, C-5'), 55.742 (4639, OMe), 27.42 (5004, Me), 25.47 (4722, Me); *EI-MS* 269.0805 (0.6%), (M-H)-C_3H_6O requires 269.07736), 225 (0.2%), 189 (0.2%), 105 (2.5%), 85 (2.8%), 84 (1.2%), 75 (0.4%), 61 (0.4%), 59 (3.0%), 58 (1.0%).

10.53 2',3'-*O*-Isopropylidene-5'-*O*-methoxymethyl-5,6-dihydro--uridine [5]

The protected uridine (1.426g, 4.34mmol) was dissolved in methanol and 5% palladium on carbon catalyst (300mg) added. The mixture was stirred under hydrogen until the required volume of hydrogen had been taken up. A sample removed, filtered free of

catalyst and the solvent removed from the filtrate. The reaction was sampled regularly and examined by ^1H n.m.r. and the reaction stopped when the doublets associated with uracil were no longer present. The catalyst was filtered from the solution and the filtrate evaporated to dryness to afford 2',3'-O-isopropylidene--5'-O-methoxymethyl-5,6-dihydrouridine (1.435g, 100%); δ_H (60MHz, MeOH-d_4, TMS) 5.7 (1H, d, J=2.0Hz, H-1'), 4.7-4.8 (2H, m, H-2' and H-3'), 4.6 (2H, s, OCH_2O), 4.2 (1H, m, H-4'), 3.8 (2H, d, J=4.0Hz, H-5'), 3.5 (2H, d, J=6.0Hz, H-5), 3.4 (3H, s, OMe), 2.7 (2H, t, J=6.0Hz, H-6), 1.7 (3H, s, Me), 1.6 (3H, s, Me)

10.54 5-Formyl-2',3'-*O*-isopropylidene-5'-*O*-methoxymethyl--5,6-dihydrouridine.

4-Methyl-2,6-ditert butylphenol (4.245g) was dissolved in dry THF (30ml). The solution was cooled to -78°C using an acetone/dry ice bath. The reaction was carried out under a nitrogen. Phenyllithium (2M,30ml) was injected into the solution and the mixture allowed to stir for 5min [6]. The protected dihydrouridine (1.3g) was dissolved in THF (20ml) and added to the mixture. The reaction mixture was allowed to warm up to -15°C. The whole mixture was cooled to -78°C and ethyl formate (6ml), dried and distilled from calcium hydride, added. The mixture was allowed to warm to 9°C and left stirring. The mixture changing colour from green/brown to yellow. Acetic acid was added and a crystalline material precipitated. Chloroform (100ml) was added and the gelatinous material filtered. The gel was dissolved in water (200ml) extracted with chloroform (100ml). The chloroform extractions were combined, dried with anhydrous sodium sulphate, filtered and the filtrate evaporated to dryness.

10.55 5-Diazo-1,6-dihydrouridine

The putative formyl derivative (see Chp.10.53) was dissolved in methanol and added to a suspension of carboxybenznesulphonyl azide in methanol and triethylamine [7]. The mixture was left stirring over the weekend and then evaporated to dryness. Three different methods were investigated in order to find a suitable method to isolate the putative product.

Work up procedure A

A sample was triturated with dichloromethane. The solution was observed to become turbid with a bright white precipitate. The precipitate was removed by filtration and the filtrate condensed on a rotary evaporator to afford a bright yellow oil which on standing gave a yellow crystalline material; *i.r* (nujol, cm^{-1}) 2120 (N_2), 870, 760, 750, 710.

Work up procedure B

A sample of the material was triturated with dichloromethane, filtered and the filtrate condensed. An attempt was made to purify the resultant yellow oil by preparative t.l.c. using dichloromethane as the mobile phase. The bands of silica were isolated, eluted with ethanol and evaporated to dryness.

product R_f=0.7

δ_H (100MHz, $CDCl_3$, TMS) 6.7-7.2 (3H, m), 6.5 (2H, s), 4.92 (2H, s), 4.64 (1H, s), 2.1 (3H, s), 1.3 (18H, s)

product R_f=0.1

i.r. (film, cm^{-1}) 3620, 2860-3180 (C-H), 2120 (N_2), 1680-1740 (CO), 1040 (O-C), 860, 770, 750, 700. δ_H (100MHz, $CDCl_3$, TMS) 7.3-7.7 (3H, m), 6.88 (1H, d, J=1.5Hz), 4.32 (1H, m), 3.84 (1H, m), 3.62 (1H, s), 3.46 (1H, s), 3.10 (7H, bs), 2.76-2.96 (6H, m, J=6.0Hz), 2.68 (2H, s), 1.08-1.3 (11H, m, J=6.0Hz)

Work up procedure C

A sample of the product was triturated with dichloromethane and filtered. The filtrate was washed with saturated sodium bicarbonate solution. The two layers were separated and the organic layer dried with anhydrous sodium sulphate, filtered and the filtrate evaporated down to afford a yellow oil, which on standing rapidly afforded yellow rhombic needles in a yellow oil (109mg crude); *high resolution mass measurement*, 313.1000 ($C_{14}H_{20}N_4O_2$+H requires 313.114795) *i.r.* 3600-3000 (NH, OH), 2100 (diazo), 1700 (CO, acid), 1720 (CO. imide), 1680 (CO, amide), 870 (p-substituted benzene); δ_H (100MHz, $CDCl_3$, TMS) 10.4 (N-H or OH), 7.70 m (Ar-H), 6.0 s (H-6?), 4.82 t (sugar), 4.34 t (sugar), 3.88 m (sugar), 3.50 bs (sugar), 3.48 m (sugar); *EI-MS* (m/z, % rel. intensity) 313

(0.6%), 285 (P-N_2), 229 (1.5%), 228 (1.6%), 182 (0.611%), 181 (14.3%), 169 (3.3%), 140 (2.2%), 139 (3.5%). 138 (0.8%) 133 (3.4%), 104 (6.3%), 90 (1.3%), 69 (15.8%), 68 (18.2%), 75 (4.8%), 74 (3.4%), 54 (3.0%), 43 (24.5%).

Carboxybenzene sulphonyl azide

i.r. (nujol, cm-1) 2310+2150 (N_3), 1690 (CO), 870, 820, 780, 770, 720, 690. σ_H (100MHz, dmso-d_6, TMS) 7.88-7.98 (4H, m, Ar-H).

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