STUDIES OF

6-DIAZOPENICILLANIC ESTERS

by

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ABSTRACT

The introduction of substituents other than nitrogen into the 6-position of penicillins and the 7-position of cephalosporins is reviewed and particular attention given to the applications of diazo compounds for these reactions.

Diazotization of a model α-aminoamide has been explored and an efficient synthesis of 6-diazopenicillanate esters has been developed.

Cyclopropanation reactions of benzhydryl 6-diazopenicillanate have been carried out with styrene and with cyclohexene. With thiophene, in addition to the expected cyclopropanation product a further adduct was obtained which provides a unique example of carbenoid ring expansion to a 2H-thiopyran. The stereochemistries of the carbenoid adducts have been established by means of ^{1}H n.O.e. difference spectroscopy.

Reactions of benzhydryl 6-diazopenicillanate with various alcohols have been shown to give rise to mixtures of 6-alkoxypenicillanates and alkoxythiazepines. The latter, and possibly the former, are formed via oxonium ylide intermediates. A different set of oxonium ylides are responsible for alkoxythiazepine formation in the reactions of the diazo compound with orthoesters. With anisole, spiro-cycloheptatrienyl adducts were obtained by Buchner ring expansion. The position of the methoxyl group in the adducts was determined by n.O.e. difference spectroscopy.

Addition of benzhydryl 6-diazopenicillanate to furan gives rise to products containing a conjugated dienal side chain at C-6, in quantitative yield. The potential of this method for the synthesis of 6-alkylated penicillanate derivatives has been explored and is exemplified by a variety of reduction, oxidation, and carbonyl group condensation reactions. Studies of the $NaBH_4$ reduction of the dienals has led to the discovery of a unique, selective, pH-dependent 1,6-reduction. This afforded 6-(alk-1-enyl)penicillanates which provide the first examples of isosteric replacement of the side chain NHCO group in penicillins by

a trans C=C bond.

Deprotection of all of the significant new penicillanate esters described in this thesis has been accomplished. None of the compounds showed antibacterial activity.

The applications of preparative HPLC to the purification of steroid esters, of a cephalosporin, and of some of the penicillin derivatives synthesised in this work, are described.

CHAPTER ONE LITERATURE REVIEW

1.1. Introduction

The penicillins were discovered over 50 years ago and still occupy a prominent position in antibacterial chemotherapy. Together with the closely-related cephalosporins, they continue to be first-line drugs in the treatment of bacterial infections. Today, β-lactams account for over half of all medically prescribed antibiotics. Despite this fact, they suffer from a number of drawbacks. Firstly, not all bacteria are susceptible to penicillins and new compounds with wider and different antibacterial spectra are always needed. Secondly, bacterial strains have developed which are resistant to the traditional penicillins and new antibiotics are needed to combat these. Other desirable properties which one might hope to see in the β-lactam drugs are less allergenicity and greater metabolic efficiency.[1-3]

It has been well established that penicillins and cephalosporins inhibit bacterial cell wall synthesis by interfering with the final cross-linkage process, which has been termed transpeptidation, and which involves an amino group in one peptidoglycan molecule and the D-alanyl-D-alanine end of the acetyl-muramyl-pentapeptide fragment in another. It has been suggested that the chemical structures of both penicillins and cephalosphorins can mimic this D-alanyl-D-alanine residue and thereby inhibit the enzyme - transpeptidase - which is responsible for the cross-linkage. However, there also appears to be a role for β-lactams in triggering the destructive activity of autolytic enzymes (murein hydrolases) and Tomasz has suggested that the situation is a complex one in which there may be a number of

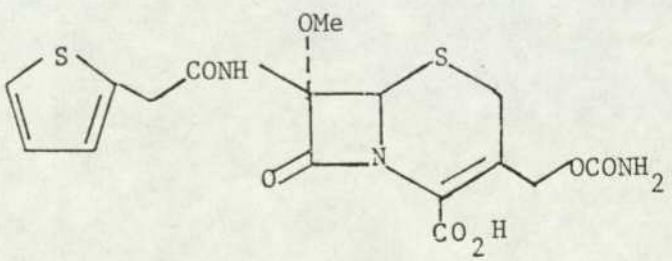
mechanisms for loss of viability and / or lysis.[4]

Resistance to β-lactam antibiotics is mainly caused by formation of enzymes, the β-lactamases, which are capable of opening the β-lactam ring common to these antibiotics, thereby inactivating them.[4]

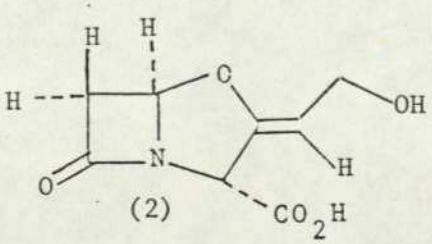
The introduction of the 7α-methoxy group into the cephem ring system was found to give compounds with considerable antibacterial activity and with a high resistance to many β-lactamases. The first medically useful outcome of this finding was the semisynthetic compound, cephoxitin (1).[5]

A new β-lactamase inhibitor with low antibacterial activity was reported in 1976.[6] This substance, named clavulanic acid (2), contains a β-lactam ring, with no side chain, fused to an oxazolidine ring.

Almost at the same time, a Japanese group discovered the nocardicin (3), a completely different type of β-lactam antibiotic. It is unusual in containing an unfused β-lactam ring, as well as a novel side chain, and in showing activity against many gram-negative bacteria.[7]



(1)



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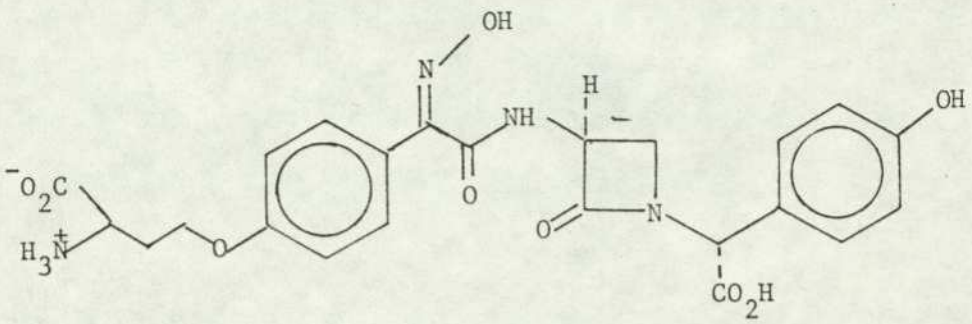
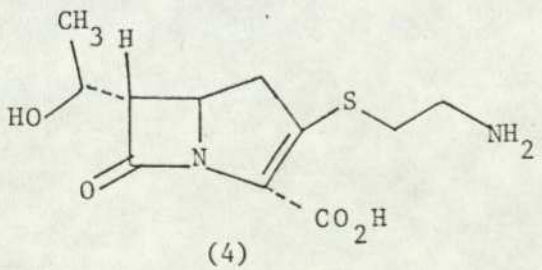


(3)

The discovery of 7α-methylcephalosporins as natural products possessing activity against some penicillin- and cephalosporin-resistant species, and the hypothesis that 6α-methylpenicillins might possess increased activity have led to the development of a wide range of methods for functionalising the penicillin or cephalosporin molecule in the 6α- or 7α-position.[3]

A further impetus to research in this area has come from the recent discoveries[8,9] of thienamycin (4) and olivanic acid (5). Although lacking the 6-amino group, thienamycin is a powerful and broadly active antibiotic and olivanic acid is an effective β-lactamase inhibitor.



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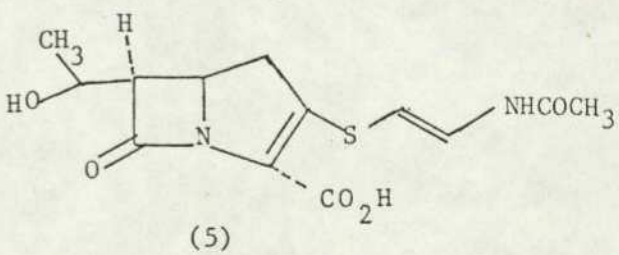
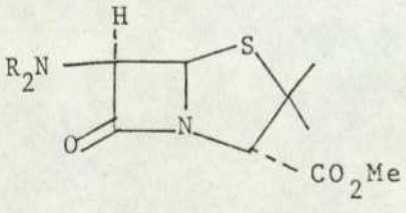


(5)

There has consequently been considerable interest in the last few years in the synthesis of penicillin analogues carrying a carbon side chain at the C-6 position. At the same time, intensive synthetic efforts have been directed to the synthesis of penem systems.[1,2,3,7]

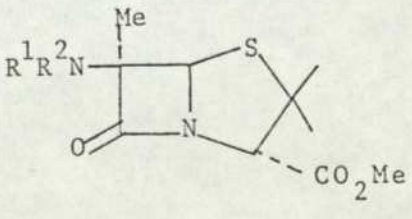
1.2. Alkylations and Other Substitution Reactions of Penicillins at C-6 and of Cephalosporins at C-7

It has been reported[10,11] that treatment of methyl 6-amino-penicillanate (6) with benzaldehyde gave the imine (7), which was reacted with one equivalent of sodium hydroxide and excess methyl iodide in dimethoxyethane at 0° to afford a mixture of epimeric 6-methyl derivatives (8a: 90%) and (8b: 5%). Hydrolysis of (8a) provided amine (9), which was acylated with phenoxyacetyl chloride to furnish the 6α-methylpenicillin V methyl ester (10). 7-Alkylcephalosphorins were reported to be obtained in the same manner.



(6) $R_2 = H_2$

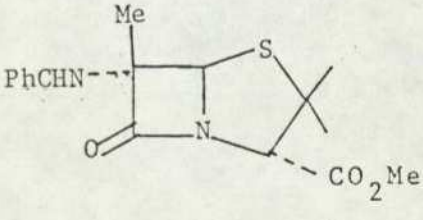
(7) R_2 = PhCH=



(8a) R^1R^2 = PhCH

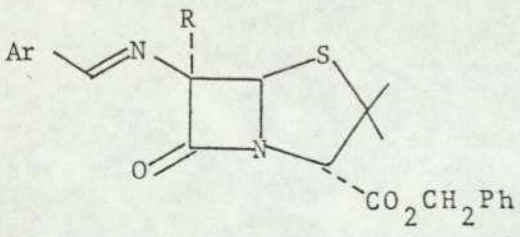
(9) $R^1R^2 = H_2$

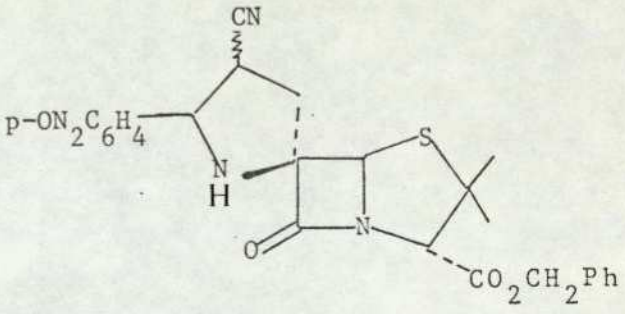
(10) R^1 = H, $R^2 = COCH_2OPh$



(8b)

Phenyl lithium can be used in place of sodium hydroxide for generation of the carbanion,[12] and in addition to condensation with alkyl halides it is also possible to obtain products of Michael addition and aldol condensation.[13] The Schiff's base (11) reacted with acrylonitrile using N,N-diisopropyl ethylamine as catalyst to give not only the Michael addition product (12) but also small amounts of the isomeric spiro-pyrrolidines (13). The imine (11) could also be alkylated with formaldehyde, acetaldehyde and ethyl chloroformate.





(11) Ar = $p\text{-}NO_2C_6H_4$, R = H

(12) Ar = $p\text{-}NO_2C_6H_4$, R = CH_2CH_2CN

(13)

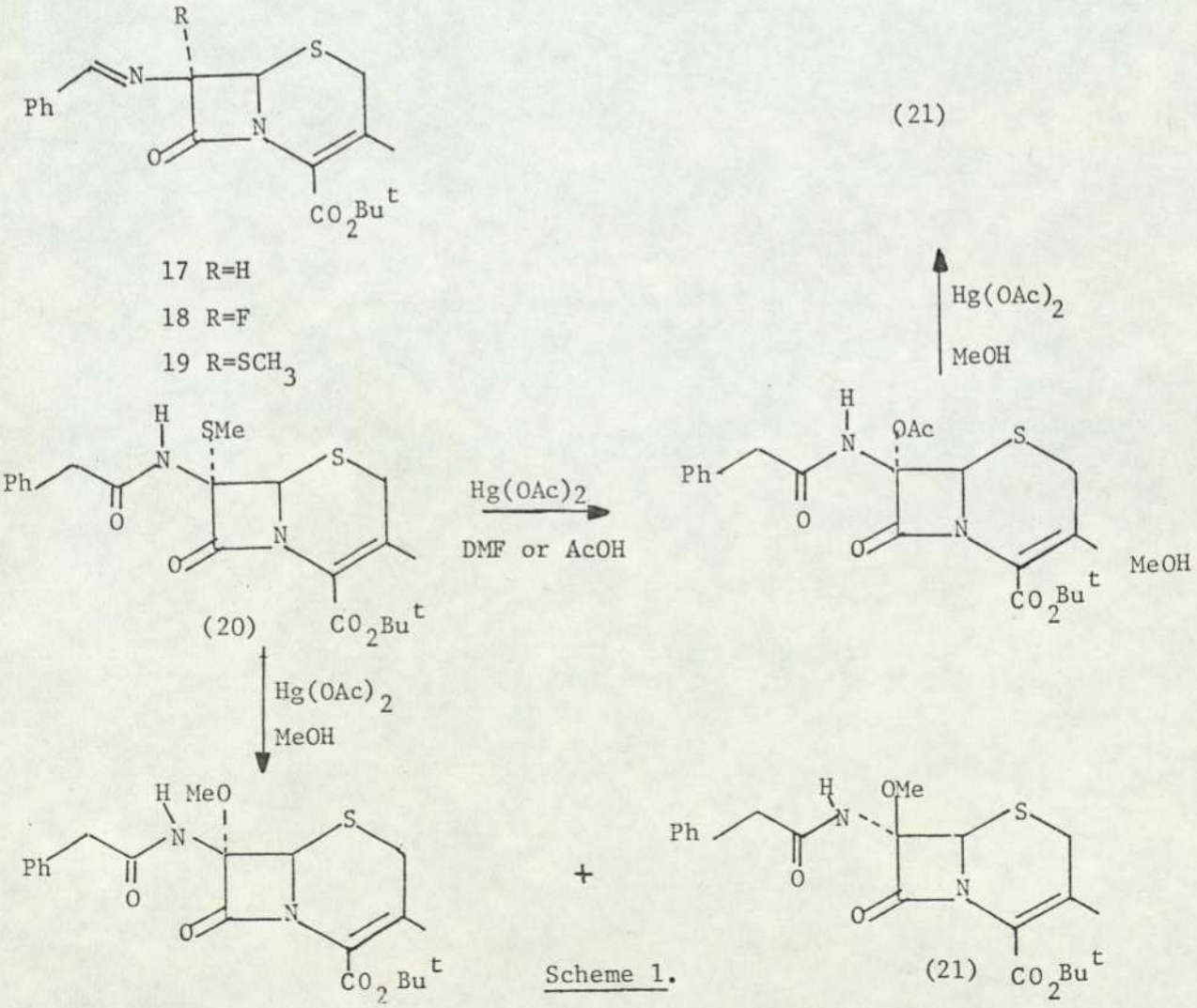
(14) Ar = Ph, R = H

(15) Ar = Ph, R = Br

(16) Ar = Ph, R = OMe

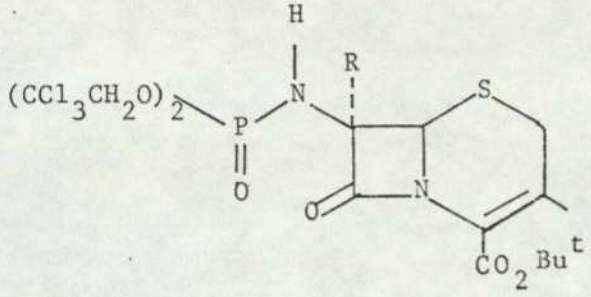
Cama and Christensen[14] showed that the Schiff's base (14) could be transformed upon reaction with phenyl lithium and N.B.S. into the 6-bromo imine (15), which was treated with methanol containing silver oxide (to neutralise the HBr) to give 6α- methoxy-6-benzaliminopenicillanate (16). Hydrolysis of the imine (16) with $PdCl_2$ gave the corresponding 6-methoxy-6-aminopenicillanate, which could then be acylated. Essentially identical conditions were used for introduction of a 7α-methoxy group into a cephalosporin.

A number of 6α-methylthiopenicillins and 7α-methylthiocephalosporins have been prepared using the Schiff's base approach.[15,16,17] Direct alkylthiolation was accomplished by reaction with methyl methanethiosulphonate ($CH_3SSO_2CH_3$) or methanesulphenyl chloride (CH_3SCl). In an alternative procedure, the Schiff's base (17) was fluorinated, using perchloryl fluoride, to give (18) which was then solvolysed with methanethiol under acidic conditions. The resulting methylthio-imine (19) was converted to the amide (20) and it was shown that mercuric acetate would catalyse the replacement of the methylthio group in (20) either by methanol or by acetic acid, depending on the choice of solvent (Scheme 1). 6α-Substituted penicillins were prepared in a parallel fashion.



Scheme 1.

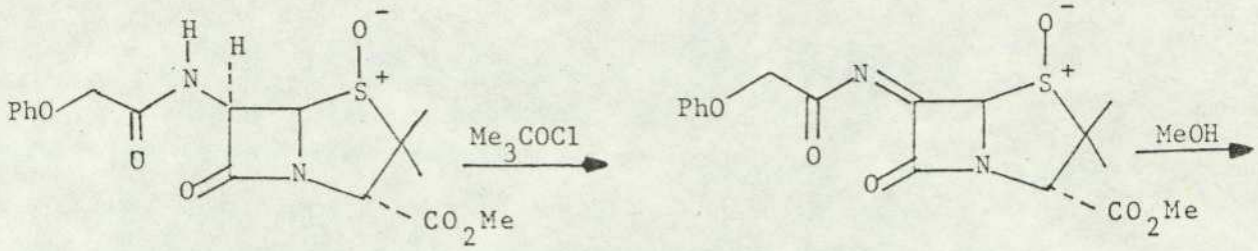
A Japanese group[18] have developed a related method in which 7-aminocephalosporanate was converted to the phosphoramidate (22) with bis-(2,2,2-trichloroethyl)-phosphorochloridate in the presence of pyridine. Reaction with t-butyl hypochlorite and lithium methoxide then gave the 7α-methoxy derivative (23).



(22) R = H

(23) R = OMe

Baldwin et.al.[19] demonstrated that methanol added to the acylimine (25), derived from sulphoxide (24) by a halogenation-dehydrohalogenation process using t-butyl hypochlorite, to produce the methoxyamide (26). This was reduced to the corresponding 6α-methoxypenicillanate (Scheme 2). The method is amenable only to penicillins protected as the sulphoxide or sulphone, because the sulphur atom of penicillin reacts vigorously with the electrophilic reagent t-butyl hypochlorite. However, by carrying out the halogenation - dehydrohalogenation in stages, the need to employ the sulphoxide is avoided and Firestone and Christensen[20] were able to prepare a series of 6α-substituted penicillanates (Scheme 3).



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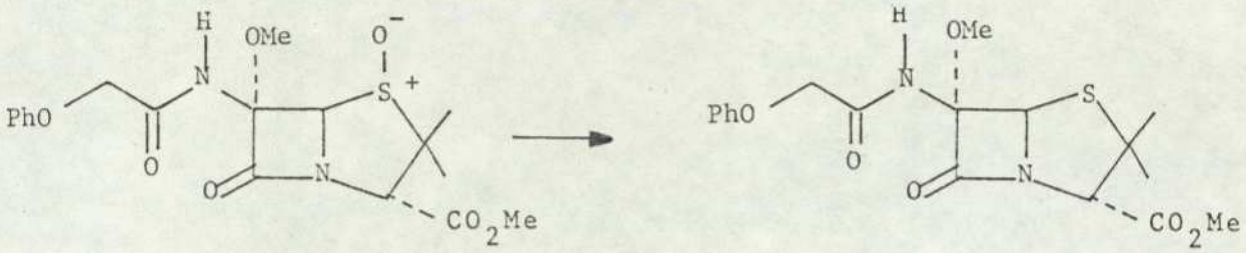
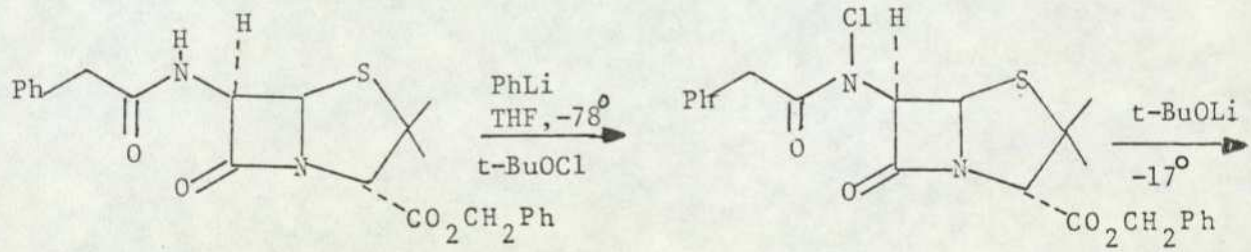
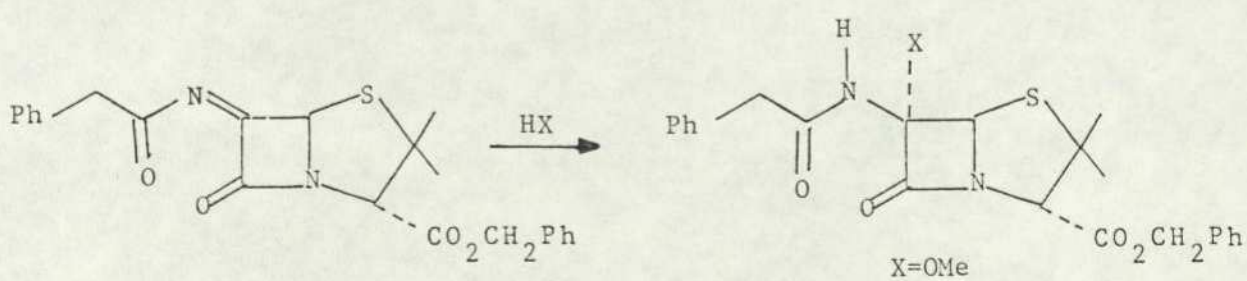


(26)

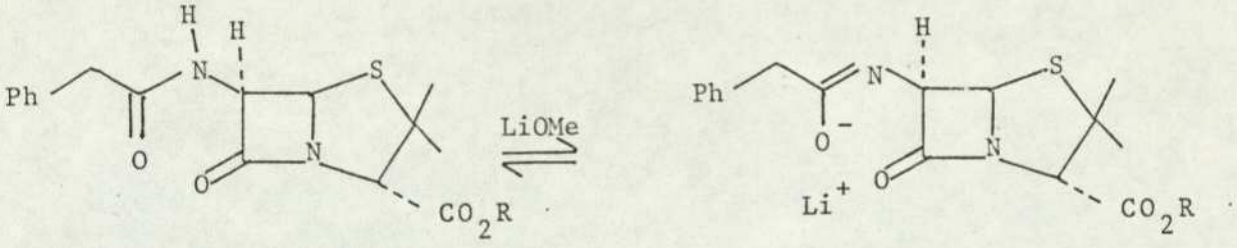
Scheme 2.



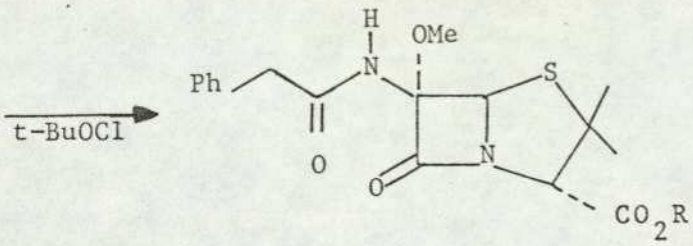


Scheme 3.

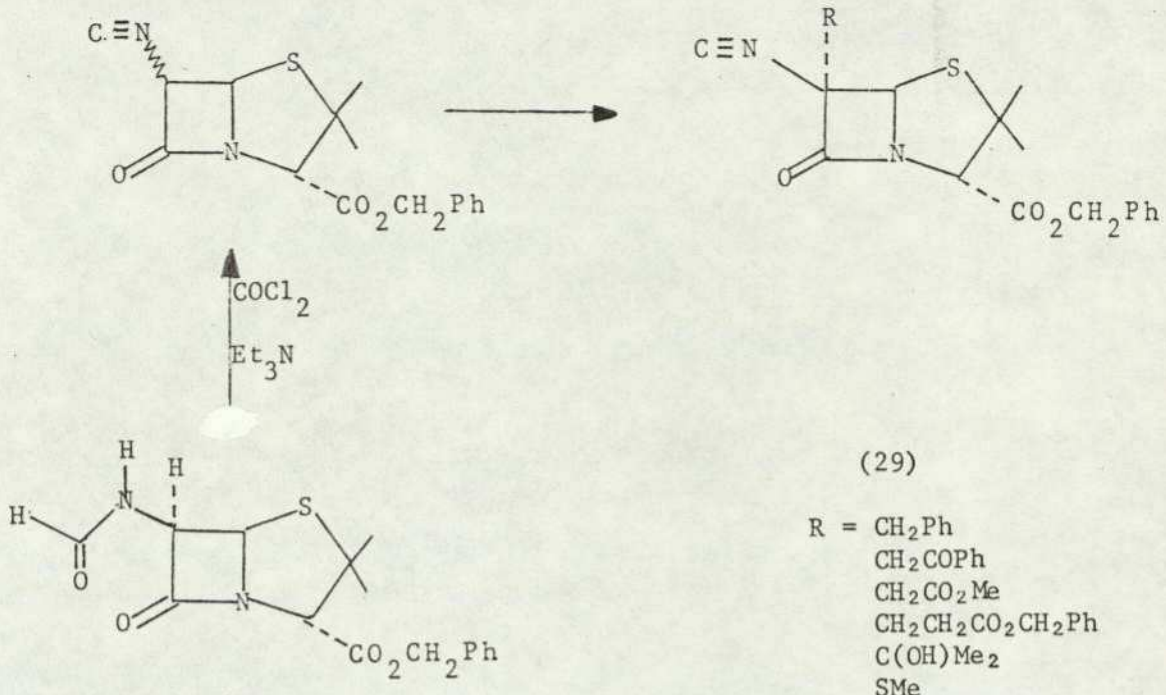
A further modification, reported by Koppel and Koehler,[21] involves treatment of the amide (27) with 3.5 equivalents of lithium methoxide in THF at -78°, followed by addition of t-butyl hypochlorite.



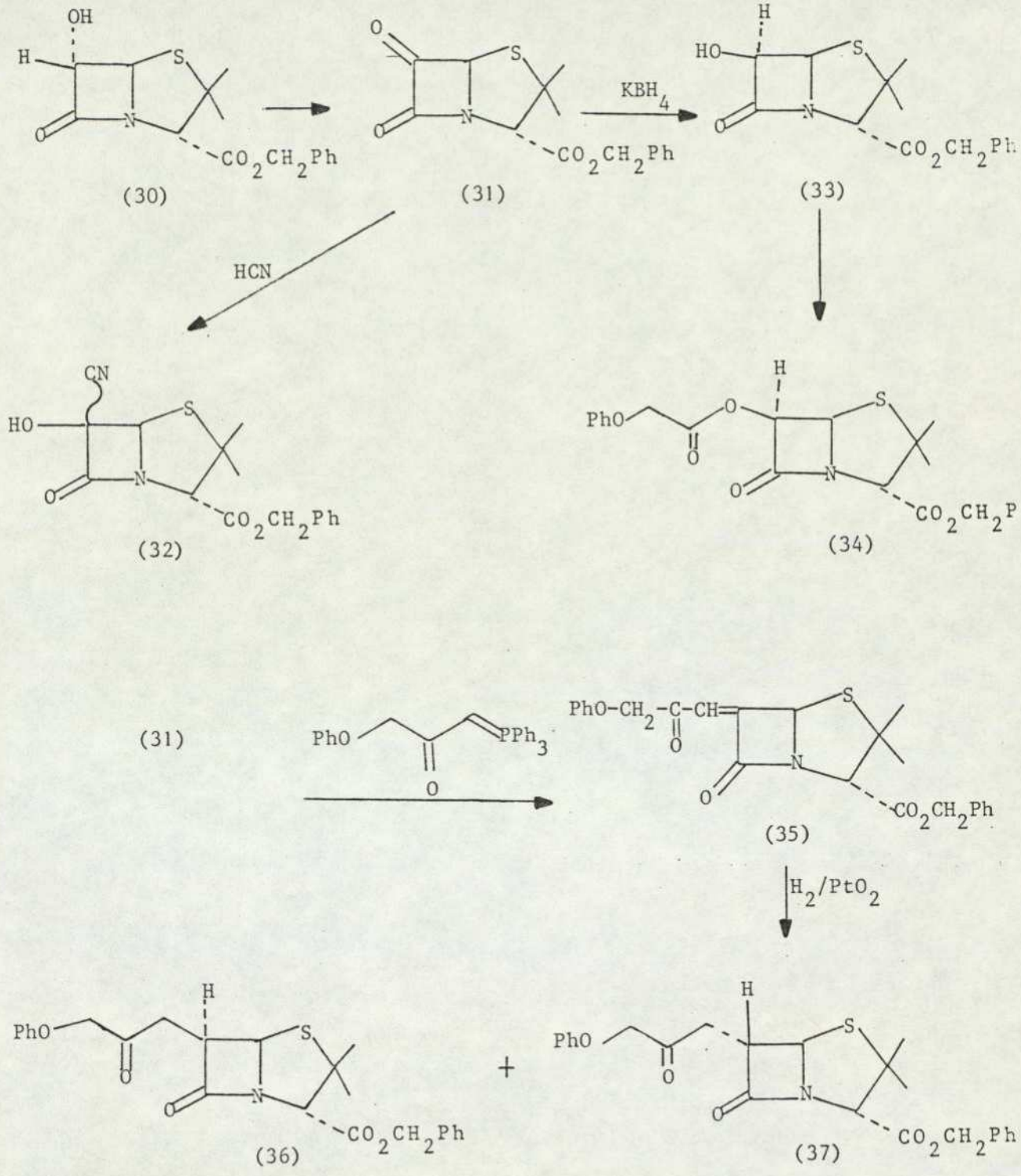
(27) R=$CH_2C_6H_4NO_2$



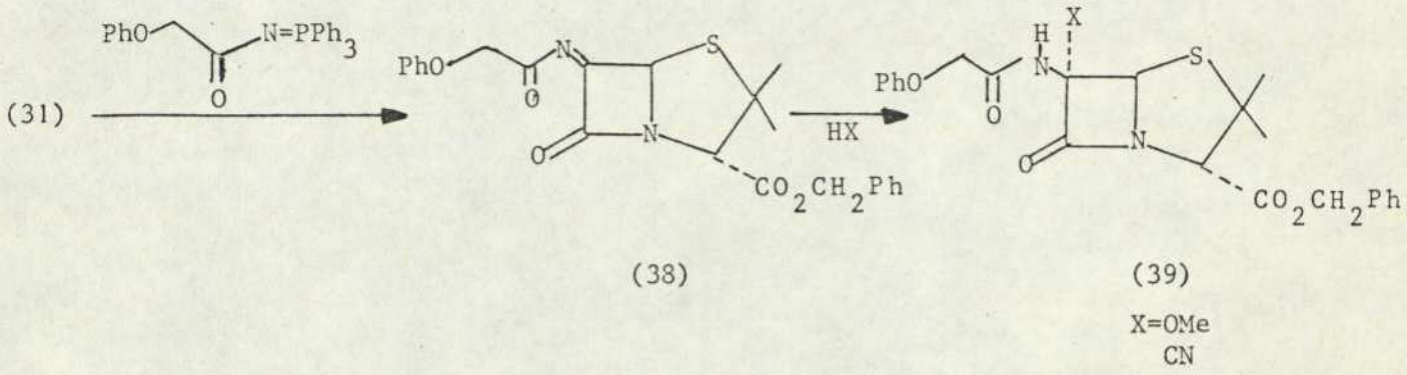
The use of 6-isocyano penicillins to introduce 6α-substituents was demonstrated by Bentley et.al.[22,23] Treatment of benzyl 6β-formamidopenicillanate (28) with phosgene at -40° in the presence of triethylamine provided a mixture of benzyl 6α-isocyanopenicillanate and the 6β-epimer in a ratio of 55:45. When the isonitrile mixture was treated in the presence of potassium carbonate with alkylating agents (benzyl bromide, phenacyl bromide or methyl bromoacetate) or with Michael acceptors (benzyl acrylate) or other electrophiles (acetone, methyl methoxycarbonyl disulphide), the 6α-substituted 6β-isocyano derivatives (29) were obtained. The isonitrile group in (29) was readily hydrolysed either to the formamide (with formic acid), or to the amine (with tosic acid), or to the urethane (with thallic trinitrate in methanol).



Sheehan and Lo[24,25] developed a completely different route to C-6 alkylated penicillanates. Benzyl 6α-hydroxypenicillanate (30) was prepared from 6-aminopenicillanic acid by the method of Hauser and Sigg.[26] Oxidation of the alcohol (30) using diisopropylcarbodiimide in DMSO gave benzyl 6-oxopenicillanate (31) which was reacted immediately with liquid hydrogen cyanide to give a crystalline cyanohydrin (32). Reduction of the ketone (31) by potassium borohydride gave the 6β-hydroxy epimer (33), which was phenoxyacetylated to give the ester (34). Further analogues were prepared by Wittig reaction on the ketone (31) with phenoxyacetylmethylene triphenylphosphorane, the resulting isomeric mixture of olefins (35) being reduced over platinum oxide to penicillanates (36) and (37). It was claimed that the free acid derived from (36), a carbon analogue of penicillin V, still retains appreciable antibiotic activity.



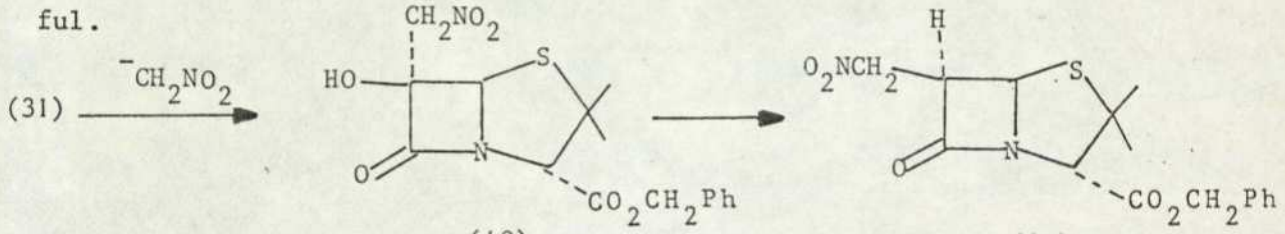
In an analogous reaction,[27] the ketone (31) was used to prepare the N-acyl imine (38), which was then reacted with methanol or liquid hydrogen cyanide to afford the 6α-substituted penicillanates (39).



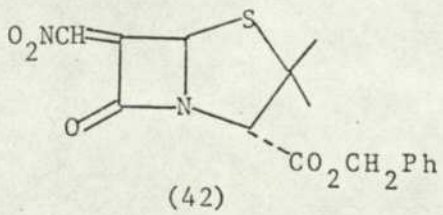
(38) (39)

X=OMe
CN

The 6-oxopenicillanate (31) has also been condensed with the anion of nitromethane.[28] The hydroxyl group in the product (40) could be removed either by the hydrogenolysis (Wilkinson's catalyst), affording 6β-nitromethyl penicillanate (41), or by dehydration to the nitroolefins (42). However, attempted catalytic reductions of the nitromethyl compound (41) to aminomethyl penicillanate was unsuccessful.



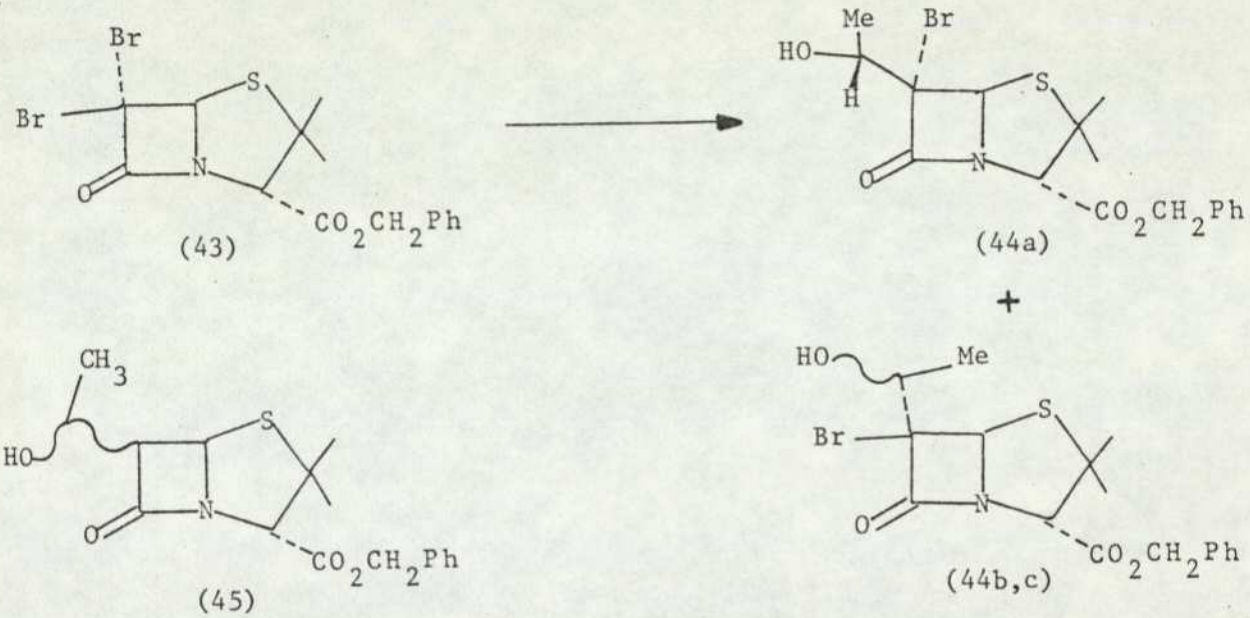
(40) (41)



(42)

DiNinno et al.[29] have reported the hydroxyethylation at C-6 of penicillanates and C-7 of cephalosporanates. They exploited the idea that enolates derived from penicillanates and cephalosporanates by metal-halogen exchange can react with acetaldehyde to yield the aldol. Treatment of anhydrous THF solution of 6,6-dibromopenicillanate (43) with one equivalent of either butyl lithium in hexane or methylmagnesium iodide in ether

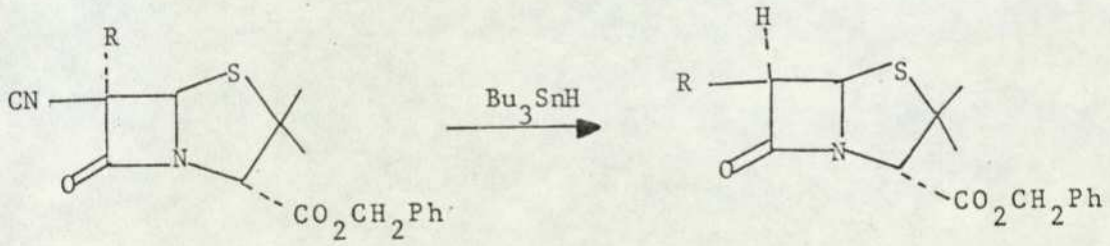
gave, after reaction with excess acetaldehyde, a mixture of three bromohydrins (44a-c). Removal of the bromine from (44) with zinc-silver couple in methanol provided the mixture of isomers (45). These could also be obtained directly from the reaction of 6-iodo-penicillanic ester with methylmagnesium bromide and acetaldehyde. The free acids derived from the isomers (45) were found to be notably less active than benzylpenicillin.



Aimetti and Kellogg[30] noted the importance of solvent effects on the stereochemistry of the aldolisation reaction. When the dibromide (43) was reacted with sec-butyl or t-butyl lithium followed by acetaldehyde, the orientation of the hydroxyethyl groups in the resulting products (44) was mainly α when the solvent used was toluene (α : β = 6 : 4), but mainly β when the solvent used was THF (α : β = 1 : 4). Solvent effects were also noted in protonation studies: the anion generated from dibromide (43) with t-butyl lithium in toluene produced, upon treatment with acetic acid, a 3 : 2 mixture of α- and β-bromopenicillanic esters. The same reaction conducted

in a mixture of THF and toluene (3 :1) yielded less than 10% of the β-epimer. In later work,[31] it was also shown that tri-n-butyl tin hydride reduction of 6-halogeno-pencillanates leads to stereoselective production of 6β-substituted penicillanates in moderate to excellent yields. It was claimed that, regardless of the stereochemistry of the bromine prior to reduction, hydrogen atom transfer takes place preferentially from the less hindered side of the molecule.

The high stereoselectivity of tri-n-butyl tin hydride was also observed in the reduction of 6α-alkyl-6β-isocyanopenicillanates.[32] The isonitriles (46) were treated with Bu_3SnH in refluxing benzene in the presence of a catalytic amount of azobisisobutyronitrile to give, in each case, a 50-70% yield of the pure 6β-alkylpenicillanate (47).



(46) R = CH_2Ph
$CH(OH)Me_2$
CH_2CO_2Me
$CH_2CH_2CO_2Me$

(47)

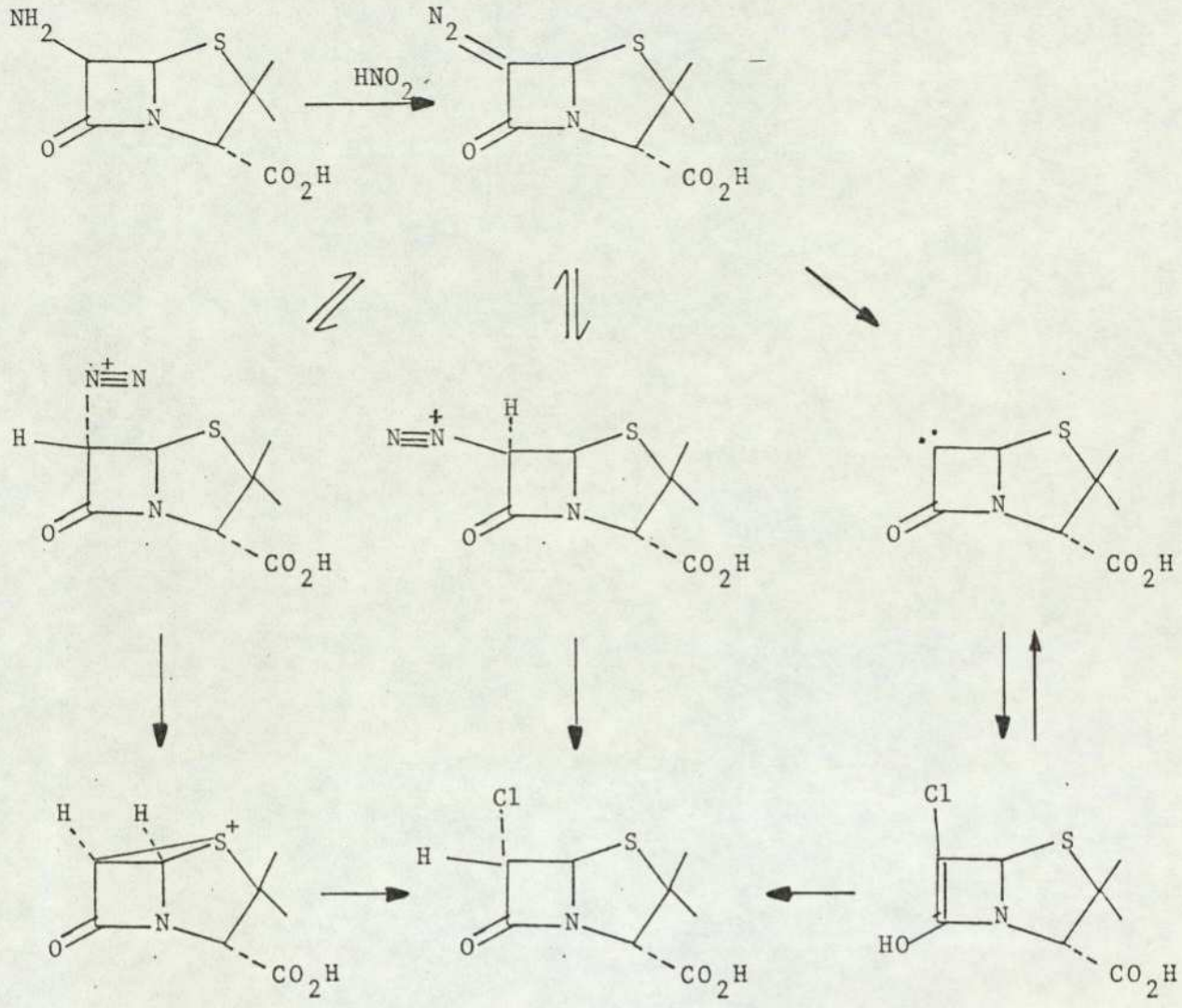
Since the discovery of the potent β-lactamase inhibiting activity of 6β-bromopenicillanic acid,[33,34] which was prepared either by epimerisation of 6α-bromopenicillanic acid or by Bu_3SnH reduction of the 6,6- dibromide. 6β-halogenopenicillanates have received considerable attention. Kemp[35] has demonstrated that 6α-hydroxylpenicil-

lanic esters can be reacted with trifluoromethanesulphonyl chloride to furnish triflates. The triflate group can then be displaced with iodide, bromide, chloride, azide and thiocyanate ions, affording the corresponding 6β-substituted penicillanates. 6β-Iodopenicillanic acid was reported to be a powerful inhibitor of several β-lactamases.

1.3. The Chemistry of 6-Diazopenicillanates and 7-Diazocephalosporanates

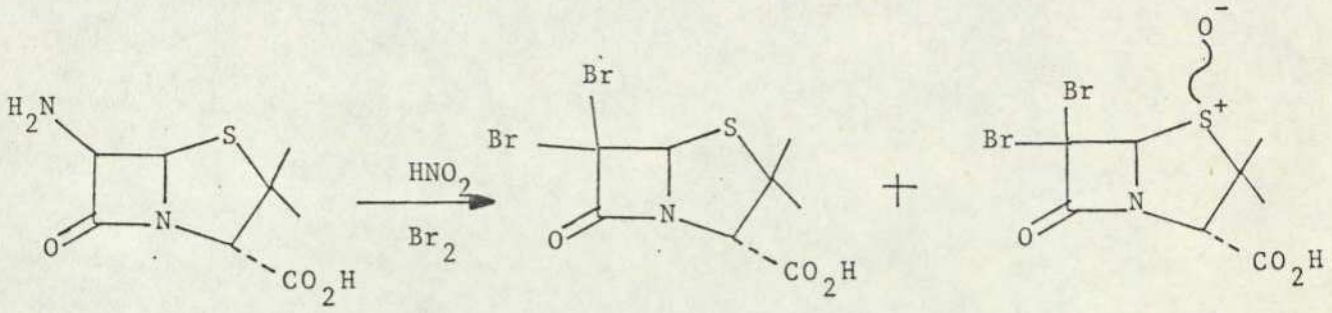
Apart from the methods described in the previous section, one of the most important routes for the introduction of substituents is to use the 6-diazopenicillanate or 7-diazocephalosporanate. These offer a versatile and easy pathway for the introduction of alkyl and other substituents.

The study of diazo derivatives was initiated by Cignarella, Pifferi and Testa[36] in 1962. They diazotised 6-aminopenicillanic acid with $NaNO_2$ in the presence of HCl or HBr to produce a crude product containing the respective 6α-halogenopenicillanic acids. The formation of the 6α—chloro compound was investigated in more detail by McMillan and Stoodley,[37] who considered three alternative pathways (Scheme 4). The intermediacy of 6-diazopenicillanic acid was established by showing that extensive incorporation of deuterium occurred when the deamination was carried out in DCl solution.



Scheme 4

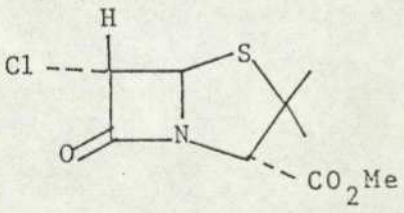
Clayton[38] observed that deamination of 6-aminopenicillanic acid by nitrous acid in the presence of sodium bromide or iodide, followed by esterification with diazomethane, led not only to the corresponding 6α-halogeno ester but also to the 6,6-dihalogeno ester. This appears to result from oxidation of the halide ions to halogen by the nitrous acid. Deamination of 6-aminopenicillanic acid in the presence of added bromine and without external cooling gave, in addition to the 6,6-dibromide, two further products which were identified as the isomeric sulphoxides (Scheme 5).



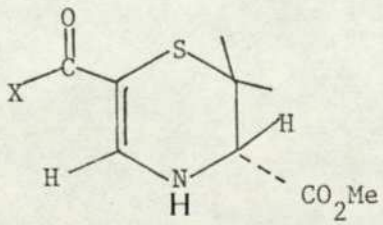
Scheme 5

The dibromide is a useful intermediate for conversion into other 6-substituted penicillins. Partial reduction by palladium gives the 6α-bromide, complete reduction affording penicillanic acid.[38] By contrast, reduction with Bu_3SnH allows selective formation of the 6β-bromide.[34]

In an attempt to carry out replacement of the halogen, the 6-chloropenicillanate (48) was reacted with sodium azide in THF.[37] Unexpectedly, the thiazine (49) was obtained. Similarly, the reaction with sodium methoxide gave the thiazine (50). These reactions are



(48)

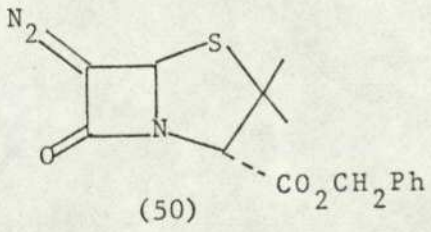


(49) x = N_3
(50) x = OMe

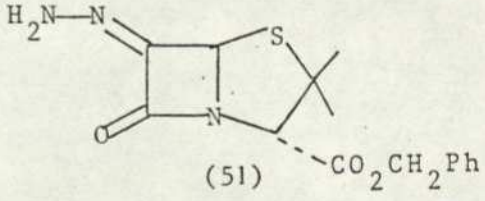
considered[37] to involve initial cleavage of the β-lactam by the nucleophile, followed by ring-expansion of the derived thiazolidines.

Deaminations of 6-aminopenicillanic acid have been carried out in the presence of a variety of acids.[26] Treatment with sodium nitrite in 90% acetic acid gave a poor yield of 6α-acetoxypenicillanate. 6α-Hydroxypenicillanate has been obtained by deamination in 1N toluene-p-sulphonic acid.

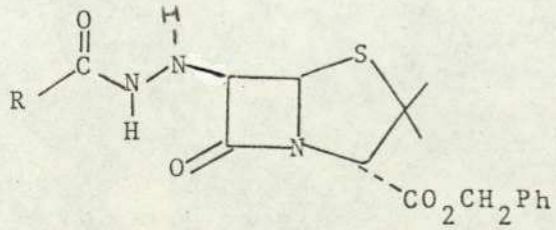
By careful control of the reaction conditions, it is possible to isolate 6-diazopenicillanic esters from the diazotisation of 6-aminopenicillanates. The diazo esters have proved to be versatile intermediates for further transformation. For example,[39] benzyl 6-diazopenicillanate (50) has been catalytically hydrogenated over palladium to give benzyl penicillanate. Reaction of the diazo compound (50) with triphenyl phosphine in wet ether gave the hydrazone (51), which could be acylated with acetic anhydride or phenylacetyl chloride and then further reduced with sodium borohydride to furnish the 6β-acylhydrazino penicillanates (52).



(50)



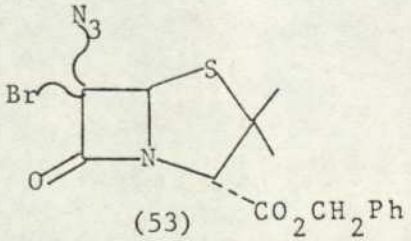
(51)



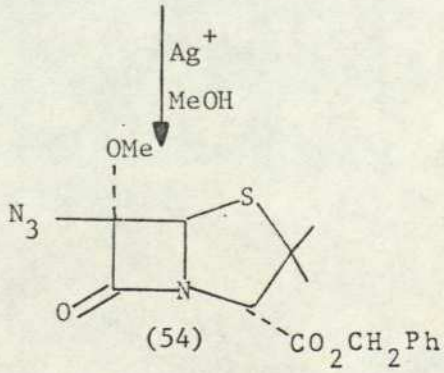
(52)

R=Me

CH_2Ph

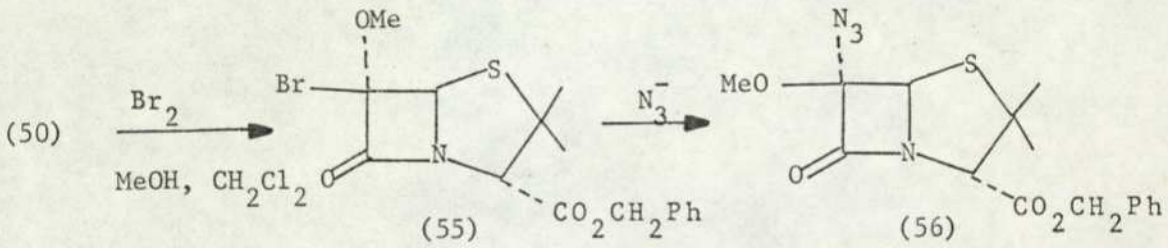


(53)

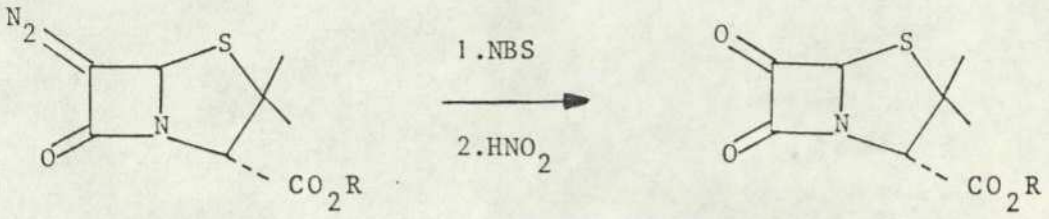


(54)

Reaction of the diazoester (50) with bromine azide and excess triethylammonium azide resulted in a mixture of epimeric 6-bromo 6-azido compounds (53), both of which gave the 6β-azido-6α-methoxy-penicillanate (54) upon reaction with $AgBF_4$ in methanol.[40] The azido group was then reduced (H_2/Pd-C) and the resulting amine acylated to furnish 6α-methoxypenicillins. A further variation of this method involves reaction of benzyl 6-diazopenicillanate (50) with NBA in the presence of methanol, giving the 6β-bromo-6α-methoxy ester (55). The bromide was then displaced from this with azide ion, furnishing the 6α-azido-6β-methoxy compound (56), from which the 6β-methoxy-epi-penicillin series could be derived.



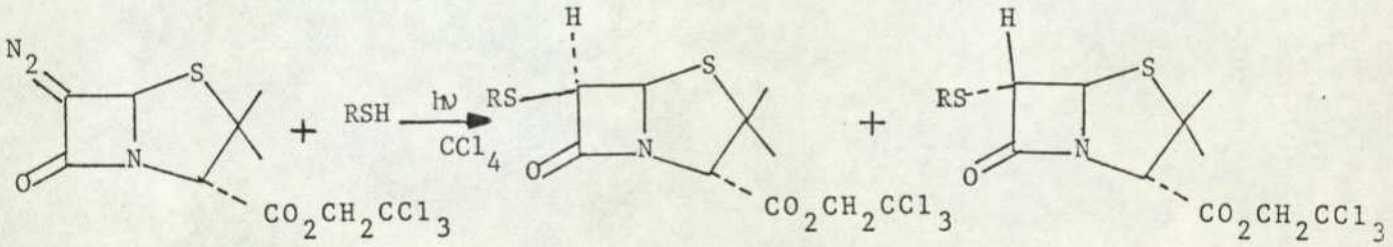
The synthetic potential of the diazo-β-lactams has been further emphasised by Sheehan and his co-workers. 6α-Hydroxypenicillanate and 7α-hydroxycephalosporanate esters were prepared by acid hydrolysis of the corresponding diazo esters.[41] The 6-diazopenicillanates(50;57) could be transformed directly into 6-ketopenicillanates by treatment with aqueous NBS followed by nitrous acid,[42] the keto compounds being key intermediates for further transformation (See Section 1.2).



(50) R=CH_2Ph

(57) CH_2CCl_3

Reaction of the trichloroethyl diazopenicillanate (57) with H_2S led to reduction to the corresponding hydrazone.[43] On the other hand, irradiation of (51) in the presence of thioacids led to mixtures of the β-and α-substituted penicillanates (58) and (59), with the former greatly predominating. Similar results were also obtained with thiophenol and benzyl mercaptan.



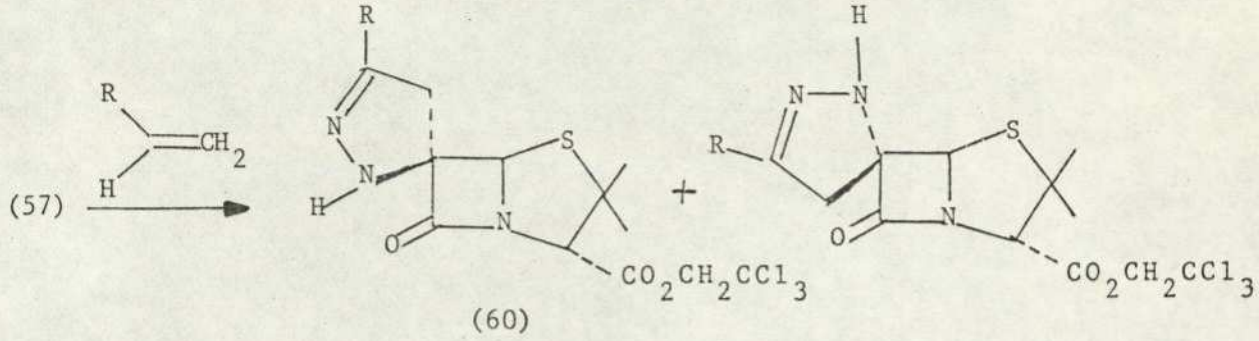
(57) (58) (59)

R = PhCO, $PhCH_2CO$, $PhOCH_2CO$, Ph, $PhCH_2$

When the diazoester (57) was irradiated in chlorothiolacetic acid, the isomeric products (58; R = $ClCH_2CO$) and (59; R = $ClCH_2CO$) were again obtained. Methanolysis of (58; R = $ClCH_2CO$) gave the mercaptan (58; R = H), which could then be acylated to give a variety of acylthiopenicillanates.

In reactions with olefins, Sheehan et al[44] observed that the diazo group took part in 1,3-dipolar additions, giving rise to isomeric

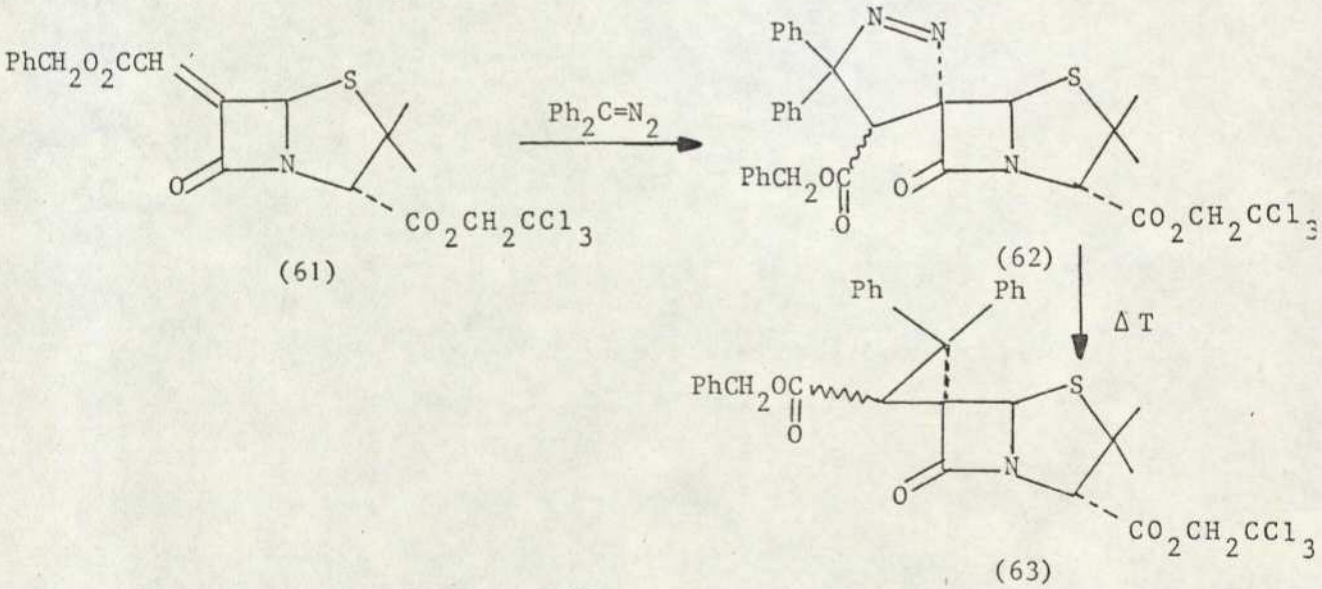
pyrazolines in which the major product (60) resulted from addition to the sterically less hindered α-face.



(60)

R=CN, CO_2Et, CO_2Bu^t, CO_2Ph.

The same type of reaction was also effected in the opposite sense, with the dipolarophile as part of the penicillin molecule. Thus, the unsaturated esters (61) reacted with diphenyldiazomethane to give $^1\Delta$-pyrazolines (62) and these were pyrolysed to give the spirocyclopropylpenicillanates (63).

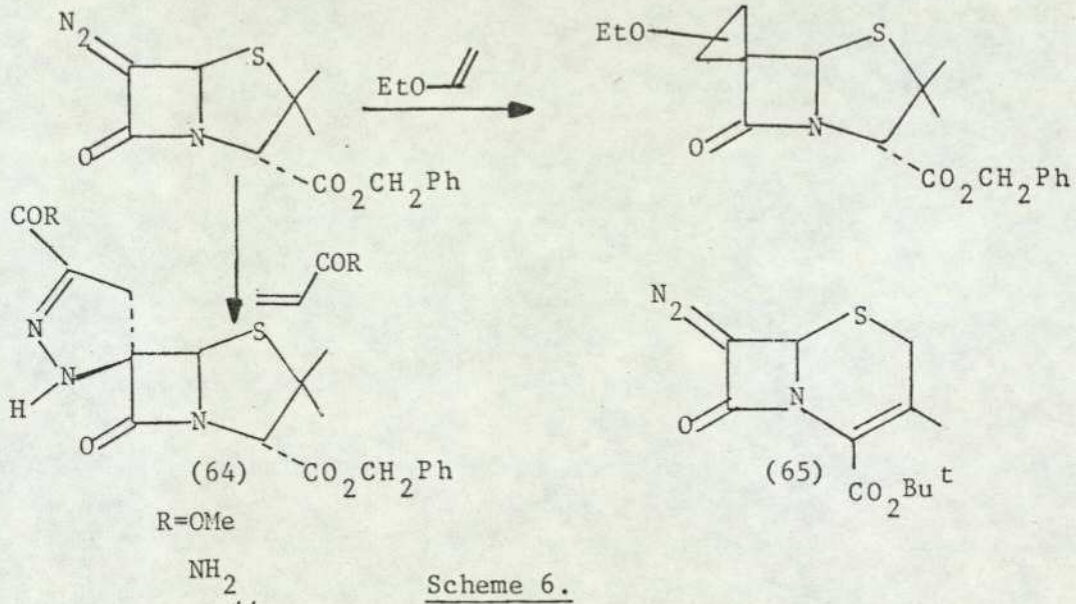


(61) (62) (63)

Campbell, Harcus and Ray[45] have also examined the reactions of diazo β-lactams with olefins. Benzyl 6-diazopenicillanate reacted with ethyl vinyl ether in the presence of $Cu(acac)_2$ to give a mixture

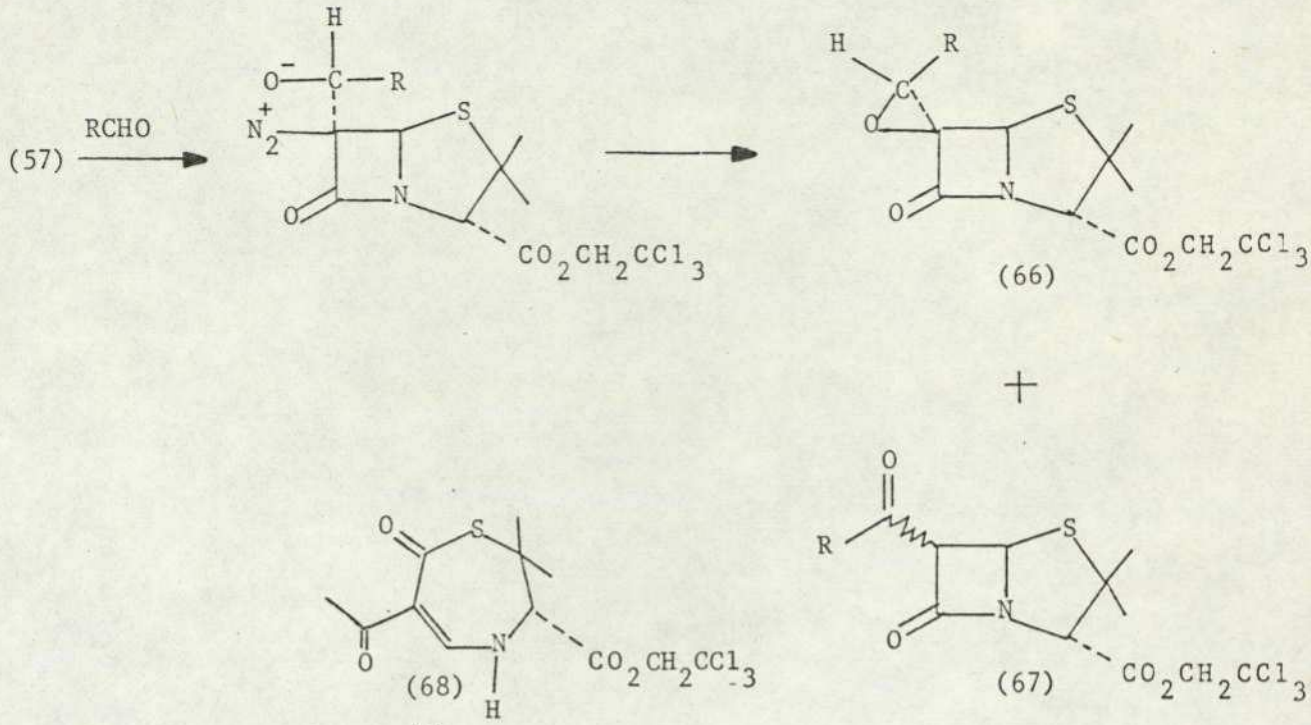
of four isomeric ethoxycyclopropanes (Scheme 6). In agreement with Sheehan's work, condensation of the diazoester with acrylic derivatives, with or without $Cu(acac)_2$ catalysis, afforded pyrazolines (64).

Similarly, the 7-diazocephem (65) afforded four isomeric ethoxycyclopropanes with ethyl vinyl ether. However, in the reaction with acrylate , a mixture of isomeric pyrazolines was obtained resulting from attacks on both faces of the β-lactam ring.

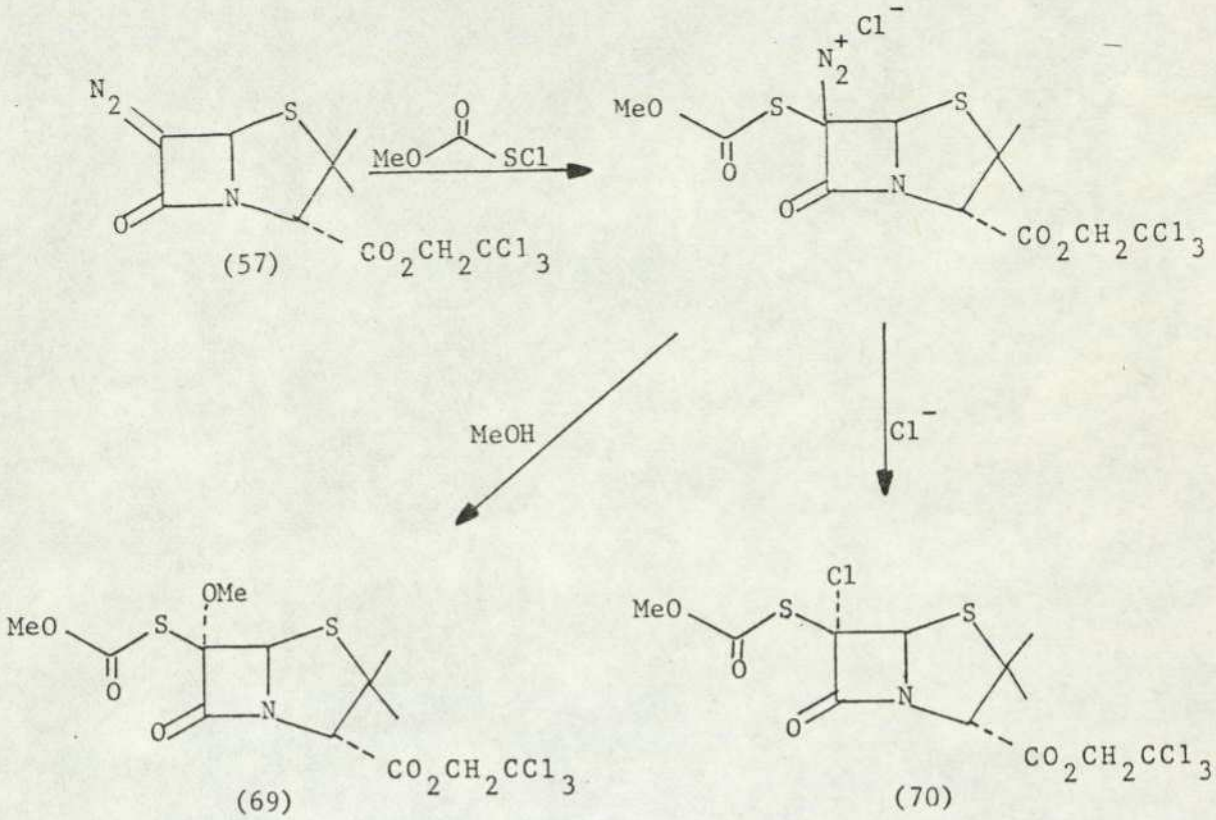


Scheme 6.

Reaction[44] of trichloroethyl 6-diazopenicillanate with acetaldehyde or phenylacetaldehyde at 0° gave the epoxides (66). At 10-25° , reaction with acetaldehyde also afforded the 6-acetylpenicillanate epimers (67). These decomposed on chromatography to give the rearranged thiazepine (68).

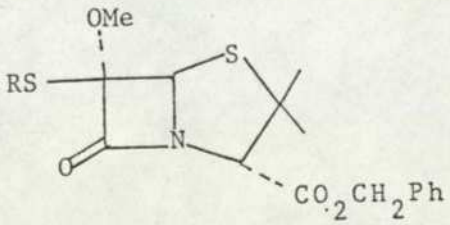


Sheehan's group[46] has also investigated reactions with sulphenyl chlorides. Treatment of the diazo ester (57) with carbomethoxysulphenyl chloride in CH_2Cl_2 containing an excess of methanol gave 6α-methoxy ester (69) along with a small amount of 6α-chloro ester (70). The latter was also obtained from the reaction with the sulphenyl chloride in the absence of methanol. A diazonium ion intermediate was proposed for these reactions, with replacement of the nitrogen by methanol or chloride ion occurring from the less hindered α-face of the β-lactam (Scheme 7).



Scheme 7.

Similar results were obtained in the reaction of benzyl 6-diazopenicillanate with 3,3,3-trichloroethoxycarbonyl sulphenyl chloride and methanol. The resulting 6α-methoxy thiol ester (71) was reduced with Zn-HOAc to give methoxy-mercaptan (72) which could then be re-acylated to furnish a variety of new thiol esters. Similar results were also obtained in the cephalosporin series.

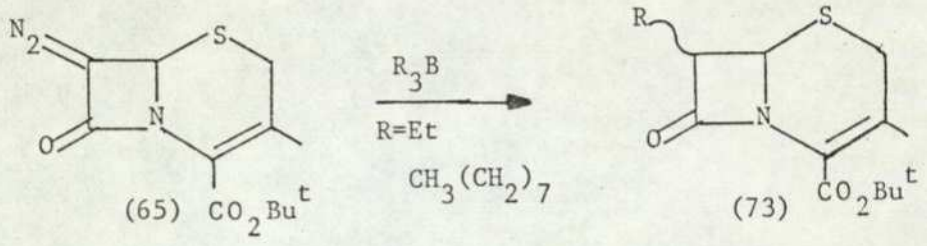


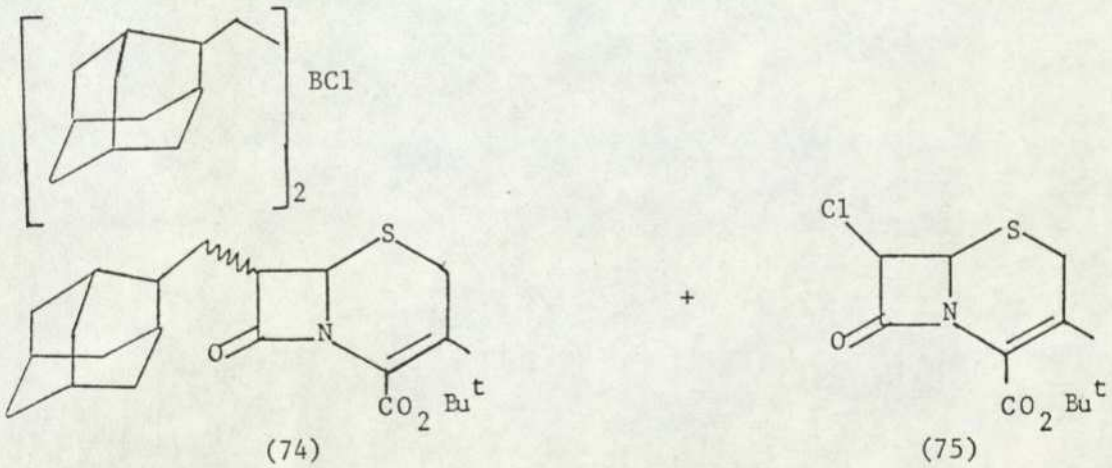
(71) R=Cl_3CCH_2OCO-

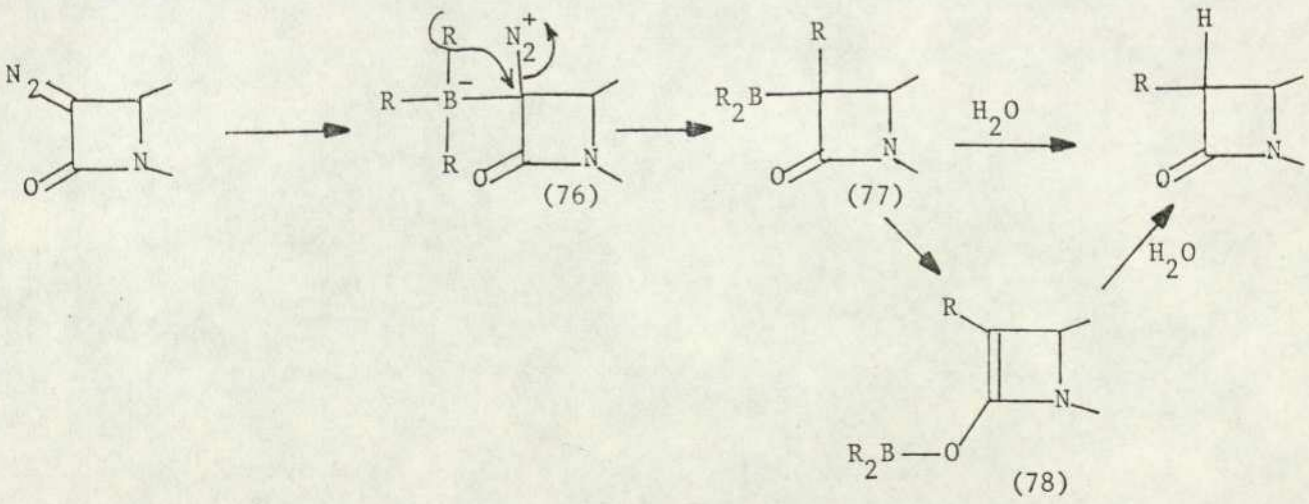
(72) R=H

Borane reagents have been used for the introduction of alkyl substituents at C-7 of cephalosporins.[47] The 7-diazoester (65) reacted under very carefully controlled conditions, with trialkyl boranes

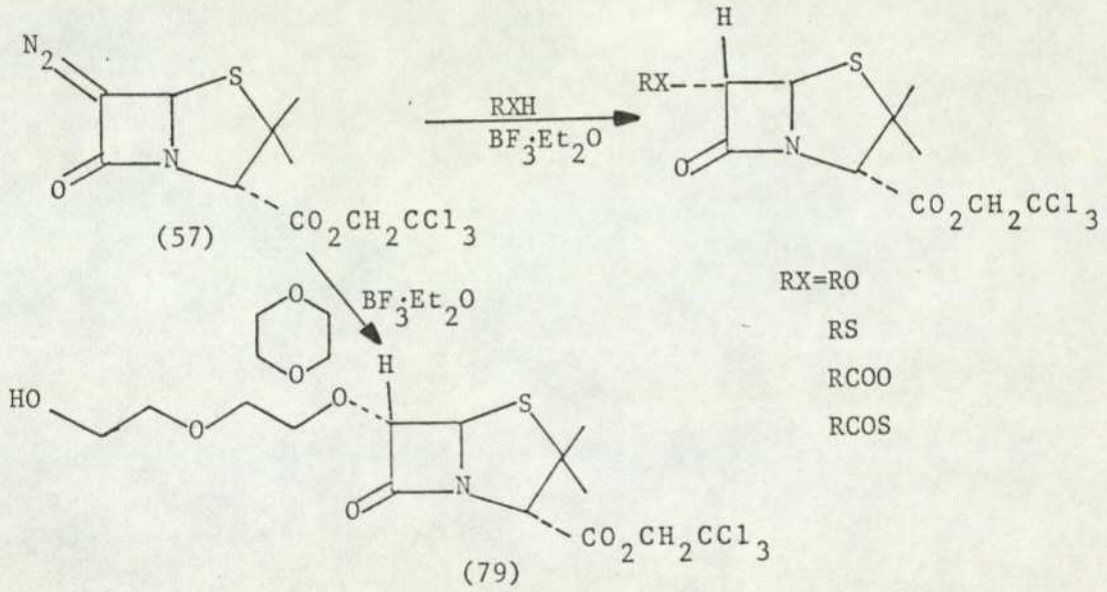
to give the 7-alkylated cephem esters (73). With bis-(2-adamantyl-methyl)chloroborane, a mixture of alkylated (74) and chlorinated (75) products was formed. The mechanism suggested for the alkylation reactions involves coordination of the boron with the diazo compound to produce the quaternary boron intermediate (76), followed by loss of nitrogen and (possibly concerted) migration. The final product is derived by hydrolysis of either the triorganoborane (77) or the alternative enol borinate form (78).







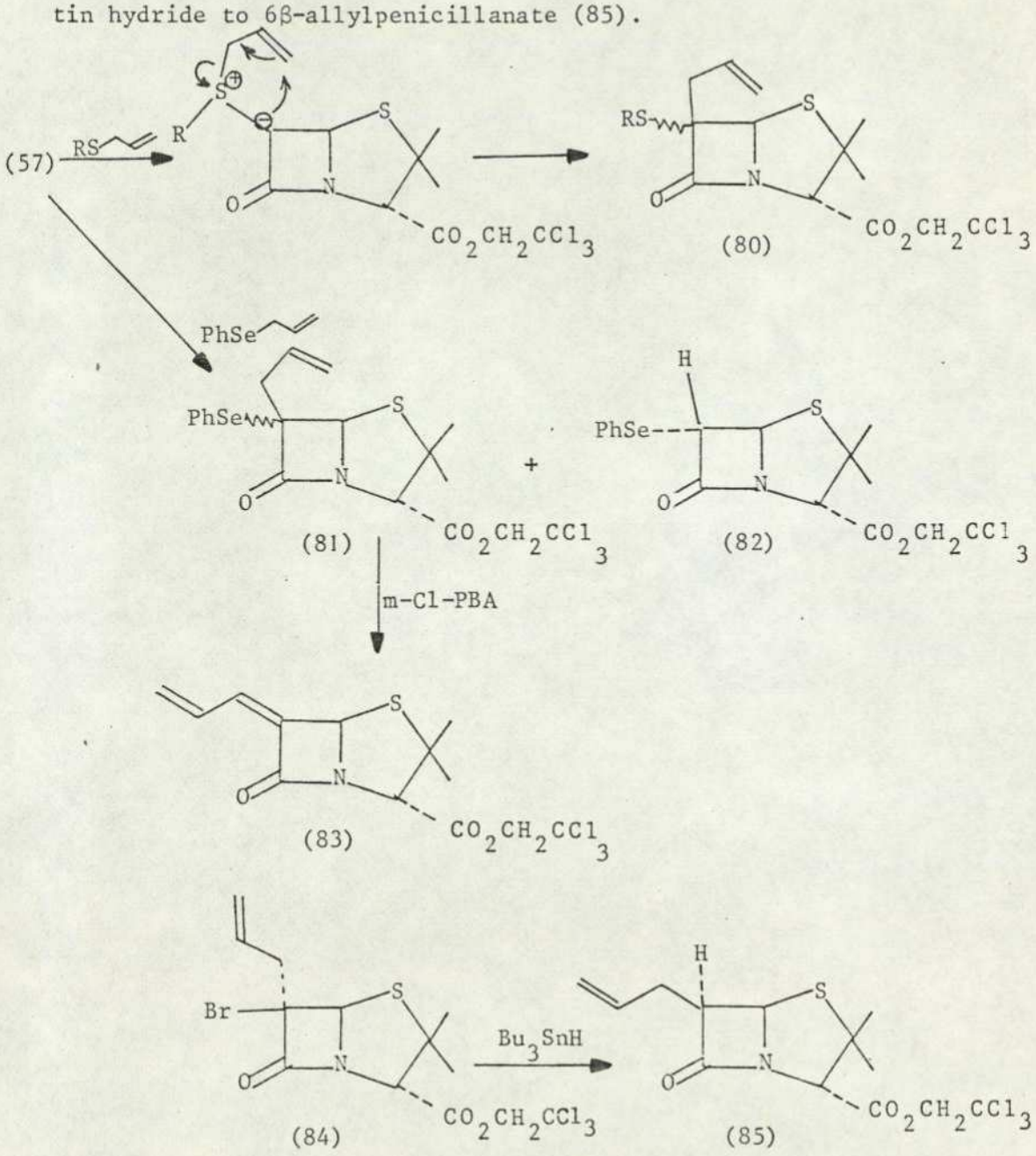
$BF_3.Et_2O$ catalysed decomposition of 6-diazopenicillanates in alcohols, thiols and related compounds has been described.[48] Alcohols gave the 6α-alkoxypenicillanates, thiols gave the 6α-alkylthiopenicillanates and acids and thioacids gave the corresponding 6α-esters and thioesters (Scheme 8). Decomposition of the diazoester (57) in dioxan led to the formation of a dioxan ring cleavage product (79) and it was similarly observed that diethyl ether was cleaved to form some 6α-ethoxypenicillanate.



Scheme 8.

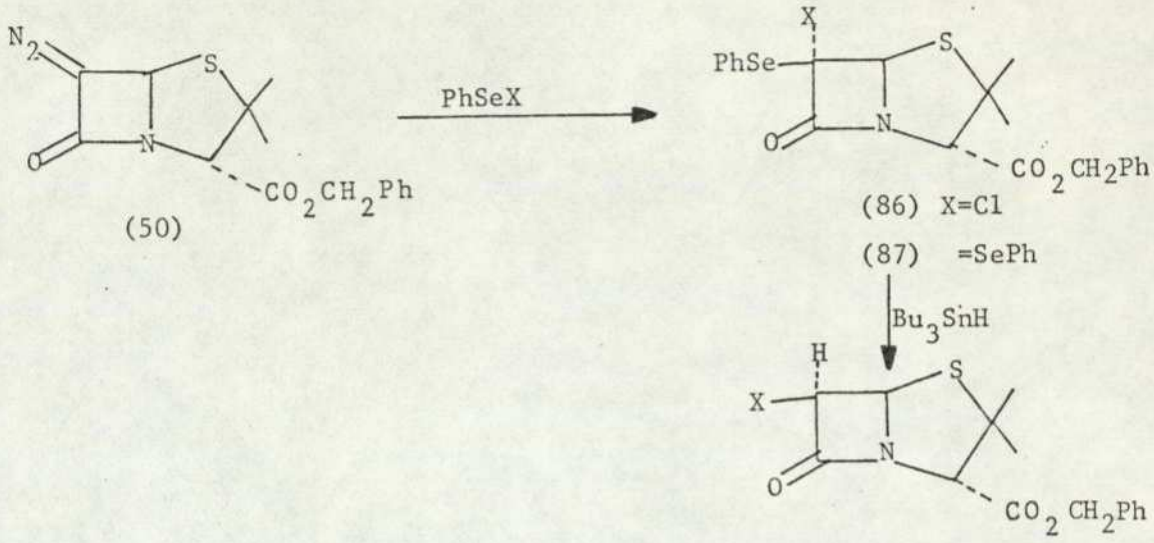
Extending this work, Thomas' group[49] examined the reactions of 6-diazopencillanates with allylic sulphides, selenides and bromides. In the presence of $BF_3.Et_2O$, allyl thioethers gave rise to the products of 2,3-sigmatropic rearrangement (80). Similarly, phenyl allyl selenide afforded the corresponding 6-allyl-6-phenylselenylpenicillanate (81), together with 6α-phenylselenylpenicillanate (82). It was noted that formation of the latter could be avoided by using $Cu(acac)_2$ as catalyst. Oxidative eliminations on the phenylseleno-allyl

penicillanates could be effected, providing a new synthesis of the diene (83). The 6-diazopenicillanate (57) was also reacted with allyl bromide in the presence of $Cu(acac)_2$ to produce the 6α-allyl-6β-bromopenicillanate (84). This was unstable and was reduced with tin hydride to 6β-allylpenicillanate (85).



It was similarly shown[50] that the phenylselenides (86) and (87), formed by reaction of benzyl 6-diazopenicillanate with PhSeCl

and PhSeSePh respectively, were selectively reduced by Bu_3SnH to give 6β-substituted penicillanates. The stereoselectivity was explained in terms of capture of an intermediate penicillanate radical by Bu_3SnH from the less hindered α-face.

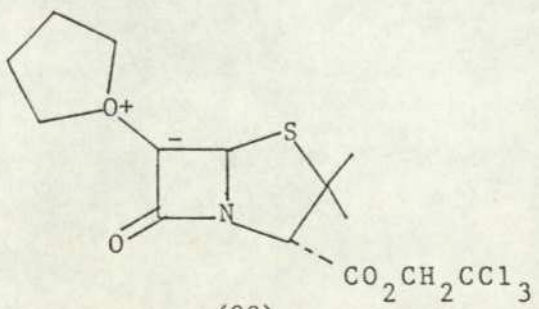


(50)

(86) X=Cl

(87) =SePh

Solvent effects were found to be important in the reaction of 6-diazopenicillanate (57) with phenylselenol in the presence of $BF_3.Et_2O$. Using dichloromethane as solvent, only the 6α-phenylselenyl penicillanate (82) was obtained, whereas addition of THF afforded a mixture of 6α- and 6β-phenylselenides, with the latter predominating. It was proposed that the THF participates by formation of an oxonium ylide (88) which then undergoes displacement with phenylselenol.



(88)

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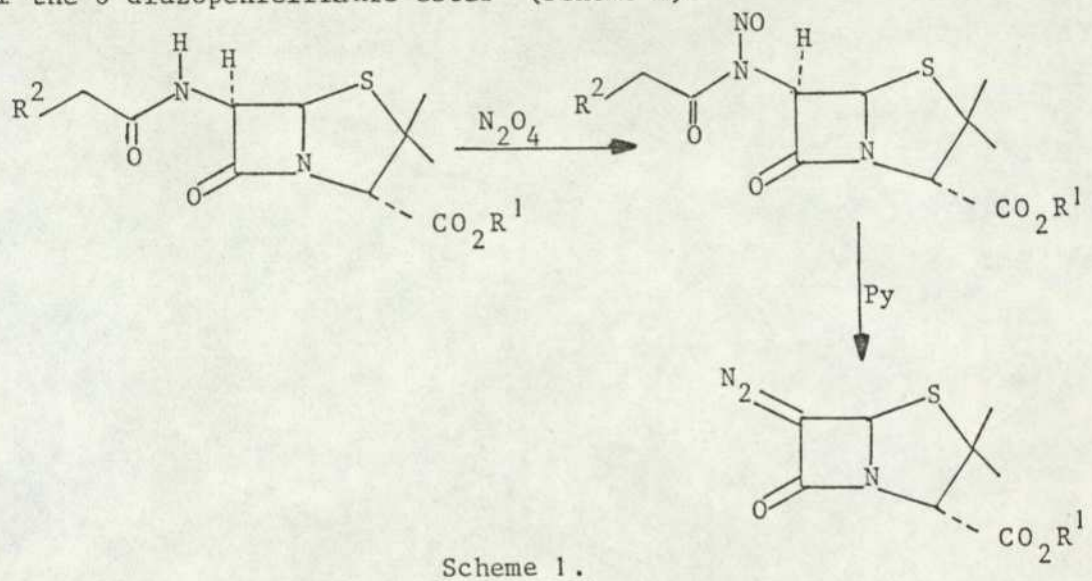
CHAPTER TWO

SYNTHESIS OF 6-DIAZOPENICILLANATES

2.1. Introduction

Synthesis of 6-diazopenicillanate has been described in the literature by two methods. In the first, penicillins were converted into N-nitrosoamides[1] using N_2O_4, and these were treated with pyridine in refluxing dichloromethane to give modest yields of the 6-diazopenicillanic ester[2] (Scheme 1).



Scheme 1.

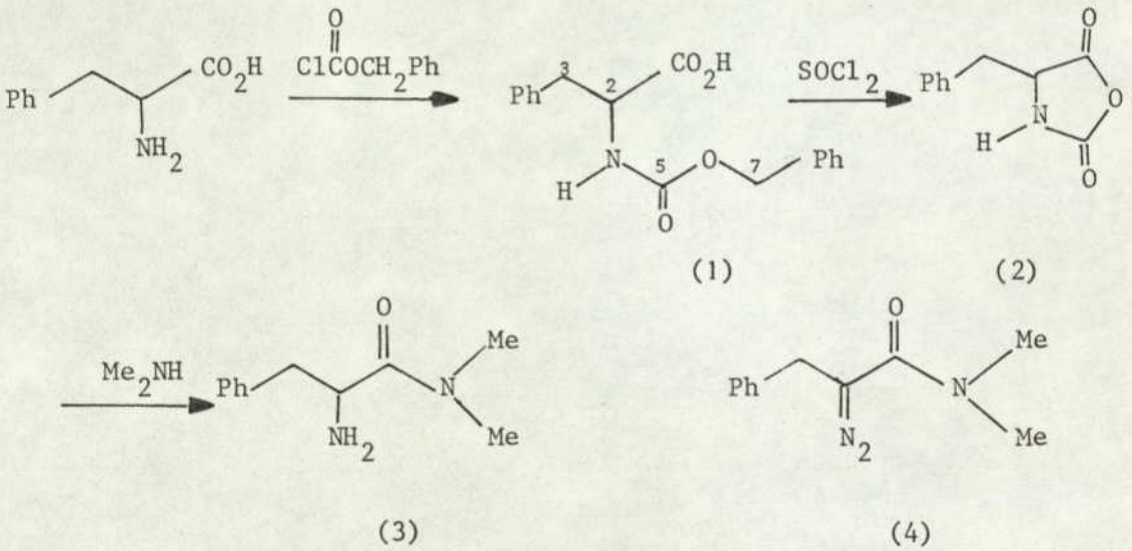
The second method involves diazotisation of 6-amino penicillanic esters by nitrous acid. In early work, this was carried out in aqueous solution, resulting in low yields. Later, the method was modified by using two phases (CH_2Cl_2/H_2O) leading to a higher yield and purer product[3,4].

Since, at the outset of this work, there was very little in the literature on α-diazo amides in general[5,6], we have carried out some model studies on the diazotisation of an α-aminoamide. We have also adopted some experimental modifications of the two-phase

technique which allows the synthesis of 6-diazopenicillanic esters to be accomplished in very high yield. The results of these investigations are presented in this chapter.

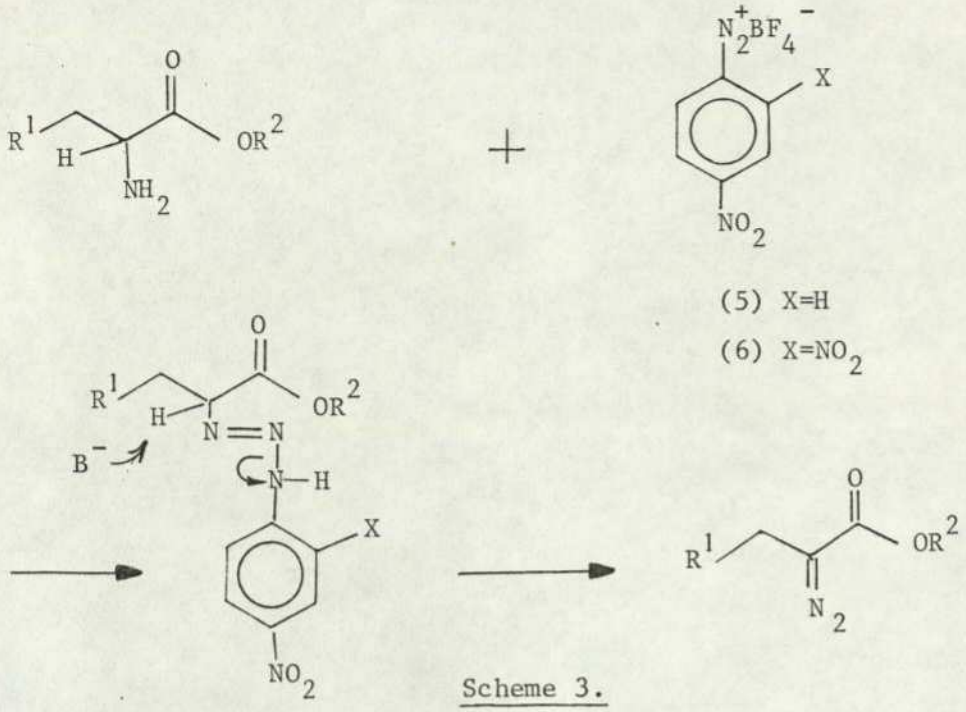
2.2. Studies with a model compound: N,N-dimethyl-2-diazo-3-phenylpropionamide.

D, L-Phenylalanine-N, N-dimethylamide (3) was prepared in three steps via the Leuch's anhydride (2) using the 'literature method'[7,8] (Scheme 2). The amide (3) was obtained as a colourless oil after distillation.



Scheme 2.

Attempts to diazotise the amine to obtain the diazoamide (4) were made by several procedures. 4-Nitrobenzenediazonium tetrafluoroborate (5) and 2,4-dinitrobenzene- diazonium tetrafluoroborate (6) have been reported[9,10] to be very successful reagents for the diazotisation of α-amino-esters (Scheme 3). However, reaction of either reagent with the amino-amide (3) only produced trace amounts (ca.1%) of the desired diazo-amide (4).



(5) X=H

(6) X=NO_2

Scheme 3.

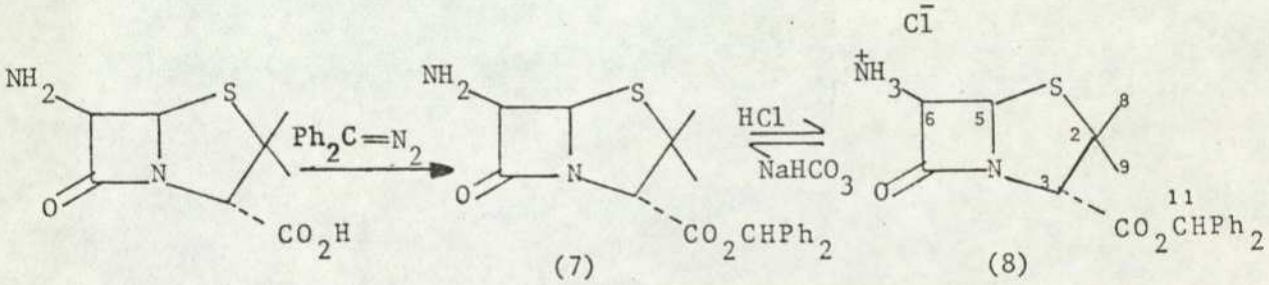
Takamura et al.[11] described an alternative route for the preparation of α-diazo esters, employing isoamyl nitrite as the nitrosating reagent. Applying this method, reflux of the α-amino amide (3) with 1.2 Eq. of isoamyl nitrite in chloroform containing 0.3 Eq. acetic acid for 15 minutes gave, after work up, a yellow oil which was submitted to chromatography on neutral alumina. This afforded the diazoamide (4) as an oil in a yield of 20%. No further effort was made to improve the yield of this reaction, but it appears to have some potential as a general route to the little-known, acyclic α-diazo amides. The chemistry of these compounds awaits exploration: it was observed that compound (4) was rather labile and acid-sensitive, decomposing even on silica gel chromatography.

2.3. Benzhydryl 6-diazopenicillanate

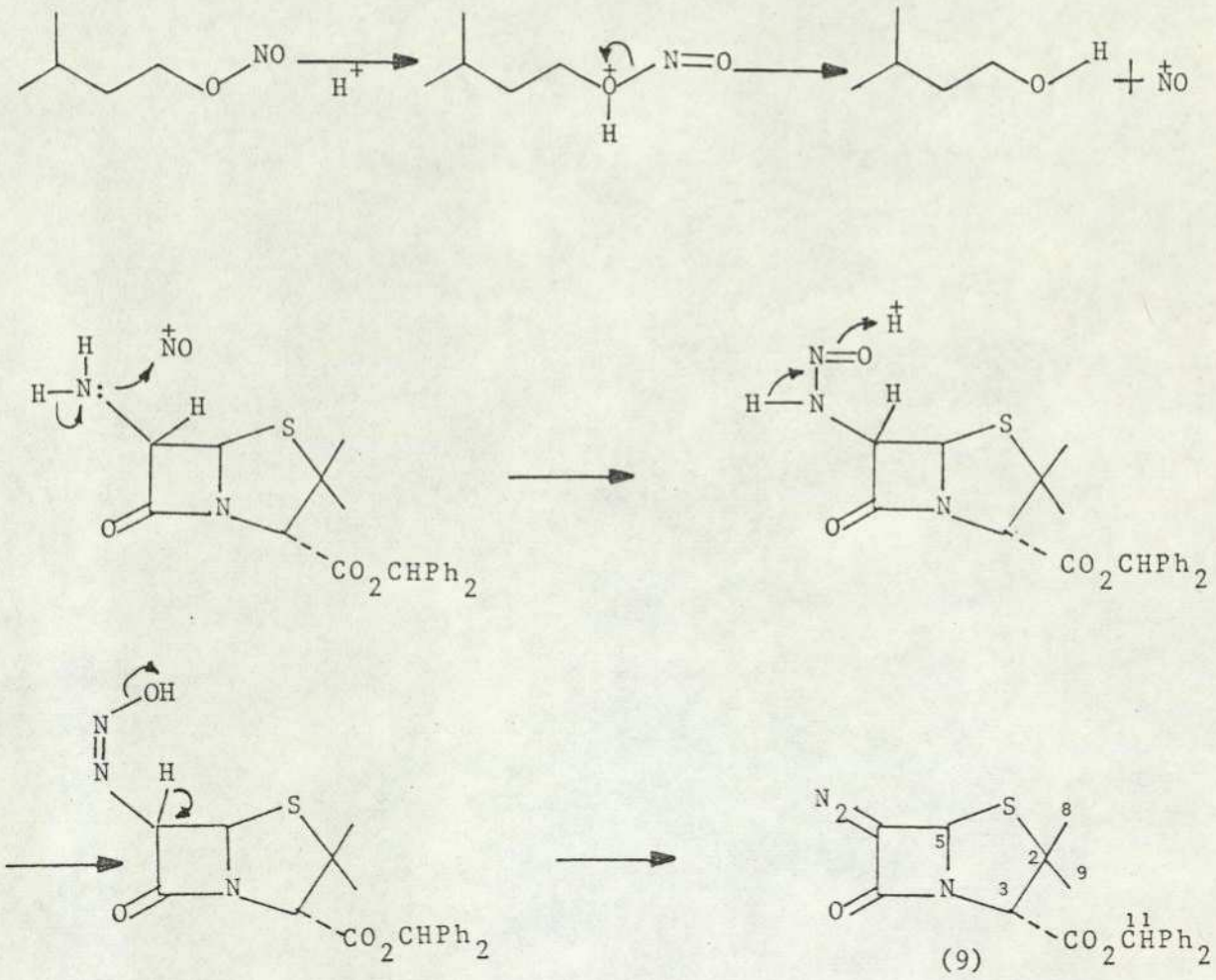
Having available 6-aminopenicillanic acid, it was first required to protect the acid group. This is necessary to facilitate subsequent handling of reaction products, and to avoid acid-catalysed decomposition of the diazo group. Initially, the benzhydryl protecting group was chosen, because (i) it can conveniently be introduced in one step without need for protection/deprotection of

the amino group (ii) it provides a good chromophore, facilitating chromatography and (iii) it can be removed under mild conditions e.g. by hydrogenolysis.

Diphenyldiazomethane was prepared by the literature method with some modifications[12,13] and was used to esterify 6-aminopenicillanic acid. The yield, based on unrecovered acid, was 85%. The ester (7) was immediately converted into the crystalline hydrochloride salt (8) by careful reaction with dry HCl in ether. This salt was found to be indefinitely stable on storage in the refrigerator and liberates the free amine by brief contact with aqueous $NaHCO_3$, as required.



The isoamyl nitrite method described above was applied to the diazotisation of the amine (7). Refluxing with 1.2 Eq. of isoamyl nitrite in dichloromethane containing 0.4 Eq. of acetic acid for 35 min. furnished, after work-up and chromatography, the 6-diazopenicillanate ester (9) in a yield of 65% (Scheme 4).



Scheme 4.

In optimising the reaction conditions for the diazotisation of the amine (7), the influence of temperature, reaction time and amount of acid catalyst were studied. The results are shown in Tables 1-3.

Solvent	Conditions	Yield (%)
CH_2Cl_2	reflux at 40° for 35-45 min	60
$CHCl_3$	reflux at 61° for 20 min	42
PhH.	reflux at 80° for 25 min	17

Table 1 Diazotisation of amine (7) with excess isoamyl nitrite in the presence of 0.3 Eq. AcOH.

Temperature(^{o}C)	Time (min)	Yield(%)
20^{o}	240	16
40-45^{o}	20	35
40-45^{o}	30	53
40-45^{o}	35-45	60
40-45^{o}	70	42

Table 2 Diazotisation of amine (7) with excess isoamyl nitrite in CH_2Cl_2 containing 0.3 Eq. AcOH.

Eq. AcOH	Yield %
0.2	42
0.3	62
0.4	65
0.5	46
0.7	40
1.0	26

Table 3 Diazotisation of amine (7) with excess isoamyl nitrite in CH_2Cl_2 for 40 min.

The optimum procedure provides a satisfactory yield of the diazoester (9) and was used throughout the early stage of our work. However, the method has one major drawback, namely, the requirement for reflux with an acid catalyst. The acid also reacts with the diazo compound, producing side-products, but to some extent these cannot be avoided. As will be clear from Table 3,

the reaction fails to proceed to completion if too little acid is used, but gives increasing quantities of side products as more is used. Thus, a yield of 65% appears to be the maximum attainable, and chromatographic clean-up of the crude product is essential.

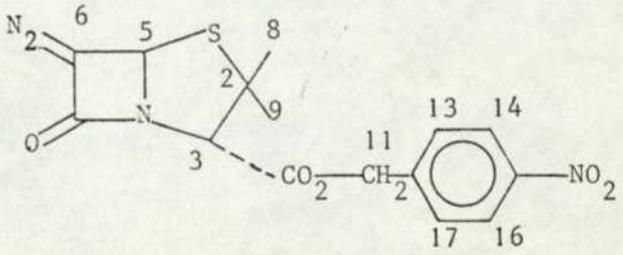
Later in the course of this work, attention was turned to the two-phase procedures mentioned above. It was found that, by manipulation of the reaction conditions an extremely efficient synthesis of benzhydryl 6-diazopenicillanate could be achieved. The amino ester (7) was stirred in dichloromethane and dilute perchloric acid with sodium nitrite for 2 h. under ice-cold conditions affording, after work-up, crystalline benzhydryl 6-diazopenicillanate (9) in a yield of >95%. The purity of this crude diazo ester is >95% and the material can be used for most purposes without any further purification.

The diazo ester (9) was found to be remarkably stable and can be stored in the freezer (-20^{o}) for long periods without considerable decomposition. In one case, a sample stored under these conditions for two years was examined by ^{1}H n.m.r. and found to have undergone very little decomposition.

2.4. p-Nitrobenzyl 6-diazopenicillanate

In a few instances, it was found to be difficult or impossible to remove the benzhydryl protecting group from penicillin derivatives prepared in this work. Consequently, the p-nitrobenzyl protecting group was also adopted, since this also provides a good chromophore for detection and offers convenient alternatives for deprotection.

p-Nitrobenzyl 6-aminopenicillanate was diazotised by the two-phase procedure described above. The diazoester (10) was obtained in 90% yield as a low-melting solid.



(10)

2.5. Experimental

N-Carbobenzoxy-D,L-phenylalanine (1). Benzyl chloroformate (9.76g; 0.057 mol) and 0.8N aqueous NaOH (80 ml) were added separately and slowly to a stirred mixture of D,L-phenylalanine (8.3g; 0.05 mol).

After 2 h stirring, the reaction mixture was washed with Et_2O, then acidified with 2N hydrochloric acid (35 ml). The white precipitate was filtered and washed with water until all chloride ions had been removed. The solid was then dissolved in absolute ethanol (75 ml) and the ethanol removed under reduced pressure at 75° to azeotropically remove water. Recrystallisation from EtOAc-Pet. Ether (60-80°) gave white crystals, yield 10.5g, 70%. M.p. 98-100° (Lit: 102°)[14] ν_{max} (nujol): 3320, 1695 cm^{-1}. $\tau(d_6$-acetone): 2.65(10H, m, aromatic H), 4.92 (2H,S, C_7-H), 5.50 (1H,m, C_2-H), 6.84 (2H, m, C_3-H).

N-Carboxy-D,L-phenylalanine anhydride (2). A mixture of carbobenzoxy-D, L-phenylalanine (50g; 0.16 mol) and thionyl chloride (100g; 1.37 mol) was stirred for 1 h at room temperature, 20 min. at 40°C and 15 min. at 60°C. Removal of the excess thionyl chloride under vacuum gave a yellow solid which was recrystallised from benzene to afford shining crystal flakes, yield 19.4g, 63%. M.p. 126-128° (Lit: 127-8°)[7]. ν_{max}(nujol): 3390, 1880, 1750 cm^{-1}. $\tau(CDCl_3)$: 2.65 (5H,m), 3.98(1H,b), 5.48(1H,m), 6.68(1H,dd, J = 15 Hz, 3.6Hz) 7.00 (1H,dd, J = 15Hz, 8Hz).

D,L-phenylalanine N,N-dimethylamide (3). A mixture of N-carboxy-D,-L-phenylalanine anhydride (19.4g; 0.1 mol) and anhydrous dimethylamine (250 ml) were stirred in an ice bath for 18 h. Removal of the excess

dimethylamine under vacuum gave an oily residue which was distilled at 145° /0.5mm to afford a clear, colourless oil. Yield 7g (36%). ν_{max}(film): 3360, 1640 cm^{-1}. $\tau(CDCl_3)$: 2.80 (5H,m), 6.08 (1H,t,7.2Hz), 7.10(1H,dd,15Hz,7.2Hz), 7.12(3H,s), 7.28(3H,s), 7.28(1H,dd), 8.39(2H,s). Adding D_2O removed the singlet at τ8.39.

N,N-Dimethyl-2-diazo-3-phenylpropionamide (4). A solution of D,-L-phenylalanine N,N-dimethylamide (192mg; 1 mol) and isoamyl nitrite (140mg; 1.2 mmol) in chloroform (6ml) was warmed to boiling and a solution of acetic acid (18mg; 0.3 mmol) in chloroform (ca. 1 ml) added. The mixture was refluxed for 15 min, then cooled and washed successively with cold sulphuric acid (1N), water, cold saturated sodium carbonate solution, water and was dried over anhydrous sodium sulphate. Evaporation under vacuum gave a crude yellow oil which was chromatographed (Al_2O_3: acetone-Pet. Ether, 4:6) to afford a golden yellow oil. Yield 40mg (20%). λ_{max}(EtOH) 266, 217 nm. $\nu_{max}(CH_2Cl_2)$, 2060, 1620 cm^{-1}. $\tau(CDCl_3)$: 2.72(5H,m), 6.28 (2H,s), 7.00 (6H,S).

Benzophenone hydrazone. A mixture of hydrazine hydrate (15ml; 0.3 mol) and benzophenone (36.4g; 0.2 mol) in n-butanol (40 ml) was refluxed for 24 h at 130°C. On cooling in ice, colourless crystals separated and were crystallised from ethanol. Yield 28.2g (72%). M.p. 98-99°(Lit:97-98°)[13]

Diphenyl diazomethane. A mixture of benzophenone hydrazone (4g: 0.02 mol), anhydrous sodium sulphate (4.6g; 0.32 mol) yellow mercuric oxide(11g: 0.049 mol) and ethanol saturated with potassium hydroxide (1.5 ml) in diethyl ether (62 ml) was stirred or shaken vigorously at room temperature for 75 min., then filtered and the filtrate evaporated at room temperature under reduced pressure. The resulting dark red

oil was dissolved in Pet. Ether (40-60°) and again filtered. Removal of the solvent gave diphenyl diazomethane as an oil, yield 3.32g (85%), which could be crystallised, M.p. 31-32°: (Lit:29-32°)[12]. ν_{max}(nujol) 2040 cm^{-1}.

Benzhydryl 6-aminopenicillanate hydrochloride (8). A mixture of 6-aminopenicillanic acid (30.7g; 0.14 mol) and diphenyl diazomethane (27.4g; 0.14M) in dichloromethane (236 ml) and methanol (94 ml) was stirred in an ice bath for 3-4 h, then at room temperature for 15 h, until all the purple colour of the diphenyl diazomethane had disappeared. The reaction mixture was diluted with Na-dried ether (700 ml), then the unreacted 6-aminopenicillanic acid (13g; 42%) was filtered off. The filtrate was kept in an ice bath and stirred as a saturate solution of HCl in dry ether was added dropwise to give a white precipitate which was filtered off and washed with ether. Yield 29.1g (85%). M.p. 150-155°. ν_{max}(nujol) 3500, 1770, 1725 cm^{-1}. $\tau(CDCl_3)$: 2.60 (10M, m, aromatic H), 3.00 (1H, s, C_{11}-H), 4.43 (1H,d, 4Hz, C_5-H), 5.44(1H, d, C_6-H), 5.46 (1H,s, C_3-H), 8.15 (2H,S,NH), 8.40 (3H,S, C_8-H), 8.72 (3H,S, C_9-H).

Benzhydryl 6-aminopenicillanate (7). The hydrochloride salt was shaken with a mixture of dichloromethane and 5% aqueous sodium bicarbonate solution. The organic layer was washed with brine and water and used immediately for diazotisation.

Benzhydryl 6-diazopenicillanate (9)

a. Diazotisation with sodium nitrite To an ice-cold mixture of benzhydryl 6-aminopenicillanate (2.18g; 5.6 mmol) in dichloromethane (400 ml) and water (400 ml) containing sodium nitrite (0.93g; 13 mmol)

was added 1N perchloric acid (12 ml) with rapid mechanical stirring. The mixture was stirred in an ice bath for 2 h, the organic layer separated, washed with cold, saturated brine, then water, and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave benzhydryl 6-diazopenicillanate (2.15g; 98%). The ^{1}H n.m.r. spectrum indicated >95% purity. The compound can be recrystallized from ether-Pet. Ether (40-60°), M.p. 85-87°. ν_{max} (nujol): 2080, 1760 cm^{-1}. $\lambda_{max}(CH_3OH)$, 5.14 nm(Log ε 5.14), 218 nm (log ε 5.08), 256 nm (log ε 4.72). ^{1}H n.m.r. τ($CDCl_3$) : 2.60 (10H, m, aromatic H), 3.00 (1H,s, C_{11}-H), 3.77 (1H,s,C_5-H), 5.50 (1H, s, C_3-H) 8.38 (3H, s, C_8-H), 8.78 (3H,s,C_9-H).

b. Diazotisation with isoamyl nitrite. A mixture of benzhydryl 6-amino penicillanate (75 mg; 0.196 mmol), isoamyl nitrite (32mg; 0.27 mmol) and acetic acid (3.5mg; 0.059 mmol) in dichloromethane (2 ml) was refluxed for 35 min. Removal of solvent under reduced pressure gave an oil which was chromatographed (SiO_2, CH_2Cl_2) to afford benzhydryl 6-diazopenicillanate as a yellow solid, M.p. 99-100°. Yield 48 mg (62%).

p-Nitrobenzyl 6-diazopenicillanate (10). Using the same procedure as method (a) aforementioned, p-nitrobenzyl 6-aminopencillanate (519 mg; 1.48 mmol) was converted with sodium nitrite (234mg; 3.39 mmol) and 1N perchloric acid (3 ml) to p-nitrobenzyl 6-diazopenicillanate (10) as a yellow solid, M.p. 39-42°.

Yield 450 mg (90%). $\lambda_{max}(CHCl_3)$: 2080, 1750, 1610, 1525, 1350 cm^{-1}. τ($CDCl_3$): 1.80 (2H, d, 9Hz, C_{14}, C_{16}-H) 2.45 (2H, d, C_{13}-H, C_{17}-H), 3.84 (1H,s, C_5-H), 4.72 (2H,s, C_{11}-H), 5.56 (1H, s, C_3-H), 8.35 (3H, s,C_8-H), 8.58 (3H, S, C_9-H).

2.6. References

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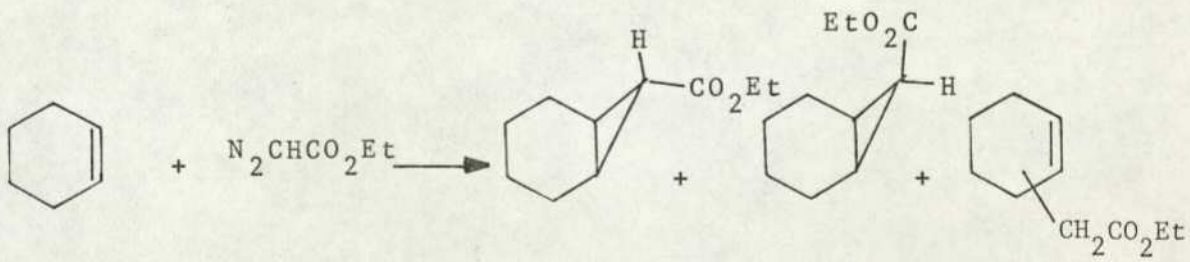
CHAPTER THREE. CYCLOPROPANATION REACTIONS WITH 6-DIAZOPENICILLANATES.

3.1. Literature Review of Cyclopropanation Reactions

The reactions of 6-diazopenicillanic esters with acrylates and with ethyl vinyl ether have been reported in the literature[15] and were reviewed in Chapter 1. Since the most detailed and pertinent studies of cyclopropanation reactions reported in the literature have concerned α-diazoacetic esters, this topic will now be reviewed before discussing our own experimental results with benzhydryl 6-diazopenicillanate.

The copper-catalysed decomposition of diazoesters is an old reaction which has been the subject of considerable investigations, e.g. Skell and Etter[1] reported that the reactive intermediate from the $CuSO_4$ catalysed decomposition of ethyl diazoacetate converts cyclohexene into cyclopropanes but does not appear to undergo insertion into carbon hydrogen bonds, and its stereoselectivity in cyclopropane formation is quite different from that of the carbene obtained by photolysis of ethyl diazoacetate.



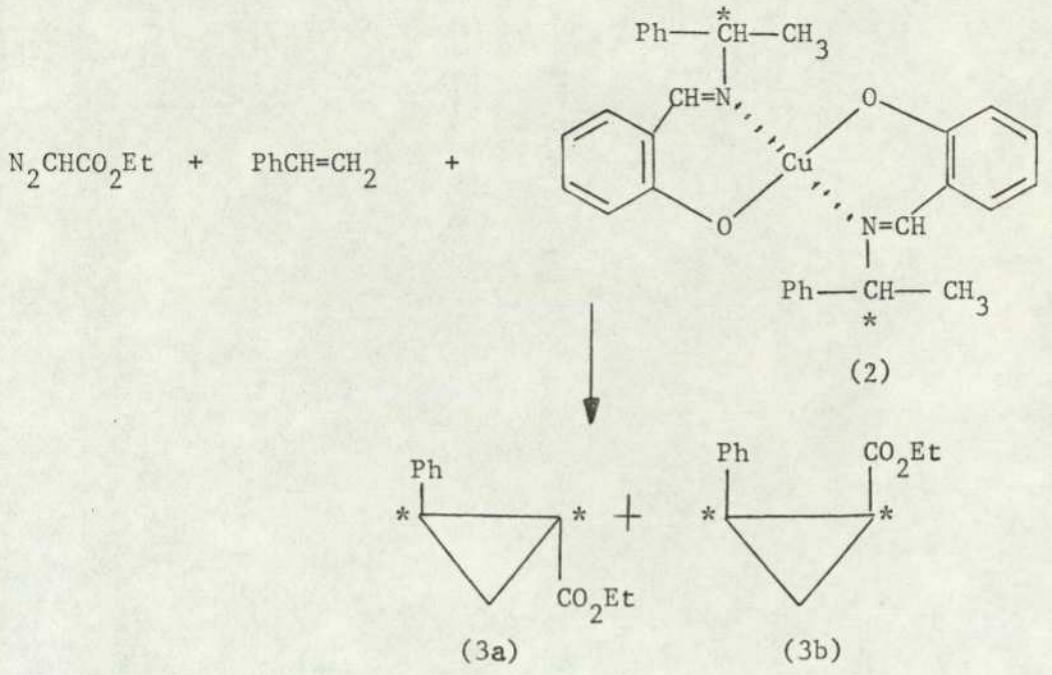
hν :	16%	10%	21%
Cu :	69%(Exo)	4%(Endo)	0%

Thus, it had been proposed that an intermediate formed in copper-induced reactions is different from the free "carbene" thought to be produced in photolysis.

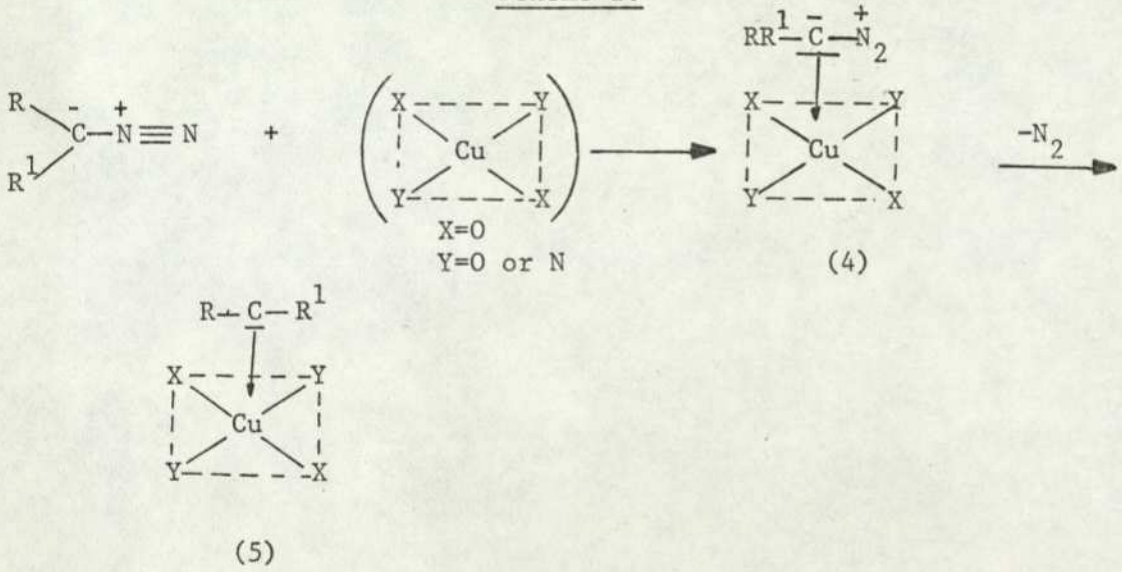
The view that a carbene-copper complex is involved in the catalysed reactions gained strong support from the observation that the decomposition of ethyl diazoacetate by the chiral complex bis—[N-(R)-α-phenethylsalicylaldiminato]copper(II) (2) in the presence of styrene, give a mixture of the optically active ethyl-2-phenylcyclopropanecarboxylate (3a) and (3b) (Scheme 1).[2]

It was postulated that the open coordination site of the square planar copper chelate wouldundergo electrophilic attack on the carbon atom of diazoalkanes yielding a complex (4) (Scheme 2). The subsequent elimination of nitrogen molecule would furnish a carbene-copper complex (or an inverse ylide) (5), in which the carbene moiety is coordinated to the copper atom as the fifth ligand. The possible back-donation from the metal atom to the vacant Pz orbital of the carbenic carbon may help to stabilize the complex. Furthermore, Nozake et al.[2] pointed out that they could not rule out an alternative possibility that the initially formed complex (4) directly reacts with the substrates. Assuming that the term "copper carbenoid" involves both species (4) and (5), it was thought to be safe to conclude that a chiral carbenoid is responsible for the accomplishment of asymmetric induction mentioned above.

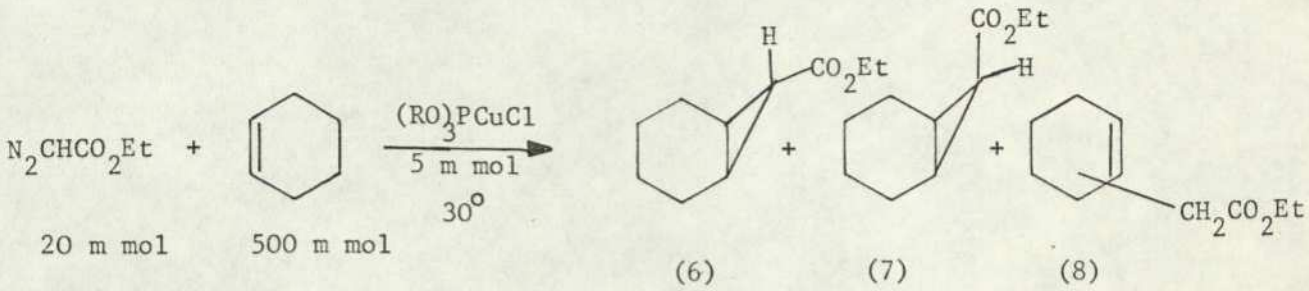
A similar olefin-metal carbene complex mechanism had been discussed by Moser[3]. The (trialkyl and triaryl phosphite) copper (I) chloride catalysed decomposition of ethyl diazoacetate in cyclohexene afforded exo and endo cyclopropanes (6) and (7) as major products, together with a trace amount of insertion adduct(8).



Scheme 1.



Scheme 2.

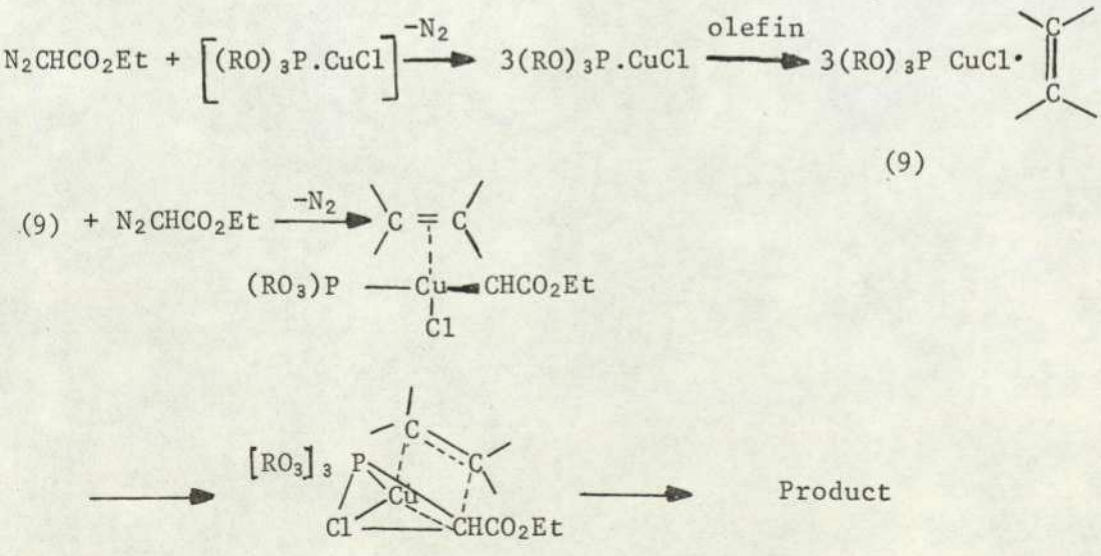


	EXO	ENDO	
Photolysis	1	1.89	2.36
Thermolysis	1	7.14	0.25
Catalysis	1	9.66	0.05

Relative Ratio

The exo and endo isomers are not isomerized under the reaction conditions, and are extremely slow to isomerize under equilibrating conditions. A tenfold change in the amount of catalyst employed afforded only 2% change in the observed ratio of the exo/endo products.

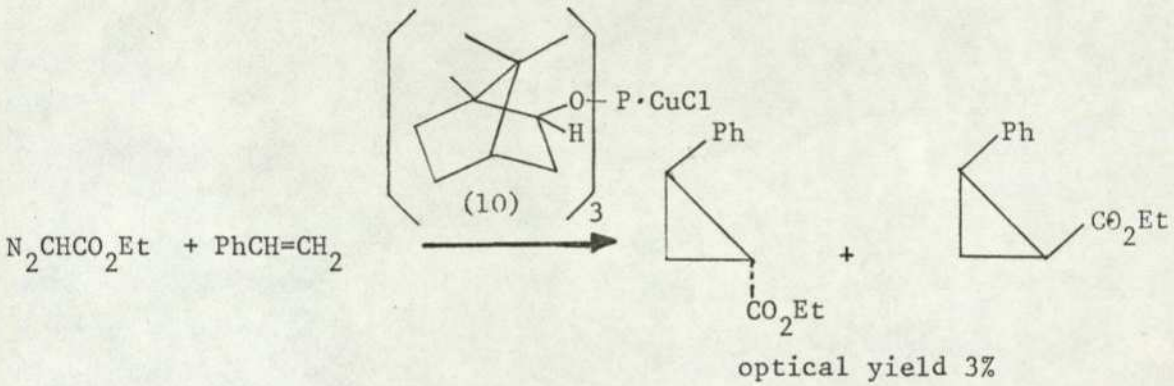
Moser's conception of the most reasonable transition state stereochemistry is depicted in Scheme (3).



Scheme 3

Moser revealed that systematic changes in the isomeric cyclopropane product distribution occurred as a function of the steric bulk and electronic effects of the ligand in the homogenous catalysed addition of ethyl diazoacetate to olefins employing (trialkyl phosphite) copper (I) chloride complex.

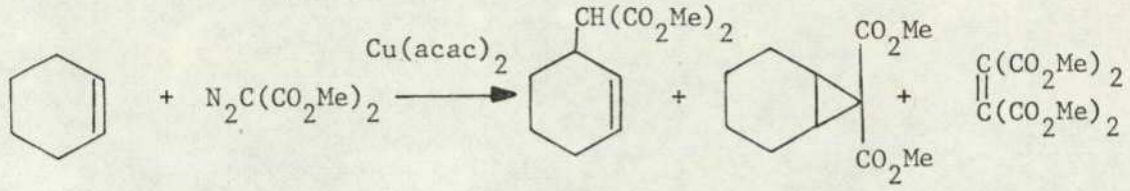
Asymmetric induction was also observed when an optical active catalyst of [(-) tribornyl phosphite] copper (I) chloride (10) was utilized to decompose ethyl diazoacetate in styrene (Scheme 4).



SCHEME (4)

The postulated mechanisms described above received a rather cold response from Wulfman.[4] He criticized that the whole copper carbenoid concept was "built upon a house of cards" in which the foundation was the assumption that various thermolyses and photolyses of diazoalkanes led to the generation of free carbenes. Therefore, the choice of a carbenoid pathway of catalysis of diazoalkanes was more a reflection of an emotional bias than empiricism, and he emphasized that some unsensitized photolyses of diazo compounds in alkene do not react solely by a "free carbene" path but rather react predominantly via some other species.

Wulfman's group[5] published a series of papers on this topic. The reaction of the dimethyl diazomalonate with cyclohexene in the presence of $Cu(acac)_2$ again gave mainly cyclopropanes.



Yield	5.92%	78.1%	12.4%

They suggested that the apparent role of copper was to act as a Lewis acid and demonstrated that copper (II) was either the actual catalyst or, at least, a far more active catalyst then either elemental copper or copper (I). The key features of copper (II) were stronger acid strength but weaker back-bonding than related copper (I) species. The copper salt catalysed addition of bis(methoxy carbonyl) carbene to olefins was highly regioselective and was stereospecific for cyclopropanation. Insertion into carbon-hydrogen bonds was of increasing importance as the degree of substitution increased and as the reaction temperature was decreased. Cyclopropanation became more important as the operating temperature was increased with all olefins. Wulfman interpreted this temperature effect as indicating that the processes were not of a free radical nature. The best catalyst for cyclopropanation was copper fluoroborate operating in homogenous mode.

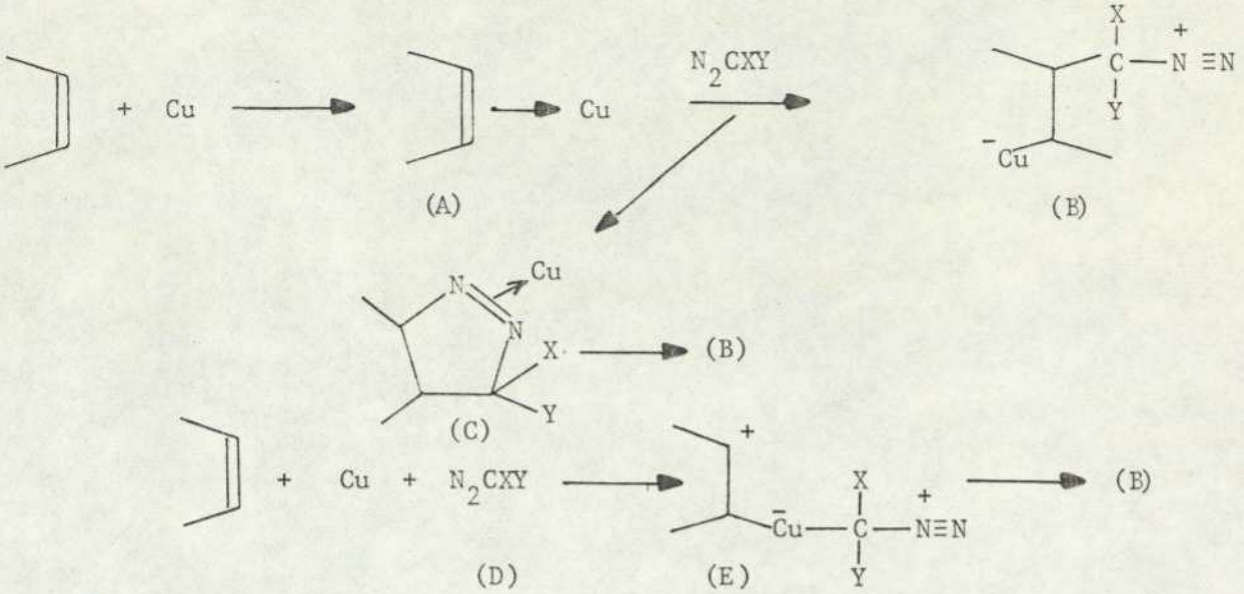
In examining the relationship between catalyst concentrations and product yields, two maxima for cyclopropanation and allylic C-H

insertion and other important features were observed in the plot of product yield versus catalyst concentration.

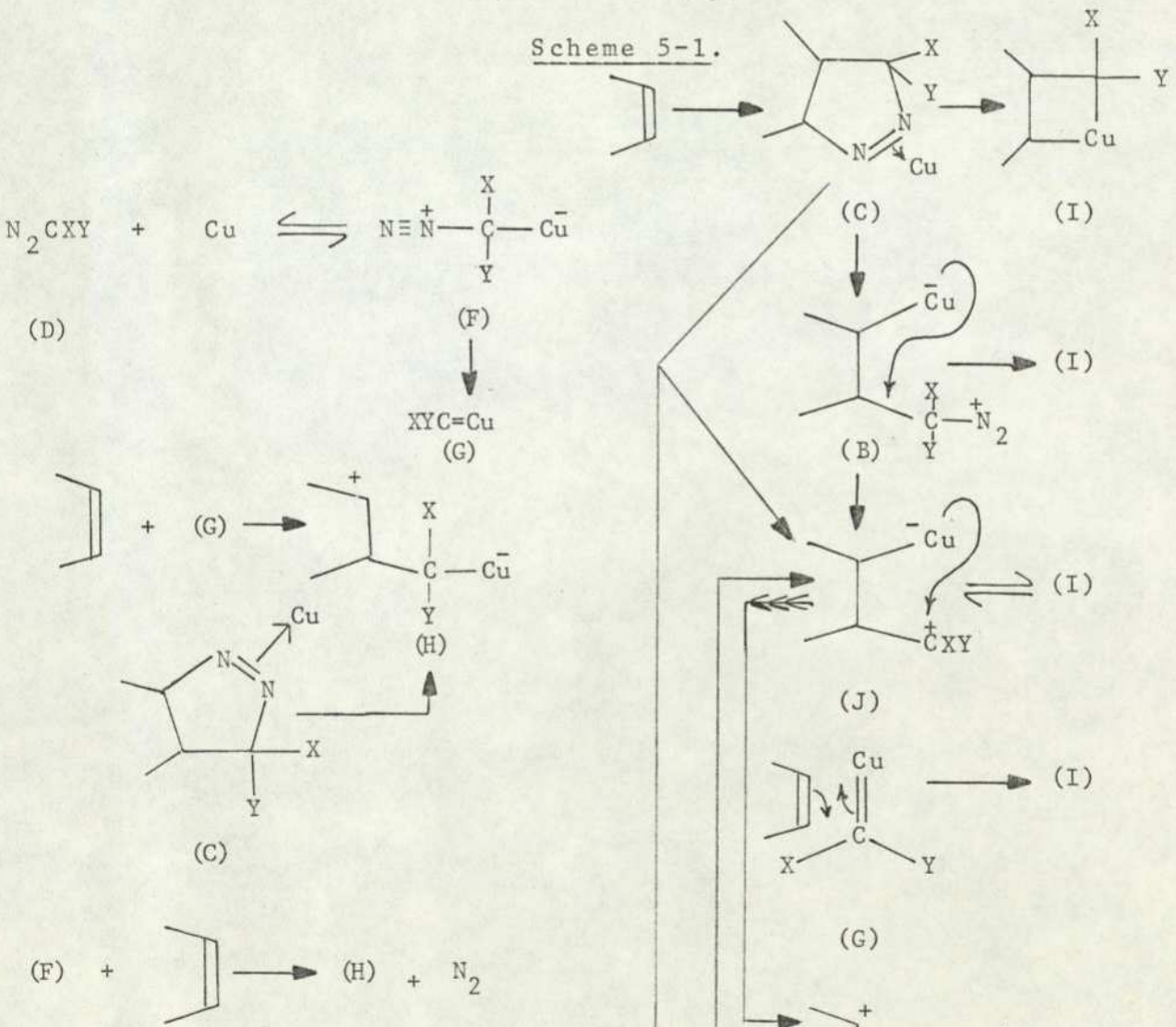
On the basis of these observations, it was concluded that there must be at least three paths to cyclopropane formation. Two of these involve a single common intermediate which is formed before product partitioning between allylic C-H insertion and cyclopropanation and involves a single molecule of catalyst. A third process involving two molecules of catalyst must be occurring at high catalyst concentrations. Alternative pathways were given. (Schemes 5-1 to 5-3)

In addition to copper complexes, several group VIII transition-metal derivatives recently received great attention in catalysed decomposition of diazocompound in olefins.

Paulissen, Hubert and Teyssie[6] reported the palladium acetate catalysed cyclopropanation of olefins which can be practically quantitative even under very mild thermal conditions.

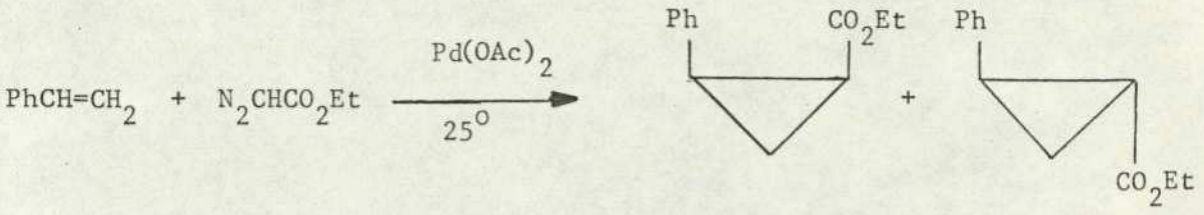


Scheme 5-1.



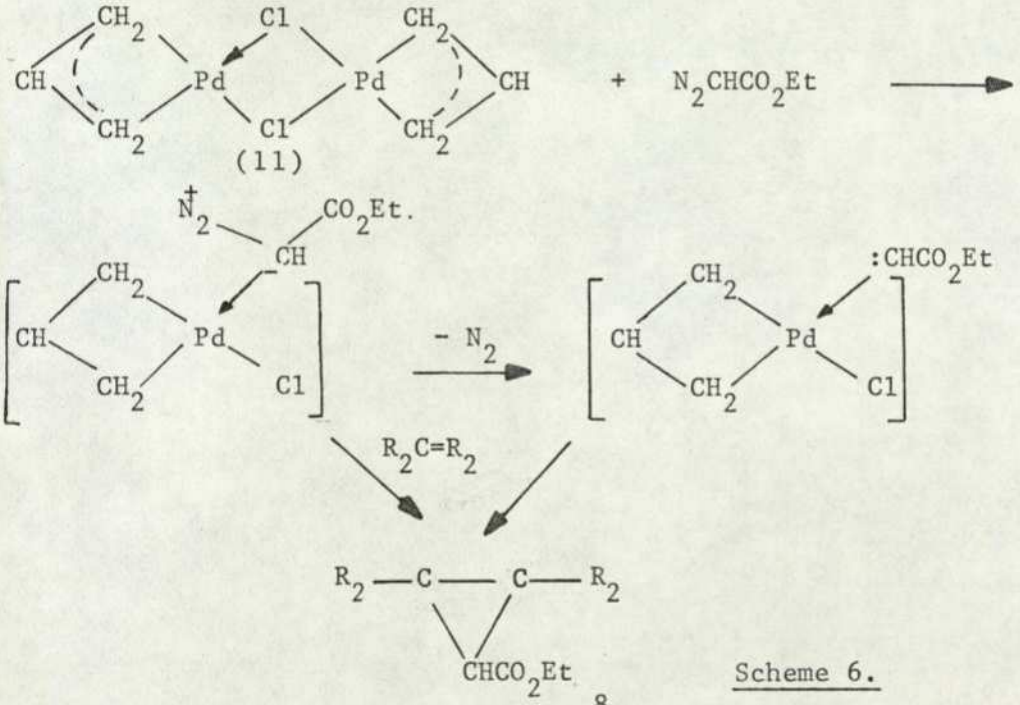
Scheme 5-2.

Scheme 5-3.



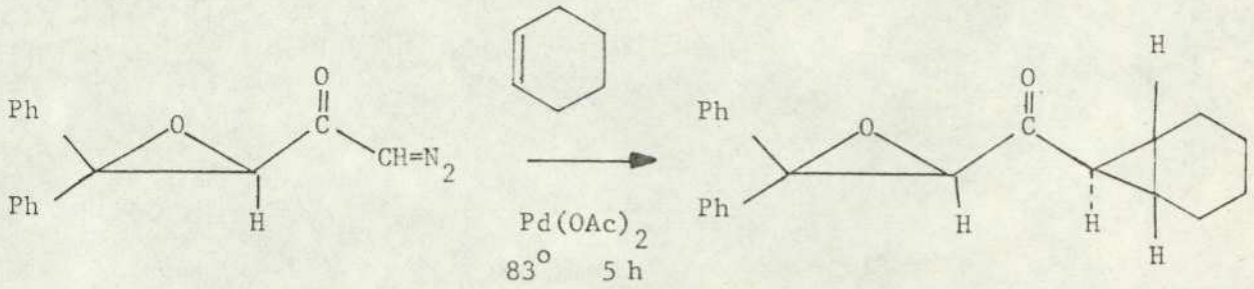
(96%) 1 : 1

Armstrong[7] described the decomposition of ethyl diazoacetate by the π-allylic palladium chloride complex (11) at low temperature (0-10°) and the possible mechanism of this reaction which appeared to involve a carbene or related intermediate. (Scheme 6)



Scheme 6.

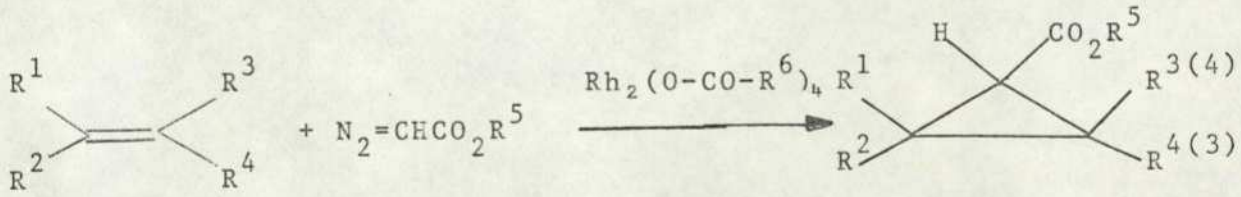
Smeets, Thijs and Zwanenburg[8] published the cyclopropanation reactions with αβ-epoxy diazomethyl ketones (12) in the presence of palladium acetate.



(12) 53.5%

Hubert et al.[9,10] have published the application of another group VIII transition metal derivative, rhodium carboxylate, in catalytic decomposition of alkyl diazoacetates.

The high efficiency of rhodium (II) carboxylate in the cyclopropanation of alkenes substituted at ethylene group had been described, which included almost any kind of alkenes (mono or poly-olefins, substituted or not). The lack of reactivity of electron-poor olefins (e.g. methyl maleate) is a notable exception. In most cases, the yields were claimed to be higher than 80-90%.

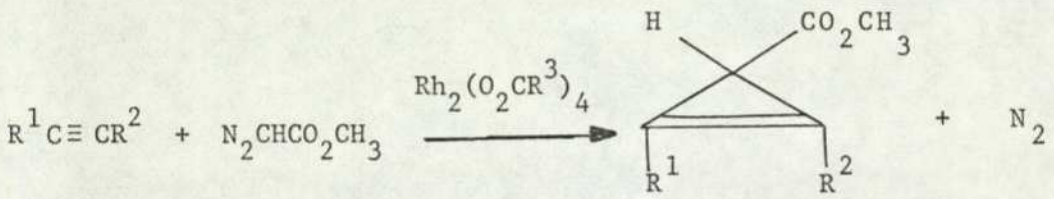


(13) (14) (15)

The efficiency of rhodium (II) derivatives depends strongly on their solubility; therefore soluble rhodium (II) carboxylates such as the butanoate and the pivalate effect high improvements in yields of cyclopropanation products. The yields of product (15) also depends strongly on the alkyl diazoacetate (14) used. The best yields are obtained with butyl diazoacetate in the presence of rhodium acetate.

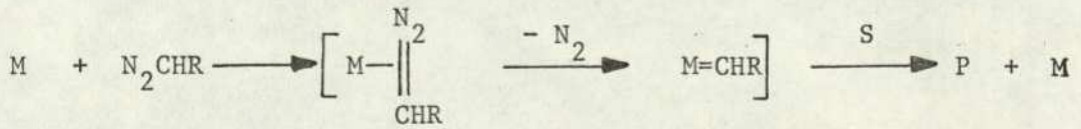
This fact may be explained by the increased solubility and stabilization of the intermediate carbenoid species. As in all coordination reactions, the influence of the electronegativity and geometry of the counter-anion are significant. Steric hindrance at the carboxylate group does not show any major effect on the yield. Moreover, the oxidation state of rhodium plays an important role (possible by controlling the overall geometry of the complex); rhodium I and rhodium III gave very low yields of cyclopropanation products.

Rhodium carboxylate can also be used to catalyse the cyclopropanation of acetylenes.[11]



(16)

Steric hindrance of substituents R on the triple bond of (16) does not significantly influence the overall yields of cyclopropenes whereas polar groups decrease it drastically. An increase in the electronegativity of the counter ions of the catalyst drastically lowers the yields. Since rhodium (II) carboxylates have only one vacant coordination site per metal, a simultaneous coordination of the olefin and diazoester (or carbene) seems unlikely. The reaction was believed to follow the pathway of an intermediate. (Scheme 7)



S=substrate, P=product

SCHEME 7

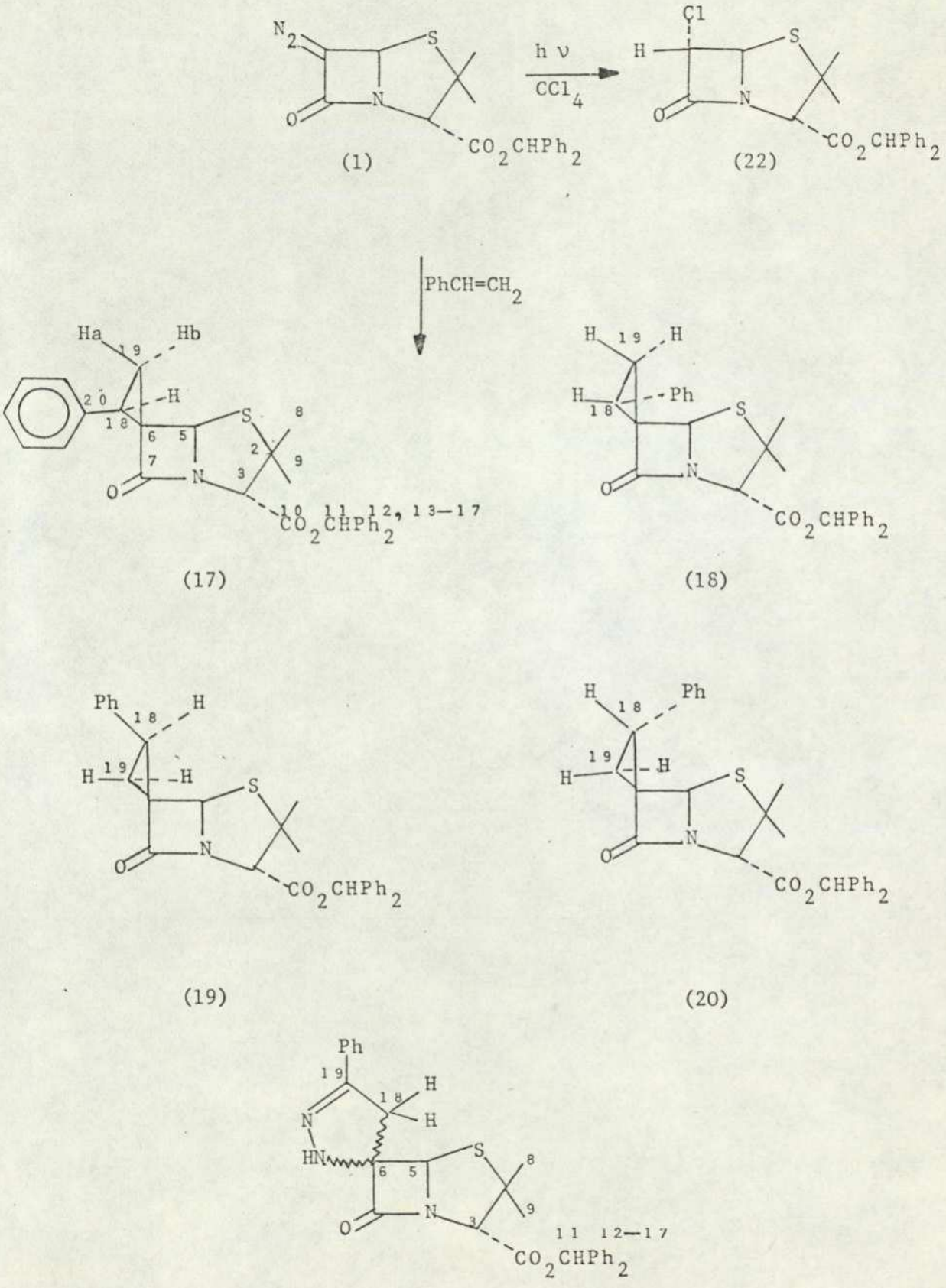
3.2. Reactions with Styrene and Cyclohexene: Results and Discussion

The investigation of the behaviour of benzhydryl 6-diazo-penicillanate (1) was initiated with its cycloaddition to styrene.

Thermolysis of benzhydryl 6-diazopenicillanate (1) in styrene was carried out by refluxing the reaction mixture in benzene overnight. After rapid conventional chromatography on silica, a mixture containing four possible isomeric cyclopropanes (17) to (20) was isolated in a very low yield (8% total) (Scheme 10). No further attempt was made to separate these four isomers. However, their structures were proved from the i.r. spectrum, showing β-lactam absorption at 1775 cm^{-1}, ester absorption at 1745 cm^{-1} and their ^1H n.m.r. spectrum, exhibiting a singlet for C_5-H at τ 4.48, 4.60, 4.70 and 4.92 respectively, together with an overlapping A.B. system for the cyclopropyl C_{18}-H at τ 7.00 to 7.30.

Copper (II) bis(acetylacetonate) decomposition of 6-diazo-penicillanate (1) in styrene in the presence of dichloromethane led to the formation of three products, which were separated by conventional chromatography on silica, and identified as the two isomers of 6-(1-phenylcyclopropano) penicillanates (17) and (18) in the ratio 1: 1.5 (overall yield 32%), together with a product which is considered to be a 3:1 isomeric mixture of spiropyrazolines (21) in a yield of 8% (Scheme 10). The isomeric purity of the isolated compounds (17) and (18) was confirmed by analytical HPLC, each showing mainly one component only.

The structure assignment for spirocyclopropanes (17) and (18) was based on their spectroscopic properties. Compound (18), with the



(1) (22) (17) (18) (19) (20) (21)

Scheme 10.

phenyl group below the plane of the β-lactam ring and pointing near C_5-H, was first recognised in these two isomers. Since in its ^1H n.m.r. spectrum the chemical shift of C_5-H appeared at higher field (τ 4.96) (Table 1) due to the influence of the shielding effect of the ring current of the phenyl group, The structure (19) was believed to be unlikely. If it formed, in the ^1H n.m.r. spectrum of (18) the chemical shift of one of its C_{19}-H would be expected to move to higher field than the (19) does due to shielding effect of thiazoline sulphur upon one of C_{19}-H of the compound (18), which was not observed in the present experiment (Table 1).

		COMPOUND	
		(17)	(18)
^1H n.m.r τ	C_5-H(s)	4.55	4.96
	C_{18}-H(d.d.)J=7.5,9.8Hz	7.24	7.07
	C_{19}-H(m)	8.20	8.20
^{13}C n.m.r δ	C_{20}	137.109	136.846

Table 1 Part of ^1H and ^{13}C n.m.r. data of compounds (17) and (18). In $CDCl_3$.T.M.S. is internal standard.

Similarly, a compound with structure (20) was also considered unlikely, since providing (20) is produced, in its ^1H n.m.r. the chemical shift of its C_{18}-H would only vary slightly from compound (18) due to the analogous magnetic environment of C_{18}-H in compounds (18) and (20). This did not match the recorded data. (Table 1)

The remaining candidate is the compound with the structure (17). In its ^1H n.m.r., the C_5-H should resonate to lower field due

to the absence of shielding effect of phenyl group and the C_{18}-H should resonate to higher field due to the absence of the deshielding effect of carbonyl group of the β-lactam, which is identical with the observations. Further evidence was obtained from the ^{13}C n.m.r. data of both isomers (Table 1), where the spirocyclopropyl phenyl carbon (C_{20}) of compound (17) was found to resonate to lower field than (18) due to the deshielding influence of carbonyl function of β-lactam. This structural assignment is in agreement with the earlier work of Sheehan proposing that the preferred mode of addition is from the sterically less hindered α side in the reaction of 6-diazo-β-lactams with dipolarophiles.[12]

Completely unambiguous assignment of the structure of (17) was achieved by employing the latest nuclear Overhauser enhancement - difference spectroscopy technique on a 400 MHz instrument, as developed by Hall and Sanders.[13]

Although the traditional n.O.e. experiment is very powerful, it is limited in scope both because the signal to be observed must be resolved and because the minimum observable effect using integration is ca. 5%. In n.O.e. difference spectroscopy, a control spectrum without n.O.e. is substracted from the spectrum with n.O.e. so that only spectral changes should appear. The signal of interest need no longer be resolved in the complete spectrum and the lower limit of observable n.O.e. is determined only by instrument stability. The whole experiment is controlled by computer. It was claimed that n.O.e.s are observable in the range of 0.5-5% with the new technique.

Inspection of molecular models shows that the distance between C_5-H and C_{18}-H in (17) is shorter than in the other three isomers. Therefore, one can only expect an appreciable n.O.e. in the isomer (17). Irradiation of the C_5-H of (17) at τ 4.35 gave an n.O.e. on C_{18}-H only. Furthermore, irradiation of C_{18}-H of (17) at τ 7.35 gave the expected n.O.e.s on C_5-H and C_{19}-H in which the C_{19}-H(a) exhibited a negative n.O.e. These results are shown in Figures 1 and 2.

Use of this technique afforded unequivocal assignments of all the proton chemical shifts of (17). Table 2.

Proton irradiated	Observed n.O.e
3	5, 8
5	18
8	3, 9
9	3, 5, 8, 11
11	12
12	-
18	5, 19b, 20
19a	18, 19b, 20
19b	18, 19a

Table 2 Proton-Proton nuclear Overhauser enhancement in (17).

Figure 1. Normal ^{1}H n.m.r. 400 MHz spectrum of (17) in $CDCl_3$.

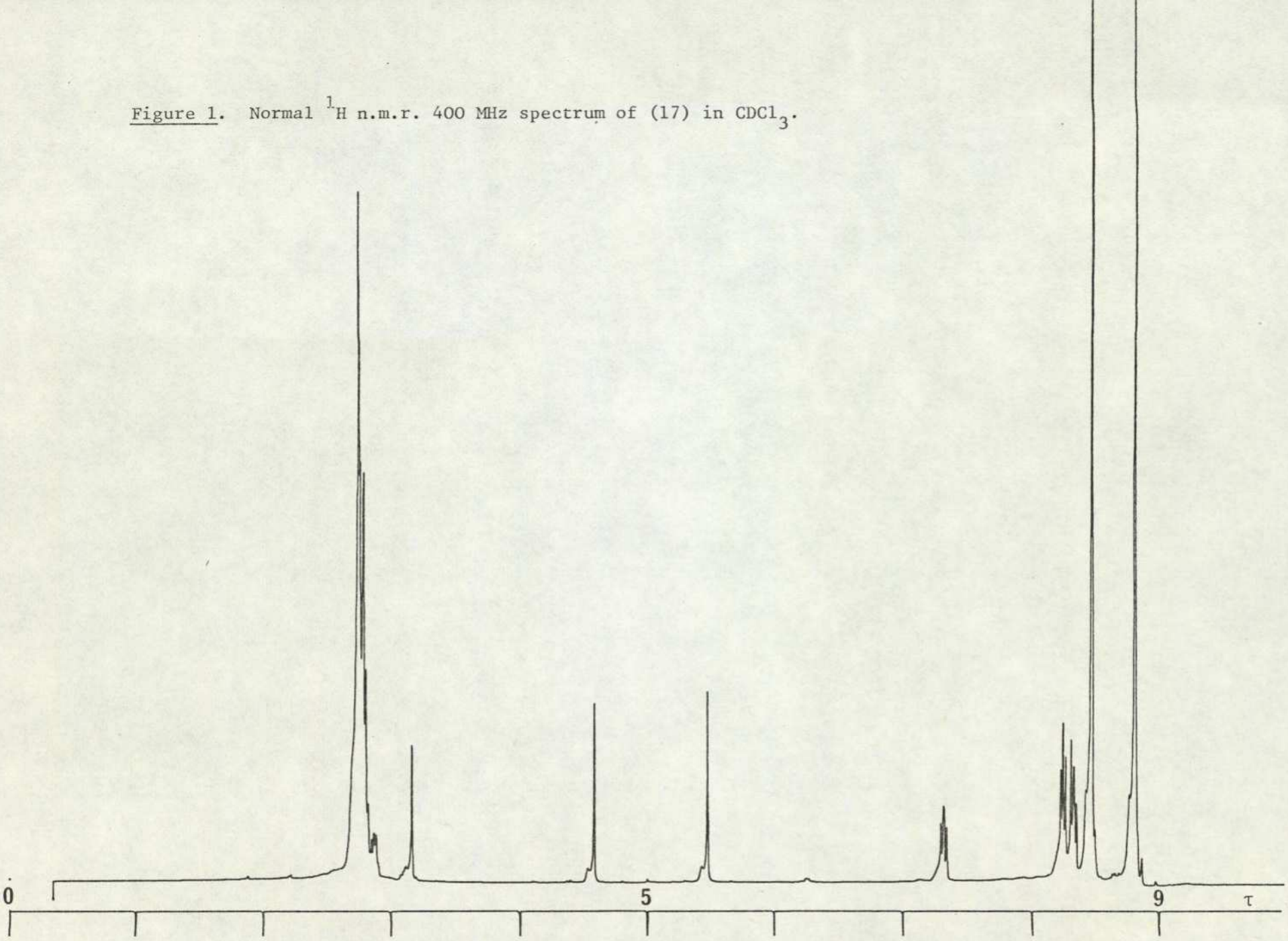
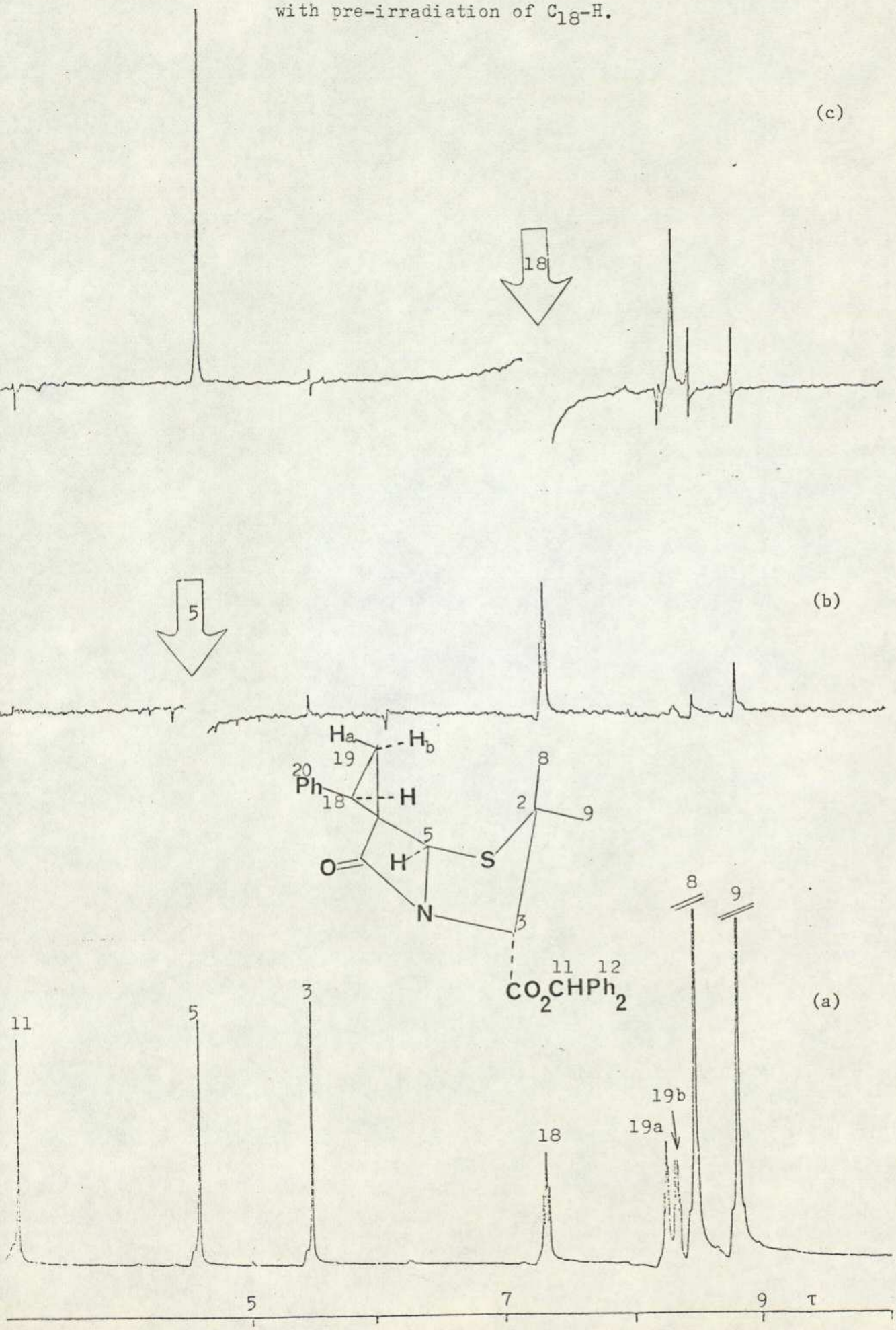


Figure 2. Partial 400 MHz spectra of (17) in $CDCl_3$. (a) Normal spectrum, (b) n.O.e. difference spectrum with pre-irradiation of C_5-H, (c) n.O.e. difference spectrum with pre-irradiation of C_{18}-H.



The possible isomeric mixture of spiropyrazolines (21) was found rather unstable; it decomposed either on the process of chromatography or upon storage, probably to give spirocyclopropanes (17) and (18) with some unidentified components (judging by analytical t.l.c.). Owing to this instability, it was difficult to fully characterize this possible isomeric mixture of spiropyrazolines. However, the assignment gained support from the spectroscopic data. The i.r. spectrum showed absorption bands at 3400 cm^{-1} (NH), 1775 cm^{-1} (β-lactam), 1745 cm^{-1} (ester) and the ^1H n.m.r. spectrum exhibited a typical A.B. system (J = 18 Hz) of gem-methylene protons of a pyrazoline at τ 6.22 and 6.56 respectively and two singlets in the ratio of 3:1 at τ 4.62, 4.71 corresponding to C_5-H of two isomers.

The catalysis of decomposition of 6-diazopenicillanate (1) in styrene was also conducted in the presence of $Cu(acac)_2$ by refluxing the reactants in benzene for 3 hours, yielding the spirocyclopropanes (17) and (18) in much lower yield (13% total), but in the identical proportion of 1:15. However, no pyrazoline was obtained from this reaction mixture.

The decomposition of 6-diazopenicillanate (1) in styrene was discovered to occur faster and furnish higher yields with a catalyst of rhodium acetate, which gave spirocyclopropanes (17) and (18) in the ratio of 1:1.2 (total yield 53%) together with a possible isomeric mixture of spiropyrazolines (21) in a yield of 14%.

Photolysis of 6-diazopenicillanate (1) with styrene in dried carbon tetrachloride was carried out by irradiating the reaction

mixture in a quartz vessel with an Hanovia medium pressure lamp under an atmosphere of dried nitrogen. After rapid chromatography, neither spirocyclopropanes nor spiropyrazolines were obtained from this reaction. Strikingly, the only isolated β-lactam was 6α-chloropenicillanate (22) in a yield of 15%. Its i.r. spectrum showed absorption bands at 1790 cm^{-1} (β-lactam), 1740 cm^{-1} (ester) and its ^1H n.m.r. spectrum exhibited two doublets (J = 1.5 Hz) at τ 4.68, 5.28 for the trans C_5-H and C_6-H respectively. It is believed that the product is formed by chlorine abstraction from the carbon tetrachloride during photolysis; the hydrogen presumably came from moisture present in the reaction.

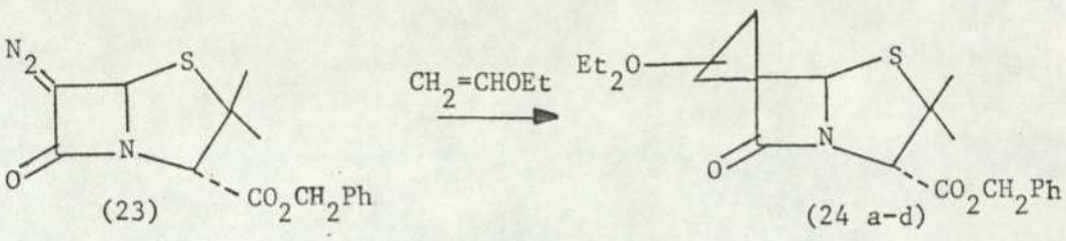
Deprotection of the spirocyclopropanes (17) and (18) was achieved by palladium black atmospheric hydrogenolysis, resulting in corresponding carboxylic acids in the yield of 75% and 43% respectively. Both of these were found to be devoid of antibacterial activity against a standard series of laboratory strains.

The results presented here clearly indicate that in the reaction with olefins the 6-diazo-β-lactam is less reactive than analogous materials, e.g. ethyl diazoacetate, and also that the reaction produces a relatively lower yield. The fact that only two isomers of the four possible ones were obtained from the metal catalysis of 6-diazopenicillanate addition to styrene also showed the stereoselectivity of this reaction.

Gilchrist and Rees[14] mentioned in their book that in the copper and other transition-metal catalysis of reactions of diazo

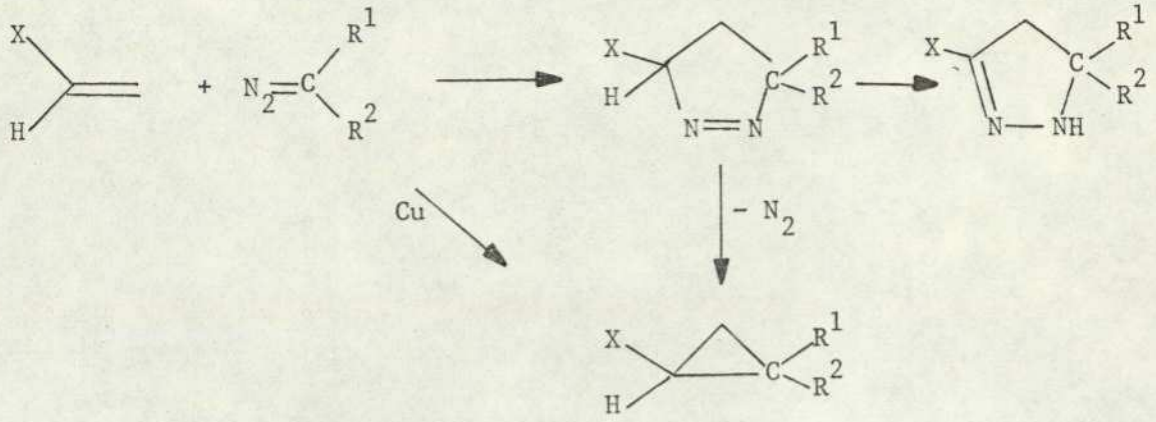
compounds, the intermediates are more selective than are free carbenes and the cycloaddition to olefins is stereospecific.

It is interesting to compare our results with those of Campbell et al.[15] Benzyl 6-diazopenicillanate (23) was reported to react with ethyl vinyl ether (29) in the presence of copper(bis-acetyl acetonate) to give in 73% total yield a mixture of four isomers of the ethoxysubstituted spirocyclopropane (24 a-d).



The difference in behaviour observed in the two cases may result from steric effects (the bulkier styrene approaching only from the α-face), or from electronic effects (the vinyl ether being more reactive), or from a combination of such factors.

The formation of pyrazolines (21) in the diazopenicillanate reaction with styrene is explained by an initial 1,3-dipolar cycloaddition followed by a prototropic rearrangement. Pyrazolines were also observed in the additions of diazopenicillanate esters to acrylic acid derivatives, reported by Campbell[15] and by Sheehan.[12] It is not clear whether $^{1}\Delta$-pyrazolines are necessary intermediates in the cyclopropanations of styrene and of acrylates, or whether there are two competing pathways (Scheme 11).

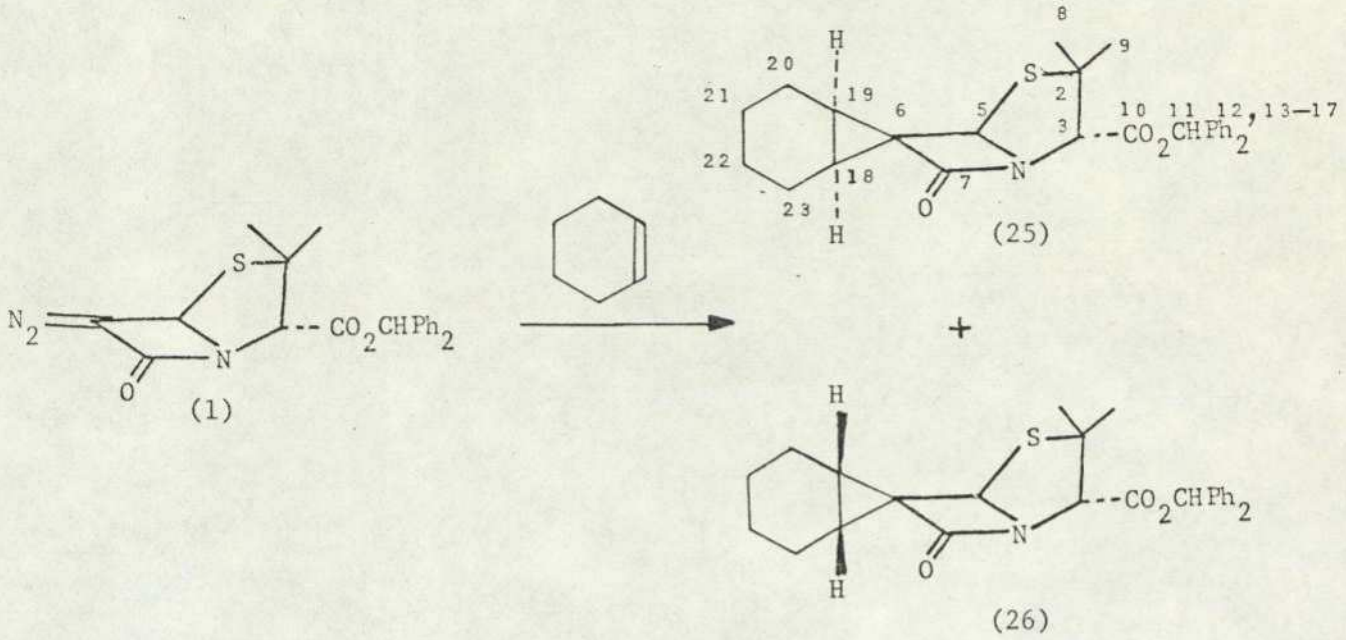


Scheme 11.

Another important characteristic of a carbene or carbenoid is its ability to insert into a C-H bond. As seen in the introduction to this Chapter, diazoacetate reacts with cyclohexene to give a cycloaddition product together with an insertion product .

Addition of benzhydryl 6-diazopenicillanate (1) to cyclohexene in dichloromethane in the presence of copper (II) bis (acetylacetonate), resulted in the rapid evolution of nitrogen and led to the formation of two major products in a total yield of 60%. After rapid conventional chromatography on silica, they were separated individually as oils and identified as 6-(bicyclo-[4,1,0]heptane) penicillanates (25) and (26) in the ratio 1.1 : 1. The i.r. spectra of samples (25) and (26) were slightly different, exhibiting β-lactam absorptions at 1760 and 1770 cm^{-1} respectively.

The ^{1}H n.m.r. data of both isomers showed an envelope of signals of τ 8∿9, corresponding to bicyclo [4,1,0]heptanyl protons. The ^{13}C n.m.r. spectra were more informative, showing signals corresponding to carbons of bicyclo [4,1,0] heptane in various positions.



The configuration of isomers (25) and (26) were tentatively deduced from their spectroscopic properties. In their ^{1}H n.m.r., the C_5-H singlet was found at lower field in (25) than in (26) (Table 3), which is presumably caused by the effect of the cyclohexyl ring facing toward the C_5-H in compound (26). A further indication of validity of this assumption may be found in ^{13}C n.m.r. data where the bicyclo [4,1,0] heptane carbons (C_{18}) and (C_{19}) appeared at lower field in sample (26) than in (25) (Table 3) presumably due to the deshielding effect of carbonyl function of β-lactam upon C_{18} and C_{19} was weakened by the cyclohexyl ring facing toward the carbonyl group in (25).

Chemical Shift		COMPOUND	
		(26)	(25)
^{1}H n.m.r. τ	C_5-H	4.90	4.72
^{13}C n.m.r. δ	C_{18} C_{19}	25.976 24.780	21.354 20.507

Table 3. Part of ^{1}H and ^{13}C n.m.r. data of compounds (25) and (26). In $CDCl_3$, with T.M.S. as internal standard.

The influence of the concentration of the catalyst $Cu(acac)_2$, upon the products of decomposition of 6-diazopenicillanate (1) in cyclohexene was briefly investigated. The results, shown in Table 4, indicated that the outcome of this reaction is relatively insensitive to a wide range of catalyst concentrations.

Mole Ratio of Catalyst to 6-Diazopenicillanate	Product and Yield* (25) (26)
0.02	31% 28%
0.2	31% 24%

Table 4. Effect of amount of $Cu(acac)_2$ upon the product yield on reaction of 6-diazopenicillanate (1) in cyclohexene.

* Product was actually isolated and characterized.

It is noteworthy that the quality of the $Cu(acac)_2$ catalyst profoundly affects the outcome of this reaction. Utilizing a poor quality of catalyst substantially reduced the yield of this reaction. Synthesis of the $Cu(acac)_2$ is a simple process involving addition of the 2,4-pentanedione to aqueous copper (II) chloride. Problems appeared to occur in the subsequent purification procedure if recrystallization was carried out with halogen-containing solvent.

Peace and Wulfman[16] have observed that in preparation of copper catalysts, if halogen-containing solvents are employed in recrystallization, either singly or with co-solvents, appreciable destruction of the catalyst can occur. This involves reaction

between the ligand and the halogenated solvent and leads to precipitation of some copper (I) salt. Catalysts resulting from this type of treatment contain varying amount of uncomplexed salt and are less effective than pure material.

The m.p. of the catalyst $Cu(acac)_2$, obtained from different sources and synthesized by various persons frequently showed appreciable deviation from the literature value, reflecting the validity of this explanation.

The catalysis of 6-diazopenicillanate (1) decomposition in cyclohexene was also carried out with rhodium acetate instead of $Cu(acac)_2$, which surprisingly gave a lower yield (total 9% only) of both isomers (25) and (26) in the ratio of 1.4 : 1. No C-H bond insertion product was detected in either case.

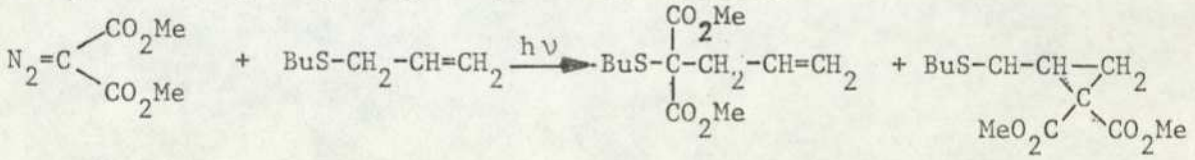
Removal of the benzhydryl group from samples (25) and (26) by palladium black hydrogenolysis at room temperature provided the corresponding carboxylic acids as white solids in yields of 75% and 50% respectively. Both of these were found to be biologically inactive.

The absence of any C-H bond insertion product in the metal catalyzed reaction of 6-diazopenicillanate (1) with cyclohexene should not be regarded as surprising. It is well documented that, for the reaction of diazoacetate and cyclohexene, photolysis favours C-H bond insertion products whereas cycloaddition predominates in the metal catalysis.

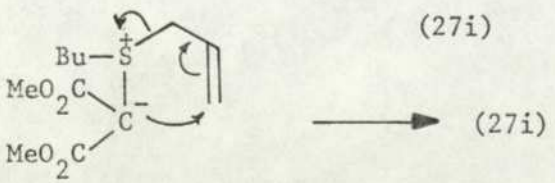
The fact that two isomers were formed in the same proportion reflects the indiscriminate addition across the carbon-carbon double bond of cyclohexene by 6-diazo-β-lactam.

3.3. Review of reactions between diazo compounds and sulphur compounds

The reactions between diazo compounds and alkenyl sulphides have been extensively investigated by Ando. The photolysis of diazomalonic ester in n-butyl allyl sulphide afforded the principal product (27-i) in 57% yield and the minor product (27-a) in 11% yield.[17] The carbene formed by irradiation attacks the sulphur atom more rapidly than the carbon-carbon double bond, leading to an ylide intermediate (28), from which the "insertion" product (27-i) is generated by a 2,3-sigmatropic rearrangement.



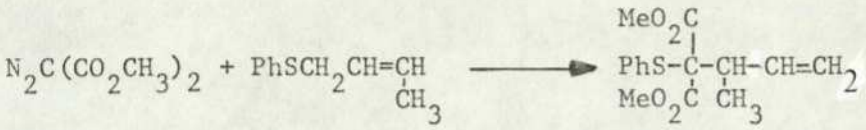
(27i) (27a)



(28)

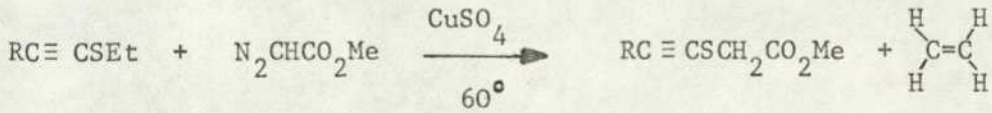
The copper sulphate catalysed decomposition of diazomalonate in allyl sulphides also yields insertion products in high yields, and no addition product is observed.[17] Thus, the reaction with but-2-enylphenyl sulphide (29) gave the rearranged insertion product (30-i) in 92% yield and no trace of γ-methylallyl thiomalonate could be detected in the reaction mixture.

Since the reaction gave the insertion product exclusively and no addition product, it was thought that the carbenoid species derived in this reaction has more electrophilic nature than that generated in photolysis. The possible structure of such a carbenoid might be represented by $X\text{-}C\bar{u}\text{-}C^{+}(CO_2CH_3)_2$.

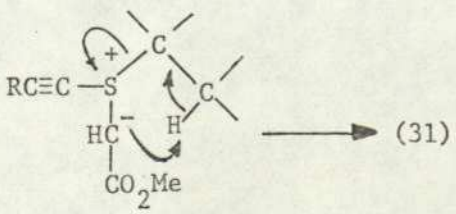


(29) (30i)

Ando, Higuchi and Migita also reported the copper-catalysed thermal reaction of acetylenic sulphides with methyl diazoacetate.[18] The formation of compound (31) may involve attack of methoxycarbonyl carbene on sulphur to form the acetylenic sulphonium ylide followed by a cyclic elimination of ethylene. (Scheme 13). The relative reactivity of the sulphur atom in the acetylenic sulphide compared with various other sulphides was investigated. The result showed that the acetylenic sulphide is the most reactive, probably owing to the contribution from the resonance structure $R\text{-}C^{+}{=}C{=}\bar{S}\text{-}Et$, which is possible because sulphur can expand its valence shell.

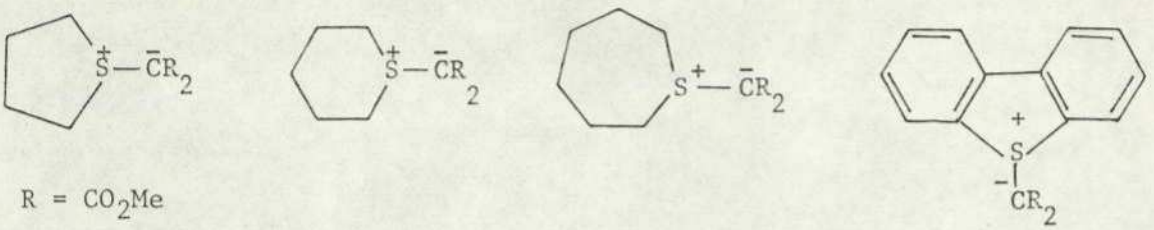


R=Ph,Me,or SEt (31)



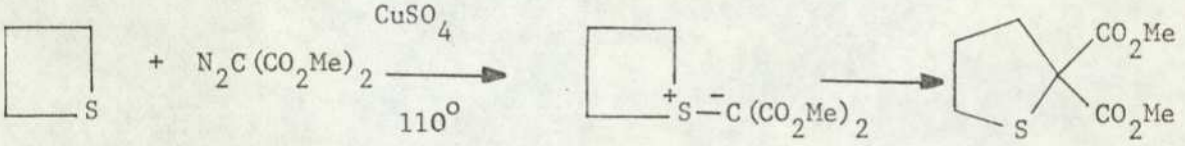
Scheme 13.

In the reaction of dimethyl diazomalonate with cyclic sulphides, the corresponding sulphonium ylides were obtained, although yields varied depending on ring size.[19] (Scheme 14).



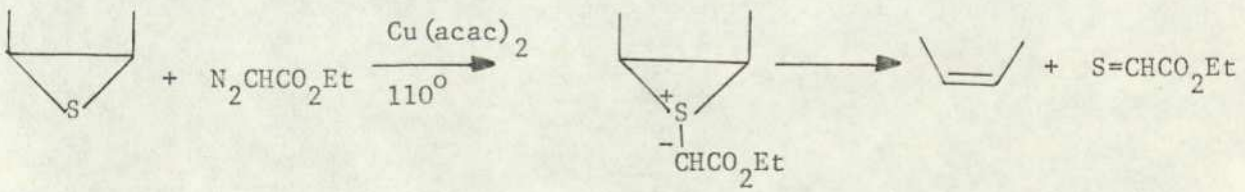
Scheme 14.

With thietane (32), dimethyl diazomalonate gave a ring expansion product (33), resulting from a facile rearrangement of the intermediate sulphur ylide.



(32) (33) 26%

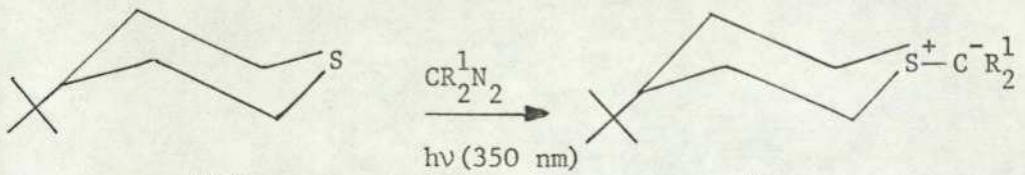
With the three-membered ring episulphide (34), ethyl diazoacetate yielded exclusive the cis-olefin (36). The episulfide probably formed ylide (35) as an initial intermediate and then decomposed stereospecifically by fragmentation into olefin (36) and thioglyoxalic ester (37).[22]



(34) (35) (36) (37)

Appleton, Bull, J.McKenna, J.M.McKenne and Walley [20] also gave some complementary results on the formation of sulphonium ylides.

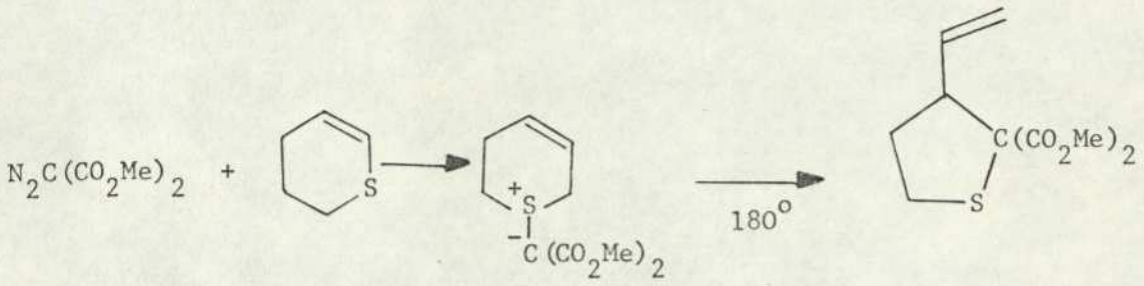
Reaction of 4-t-butylthiacyclohexane (38) with the carbenes CR^1_2 (R^1 = COMe, CO_2Me, or CO_2Et), photochemically generated from the corresponding diazo-compounds, gave in each case a single sulfonium ylide formulated as the product (39) of equatorial addition of the carbene.



(38) (39)

An analogous result (one product, (39); R^1 = CO_2Me) was obtained from the Cu(II) catalysed decomposition of diacetyl-diazomethane in 4-t-butylthiacyclohexane.[21]

A study of the reaction of carbenes with cyclic allyl sulphides was also carried out by Ando[22,23] and gave particularly interesting results. Photolysis of dimethyl diazomalonate in Δ^3-dihydrothiopyran (40) gave a white solid which was characterized as sulfonium ylide (41). It was claimed that the ylide (41) was stable enough to be isolated and rearranged only at high temperature to give (42) while the ylide derived from the open chain allylic sulphides could not be isolated, perhaps being easily converted into rearranged product at room temperature.

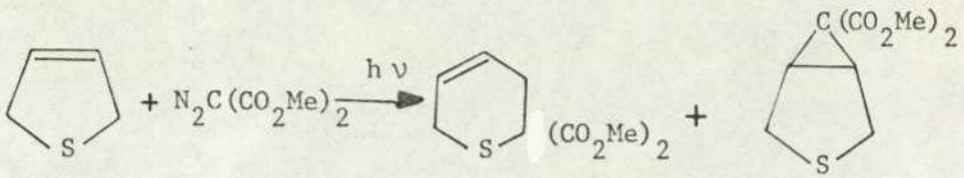


(40) (41) (42)

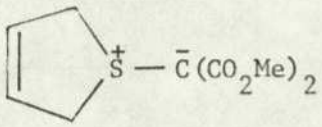
hν 30%

(110°) $CuSO_4$ 68%

In contrast to the six membered cyclic sulfide, 2,5-dihydrothiophene was found to give rise to 2,2-bis(methoxycarbonyl)thiopyran (43) and an adduct (44), but the corresponding sulfonium ylide (45) could not be isolated. The product (43) was suggested to be derived from the intermediate sulfonium ylide (45), which may be unstable and therefore undergo Stevens type rearrangement, because it would inevitably have an eclipsed conformation, and the interaction between the $-C(CO_2CH_3)_2$ group and neighbouring methylene carbon atom will promote C-S bond fission by either concerted or radical-pair processes. Only ring-expansion product (43) was isolated (51% yield) from the $CuSO_4$-catalyzed reaction of dimethyl diazomalonate in 2.5-dihydrothiophene.

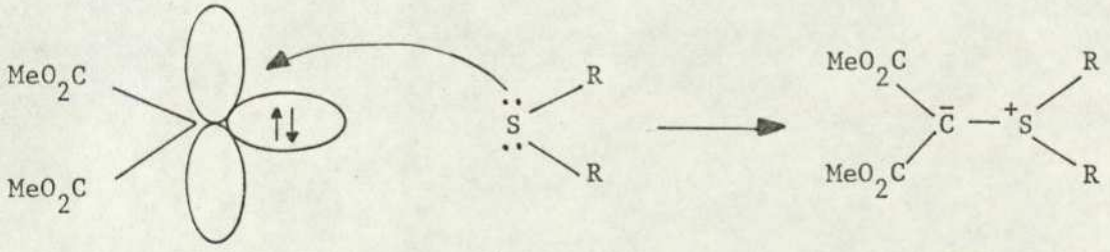


(43) (44)



(45)

All the reactions discussed above are assumed to occur by conversion of the diazo compound into an electrophilic singlet carbene which attacks the non-bonding electron pair of a sulphur atom giving a sulphur ylide. (Scheme 15). Thermolysis of diazo compound in the presence of a copper salt probably involves a carbene-copper complex which also exhibits electrophilic character.

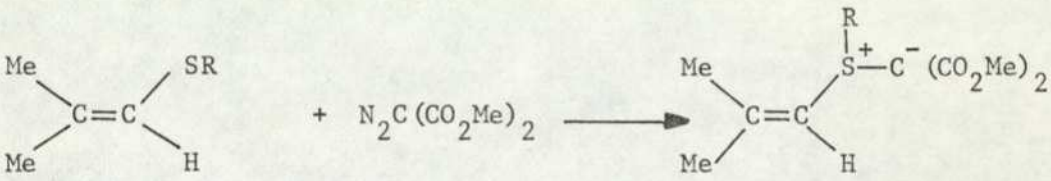


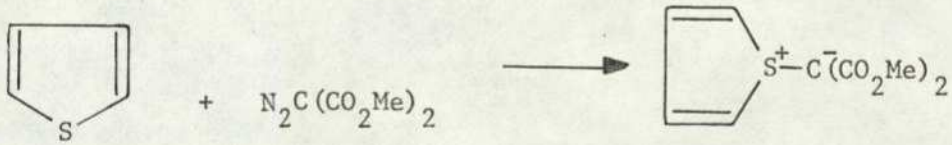
Scheme 15.

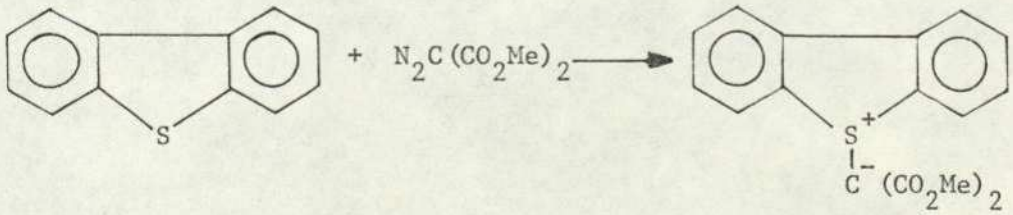
It has been clearly demonstrated that a carbene attacks a sulphur atom more rapidly than a carbon-carbon double bond.

Repetition of reaction of dimethyl diazomalonate and dimethy sulfide in the presence of cyclohexene indicated that the sulphide was about six times as reactive as the olefin towards biscarbomethoxy-carbene. Similar high reactivity of sulphide towards carbenes was also observed in other competitive experiments carried out by Ando.[22]

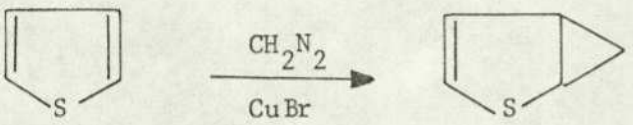
It was shown that vinyl sulfides, thiophene and dibenzothiophene in which the lone pair of sulphur is highly delocalized, are efficient traps for carbenes and form the corresponding stable sulphonium ylides on reaction with dimethyl diazomalonate under either thermal or photochemical conditions.





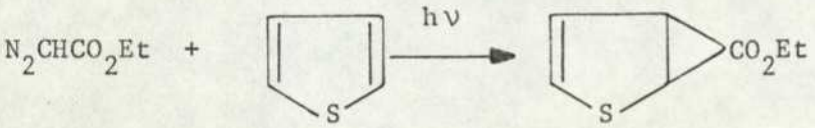


The reactions of diazo compounds with thiophene to give cyclopropanated products are well documented processes, e.g. Muller, Kessler, Fricke and Suhr[24] reported the catalysis of diazomethane addition to thiophene, which gave 2-thiabicyclo [3, 1, 0] hex-3-ene (46) in 22% yield.



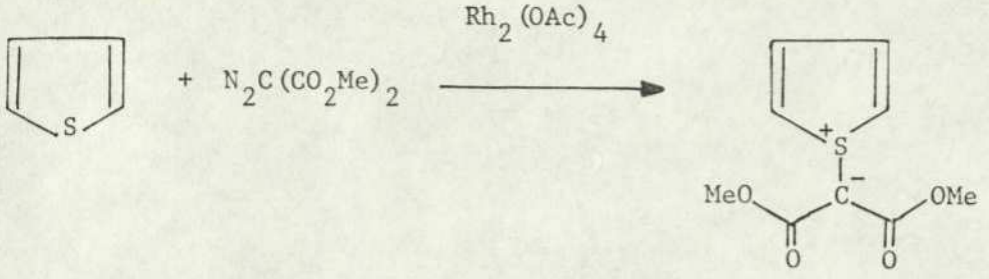
(46)

Irradiation of ethyl diazoacetate in thiophene leads to 2-thiobicyclo [3,1,0] hex-3-ene-6-carboxylic acid ethyl ester (47), which was characterized by transformation into its amide.[25]



(47)

Since the recent introduction of rhodium acetate as a catalyst, there has been growing interest in its application to the decomposition of diazo compounds in different reactants. Gillespie, J.M. Rust, P.M. Rust and Porter[26] revealed the utilization of rhodium acetate on addition of dimethyl malonate to thiophene and substituted thiophenes. Reaction of dimethyl diazomalonate and thiophene in the presence of $Rh_2(OAc)_4$ at room temperature produces an ylide (48) in quantitative yield.

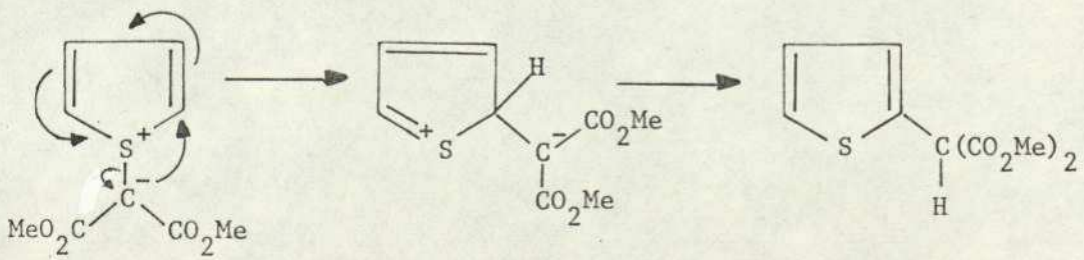


(48)

The same reaction was found to be impractically slow in the presence of a copper catalyst. When thiophene carries substituents with a +I or + M effect, ylides are formed in high yields (e.g. 2,5-Cl_2, 2-$HOCH_2$, 2-Br, 3-Me, 2-Me, 2-Br). When substituents with a -M effect are present then no reaction occurs, as typified by the failure of 2-acetyl, 2-cyano-, and 2-formyl-thiophene to undergo reaction, presumably due to the reduced availability of the lone pair of electrons on the ring sulfur atom as a consequence of mesomeric interaction with the substituent.

Other systems which are capable of stabilizing ylide structures do not form thiophenium ylides. Ethyl diazoacetoacetate, ethyl diazoacetate, and diazoacetophenone under the same condition give rise to the corresponding cyclopropanes.

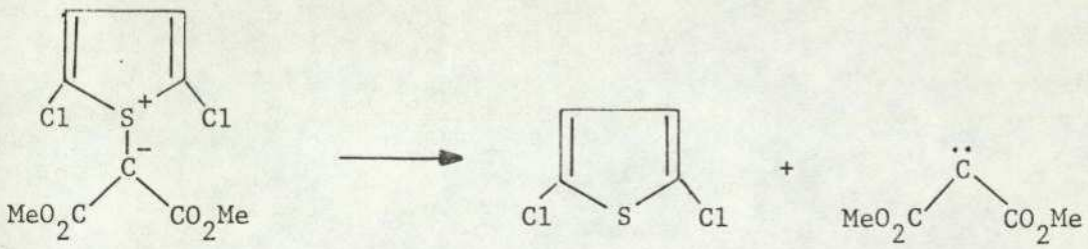
Thermolysis of thiophenium bis(methoxycarbonyl)methylide (49) results in a ready rearrangement to dimethylthiophene-2-malonate (50).[27]



(49)

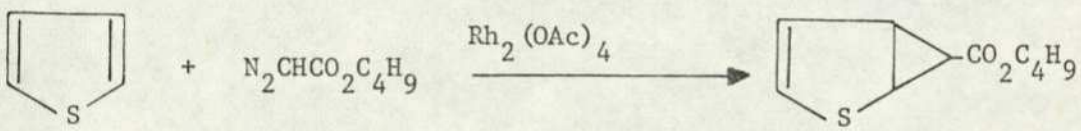
(50)

Pyrolysis of 2,5-dichlorothiophenium bismethoxycarbonyl methylide results in an alternative pathway, in which dissociation occurs into dichlorothiophene and carbene (51), which was trapped with a number of olefins. 2,5-Dichlorothiophenium bismethoxycarbonyl-methylide has been recommended as a convenient source of bis-methoxycarboxylcarbene, which can react with a number of olefins, pyrrole and activated aromatic substrates to yield the corresponding cyclopropanes and aryl malonates in good to excellent yields.[28]



(51)

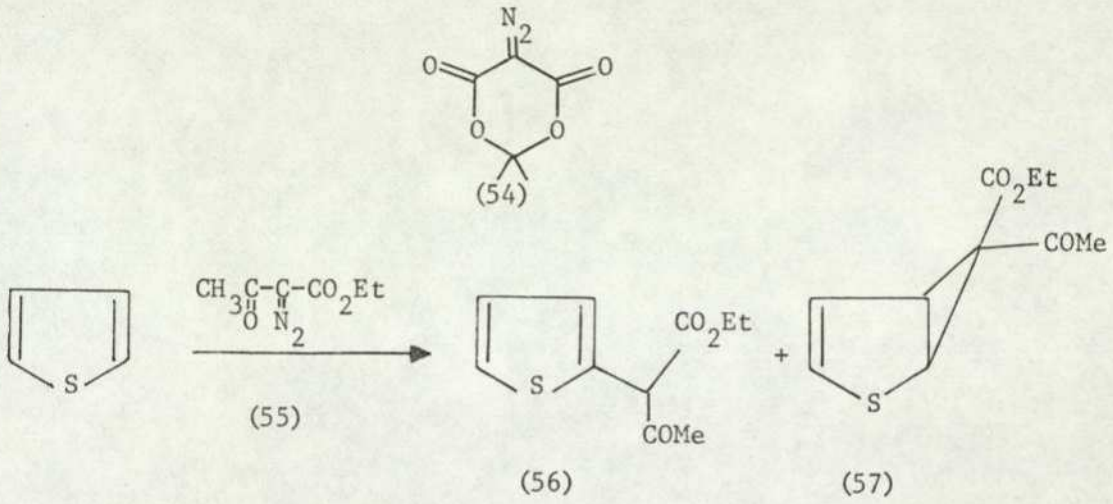
In the synthesis of 2-thiobicyclo[3, 1 ,0]hex-3-enecarboxylates (53) from the reaction of thiophene and n-butyl diazoacetate (52), a rhodium (II) catalyst was shown to be far superior to a copper catalyst. In the case of the copper (I) chloride, only 17.2% yield was obtained, whereas the rhodium II catalyst give 71% yield.[29]



(52) (53)

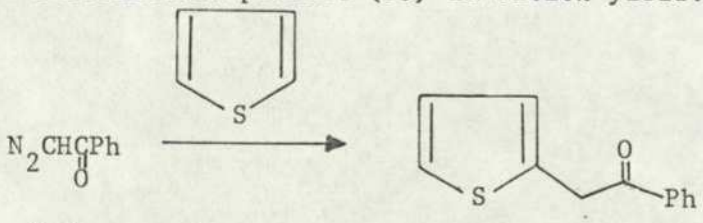
Both dimethyl diazomalonate and diethyl diazomalonate give stable ylides under the same conditions, but the rhodium II-

catalysed reaction of the Meldrum's acid diazo derivative (54) with thiophene proved to be an exception. Addition of the diazoacetoacetate (55) to a refluxing solution of the rhodium catalyst in thiophene results in the 2-substitution product (56) (67%) together with a product claimed to be a mixture of exo and endo isomeric cyclopropanes (57) (13%).



(56) (57)

By contrast, the reaction with diazoacetophenone (58) only affords the 2-substitution product (59) in ca.20% yield.



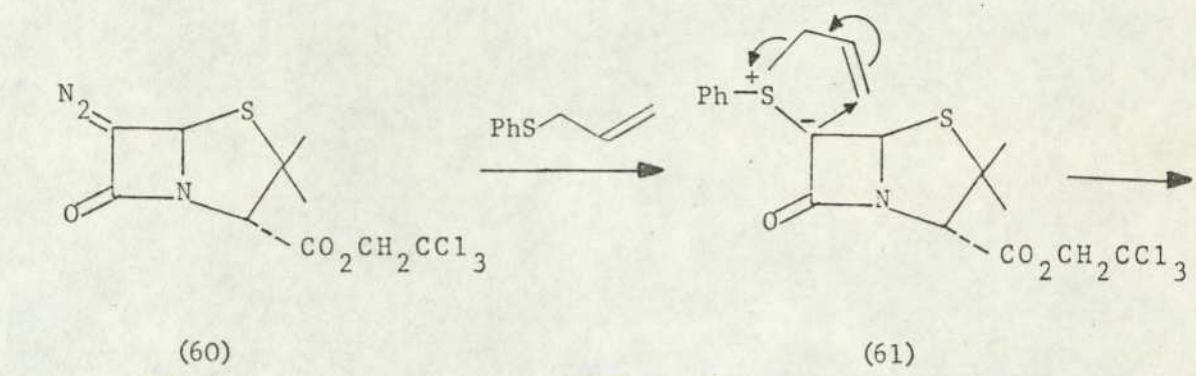
(58) (59)

It was proposed that these products can be rationalized in terms of a single mechanistic scheme involving initial ylide formation in all cases.

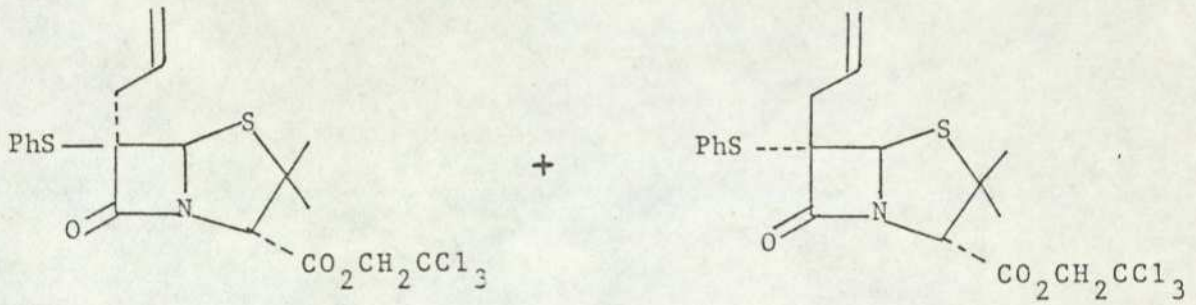
Studies of the reactions of 6-diazopenicillanates with allylic sulphides have recently been reported by Giddings John and

Thomas.[30] They observed that reaction between phenylallyl sulfides and 2,2,2-trichloroethyl 6-diazopenicillanate (60) in the presence of catalyst of $Cu(acac)_2$, afforded 6-α-phenylthiopenicillanates (62) and (63). (Scheme 16) (for more details see Chapter 1). The formation of this insertion product was suggested to involve a [2, 3] sigmatropic rearrangement of an intermediate ylide (61). (Scheme 16)

Evidence was given to favour this mechanism. Treatment of 6-diazopenicillanate (60) with unsymmetric 3-methylbut-2-en-1-yl phenyl sulfide (64) in the presence of $Cu(acac)_2$ catalyst, led to the formation of one major product, 6,6-disubstituted penicillanate (66) in 70% yield. By contrast, the analogous reaction with 3-methylbut-1-en-3-yl phenyl sulfides (65) gave two isomers of penicillanates (67) and (68). No cross over of products was detected suggesting that the [2,3]-sigmatropic rearrangement pathway is the exclusive mode of product formation. (Scheme 17).

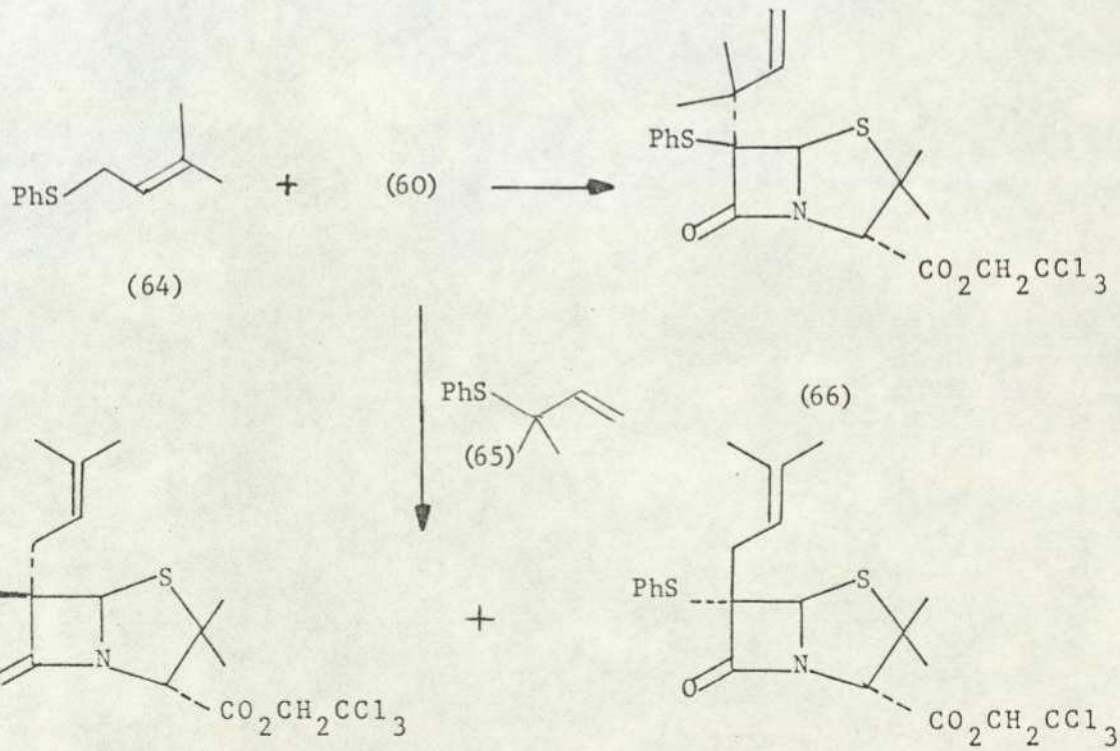


(60) (61)



(62) (63)

Scheme 16.



(64) (66)

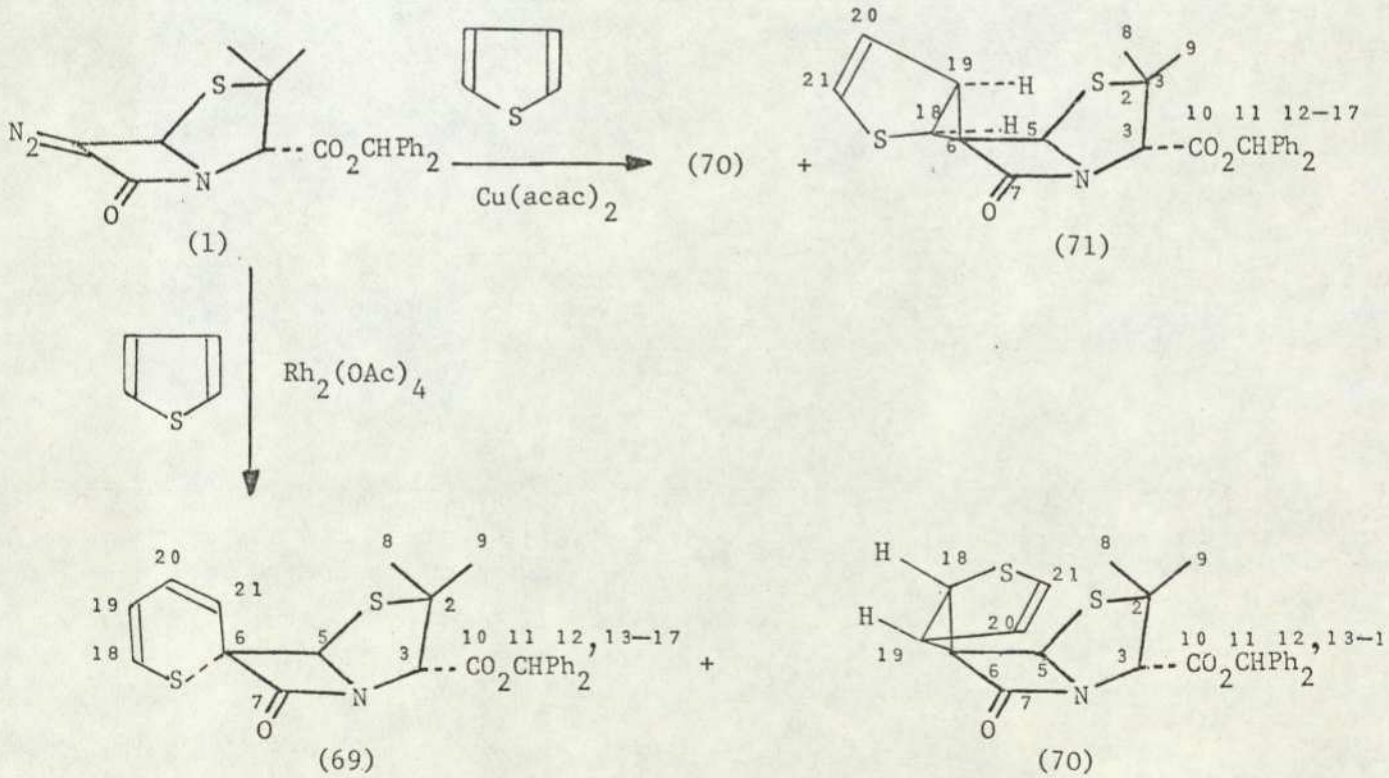
(65)

(67) (68)

Scheme 17.

3.4 Reaction with Thiophene: Results and Discussion

Porter's results stimulated us to investigate the behaviour of 6-diazopenicillanates with thiophene. Addition of benzhydryl 6-diazopenicillanate (1) to neat thiophene in the presence of rhodium acetate resulted in the rapid evolution of nitrogen, and led to the formation of two products, which were separated by conventional chromatography on silica. The more polar component was found to be the cycloaddition product (70) in a yield of 22% and the less polar component was proved to be a very interesting ring expansion adduct, the 2H-thiopyran (69) in a yield of 11%. (Scheme 18).



Scheme 18.

When the catalysis was conducted with $Cu(acac)_2$ instead of $Rh_2(OAc)_4$, the reaction proceeded more slowly and yielded two major products in 30% yield. After conventional chromatography on silica they were isolated as a mixture which was identified to be two isomeric 2-thiobicyclo [3, 1, 0] hex-3-enes, (70) and (71), in the ratio of 1 : 1. No ring expansion product (69) was detected.

Rhodium trifluoroacetate has been recently described to be a highly efficient catalyst in the synthesis of ring expansion products with diazo compounds and aromatic substrates.[31] However, when it was applied to the catalysis of 6-diazopenicillanate (1) decomposition in thiophene, the reaction was found to go less efficiently. It gave the cyclopropanation product (70) in 9% yield, together with ring expansion adduct (69) in 8% yield. Another rhodium compound, rhodium ethylenediamine dichloride, was found to be totally inactive in this reaction.

The decomposition of 6-diazopenicillanate (1) in thiophene was also carried out thermally in the absence of any catalyst, which did not produce any of the above mentioned products. The characteristic diazo i.r. band at 2084 cm^{-1} was still present after the reaction mixture had been stirred at room temperature for 18 hours. When the reaction mixture was heated under reflux at 80°C for one hour, only a trace amount of unreacted 6-diazopenicillanate (1) could be recovered but none of the above-mentioned products could be isolated.

The identity of the cycloaddition product (70) was deduced from its spectroscopic data. Analysis of its ^{1}H n.m.r. spectrum showed that two low field multiplets centered at τ 3.76 and 4.71 correspond to two A.B. quartets. The large (1.5 Hz) four bond coupling between C_{18}-H and C_{21}-H almost certainly arises from the W (molecular model) arrangement of these two protons. The appearance of a similar pattern of quartets of τ 6.24 and 6.52 corresponding to C_{18}-H and C_{19}-H is consistent with the cycloaddition product structure (70). Examination of its ^{13}C n.m.r. spectrum revealed signals at δ 128.333, 120.644, 39.973 and 35.286 corresponding to C_{21}, C_{20}, C_{18} and C_{19} respectively.

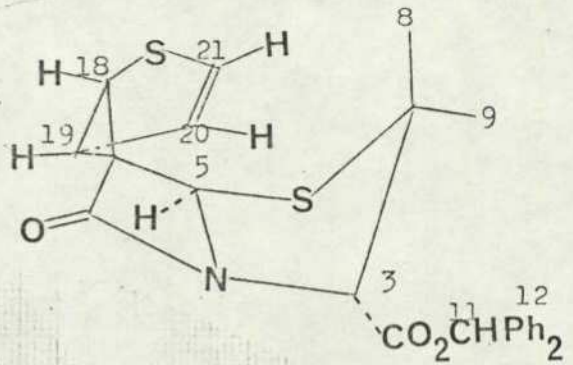
The isomeric purity of this cycloaddition product was confirmed chromatographically and spectroscopically. In the ^{1}H n.m.r. spectrum, gradual introduction of europium shift reagent, Eu (fod)$_3$ into the $CDCl_3$ solution did not cause any signal splitting which would be expected to occur with some of the protons if more than one isomer was present (Figure 3).

Analysis of this cyclopropanation product by analytical H.P.L.C. on both normal phase (Hypersil) and reverse phase (O.D.S. Hypersil) columns showed one component only.

Determination of the configuration of the isomers (70) and (71) was fraught with difficulty due to the close resemblance of their ^{1}H n.m.r. spectra.

Potentially, four isomeric cyclopropanation products may be formed in the decomposition of 6-diazopenicillanate (1) in

Fig. 3 Chemical shift for compound (70) in $CDCl_3$ with increasing concentration of $Eu(fod)_3$ in ^1H n.m.r., temperature: ambient.



thiophene, so that in principle six combinations of pairs of isomers would be considered. (Table 6). However, careful examination of molecular models of all four possible isomers indicated that in their ^1H n.m.r. the variation of deshielding effects of the carbonyl group of the β-lactam on C_{18}-H, C_{19}-H, C_{20}-H, C_{21}-H, and the shielding influence of the thiazoline sulfur on C_{19}-H and the shielding effect of the 2-thiobicyclo [3, 1, 0] hex-3-ene on C_5-H were harmonious solely in the combination (B). (Table 5) Contradictions were found in the rest of five possible combinations, e.g. for combination (A) (Table 6) under the influence of the aforementioned effects, in their ^1H n.m.r. one of the isomers would be expected to have C_5-H at higher field together with C_{18}-H at lower field than other isomer, which is not in agreement with the data recorded. (Table 5) Analogous disagreements were seen in combinations of (C), (E) and (F). In the combination (D) the chemical shift of C_5-H of both isomers would be expected to be very close due to the resemblance of magnetic environment, which was also contradictory to the experimental data. (Table 5)

		COMPOUND		
		(70)		(71)
τ	C_3-H	5.60	High	5.50
	C_5-H	5.22	High	5.00
	C_{18}-H	6.25	High	6.16
	C_{19}-H	6.52	Low	6.65
	C_{20}-H	4.12	High	4.04
	C_{21}-H	3.78		3.78

Table 5. Part of ^1H n.m.r. data for compounds (70) and (71). In $CDCl_3$ with T.M.S. as internal standard.

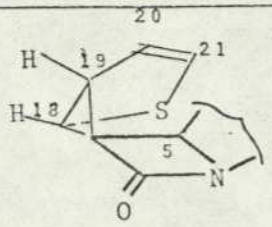
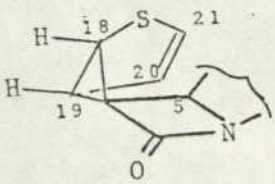
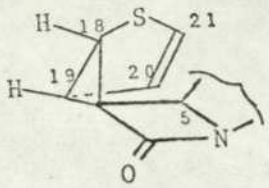
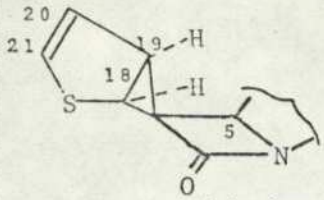
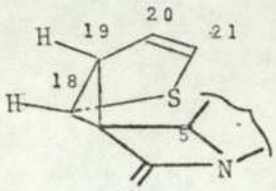
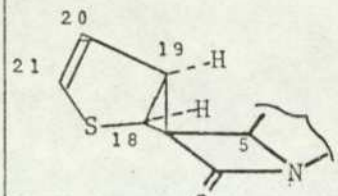
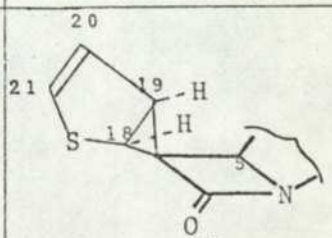
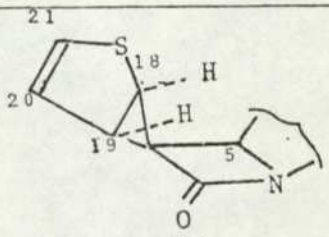
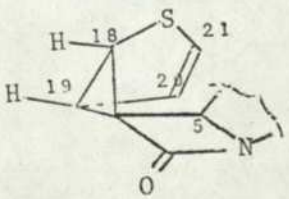
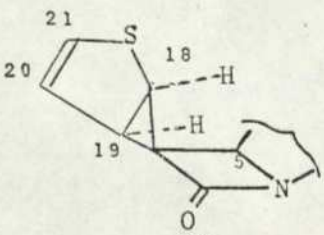
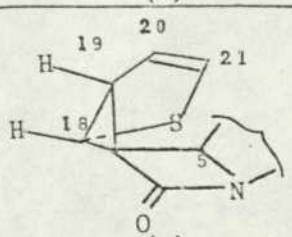
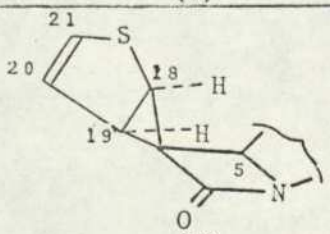
A	* C_5-H(high) C_{18}-H(low)	 (a)	 (b)
B	* C_5-H(high) C_{18}-H(high) C_{19}-H(low)	 (a) (70)	 (b) (71)
C	* C_5-H(high) C_{18}-H(low)	 (a)	 (b)
D	* C_5-H (no change)	 (a)	 (b)
E	* C_5-H(high) C_{18}-H(low)	 (a)	 (b)
F	* C_5-H(high) C_{18}-H(low)	 (a)	 (b)

Table 6. Possible combinations of 6-(2-thiobicycle[3,1,0]-hex-3-ene)-penicillanates.

* Expected chemical shift of isomer (a) in ^{1}H n.m.r. compared with isomer (b).

Assignment of compound (70) with the structure where the 2-thiobicyclo [3, 1, 0] hex-3-ene is facing towards C_5-H was based on the ^1H n.m.r. data where the C_5-H of (70) resonates at higher field than the C_5-H in (71) due to the shielding effect of 2-thiobicyclo-[3, 1, 0] hex-3-ene. (Table 5).

Applying the previously described nuclear Overhauser enhancement - difference technique provided unarguable evidence supporting the configuration of (70). As shown in Figures (4) and (5), and Table (7) irradiation of C_5-H of (70) at τ 5.4 gave greater n.O.e. on C_{20}-H than C_{21}-H since the former approached C_5-H more closely than C_{21}-H did. When C_{20}-H was irradiated n.O.e.s were found on C_{21}-H, C_5-H and C_{19}-H. Similarly, n.O.e.s were seen on C_5-H, C_{20}-H and C_{19}-H on irradiation of C_{21}-H.

Proton irradiated	Observed n.O.e.
3	8, 9
5	9, 20, 21
8	9, 3
9	8, 5, 3
11	12
18	21, 20
19	18, 20
20	21, 5, 19
21	5, 20, 19

Table 7. Proton - Proton n.O.e. in (70).

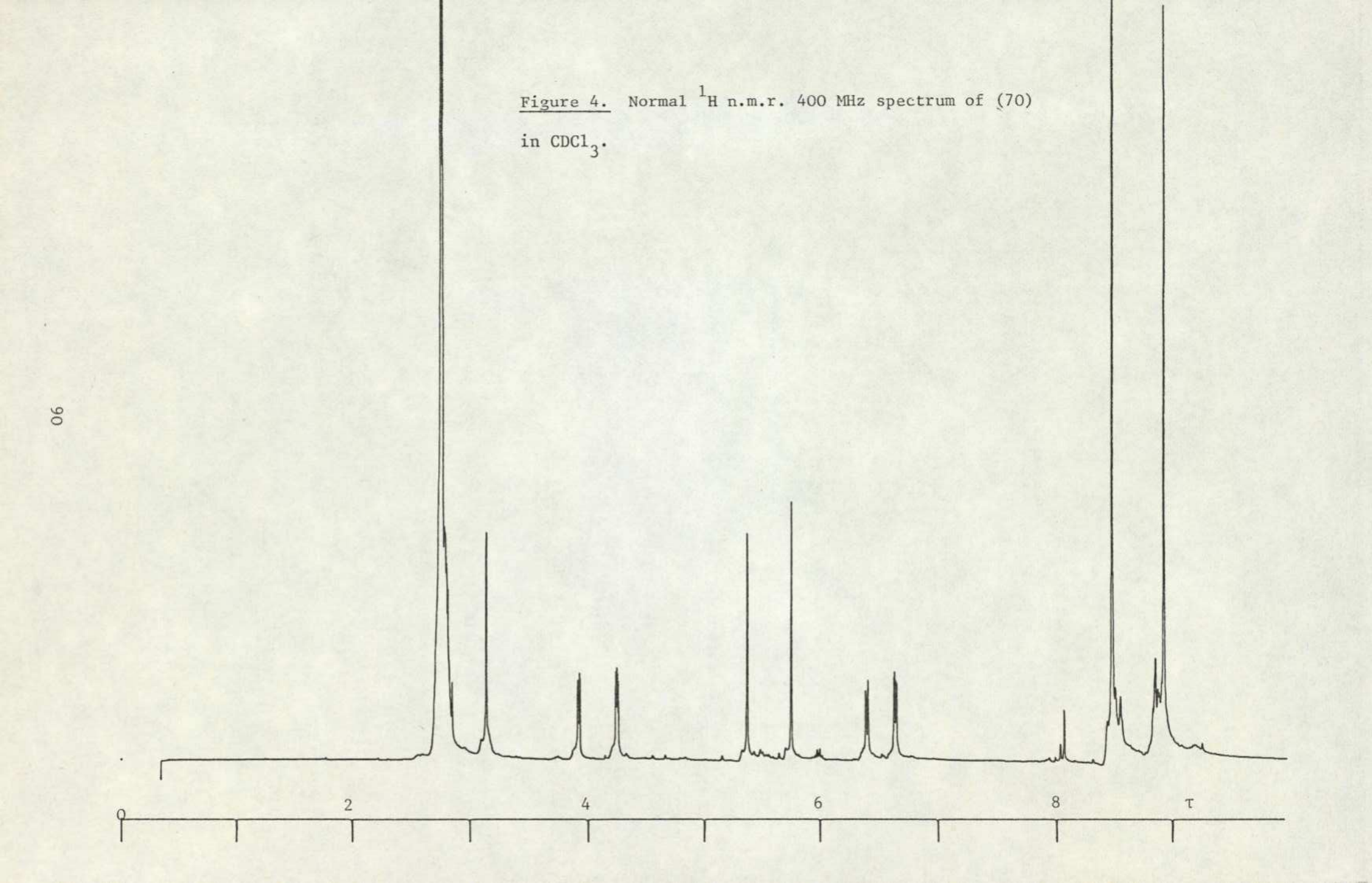


Figure 4. Normal ^{1}H n.m.r. 400 MHz spectrum of (70) in $CDCl_3$.

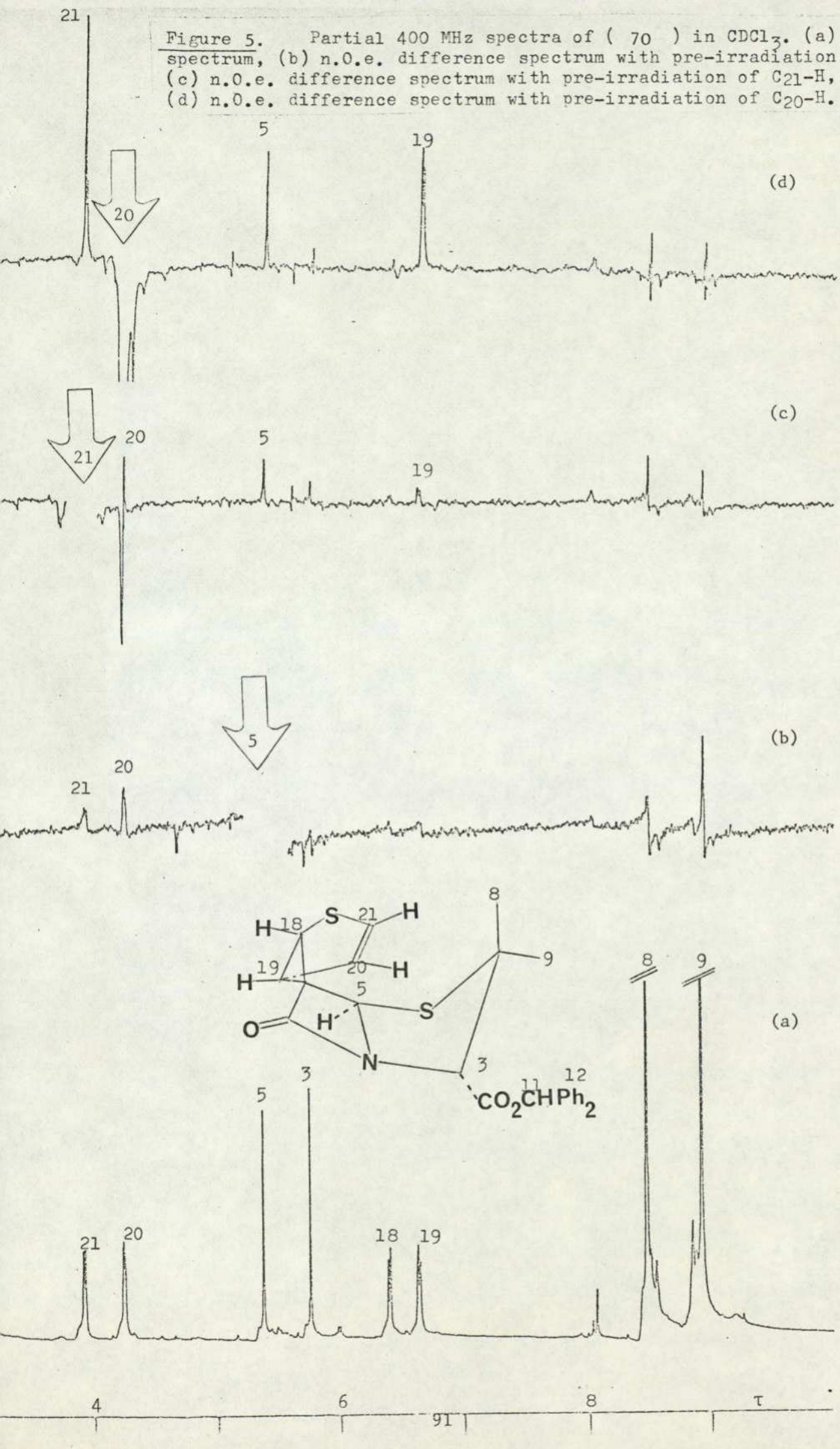


Figure 5. Partial 400 MHz spectra of (70) in $CDCl_3$. (a) Normal spectrum, (b) n.O.e. difference spectrum with pre-irradiation of C_5-H, (c) n.O.e. difference spectrum with pre-irradiation of C_{21}-H, (d) n.O.e. difference spectrum with pre-irradiation of C_{20}-H.

Identification of compound (69) was also made from its spectroscopic data. It was initially anticipated that an ylide (72) might be isolated from the reaction. However, it was clear from the ^{1}H n.m.r. spectrum of the product that it lacked the symmetry of this structure. Moreover, benzhydryl-6-diazopenicillanate failed to give a recognisable product with 2,5-dichlorothiophene This suggests that stable ylide formation would not be observable by comparison with Porter's results. The ring expanded 2H-thiopyran structure (69) was then proposed, based on the available spectroscopic data.

The i.r. spectrum of compound (69) showed absorption at 1774 and 1740 cm^{-1} typical of a β-lactam ester, and the ^{1}H n.m.r.(100 MHz) spectrum exhibited two very complicated multiplets at τ 3.64 (3H) and 4.24 (1H) corresponding to the thiopyran protons. The ^{13}C n.m.r. data was more informative, showing signals at δ 126.692 (C_{18}), 120.765 (C_{19}), 120.572 (C_{20}) and 112.890 (C_{21}) corresponding to the thiopyran carbons.

The isomeric purity of sample (69) was confirmed by analytical HPLC on both normal phase (Hypersil) and reversed phase (O.D.S. Hypersil) columns, showing one component only.

The structure and stereochemistry of sample (69) were determined decisively by the n.O.e. difference technique. The results of this experiment are shown in Table (8), Figures (6) and (7). Irradiation of C_{21}-H of (69) at τ 4.45 gave n.O.e.s on C_8-H, C_{20}-H, C_{19}-H and C_3-H while irradiation of C_8-H provided n.O.e.s on C_{21}-H, C_3-H, C_5-H and C_9-H, which are consistent with the configuration of (69) where the sulfur atom of the thiopyran is located below the β-lactam ring.

Proton irradiated	n.O.e. observed
3	5, 8
5	-
8	3, 21, 9, 5
9	5, 3, 8, 11
11	12
12	-
18	19, 20
19, 20	21, 18
21	20, 19, 3, 8

Table 8. Proton-Proton n.O.e. in (69).

Figure 6. Normal ^{1}H n.m.r. 400 MHz spectrum of (69) in $CDCl_3$.

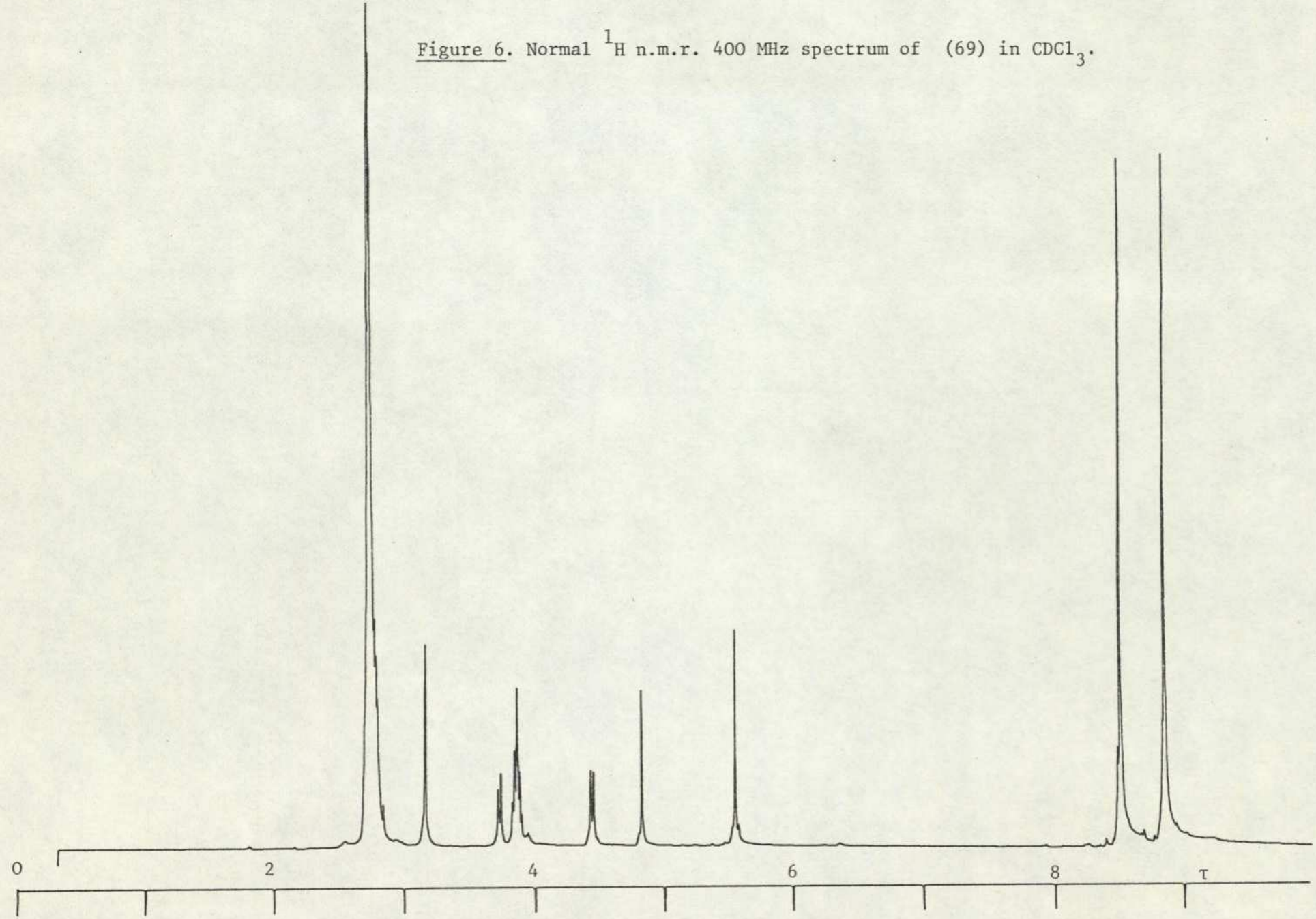
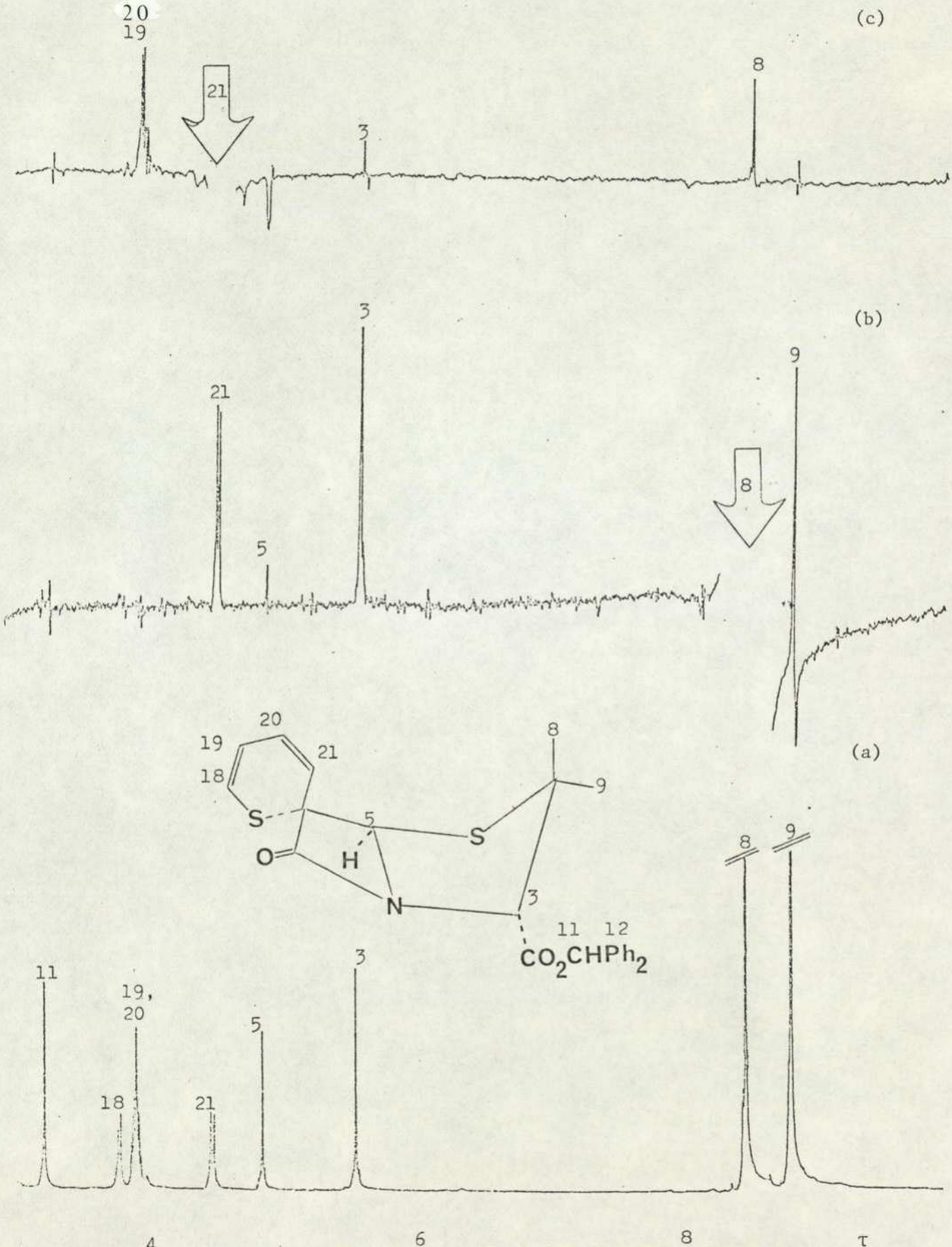
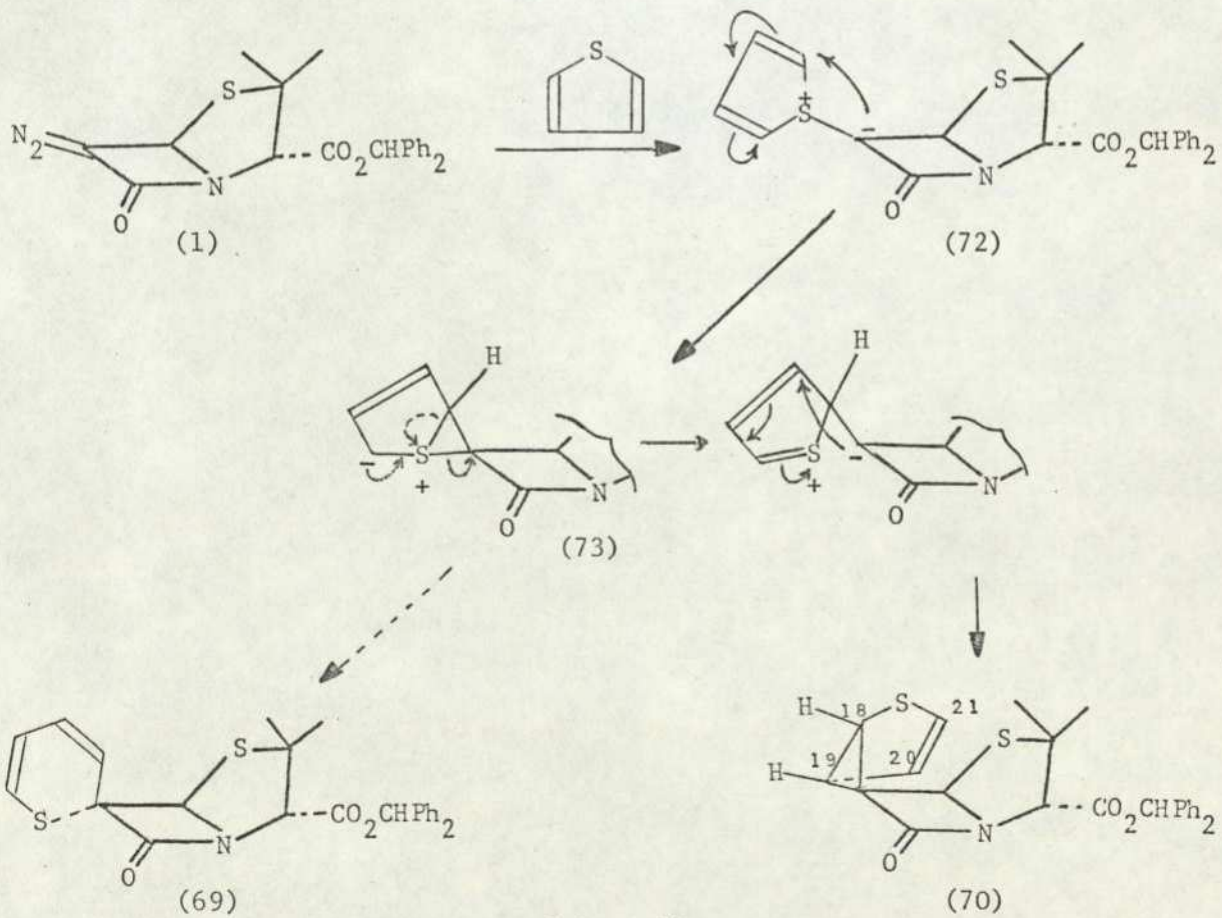


Figure 7 Partial 400 MHz spectra of (69) in $CDCl_3$. (a) Normal spectrum, (b) n.O.e. difference spectrum with pre-irradiation of C_8-H ,(c) n.O.e. difference spectrum with pre-irradiation of C_{21}-H.



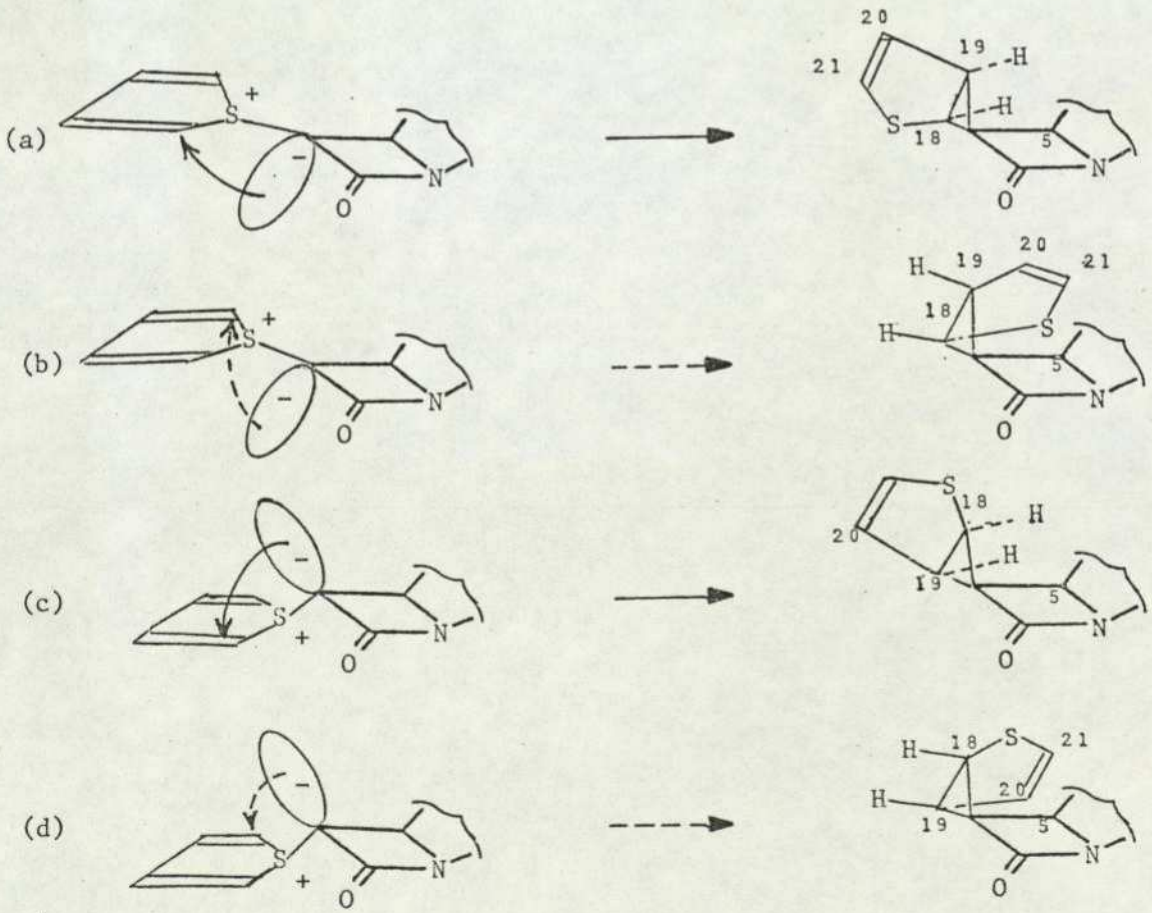
The literature evidence presented in Section 3.3 indicates that sulphur is far more efficient than carbon-carbon double bonds at attacking electrophilic carbenoids, and that in some cases thiophenium ylides can be formed quantitatively. Thus, it seems reasonable to explain the formation of both cyclopropanation product (70) and the ring expansion product (69) in the rhodium-catalysed reaction in terms of a common ylide intermediate (72) which closes to the zwitterion (73). This can then undergo a cleavage of the internal bond in the 3-membered ring to furnish the 2H-thiopyran, or a migration reaction involving cleavage of the external C-S bond, to give the cyclopropane (Scheme 19). It must be recognised, however, that the cyclopropane could arise independently by direct attack of the diazo compound or of a carbenoid species on the 2,3-bond of thiophene.



Scheme 19.

If the formation of the cyclopropane does proceed via the thiophenium ylide, the four possible geometries of the product may be determined by the direction of ring closure in the ylide, as shown in Scheme 20. For example, the product (70) obtained in the rhodium catalysed reaction can be pictured as

by a movement of the thiophene ring towards the lower face of the β-lactam ring from the normal position in the ylide, followed by attack of the C_6-anion on the rear C_α-position of the thiophene ring, as shown in Scheme 20(d). The second product formed in the copper-catalysed reaction would similarly arise via the processes shown in Scheme 20(a).

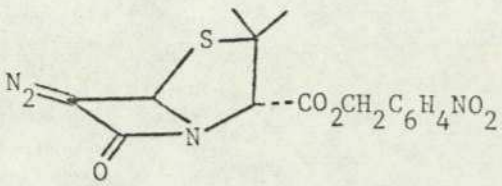


Scheme 20.

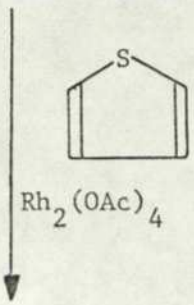
Deprotection of ring expansion product (69) was readily accomplished by brief contact with T.F.A. and anisole, and the corresponding carboxylic acid could be isolated in 80% yield. It was found to be devoid of anti-bacterial activity.

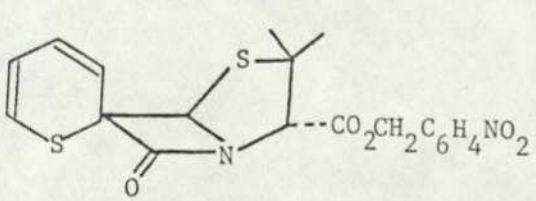
Deprotection of cyclopropanation product (70) encounted a lot of problems. Normal and modified methods employing combinations of T.F.A. and anisole failed to deblock the benzhydryl group, in all cases the cyclopropyl ring and the β-lactam ring being destroyed. Application of the latest reagents including aluminium trichloride and iodotrimethylsilane to cleave the ester function was also unsuccessful. Alternatively, hydrogenolysis of sample (70) resulted in saturation of the carbon-carbon double bond of the 2-thiobicyclo [3, 1, 0]hex-3-ene without removing the benzhydryl function.

The alternative p-nitrobenzyl protecting group was then introduced to replace the benzhydryl function. P-nitrobenzyl 6-diazopenicillanate (74) was synthesized (refer to Chapter 2) and reacted with thiophene in the same manner described above to give the analogous cycloaddition product (75) and ring expansion adduct (76) in lower yields, 6.5% and 15% respectively. Removal of the p-nitrobenzyl group from cyclopropanation product (76) was achieved by stirring the mixture of compound (76) and sodium sulphide in cooled, wet, T.H.F., and the corresponding carboxylic acid was isolated in 50% yield. It was found to be devoid of anti-bacterial activity.

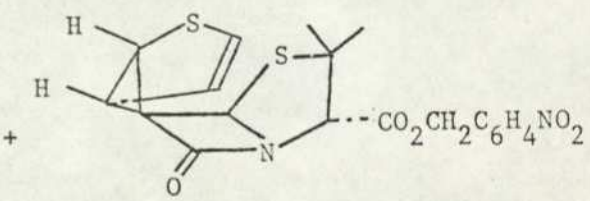


(74)





(75)



(76)

3.5. EXPERIMENTAL

Reaction of Benzhydryl 6-Diazopenicillanate (1) with Styrene

a. Cu(acac)$_2$ catalysis. The mixture of styrene (0.5 ml) and copper bis(acetylacetonate)(15 mgm) in CH_2Cl_2(2 ml) was cooled and stirred in an ice bath and the solution of benzhydryl 6-diazo penicillanate (400 mgm, 1 m mol) in styrene (3 ml) was added dropwise in a period of 2 hours. The reaction mixture was allowed to warm to room temperature and stirred for 10 hours. The crude product was submitted to prep. t.l. c. (SiO_2, CH_2Cl_2/Pet. ether 75/25). Isolation of the fastest moving fraction gave the spiro-cyclopropyl penicillanate (17) as a pale yellow solid, 60 mgm (13%), m.p. 69-100°, λ_{max} (C_2H_5OH) 210 nm ,(log ε 4.28), ν_{max} (CH_2Cl_2) 1775, 1745, 1200, 1170 cm^{-1}, ^1H n.m.r. ($CDCl_3$) τ 2.73 (15H, aromatic H), 3.15 (1H, s, C_{11}-H), 4.55 (1H, s, C_5-H), 5.42 (1H, s, C_3-H), 7.24 (1H, d.d, J = 9.8 Hz, 7.5 Hz, C_{18}-H), 8.20 (2H, m, C_{19}-H), 8.40 (3H, s, C_8-H), 8.75 (3H, s, C_9-H), ^{13}C n.m.r. δ ($CDCl_3$), 177.343 (C_{10}), 167.578 (C_7), 139.583 (C_{12}), 137.109 (C_{20}), 128.775, 128.515, 127.734, 127.343, 127,083 (aromatic C), 78.385 (C_5), 71.223 (C_{11}), 70.182 (C_3), 64.452 (C_2), 48.567 (C_6), 33.463 (C_8), 31.540 (C_{18}), 26.041 (C_9), 19.400 (C_{19}), (Found : C, 73.96; H, 5.96; N, 2.94%; $C_{20}H_{27}NO_3S$ requires C, 74.17; H, 5.79; N, 2.98%), m/e: 469 (M^+; 0.6%), 302 (14.3%), 167 (100%), 158 (0.4%), 99 (2.8%), 59 (4.4%), 144 (3%), 77 (8.3%), 67 (2.1%). Isolation of slower moving fraction gave the cyclopropane ester isomer (18) as a pale yellow solid, 90 mgm (19%), m.p. 40-55°, λ_{max} (C_2H_5OH) 208 nm (log ε 4.40), ν_{max} (CH_2Cl_2) 1775, 1745, 1200, 1170 cm^{-1}, ^1H n.m.r. ($CDCl_3$) τ 3.1 - 2.76 (15H, aromatic H), 3.18 (1H, s, C_{11}-H),

4.96 (1H, s, C_5-H), 5.14 (1H, s, C_3-H), 7.07 (1H, dd, J = 9.8, 7.5 Hz, C_{18}-H), 8.20 (2H, m, C_{19}-H), 8.40 (3H, s, C_8- H), 8.78 (3H, s, C_9-H), ^{13}C n.m.r. ($CDCl_3$) δ 178.510 (C_{10}), 167.447 (C_7), 139.584 (C_{12}), 136.848 (C_{20}), 129.036, 128.776, 127.864, 127.213 (aromatic C), 78.385 (C_5), 70.182(C_3), 68.098 (C_{11}), 64.843 (C_2), 48.697 (C_6), 33.330 (C_8), 28.645 (C_{18}), 25.780 (C_9), 17.708 (C_{19}), (Found: C, 73.93; H, 5.89; N 2.72%; $C_{29}H_{27}NO_3S$ requires C, 74.17; H, 5.79; N 2.98%) m/e: 469 (M^+; 0.2%), 302 (6.6%), 167 (100%), 158 (0.5%), 99 (3.4%), 59 (3.4%), 144 (2.3%), 77 (6.9%), 67 (1.8%). Isolation of the slowest moving fraction gave the pyrazoline (21) as an oil, 49 mgm (8%), ν_{max} (CH_2Cl_2) 3400 (NH), 1775, 1745, 1720 (shoulder) 1200, 1175 cm^{-1}, τ ($CDCl_3$) 2.60 (15H, aromatic H), 3.02 (1H, s, C_{11}-H), 4.62 ($\frac{3}{4}$ H, s, C_5-H), 4.71 ($\frac{1}{4}$H, s, C_5-H), 5.31 ($\frac{1}{4}$H, s, C_3-H), 5.37 ($\frac{3}{4}$ H, s, C_3-H), 6.22, 6.56 (2H, AB, J_{gem}= 18 HZ, C_{18}-H), 8.41 (3H, s, C_8-H), 8.69 (3H, s, C_9-H).

b. Rhodium acetate catalysis. Reaction of 6-diazopenicillanate (1) (550 mgm 1.4 mmol), rhodium acetate hydrate (5 mgm) and excess of styrene was carried out in the same manner as described in (a) above, which gave compound (17) 160 mgm (24%),compound (18) 190 mgm (29%) and compound (21) 100 mgm (14%).

Deprotection of compounds (17) and (18)

The hydrogenolysis technique was used to remove the benzhydryl function from these two samples (general procedure is given in Chapter 6).

48 mgm (0.1 mmol) of cyclopropane ester (17) afforded 24 mg (75%) of the corresponding carboxylic acid as white solid, m.p. 140-145°, (Found: C, 63.28; H, 5.89; N, 4.32%; $C_{16}H_{17}O_3SN$ requires C, 63.35; H, 5.65; N, 4.62%).

110 mgm (0.23 mmol) of cyclopropane ester isomer (18) furnished 40 mgm (43%) of the corresponding carboxylic acid as an oil.

Copper (II)bis(acetylacetonate) catalysed reaction of benzhydryl 6-diazopenicillanate (1) with cyclohexene

The mixture of $Cu(acac)_2$ (1 mgm, 3.8×10^{-3} mmol) in cyclohexene (1 ml) and CH_2Cl_2 (0.5 ml) was cooled and stirred in an ice bath and the solution of benzhydryl 6-diazopenicillanate (1) (80 mgm 0.2 mmol) in benzene (2 ml) was added dropwise in a period of 30 minutes. The mixture was stirred in an ice bath for one hour and then was stirred at room temperature overnight. The excess of cyclohexene, dichloromethane and benzene were removed under reduced pressure. The residual yellow oil was submitted to prep. t.l.c. on silicic acid using CH_2Cl_2 as an eluent. Isolation of first and second fractions produced two isomers of 6-(bicyclo-[4, 1, 0] heptane) penicillanates (25) and (26). Compound (25) is a white solid, yield 29 mg (31%), m.p. 60-70°, λ_{max} (C_2H_5OH) 207 nm (log ε 4.2), ν_{max} (CH_2Cl_2) 1770, 1745, 1175, 2930 cm^{-1} (C-H stretching of cyclohexane), ^1H n.m.r. ($CDCl_3$) τ 2.85 (10H, aromatic H), 3.25 (1H, s, C_{11}-H), 4.90 (1H, s, C_5-H) 5.55 (1H, s, C_3-H), 8-9 (16H, signals overlapping

C_{18}-C_{23}, C_8 and C_9-H), ^{13}C n.m.r. ($CDCl_3$) δ 178.77 (C_{10}), 167.838 (C_7), 139.71 (C_{12}), 128.776, 128.447, 128.317, 127.864, 127.343 (C_{13}-C_{17}), 78.254 (C_5), 72.265 (C_{11}), 70.247 (C_3), 63.60 (C_2), 50.19 (C_6), 33.590 (C_8), 25.976 (C_{18}), 25.780 (C_{19}), 25.325 (C_9), 21.158, 20.898, 19.986 (C_{20}-C_{23}), (Found: C, 72.34; H, 6.73; N, 3.02%; $C_{27}H_{29}O_3NS$ requires C, 72.45; H, 6.53; N, 3.13%), m/e: 447 (M^+, 0.2%), 280 (4.2%), 167 (100%), 158 (0.2%), 99 (2.4%), 59 (17.8%), 122 (5.9%), 66 (11.6%), 56 (5.1%). Compound (26) is also a white solid, yield 26 mgm (28%), m.p. 78-83°, λ_{max} (C_2H_5OH) 206 nm (log ε 4.16), ν_{max} (CH_2Cl_2) 2920, 1760, 1735, 1175 cm^{-1}, ^{1}H n.m.r. ($CDCl_3$) τ 2.84 (10 H, aromatic H), 3.22 (1H, s, C_{11}-H), 4.72 (1H, s, C_5-H), 5.65 (1H, s, C_3-H), 8-9 (16H, signals overlapping, C_{18}-C_{23}, C_8 and C_9-H). ^{13}C n.m.r. ($CDCl_3$) δ 180.648 (C_{10}), 168.030 (C_7), 139.713, 139.583 (C_{12}), 128.776, 128.317, 127.994, 127.276 (C_{13}-C_{17}), 78.254 (C_5), 69.075 (C_3), 67.382 (C_{11}), 63.866 (C_2), 45.312 (C_6), 31.444 (C_8), 26.171 (C_9), 21.354 (C_{18}), 20.507 (C_{19}), 21.354, 20.312, 19.140 (C_{20}- C_{23}), (Found: C, 72.19, H, 6.60; N, 3.06%; $C_{27}H_{29}O_3NS$ requires C, 72.45; H, 6.53; N, 3.13%), m/e: 447 (M^+; 0.6%), 280 (2.6%), 167 (52.2%), 99 (0.3%), 59 (1.6%), 122 (0.9%), 66 (0.9%), 56 (0.5%).

Deprotection of compounds (25) and (26)

The benzhydryl function was removed by hydrogenolysis (general procedure is shown in Chapter 6).

42 mgm (0.93 mmol) of ester (25) gave 20 mgm (75%) of the corresponding carboxylic acid as a solid m.0. 140-142°, (Found: C, 59,52; H, 6.96; N, 4.79%; $C_{14}H_{19}O_3NS$ requires C, 59.76; H, 6.81; N, 4.98%).

45 mgm (0.1 mmol) of ester (26) gave 15 mg (50%) of corresponding carboxylic acid as a solid, m.p. 138-160°.

Reaction of benzhydryl 6-diazopenicillanate (1) with thiophene

a. $Rh_2(OAc)_4$ catalysis. The solution of rhodium acetate hydrate (5 mgm) in thiophene (5 ml) was kept in the ice bath, and the solution of benzhydryl 6-diazopenicillanate (750 mgm, 1.9 mmol) in thiophene (5 ml) was added dropwise in a period of 2 hours. The mixture was stirred at room temperature overnight under nitrogen. The excess of thiophene was removed under vacuum. The residual red oil was submitted to prep. t.l.c. (SiO_2, CH_2Cl_2). Isolation of the more polar fraction gave cyclodaddition product ester (70) as a solid, m.p. 45-50°, 187 mgm (22%), λ_{max} (CH_3OH) 220 nm (log ε 4.17), ν_{max} (CH_2Cl_2) 1770, 1745 cm^{-1}, ^1H n.m.r. τ ($CHCl_3$) 2.56 (10H, aromatic H), 2.95(1H,s,C_{11}-H) 3.76 (1H, dd, J_{21-20} = 5.7 Hz, J_{21-18} = 1.5 Hz, C_{21}-H), 4.71 (1H, q, J_{20-19} = 3.8 Hz, C_{20}-H), 5.17 (1H, s, C_5-H), 5.56 (1H, s, C_3-H), 6.24 (1H, dd, J_{18-19} = 7.8 Hz, C_{18}-H), 7.81 (1H, q, C_{19}-H), 8.33 (3H, s, C_8-H), 8.77 (3H, s, C_9-H), ^{13}C n.m.r. δ ($CDCl_3$), 178.250 (C_{10}), 167.968 (C_7), 139.713 (C_{12}), 128.906, 128.645, 128.124, 127.343 (C_{13}-C_{17}, C_{21}), 120.702 (C_{20}), 78.515 (C_5), 68.880 (C_3), 65.754 (C_{11}), 62.369 (C_2), 41.406 (C_6), 39.973 (C_{18}), 35.286 (C_{19}), 30.338 (C_8), 26.562 (C_9), (Found: C, 66.84; H, 5.36; N, 2.95%; $C_{25}H_{23}O_3NS_2$ requires C, 66.79; H, 5.16; N, 3.12%), corresponding carboxylic acid, m.p. 121-123°, (Found: C, 51.02; H, 4.86; N, 4.86%; $C_{12}H_{13}NS_2O_3$ requires C, 50.87; H, 4.62; N, 4.94%), m/e: 449 (M^+; 2.6%), 167 (100%), 99 (0.8%), 59 (1.8%), 66 (0.2%), 58 (0.6%).

Isolation of less polar fraction gave ring expansion adduct (69), oil, 93 mgm (11%), λ_{max} (CH_3OH) 213 nm (log ε 4.16), ν_{max} (CH_2Cl_2) 1775, 1740 cm^{-1}, ^1H n.m.r. ($CDCl_3$) τ 2.56 (10H, aromatic H), 2.97 (1H, s, C_{11}-H), 3.64 (3H, m, C_{18-20}-H), 4.24 (1H, m. C_{21}-H), 4.64 (1H, s, C_5-H), 5.38 (1H, s, C_3-H), 8.38 (3H, s, C_8-H), 8.72 (3H, s, C_9-H), ^{13}C n.m.r. ($CDCl_3$) δ 171.739 (C_{10}), 166.660 (C_7), 139.352 (C_{12}), 128.776, 128.515, 127.734, 127.343, (C_{13}-C_{17}), 126.562 (C_{18}), 120.765 (C_{19}), 120.572 (C_{20}), 112.890 (C_{21}), 79.556 (C_{11}), 78.515 (C_5), 69.140 (C_3), 64.452 (C_6), 60.026 (C_2), 32.942 (C_8), 26.041 (C_9), (Found: C, 66.59; H, 5.22; N, 3.10%; $C_{25}H_{23}NO_3S_2$ requires C, 66.79; H, 5.16; N, 3.12%), m/e: 449 (M^+; 0.2%), 282 (1.2%), 167 (43.8%), 99 (2.6%), 59 (2.5%), 124 (3.1%), 84 (20.4%), the corresponding carboxylic acid m.p. 115-120°.

b. Cu(acac)$_2$ catalysis. Treatment of benzhydryl 6-diazopenicillanate (1) (97 mgm 0.25 mmol) with Cu(acac)$_2$ (4 mgm) and thiophene in the same manner as described above afforded a 1 : 1 isomeric mixture of cycloaddition products (70) and (71), 30 mgm (30%), which was homogeneous on conventional chromatography ν_{max} (CH_2Cl_2), 1765, 1740 cm^{-1}, τ ($CDCl_3$) 2.60 (10H, aromatic H), 3.00 (1H, s, C_{11}-H), 3.78 (1H, dd, J_{21-20} = 5.7 Hz, J_{21-18} = 1.5 Hz, C_{21}-H), 4.04 (½H , q, J_{20-19}=3.8 Hz, C_{20}-H), 4.12 (½H, q, J = 3.8 Hz, C_{20}-H), 5.00 (½H, s, C_5-H), 5.22 (½H, s, C_5-H), 5.50 (½H, s, C_3-H), 5.60 (½H, s, C_3-H), 6.16 (½H, dd, C_{18}-H), 6.25 (½H, dd, C_{18}-H), 6.52 (½H, q, J_{19-18} = 7.5 Hz, C_{19}-H), 6.65 (½H, q, J_{19-18} = 7.8 Hz, C_{19}-H), 8.36 (½H, s, C_8-H), 8.39 (1½H, s, C_8-H), 8.72 (1½H, s, C_9-H), 8.78 (1½H, s, C_9-H).

Deprotection of ring expansion product (69). The benzhydryl function was removed from compound (69) by the standard method using trifluoroacetic acid. The experimental procedure is shown in Chapter 6. 63 mgm (0.14 mmol) of compound (69) afforded 32 mgm (80%) of corresponding carboxylic acid, m.p. 115-120°.

Deprotection of cycloaddition product (76). Sodium sulphide ($Na_2S.9H_2O$) (41 mgm, 0.17 mmol) in water (1 ml) was added to the mixture of compound (76) (70 mgm, 0.17 mmol) in redistilled THF (2.5 ml) and water (1.1 ml) in ice bath. The mixture was stirred at 0° for 25 minutes, then 1 N HCl (0.17 ml) was added. After removal of the THF under vacuum the aqueous solution rendered alkaline to pH 8.5 by aqueous $NaHCO_3$(5%), then washed with EtOAc twice, acidified with HCl (1 N) to pH 2.5, extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, the solvent evaporated off in vacuum. 24 mgm (50%) of corresponding carboxylic acid was obtained, a pale-yellow solid, m.p. 121-123°.

3.6. References

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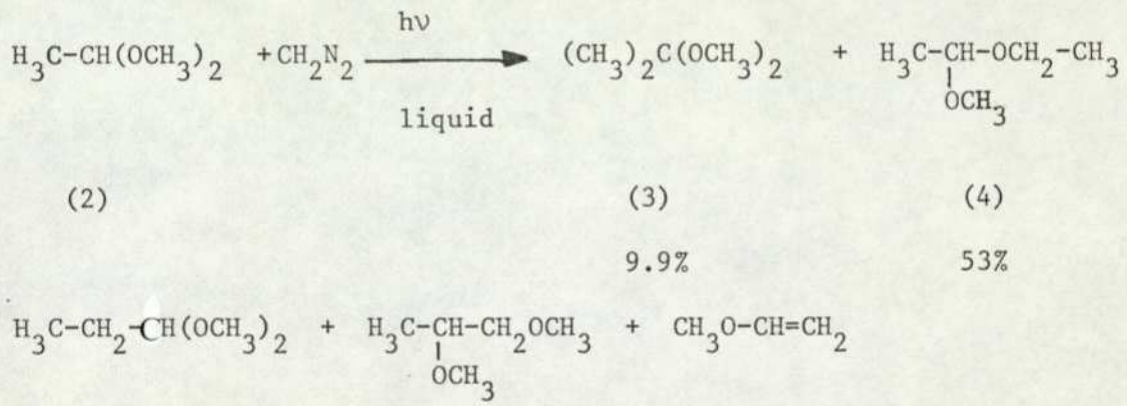
CHAPTER FOUR

REACTIONS OF 6-DIAZOPENICILLANATES WITH OXYGEN-CONTAINING COMPOUNDS

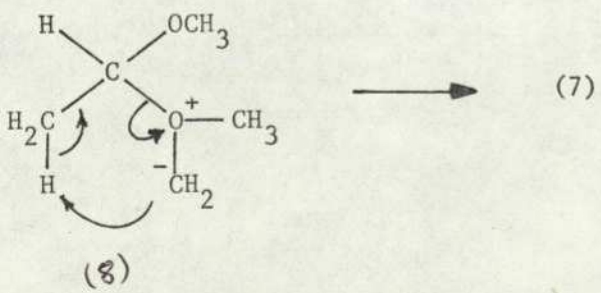
4.1. Review of reactions of carbenes with oxygen-containing compounds.

The reactions of diazomethane with acetals and orthoesters have been described by Kirmse and Buschhoff[1]. Photolysis of diazomethane in 1, 1-dimethoxy ethane (2) gave insertion products (3)–(7). The formation of compound (7) was rationalized in terms of the oxonium ylide (8).



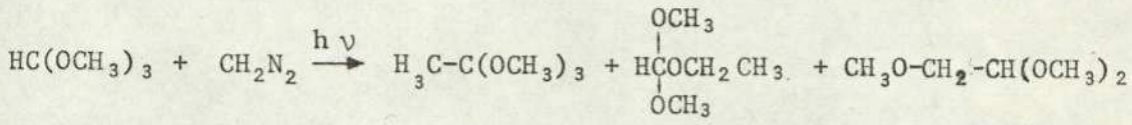
(2) (3) 9.9% (4) 53%

(5) 24.2% (6) 11.3% (7) 1.6%



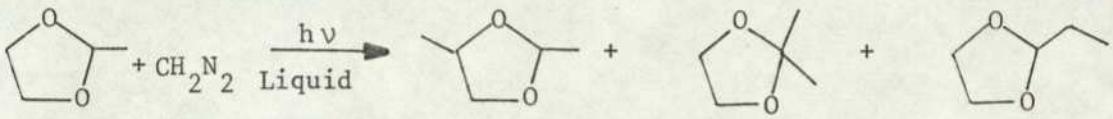
(8) → (7)

Photolysed decomposition of diazomethane in trimethyl orthoformate yielded insertion products (9-11).



	(9)	(10)	(11)
Liquid	11.1%	86.2%	2.6%

The irradiation of diazomethane in 2-methyl-1,3-dioxolane(12) produced compounds(13-18)and the oxonium ylide intermediate (19) was again suggested.

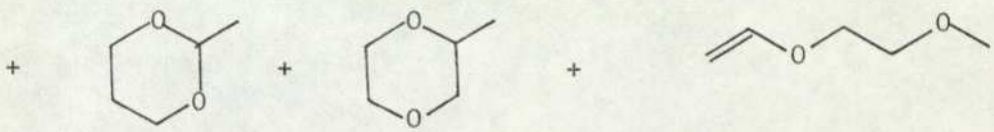


(12)

(13) 42.2%

(14) 16.8%

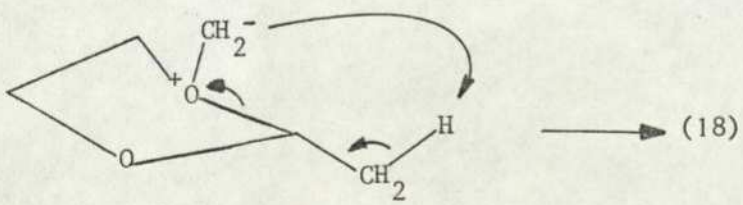
(15) 23.4%



(16) 0.3%

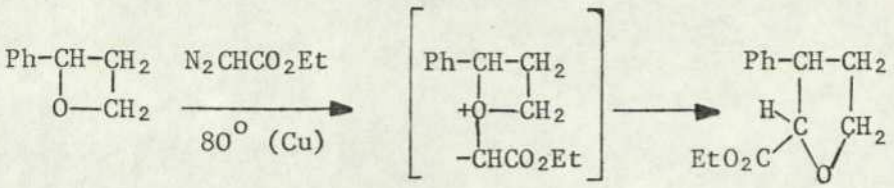
(17) 3.6%

(18) 13.7%



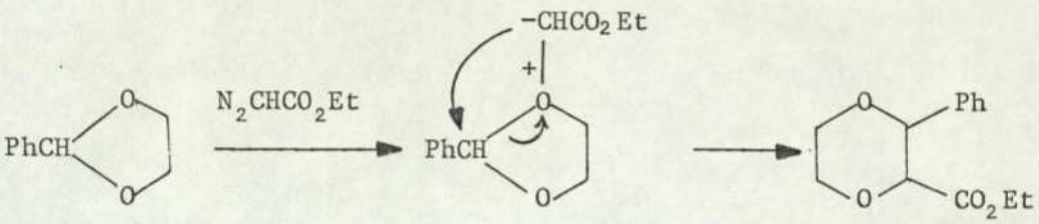
(19)

Nozaki, Takaya and Noyori[2] investigated the copper-catalysed decomposition of ethyl diazoacetate in the oxetane(20),which resulted in an 0.3:1 mixture of cis and trans isomers of 2-carbethoxy-3-phenyltetrahydrofuran(22)via a proposed oxonium ylide intermediate (21).



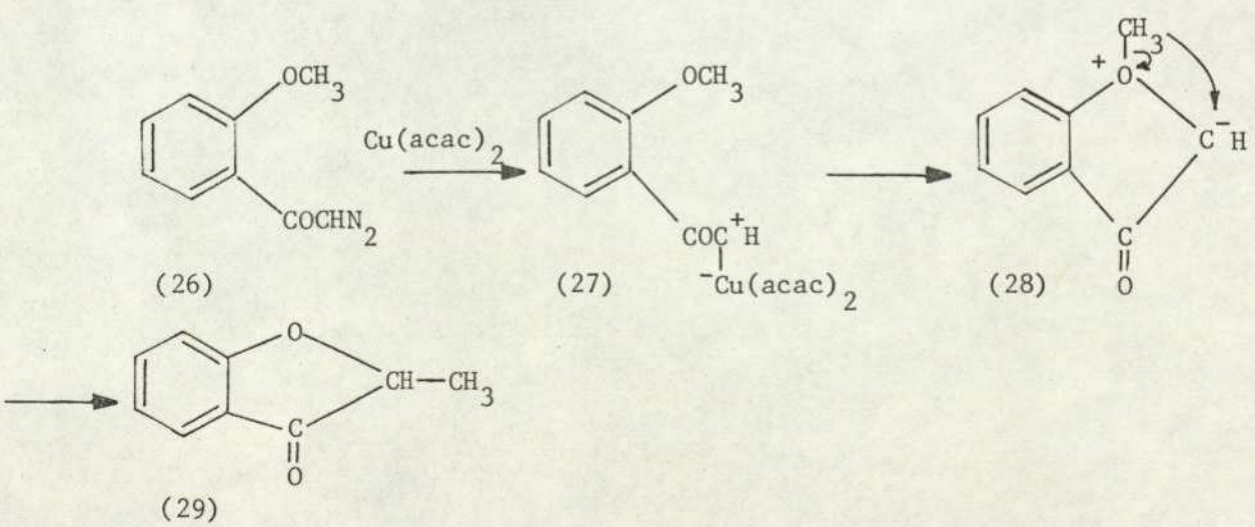
(20) (21) (22)

It was reported that 2-phenyl-1,3-dioxolane(23)and ethyl diazoacetate reacted at 130-150° to give 48.5% of product(25),which was supposed to involve oxonium ylide intermediate(24).[3]



(23) (24) (25)

The decomposition of 2-methoxy-α-diazoacetophenone (26) in benzene solution containing $Cu(acac)_2$ was carried out by Ibata, Ueda and Takebayashi[4] giving 2-methylcoumaranone (29)(52%). Formation of (29) was explained by the intermediacy of an oxonium ylide (28) produced by intramolecular electrophilic attack of the carbenoid carbon on the ether oxygen atom, followed by methyl migration.



(26) (27) (28) (29)

In these reactions of carbenes with compounds containing ethereal oxygen linkages, products of net C-O and C-C bond insertion are observed. By contrast, the reactions of carbenes with hydroxy compounds generally lead to predominant or exclusive O-H insertion.

When diazomethane/t-butanol mixtures were photolysed, the major products were t-butyl methyl ether (30) and 2-methylbutan-2-ol (31). From the amounts formed, it was calculated that in t-butanol the OH bond is 10.9±0.5 times as reactive as a CH bond towards insertion[5].

$$CH_2N_2 + (CH_3)_3COH \longrightarrow (CH_3)_3COCH_3 + CH_3-\overset{\overset{CH_3}{|}}{\underset{\underset{OH}{|}}{C}}-CH_2CH_3$$

(30) (31)

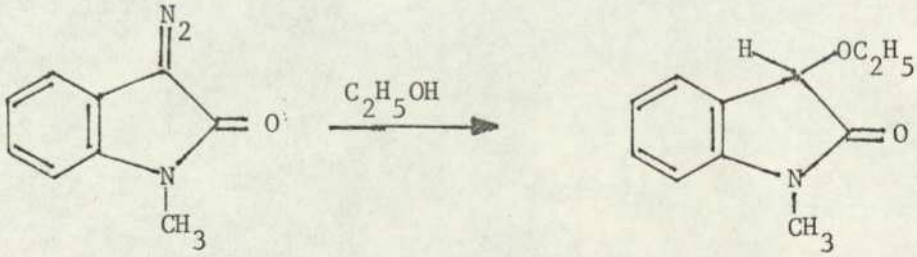
The reaction of α-diazoketone (32) with alcohols in the presence of copper was shown by Yates[6] to occur without rearrangement to give α-alkoxyketones (33).

$$C_{17}H_{35}COCHN_2 + R^1OH \xrightarrow{Cu} C_{17}H_{35}COCH_2OR^1$$

(32) (33)

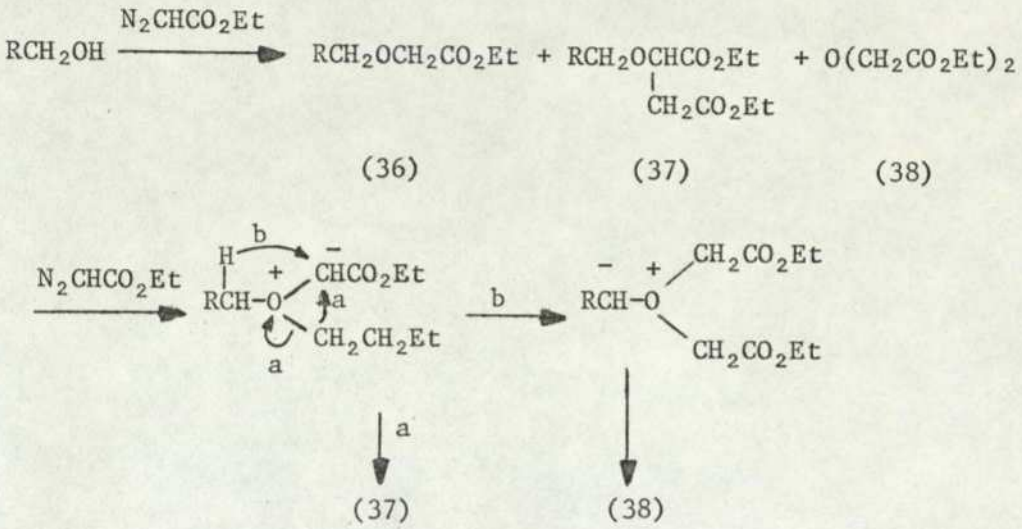
R^1 = CH_3, Et, or t-Bu

Photolysis of a very dilute solution of 1-methyl-3-diazo oxindole(34) in ethanol resulted in the formation of 3-ethoxyl-1-methyl-oxindole(35) in 24% yield[7].



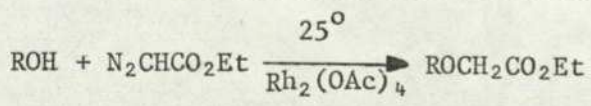
(34) (35)

Saegusa, Ito, Kobayashi, Hirota and Shimizu[8] reported the copper-catalysed reaction of alcohols with diazoacetate yielding the products (36)-(38) and proposed the mechanism shown (Scheme 1). No significant difference in catalyst was observed by them among cupruous and cupric compounds with various ligands in this reaction.



Scheme 1.

Paulissen, Reimlinger, Hayez, Hubert and Teyssie[9] reported the homogenous rhodium-catalysed insertion of ethyl diazo-acetate in hydroxylic bond of alcohols, water and weak acid (Scheme 2).

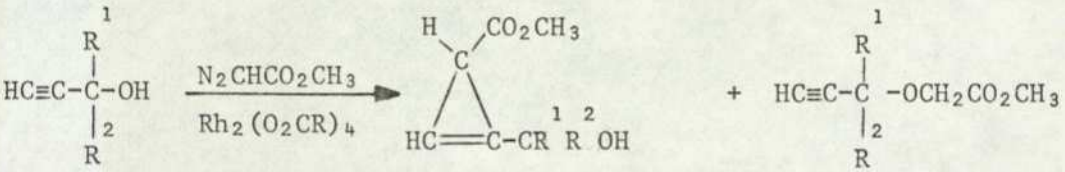


R	Yield %
C_2H_5	88
$(CH_3)_2CH$	83
$(CH_3)_3C$	82
H	80
CH_3CO	93

Scheme 2.

The relative reactivity of O-H bonds towards attack by carbethoxycarbene produced in this catalytic reaction was given: ethanol 2.12, propan-2-ol 1.20, t-butanol 1.00. This is also the order of decreasing acidity of the alcohols as well as the order of increasing steric hindrance.

The same authors[10] also described the regioselectivity in the intramolecular competition with various acetylenic alcohols(Scheme 3), which seemed directed by a higher sensitivity to steric hindrance of the insertion reaction versus cyclopropanation.



Alcohols	Yield %	
Propargyl alcohol	12	60
3-Butyn-1-ol	19	50
1-Pentyn-3-ol	21	54
3-Methyl-1-pentyn-3-ol	36	37
1-Ethynylcyclohexan-1-ol	12	10
4-Methyl-1-pentyn-4-ol	29	25

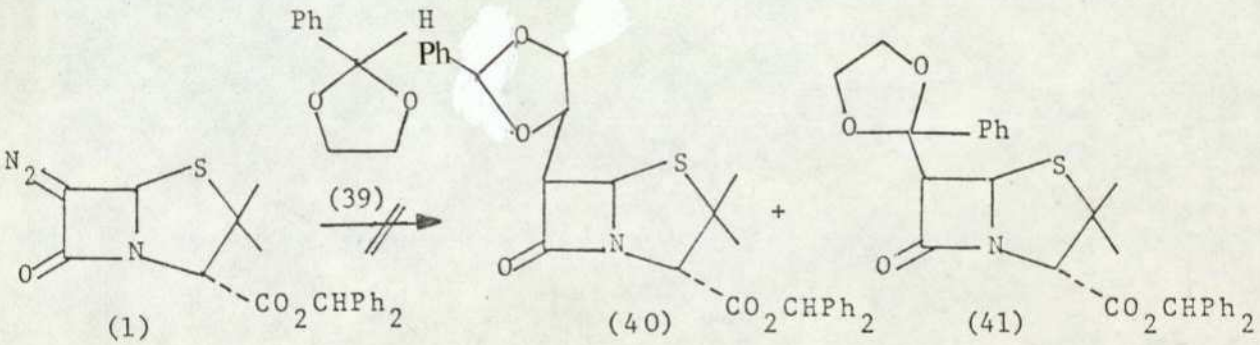
Scheme 3

These results were explained by a bimolecular carbenoid mechanism, as the more nucleophilic functional group (OH) was preferentially attacked.

4.2 Reactions with compounds containing oxygen: results and discussion.

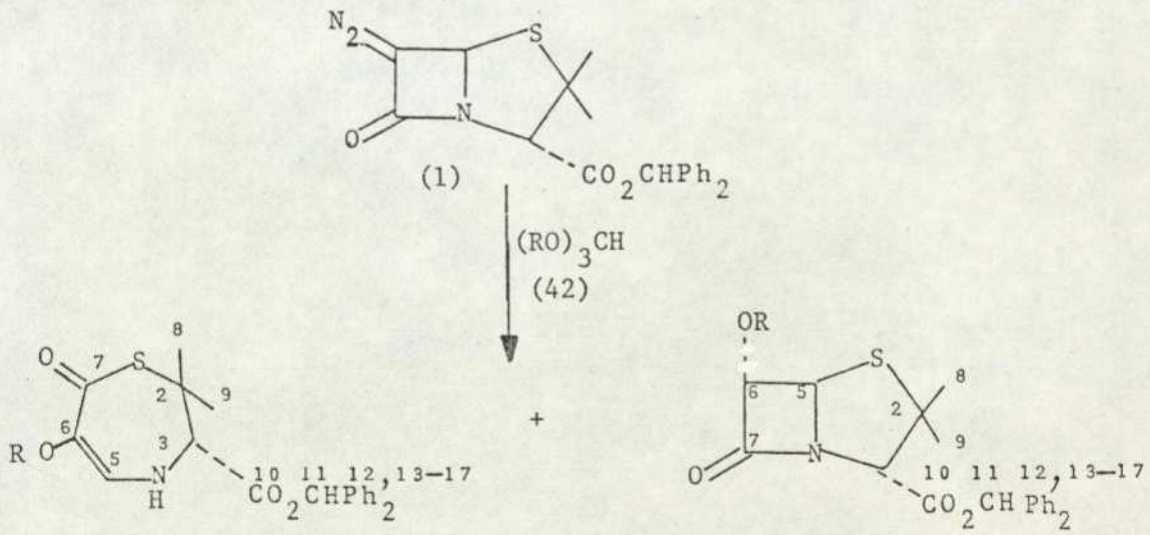
A number of reactions between benzhydryl 6-diazopenicillanate and acetals and orthoesters were examined, with the initial objective of obtaining C-H insertion products.

2-Phenyl-1,3,-dioxolane (39), synthesized according to the literature method[11], was the first candidate chosen to react with 6-diazopencillanate (1). The reaction was carried out in the presence of either $Cu(acac)_2$ or $Rh_2(OAc)_4$ at room temperature. Chromatography of the product mixture on silica gave a number of compounds, none of which corresponded to the expected insertion product (40),(41) or relevant adducts.



Reactions with orthoesters were then examined. The addition of 6-diazopenicillanate (1) in trimethylorthoformate (42a) to the neat trimethylorthoester (42a) containing copper (II) bis(acetylacetonate) resulted in evolution of nitrogen, and led to the formation of a white precipitate. This was filtered off and recrystallized to give analytically pure methoxythiazepine (43a) in a yield of 54%. The identification of compound (43a) was based on the spectroscopic analysis. The i.r. spectrum showed absorptions at cm^{-1} 3250 (NH),

1740, , 1620 (unsaturated thiolactone) 1545 (C=C), the ^1H n.m.r. spectrum exhibited singlet at τ 5.46 (methoxy protons), broad signal at τ 4.48 (NH), doublet at τ 3.00 (C_5- H,8Hz), doublet at τ 5.70 (C_3-H, 6Hz). The signal for NH disappeared upon addition of D_2O to the sample and the doublets collapsed to singlets. The ^{13}c n.m.r. data was also in a good agreement with the structure (43a). Chromatography of the mother liquor solution on silica afforded another compound in an extremely low yield (1%), which was identified as 6α-methoxypenicillanate (44a). The structure of (44a) was confirmed by spectroscopic methods, the low value of the C_5-H and C_6-H coupling constant (1.5Hz) being consistent with the assigned α-configuration at C_6.



a R=CH_3

b R=CH_2CH_3

e R=$-CH_2-CH=CH_2$

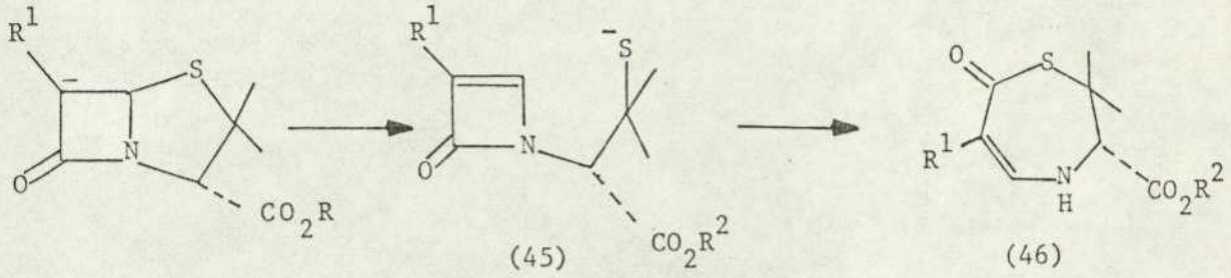
The catalysis was also carried out with different metal derivatives showing that rhodium acetate is most efficient both in terms of yield and reaction rate (Table 1).

	(43a) Yield % Thiazepine	(44a) 6α-Methoxy-penicillanate
$Cu(acac)_2$	54	1
$Cu\ SO_4$	41	0-1
$Rh_2(OAc)_4 2H_2O$	60	0-1
$Rh_2(OAc)_4 2H_2O$, with D.B.N.	18	29
$Rh_2(OAc)_4 2H_2O$, with $(CH_3)_3SiCl$ and $((CH_3)_3Si)_2NH$	16	0

Table 1 Effect of catalyst and additives on reaction of 6-diazo-penicillanate (1) with $(CH_3O)_3CH$.

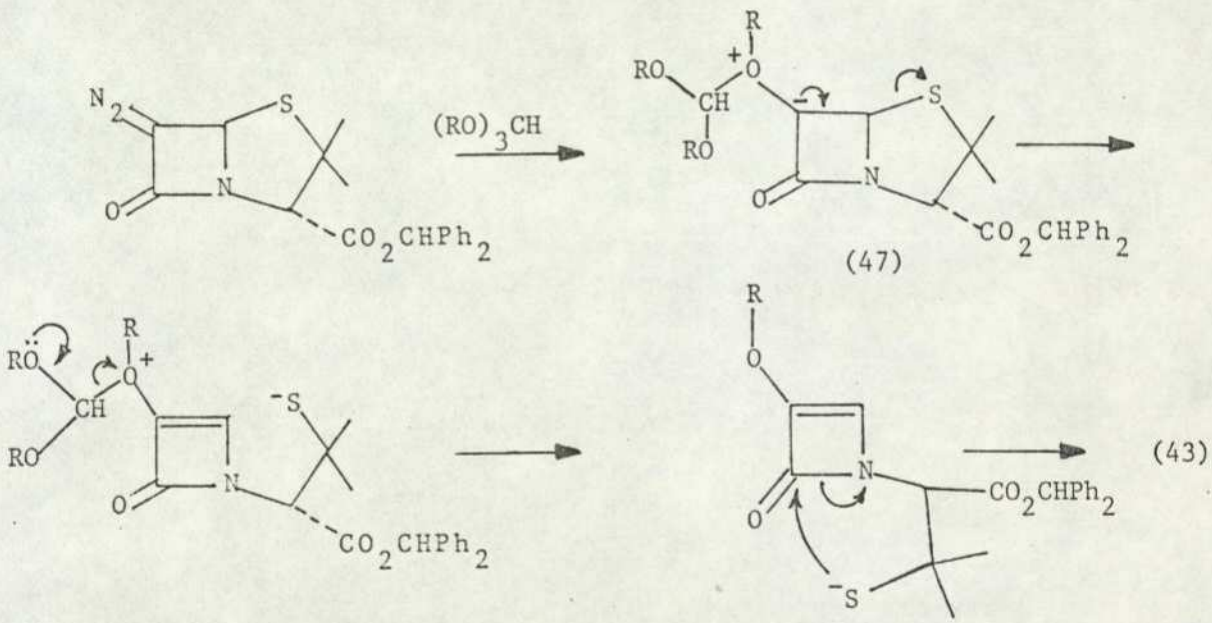
As expected by analogy with trimethyl orthoformate, the reaction of 6-diazopenicillanate (1) with triethylorthoformate (42b) in the presence of $Rh_2(OAc)_4$ gave ethoxythiazepine (43b) but in a better yield (75%). The spectroscopic data of compound (43b) was analogous with (43a). Perhaps one thing is noteworthy: in the ^1H n.m.r. spectrum of (43b) the signal of the methylene protons of ethoxy group appeared at the normal position τ6.33 but in an unusually complicated pattern rather than the expected quartet, due to an unknown reason. The ^{13}c n.m.r. data of (43b) did not indicate any isomers to be present.

Thiazepine products have been reported by Sheehan et al.[12] (refer to Chapter 1) and also have previously been observed during the base-catalysed epimerisation of 6β-aminopenicillanate derivatives to the 6α-isomers, involving deprotonation at 6-position[13,14,15]. It was postulated that the thiazepine(46) arises by cleavage of the 1,5-bond of the penam nucleus to give an enethiolate species (45) followed by intramolecular attack on the β-lactam ring to break the 4,7-bond.



(45) (46)

Thus in the metal-catalysed reaction of 6-diazo-β-lactam in orthoester, the formation of thiazepine (43) is consistent with the intermediacy of the oxonium ylide (47).

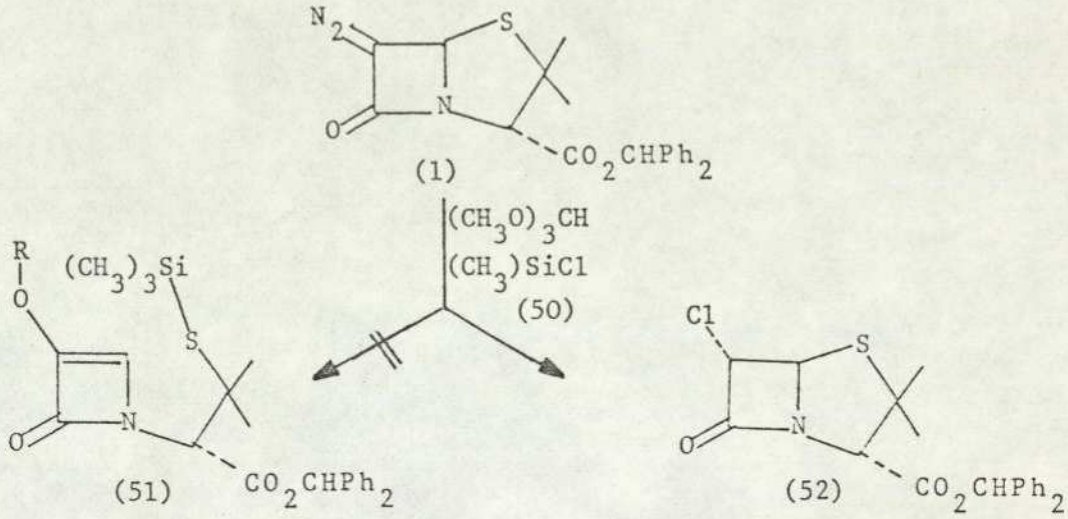


(47) (43)

(48) (49)

The metal-catalysed reaction of 6-diazopencillanate (1) with the trimethyl orthoformate in the presence of a trapping agent was studied in an effort to provide an alternative mode of reaction for the enethiolate (48) or (49). It was found that treatment of 6-diazopencillanate (1) with $(CH_3O)_3CH$ in the presence of redistilled trimethylsilylchloride (50) and $Rh_2(OAc)_4$ catalyst resulted in rapid decomposition of (1) with evolution of nitrogen. After removal

the excess trimethylsilylchloride under vacuum the ^1H n.m.r. spectrum of the crude product mixture was immediately recorded, showing the absence of the expected (51) or any other silylated compound. Chromatography of the crude product on silica furnished one major product, which was identified to be 6α- chloropenicillanate (52) in a yield of 35%. The thiazepine adduct was not detected in this reaction.



It was initially assumed that the formation of compound (52) was attributable to the reaction of 6-diazopenicillanate (1) with the chloride group in the trimethylsilyl chloride (50).

A parallel reaction was carried out in an attempt to prove this suggestion. t-Butyl chloride which was prepared by literature method[16] reacted with 6-diazopenicillanate (1) in the presence of $Rh_2(OAc)_4$ in the same manner, leading to the formation of a number of products in very small amounts, which were separated by conventional chromatography on silica and characterized. None of them was recognised to be the 6α-chloropenicillanate (52). The lack of detectable 6α-chloro-penicillanate(52) in this reaction ruled out the possibility that compound (52) was formed by the reaction of 6-diazopenicillanate (1)

with the chloride group of $(CH_3)_3SiCl$.

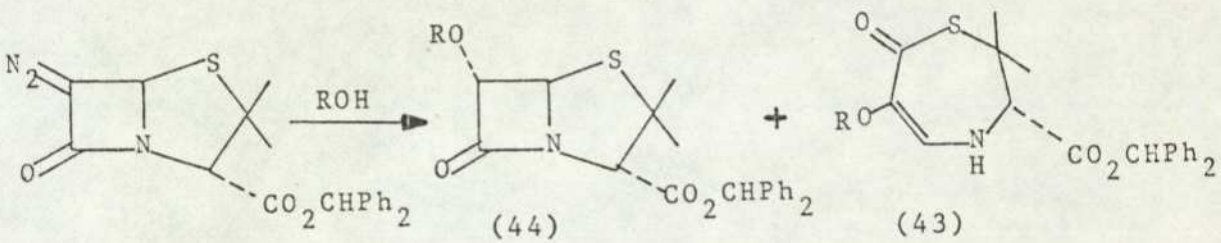
It was eventually realized that residual HCl, which either derived from contaminant or decomposition of trimethylsilyl chloride, was responsible for the formation of 6α-chloropencillanate (52). The evidence arose from the direct reaction of 6-diazopenicillanate (1) and trimethylsilyl chloride without any catalyst, which afforded the 6α-chloropenicillanate (52) in a yield of 60% after separation of the product mixture by conventional chromatography on silica.

In order to avoid this interference caused by the residual HCl, trimethylsilyl chloride was mixed with bis(trimethylsilyl) amine prior to reaction with 6-diazopenicillanate (1). The rhodium acetate catalysed decomposition of (1) in trimethylorthoformate (42a) containing trimethylsilyl chloride and trimethysilyl amine was accomplished in the same manner. After removal of unreacted $(CH_3)_3SiCl$ and $((CH_3)_3Si)_2NH$ in vacuum, the ^1H n.m.r. spectrum of the crude product was recorded immediately, showing the absence of any silylated adduct, which indicated lack of success in this attempt to trap the intermediate (48) or (49) by silylation. However the yield of methoxythiazepine (43a) was found to be reduced substantially (16%) and the 6α-methoxypenicillinate was not observed in this experiment. The fact that the yield of thiazepine was substantially lower may be an indication that silylation of an intermediate enethiolate was occurring, but that the product was very unstable and rearranged further.

In view of the great ease with which orthoesters hydrolyse in the presence of acid catalysts, it was considered important to examine the possibility that the observed products were arising from condensation of

the diazopenicillanate with free alcohol. The reactions of the diazo compound (1) with alcohols in the presence of catalysts were therefore studied.

Addition of 6-diazopenicillanate (1) to the alcohols ROH (R=Et, t-Bu, $PhCH_2$ or $CH_2=CHCH_2$) in the presence of rhodium acetate resulted in the evolution of nitrogen and led in each case, to the formation of two products, which were isolated by conventional chromatography on silica and were identified to be the alkoxythiazepine (43) as the major product together with the 6α-alkoxypenicillanate (44) as minor product (Table 2).



a CH_3

b CH_2CH_3

c $(CH_3)_3C$

d $CH_2=CHCH_2$

e $PhCH_2$

When the catalytic decomposition was carried out with copper (II) bis(acetylacetonate) analogous results were obtained (Table 2).

Reactions of 6-diazopenicillanate (1) with methanol were studied under a variety of conditions.

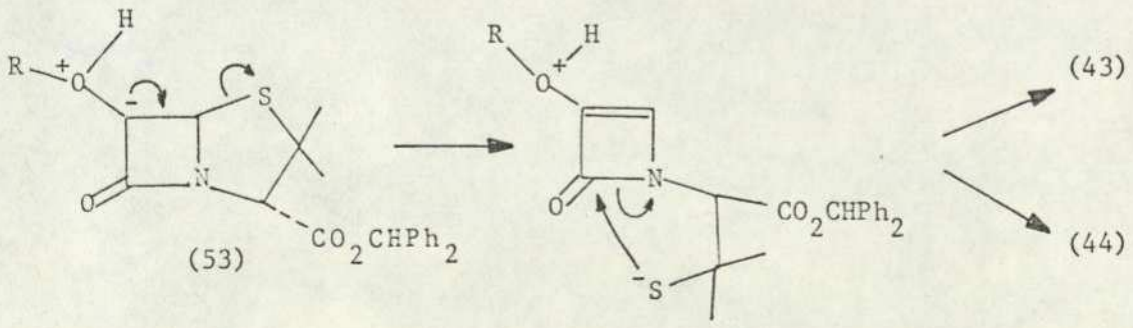
Use of BF_3Et_2O gave a high yield (72%) of 6-methoxypenicillanate (44a) and no thiazepine was detected.

Use of TsOH* as catalyst gave an identical result. On the other hand, catalysis by both $Rh_2(OAc)_4$ and $Cu(acac)_2$ led to the formation of methoxythiazepine as well as methoxypenicillanate. (Table 2).

	$Rh_2(OAc)_4$		$Cu(acac)_2$	
	%	%	%	%
Alcohols	(44)	(43)	(44)	(43)
MeOH	55	19	56	23
EtOH	12	75	20	29
t-BuOH	6	72		
$PhCH_2OH$	<2.5	67		
EtOH-D.B.N.	55	20		
$CH_2=CHCH_2OH$	<2.5	70	12	56

Table 2. Reactions of benzhydryl 6-diazopenicillanate (1) with alcohols.

The formation of thiazepine (43) was analogously rationalized in terms of the oxonium ylide intermediate (53). The 6α-alkoxypenicillanate (44) could arise either via proton transfer in the ylide (53) or by an independent pathway.



* p-Toluenesulfonic acid monohydrate

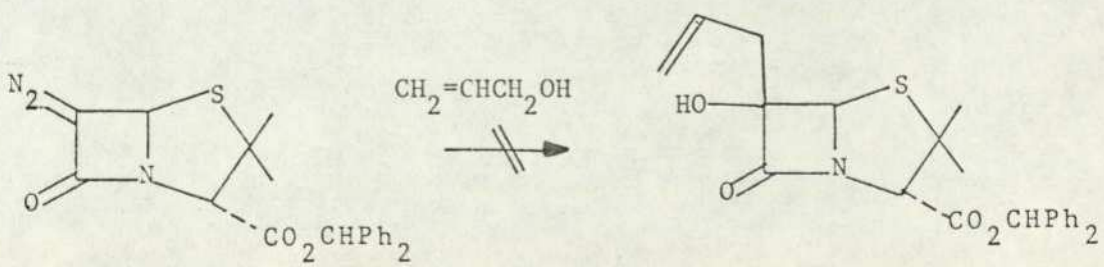
The trend in product ratios for methanol, ethanol and t-butanol with $Rh_2(OAc)_4$ catalyst parallels the trend in acidity (MeOH>EtOH>t-BuOH) and is in the same order as the relative rates of rhodium-catalysed O-H insertion reactions of diazoacetic esters (refer to the review). An explanation consistent with these observations is that the product ratio is kinetically controlled by the relative rate of rearrangement of the oxonium ylide (53) and a competing proton transfer pathway, which may involve (53) as a common intermediate. In agreement with this mechanism, when the 6-diazopenicillanate (1) was added to 0.1 equivalent of 1,5-diazabicyclo [4,3,0] non-5-ene (D.B.N.) in ethanol containing $Rh_2(OAc)_4$, there was a substantial change in product yields, with the ethoxypenicillanate now being favoured, (Table 2). In ethanol alone or ethanol-D.N.B, in the absence of rhodium acetate catalyst, the 6-diazopenicillanate showed little decomposition after 48 h and neither ethoxypenicillanate nor ethoxythiazepine could be detected. The effect on product ratios of changing the metal catalyst is also noteworthy (Table 2) and implies a role for the metal either in assisting proton transfer or in co-ordinating to ylide (53).

It will be noted from Table 1 that the effect of adding DBN to the rhodium-catalysed reaction of 6-diazopenicillanate with trimethyl orthoformate was also to promote formation of 6α-methoxypenicillanate at the expense of methoxy-thiazepine.

It is clear that the metal-catalysed reactions of benzhydryl 6-diazopenicillanate with alcohols give rise to mixtures of 6α-alkoxy-penicillanate and alkoxythiazepine, the former product predominating in the case of methanol. However, reaction with methyl and ethyl

orthoformate esters give alkoxythiazepines only Thus, it can be concluded that the reactions with orthoesters do not involve free alcohol produced by chance hydrolyses, but proceed via direct combination between the metal carbenoid and the orthoformate. Both alcohols and orthoesters give rise to related, but different, oxonium ylide intermediates in the metal-catalysed reactions. The fate of these intermediates can be influenced by the presence of an agent such as DBN, which assists proton transfer and favours the production of 6α-alkoxypenicillanate.

In an attempt to provide an alternative pathway for the intermediate oxonium ylide rearrangement the reaction with allyl alcohol was studied. Allyl-substitute ylides of sulphur, selenium, (refer to Chapter 1) and nitrogen[17] are known to undergo 2,3-sigmatropic shifts affording 6-allylpenicillanates. However, both the rhodium- and copper-catalysed reactions of the 6-diazopenicillanate (1) with allyl alcohol gave mainly allyloxy-thiazepine (43e) and the 6-allyl-6-hydroxypenicillanate (54) was not observed. In contrast with these results (Table 2), the $BF_3{\cdot}Et_2O$ -catalysed reaction with allyl alcohol gave 6α-allyloxypencillanate (44e) in 70% yield and no thiazepine could be detected.



(1) (54)

The behaviour of photolytic decomposition of 6-diazopenicillanate in alcohol was also studied briefly.

The mixture of 6-diazopenicillanate (1) with ethanol in a quartz vessel was irradiated under nitrogen atmosphere with a medium-pressure Hanovia light source. Removal of the excess ethanol under vacuum and chromatography of the crude mixture on silicic acid gave 6α-ethoxy-penicillanate (44b) in 4% yield, together with a major product in 50% yield, which has not yet been fully characterized. The i.r. data showed an absorption band at 1740 cm^{-1}, the ^1H n.m.r. spectrum exhibited signals for ethoxy protons two doublets (J=2Hz) at τ6.16 (1H), 7.30 (1H), singlet at τ6.48 (1H), two very close (δ= 0.1) singlets (2 x CH_3) at τ8.60, 8.70, indicating this is a rearrangement product other than a β-lactam. No thiazepine could be isolated from the product mixture, implying that a different pathway is involved in the photolysis of 6-diazo-β-lactam in alcohol.

The rhodium acetate catalysed decomposition of 6-diazopenicillanate (1) in diethyl ether was also investigated briefly, which gave ethoxy - thiazepine (43b) in a 6% yield and no ethoxypenicillanate was detected.

The Lewis acid BF_3Et_2O catalysed reaction of 2,2,2,-trichloroethyl 6-diazo-penicillanate with alcohols (MeOH,t-BuOH or $PhCH_2OH$) or diethyl ether has been previously reported[18], (refer to Chapter 1) where 6α-alkoxypenicillanates were claimed to be the major products. This result has been confirmed by us for two cases (methanol and allyl alcohol) where no thiazepines were observed.

Thomas et al.[18] proposed three possible mechanisms for BF_3Et_2O catalysis of reactions between alcohols and 6-diazo-β-lactam: the

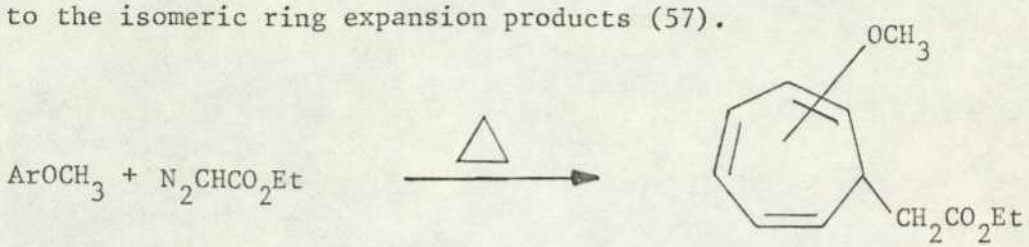
$BF_3{\cdot}Et_2O$ may coordinate with the alcohol which then protonates the 6-diazopenicillanate. Alternatively, the $BF_3{\cdot}Et_2O$ may coordinate with the 6-diazopenicillanate so making it more electrophilic and more susceptible to attack by a nucleophile. A third possibility is the participation of a BF_3—complexed carbene formed by N_2 loss from BF_3 co-ordinated 6-diazopenicillanate. Whatever the mechanism involved in Thomas's work, it is clearly different to that operating in the metal-catalysed reactions we have studied. The similarily between the results of BF_3 catalysis and the effects of TsOH as catalyst seem to argue in favour of a mechanism involved protonation of the diazo compound.

Finally, the reaction of benzhydryl 6-diazopenicillanate with an aryl alkyl ether (anisole) was also examined. The results discussed so far provide compelling evidence that the rhodium-carbenoid species,whatever its precise structure, exhibits distinct electrophilic behaviour and reacts particularly effectively with nucleophilies. Anisole was therefore selected as a reagent which, in addition to possessing lone pairs in the ether oxygen, has the potential to act as a carbon nucleophile via activation of the benzene ring. The literature on reactions of diazo compounds with anisole provides evidence that it can function as both an O- and C-nucleophile, the former giving rise to products of O-C and C-H in the side chain, and the latter to ring substitution and ring expansion products.

Johnson, Langemann and Murray[19] reported that anisole (55) gave rise to aryloxyacetate (56) when heated with ethyl diazoacetate.

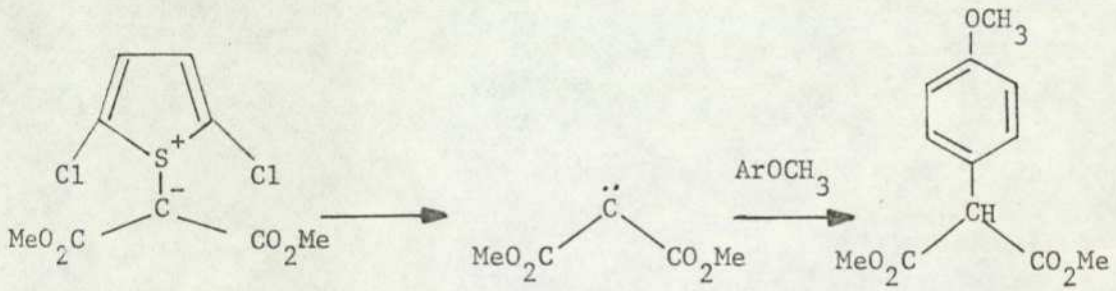
$$\underset{(55)}{Ar\text{-}OCH_3} + N_2CHCO_2Et \xrightarrow{\Delta} \underset{(56)}{ArOCH_2CO_2Et}$$

Bartels-Keith, Johnson and Langemann[20], reported that the thermal reaction of anisole with ethyl diazoacetate probably gave rise to the isomeric ring expansion products (57).



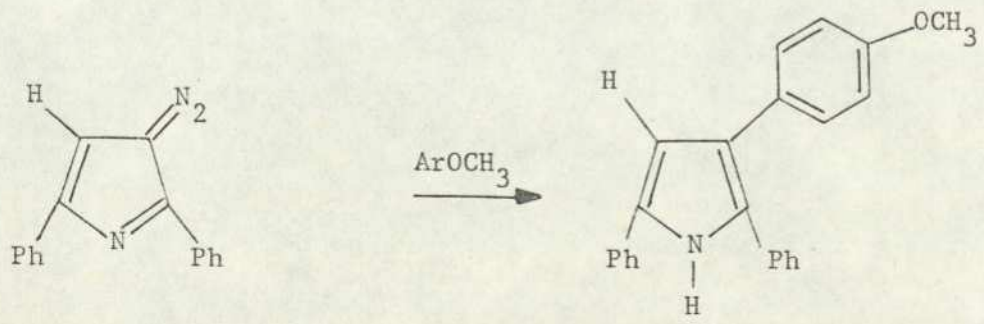
(57)

Gillespie and Porter[21] demonstrated that the aryl malonate (60) can be prepared by the reaction of anisole with bismethoxy-carbonyl-carbene (59), which was generated from the metal catalysed decomposition of 2,5-dichlorothiophenium ylide (58).



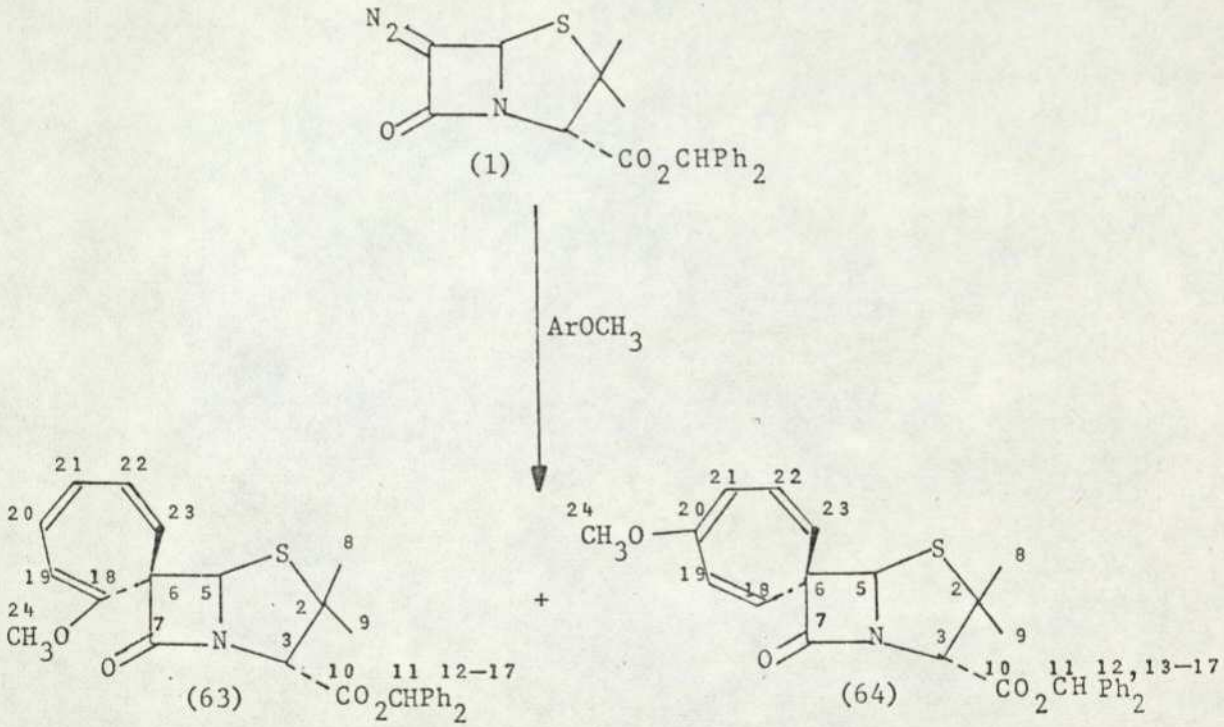
(58) (59) (60)

The thermal decomposition or irradiation of 3-diazo-2,5-diphenyl-pyrrole(61) in anisole resulted in the substitution product 3-(p-methoxy-phenyl)-2, 5-diphenyl pyrrole (62)[22]



(61) (62)

We observed that addition of 6-diazopenicillanate (1) in anisole to a mixture of anisole and dichloromethane in the presence of rhodium acetate led to the slow evolution of nitrogen and to the formation of a number of products, which were separated by conventional chromatography or preparative HPLC and characterized. Two of them were identified to be the isomeric pair of ring expansion products (63) and (64) in 1% and 4% yields, respectively. Neither C-H nor C-O bond insertion products were detected. Use of rhodium trifluoroacetate instead of rhodium acetate in the same reaction furnished identical products (63) and (64) in improved yields (2 and 13% respectively).



Spectroscopic data established the structures of these isomers. The i.r. spectra were almost identical, showing β-lactam absorption at 1770 cm^{-1}.

The 100 MHz n.m.r. spectrum of (64) exhibited a singlet at τ6.36 for methoxy proton and partial overlapped signals at τ 3.5-4.6 for heptatriene protons which were not differentiated due to the limitation of the 100 MHz instrument. ^{13}C n.m.r. data were more informative, showing distinguishable signals for 3-methoxyheptatriene carbons of (64) (Table 3).

However the stereochemistry of (64) was resolved by application of n.o.e. difference spectroscopy, together with the latest spin-decoupling difference n.m.r. spectroscopy[23]. In the latter, a computer is used to subtract a control spectrum from a decoupled one, so that only those resonances directly coupled to the irradiated proton appear in the difference spectrum. Figure 1(c) shows the effect of irradiating C_{18}-H; response occurs only from C_{19}-H. Figure 1(b) illustrates the influence of the irradiation of C_{19}-H; responses appear only from C_{18}-H and C_{21}-H. The similar experiment was carried out with C_{21}-H, C_{22}-H and C_{23}-H(Figures 2 and 3). The result of such experiments is an over-determined, completely unambiguous assignment of all the proton chemical shifts of 3-methoxy-heptatriene of (64) (Table 3).

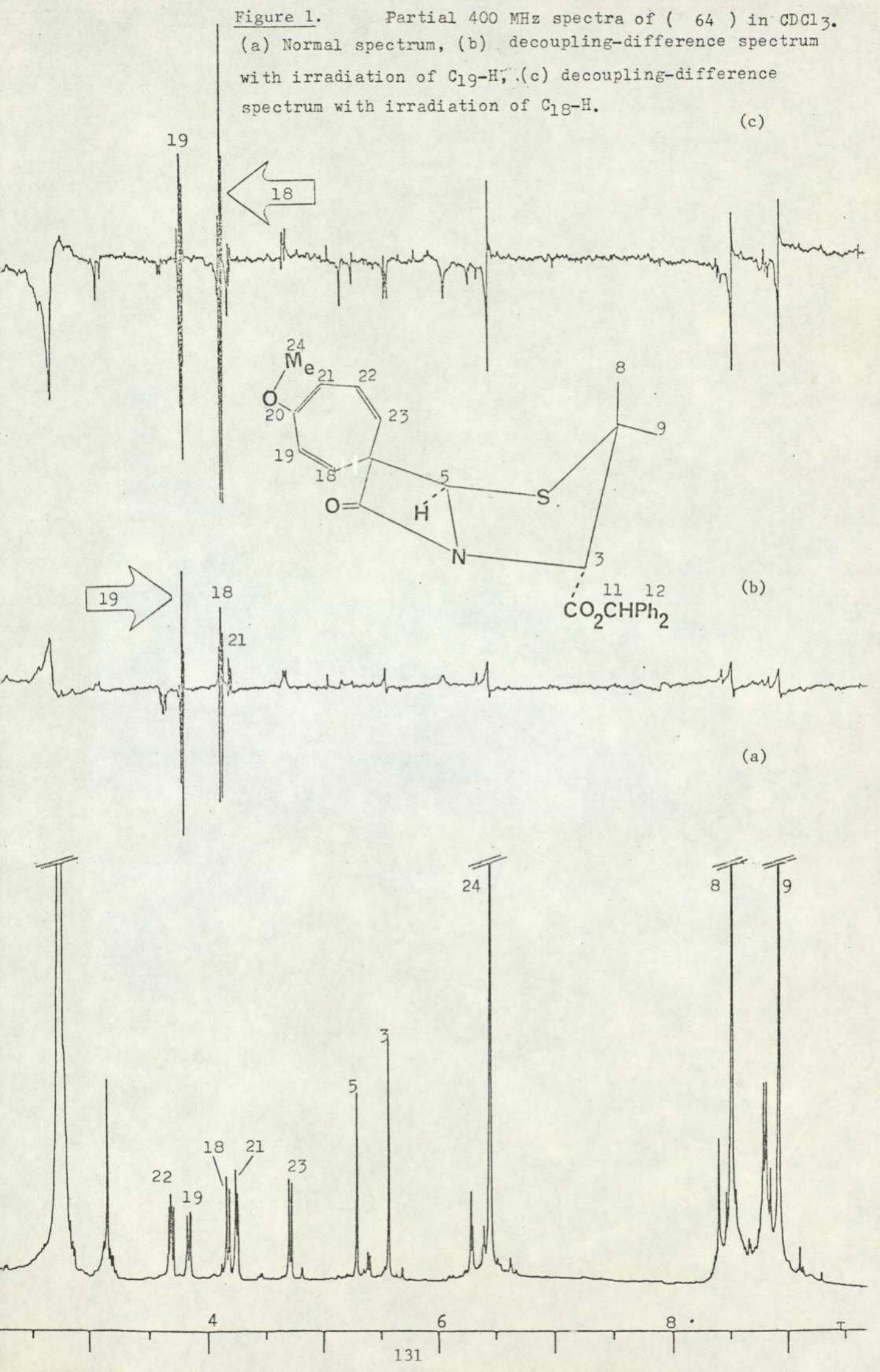
	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}
^1H τ 400MHz	4.20 $J_{18,19}$=10Hz	3.80 $J_{19,21}$=2Hz	-	4.30 $J_{21,22}$=7.3Hz	3.70 $J_{22,23}$=9.5Hz	4.70
^{13}C δ	123.060	124.100	159.836	114.743	125.139	104.868

Table 3. Part of ^{13}C and ^1H n.m.r. data of (64). In $CDCl_3$.

The result of n.O.e. - difference spectroscopy is shown in Figure (4) and Table (4). As seen in Figure 4(c) n.O.e. was found between C_5-H and C_{18}-H, and the Figure 4(b) displays the n.O.e. between C_8-H and C_{23}-H , which confirmed the stereochemistry of (64).

Analogously, by employing the combination of n.O.e.- difference and spin decoupling-difference n.m.r. spectroscopy, the configuration

Figure 1. Partial 400 MHz spectra of (64) in $CDCl_3$. (a) Normal spectrum, (b) decoupling-difference spectrum with irradiation of C_{19}-H, (c) decoupling-difference spectrum with irradiation of C_{18}-H.



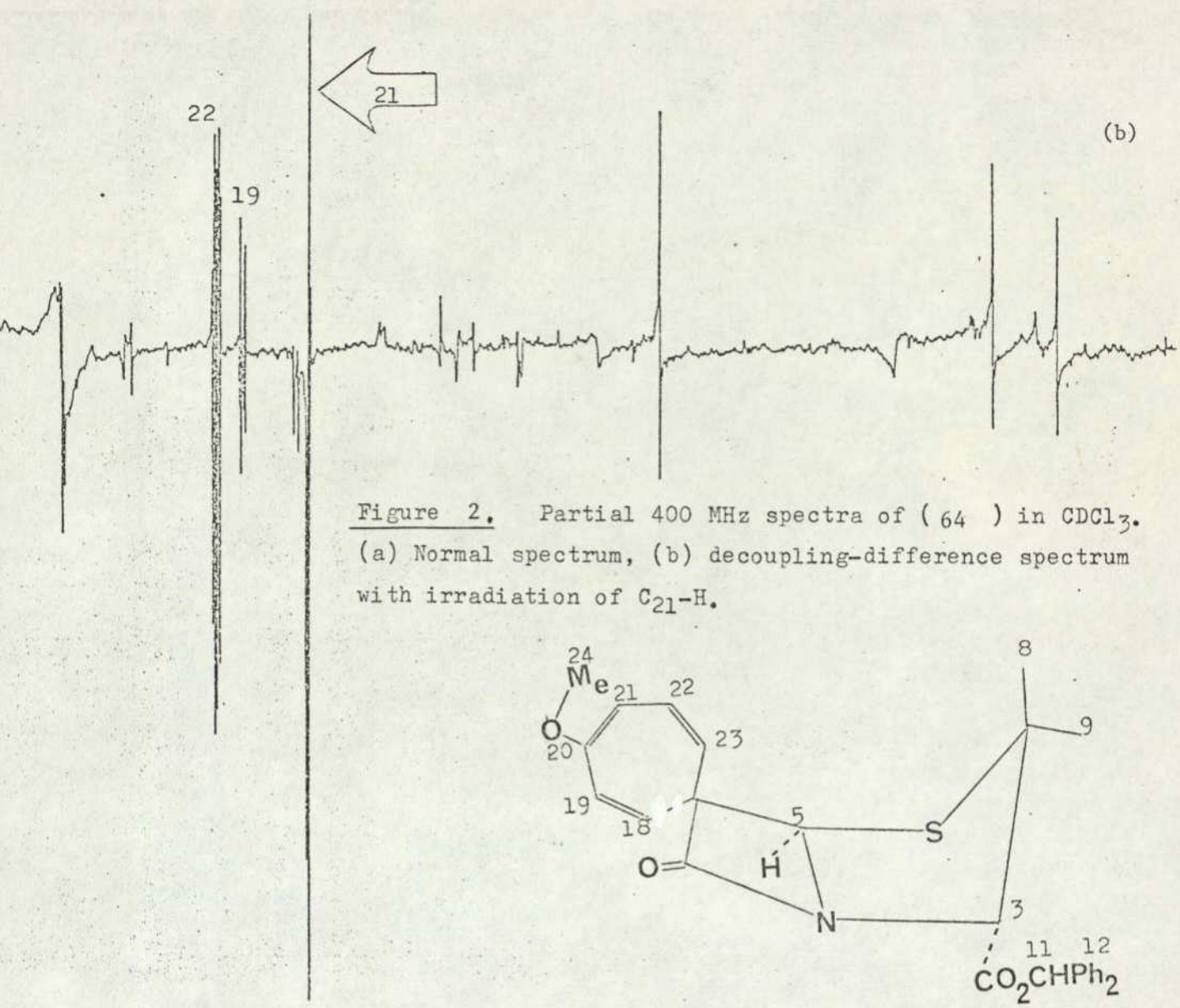
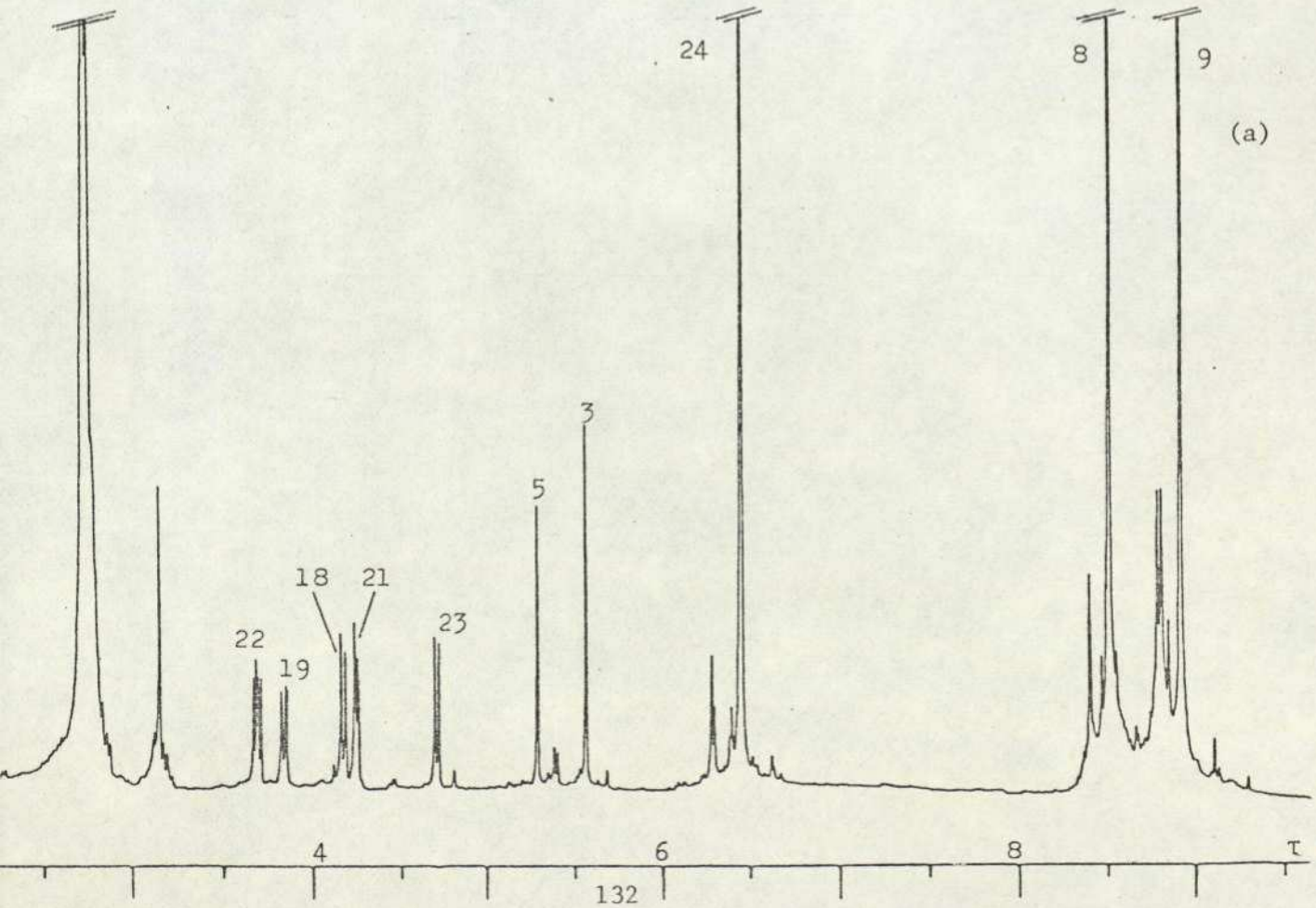


Figure 2. Partial 400 MHz spectra of (64) in $CDCl_3$. (a) Normal spectrum, (b) decoupling-difference spectrum with irradiation of C_{21}-H.



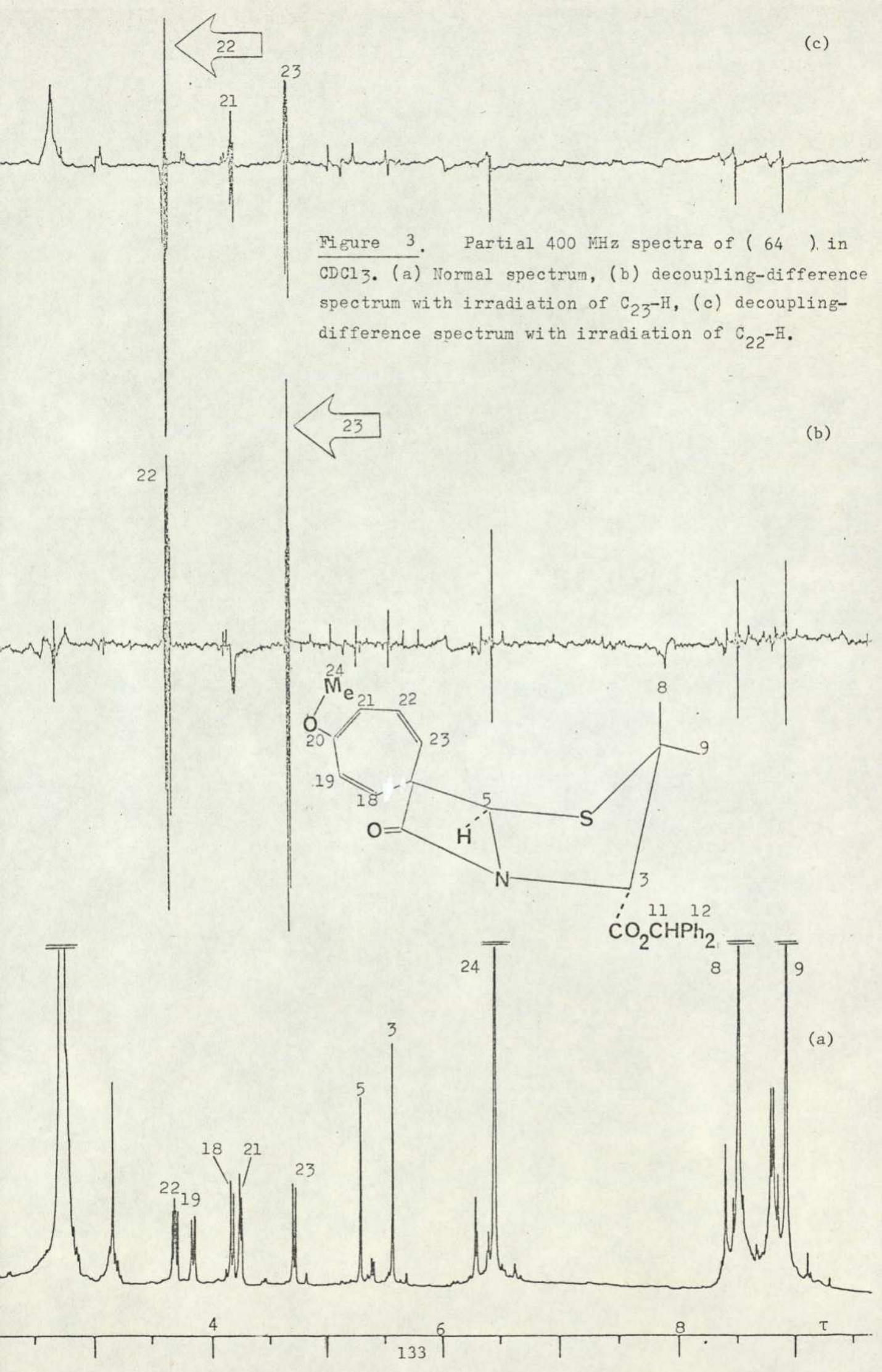
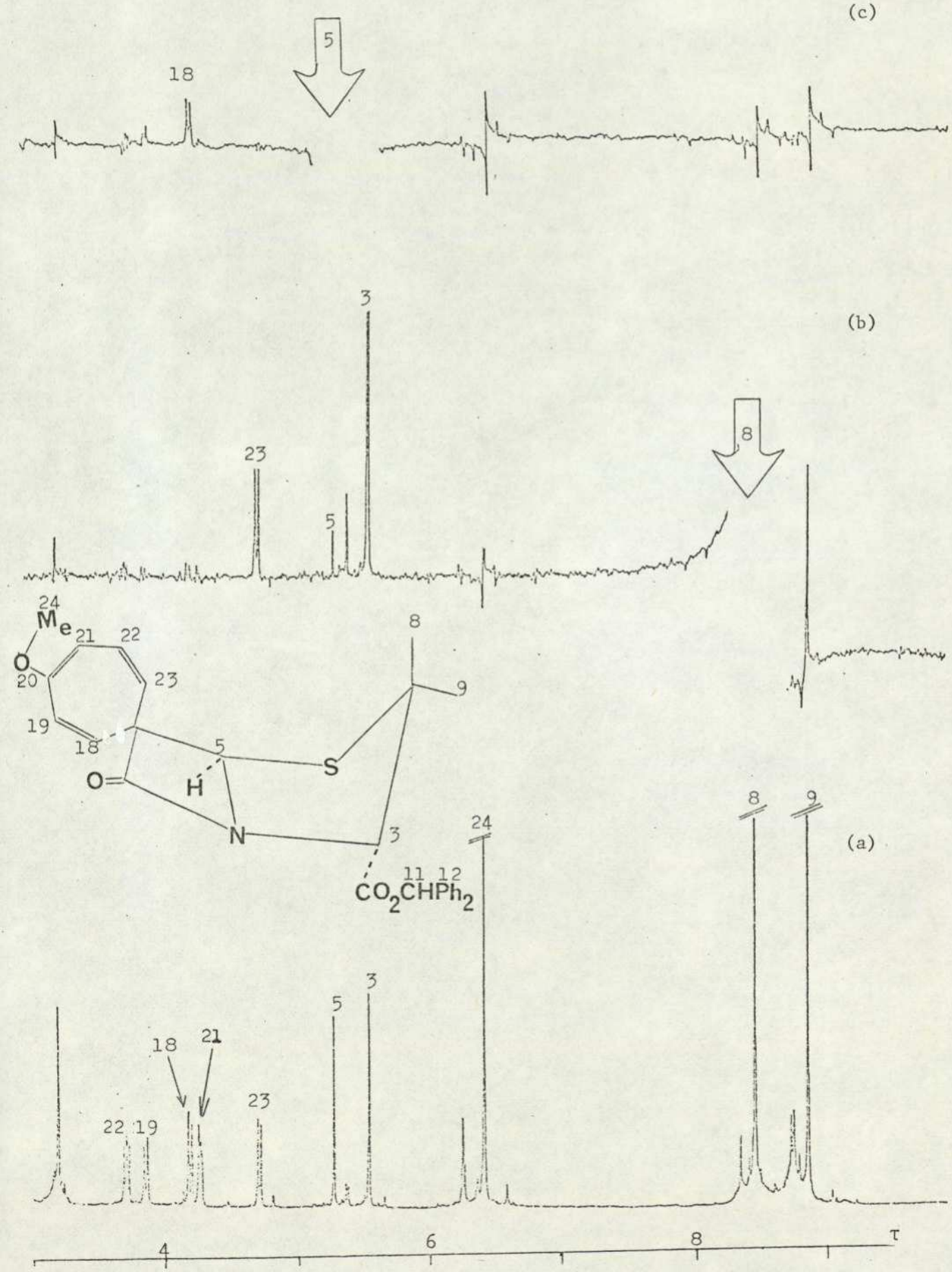


Figure 3. Partial 400 MHz spectra of (64) in $CDCl_3$. (a) Normal spectrum, (b) decoupling-difference spectrum with irradiation of C_{23}-H, (c) decoupling-difference spectrum with irradiation of C_{22}-H.

Figure 4. Partial 400 MHz spectra of (64) in $CDCl_3$. (a) Normal spectrum, (b) n.O.e. difference spectrum with pre-irradiation of C_8-H, (c) n.O.e. difference spectrum with pre-irradiation of C_5-H.



of isomer (63) was successfully assigned. (Table 5).

Proton irradiated	Observed n.O.e.
3	8, 9
5	18
9	5, 11, 3
11	12
18	19, 5
19	18
21	22, 24
22	21, 23
23	22

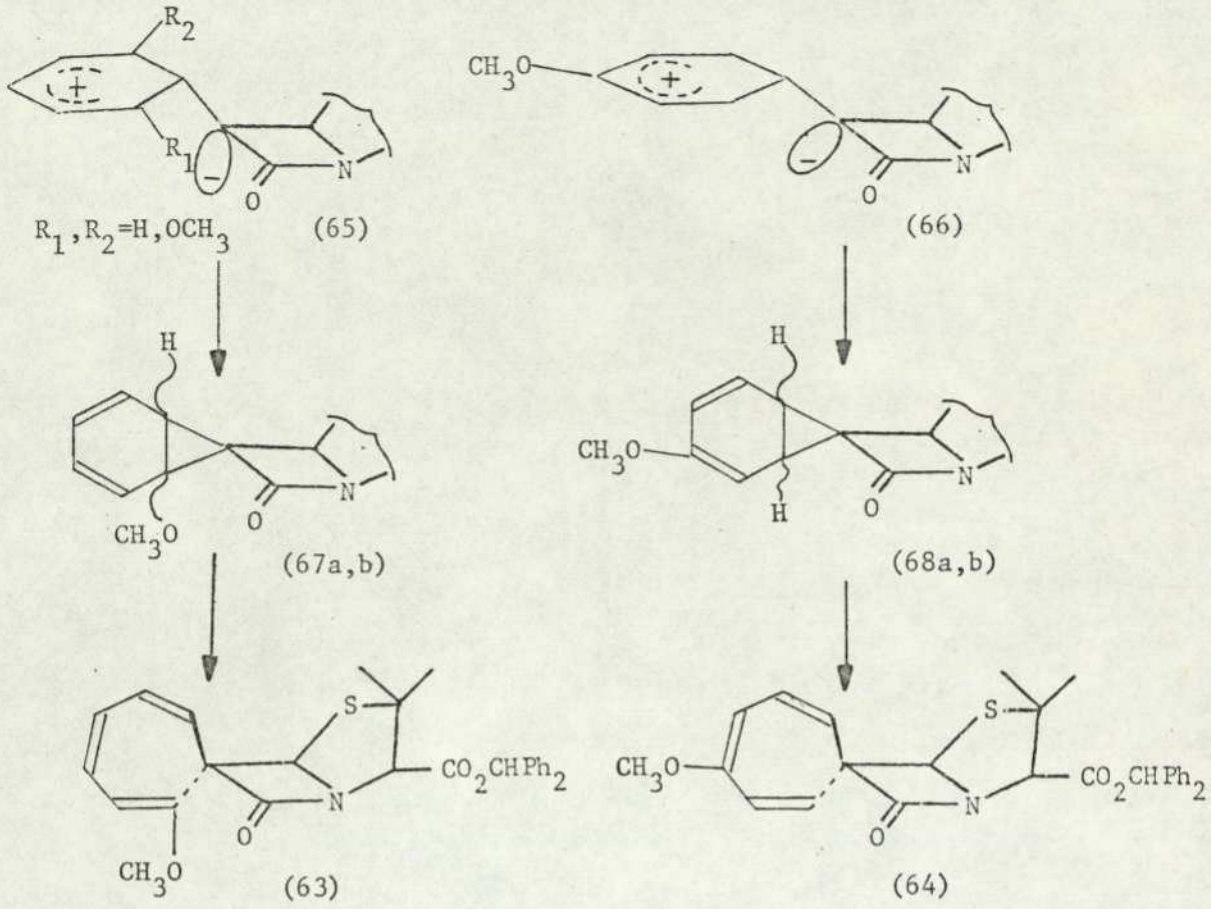
Table 4. Proton-Proton nuclear Overhauser enhancement in (64).

Proton irradiated	Protons decoupled	Protons in n.O.e.
9	-	8 ,3,5,11,12
8	-	9 ,3,23
3	-	8
5	-	-
11	-	12
12	-	-
23	22	22
22,20	23,21,19	23,21,19
21	22,20	22,20
19	20,24	20,24
24	-	19,5

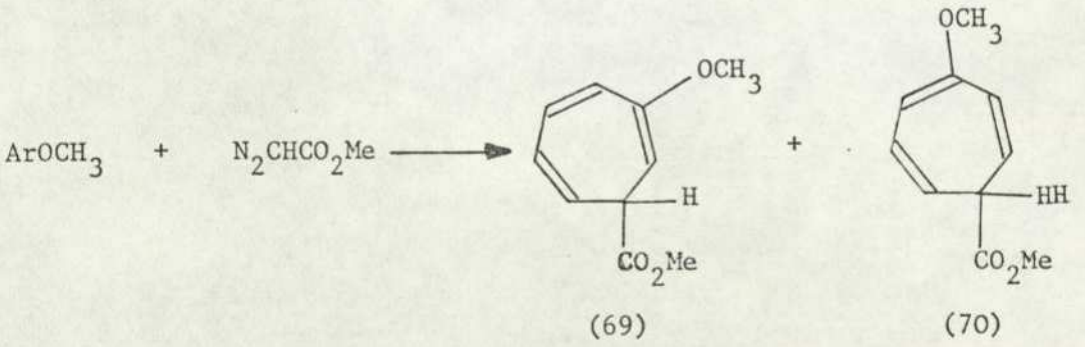
Table 5. Proton-proton n.O.e. and proton-proton decoupled in (63).

The formation of both the observed products is explained by the attack of anisole (o- or p-) on the upper face of a rhodium-complexed carbenoid to give the dipolar species (65) and (66). Collapse of each of these can take place to give two possible norcaradienes (67a,b) and (68a,b) respectively, both the subsequent ring opening of each pair of stereoisomers leads to a single cyclo-heptatriene (Scheme 3). Thus, the observed specificity in the formation of the products (63) and (64) stems entirely from the initial attack of anisole from the β-face of the penicillanate. Presumably, the bulky rhodium catalyst is complexed at the less hindered α-face of the β-lactam ring, preventing attack from this side, and collapse of the intermediate dipolar species (65) and (66) is faster than inversion at C_6. This contrasts with the thiophene reaction, in which the thiophene must also be presumed to attack from the upper face of the β-lactam but the greater stability of the thiophenium ylide allows time for inversion through to the lower face as the reaction proceeds (Scheme 3). (see chapter 3)

Whilst this work was in progress, Hubert et al.[24] published a very closely related result which is in agreement with the present work. The rhodium trifluoroacetate catalysed reaction of methyl diazoacetate with anisole was reported to afford the isomeric ring expansion adducts (69) and (70), and the later 4-methoxy isomer was the major product (Scheme 4).



Scheme 3.



Scheme 4.

It was claimed that benzene can also react in the same manner as anisole to give the ring expansion product in quantitative yield, and the effiency of the rhodium catalyst is strongly dependent on the electron withdrawing ability of carboxylate ligand(Table 6).

Ligand	CF_3CO_2H	$C_6F_5CO_2H$	$MeOCH_2CO_2H$	$MeCO_2H$
pKa	0.23	1.48	3.57	4.76
Yield %	100	89	30	7

Table 6 The influence of various rhodium carboxylates upon the yield of reaction of methyl diazoacetate with benzene.

Encouraged by Hubert's result, an investigation on the rhodium trifluoroacetate catalysed decomposition of 6-diazopencillanate in benzene was carried out.

It was discovered that, after stirring the mixture of 6-diazo-penicillanate (1) in benzene containing a catalytic amount of $Rh_2(CF_3CO_2)_4$ at room temperature for 16 h, the characteristic 6-diazo-β-lactam absorption band at 2080 cm^{-1} was still present in the i.r. spectrum of the product mixture. The 6-diazopenicillanate (1) disappeared when the reaction mixture was refluxed in benzene for 2 h, but no corresponding ring expansion adduct could be detected following work up of the reaction mixture and silica gel chromatography. Hence 6-diazo-β-lactam shows once more a significantly lower reactivity than the alkyl diazoacetates.

4.3. Experimental

2-phenyl-1,3-dioxolane (39).

The mixture of ethylene glycol (6.67g 0.107 mol), benzaldehyde (11.35g 0.107 mol) and oxalic acid dihydrate (200 mg 1.59 mmol) in benzene (100 ml) was refluxed for 20 h, then the product mixture was cooled down and solid K_2CO_3(219 mgm 1.59 mmol) was added with shaking. After removal of the benzene on a rotary evaporator, the residue was subjected to fractional distillation under vacuum with a Vigreaux column. A fraction was collected at 94-96°/4mm to give 6g (40%) of colourless oily 2-phenyl-1,3-dioxolane (39), ν_{max}(liquid film) 2880 (CH_2), 1090, 1070 cm^{-1}(C-O), $\tau(CDCl_3)$ 2.65(5H, m, Ph-H), 4.28 (1H,s,CH), 6.08 (4H, m, 2xCH_2).

Reaction of 6-diazopenicillanate (1) with dioxolane (39).

The solution of 6-diazopenicillanate (1) (500 mgm 1.27 mmol) in benzene (5 ml) was added dropwise in a period of one hour to the mixture of dioxolane (39) (2 ml; excess) containing either $Rh_2(OAc)_4$ or $Cu(acac)_2$ (5 mgm), in an ice bath with stirring. The mixture was stirred at room temperature overnight, then submitted to prep.t.l.c. (SiO_2), CH_2Cl_2/Et_2O, 95/5). Five components were isolated, none of which corresponded to compounds (40) or (41).

General procedure for the reaction between benzhydryl 6-diazopenicillanate (1) with alcohols, orthoesters or diethyl ether.

The 6-diazopenicillanate (1) in alcohol, orthoester or diethyl ether[a], was added dropwise in a period of time ($\frac{1}{2}$-2 hours) to the ice cooled alcohol, orthoester or diethyl ether[b] containing catalyst

$(Rh_2(OAc)_4$, $Cu(acac)_2$, TsOH, $BF_3{:}Et_2O$, $Rh_2(OAc)_4$ + D.B.N. or $Rh_2(OAc)_4$ + $(CH_3)_3SiCl$ +$((CH_3)_3Si)_2NH_2$ with stirring. When all the diazo compound had disappeared, the crude mixture[d] was submitted to prep.t.l.c or column (SiO_2 , eluent CH_2Cl_2 first, then CH_2Cl_2/Et_2O 95:5). The less polar component was 6α-alkoxy-penicillanate (44) and the far more polar component was alkoxythiazepine (43).

a. All the alcohols were treated with anhydrous Na_2CO_3 and redistilled prior to use.

b. In benzyl and allyl alcohols, cosolvent CH_2Cl_2 was employed.

c. Amount of catalyst: $Rh_2(OAc)_4$ 0.01 wt %, $Cu(acac)_2$ 0.02 wt %, TsOH 5 wt %, $BF_3{:}Et_2O$ 10%, $Rh_2(OAc)_4$ + D.N.B., 1% + 3%, $Rh_2(OAc)_4$ + $(CH_3)_3SiCl$+$((CH_3)_3Si)_2NH$, 2% + 30% + 30%.

d. If any precipitate of thiazepine was formed, this was filtered off first, then combined with the portion isolated from chromatography.

Methoxythiazepine (43a).

White solid, m.p. 204-208°, $\lambda_{max}(CH_3OH)$ nm 215 (log ε 4.05), 262 (log ε 3.61), 330 (log ε 3.93), ν(KBr) cm^{-1} 3250 (NH), 1740 (C=O), 1620 (unsaturated thiolactone), 1545 (C=C), ^1H n.m.r. ($CDCl_3$)τ 264 (10H, m, aromatic H), 3.00 (1H, d, 8Hz, C_5-H), 3.10 (1H, s, C_{11}-H), 4.48 (1H, m, NH), 5.70 (1H, d, 6Hz, C_3-H), 6.46 (3H, s, CH_3O), 8.54 (3H, s, C_8-H), 8.70 (3H, s, C_9-H). Addition of D_2O to the solution caused the signal at τ 4.48 to disappear and the doublets to collapse to singlets. ^{13}C n.m.r. ($CDCl_3$) δ 186.062(C_7), 168.812 (C_{10}), 139.192 (C_6), 138.280 (C_5), 135.026 (C_{12}), 128.968, 127.926,

127.213 (C_{13-17}), 79.361 (C_{11},masked by $CDCl_3$, but can be seen in $(CD_3)_2CO$), 68.359 (C_{18}), 62.694 (C_3), 48.372 (C_2), 27.799 (C_8), 25.780 (C_9), (Found: C, 66.57; H, 5.86; N, 3.59; $C_{22}H_{23}NO_{45}$ requires C, 66.67; H, 5.80; 3.53%), m/e: 396(M^+; 0.4%), 167 (15.5%), 114 (4.5%), 86 (1.1%).

Ethoxythiazepine (43b).

White solid, m.p. 175-177°, ν_{max} (KBr) cm^{-1} 3230, 1740, 1620, 1540, λ_{max} nm 215 (log ε 4.11), 260 (log ε 3.68), 330 (log ε 4.02), ^1H n.m.r. ($CDCl_3$) τ2.75 (10H, m, aromatic H), 3.04 (1H, d, 8Hz, C_5-H), 3.12 (1H, s, C_{11}-H), 4.00 (1H, m, NH), 5.75 (1H, d, 6Hz, C_3-H), 6.33 (2H, m, $CH_3\underline{CH_2}O$), 8.55 (3H, s, C_8-H), 8.62 (3H, s, C_9-H), 8.85 (3H, t, $\underline{CH_3}CH_2O$), addition of D_2O caused the signal at τ 4.00 to disappear and the doublets to collapse to singlets, ^{13}C n.m.r. $(CD_3)_2CO$ δ 185.156 (C_7), 169.072 (C_{10}), 141.276 (C_6), 140.166 (C_5), 133.463 (C_{12}), 129.556, 129.166, 128.906, 128.385, 128.056 (C_{13-17}), 79.361 (C_{11}), 69.986 (C_{18}), 68.750 (C_3), 47.070 (C_2), 29.166 (C_8), 27.538 (C_9), 15.624 (C_{19}), (Found: 67.20; 6.15; 3.34; $C_{23}H_{25}NSO_4$ requires 67.13; 6.12; 3.40%), m/e: 411 (M^+; 0.2%), 382 (17%), 244 (0.1%), 167 (48%), 383 (1.1%), 216 (3.1%), 114 (2.2%), 86 (0.6%).

t-Butoxy thiazepine (43c).

Off white solid, m.p. 165-166°, ν(nujol) cm^{-1} 3280, 1740, 1625, 1570, ^1H n.m.r. ($CDCl_3$) 2.44, (10H, m, aromatic H),2.86 (1H, s, C_{11}-H), 2.95 (1H, d, 9Hz, C_5-H), 3.08 (1H, m, NH), 5.52 (1H, d, 6Hz, C_3-H), 8.50 (3H, s, C_8-H), 8.64 (3H, s, C_9-H), 8.76 (9H, s, t-butyl H), addition of D_2O to the solution caused the signal at τ 3.08 to disappear and the doublets to collapse to singlets, (Found: C, 68.43; H, 6.80; N,3.02; $C_{25}H_{29}O_4NS$ requires C, 68.31; H, 6.65; N, 3.19%), m/e: 383 (0.4%),

56 (2.2%), 114 (4.4%), 86 (2.6%), 131 (0.1%), 69 (0.5%), 102 (4.2%), 85 (3.5%).

Allyloxythiazepine (43d) - Off white solid, m.p. 163-165° ν(nujol) cm^{-1} 3230, 1745, 1620, 1520, 1170, ^1H n.m.r. ($CDCl_3$) τ 2.46 (10H, m, aromatic H), 2.80 (1H, d, 7.5Hz, C_5-H), 2.96 (1H, s, C_{11}- H), 3.94 (1H, m, CH_2=CH-CH_2-O-), 4.46 (1H, m, NH), 4.54-4.72 (2H, m, CH_2 =CH-CH_2-O-), 5.52 (1H, d, 6Hz, C_3-H), 5.64 (2H, d, 6Hz, CH_2=CH-CH_2-O-), 8.50 (3H, s, C_8-H), 8.66 (3H, s, C_9-H), addition of D_2O to the solution caused the signal at τ 4.46 to disappear, and doublets at τ 2.80 and 5.52 to collapse to singlets, (Found: C, 68.33; H, 5.97; N, 3.09; $C_{24}H_{25}NO_4S$ requires C, 68.06; H, 5.95; N, 3.31%), m/e: 423 (M^+; 0.6%), 256 (0.2%), 167 (100%), 114 (1.8%), 102 (0.3%), 131 (0.2%), 85 (2.4%), 86 (2%), 69 (4.7%), 56 (2.7%).

Benzyloxythiazepine (43e) - Off white solid, m.p. 136-139°, ν(nujol) cm^{-1}, 3280, 1730, 1625, 1550, 1520, ^1H n.m.r. ($CDCl_3$) τ 2.50 (15H, m, aromatic H), 2.92 (1H, s, $C_{11}H$), 3.10 (1H, d, 9Hz, C_5-H), 4.40 (1H, m, NH), 5.12, 5.24 (2H, dd AB, 12Hz, $PhCH_2O$), 5.68 (1H, d, 6Hz C_3-H), 8.60 (3H, s, C_8-H), 8.72 (3H, s, C_9-H), addition of D_2O to the solution caused the signal at τ 4.40 to disappear and the doublets at τ 3.10, 5.68 to collapse to singlets, (Found: C, 71.02; H, 5.74; N, 2.96; $C_{28}H_{27}NO_4S$ requires C, 71.01; H, 5.75; N, 2.96%), m/e: 473 (M^+; 0.2%), 167 (54.3%), 114 (2.7%), 86 (2.1%), 131 (0.2%), 69 (0.1%), 102 (3.32%), 85 (1%), 91 (100%).

6α-methoxypenicillanate (44a) - Colourless oil, ν($CHCl_3$) cm^{-1} 1770, 1740, τ($CDCl_3$) 2.50 (10H, s, aromatic H), 2.93 (1H, s, C_{11}-H), 4.75 (1H, d, 1.5Hz, C_5-H), 5.28 (1H, d, C_6-H), 5.30 (1H, s, C_3-H), 6.40 (3H, s, CH_3-O-), 8.70 (3H, s, C_8-H), 8.40 (3H, s, C_9-H), m/e: 397 (M^+, 6%), 158 (0.7%), 167 (100%), 99 (1.5%), 59 (5.1%), 72 (3.8%), 31 (17.4%), 41 (5.5%), 57 (4.3%), 44 (3.1%).

6α-ethoxypenicillanate (44b) - Colourless oil, $\nu(CHCl_3)$ cm^{-1} 1770, 1740, τ ($CDCl_3$) 2.70(10H, m, aromatic H), 3.13 (1H, s, C_{11}-H), 4.70 (1H, d, 1.5 Hz, C_5-H), 5.40 (1H, d, C_6-H), 5.45 (1H, s, C_3-H), 6.30 (2H, m, $CH_3\underline{CH_2}O$), 8.45 (3H, s, C_8-H), 8.75 (3H, s, C_9-H), 8.75 (3H, t, 7.5 Hz, $\underline{CH_3}CH_2O$), m/e: 411 (M^+; 10.5%), 167 (100%), 382 (0.2%), 99 (0.5%), 59 (1.1%), 86 (1%), 58 (0.8%), 71 (0.3%), 57 (1%).

6α-t-Butoxypenicillanate (44c) - Oil, $\nu(CHCl_3)$ cm^{-1} 1770, 1740, τ ($CDCl_3$) 2.64 (10H, m, aromatic H), 3.12 (1H, s, C_{11}-H), 4.84 (1H, d, 1.5 Hz, C_5-H), 5.34 (1H, d, C_6-H), 5.44 (1H, s, C_3-H), 8.44 (3H, s, C_8-H), 8.72 (12H, s, C_9-H overlapped with t-butyl protons), m/e: 439 (M^+; 2.5%), 382 (6.1%), 167 (100%), 9.9 (1.9%), 59 (1.6%), 57 (18%), 57 (0.2%), 114 (3.4%), 99 (1.9%).

6α-allyloxypenicillanate (44e) - Oil, $\nu(CHCl_3)$ cm^{-1} 1770, 1745, τ ^1H n.m.r. τ ($CDCl_3$) 2.70 (10H, m, aromatic H), 3.10 (1H, s, C_{11}-H), 3.95~4.30 (1H, m, $CH_2=\underline{CH}CH_2O$), 4.68 (1H, d, 1.5 Hz, C_5-H), 4.62~4.80 (2H, m, $\underline{CH_2}=CHCH_2O$), 5.36 (1H, d, C_6-H), 5.40 (1H, s, C_3-H), 5.86 (2H, m, $CH_2=CH\underline{CH_2}O$), 8.45 (3H, s, C_8-H), 8.72 (3H, s, C_9-H), ^{13}C n.m.r. δ ($CDCl_3$) 169.854 (C_{10}), 166.796 (C_7), 139.452 (C_{12}), 133.463 (C_{19}), 128, 838, 127.734, 127,343 (C_{13-17}), 119.140 (C_{20}), 91.078(C_6), 78.515 (C_5), 71.874 (C_{18}), 69.530, 69.270 (C_3, C_{11}), 64.452 (C_2), 33.984 (C_8), 25.390 (C_9), m/e: 423 (M^+; 0.7%), 382 (1.4%), 167 (100%), 99 (0.5%), 59 (1.7%), 57 (03%), 84 (31.8%), 56 (3.1%).

Reaction of 6-diazopenicillanate (1) with anisole. The 6-diazopenicillanate (1)(400 mgm 1mmol) in CH_2Cl_2 (5 ml) was added dropwise in a period of one hour to the mixture of anisole (1 ml) and rhodium trifluoroacetate (10 mgm) at room temperature with stirring. The reaction was complete in two hours. The excess anisole was removed by column chromatography (SiO_2 pet. ether 40-60°). The crude product was submitted to prep.t.l.c. (SiO_2, CH_2Cl_2) or prep. H.P.L.C. (Lichroprep. Si60, CH_2Cl_2). Isolation of the less polar fraction gave 6-spiro(2'-methoxycycloheptatrienyl) penicillanate (63) (10 mg; 2%) as oil, λ_{max} (CH_3CN) 224 nm(log ε 4.16), 297 nm (log ε 3.49), ν ($CHCl_3$) cm^{-1} 1775,1730, 1610, 1260, 1180, 1155, 750, 665 cm^{-1}, ^1H n.m.r. (400 MHz) ($CDCl_3$) τ 2.75(10H,m, aromatic H), 3.18(1H, s, C_{11}-H), 3.76(1H, C_{21}-H), 3.65(2H, C_{22},C_{20}-H), 4.49(1H, C_{19}-H), 4.58(1H, C_{23}-H), 5.35(1H, s, C_5-H), 5.53(1H, s, C_3-H), 6.40(3H, s, C_{24}-H), 8.53(3H, s, C_8-H), 8.96(3H, s, C_9-H), m/e: 473 (M^+; 10.3%), 325 (0.3%), 167 (100%), 158 (0.3%), 99 (1.4%), 59 (2.6%), 148 (88.3%), 118 (1.3%), 105 (14%).

Isolation of a slightly more polar fraction gave 6-spiro (4'-methoxycycloheptatrienyl) penicillanate (64) (62 mgm 13%) as oil, λ_{max} (CH_3CN) 225 nm (log ε 4.25), $\nu(CHCl_3)$ 1770, 1740, 1620, 1220, 750, 665 cm^{-1}, ^1H n.m.r. 400 MHz ($CDCl_3$) τ 2.60 (10H, m, aromatic H), 3.20 (1H, s, C_{11}-H), 3.70 (1H, dd, $J_{22,23}$ = 9.5 Hz, $J_{22,21}$ = 7.3 Hz), 3.80 (1H, dd, 11 Hz, 2Hz, C_{19}-H), 4.20 (1H, d, C_{18}-H), 4.30 (1H, dd, 2Hz, 7.3 Hz, C_{21}-H), 4.70 (1H, d, 9.5 Hz, C_{23}-H), 6.50 (3H, s, C_{24}-H), 8.55 (3H, s, C_8-H), 8.90 (3H, s, C_9-H), 5.30 (1H, s, C_5-H), 5.60 (1H, s, C_3-H),

^{13}C n.m.r. $\delta(CDCl_3)$ 174.910 (C_{10}), 166.983 (C_7), 159.836 (C_{20}), 139.174 (C_{12}), 128.518, 127.608, 126.959 (C_{13-17}), 125.139 (C_{22}), 124.100 (C_{19}), 123.060 (C_{18}), 114.743 (C_{21}), 104.868 (C_{23}), 78.228 (C_5), 75.239 (C_{11}), 69.132 (C_3), 63.674 (C_6), 63.414 (C_2), 54.837 (C_{24}), 31.576 (C_8), 26.119(C_9), m/e: 473 (M^+; 2.6%), 329 (0.5%), 167 (100%), 158 (0.2%), 99 (0.4%), 148 (33.3%), 118 (0.2%), 105 (5.9%).

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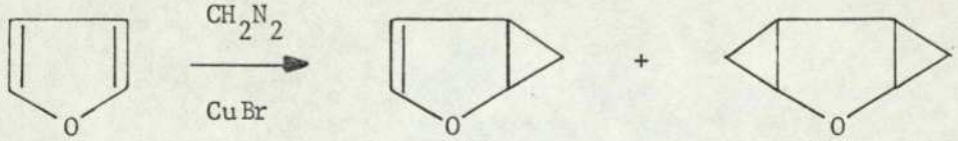
CHAPTER FIVE

SYNTHESIS AND TRANSFORMATIONS OF THE FURAN ADDUCTS OF BENZHYDRYL 6-DIAZOPENICILLANATE.

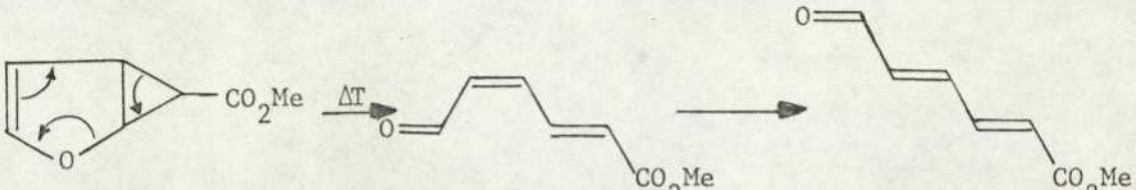
5.1. Review of reactions between carbenes and furans.

It has been shown that diazomethane reacts with furan in the presence of CuBr to give either monocyclopropyl (1) or dicyclopropyl (2) adducts, depending on the conditions[1].



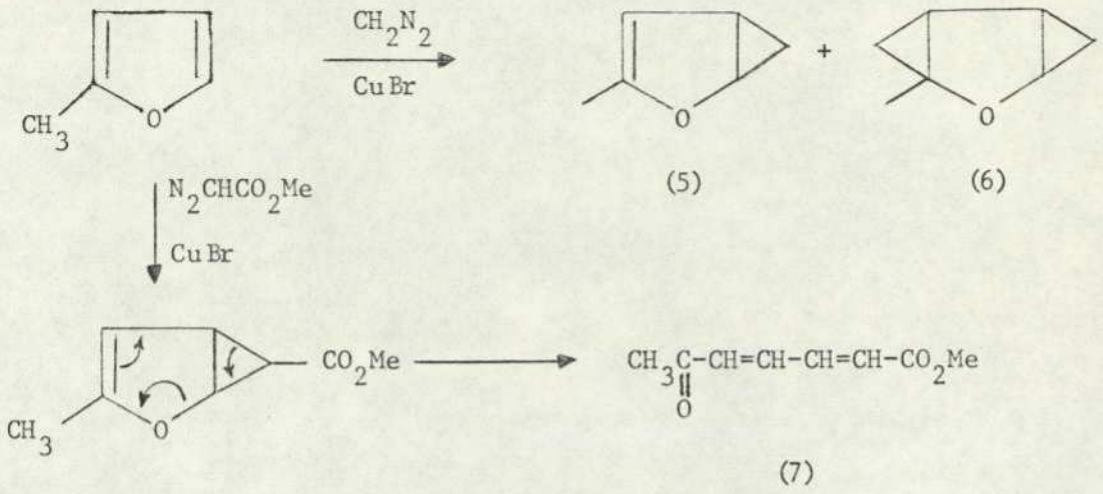
(1) (2)

Similarly, 2-oxabicyclo [3,1,0] hex-3-ene-6-carboxylate (3) is obtained from the addition of methyl diazoacetate to furan. Thermolysis of this product (neat or in solution at 140-150°) results in quantitative rearrangement to the dienal (4a), which is transformed into isomer (4b) by irradiation in the presence of iodine or treatment with HCl[2].



(3) (4a) ZE (4b) EE

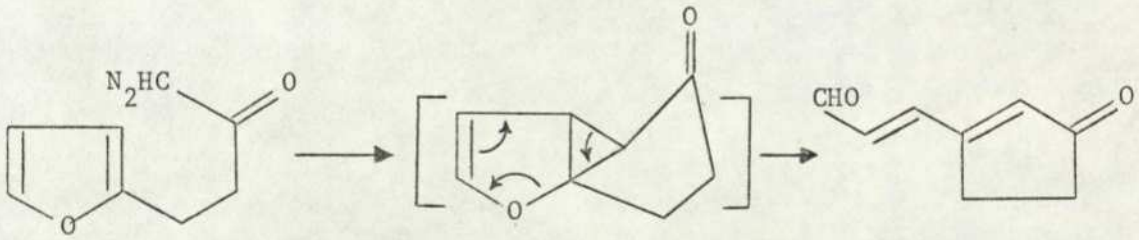
The CuBr-catalysed addition of diazomethane to 2-methylfuran affords mainly the monoadduct (5; 20%), together with some bisadduct (6; 7%), whereas methyl diazoacetate gives only methyl 6-oxo-2,4-heptadienoate (7; no yield quoted)[3]. Clearly, attack on the less substituted side of the furan ring is sterically favoured and the formation of the product (7) was interpreted as resulting from ring opening of a cyclopropane intermediate (Scheme 1).



Scheme 1.

It is characteristic in these reactions that the 2,3-double bond in the initially formed dienal has cis-stereochemistry, because it arises from the ring-opening process, e.g. (3).

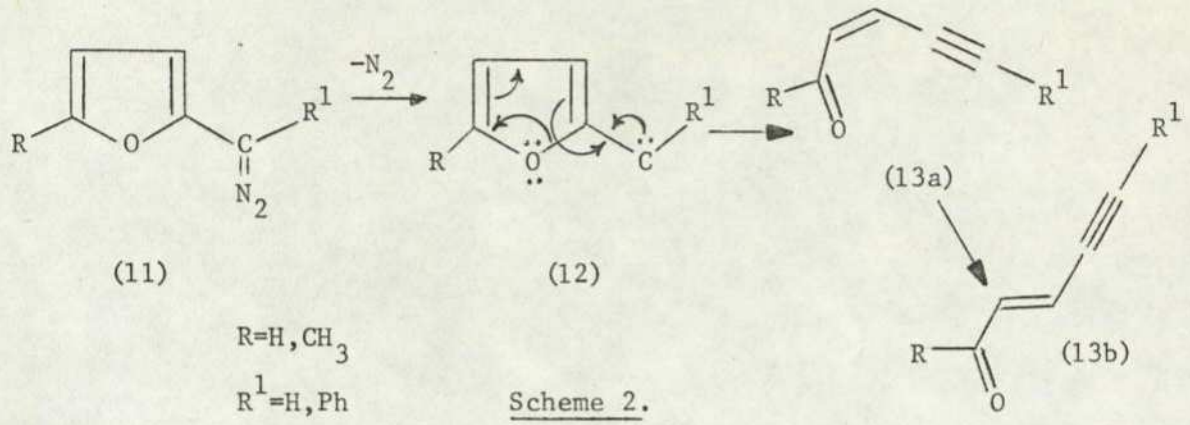
Nawaji and Onyiriuka[4] demonstrated the formation of the cyclopenteno-aldehyde (10;60%) from anhydrous copper II sulphate catalysed decomposition of a 0.05M solution of diazoketone (8) in refluxing cyclohexane.



(8) (9) (10)

Formation of compound (10) can be rationalised on the basis of an initial intra-molecular addition of the ketocarbene to the furan to give the intermediate (9) followed by ring opening of the heterocyclic system. The trans-stereochemistry of the enal side chain is assumed to arise from a thermal or metal-catalysed isomerisation subsequent to the ring opening.

Hoffman and Shechter[5] reported that furfurylidenes (12) as generated by thermolysis of 1-diazo-1-(2-furyl) alkanes (11) undergo reorganization (Scheme 2) to cis-and trans-γ-δ-acetylenic α,β-olefinic aldehydes and ketones (13a) and (13b).



(11) (12) (13a) (13b)

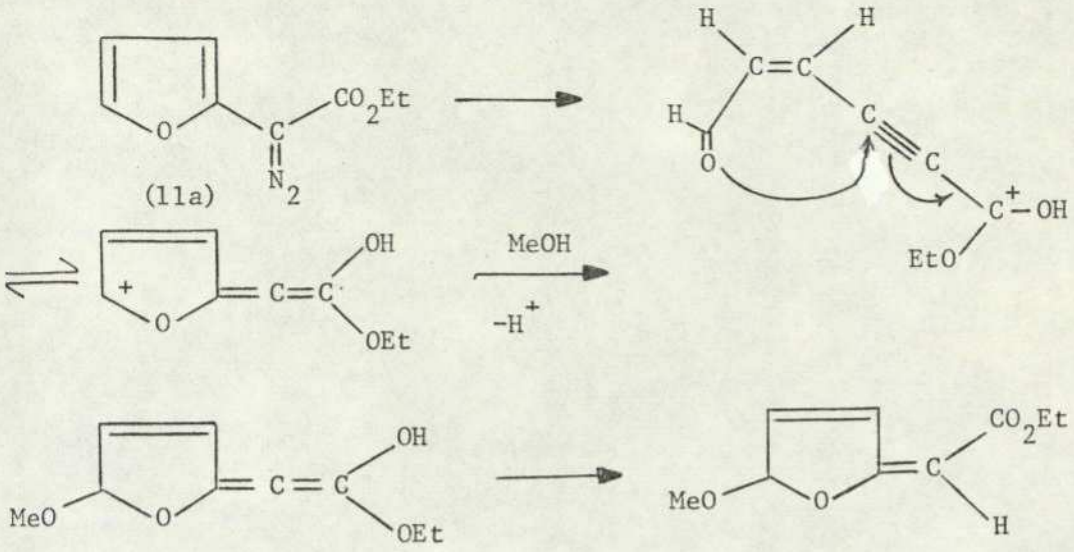
R=H,CH_3

R^1=H,Ph

Scheme 2.

Since (13a) isomerizes readily to (13b) at elevated temperatures, it is likely that carbenic decomposition of (11) occurs stereospecifically by a singlet electrocyclic process to give initially the cis ring-opened product (13a). Isomers (13a) and (13b) were isolated gas chromatographically in preparative quantities and converted to 2,4-dinitrophenylhydrazones for analysis.

In a later, more detailed study, carbenic, cationic, metal ion catalysed, and photolylic decompositions of ethyl(2-furyl) diazoacetate (11a) were fully investigated. A very interesting ring reclosing pathway was observed in the reaction between (11a) and methanol (Scheme 3).

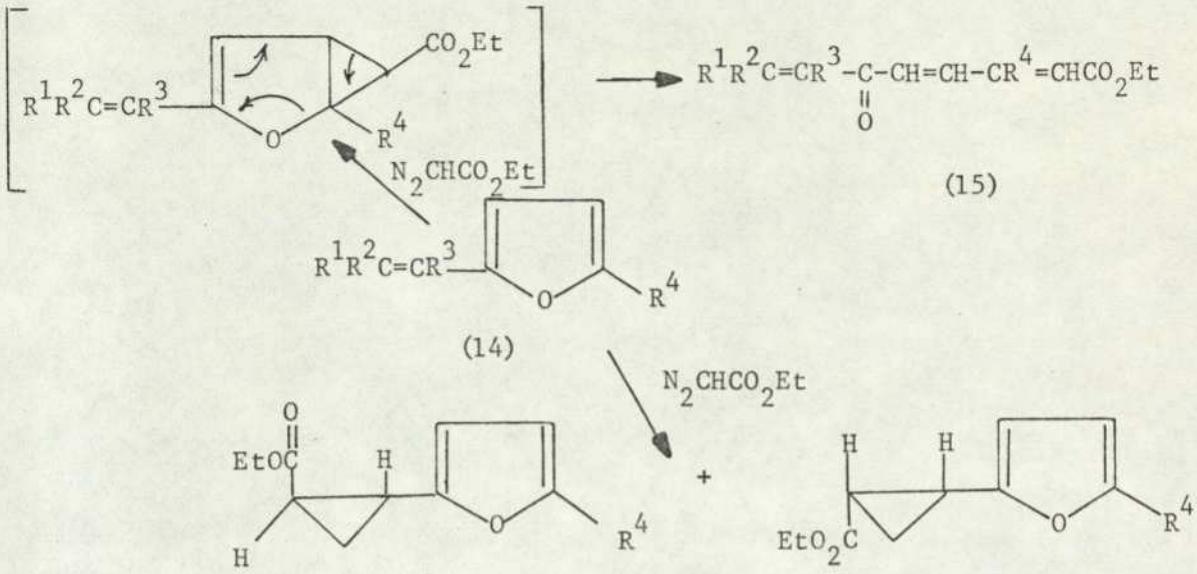


Scheme 3.

Alkenylfurans (14a), (14b) and (14g) react with ethyl diazoacetate to give as the major products ethyl esters (15), together with minor adducts (16). (Scheme 4) (14g) provides (15g) in highest yield (58%)[6]. Neither the reaction temperature nor the manner of decomposition of the ethyl diazoacetate(metal catalysis, thermolysis or photolysis) influences the reaction path.

In contrast, reaction of (14c) and (14d) with ethyl diazoacetate leads mainly, and in the case of (14e) and (14f) exclusively, to the formation of ethyl ester (16). The different behaviour of (14a), (14b) and (14g) on one hand, and of (14c) to (14f) on the other towards ethylacetate correlates with the fact that the ratio of rate constants for addition to the vinyl double bond and for the addition to the double bond remote from substitutents in the furan ring decreases with increasing number of methyl substituents on the vinyl group. It can be largely explained in terms of steric factors. The double bond of the furan ring(remote from alkenyl substituents) in (14a) and (14b) is more reactive than the double bond in hexene.

The formation of ester (15) can be explained by participation of the double bond of the furan ring remote from alkenyl substituents.



(15)

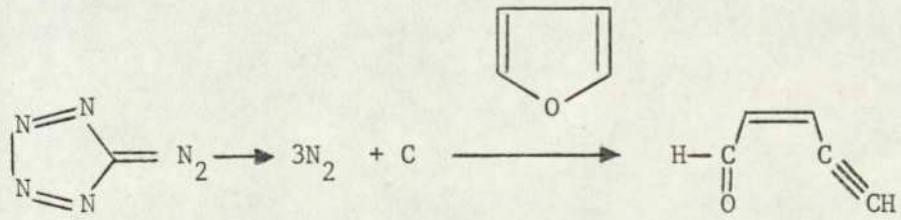
(14)

(16) trans (16) cis

	R^1	R^2	R^3	R^4
a	CH_3	CH_3	H	H
b	CH_3	H	H	H
c	H	H	H	H
d	H	H	H	CH_3
e	H	H	H	Cl
f	H	H	H	Br
g	CH_3	H	CH_3	H

Scheme 4.

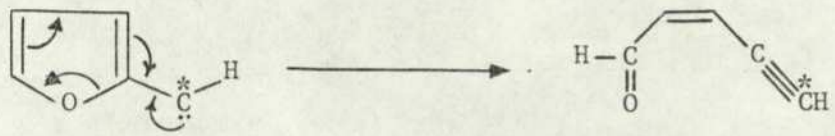
Recently, Dyer and Shevlin[7] have shown that when carbon atoms are generated by the pyrolysis of 5-diazoterazole (17) in the presence of gaseous furan, the major volatile organic product is cis-2-penten-4-ynal (18).



(17) (18)

Two mechanisms were discussed in this paper. One could rationalise the formation of (18) in the reaction of carbon atoms

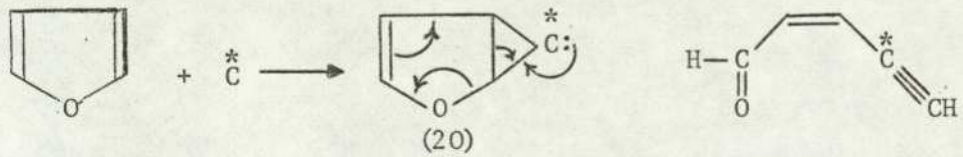
with furan by proposing C-H insertion to generate (19) which subsequently rearranges (Scheme 5).



(19) (18)

Scheme 5.

An alternative mode of attack is initial addition of carbon to one of the double bonds of furan to generate cyclopropylidene intermediate (20). (Scheme 6).



(20)

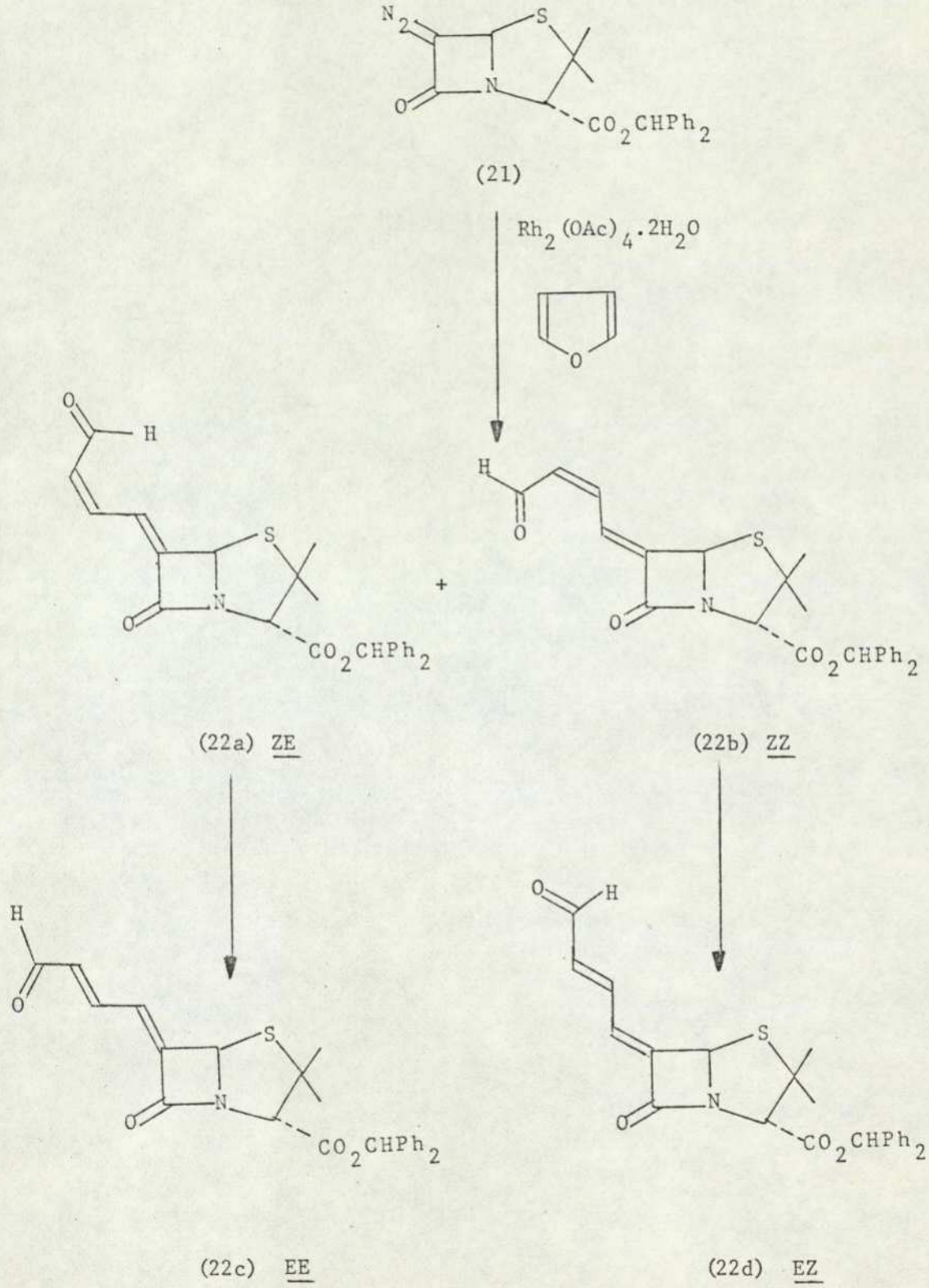
Scheme 6.

The mode of formation of (18) may be differentiated through the use of labelled carbon atoms (Schemes 5 & 6), by ^{13}C n.m.r. It was found that unsaturated aldehyde (18) was 85% labelled at C_4. Therefore a conclusion was drawn that only a small amount of (18) arises by C-H insertion while the remainder probably resulted from addition to the double bond followed by the rearrangement shown in Scheme 6.

5.2. The reaction of benzhydryl 6-diazopenicillanate with furan: results and discussion.

Addition of benzhydryl 6-diazopencillanate (21) to neat furan containing 0.009 equivalent of rhodium acetate led to the rapid and quantitative formation(as judged by t.l.c. and by ^1H n.m.r.) of the total product of a 2:1 mixture of the ZE and ZZ isomeric dienals (22a) and (22b). The condensation could also be effected by copper catalysts, $Cu_2(CF_3OAc)_4$ or $Cu(acac)_2$ but less cleanly and interestingly resulting in a different 1.2:1 ratio of the dienals (22a) and (22b). (Scheme 7).

The mixture of dienals (22a) and (22b) was separated by chromatography on silica, but during this process some isomerization to dienals (22c) and (22d) occurred.


N2
S
O
N
CO2CHPh2
(21)
Rh2(OAc)4.2H2O
O
H
O
S
N
O
CO2CHPh2
+
H
O
S
N
O
CO2CHPh2
(22a) ZE
(22b) ZZ
H
O
S
N
O
CO2CHPh2
O
H
S
N
O
CO2CHPh2
(22c) EE
(22d) EZ

Scheme 7.

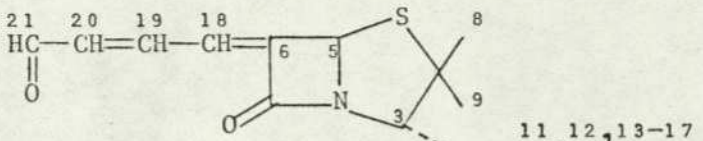
The isomerisation also occurred slowly on stirring a solution of (22a) and (22b) in CH_2Cl_2 with t.l.c. grade silica (conversions: 24h, 9%; 72h, 47%; 144h, 62%). Interestingly a batch of column-grade silica did not transform the isomers at all, presumably because it was less acidic. The use of acid solution produced very fast isomerisation to a mixture of all four dienals (22a-d), the ratios of isomers depending on the conditions. At elevated temperatures, the 2:1 mixture of dienals (22a) and (22b) could be completely converted into the isomers (22c) and (22d) in a 1.5:1 ratio. Strikingly, basic conditions also favoured the transformation of isomers very efficiently. Adding a solution of sodium hydroxide to the mixture of dienals (22a) and (22b) in acetonitrile resulted in immediate conversion into a 1.2:1 mixture of dienals (22c) and (22d)(this result is discussed in detail in Section 5.3). Isomerisation was also noticed upon storage of the mixture of dienals (22a) and (22b) in the refrigerator.

The four isomers could be isolated individually by rapid chromatography in two steps. The separation between (22a) and (22b) or between (22c) and (22d) could be achieved by conventional chromatography, or more efficiently by prep. HPLC. However, (22b) had almost identical polarity with (22d), the separation factor α between them being 1.005 (Hypersil 5μm, CH_2Cl_2/Et_2O 98.5 : 1.5). Therefore, single-stage adsorption chromatography, even prep. HPLC, was unable to resolve them completely.

The four isomeric dienals were readily distinguished by their ^1H and ^{13}C n.m.r. spectra. (Table 1 and 2). Application of the

Tobey-Simon rules assisted in the assignment of ^1H n.m.r. spectra of the dienals (22a-d). As shown in Table 3, for the proton on C_{20} of the four isomers the observed chemical shift values reasonably fit into the Tobey-Simon rules' prediction, but a lot of deviations between the recorded figures and the calculation value are seen at the protons on C_{18} and C_{19} of four dienals. These deviations probably stem mainly from the uncertain contributions of the β-lactam ring. Table 3 shows three ways of treating C_5, none of which is ideal, and the treatment of the carbonyl group as an amide carbonyl is probably also unsatisfactory. Not withstanding these limitations, the predicted trends in chemical shift values for the olefinic protons, taken with the information from coupling constants, allow the geometrical assignments to be made with a reasonable degree of certainty. 11.2 and 10.7 Hz proton-proton coupling constants for the C=C bond adjacent to the aldehyde function of dienals (22a) and (22b) led to the unambiguous Z configuration assignment. Differentiation of (22a) and (22b) was based on the different chemical shifts of the proton on carbon-carbon double bond connected to the β-lactam ring. The proton on C_{18} of dienal (22b) exhibited a lower field chemical shift due to the deshielding effect of carbonyl function of β-lactam, which the proton on C_{18} of dienal (22a) did not suffer.

The proton-proton coupling constants for the C=C bond adjacent to the aldehyde function of dienals (22c) and (22d) were 15.9 and 14.3 Hz respectively, characteristic of the E configuration. Dienals (22c) and(22d) were differentiated in a similar manner to above.

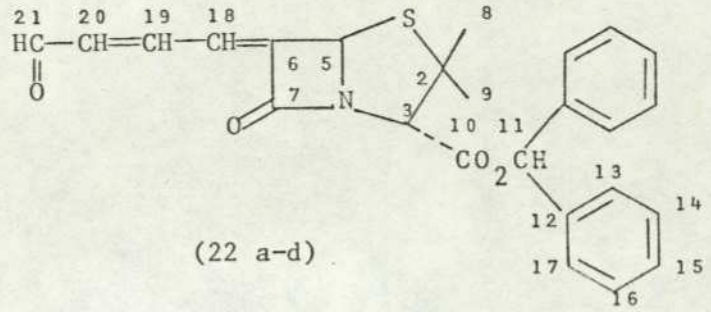


(22 a-d)

	PROTONS ON CARBON									
	C_3 (s, 1H)	C_5 (s, 1H)	C_{18} (d, 1H)	C_{19} (d.d, 1H)	C_{20} (d.d, 1H)	C_{21} (d, 1H)	C_{11} (s, 1H)	C_{12-17} (m,10H)	C_8 (s, 3H)	C_9 (s, 3H)
(22a) ZE	5.30	4.17	2.72 (J=12.7 Hz)	2.38 (J_{19-20}=11.2Hz)	3.86 (J_{20-21}=6.8Hz)	-0.16	3.02	2.62	8.41	8.70
(22b) ZZ	5.30	4.04	2.42 (J=12.7 Hz)	3.16 (J_{19-20}=10.7Hz)	3.74 (J_{20-21}=6.8Hz)	-0.24	2.98	2.56	8.40	8.66
(22c) EE	5.30	4.16	3.49 (J=11.9 Hz)	2.20 (J_{19-20}=15.9Hz)	3.62 (J_{20-21}=7.9Hz)	0.32	2.98	2.58	8.40	8.66
(22d) EZ	5.30	4.04	3.15 (J=10.4 Hz)	2.86 (J_{19-20}=14.3Hz)	3.56 (J_{20-21}=7.9Hz)	0.30	2.98	2.58	8.40	8.68

Table 1 ^1H n.m.r. data of dienals (22a-d)

In $CDCl_3$, τ values referred to T.M.S.



(22 a-d)

Compound	C_2	C_3	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}-C_{17}	C_{18}	C_{19}	C_{20}	C_{21}
(22a) ZE	65.8	70.6	78.7	149.8	167.4	33.7	25.6	167.1	68.2	139.5	128-127	131.5	138.8	124.3	190.0
(22b) ZZ	66.0	70.5	78.6	150.5	167.8	33.7	25.5	167.1	67.4	139.5	127-128	133.7	138.0	119.8	189.7
(22c) EE	65.7	70.6	78.6	149.5	167.3	33.7	25.5	167.1	68.2	139.4	127-128	136.8	144.0	127-128	193.6
(22d) EZ	66.0	70.5	78.6	150.5	167.3	33.7	25.5	167.1	67.6	139.4	127-128	138.2	142.6	123.8	192.8

Table 2. ^{13}C n.m.r. data of dienals (22a-d)

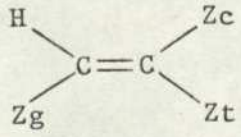
In $CDCl_3$, δ values referred to T.M.S.

	C_{18}-H		C_{19}-H		C_{20}-H	
	Observed	Calculated	Obs.	Cal.	Obs.	Cal.
22a ZE	2.72	*2.91 **2.80 ***2.70	2.38	2.25	3.86	3.70
22b ZZ	2.42	*2.62 **2.48 ***2.56	3.16	2.25	3.74	3.70
22c EE	3.49	*2.91 **2.80 ***2.70	2.20	2.49	3.62	3.61
22d EZ	3.15	*2.62 **2.48 ***2.56	2.86	2.49	3.56	3.61

Table 3. Comparison of the chemical shifts of protons on C_{18}, C_{19} and C_{20} of dienals (22a-d) in ^1H n.m.r. spectra between the observed values and the calculated figures derived from Tobey-Simon rules. In $CDCl_3$, τ values referred to T.M.S.

Tobey-Simon rules[8]:

$\delta = 5.28 + Z_{gem} + Z_{cis} + Z_{trans}$ (for the chemical shift of a proton on a double bond.)

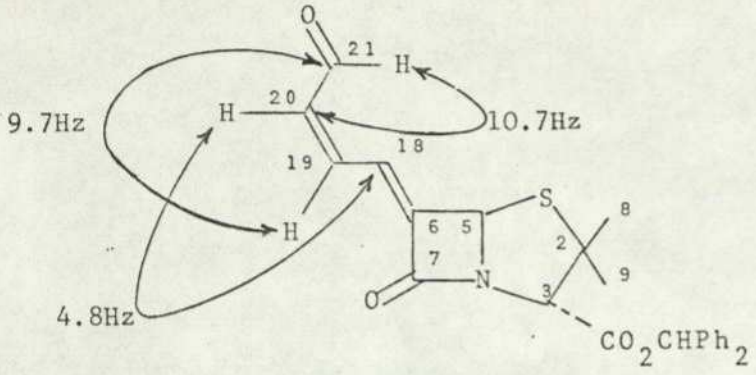


Notes: * Z is regarded as alkyl subsitutent

** " " " " CH_2S "

*** " " " " CH_2N "

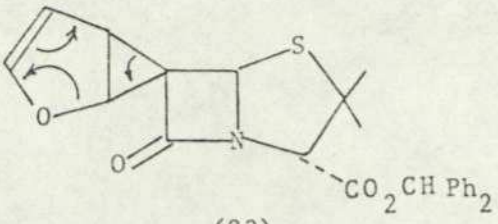
In assignments of the ^{13}C n.m.r. spectrum of dienal (22a), the two bond and three bond C-H couplings allow an unequivocal assignment of C_{18}, C_{19} and C_{20}.[9]



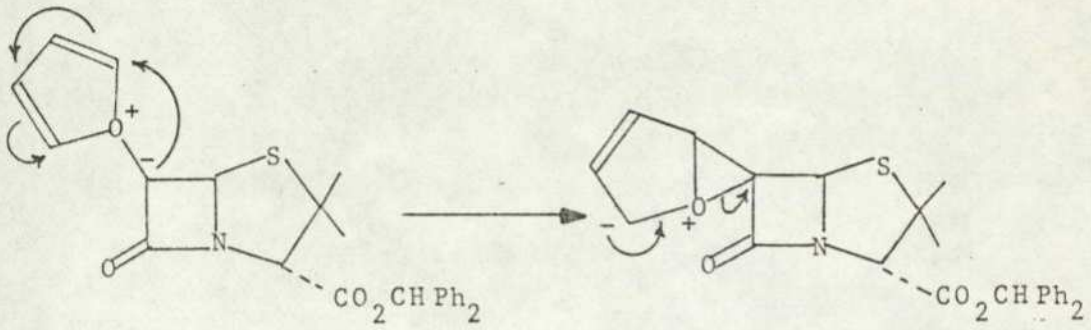
(22a) ZE

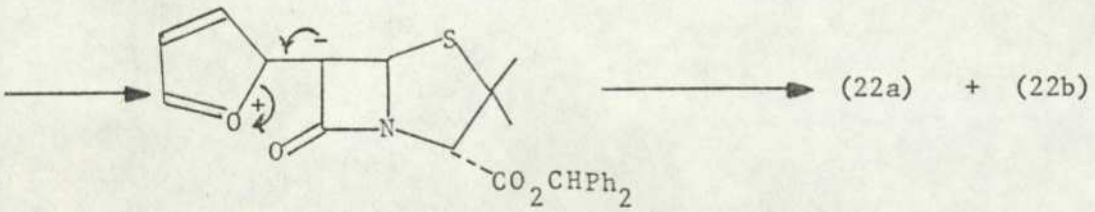
The four isomeric dienals have very similar u.v. spectra, with λ_{max} in the region 277-279. Thus, the isomers show less distinctive u.v. behaviour than the ZE and EE isomers (4a) and (4b), for which λ_{max} 268 nm (log ε 4.51) and λ_{max} 260 nm (log ε 4.40), respectively, were reported. In the penicillanate derivatives, u.v. does not appear to be very useful in distinguishing between the isomers.

A final point should be made regarding the mechanism of formation of the dienals (22). The condensation of benzyhydryl 6-diazo-penicillanate with furan can be considered to proceed via the ring opening of an intermediate cyclopropa-dihydrofuran (23), as suggested by the earlier literature discussions. However, an alternative process which by-passes cyclopropane formation (Scheme 8) cannot be ruled out on the presently available evidence. This pathway has the merit of closely resembling the mechanism suggested for thiophene ring expansion to the 2H-thiopyran in Chapter 3.


S
O
O
N
$CO_2CH\,Ph_2$

(23)


O
S
N
O
$CO_2CH\,Ph_2$
O
S
N
O
$CO_2CH\,Ph_2$


O
S
N
O
CO_2CHPh_2
(22a) + (22b)

Scheme 8.

Deprotection of the ester function in the dienals(22) was readily accomplished by brief dissolution in trifluoroacetic acid-anisole, in quantitative yield (100%), the acids being formed with concomitant geometric isomerization about the side chain double bonds. The acids showed no significant antibacterial activity.

The diazopenicillanate (21) is obtainable in high yield from 6-aminopenicillanic acid, as described in Chapter 2. The discovery of the extremely efficient condensation reaction with furan therefore means that, overall, a simple and high-yield process is available for replacing the C_6-N by a C_6-carbon chain. Moreover, the high degree of functionality incorporated in the side chain of the dienals makes them attractive intermediates for further modification.

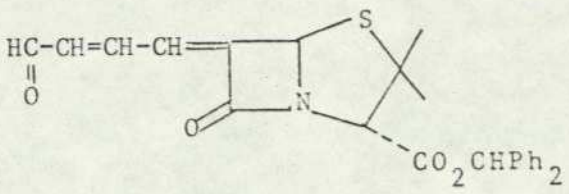
With the aim of exploring the synthetic potential of the dienals, a number of their reactions have been examined, including reductions, oxidation, and condensations of the carbonyl and olefinic groups. These are discussed in the remainder of this chapter.

5.3. Reduction reactions of the dienals (22)

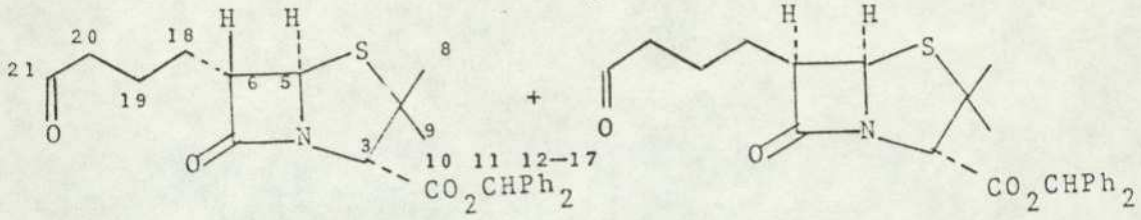
Hydrogenation of aliphatic α,β-unsaturated aldehydes to saturated aldehydes is readily achieved with a variety of metal catalysts (Pd, Pt, Rh, Ru) on supports in the presence of hydrogen gas[10]. In most cases, palladium is the metal of choice, being highly active for C=C bond reduction but the least active of these metals generally for aliphatic carbonyl reduction. Thus, except under vigorous conditions, with palladium the reduction usually stops after one equivalent of hydrogen has been absorbed. With increasing conjugation and increasing substitution of the C=C bond, reduction proceeds less readily and larger amounts of catalyst are required.

When hydrogenation of the 2:1 mixture of dienals (22a) and (22b) was attempted, even with 25% by weight of 5% palladium on carbon, in EtOAc and MeOH for 4 hours, it was observed that the starting materials were recovered unchanged. However, when the amount of catalyst was increased to over 100% by weight, the reaction afforded a 1:1 mixture of α- and β-6-(4'-oxobutanyl) penicillanates (24) and (25) in 35% yield. The isomers were resolvable by HPLC.

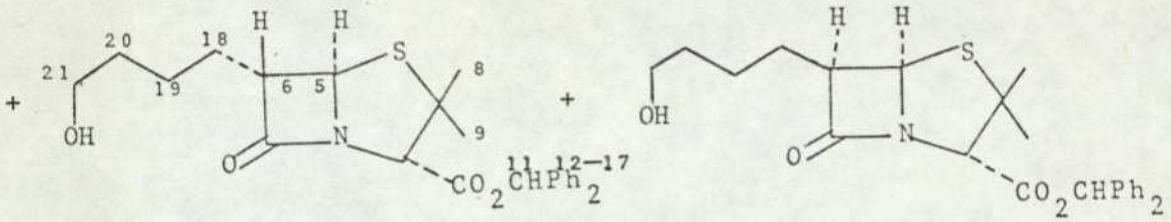
Interestingly, when the reduction was carried out with palladium black, a catalyst which usually results in hydrogenolysis of the benzhydryl ester function, no carboxylic acids were isolated. Instead, the major products were the saturated aldehydes (24) and (25) and in addition a small amount of a 1.75:1 mixture of α and β 6-(4'-hydroxybutyl) penicillanates (26), (27) was also obtained.



(22a) ZE
(22b) ZZ
Pd black



(24) α (25) β

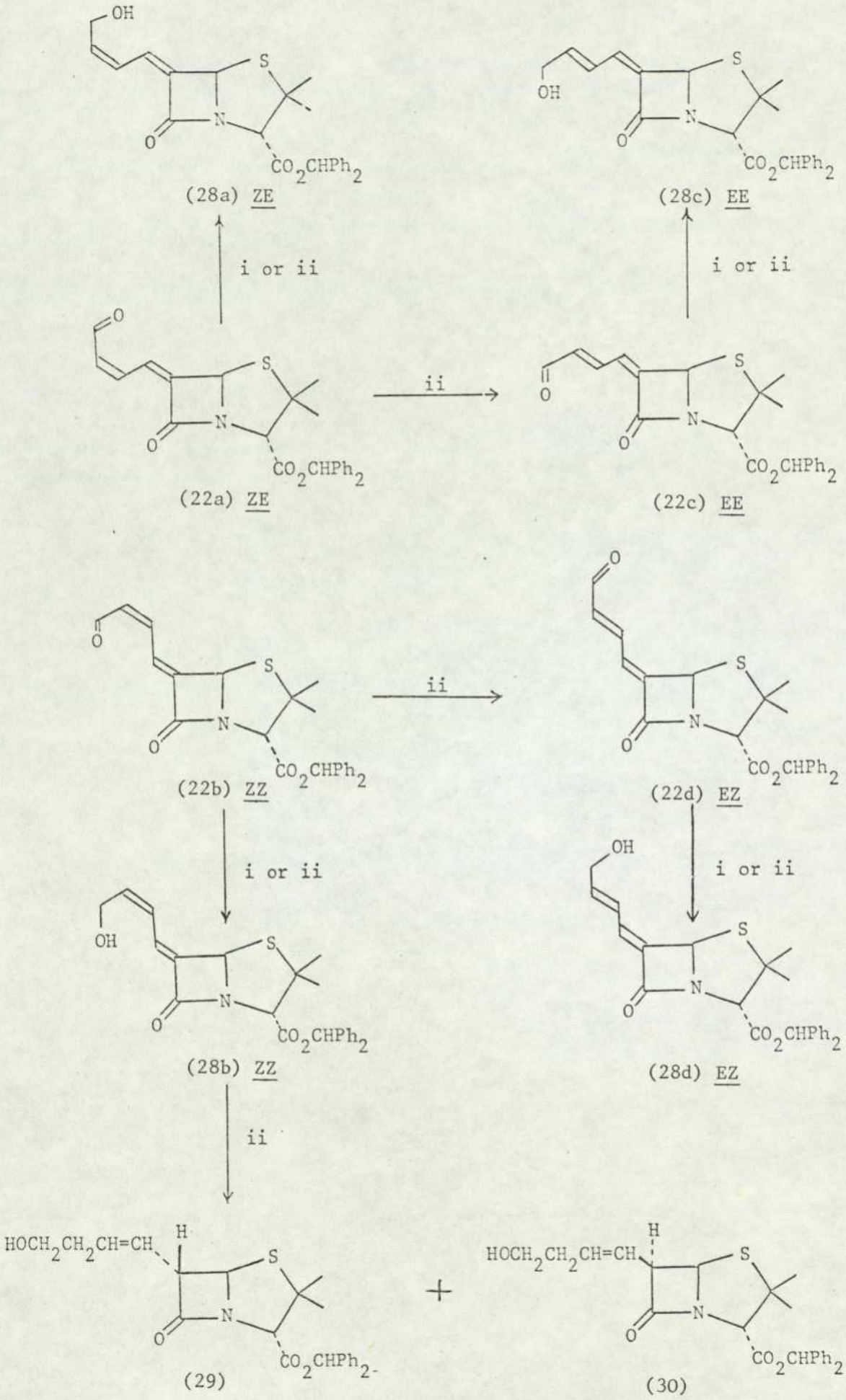


(26) α (27) β

The results confirm the highly selective nature of the Pd-C catalyst and the slightly lower selectivity of the more powerful Pd black. Evidently, reduction of the olefinic C=C bonds is considerably faster than hydrogenolysis of the benzhydryl ester function. The stereochemical results in these reductions are difficult to interpret: the observed ratio of α and β isomers may reflect a combination of effects, including competing cis-hydrogenation from the α and β faces, competing cis and trans hydrogenation (the latter can become a significant process in protic solvents), and epimerisations subsequent to reduction.

The 1:1 mixture of alkanals (24) and (25) was deprotected by treatment with trifluoroacetic acid and anisole, furnishing a 1.6:1 mixture of the corresponding 6-(4'-oxobutyl) penicillanic acids. These were found to be devoid of biological activity.

Attention was then turned to the reduction of the carbonyl group of the dienals. Sodium borohydride is a widely used reducing agent for aldehydes and ketones. It has the merits of generally being mild and selective, and does not normally reduce isolated olefinic bonds, esters or amides. Reduction of dienals (22) by sodium borohydride was expected to give simply the corresponding dienyl alcohols, but proved to be more complicated. The individual isomers (22a-d) were separated chromatographically and each was reacted with $NaBH_4$ in wet acetonitrile. By monitoring the reactions by HPLC (Hypersil 5μm SiO_2, $MeCN/CH_2Cl_2$, 7:93), it was established that the ZE and ZZ dienals (22a) and (22b) each undergo two competing reactions with $NaBH_4$ in wet acetonitrile - isomerisation of the Z double bond adjacent to the aldehyde function and reduction of the aldehyde to primary alcohol. The isomeric EE and EZ dienals (22c) and 22(d) were themselves rapidly reduced to the corresponding dienyl alcohols. The isomerisations and carbonyl group reductions are all fast processes: all the dienals (22a-d) were consumed within 1-2 min. of mixing with excess $NaBH_4$. At a rather slower rate (10-20 minutes reaction time), the ZZ dienyl alcohol (28b) was reduced to a 6:4 mixture of α and β 6-(4'-hydroxybut-1'-enyl) penicillanates (29) and (30). The ZE isomer (28a) was not significantly reduced under these conditions, but it was found that with a large excess of $NaBH_4$ in wet acetonitrile there was some reduction to alkenols (29) and (30) after 2 h.



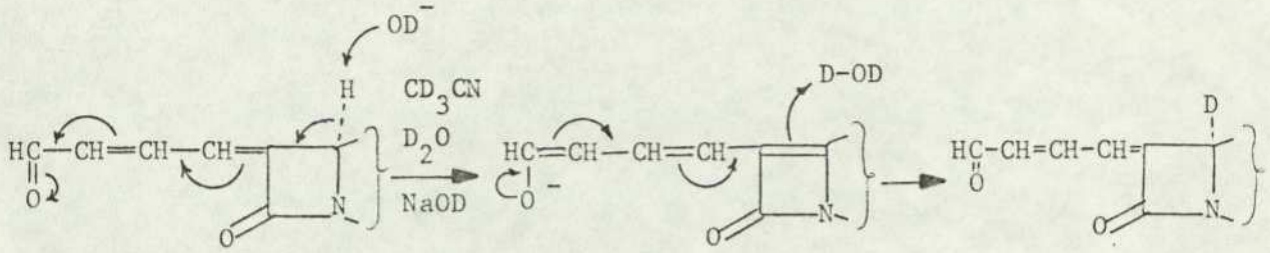
i. $NaBH_4$ in aq. MeCN buffered to pH 7.0

ii. $NaBH_4$ in aq. MeCN

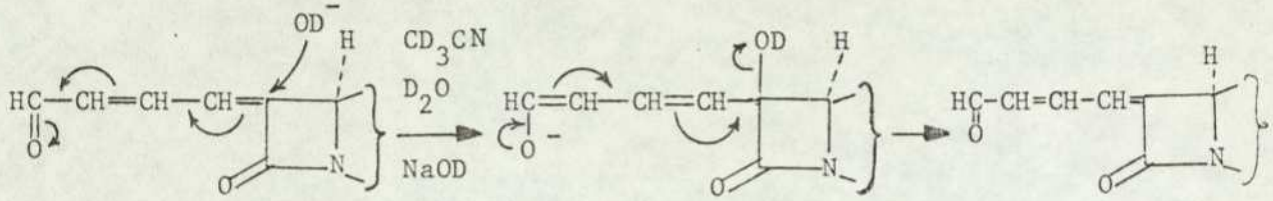
The remaining two isomers (28c) and (28d) showed no conversion at all to alkenols, even on prolonged reaction.

The observation of isomerisation of the dienals (22a) and (22b) during these borohydride reductions was unexpected - we had not anticipated that such a reaction would take place readily in the absence of acid. It was initially suspected that interaction between the dienals and sodium ions was responsible, but addition of aqueous sodium chloride to the dienals (22a) and (22b) in acetonitrile caused no change. The effect of adding aqueous sodium hydroxide was then examined, and this resulted in the rapid appearance of isomers (22c) and (22d), the isomerisation being followed by HPLC (the results are discussed also in Section 5.2). Thus, it is the high pH of the $NaBH_4$ solution which is responsible for isomerisation during the reductions.

Two mechanisms were considered possible to account for the base-catalysed isomerisations, one involving enolisation towards C_5 (Scheme 9a) and the other a conjugate addition-elimination pathway (Scheme 9b) In an experiment carried out in an n.m.r. tube, it was observed that on treatment of dienals (22a) and (22b) with $NaOD/D_2O$ in CD_3CN, rapid isomerisation to dienals (22c) and (22d) occurred (clearly visible from the appearance of new aldehyde signals - c.f. Table 1), without any D-incorporation at C_5. This result is inconsistent with enolisation towards C_5 and leaves an addition-elimination pathway for consideration. Since it was observed that the dienols (28) were not isomerised under basic conditions, the addition of hydroxide ions is assumed to occur towards the aldehyde function in the dienals by attack on the γ,δ-bond as shown in Scheme 9b.



Scheme 9a



Scheme 9b

When $NaBH_4$ reductions of the ZE and ZZ dienals (22a) and (22b) were carried out in aqueous MeCN buffered to pH 7.0, there was no isomerisation observed and only the corresponding ZE and ZZ dienyl alcohols, (28a) and (28b) respectively were formed. Even on prolonged exposure to buffered $NaBH_4$, the ZZ isomer (28b) did not suffer appreciable reduction to the alkenols (29) and (30). This confirmed that a fast, base-catalysed isomerisation of dienals was responsible for the formation of the complex mixture formed in the unbuffered conditions and also allowed a method to be devised for optimising the synthesis of the intriguing alkenols (29) and (30). To accomplish this, a sequence of buffered and unbuffered reductions was carried out. The readily obtained 2:1 mixture of dienals

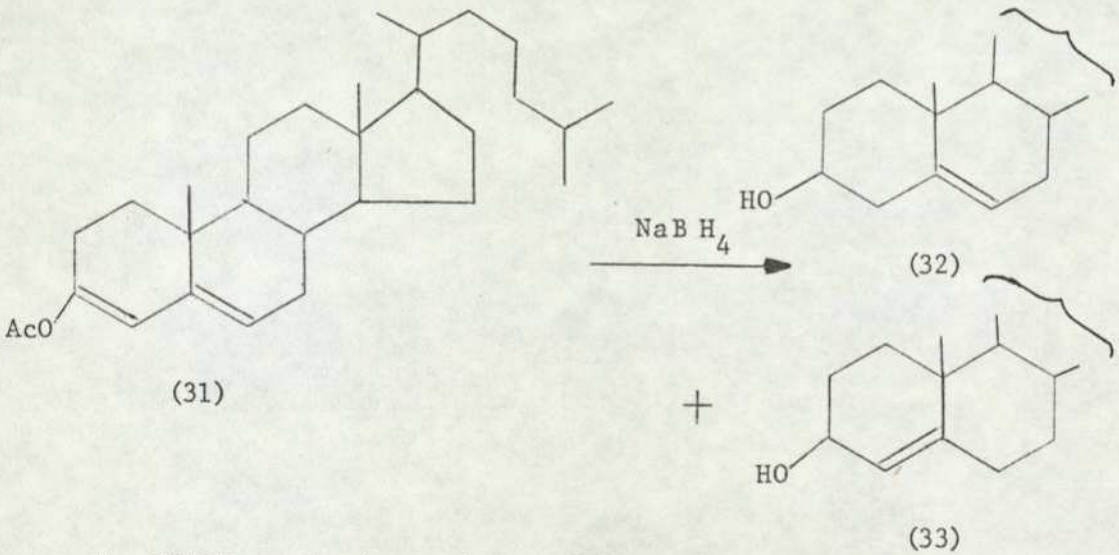
(22a) and (22b) was treated with buffered $NaBH_4$, to avoid isomerisation, affording a 2:1 mixture of dienols (28a) and (28b) after extraction. This mixture was then further treated with unbuffered $NaBH_4$ to give a mixture of unreacted dienol (28a) and alkenols (29) and (30). The latter isomers were isolated in a combined, overall yield of 35% based on benzhydryl 6-diazopenicillanate and were separated by preparative HPLC.

The orientations of the side-chains at C_6 in two isomers (29) and (30) were apparent from the C_5-H-C_6-H couplings in their ^1H n.m.r. spectra (1.5 Hz in the α-isomer and 4.5 Hz in the β-isomer). The geometries of the double bonds in the two isomers were not immediately apparent, since in each case the ^1H n.m.r. spectrum showed a deceptively simple pattern due to accidental magnetic equivalence of the two vinyl protons[11]. However, addition of $Eu(fod)_3$ led to a good separation of the chemical shifts of the vinyl protons in each of the two compounds, allowing the vicinal olefinic couplings to be measured. Both of the isomers (29) and (30) showed a coupling of 15 Hz, demonstrating that both possess the trans configuration in their side-chains. A similar technique is demanded to clarify the complex olefinic patterns in the ^1H n.m.r.spectra of the dienals (28b-28d).

The formation of the alkenals (29) and (30) appears to be a unique example of the selective reduction of one of a set of four isomeric 2,4-dienyl carbonyl compounds, the reaction occurring only under alkaline conditions. There are numerous precedents in the literature for the conjugate reduction of α,β-unsaturated carbonyl compounds, including aldehydes, ketones and esters, and also for the

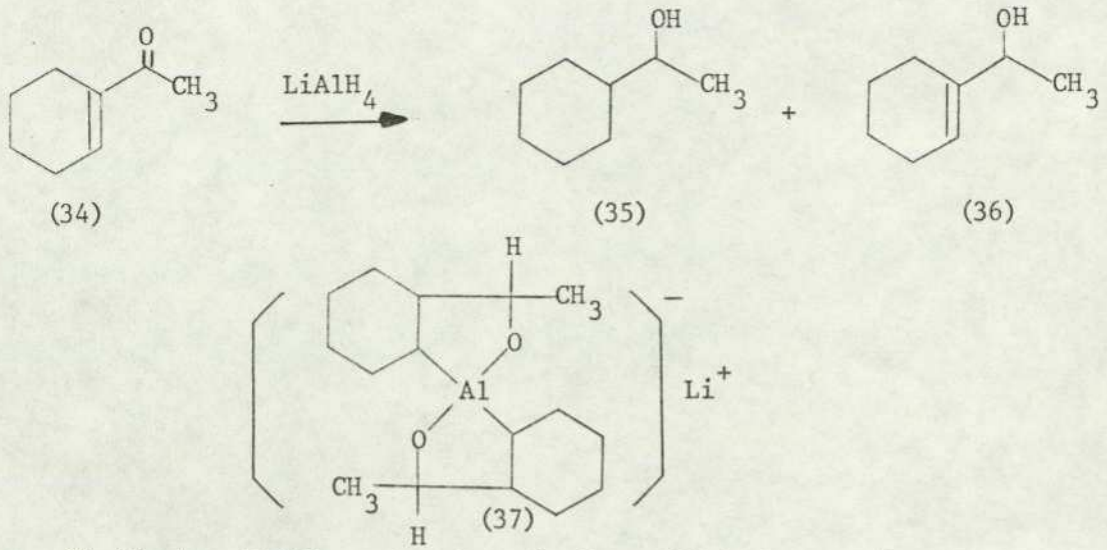
C=C bond reduction of nitroalkenes and enol acetates with metallic hydrides. It is relevant to consider some of these reports in relation to our own results.

The reduction of cholestenone enol acetate (31) with $NaBH_4$ in methanol-ether or in aqueous ethanol led to cholesterol (32) as the major product (58-75%) and cholest-4-ene-3-ol (33) as the minor product (10%)[12,13]. Since $NaBH_4$ does not ordinarily attack esters, the initial step in the reduction of (31) was postulated to be solvolysis of the enol acetate to give cholest-5-en-3-one, followed by reduction to the major product (32) or (slow) conjugation and reduction to the minor one (33).



(31) (32) (33)

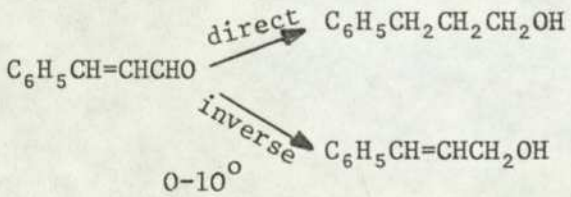
The $LiAlH_4$ reduction of 1-cyclohexenyl methyl ketone (34) was shown to give more of the saturated alcohol (35) than the unsaturated one (36), when the normal technique of adding ketone to hydride was employed[14]. The proportions of products were reversed when inverse addition of the $LiAlH_4$ was used. An internal hydroalumination of the C=C bond to give an intermediate complex (37) was suggested to account for the results.



(34) (35) (36)

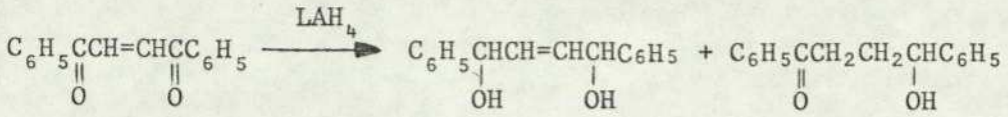
(37)

Similarly, $LiAlH_4$ reduction of cinnamaldehyde was shown to furnish either cinnamyl alcohol or hydrocinnamyl alcohol, depending on the mode of addition[15]. (Scheme 10). It was argued that the reduction proceeds in two stages at distinctly different rates, the carbonyl group reacting faster than the double bond, so that the outcome depends on the conditions used.



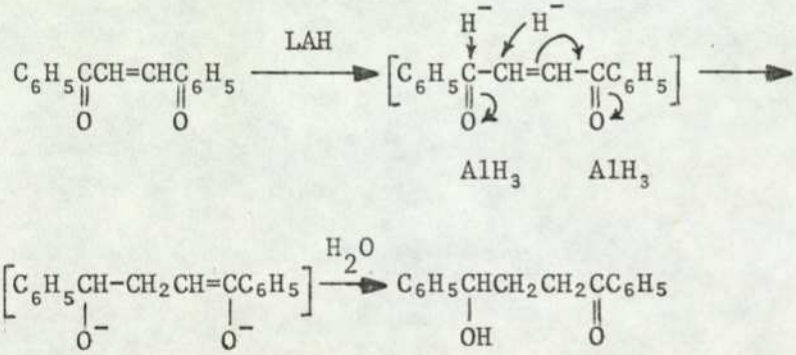
Scheme 10

Lutz and Gillespie[16] observed that the $LiAlH_4$ reduction of cis- and trans-dibenzoylethylene (38) gave 10% of trans-1,4-dipheyl-2-buten-1,-4-diol (39) and 88% of 1,4-dipheylbutan-4-ol-1-one (40)



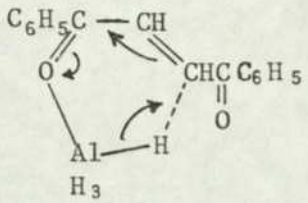
(38) (39) (40)

The same products were obtained from both isomers doubtless due to isomerization before reduction. The unsaturated glycol (39) was the expected result of reduction of the two carbonyl groups. The hydroxy ketone (40) resulted from 1,4 addition of $LiAlH_4$ to the α,β-unsaturated ketone with prior or subsequent 1,2,-reduction of the carbonyl group independently of the other carbonyl group.



(40)

An intermediate cyclic transition state (41) analogue to those proposed in Grignard reactions and in aluminum alkoxide reductions was postulated as applicable to this $LiAlH_4$ reduction.



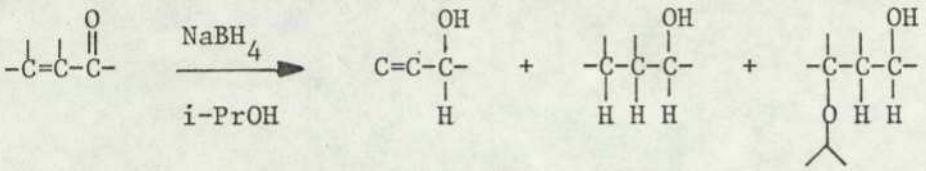
(41)

Johnson and Rickborn[17] have published results on the sodium borohydride reduction of conjugated aldehydes and ketones, some of which are shown in Table 4. These results show the steric effect on the reduction of conjugated aldehydes. One β-methyl group (43) lowers the amount of 1,4 reduction, but interestingly, the effect is not so great as that of a single α-methyl substituent (44), where only carbonyl reduction is observed.

	Yield	>C=C-CH(OH)	H-C-C-C(OH)-H
(42) $CH_2=CHCH(=O)$	70%	85%	15%
(43) $CH_3CH=CHCH(=O)$	91%	92	8
(44) $CH_2=C(CH_3)-C(=O)H$	100%	≥99%	≤1%
(45) $(CH_3)_2C=CH-CH(=O)$	95%	≥99%	≤1%

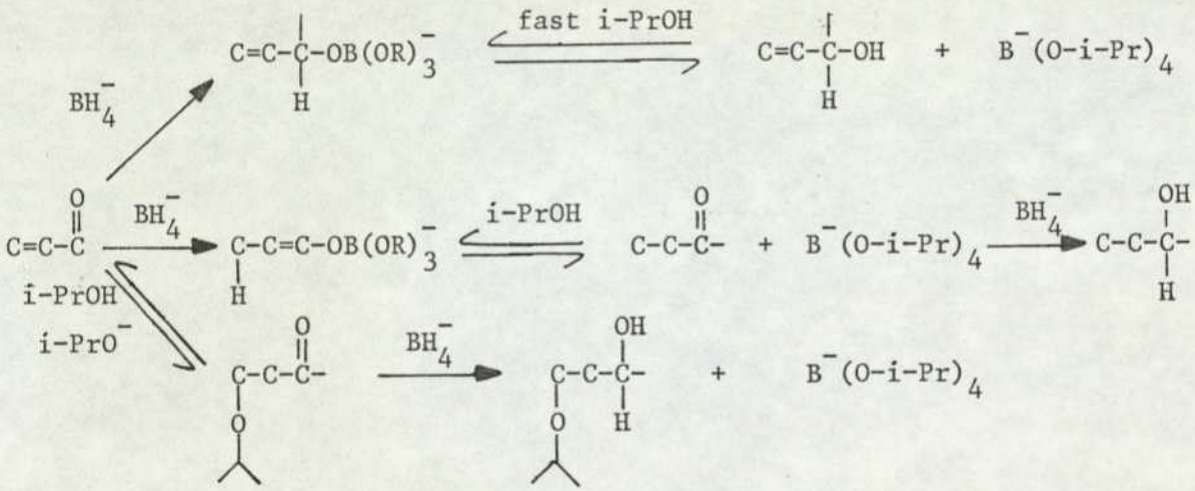
Table 4. Reduction of conjugated aldehydes with sodium borohydride (the aldehydes were added to the $NaBH_4$ 50% aqueous ethanol)

An unprecedented formation of saturated 3-isopropoxy alcohol product (45) in the reduction of conjugated carbonyl compounds by sodium borohydride in isopropyl alcohol(i-PrOH) as solvent has been reported.



(45)

The following mechanism was proposed:



A similar solvent effect was discovered in the reduction of crotonaldehyde by $NaBH_4$ in a number of common alcohols as solvent. These results are shown in Table 5.

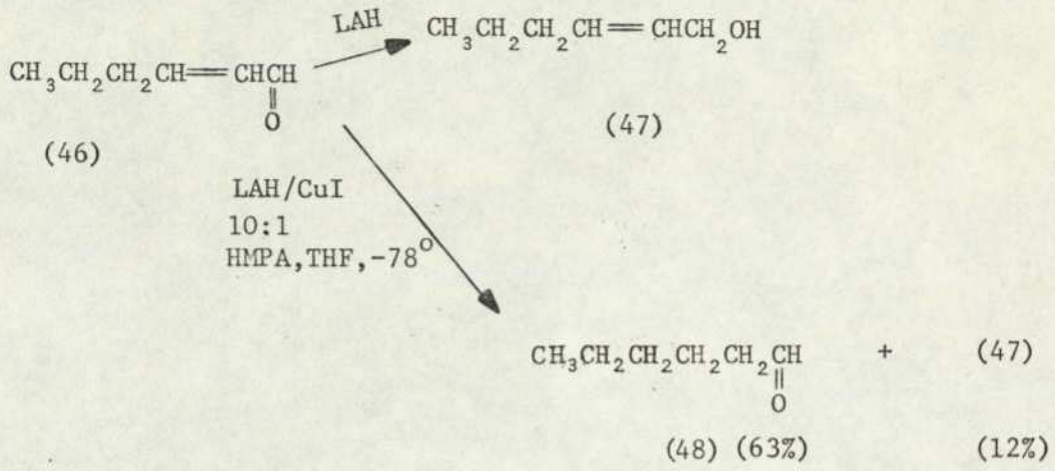
It was noted that reductions in water or aqueous alcohol gave high yields of carbonyl and conjugate reduction products and consequently solvent addition could not be an important pathway under these conditions.

Solvent ROH R	Products, %		
	$CH_3CH_2CH_2CH_2OH$	$CH_3CH{=}CHCH_2OH$	$CH_3\underset{\displaystyle OR}{\underset{|}{C}}HCH_2OH$
Me	7	56	37
Et	9	84	7
n-Pr	11	63	26
i-Pr	15	59	26
n-Bu		79	21
i-Bu	4	68	28
sec-Bu	2	86	12
t-Bu	7	93	0
allyl	4	85	11

Table 5. Sodium borohydride reduction of crotonaldehyde in various alcohol solvents.

A recent report in this field comes from a Japanese group[18]. They discovered that selective 1,4 reduction of α,β unsaturated conjugated carbonyl compounds could be achieved by means of combination of $LiAlH_4$ with copper iodide (CuI) in the presence of hexamethyl phosphoric triamide (HMPA) as a co-solvent. One example was the reduction of trans-hex-2-enal (46), which, on treatment with $LiAlH_4$ under normal conditions mainly gave the 1, 2 reduction product trans-hex-2-en-1-ol (47). However, the same reduction in the presence of CuI as well as HMPA as a co-solvent gave hexanal (48) as the major product,

which derived from 1,4 reduction of trans-hex-2-enal.



A recent example of high stereoselectivity in the reduction of an α-alkoxy - β-ketoester has been reported[19].

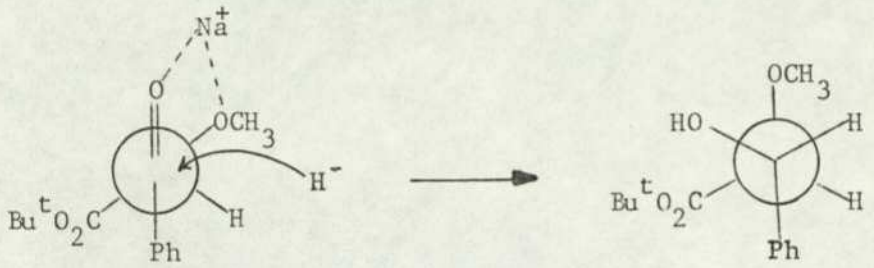
When methoxy ester (50) was prepared by reduction of t-butyl α-methoxy- β-keto carboxylate (49) by $NaBH_4$ in propanol, the diastereomer (erythro-threo) product ratio was 20:1.

A crucial role was suggested for the sodium cation in sodium borohydride reductions in 2-propanol.

(49) (50)

The explanation of the stereochemical results was that reduction of compound (49) proceeded via a chelate in which the sodium ion is coordinated by the carbonyl oxygen atom and the oxygen atom of the α-methoxy group. Delivery of hydride from the less hindered

direction generated the erythro diastereomer as shown in Scheme 11.



Scheme 11.

The selective reduction of the dienol (28b) to alkenols (29) and (30) can now be considered in relation to this literature information. In attempting to formulate a mechanism, several questions arise: (i) why does only one of the four dienol isomers undergo a relatively rapid reduction to alkenols? (ii) what is the role of pH in this reaction, which does not take place at all under neutral conditions? (iii) what controls the apparently sterospecific generation of E-geometry in the alkenols? Answers to these questions cannot be provided at this stage. A number of variations in experimental conditions were made in the hope of gaining further insight into the processes involved in the reduction. The use of potassium borohydride in place of sodium borohydride gave an identical result. In contrast to some of the literature reports discussed above, the use of inverse addition of $NaBH_4$ to the dienals (22a) and (22b) in MeCN instead of the normal mode of addition, did not affect the outcome. Both procedures resulted in formation of the dienols (28a),(28c), and (28d) in 58% yield and alkenols (29) and (30) in 10% yield. Solvent effects on the reduction were only briefly investigated. Reduction of dienals (22a) and (22b) in

wet THF gave a better yield (>20%) of alkenols (29) and (30), with lower yields (<40%) of the isomeric dienols. This last result can probably be explained by a reduction in the amount of isomerisation occurring in wet THF, compared with wet MeCN.

The alkenols (39) and (30) are interesting from the structural point of view in that they are the first examples of penicillin derivatives in which the normal side-chain NHCO linkage has been isosterically replaced by a trans C=C bond. Such isosteric replacements have attracted considerable attention recently, particularly in the field of bio-active peptides. It has recently been shown that in enkephalin analogues in which one peptide linkage has been replaced by a trans-olefinic bond, considerable biological activity is retained[20]. It was therefore of interest to see what activity would result in the penicillin analogues.

Deprotection of each of the benzhydryl esters of (29) and (30) was readily accomplished by brief contact with trifluoroacetic acid-anisole. Fortunately, neither compound underwent isomerization under these conditions and the corresponding carboxylic acid could be isolated in yields of 70-80%. Both of these acids were found to be devoid of antibacterial activity against a standard series of laboratory strains.

Deprotection of dienol (28a) with the same method was also successful, but a carboxylic acid mixture isomeric about the double bonds of side-chain at C_6 was obtained due to the acid catalysed isomerization. This mixture was also devoid of activity.

5.4. Oxidation of the dienals (22).

Oxidation of aldehydes to carboxylic acids can generally be accomplished with a wide variety of oxidising agents, amongst the most popular of which have been $KMnO_4$ (in acid, base or neutral solution), chromic acid and silver oxide.

Oxidation of the dienals (22a) and (22b) was first attempted using $KMnO_4$, but under either basic or neutral conditions no oxidisation products were obtained. The only observable reaction was isomerisation to the expected isomers (22c) and (22d).

Silver oxide has been used under alkaline conditions for the oxidation of thiophene-3-aldehyde to thiophene-3-carboxylic acid.[21] Following an identical procedure, a 2:1 mixture of dienals (22a) and (22b) gave only isomerisation and decomposition products.

Holum[22] has reported that chromium trioxide-pyridine complex is a good oxidant for primary and secondary alcohols and does not attack other readily oxidized groups such as olefinic double bonds.

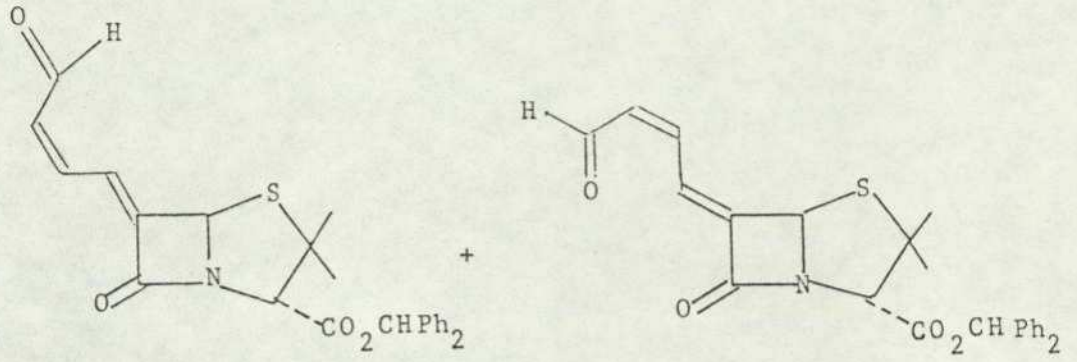
When chromium oxide-pyridine complex was employed to oxidize the dienals (22), the result was negative. It was found that after 18 hours stirring the 2:1 mixture ZE:ZZ of dienals (22a) and (22b) in the presence of chromium oxide-pyridine complex at room temperature, the major components isolated from the product mixture were an isomeric 2:1 mixture of EE and EZ dienals (22c) and (22d). None of the desired carboxylic acids were obtained.

Bowden, Heilbron, Jones and Weedon[23] introduced the famous Jones reagent, which provided a convenient route to acetylenic ketones by oxidizing the corresponding secondary alcohols, without

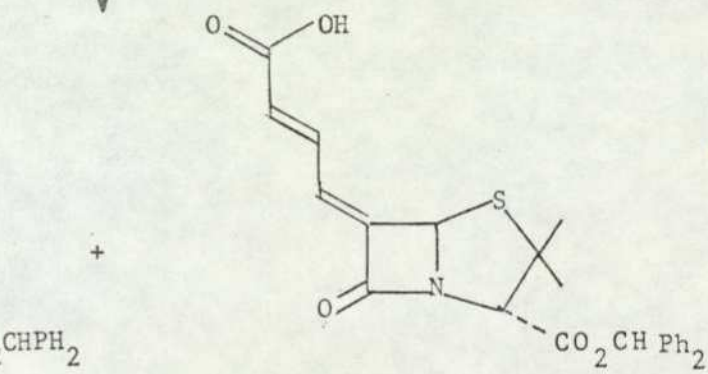
damage to the centres of unsaturation. Since then, the Jones reagent has been widely used, especially in steroid synthesis where hydroxyl groups are frequently required to be converted to ketone functions. The Jones reagent has also been demonstrated to be capable of oxidizing aldehydes to acids.

With normal amounts of oxidant, Jones reagent could not totally convert the dienal (22) to dienoic acid. Complete disappearance of dienal (22) in this reaction required sixfold excess over the usual quantity of Jones reagent and prolonged reaction time or heating. Under these severe reaction conditions, the β-lactam ring did not survive well, a lot of decomposition occurring. Therefore, the oxidation of dienal (22) by this method turned out to be a low yield synthesis. However, an impure 1:1 EZ:EE mixture of 6-(4'-carboxybut-2'-enylidene) pencillanates (51) and (52) could be isolated (Scheme 12), which was unstable and difficult to purify. The structural assignments are based on spectroscopic evidence (i.r. and ^{1}H n.m.r.).

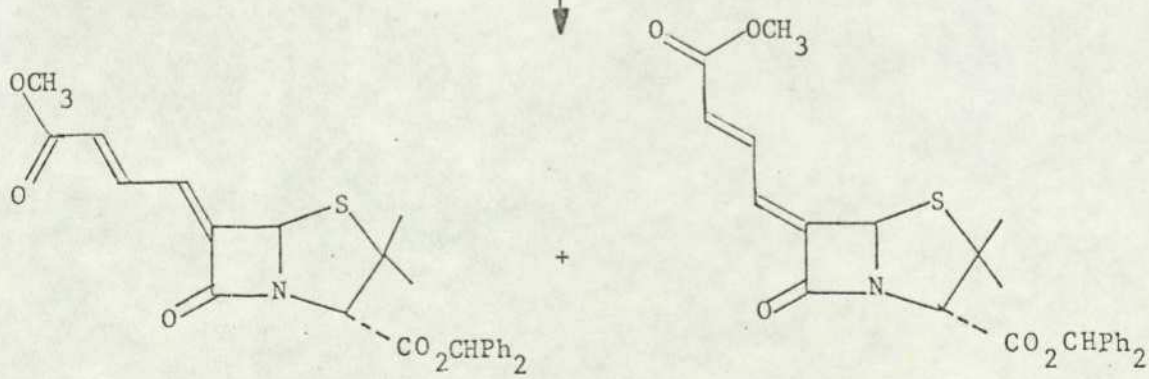
Attempts were made to convert the dienoic acids (51) and (52) to their corresponding esters (53) and (54) for easier purification and storage, by reaction with diazomethane. This reaction proceeded in extremely poor yield and proved to be impractical as a synthetic route to the esters (53) and (54).



(22a) ZE 2 : 1 (22b) ZZ



(51) EE (52) EZ

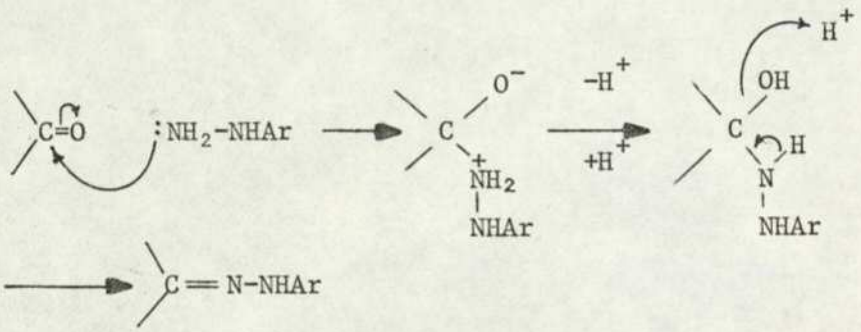


(53) (54)

Scheme 12.

5.5. Carbonyl group condensations of the dienals (22).

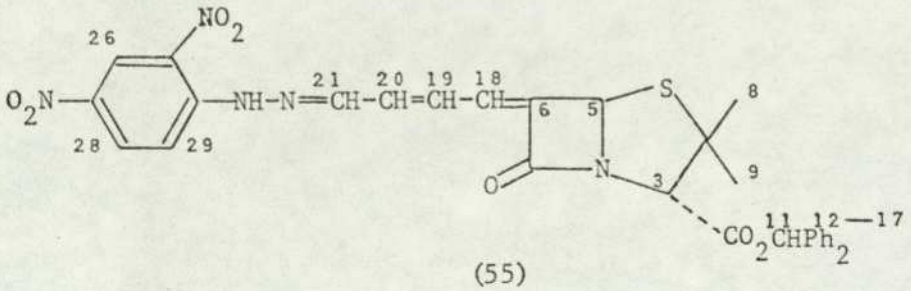
2,4-Dinitrophenylhydrazine has long been used as a reagent for preparing derivatives of aldehydes and ketones. The conventional method, as applied for example[24] to the preparation of derivatives of steroidal ketones, is to carry out the reaction in alcohol solution in the presence of a small amount of strong mineral acid. Johnson[25] discovered that a phosphoric acid-ethanol solution of 2,4-dinitrophenylhydrazine was indefinitely stable and preferable to solutions containing sulphuric acid. The general mechanism of hydrazone formation is shown in Scheme 13.



Scheme 13

When the mixture of dienals (22a) and (22b) was treated with 2,4-dinitrophenylhydrazine using Johnson's procedure, the hydrazone (55) was isolated in good yield (73%), as a complex mixture of isomers. In view of the necessity to use acid conditions for the condensation, it was not surprising that double bond isomerisation should take place. In addition, syn and anti isomerism is possible about the C=N bond in the hydrazone, so that two isomeric hydrazones can be formed for each of the four dienals (22a) — (22d), leading to a total of eight possible products. The product mixture

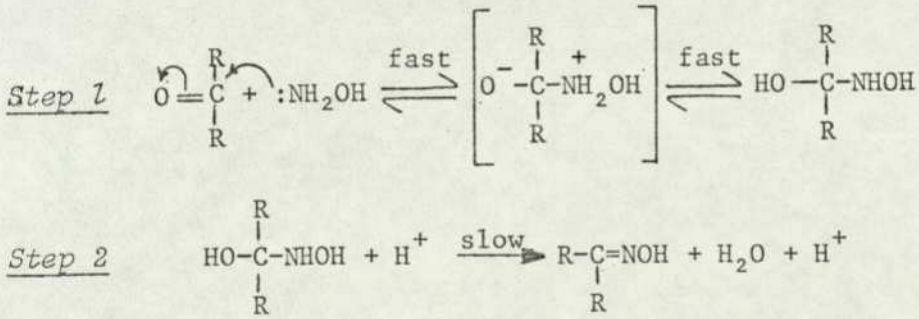
obtained showed two broad singlets in the ^1H n.m.r. at τ -1.30 and -1.48, characteristic of hydrazone =NH groups. Analysis by HPLC (Hypersil 5μm SiO_2, CH_2Cl_2/Et_2O, 99.75 : 0.25) showed that at least five principal components were present. No attempt was made to isolate these individually, although such a separation appears to be possible by preparative HPLC.



(55)

The reaction of hydroxylamine with a carbonyl compound is also a widely used method of preparing derivatives. As expected, aldehydes usually react more rapidly than ketones and aliphatic aldehydes are more reactive than aromatic aldehydes in the synthesis of oximes.

Jencks[26] demonstrated that the rate of formation of oxime is at a maximum at a pH which depends on the substrate but is usually about pH4. The reaction is supposed to follow the mechanism shown in Scheme (14).



Scheme 14

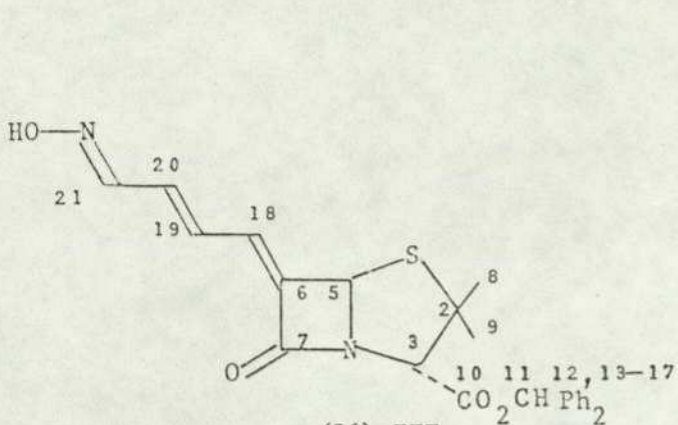
At the lower pH values step 2 is rapid (because it is acid-catalysed) and step 1 is slow (and rate determining) because under these acidic conditions most of the NH_2OH molecules have been converted to the conjugate NH_3OH^+ ions, which cannot attack the substrate. As the pH is slowly increased, the fraction of free NH_2OH molecules increases and consequently so does the reaction rate, until the maximum rate is reached at about pH4. As the rising pH has been causing an increase in the rate of step 1, it has also been causing a decrease in the rate of the acid-catalysed step 2, although this later process has not affected the overall rate since step 2 is still faster than step 1. However, when the pH goes higher still, step 2 becomes rate-determining, and although the rate of step 1 is still increasing (as it will until essentially all the NH_4OH is unprotonated), it is now step 2 which determines the rate, and this step is slowed by the decrease in acid concentration. Thus the overall rate decreases as the pH rises beyond about 4. It is likely that similar conditions apply to the reaction of aldehydes and ketones with amines, hydrazines and other nitrogen nucleophiles. When the oximes are to be prepared for identification purposes, the usual method of preparation simply involves treatment of the carbonyl compound with hydroxylamine in an aqueous medium. If necessary, the pH may be adjusted, and ethanol may be added to enhance mutual solubility of reagents. The reactants may simply be shaken at room temperature or warmed gently on a steam bath.

While the free base, hydroxylamine may be used directly to prepare oximes, because of storage problems it is more usual to prepare hydroxylamine as required <u>in situ</u> from its hydrochloride

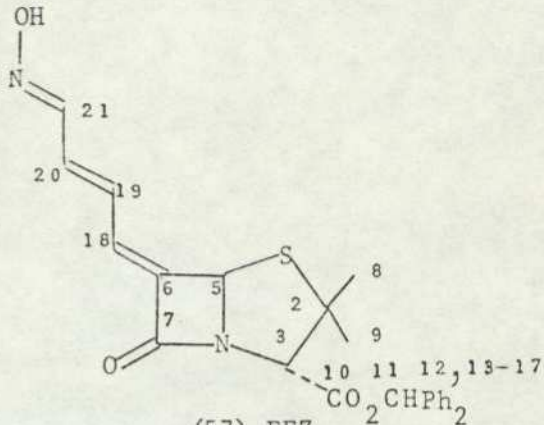
or sulphate salts with a variety of alkaline reagents. (Na_2CO_3, NaOH, NH_4OH, $NaOCOCH_3$, K_2CO_3, pyridine). The importance of pH in the synthesis of oxime was reflected in our experiments. Treatment of dienal (22) with hydroxylamine hydrochloride and pyridine in ethanol on a water bath for 10 minutes did not provide any aldoxime product. Successful preparation of aldoxime was only achieved by reacting the dienal (22) with hydroxylamine hydrochloride and the base sodium acetate instead of the pyridine. Although the pH of two reaction mixtures was not actually measured, it is known that sodium acetate is a stronger base than pyridine. This difference influenced the pH of reaction medium, which was vital for the formation of oxime.

In view of the requirement for both heat and base, it is to be expected that considerable isomerisation will take place during oxime formation from the dienals (22). As in the case of hydrazone formation, eight possible isomers can be formed, in principle. In practice, the crude product isolated after heating the mixture of dienals (22a) and (22b) with hydroxylamine hydrochloride and sodium acetate in aqueous THF was an oil. Preparative t.l.c. gave a main fraction which proved to be a 2:1 mixture which was difficult to separate even by preparative HPLC, but which was identified as a pair of oxime adducts. A minor product fraction was also isolated which contained some unknown compounds which may have included further oxime isomers. The two major oxime products are believed to be the EEE and EEZ isomers (56) and (57). The formation of EE isomers on oximation of α,β-unsaturated aldehydes is to be expected on the basis of literature reports[27,28], and experience with the

dienals (22) leads to the expectation of isomerizations about the α, β-C=C bond, but not the γ, δ- C=C bond, under conditions used.



(56) EEE

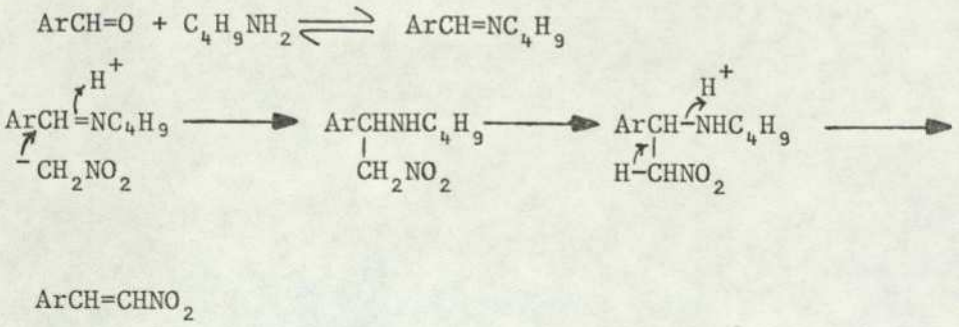


(57) EEZ

Finally, having demonstrated the ability of the carbonyl group of the dienals (22) to condense with nitrogen nucleophiles, attention was turned to the possibility of further extending the carbon side chain by condensation with carbon nucleophiles. As an example of such a reaction, the Knoevenagel condensation was chosen. This is, in general, a reaction between an aldehyde or ketone and an active methylene compound such as malonic acid or its derivatives, usually carried out with piperidine as catalyst.

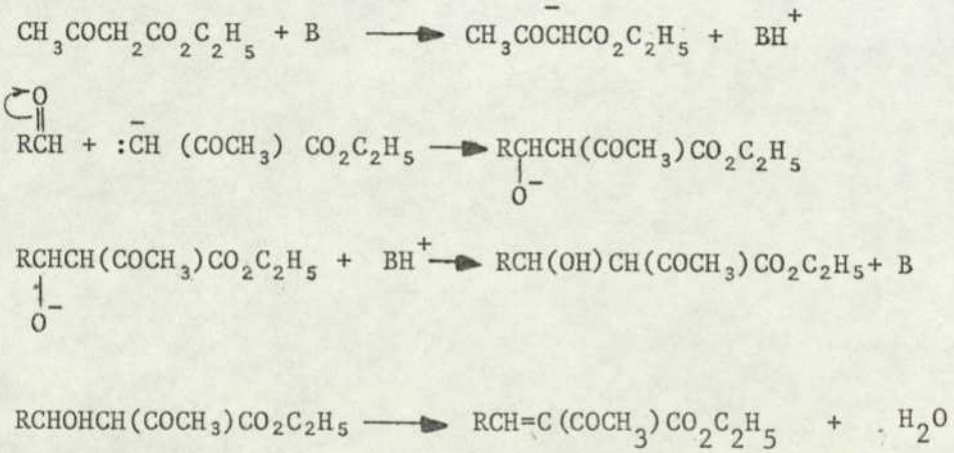
The mechanism of Knoevenagel condensation has been the subject of much discussion. Two principal mechanisms have been discussed in an excellent review by Jones[29]. The first one was suggested by Knoevenagel. He assumed that the amines do not function as simple bases, but instead, are involved in prior reaction with carbonyl compound as well. The fact that such condensations are often most effectively catalysed when a weak acid is present in addition to the amine favours this proposal. Kinetic evidence in support of such a mechanism in the condensation of aromatic aldehydes with nitro-

methane was reported[30]. It has been suggested that the amine reacts with carbonyl compound to form a Schiff base which subsequently condenses with nitromethane to yield the final product, (Scheme 15), and it was necessary to obtain a value for the rate of Schiff base formation in the presence of one equivalent of acetic acid.



Scheme 15

Hann and Lapworth[29] proposed an alternative mechanism in which the function of the base was to remove a proton from active methylene component forming a carbanion which then added to the aldehyde (Scheme 16).

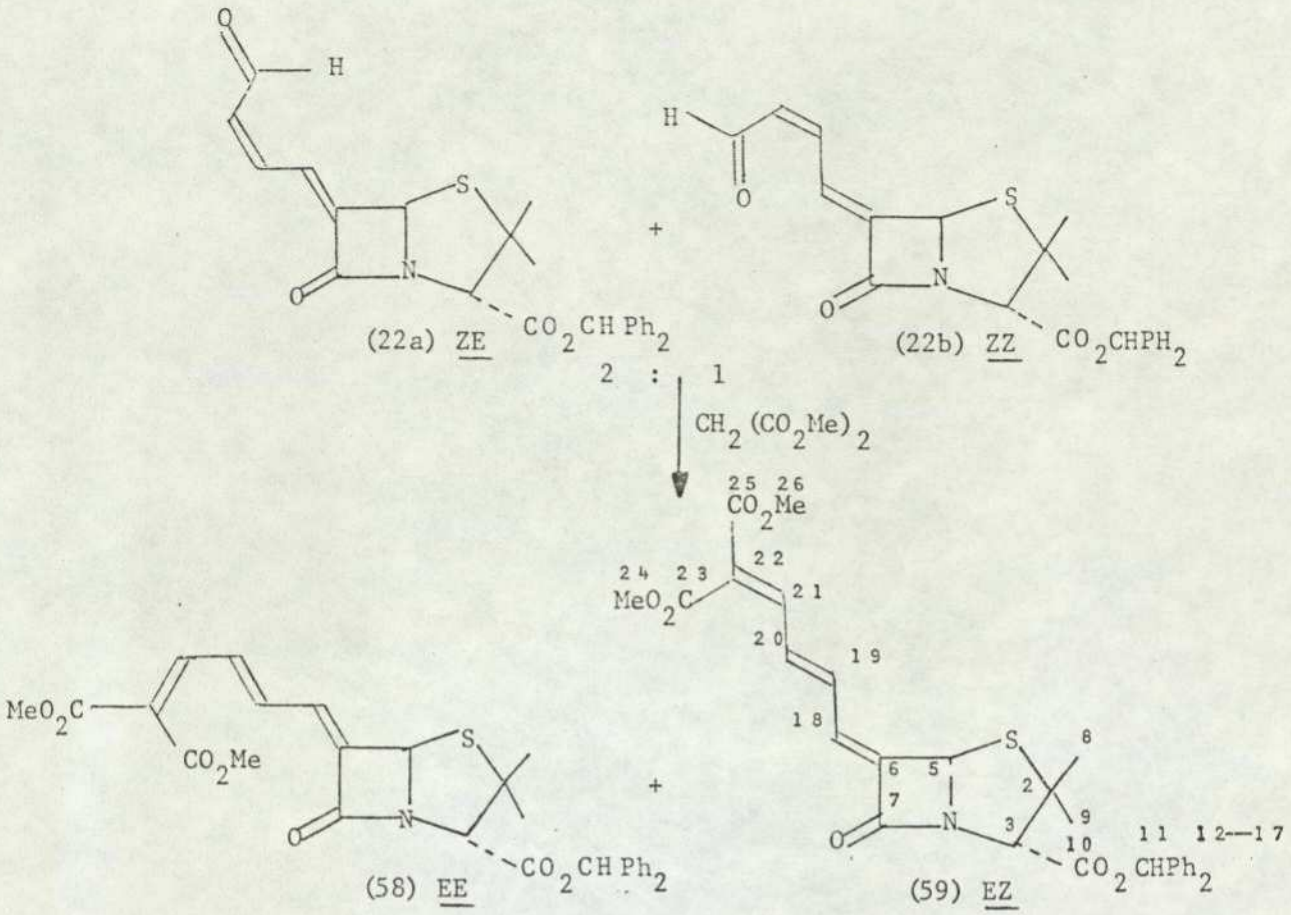


Scheme 16

Jones gave his general conclusion that a single mechanism is unlikely for the wide range of Knoevenagel condensations. The two mechanisms depend on the type of amine used. Condensation in polar media with tertiary base e.g. pyridine as catalyst appears on the whole to favour the Hann and Lapworth mechanism, while the use of primary amines or ammonia favours the intermediate formation of imines or substituteed imines.

The dienals (22) proved to be slow in their reaction with dimethyl malonate. Refluxing in benzene with piperidine and acetic acid for 24 hours led to recovery of considerable amounts of isomers of starting material. With increased amounts of piperidine and use of refluxing toluene, complete conversion was achieved within one hour and a 1.5 : 1 mixture of EE and EZ products (58) and (59) could be isolated in 56% yield and were separated by preparative t.l.c. Once again, isomerisation of the side-chain double bonds is a noticeable feature of the reaction, with the more stable E geometry being favoured for the bond which is α,β in the dienals.

Deprotection of the condensation products (58) and (59) caused a lot of problems. Treatment of compounds (58) and (59) with T.F.A. and anisole failed to produce any of the corresponding carboxylic acids. It is postulated that the additional unsaturated centre $[(CH_3CO_2)_2C{=}C]$ in the condensation adducts (58) and (59) makes the side chain at C_6 of the pencillanate more acid labile. The T.F.A. reagent might attack the centres of unsaturation prior to cleaving the benzhydryl ester function.



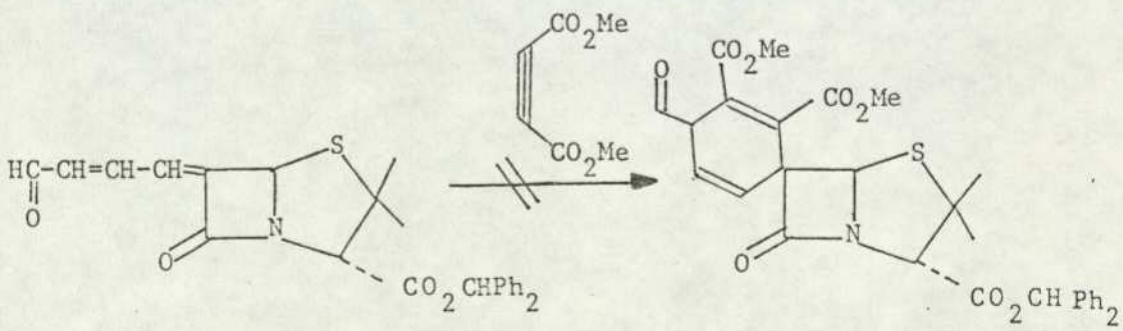
The alternative technique, hydrogenolysis, was not considered due to the presence of numerous unsaturated centres. Attempts were therefore made to deblock the dienals (22) prior to Knoevenagel condensation. Deprotection of the mixture of dienals (22a) and (22b) with T.F.A. and anisole resulted quantitatively in the corresponding carboxylic acid isomeric mixture, which was reacted directly with the dimethyl malonate under similar conditions. The effort was not successful, no desired aduct being detected in the product mixture.

It was discovered that even at room temperature the carboxylic acids were rather unstable. When the reaction temperature had to be raised sufficiently for the Knoevenagel condensation, the carboxylic acids decomposed before the condensation actually started.

5.6 Attempted Diels-Alder reactions with the dienals (22).

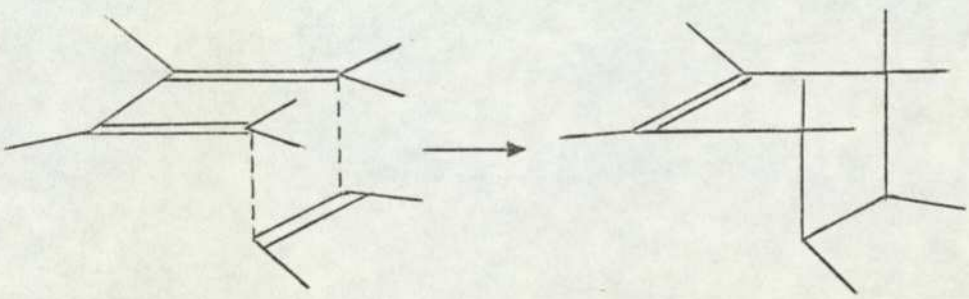
The Diels-Alder reaction[31,32,33,34] consists of the addition of a compound containing a double or triple bond (Dienophile) to the 1,4-positions of a conjugated diene system, leading to the formation of a six-membered hydroaromatic ring. Most dienophiles are substituted by at least one electron - withdrawing group (e.g. C=O, C≡N, NO_2) and react particularly effectively with electron-rich dienes (e.g. those with alkyl or alkoxy substituents). However, other dienophiles such as -C≡N, -N=N, O=N- and singlet oxygen have been used in heterocyclic syntheses, and reverse-polarity reactions are also known between electron-poor dienes and electron-rich dienophiles.

Dimethyl acetylenedicarboxylate (D.M.A.D.) is regarded as one of the most powerful dienophiles, which reacts with a very wide range of dienes. It was the first dienophile chosen to react with the dienal (22). However, attempts to synthesize the cycloaddition adduct (60) were unsuccessful. Addition of D.M.A.D. to dienal (22) at 130° in toluene or neat form did not produce any of the desired product.



(22) (60)

Three factors may be responsible for the failure of this reaction. Firstly, D.M.A.D. is a very electron-poor dienophile. However, the dienals (22) are certainly not electron-rich dienes and the relative energies of the orbitals in the reaction partners may therefore be inappropriate. Secondly, the dienals are effectively tri-substituted at the two ends of the diene system, and it is known that substitution at the ends of dienes tends to disfavour the Diels-Alder reaction sterically. Thirdly, the (4 + 2) cycloaddition reaction requires a syn-periplanar arrangement of the reaction partners (61) in order to achieve orbital overlap and this may be sterically difficult for the isomeric dienals (22) to achieve.



(61)

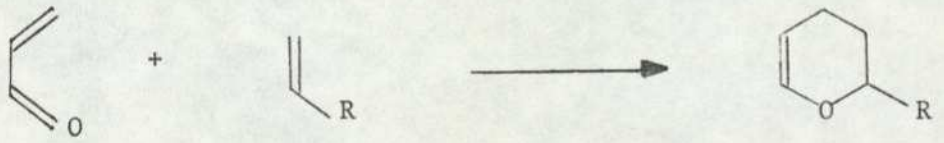
Literature reports of successful Diels-Alder reactions between electron-poor dienes such as dimethyl hexa-2,4-dienoate and maleic acid derivatives encouraged us to make further attempts. Reactions between the dienals (22) and dimethyl maleate and maleic anhydride were attempted, but again with negative results.

Styrene and vinyl acetate have been used as an electron-rich olefin for (4 + 2) cycloaddition reactions e.g. with α,β-unsaturated aldehydes, leading to dihydropyrans (Scheme 17).

Attempted condensations of styrene and of vinyl acetate with the dienals(22) again proved fruitless.

Thus, it does not appear to be possible to effect (4 + 2) cycloaddition reactions with the dienals (22). This failure is regarded as being due to a combination of steric factors, arising from the terminal substitution of the diene and the difficulty of its achieving the required cisoid conformation.

It should be noted that in any of the above attempted cycloaddition reactions, the maximum temperature employed was 130°. Above this temperature, the dienals (22) are unstable and rapidly decompose.



R=Ph

OAc

Scheme 17.

5.7 Experimental

6-(4'-oxobut-2'-enylidene) penicillanates(22a)ZE and (22b)ZZ.-The solution of benzhydryl 6-diazopenicillanate (21) (4g, 0.01 mmol) in furan (15 ml) was added dropwise with stirring to ice-cooled furan (10 ml) containing rhodium acetate hydrate (20 mgn, 9.05×10^{-5} mmol) in a period of one hour. The unreacted catalyst was filtered off. Removal of excess furan under vacuum gave a yellow-solid (100%), m.p. 54-60°, a 2:1 mixture of ZE and ZZ dienals (22a) and (22b). The adducts were pure enough to be used as starting material in the subsequent experiments without any purification. Separation of the two isomers could be achieved by prep. t.l.c. (CH_2Cl_2/Et_2O 99:1) (22a), yellow solid, mp 54-60°, (22b) oil.

6-(4'-oxo-but-2-enylidene penicillanates (22c) EE and (22d) EZ. To a solution of the 2:1 mixture of ZE and ZZ dienals (22a) and (22b) in CH_3CN was added aqueous NaOH (0.4-0.5 equivalent). After working up, a 1.2:1 mixture of EE and EZ dienals (22c) m.p. 185° and (22d) m.p. 70° was recovered. The two isomers could be separated by prep. t.l.c. (CH_2Cl_2/Et_2O 99:1) or more efficiently by prep. HPLC (LichroPrepSI60 15-25μm, CH_2Cl_2/CH_3CN 99:1). The polarity of 4 isomers were in the order: ZE (22a) <EE(22c) <EZ(22d) <ZZ(22b). The spectroscopic data of 4 isomers are shown in Tables 1, 2, 6, 7 and 9. Their microanalytical data are shown in Table 8.

Deprotection of dienals (22a) and (22b)* To a solution of the 2:1 mixture of dienals (22a) and (22b) (217 mgm 0.5 mmol) in anisole (0.5 ml) cooled in an ice bath was added cold trifluoroacetic acid (1.5 ml) with stirring. After 2 minutes the reaction was quenched immediately with aqueous $NaHCO_3$ (5% 40ml), washed with CH_2Cl_2 three times, EtOAc

once, then acidified by aqueous citric acid (37.5 ml 5%), extracted with EtOAc, washed with brine, and dried over anhydrous magnesium sulphate. Evaporation of the solvent in vacuum gave a brown oil, 130 mgm, (99%), a mixture of geometric isomers about the side-chain double bonds, which could be recrystallized from ethylacetate and cyclohexane as a very unstable solid.

*The same deprotection procedure has been used throughout this Chapter.

Compound	Main u.v. Band λ_{max} nm (Logε)
(22a)	277 (4.28)
(22b)	277 (4.33)
(22c)	279 (4.47)
(22d)	279 (4.30)

Table 6. U.v. data of dienals (22a-d). In CH_3CN. All the compounds show shoulders at 242, 248, 254 and 256 nm.

	22a (nujol) cm^{-1}	22b (nujol) cm^{-1}	22c (nujol) cm^{-1}	22d ($CHCl_3$) cm^{-1}
C-H stretching	3090, 3060	3060, 3030	3050, 3030	
in alkene	3030			
β-Lactam	1765	1770	1762	1765
ester	1740	1740	1740	1740
unsaturated aldehyde	1675	1675	1680 1670	1685

Table 7 I.r. data of dienals (22a-d).

$C_{25}H_{23}NO_4S$

	calculated	22a	22b
C%	69.26	68.97	68.97
H%	5.35	5.41	5.40
N%	3.23	2.98	2.97

Table 8. Microanalytical data of dienals (22a-b)

$C_{25}H_{23}NSO_4$ MW: 433

	Intensity %			
$^m/_e$	(22a)ZE	(22b)ZZ	(22c)EE	(22d)EZ
433(M^+)	1.2	0.3	0.1	0.6
266	2.9	0.5	0.5	1.8
167	100	100	82.3	100
108	1.3	1.2	2.1	1.2
99	2.3	0.6	3.9	0.6
59	2.2	1.9	5.3	0.9
79	0.9	2.5	3.1	0.6
56	0.7	2.0	14.3	*
52	2.2	2.0	3.8	*

Table 9. M.S. data of dienals (22a-d).

*Below 50 no data were available.

Benzhydryl 6-(4'-oxobutanyl) penicillanates (24) and (25). A solution of the 2:1 mixture of dienals (22a) and (22b)(200 mgm 0.46 mmol) in ethyl acetate (5 ml) was hydrogenated in the presence of 5% palladium on charcoal (250 mgm) at room temperature and atmospheric pressure for 18 hours. After removal of catalyst with filter aid (ayflo), washings and concentration of filtrate, the crude product was submitted to prep. t.l.c. (CH_2Cl_2/Et_2O 97:3). A 1:1 mixture of α and β epimeric saturated aldehydes (24) and (25) was isolated, which was not separable on conventional chromatography, but can be resolved by HPLC (Hypersil, 5μm, CH_2Cl_2/Et_2O 100:3) polarity: α<β. The mixture was an oil, 70 mgm (35%), $\lambda_{max}(CH_3OH)$

215 nm, ν_{max}($CHCl_3$) 1765, 1740 (shoulder) cm^{-1}, (24)$\underline{\alpha}$ τ ($CDCl_3$) 0.30(1H, t, J=1.2Hz, C_{21}-H), 2.64 (10H, m, aromatic H), 3.08 (1H, s, C_{11}-H), 4.84 (1H,d, J=1.5Hz, C_5-H), 5.44 (1H, s, C_3-H), 6.72 (1H, m, C_6-H), 7.50 (2H, t, d, J=4.8, 1.2Hz, C_{20}-H), 8.20 (4H, m, C_{18} and C_{19}-H),8.36(3H, s, C_8-H), 8.72 (3H, s, C_9-H), (25) $\underline{\beta}$ τ ($CDCl_3$) 0.3 (1H, t, J=1.2Hz), C_{21}- H), 2.64 (10H, m, aromatic H),3.08 (1H, s, C_{11}-H), 4.76 (1H, d, J=4.4Hz , C_5-H), 5.52 (1H, s, C_3-H), 6.44 (1H, m, C_6-H), 7.50 (2H, t d, J=4.8, 1.2Hz, C_{20}-H), 8.20 (4H, m, $C_{18}$and C_{19}-H), 8.36 (3H, s, C_8-H), 8.72 (3H, s, C_9-H), (Found, C, 67.77; H 6.18; N, 3.14%; $C_{25}H_{27}NO_4S$ requires C, 68.63; H, 6.22; N,3.20%). m/e: 158(0.7%), 167 (81.4%), 99 (4.2%), 59(3.9%), 112 (3.9%), 68 (7.2%). Deprotection of the 1:1 mixture of (24) and (25) (87 mgm 0.2 mmol) with T.F.A. and anisole furnished the corresponding carboxylic acids, a 1.6:1 mixture of α and β isomers 43 mgm (80%).

Benzhydryl 6-(4'-hydroxybutanyl)penicillanates (26) and (27). A solution of the 2:1 mixture of dienals (22a) and (22b) (87 mgm, 0.2 mmol) in THF (1 ml) and CH_3OH (3 ml) was hydrogenated in the presence of palladium black (87 mgm) at room temperature and atmospheric pressure for 14 hours. After removal of catalyst with filter-aid (afylo), washing and concentration of filtrate, the crude product was submitted to prep. t.l.c. (CH_2Cl_2/Et_2O 97:3). In addition to the less polar alkanals (24) and (25) 3o mgm (34.5%), an isomeric 2:1 mixture of α and β epimeric saturated alcohols (26) and (27) (11 mgm, 13%) was eluted, which was non-separable on conventional chromatography but resolved on analytical HPLC (Hypersil 5μm, CH_3CH/CH_2Cl_2 5:95, 230 nm), λ_{max} (CH_3CN) 220 nm (log ε4.16) ν_{max}($CHCl_3$) 3480, 1765, 1740 cm^{-1}, (26) $\underline{\alpha}$ τ ($CDCl_3$) 2.65 (10H, m, aromatic H), 3.10 (1H, s, C_{11}-H), 4.95 (1H, d, J=1.5Hz, C_5-H), 5.44 (1H, s, C_3-H), 6.36 (2H, t, J=6.3Hz C_{21}-H), 6.72 (1H, m, C_6-H), 8.20-8.40 (6H, m, C_{18}, C_{19} and C_{20}-H), 8.38 (3H, s, C_8-H),

8.75 (3H, s, C_9-H), (27) β ($CDCl_3$) 2.65 (10H, m, aromatic H), 3.10 (1H, s, C_{11}-H), 4.60(1H,d,J=4.1Hz,C_5-H), 5.53 (1H, s, C_3-H), 6.72 (2H, t, J=7.2Hz, C_{21}-H), 6.36 (1H, m, C_6-H), 8.20-8.40, (6H, m, C_{18}, C_{19},C_{20}-H), 8.38 (3H, s, C_8-H), 8.75 (3H, s, C_9-H).

m/e : 439 (M^+; 0.04%), 325 (0.14%), 99 (0.6%), 59 (0.2%), 167 (100%), 114 (3.7%), 96 (0.4%), 67 (0.6%).

Benzhydryl 6-(4'-hydroxybut-2'-enylidene)penicillanates (28a-d). The buffer solution, pH7, was prepared by dissolving 1 pack of E.M.I. powder into 200 ml of distilled water. Its pH value was checked with a pH meter.

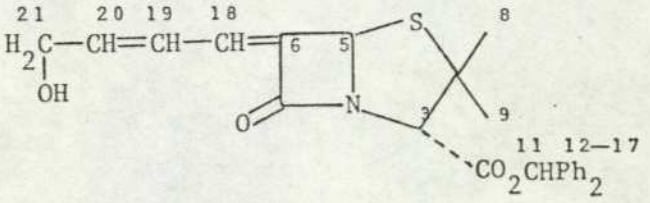
Aqueous buffer solution (pH7) was added to the solution of dienal mixture of (22a) and (22b) (2:1),(216 mgm, 0.5 mmol) in acetonitrile (15 ml). Sodium borohydride (95%, 10mg, 0.25 mmol) in water (0.25 ml) was added dropwise to the above solution with stirring at room temperature for 10 minutes. The reaction mixture was diluted with methylene chloride (20 ml), washed with brine several times and dried over anhydrous magnesium sulphate. After evaporation of the solvent under vacuum, the yellow oil was submitted to preparative HPLC, (30 x 2.1 cm. LichroPrep. SI60 15-25μm, flow rate 43 ml/m, eluent CH_3CN/CH_2Cl_2 5:95, u.v. 275 nm). Dienals (28a) and (28b) were isolated individually as pure compounds, isomers (28c) and (28d) came out as a mixture which might be separated by changing the HPLC conditions, however analytical HPLC (Hypersil 5μm, CH_3CN/CH_2Cl_2 , 9:91, u.v. 275 nm) showed the resolution of these two isomers. The polarity of 4 isomers being in the order:

ZE (28a)<EZ (28d)<EE (28c)<ZZ (28b).

Dienol (28a), 87 mgm (40%), mp 54-58°, λ_{max}(CH_3CN) 271 nm (log ε 4.20)

ν_{max} ($CHCl_3$) 3400, 1745 (shoulder) 1670, 1170 (C-O-C, and C-OH) cm^{-1}, data of ^1H and ^{13}C n.m.r. are shown in Tables 10 and 11, m/e: 435(M^+;0.22%)268(0.4%), 167 (100%), 110 (0.9%), 158 (0.5%) 92 (0.4%). Dienol (28b), 48 mgm (22%), m.p. 48-50°C, λ_{max}(CH_3CN) 271 nm (log ε 4.1), ν_{max} (CH_2Cl_2) 3600, 3480, 1745, (shoulder) 1670, and 1170 (C-O-C, and C-OH) cm^{-1}, data of ^1H and ^{13}C n.m.r. are shown in Tables 10 and 11. (Found: C, 68.71; H, 5.60; N, 3.19%; $C_{25}H_{25}NO_4S$ requires C, 68.94; H, 5.60; N, 3.21%), m/e: 280 (0.2%), 167 (29.5%), 251 (0.3%), 158 (0.2%), 99 (41.2%), 59 (2.1%), 107 (0.9%), 110 (1.2%), 92 (7.7). Mixture of dienals (28c) and (28d), 20 mgm (9.3%), ratio of EE/EZ is 1:1.7, λ_{max} (CH_3CN) 269 nm (log ε 4.35), ν_{max} ($CHCl_3$) 3600-3100, 1745 (shoulder), 1670, 1260 cm^{-1} (C-O-C, C-OH), data of ^1H and ^{13}C n.m.r. are shown in Tables 10 and 11. m/e: 435 (M^+; 4.7%), 268 (17%)., 167 (100%), 110(0.3%), 92(0.3%), 99(0.9 %),107(0.6 %), 59(1%).

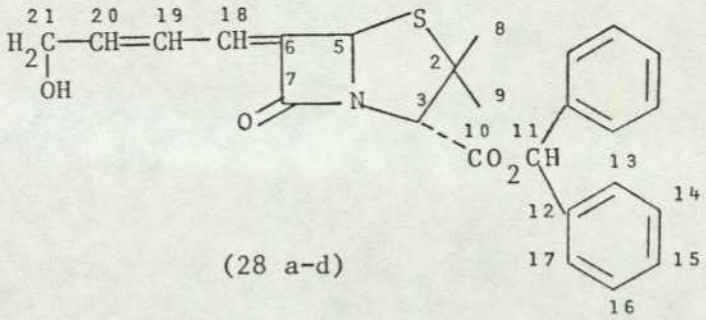
Deprotection of dienol (28a) (87 mgm 0.2 mmol) by trifluoroacetic acid-anisole gave the corresponding carboxylic acid (20 mgm 37%) which was a mixture of geometric isomers about the side-chain double bonds at C_6 of penicillin.



(28 a-d)

	PROTONS ON CARBON										
	C_3 (s,1H)	C_5 (s,1H)	C_8 (s,3H)	C_9 (s,3H)	C_{11} (s,1H)	C_{12-17} (m,10H)	C_{18} (d, 1H)	C_{19} (d,d, 1H)	C_{20} (d,t, 1H)	C_{21} (d, 2H)	OH (s, 1H)
(28a)ZE	5.28	4.16	8.40	8.70	2.94	2.52	3.10 ($J_{18=19}$ 13.5Hz)	3.16 ($J_{19=20}$ 10.5Hz)	3.92	5.52 (J=6.9Hz)	8.0
(28b)ZZ	5.30	4.08	8.40	8.70	2.94	2.52	2.88	*Around 3.8	*Around 3.8	5.52 (J=4.8Hz)	7.68 (broad)
(28c)EE	5.28	4.16	8.40	8.70	2.92	2.52	*Around 3.66	3.82	*Around 3.66	5.62	7.44 (broad)
(28d)EZ	5.28	4.05	8.40	8.70	2.92	2.52	*Around 3.66	3.16	*Around 3.66	5.62	7.44 (broad)

Table 10. ^{1}H n.m.r. data of dienols (28a-d). In $CDCl_3$, τ values referred to T.M.S.
*signals were overlapped as a deceptively simple pattern.



(28 a-d)

	C_2	C_3	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13-17}	C_{18}	C_{19}	C_{20}	C_{21}
(28a)ZE	65.56	70.44	78.52	142.45	168.94	33.60	25.72	167.58	68.29	139.58	128.91 128.65 128.45 127.80 127.34	126.36	137.50	125.19	58.85
(28b)ZZ	65.43	70.18	78.45	143.36	169.46	33.46	25.65	167.45	67.51	139.52	128.84 129.58 128.39 127.80 127.27	122.07	139.97	123.44	59.11
(28c)EE	65.36	70.25	78.39	141.73	168.94	33.52	25.65	167.58	68.23	139.52	128.78 129.52 128.39	130.92	141.28	126.95	62.89
(28d)EZ	65.36	70.25	78.39	141.73	169.66	33.52	25.65	167.58	67.71	139.52	127.73 127.34	125.65	143.29	123.50	62.89

Table 11. ^{13}C n.m.r. data of dienols (28a-d). In $CDCl_3$, δ values referred to T.M.S.

Benzhydryl 6-(4'-hydroxybut-1'-enyl) penicillanates (29) and (30).

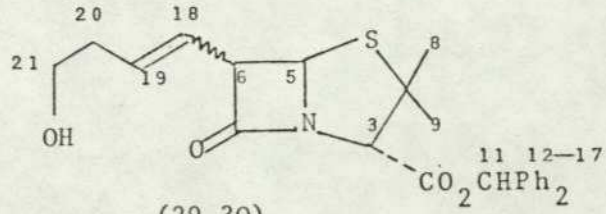
Sodium borohydride (95%, 15 mgm, 0.375 mmol) in water (0.25 ml) was added to the mixture of dienals (22a), (22b) (2:1, 324 mgm, 0.75 mmol) in acetonitrile (20 ml) and aqueous buffer solution (pH7 4.5 ml) with stirring. The mixture was stirred for 15 minutes at room temperature, then diluted with EtOAc (50 ml), washed by brine three times, and evaporated down under vacuum to give a yellow oil. The yellow oil in acetonitrile (7 ml) was added to a solution of sodium borohydride (95%, 300 mgm, 7.5 mmol) in water, stirred for one hour at room temperature, then an additional batch of sodium borohydride (95%, 150 mgm, 3.75 mmol), was added and the stirring was continued for another hour. The product mixture was diluted with EtOAc, washed with brine and dried over anhydrous magnesium sulphate. After removal of the solvent under vacuum, the crude product was first prepurified by open column chromatography (1.4 x 14 cm, silica gel 63-250 mesh 10g, eluent: CH_3CN/CH_2Cl_2 1:99, 150 ml, CH_3CN/CH_2Cl_2 13:87, 100 ml), then was submitted to preparative HPLC, (30 x 2.1 cm, LichroPrep Si60, 15-25μm, 230 nm, CH_3CN/CH_2Cl_2, 7~8/92~93). The less polar component was (29) α, oil, 43 mgm (13%), λ_{max} (CH_3CN) 224 nm (log ε 3.84), ν_{max}($CHCl_3$) 3440, 1765, 1745, 1175 (C-O-C, C-OH), 968 (C=C trans) cm^{-1}, data of ^1H and ^{13}C n.m.r. are shown in Tables 12 and 13, $^m/e$: 437 (M^+; 0.14%), 167 (100%), 270 (0.6%), 99 (2.9%), 59 (0.4%), 112 (5.6%), 94 (0.6%), 66 (2%). The more polar componentwas (30) β, oil, 59 mgm (18%), λ_{max} (CH_3CN) 220 nm (log ε 4.01), ν_{max} ($CHCl_3$) 3440, 1765, 1740, 1175, 968 cm^{-1}, Data of ^1H and ^{13}C n.m.r. are shown in Tables 12 and 13, $^m/e$: 437

(M^+; 0.2%), 167 (100%), 270 (0.6%), 99 (1.7%), 59 (1%), 112 (4.7%), 94 (0.5%), 66 (0.5%). Deprotection of (29) (55 mgm. 0.129 mmol) by trifluoroacetic acid-anisole gave the corresponding carboxylic acid (29 mgm, 85%), and deprotection of (30) (41 mgm, 0.1 mmol) produced the corresponding carboxylic acid (23 mgm 90%), m.p. 45-50°.

General procedure for monitoring the $NaBH_4$ reduction of dienals (22). 3 mgm .(6.9×10^{-6} mole) of the 2:1 isomeric mixture of dienals (22a) and (22b) was dissolved in CH_3CN (0.2 ml). The concentration of aqueous sodiumborohydride solution was 0.031 mgm (7.75×10^{-7} mole) per drop. After adding every drop of $NaBH_4$ solution, 1-2µl of product mixture was analysed by HPLC. Conditions for analytical HPLC: column: 25 x 0.45 cm i.d., Hypersil 5µm, eluent: CH_3CN/CH_2Cl_2 9:91, u.v.: 215 nm for dienols analysis, 230 nm for alkenols analysis.

Procedure for investigation of mechanism of base-catalysed isomerization of (22a) and (22b). A solution of NaOD was prepared by adding sodium lead alloy to the D_2O, then the unreacted metal was filtered off by glass wool.

20 mgm (0.046 mmole) of 2:1 isomeric mixture of (22a) and (22b) was dissolved in 0.2 ml of CH_3CN, which was placed into a ^1H n.m.r. tube. The NaOD solution was directly added to the dienals mixture in several portions with a microsyringe. The product mixture was monitored by ^1H n.m.r. The ratio of C_5-H and C_3-H remained 1:1 all the time, a result indicated no any D-incorporation at C_5.

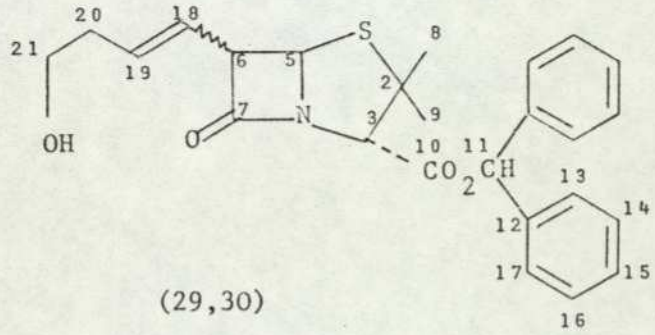


(29,30)

	PROTONS ON CARBON											
	C_3 (s,1H)	C_5 (d,1H)	C_6 (d,d,1H)	C_8 (s,3H)	C_9 (s,3H)	C_{11} (s,3H)	C_{12-17} (m, 10H)	C_{18} (d,d, 1H)	C_{19} (d,t, 1H)	C_{20} (d,t,2H)	C_{21} (t,2H)	OH
(29)(6α)	5.30	4.75 (J=1.5Hz)	6.00 ($J_{C_6C_{18}}$ 7.5Hz)	8.32	8.70	2.95	2.52	*4.20 ($J_{C_{18-19}}$ 15.8Hz)	*4.20 ($J_{C_{19-20}}$ 6.8Hz)	7.60 ($J_{C_{20-21}}$ =6.0Hz)	6.23	8.12
(30)(6β)	5.38	4.40 (J=4.2Hz)	5.62 ($J_{C_6C_{18}}$ 7.5Hz)	8.32	8.65	2.95	2.54	*4.20 ($J_{C_{18-19}}$ 15.8Hz)	*4.20 ($J_{C_{19-20}}$ 6.8Hz)	7.60 ($J_{C_{20-21}}$ =6.0Hz)	6.25	8.16

Table 12. ^{1}H n.m.r., data of alkenols (29) and (30). In $CDCl_3$, τ values referred to T.M.S.

*signals were overlapped as a deceptively simple pattern. Addition of Eu(fod)$_3$ or changing the $CDCl_3$ to C_6D_6 led to a good separation.



(29,30)

Compound	C_2	C_3	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13-17}	C_{18}	C_{19}	C_{20}	C_{21}
(29)(6α)	65.36	69.79	78.45	64.19	167.19	33.07	26.04	172.92	67.71	139.45	128.78 128.39 127.80 127.21	124.93	132.81	35.31	61.65
(30)(6β)	64.71	69.79	78.52	56.77	167.51	31.97	26.43	174.22	67.38	139.52	128.91 128.43 127.28 127.93	124.15	133.53	36.13	61.59

Table 13. ^{13}C n.m.r. data of alkenols (29) and (30). In $CDCl_3$, δ values referred to T.M.S.

Benzhydryl 6-(4'-carboxybut-2-enylidene) penicillanates (51) and (52).

Jones reagent. Chromium trioxide (6.7g) was dissolved in conc. sulphuric acid (6ml) and distilled water, which made up the volume of the solution to 50 ml.

To a solution of the 2:1 mixture of ZE:ZZ dienals (22a) and (22b) (100 mgm, 0.23 mmol) in acetone (1 ml) was added the Jones' reagent (0.5 ml) dropwise with stirring at room temperature. After 10-15 minutes, the reaction mixture was mixed with EtOAc (10 ml), washed with brine, then made alkaline with aqueous sodium bicarbonate solution (5%). The aqueous layer was washed with methylene chloride twice, then acidified by aqueous citric acid solution (5%), extracted with EtOAc, and the latter washed with brine and dried over anhydrous magnesium sulphate. After evaporation of the solvent under vacuum, it gave a yellow oil, 20 mgm, (19%). Analytical t.l.c. showed mainly two spots, (EtoAc/H_2O/$(CH_3)_2CO$ 2.5:0.5:2.5), ν_{max} ($CHCl_3$) 3500-3000 (broad), 1750, 1720 (shoulder) 1695 (shoulder) cm^{-1}, τ ($CDCl_3$) 1.9-2.27 (signals overlapped, OH and C_{19}-H), 2.64 (10H, m, aromatic H), 3.05 (1H, s, C_{11}-H), 3.44-4.56 (3H, signals overlapped, C_5, C_{18}, C_{20}-H), 5.24 ($\frac{1}{2}$H, s, C_3-H of (51)), 5.35 ($\frac{1}{2}$H, s, C_3-H of (52), 8.30 (3H, s, C_8-H), 8.44 (3H, s, C_9-H), m/e for the mixture of (53) and (54): 463(M^+;0.6%), 167(100%), 99(1.3%), 59(3.2%), 138 (2.5%), 107(1.3%).

Benzhydryl 6-(4'-oxobut-2'-enylidene) pencicillanate 2,4-dinitrophenylhydrazones (55). 2,4-Dinitrophenylhydrazine (2g, 0.01m) was dissolved in phosphoric acid (85% 60ml) and diluted to 100 ml with methanol. To a solution of the 2:1 mixture of ZE and ZZ of dienals (22a) and (22b) (100 mgm 0.23 mmol) in THF (2 ml) was added the 2,4-dinitrophenyl hydrazine reagent (2.5 ml) at room temperature with stirring. The deep orange coloured precipitate which formed immediately was filtered, washed with methanol, then recrystallized from ethyl acetate and cyclohexane. Yield 103 mgm (73%), m.p. 115-118°, $\lambda_{max}(CH_3CN)$ 394 mm (log ε 4.53), ν_{max} (nujol) 330 (NH), 1765, 1745, 1680 (N=C-C=C), 1620 (NO_2) cm^{-1}, τ ($CDCl_3$) -1.48 and -1.30(totally 1H , broad, NH of different isomers), 0.91 (1H, d, J=2.4 Hz, C_{26}-H), 1.64 to 3.58 (signals overlapped, C_{18}, C_{19}, C_{20}, C_{21}, C_{28}, C_{29}-H), 2.64 (10H, m, C_{12}-C_{17}-H), 3.04 (1H, s, C_{11}-H), 4.20, 4.18 (totally 1H, s, C_5-H of different isomers) 5.32 (1H, s, C_3-H), 8.4 (3H, s, C_8-H), 8.7 (3H, s, C_9-H), (Found: C, 60.47; H, 4.67; N, 11.24%,$C_{31}H_{27}N_5O_7S$ requires C, 60.68; H, 4.43; N 11.41%), $^m/e$: 432 (0.8%), 181 (0.7%), 167 (100%), 158 (0.4%), 107 (2.6%), 99 (1.4%), 79 (10.4%),

Benzhydryl 6-(4'-oxobut-2'-enylidene) penicillanate oximes (56) and (57). The 2:1 mixture of ZE and ZZ dienals (22a) and (22b) (200 mgm, 0.46 mmol) was added to a solution of hydroxylamine hydrochloride (200 mgm, 2.9 mmol) and sodium acetate (300 mgm, 2.2 mmol) in water (1 ml), THF (3 ml) and CH_3CN (2 ml). The mixture was warmed at 100° with stirring for 5 minutes, extracted with EtOAc (15 ml), the organic phase was washed with brine and dried over anhydrous magnesium sulphate. After removal of the solvent under vacuum, the crude product, a yellow oil, was submitted to prep. t.l.c. (SiO_2, CH_2Cl_2/Et_2O 95:5). Two fractions were isolated. The less polar fraction, yellow oil, 95 mgm (46%), was an isomeric mixture of aldoximes (56) and (57), analytical HPLC showed a 2:1 ratio of them (Hypersil 5μm, CH_3CN/CH_2Cl_2 7:93, 275 nm), $\lambda_{max}(CH_3CN)$ 302 nm (log ε 4.44), $\nu_{max}(CHCl_3)$ 3570 (free OH in dilute solution), 3370 (bonded OH), 1750 (broad), 1205 (C-O-C, C-OH) cm^{-1}, ^1H n.m.r. τ ($CDCl_3$) 0.56 (1H, broad, OH), 1.65 (1H, d, J=10Hz, C_{21}-H), 2.50 (10H, m, aromatic H), 2.60 to 3.80 (3H, signals overlapped, C_{18}, C_{19}, C_{20}-H), 2.96 (1H, s, C_{11}-H), 4.08 (1/3 H, s, C_5-H of (57) EEZ), 4.16 (2/3H, s, C_5-H, of (56) EEE), 5.26 (1H, s, C_3-H), 8.42 (3H, s, C_8-H), 8.72 (3H, s, C_9-H), ^{13}C n.m.r. ($CDCl_3$) δ 169.07 (C_7 of (57) EEZ), 168.42 (C_7 of (56) EEE), 167.45 (C_{10}), 147.07 (C_6 of EEZ), 146.87 (C_6 of EEE), 144.92 (C_{19} of EEZ), 144.07 (C_{19} of EEE), 139.52 (C_{12}), 130.78 (C_{18}), 125.19 (C_{20}), 128.91, 128.65, 127.80, 127.41 (C_{13} - C_{17}), 122.20 (C_{21} of EEZ), 121.29 (C_{21} of EEE), 78.58 (C_5), 70.51 (C_3), 68.36 (C_{11} of EEE), 67.64 (C_{11} of EEZ, 65.62 (C_2), 33.66 (C_8), 25.65(C_9), (Found: C, 66.93; H, 5.69; N, 6.01%; $C_{25}H_{24}N_2O_4S$ requires C, 66.95; H. 5.39; N, 6.25%), m/e: 431 (M^+-OH; 0.2%), 430 (0.9%),

158 (0.4%), 99 (1%), 59 (2.3%), 167 (100%), 123 (1.3%), 106 (4.2), 105, (5.6%). The more polar, minor, fraction was a rather complicated mixture which contained some unknown compounds and some possible isomeric aldoximes. The quantity was too small to be identified.

Deprotection of the 2:1 mixture of EEE and EEZ aldoximes (56) and (57) (49 mgm, 0.1 mmol) by T.F.A. and anisole afforded a corresponding mixture of carboxylic acids, 12 mgm (50%), as a brown oil, which was an isomeric mixture about the three unsaturated centres of the side chain at C_6 of the penicillin.

Benzhydryl 6-(5', 5'bis-methoxycarbonyl penta-2',4'-dienylidene) penicillanates (58) and (59). The mixture of dienals (22a) and (22b) (2:1, 325 mgm, 0.75 mmol), dimethyl malonate (200 mgm, 1.5 mmol), piperidine (10 mgm, 0.12 mmol) and acetic acid (26 mgm, 0.43 mmol) in toluene (1 ml) was refluxed at 120-130° for ca. one hour. Unreacted dimethylmalonate, acetic acid and piperidine and toluene were removed under vacuum with heating. The crude mixture was first purified by column chromatography (silica gel 70~230 mesh, Merck, 50g). After elution with CH_2Cl_2(500 ml) which was discarded, the fractions were collected with an eluent of CH_2Cl_2/Et_2O (98/2) (400 ml). After removal of solvent in vacuum the crude product (350 mgm) was submitted to prep. t.l.c. SiO_2 CH_2Cl_2/Et_2O). Two compounds (232 mgm. 56%) were isolated. The less polar component (58) EE a solid,m.p. 175-185°, (132 mgm. 32%) ν_{max} ($CHCl_3$) 1760, 1740, 1680, 1260 cm^{-1} (stretch of C-O in -C=C-CO-O), λ_{max} (CH_3CN). 278.5 nm (log ε 4.57); ^1H n.m.r. ($CDCl_3$). 2.42-3.20 (3H, signals overlapped, C_{19}, C_{20}, C_{21}-H), 2.60 (10H, m, aromatic H), 3.00 (1H, s, C_{11}- H), 3.57 (1H, d, J=11.26Hz, C_{18}-H), 4.17 (1H, s, C_5-H), 5.33 (1H, s, C_3-H), 6.10 (3H, s, C_{24}- H), 6.16 (3H, s, C_{26}-H), 8.42 (3H, s, C_8-H), 8.71 (3H, s, C_9- H), ^{13}C n.m.r. ($CDCl_3$) δ 168.03 (C_{10}), 167.25 (C_7), 165.43 (C_{23}), 164.78 (C_{25}), 146.23 (C_6), 143.94 137.89, 133.40, 129.56 (C_{18}-C_{22}), 139.52 (C_{12}), 128.84, 128.58, 127.73, 127.34, (C_{13}-C_{17}), 78.52 (C_5), 70.51 (C_3), 68.29 (C_{11}), 65.56 (C_2), 52.61 (C_{24},C_{26}), 33.59 (C_8), 25.52 (C_9), $^m/e$: 547 (M^+; 0.3%), 167 (50%), 380 (2.8%), 99 (0.3%), 59/0.6%), 222 (0.5%), 104 (0.2%), 59 (3.8%), 82 (100%).

The more polar component (59) EZ was an oil, (100 mgm 24%) λ_{max} (CH_3CN) 300 nm (log ε 4.37), ν_{max} ($CHCl_3$) 1765, 1735, 1275, 1250, 1210, 1175 cm^{-1},

^1H n.m.r. ($CDCl_3$) τ 2.44-3.52(4H, signals overlapped, C_{18}-C_{21}-H), 2.60 (10H, m, aromatic H), 2.98 (1H, s, C_{11}-H), 4.08 (1H, s, C_5-H), 5.31 (1H, s, C_3-H), 6.08 (3H, s, C_{24}-H), 6.13 (3H, s, C_{26}-H), 8.42 (3H, s, C_8-H), 8.68 (3H, s, C_9-H), ^{13}C n.m.r. ($CDCl_3$) δ 168.42 (C_{10}), 167.32 (C_7), 165.30 (C_{23}), 164.84 (C_{25}), 147.27 (C_6), 139.52 (C_{12}), 143.36, 136.52, 134.96, 125.65 (C_{18} - C_{22}), 128.91, 128.65, 127.80,127.41 (C_{13}-C_{17}), 78.58 (C_5), 70.51 (C_3), 67.77 (C_{11}), 65.69 (C_2), 52.73 (C_{24}, C_{26}), 33.72 (C_8), 25.59 (C_9), ^{m}/e: 547 (M^+; 0.3%), 380 (2.2%), 167 (41.6%), 99 (0.5%), 59 (1.3%), 222 (0.5%), 104 (0.3%), 59 (1.3%), 82 (100%).

Reactions of dienals (22) with dienophiles.

a) With dimethyl acetylene dicarboxylate a 2:1 mixture of dienals (22a) and (22b) (217 mg 0.5 mml) was refluxed with D.M.A.D. (1 mmol) in toluene at 110°. Analytical t.l.c. was employed to follow the reaction. After 40 hours, starting materials had disappeared. After working up and chromatography, no desired product was isolated.

A similar negative result was observed when the dienal mixture (22a) and (22b) was heated with neat D.M.A.D. (excess) in a sealed tube at 130° for 13 hours.

b) With maleic anhydride. A 2:1 mixture of dienals (22a) and (22b) (0.25 mmol) was refluxed with maleic anhydride (0.5 mmol) in benzene at 60° for one hour. T.l.c. showed that other than isomerization of (22c) and (22d), no product was formed. When the reflux was continued in toluene at 120°C for 12 hours, all the dienals reacted but no cycloaddition adduct could be obtained.

c) With dimethyl maleate A 2:1 mixture of dienals (22a) and (22b) (110mg 0.25 mmol) was refluxed with dimethyl maleate (0.5 mmol) in toluene at 110° for 16 hours. Most of the dienals were still present in the isomeric forms of (22c) and (22d). The reflux was carried on at 130°C for another 3 hours until all the dienals (22) were disappeared, but no appropriate product was detected.

d) With styrene A 2:1 mixture of dienals (22a) and (22b) (80 mgm. 0.19 mmol) (60 mgm 0.6 mmol) which containing stabilizer (hydroquinone) was refluxed at 110° in toluene for 12 hours. After working up and chromatography, no starting materials or desired product could be recovered.

e) With vinyl acetate Treatment of vinyl acetate (1 equivalent) with the 2:1 mixture of dienals (22a) and (22b) in toluene at 115° for 12 hours only provided a mixture of isomeric dienals (22c) and (22d) which survived a further 12 hours refluxing at 125° without the presence of any detectable cycloaddition product.

A 2:1 mixture of dienals (22a) and (22b) with neat vinyl acetate (4 equivalent) in a sealed tube was heated at 130° for 12 hours. After working up and chromatography neither starting material (22) nor desired product could be isolated.

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CHAPTER SIX DEPROTECTION OF THE PENICILLANATE ESTERS

6.1. Results and Discussion

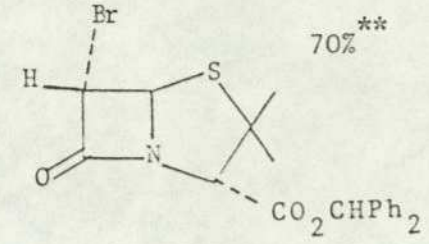
All the important new compounds synthesized in this project were successfully deprotected for biological testing (Table). Six different methods have been used in the course of deprotection.

Removal of the benzhydryl function from β-lactam esters with trifluoroacetic acid is a well known and popular technique, which involves stirring the mixture of the β-lactam ester in trifluoroacetic acid and anisole in an ice bath for a few minutes followed by immediate quenching of the acid with alkaline solution.[1]

This method was very successfully applied to the deprotection of most of our new compounds. The yield was found to be good to quantitative.

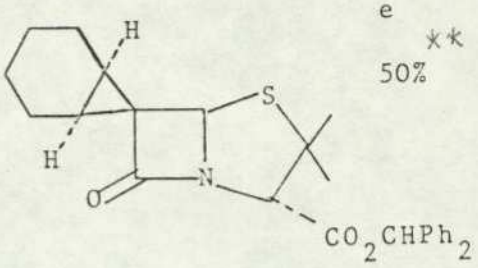
The high acidity of T.F.A. is the main disadvantage of this technique, which is unsuitable for any acid-labile materials. Thus, it failed in the deprotection of the benzhydryl 6-spiro-cyclopropyl-penicillanates (2)(3)(4)(5) and (6; R = $CHPH_2$). Instead, the trifluoroacetic acid disrupted the β-lactam structure of these adducts. The T.F.A. would also occasionally accelerate isomerization in the unsaturated centres of the compounds deprotected.

Sheehan and Commons[2] modified the T.F.A.-anisole method by conducting the experiment at -10^{o} , combined with the freeze drying technique. This modified procedure showed no difference in our attempts to deblock the benzhydrylgroup from 6-spiro-cyclopropyl penicillanates (2-5) and (6 R = $CHPh_2$). Once again the β-lactam ring was cleaved by T.F.A. even under very cold reaction conditions.



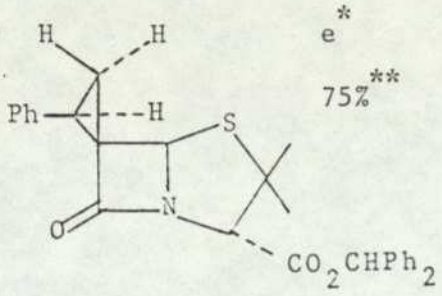
a* 70%**

(1)



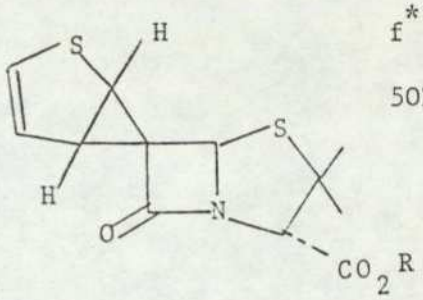
e* 50%**

(5)



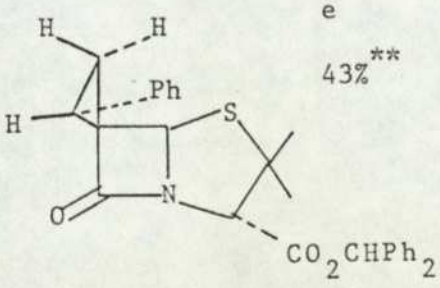
e* 75%**

(2)



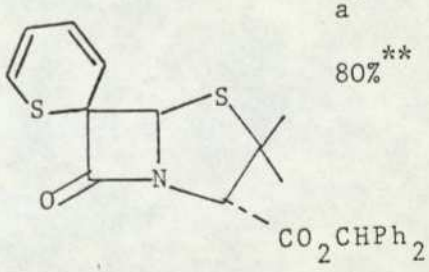
f* 50%**

(6)



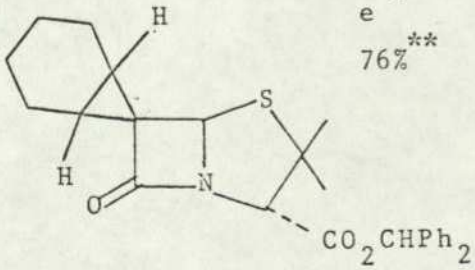
e* 43%**

(3)



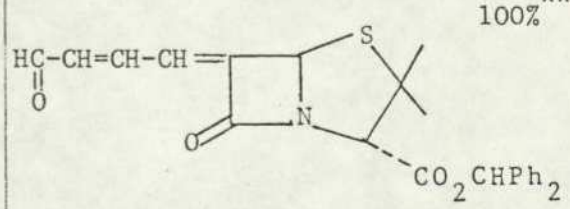
a* 80%**

(7)



e* 76%**

(4)



a* 100%**

(8)

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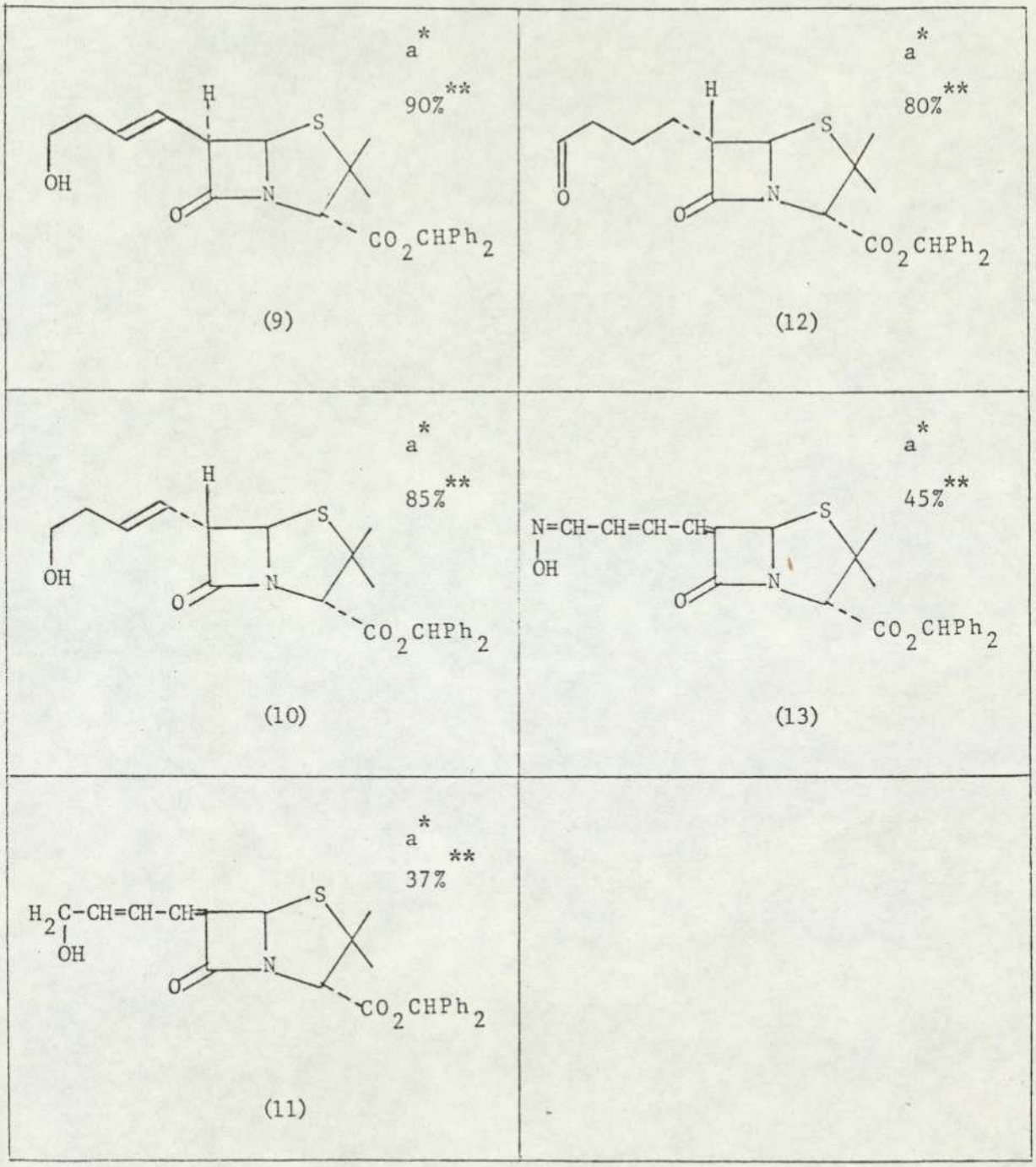
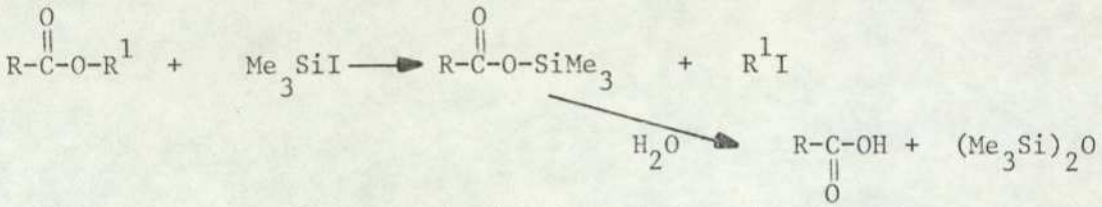


Table Compounds successfully deprotected in this project.

* Methods of deprotection: for details of methods a-f, see Experimental section.

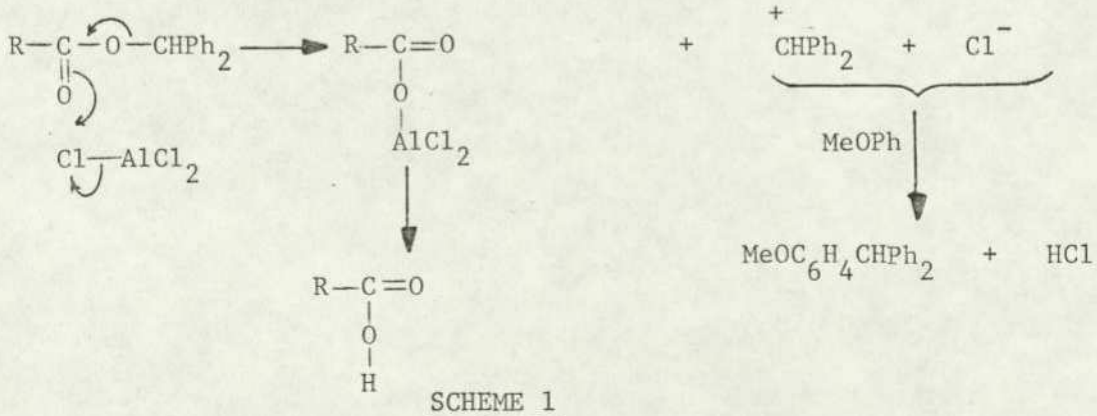
** Yield of the deprotection.

The use of organosilicon reagents has become significant during recent years in organic synthesis. The high bond energy of the silicon—oxygen bond (90-110 Kcal/mol) makes it thermodynamically very favourable to use a reagent with a weak Si-X bond and react it with an appropriate oxygen-containing organic molecule to form a silicon-oxygen bonded intermediate, which then can be transformed to another product in a subsequent step. One such reagent is iodotrimethylsilane, which has gained use in the cleavage of esters.[3,4]



Our attempt to cleave the benzhydryl function from 6-spiro-cyclopropylpenicillanate (6; R = $CHPh_2$) with iodotrimethylsilane reagent was thwarted by the fact that the β-lactam ring was opened by iodotrimethylsilane.

The latest route to remove the benzhydryl function from β-lactam esters was reported by Tsuji et al.[5] They suggested that aluminium trichloride, a strong Lewis acid having a high affinity for oxygen, would coordinate with the carbonyl oxygen to assist generation of a benzhydryl cation which, in turn, would be trapped by anisole, as shown in Scheme 1.



SCHEME 1

Attempted removal of the benzyhydryl protective group from 6-cyclopropylpenicillanates and 6α-bromopenicillanate by aluminium chloride was unrewarding. The β-lactam structures of these compounds were found to be destroyed under the experimental conditions described.

It was eventually realized that any deprotection procedures which involved acid media cannot be applied to benzhydryl 6-spiro-cyclopropylpenicillanates, because the β-lactam ring of these derivatives would be ruptured. Presumably, the cyclopropyl ring at the 6 position of penicillanates increases the strain on the β-lactam ring, which renders it extremely labile towards acid.

Interestingly, when the spiro structure at C_6 position of penicillanates is a 6 membered-ring rather than a cyclopropyl ring the compound is apparently quite stable in acidic conditions and can even be deprotected by the T.F.A. method.

Deprotection of benzhydryl 6-spiro-cyclopropylpenicillanates (2-5) was finally achieved by catalytic hydrogenolysis. Hydrogenation is a well documented method.[6] Esters, with a few exceptions, are reduced with difficulty and survive most catalytic hydrogenolysis. Cleavage is accomplished readily only in those molecules whose special structure features render the ester exceptionally prone to hydrogenolysis, as exemplified by the reduction of the benzhydryl ester where the benzhydryl groups weakens the R—O bond.

Deprotection with hydrogenolysis is a reliable method which gives a good yield and very clean product. Applied to deprotection

of esters of β-lactam derivatives, prolonged reaction time and larger amount of metal catalyst are normally required, owing to poisoning by the sulfur atom at position 1 of the penicillin.

Palladium is claimed to be a good catalyst which has widely been used in β-lactam work. It was found in our experiments that under an atmospheric pressure of hydrogen, an equal weight of 5% palladium on carbon was not strong enough to remove the benzhydryl function completely. Only an equal weight of palladium black 100% could do the job successfully.

Solvent effects on the palladium-catalysed hydrogenolysis of benzhydryl 6-spiro-cyclopropylpenicillanates (2-5) were briefly investigated, showing that a combination of methanol, ethyl acetate and aqueous $NaHCO_3$ gave the most satisfactory result.

The limitation of the hydrogenolysis method is that it is unsuitable for any material containing C=C bonds, which would be normally reduced as well during the process of deprotection. This phenomenon was observed in the attempted deprotection of compound (6; R = $CHPh_2$) by palladium black hydrogenolysis, where the double bond of the 2,3-dihydrothiophene ring was saturated.

To obtain the corresponding carboxylic acid from thiophene adduct (6), the protecting group of the starting material had to be changed to the p-nitrobenzyl function, which was easily cleaved by the sodium sulphide method.

Removal of the p-nitrobenzyl ester protective group from

β-lactam derivatives by sodium sulphide was described by Lammert Ellis, Chauvette and Kukolja.[7] The procedure reported involves stirring the mixture of β-lactam ester to be deprotected with sodium sulphide in aqueous T.H.F in an ice bath for 25-35 minutes. This technique was effective in removing the p-nitrobenzyl group from compound (6; R = $CH_2C_6H_4NO_2$), resulting in the corresponding acid in 50% yield.

6.2. Experimental

The following methods of deprotection were examined:-

a. Standard trifluoroacetic acid method. The benzhydryl ester (0.1 mmol) was dissolved in anisole (0.1 ml) and kept in an ice bath with stirring. Ice-cold trifluoroacetic acid (0.3 ml) was added, the mixture was stirred for 2 minutes, then immediately quenched by adding aqueous sodium bicarbonate (8 ml, 5%). The reaction mixture was washed with ethyl acetate three times, then was acidified by aqueous citric acid (7.5 ml, 5%) and extracted with ethyl acetate several times. The organic layer was washed with brine several times, then water, and dried over anhydrous magnesium sulphate. Removal of the solvent under reduced pressure afforded the corresponding acid.

b. Modified T.F.A. method. The benzhydryl ester was dissolved in dichloromethane (10 ml) and the solution was cooled at -77° before adding trifluoroacetic acid (0.5 ml). The solution was warmed to -10° and stirred at that temperature for 5-6 hours.

The solution was then cooled again to -77°C and benzene (75-100 ml) added. After the contents of the flask solidified, the product was isolated by freeze drying.

c. Iodotrimethylsilane method. The benzhydryl ester (0.156 mmol) was dissolved in CCl_4 (3 ml) and kept in an ice bath, then dried nitrogen gas passed through the solution and trimethylsilyl iodide (50 µl) was introduced by a microsyringe. The reaction mixture was stirred for one hour in an ice bath, then water (2 ml) was added. The mixture was diluted with ethyl acetate, then was basified with aqueous $NaHCO_3$ (5%), and washed with ethyl acetate. The aqueous layer was acidified with HCl (1 N) and extracted with ethyl acetate. The extract was washed with water, then dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give the product.

d. Aluminium trichloride method. A solution of $AlCl_3$ (950 mgm) in nitromethane (20 ml) was added to a solution of benzhydryl ester (1 g) and anisole (1.5 g) in dichloromethane (20 ml) under ice cooling, and the mixture was stirred for 5 hours at room temperature. The reaction mixture was diluted with ethyl acetate, washed with dilute HCl and extracted with aqueous $NaHCO_3$ (5%). The aqueous extract was acidified with dilute HCl and extracted with ethyl acetate. The extract was washed with water, dried and evaporated to give the product.

e. Hydrogenolysis. The benzhydryl ester (0.1 mmol), with an equal weight of palladium black and one equivalent of $NaHCO_3$ (10 mg) in a mixture of CH_3OH (1.5 ml), H_2O (0.5 ml) and EtOAc (0.5 ml) was

hydrogenolyzed at room temperature under an atmospheric pressure of hydrogen with stirring. After 12 hours, the catalyst was filtered off by filter aid (Ayflo) and washed with aqueous $NaHCO_3$, methanol and ethyl acetate.

The organic solvent was removed under reduced pressure. The aqueous solution was washed with ethyl acetate, then acidified to pH 2 with phosphoric acid (10%) and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous $MgSO_4$ and evaporated to afford the corresponding acid.

f. Sodium sulphide method. The p-nitrobenzyl ester (0.17 mmol) in redistilled T.H.F. (25 ml) and water (1.1 ml) was cooled in an ice bath and sodium sulphide ($Na_2S.9H_2O$) (41 mgm, 0.17 mmol) in water (1 ml) was added. The mixture was stirred at 0°C for 25 minutes, then HCl (1N, 0.17 ml) was added and the T.H.F. was removed under reduced pressure. The aqueous solution was basified with aqueous $NaHCO_3$ (5%) to pH 8.5, and washed with ethyl acetate twice, then was acidified with HCl (1N) to pH 2.5, then extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous $MgSO_4$, and evaporated to give the corresponding acid.

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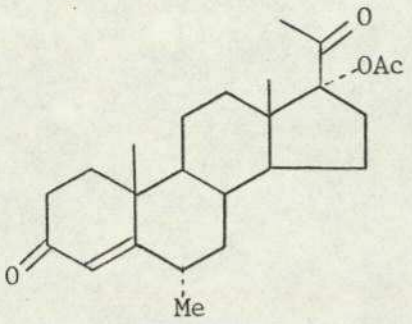
CHAPTER SEVEN. PREPARATIVE HPLC OF STEROIDS AND β-LACTAMS

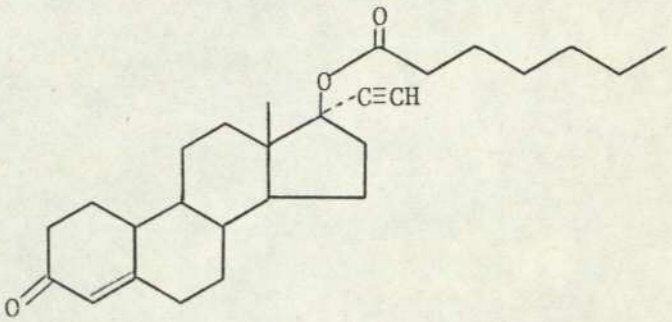
7.1. Introduction

During the entire 3-year period of the research work described in this thesis, a second project was also being undertaken on a part-time basis. This second project concerned the Quality Control analysis and purification of steroid esters sent to us by the World Health Organisation (WHO) in Geneva.

In 1976 the WHO instituted a multi-national research programme to develop new, long-acting, injectable contraceptive agents, particularly for application in Third World Countries. At that time, only two such agents were known: medroxyprogesterone acetate (1) and norethisterone enanthate (2). The latter compound exerts its effect by slow release from the site of injection, followed by enzymatic hydrolysis to norethisterone (NET), which is the actual biologically active progestogen responsible for the contraceptive effect. The intention of the WHO programme was to examine a large number of other esters analogous to NET enanthate in the hope of obtaining improved activity. To this end, laboratories in about 14 countries, mainly in the Third World, were given commissions to synthesise specific steroid esters. All of these compounds were forwarded to our laboratory in London for Quality Control, prior to despatch for formulation by a pharmacist [redacted]



(1)



(2)

In all, about 300 samples were received for examination, generally in 1-5 g quantities. For the preliminary biological evaluation, ca. 500 mg of pure material was considered a desirable minimum. Because the release rate of the steroid ester would depend on its physical form, which might be strongly influenced by the presence of minor impurities, the WHO set a maximum limit of 0.5% detectable impurities.

Using analytical HPLC as a major element of our Quality Control procedure, it was not difficult to carry out high resolution separations of the steroid esters from other steroidal impurities and to detect the latter at levels below 0.1%. However, the requirement to produce samples of pure material meeting the strict test of 99.5% purity posed considerable difficulties. Frequently, the procedures of recrystallization and conventional preparative chromatography (column or thin layer) were ineffective and it was necessary to resort to preparative HPLC. The challenge of meeting the stringent purity requirements forced us to press the capabilities of preparative HPLC to their limit.

In this Chapter, the essential features of preparative HPLC and examples of its application to the purification of steroid esters are

discussed. In addition, the experience in analytical and preparative HPLC gained in the WHO programme proved to be of great benefit in the β-lactam works described in this thesis. The use of analytical HPLC to follow reactions and to examine products has been mentioned repeatedly in previous Chapters. In this Chapter, some of the applications of preparative HPLC to the isolation and purification of β-lactam derivatives are described in detail.

7.2. Literature Review of Preparative HPLC

The term "preparative HPLC" is taken here to mean fast, automated and efficient liquid column chromatography, on a scale significantly larger than that associated with analytical HPLC. The overloading of analytical HPLC columns can increase their capacity from roughly microgram to milligram loadings. True preparative HPLC is concerned with milligram-to-gram scale separations and this requires an increase in column size and a readjustment of many, if not all, of the running parameters.

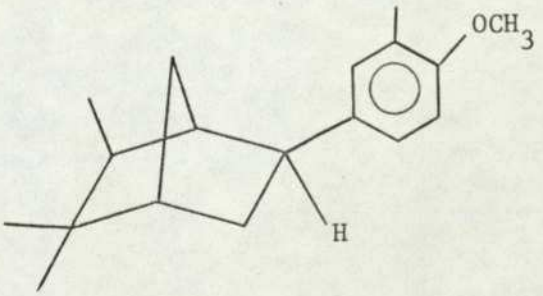
Preparative HPLC is considerably less well developed than the analytical form of the technique, both in terms of theory and available equipment. Few systematic studies of operating conditions have been reported and most of the literature in the field is concerned with the application of arbitrary methodoly to solve specific separation problems.

Baker et al.[1] have demonstrated the purification of 1 g of cholesteryl phenylacetate on two 50 x 2.3 cm columns packed with

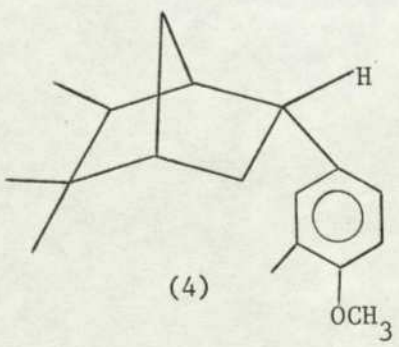
Spherosil XOA-400 and connected in series, with a step elution from dry hexane to dichloromethane containting 0.1% methanol. They also separated 2 mg of Pyrethrum extract on one 50 x 2.3 cm column packed with Permaphase O.D.S., eluent methanol/water (50/50) at 50°.

Examples were given by Godbille and Devaux[2] of the use of a home made system consisting of an 8 cm I.D. column packed with t.l.c. grade silica (silica gel H Type 60 Merck). They separated 600 mg of a mixture of corticosteroids. The most original part of this unit is composed of a piston which is moved up and down with a pneumatic jack to compress the column bed. A constant packing pressure is then maintained throughout the chromatographic procedure. This system is use in the commercial Jobin-Yvon equipment.

A separation of a 2 g of mixture of exo and endo isomers (3) and (4) was reported,[3] using a 3.3 ft x ⅜ in. I.D. column dry packed with Porasil T, and a solvent flow of 9.9 ml/min.

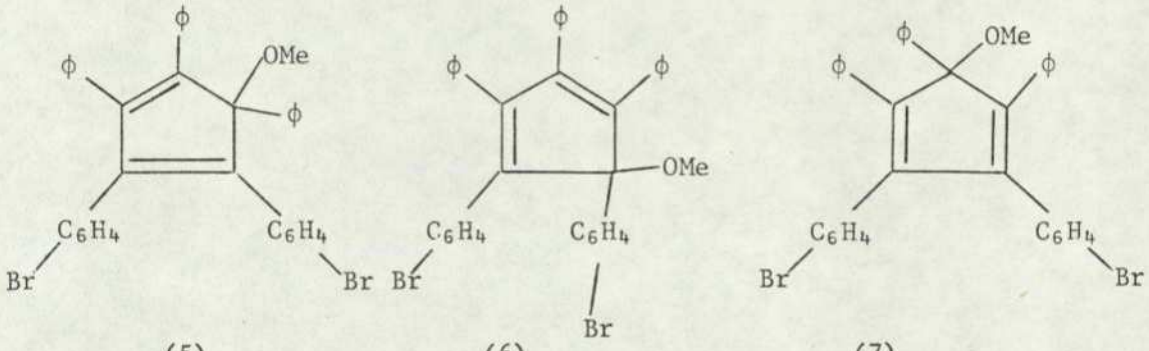


(3)



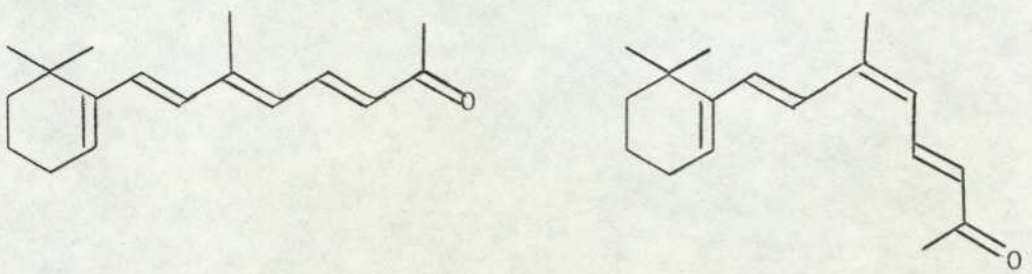
(4)

Berg[4] reported the separation of a 1.5 mg of isomeric mixture of compounds (5),(6) and (7) on a 20 cm x ½ in. O.D. column packed with Lichrosorb S.I. 100 10 µm.



(5) (6) (7)

Some successful separations of natural products were demonstrated by Pettei et al.[5] and by Adams et al.[6] By using the Waters system (Waters Prep 500), 820 mgm of compounds (8) and (9) were separated on a 30 x 5 cm silica column in 16 minutes(eluent ether 11% in hexane, flow rate:250 ml/min).



(8) (9)

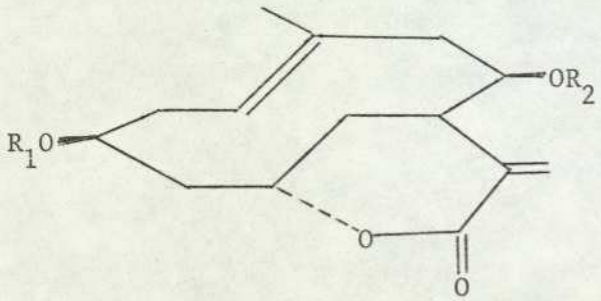
450 mg of a mixture of trans and cis (10) was separated on a prepared 10% $AgNO_3$ on silica column (30 x 5 cm, flow rate: 100 ml/min) in half an hour. The commercial column was modified, because it was found that the use of the only commercially available cartridge from the Waters Prep 500 instrument gave a poor separation of this mixture. It is known that $AgNO_3$ coated silica gel greatly improves the separation of cis/trans isomers. So a new cartridge was prepared

by emptying a commercial one, impregnating its silica gel particles with 10% $AgNO_3$, refilling by dry-packing, and finally refitting the cartridge with the end frits. It was claimed that a far better separation was achieved in significantly less time with a shorter column length.

$$THPO - (CH_2)_5CH = CH(CH_2)_3CHO$$

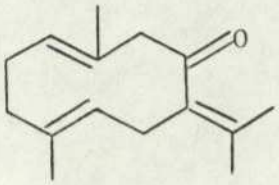
(10)

It was also reported that by using the Jobin-Yvon Prep 100 machine packed with 1 kg of C_{18} stationary phase, eluent methanol/water (53/47), flow rate 105 ml/min, gram quantities of pure compounds (11) and (12) were obtained from 6.6 g of sample of a crude extract of Schkuhria Pinnata.

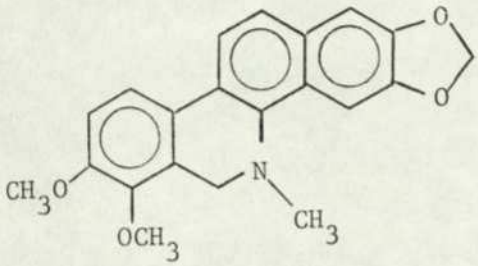


	R_1	R_2
(11)	$OC\text{-}CH\text{-}CH - CH_3$ $\quad \| \quad \| \quad \|$ $\quad O \quad OH \quad CH_3$	$OC - C = CH(CH_2OH)$ $\quad \| \quad \|$ $\quad O \quad CH_2OH$
(12)	$OCO\,CH_3$	H

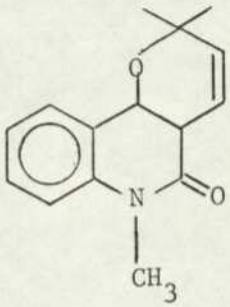
The same authors also isolated compounds (13), (14) and (15) and other components from the hexane extract of dried bark of the African and Indian medicinal plant Tagara Chelybea Engl with the Waters system (silica column; eluent ether/hexane, 1 : 9).



850 mg
(13)

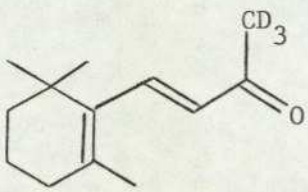


120 mg
(14)

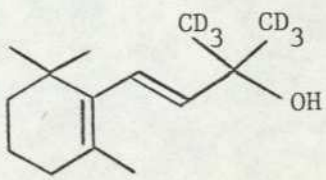


125 mg
(15)

17.5 g of d_3-β-ionone (16), a key intermediate in synthesis of deuterated Vitamin A compounds, was separated from its main synthetic contaminant d_6- tert -alcohol (17) in a manner similar to those described above.

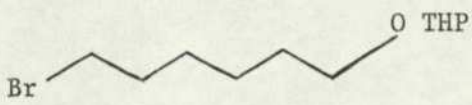


(16)



(17)

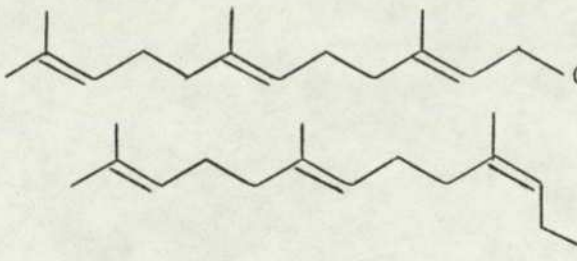
Similarly, 14 g of the bromotetra-hydropyranyl ether (18) could be easily separated from numerous trace impurities.



(18)

The separation of 3 g of mixture of trans farnesol (19) and its isomer (20) was obtained by using two 30 x 5 cm silica

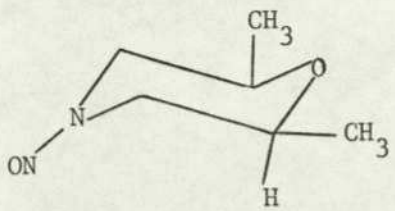
columns (Waters Prep 500, flow rate 250 ml/min, eluent 10% ether in hexane). Exactly the same separation was also given by another group.[7]



(19)

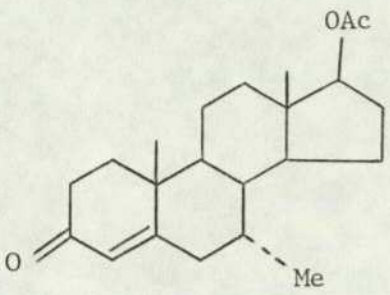
(20)

Waddell[8] has used the Waters Prep 500 system to separate products of various photochemical reactions. HPLC has also been applied to the separation of a mixture of cis-2,6-dimethyl-N-nitroso-morpholine (21) and its trans isomer by using the recycling technique. (Waters Prep 500; 2 x 30 cm x 5 cm silica columns, step elution: $CHCl_3/C_6H_{14}$, CH_3CO_2Et/C_6H_{14}, 250 ml/min).



(21)

Gasparrini et al.[9] have shown that an isomeric mixture of 7α- and 7β-methyl-17β-acetoxy-3-oxoandrost -4-enes (22) and (23) could be separated on the Jobin-Yvon Miniprep LC (2 cm I.D. column) or Chromatospac Prep 10 Chromatography (4 cm I.D. column) packed with Lichroprep L.C. R.P-18. The loading was 120 mgm on the 2 x 20 cm column, 700 mgm on the 4 x 20 cm column.



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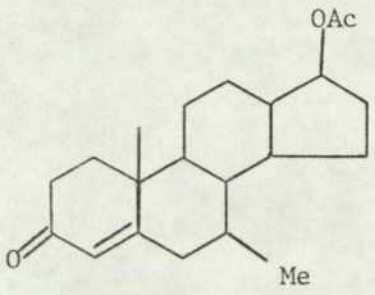


(23)

7.3. Purification of Steroid Esters: Results and Discussion

HPLC analysis of each of the steroid esters was performed on two column systems: (i) silica (Hypersil 5 μm) eluted with $MeCN/CH_2Cl_2$, (ii) reverse phase (ODS Hypersil) eluted with $MeOH/H_2O$. About half of all the esters analysed failed Quality Control and required purification. About 60 samples were handled by preparative HPLC on silica columns, and were roughly evenly split between each of three systems - two commercial and one laboratory made.

In early phases of the work, the system employed was the Jobin-Yvon Prep 100 machine. This consists of an 8 cm diameter column connected via a system of valves to a 250 ml sample reservoir and a 10 litre solvent reservoir, both of which are gas pressurised. The column is loaded with a loose slurry of silica (0.5 to 3 kilos), which is then compressed by a gas-driven piston ram fitted to its lower end. This ensures a tight, homogeneous column bed, which is essential for efficient chromatography. Compression of the column

must be maintained: depressurisation causes cracking of the bed and it then needs re-packing. The operating elution pressure is limited to ca 150 psi by the equipment design and consequently only a relatively coarse grade of silica can be used at acceptable flow rate (50 - 200 ml/min).

In practice, we used a cheap and reasonably efficient grade of t.l.c. silica - Merck Type H60. The column was normally packed with 0.5 - 1 kilo, which was then discarded after each purification was complete. This was considered to be more economical than extensive scrubbing with large volumes of solvent to avoid cross-contamination of the steroid esters.

The Jobin-Yvon instrument is not supplied with a detector and we therefore adapted an analytical HPLC detector for use with the preparative machine. The Cecil 272 monitor was found to be highly suitable for this purpose. Being a variable wavelength detector, the wavelength control can be used as an additional attenuator, by "tuning off" the λ_{max} of the compound eluted. This makes it relatively easy to cope with detector overload, which is usually one of the most serious difficulties in preparative work. The Cecil detector was modified for high-flow conditions either by incorporation of a split-stream T arrangement after the column, or more conveniently by incorporation of a special 1-mm pathlength high-flow preparative flow cell supplied by Cecil Instruments Ltd. Signal output from the Cecil was transmitted to a conventional strip-chart recorder.

Fractions of eluate were manually collected for re-inspection by analytical HPLC prior to combination and evaporation. Since the resolution of preparative systems is inherently lower than that of analytical ones, it was found essential to split even apparently homogeneous peaks on the preparative run into numerous fractions and to determine which of these were pure by re-analysis.

An example of a typical purification of a steroid ester using the Jobin-Yvon equipment is shown in Figure 1. Analytical HPLC of the levonorgestrel ester (24) revealed the presence of several impurities totalling 4% (Fig. 1a). To allow for the expected loss of resolution on scale-up, the polarity of the mobile phase was reduced for the preparative run. An injection of 1.3g of crude ester gave the result shown in Figure 1b. To provide a clear trace of the minor impurities, this run was commenced with a sensitive detector setting of 243 nm, 0.5 AUf.s.d. (λ_{max} for the steroid 4-en-3-one). When the detector overloaded on the main peak, it was tuned off (260 nm; 20 AU f.s.d.). Six fractions were collected across the main peak and these were re-analysed by analytical HPLC. It can be seen from the results (Fig. 1c) that resolution is considerably lower on the preparative column than in the original analysis. However, by fraction cutting it has been possible to achieve the objective of purification: combination of fractions 2-6 and evaporation under reduced pressure yielded 700 mg of material whose final analysis showed $< 0.5\%$ of one impurity.

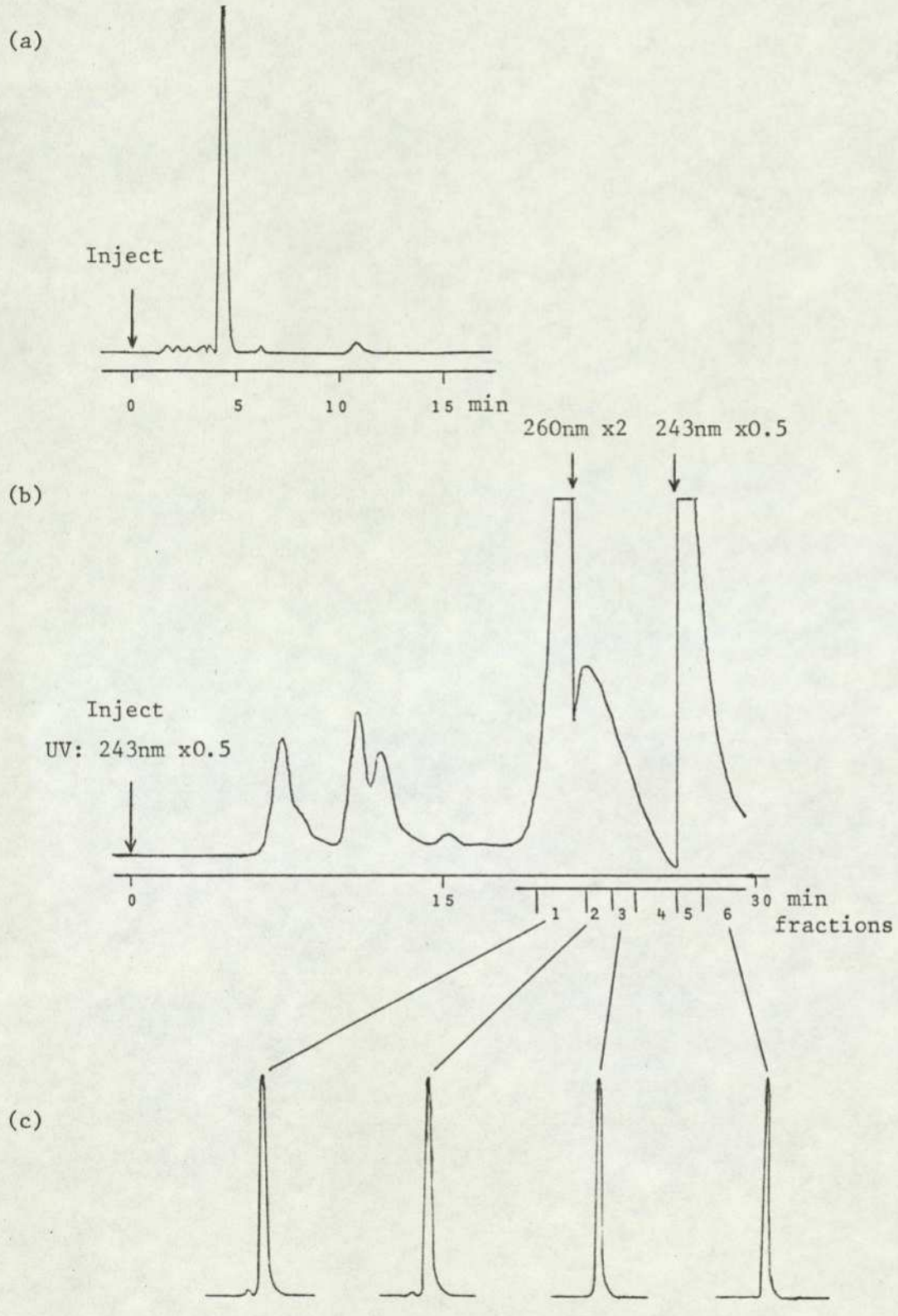
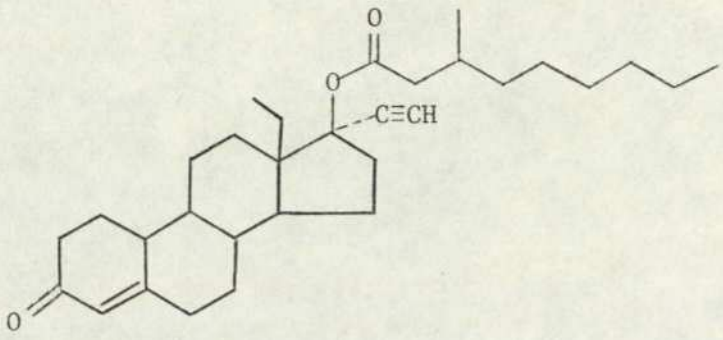


Figure 1 HPLC of levonorgestrel 3-methylnonanoate (24).

a. Analysis on 20cm x 4.5mm i.d. Merckosorb SI60 (10μm) eluted with MeCN-CH_2Cl_2, 10:90, at 2.0 ml/min. Detector, 243nm x 0.5AU fsd. Injection, 3μl of CH_2Cl_2 solution.

b. Preparative HPLC on Jobin-Yvon Prep 100 loaded with 600g Merck Type H60 tlc silica, eluted with MeCN-CH_2Cl_2, 5:95, at 26ml/min. Detector, UV. Injection, 1.3g in 25 ml CH_2Cl_2

c. Analysis as in a.



(24)

The large loading capacity and economic operation of the Jobin-Yvon make it a suitable instrument for routine, large-scale purifications, and we used it for about 20 compounds in the WHO programme. However, the pressure limitation (10 atmospheres) and over-large sample capacity (up to 50 g), together with the inconvenient solvent delivery system mean that it is far from ideal for the requirements of high resolution work on a 0.5—5 g scale.

Later in our work, we were able to use a Waters Prep 500 machine for about 20 purifications. This machine differs from the Jobin-Yvon instrument in a number of important details. Solvent delivery is by means of an electro-mechanical piston pump, which operates at up to 0.5 litre/minute and 25 ats. The column consists of one or two plastic cartridges pre-loaded with a coarse (up to 80 μm), ground silica, which are placed into steel cylinders where they are radially compressed by pneumatic action prior to passage of eluent through the bed. The effect of radial compression, as in the Jobin-Yvon system, is to ensure close-packing of the bed. Owing to permanent distortion of the plastic cartridge, the column must be

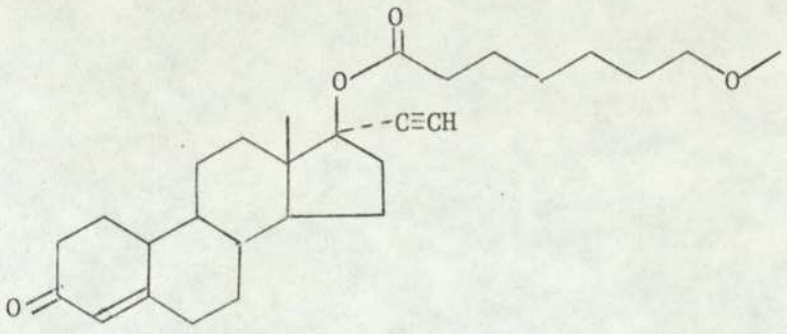
discarded after decompression. This feature makes the system expensive to operate, and at present there is little choice in column packings (Porasil or C-18 Porasil only).

Detection is achieved in the Waters Prep 500 by means of a built-in refractive index (RI) monitor. Such detectors are inherently less sensitive than UV, which is considered an advantage for preparative work. However, they are also non-specific and give a large response to the solvent of injection and are sensitive to changes in temperature. Worst of all, they make it impossible for step or gradient changes in the mobile phase to be accomplished.

We used a system containing a single Prep-Pak silica cartridge for the steroid purifications. After each run, the cartridge was thoroughly scrubbed with a polar solvent mixture (50 : 50 MeCN-CH_2Cl_2) before regeneration for the next injection. Despite this, a gradual rise in a back pressure was observed on continued use, so that after 5 - 10 injections of esters (normally > 90% pure and free of extremely polar contaminants) the back pressure was too high for continued operation and the column was discarded. During 20 purifications in the course of one month, three silica cartridges and several hundred litres of solvent were consumed. Even with solvent recycling of as much as possible of the eluent (fractional distillation of the MeCN-CH_2Cl_2 mixture and GLC analysis of the distillate composition), this instrument proved to be exceedingly expensive to operate.

A typical result with the Waters Prep 500 is shown in Figure 2, for the purification of the norethisterone ester (25). Analysis of

the material submitted had revealed the presence of 3% of a single, less polar contaminant, with a separation factor $\alpha = 1.28$. As shown in Figure 2a, the impurity was a poorly resolved shoulder when a 6 g injection was made. Ten fractions were collected, and the impurity was found to be confined mainly to the first four fractions (Figure 2b). Combination of fractions 5 -10 yielded 3 g of acceptably pure material.



(25)

The Waters system shares with the Jobin-Yvon equipment the characteristic of inherently poor resolution. Both instruments have been optimised for large throughput-per-unit time and in doing so the operating pressure and column efficiency have been heavily compromised. The Waters system has the advantage, however, of incorporating a pumping system designed for recycle operation. In this technique, the column eluate containing poorly resolved peaks is returned to the inlet and sent repeatedly through the column until sufficient resolution is obtained. In practise, the peak broadening produced by the system plumbing is extensive and after a maximum of 2-3 recycles it becomes essential to "shave" the outer edges of the peaks to reduce their overall width and prevent the leading edge

a. Preparative HPLC on Waters Prep 500 with one Prep Pak silica cartridge, eluted with MeCN-CH_2Cl_2, 7:93. Injection, 6g in 30ml CH_2Cl_2.

b. Analysis on 12.5cm x 4.5mm i.d. Hypersil 5µm column, eluted with MeCN-CH_2Cl_2, 12:88, at 2.0ml/min. Detector, 243nm x 0.5AU fsd. Injections, 10µl of eluted fractions.

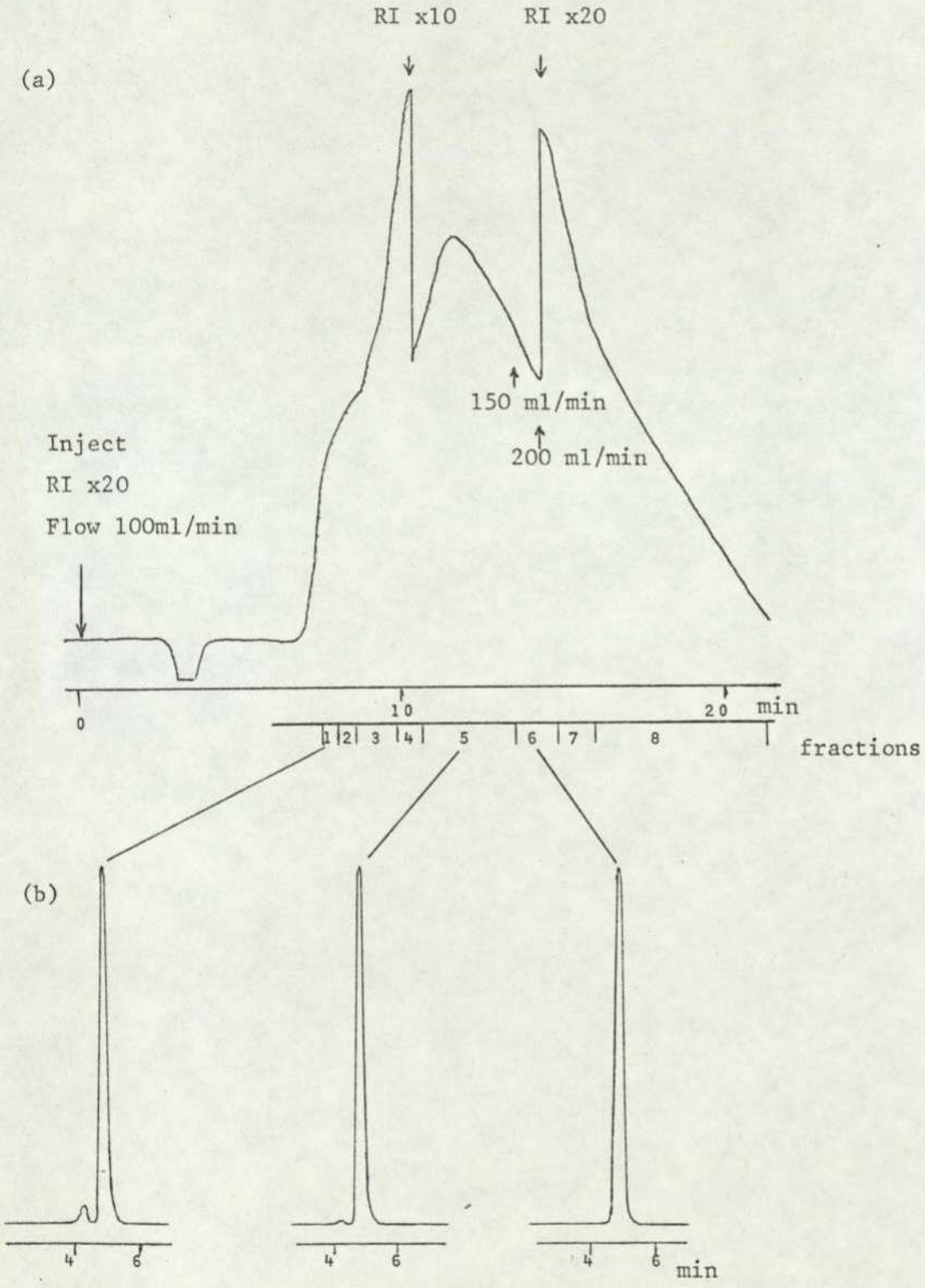
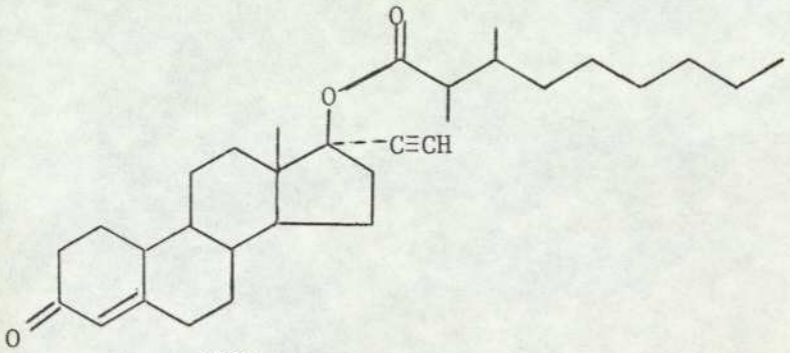


Figure 2 HPLC of norethisterone 7-methoxyheptanoate (25).

from overtaking the tailing edge.

The NET ester (26) was found analytically (Figure 3a) to contain four impurities totalling 16%, one of which ran very close to the main peak. A single pass of 3 g on the Waters Prep 500 was unsatisfactory (Figure 3 b) . All the fractions collected across the main peaks were contaminated with the close-running impurity (Figure 3c). Fractions 2-8 were recombined to give 700 mg and re-injected. The material was then recycled (Figure 4a) with careful fraction collection to yield a number of fractions with the required purity (Figure 4 b) , Combination of fractions 2, 3 and 5 gave 500 mg of pure compound (26).



(26)

In view of the operational deficiencies of both of the available commercial preparative machines, we eventually decided to assemble our own preparative chromatograph. The guiding principle was that a high pressure, high flow rate pump would allow the use of large columns packed with a fine grade of silica, thus providing high efficiency separations. The use of steel columns packed directly by a high-pressure slurry technique, as in analytical work, would provide columns which could be re-used at convenience, minimising expense.

a. and c. Analysis conditions as Figure 2b, except mobile phase MeCN-CH_2Cl_2, 7:93.

b. Preparative HPLC as Figure 2a, except mobile phase MeCN-CH_2Cl_2, 5:95, at 150ml/min. Injection, 3g in 20ml CH_2Cl_2.

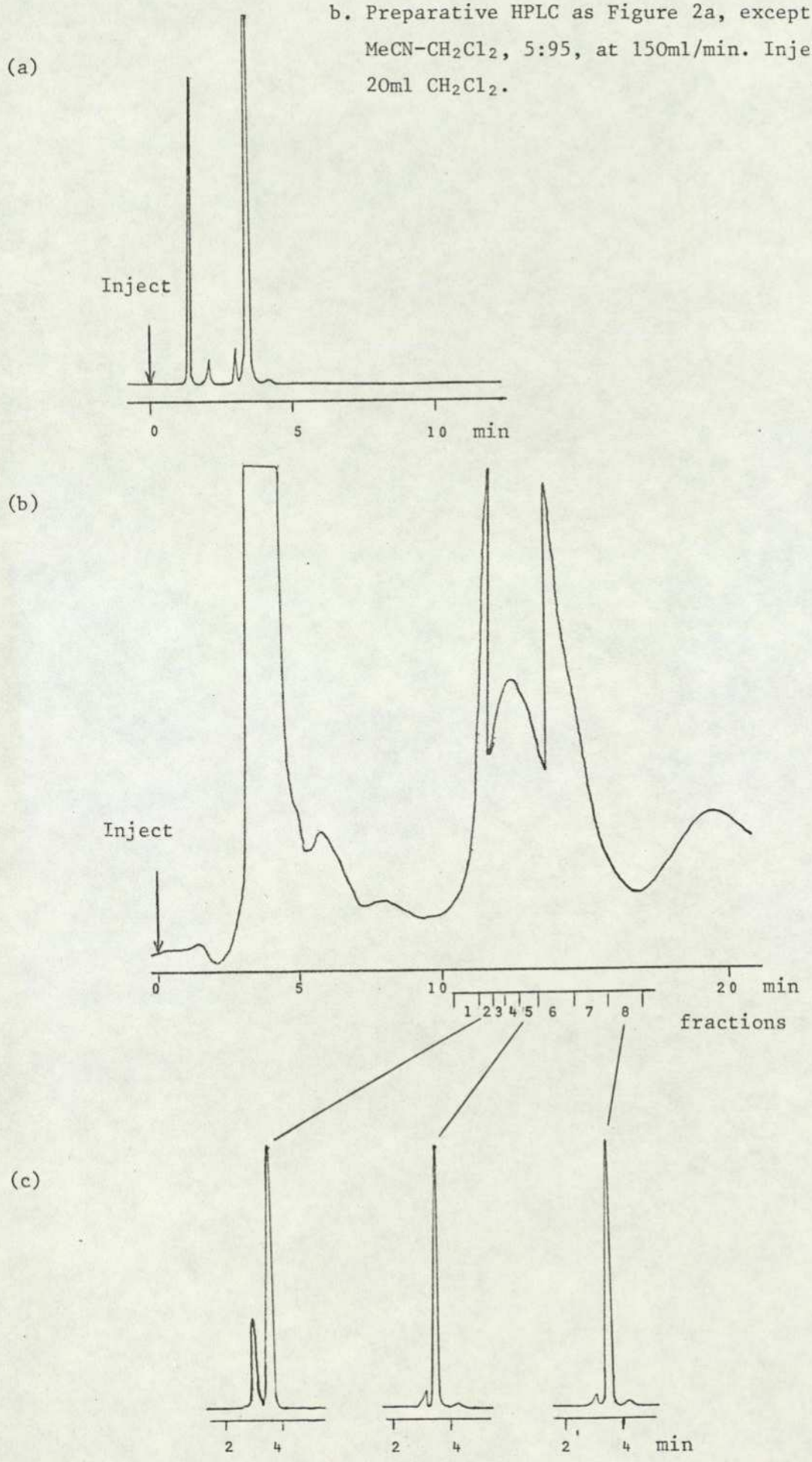


Figure 3 HPLC of norethisterone 2,3-dimethylnonanoate (26).

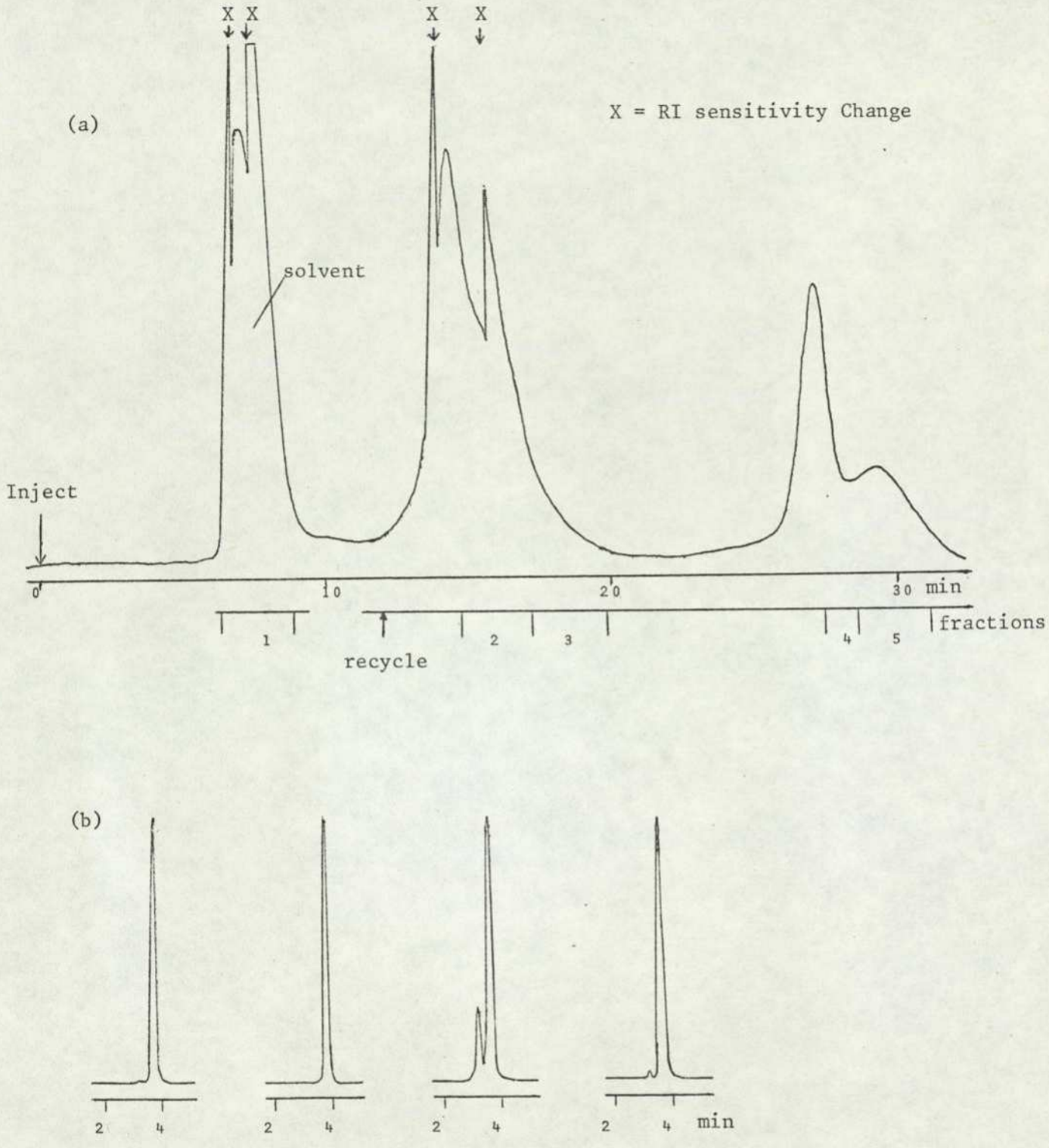


Figure 4 HPLC of norethisterone 2,3-dimethylnonanoate (26).

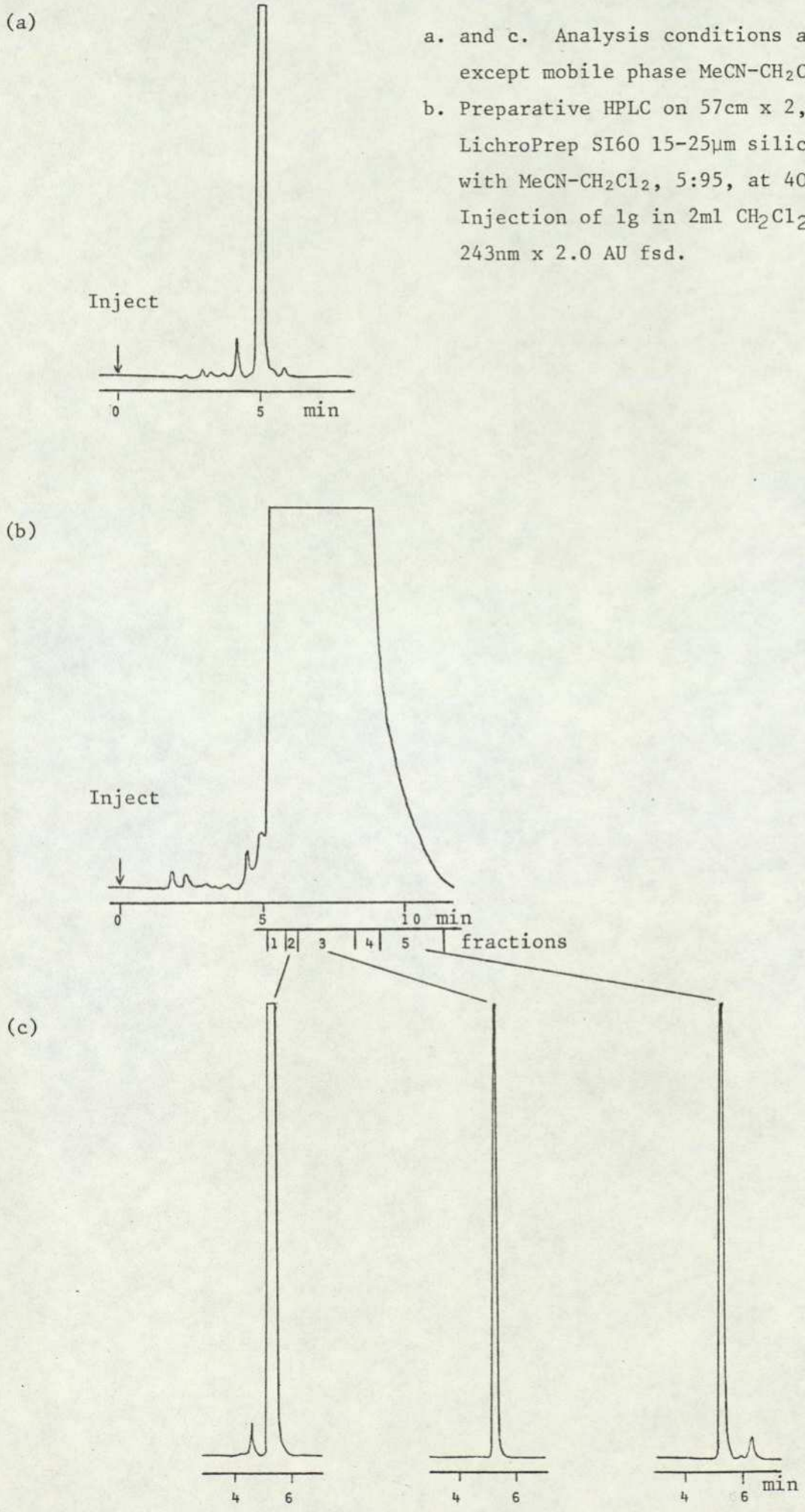
a. Preparative HPLC with recycle, conditions as for Figure 3b, except flow rate 100ml/min. Injection, 700mgm in 10ml CH_2Cl_2.

b. Analysis conditions as Figure 3a.

The largest fittings readily available at a reasonable price are for 1" diameter columns and such columns, 1-2ft.long, have a capacity for ca. 1 g loadings. The columns were high pressure slurry packed (see Experimental) with Lichro Prep S.I. 60, a 15-25 μm irregular silica recently introduced by Merck. The packed column was fitted with a commercial valve-loop injector, which was modified by attachment of hand-made 4-10 ml loops for sample introduction, and connected to a twin-piston reciprocating pump capable of solvent delivery at up to 100 ml/min and 1000 psi. The same detection system was used as that adopted for the Jobin-Yvon system, i.e. a Cecil detector fitted with a preparative flow cell.

This laboratory-assembled system was considerably more economical in initial outlay (ca. £3,500) than either the Jobin-Yvon or Waters equipment. Because of the smaller, re-usable columns it is also considerably cheaper to operate for 1 g-scale separations. Most important of all, it is capable of at least an order of magnitude better resolution then either commercial system. Finally, it is a flexible system which can readily be improved further as technology advances. The efficiency-limiting component is the column and as better end fittings and better packings become available they can be readily incorporated.

An example of the use of the new system is in the purification of the NET ester (27). This was found to have several minor impurities totalling 2% on HPLC analysis (Figure 5a). The separation is a challenging one in that the α values between the closest impurities

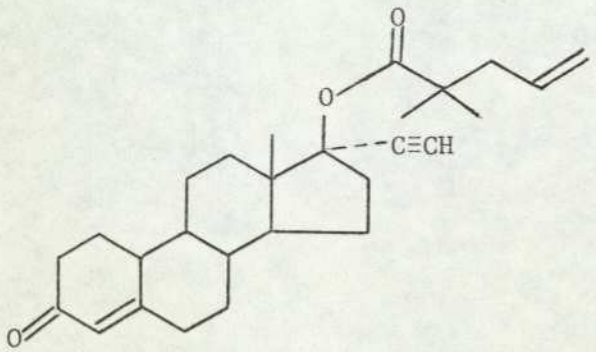


a. and c. Analysis conditions as Figure 2b, except mobile phase MeCN-CH_2Cl_2, 5:95.

b. Preparative HPLC on 57cm x 2,1cm i.d. LichroPrep SI60 15-25µm silica, eluted with MeCN-CH_2Cl_2, 5:95, at 40ml/min. Injection of 1g in 2ml CH_2Cl_2. Detector, 243nm x 2.0 AU fsd.

Figure 5 HPLC of norethisterone 2,2-dimethylpent-4-enoate (27).

before and after the main peak are 1.17 and 1.29 respectively. From experience, such a separation would be impractical on the Waters Prep 500 without multiple recycling. Injections of 1 g on a 1" x 60 cm home-made column of Lichro Prep SI60 gave a separation in under half an hour (Figure 5 b). The fractions collected were re-analysed and showed a satisfactory result (Figure 5c), with a pure, central portion to the main peak. Combination of fractions 3 and 4 gave a final sample of 400 mg lacking any detectable impurities. This purification was achieved with the expenditure of 1 litre of mobile phase: a further 1 litre of 1 : 1 MeCN-CH_2Cl_2 was used for column scrubbing and 0.5 litre of the next solvent system for re-equilibration.

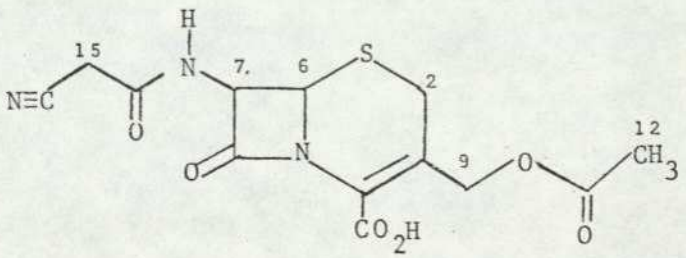


(27)

Further experience with this column has shown that base-line resolution can be achieved at a 1 g (9 mg/g packing) loading for α = 1.8 and at 0.5 g loading for α = 1.27. After the column had been used for 10 - 20 purifications it was repacked with fresh silica.

7.4. Purification of a Cephalosphorin on a Home-Made Bonded Phase: Results and Discussion

During the course of the research work, we were presented with a problem concerning a commercial sample of the antibiotic cephacetrile (28). The sample was known to be contaminated with small amounts of impurities (<5% total) and two specific objectives were set: (i) to purify the major component completely and (ii) to establish whether any of the minor impurities was a β-lactam.



(28)

The problem was therefore somewhat different to that encountered with the steroids: it would not be sufficient to fraction-cut across a poorly resolved group of peaks, since this would not yield pure samples of the minor components. Moreover, the small amounts of the minor components meant that a large batch would have to be chromatographed to provide sufficient material for spectroscopic examination.

A survey of the literature revealed that there were no reports of preparative HPLC of cephalosphorins on a scale greater than a few milligrams, although there were numerous reports of analytical separations.[10] In terms of the chromatographic phases

employed, these could be grouped as follows:

1. Ion exchange chromatography, mostly on strong anion exchange resins, eluted with buffered aqueous solutions.

2. Reverse phase HPLC on long-chain alkyl silicas, usually eluted with aqueous methanol containing ammonium carbonate or other salt buffers.

3. Reverse phase HPLC on a phenyl silica eluted with H_2O/MeOH/AcOH, or on an alkyl-phenyl silica eluted with a mixture of MeCN and an aqueous phosphate buffer.

4. Chromatography on an aminopropyl silica eluted with H_2O/MeOH/MeCN/AcOH.

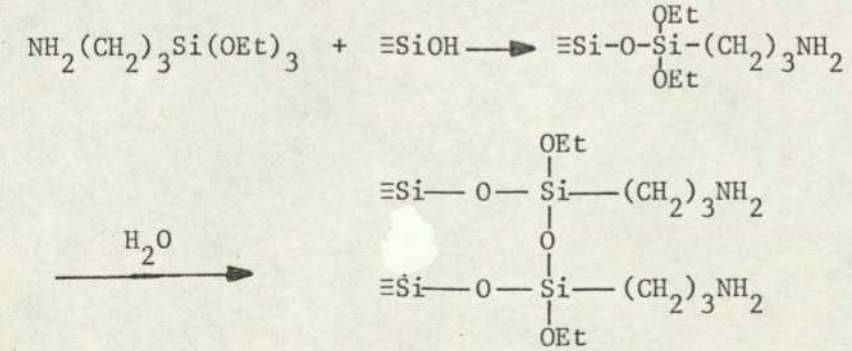
In selecting our own system, we were concerned to avoid the use of buffered aqueous solutions containing involatile salts which would complicate the isolation of eluted components and might also contribute to their decomposition. The use of an aminopropyl phase with only volatile components in the mobile phase therefore appeared attractive. It was established that a good separation between the cephacetrile and its impurities could be obtained on such a system under analytical conditions (Figure 6a) and the question of scale-up of this system was therefore considered.

Increase of loading of the analytical system to the point where base-line resolution was lost permitted injections of ca. 3 mg to be made. It was evident that accumulation of sufficient quantities of pure material by this technique would be excessively tedious.

After consideration of the considerable expense that would be involved in packing a large column with the commercially available, analytical grade of aminopropyl silica, attention was turned to the synthesis of a large batch from cheap raw materials.

The bonding of aminopropyl triethoxysilane to silica is readily accomplished under well documented conditions.[11] Three different grades of silica were examined: (i) Merck t.l.c. grade silica Type H60, (ii) Merck Type H60, 15 μm (average) and (iii) LichroPrep S.I. 60 15-25 μm HPLC silica. (Scheme 1)

Reaction of each type of silica with aminopropyl triethoxysilane (2 : 1 W/W) followed by exhaustive washing and drying gave an aminopropyl silica which was subjected to combustion analysis. The three materials gave comparable loadings ((i) 8.3%, (ii) 10.2%, (iii) 9.7%). For preliminary evaluation, a sample of each phase was high pressure slurry packed into an analytical HPLC column. The first phase generated such a high back pressure that it was impractical to carry out a separation on the column and work with this phase was discontinued. The high back pressure is probably due to the presence of fines in the t.l.c. silica causing blocking of the column end frit.



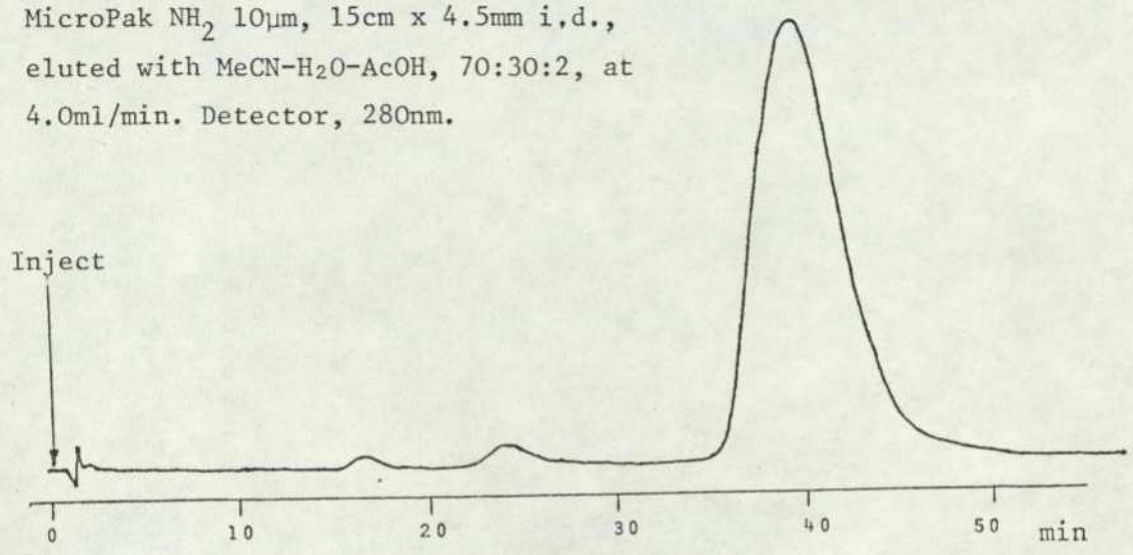
Scheme 1.

The results with the other two phases are shown in Figures 6b, 6c . For analytical purposes, both phases give adequate results with base-line separation being achieved between the main component and its nearest impurity. However, the magnitude of this separation is greater on phase (iii) (α = 2.1) than on phase (ii) (α = 1.47). Since the larger α value will permit a higher preparative loading before base-line resolution is lost, the Lichro Prep S.I.60 silica was adopted for the final scale-up. The difference in performance between phases (ii) and (iii) may reflect the difference in particle size distributions (type (ii) is smaller but less closely sized) or the slight difference in phase loadings. It has been observed previously with analytical aminopropyl silicas that above a certain limit (8.5%) increased phase loading does not contribute to increased retention but rather to greater peak broadening, probably because of a thickening polymeric matrix.[11]

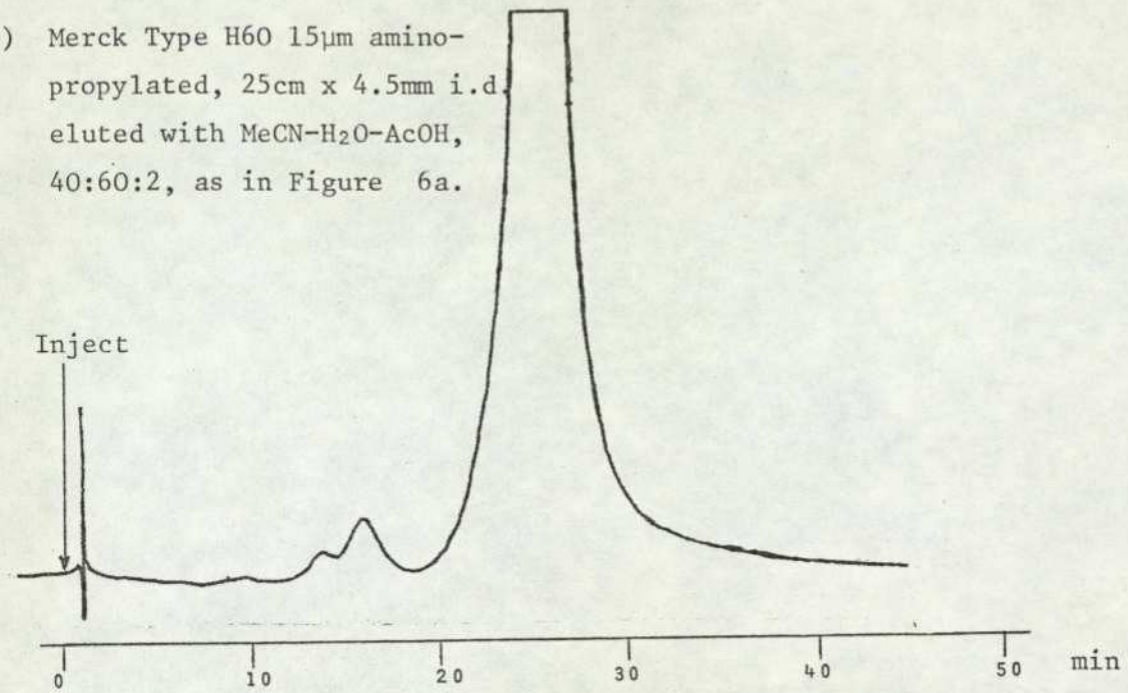
A 100g batch of the Lichro Prep S.I. 60 silica was bonded with aminopropyl triethoxysilane and the product high pressure slurry packed into a 30 cm x 1" o.d. column. A 500 mg loading of impure cephacetrile gave the result shown in Figure 7a. On this large scale, the presence of three rather than two impurities became apparent. Complete separation of the cephacetrile from its contaminants was achieved at this loading.

The fractions collected from the preparative run were evaporated in a freeze dryer to minimise the risk of hydrolysis of the sensitive β-lactam function. The major peak yielded 450 mg of recovered cephacetrile which was completely free of impurities and was

(a) MicroPak NH_2 10μm, 15cm x 4.5mm i.d., eluted with MeCN-H_2O-AcOH, 70:30:2, at 4.0ml/min. Detector, 280nm.



(b) Merck Type H60 15μm aminopropylated, 25cm x 4.5mm i.d. eluted with MeCN-H_2O-AcOH, 40:60:2, as in Figure 6a.



(c) LichroPrep SI60 15-25 μm aminopropylated 25cm x 4.5mm i.d., conditions as for Figure 6a.

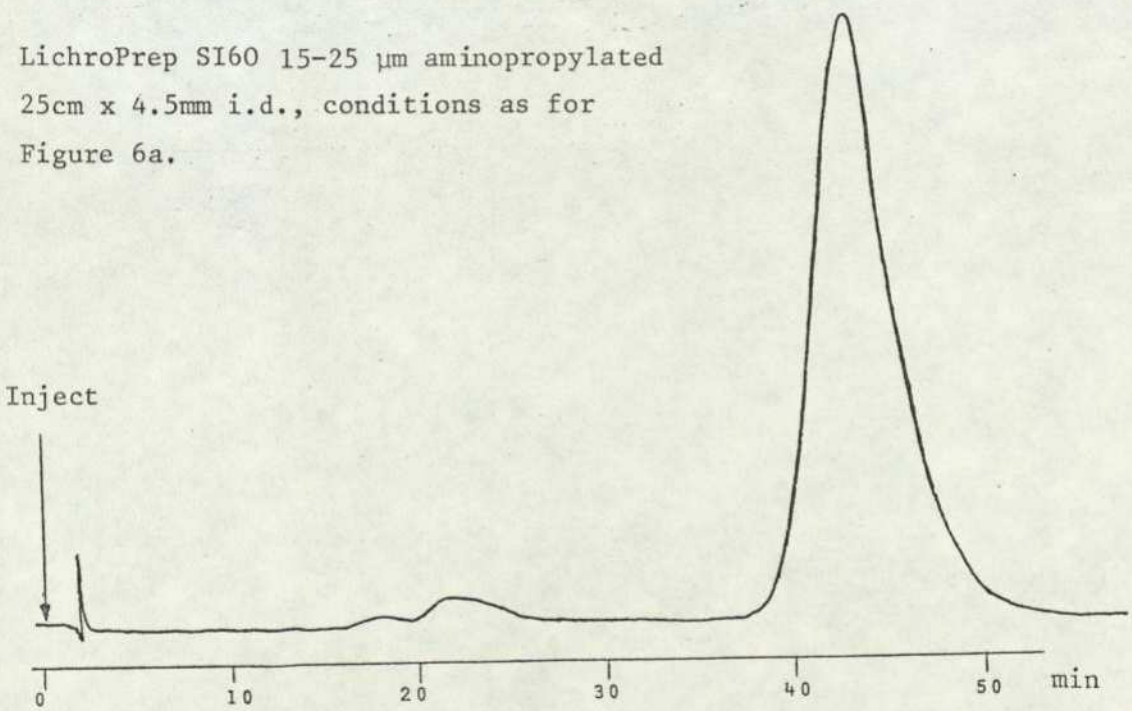


Figure 6 HPLC of cephacetrile (28) on aminopropyl silicas.

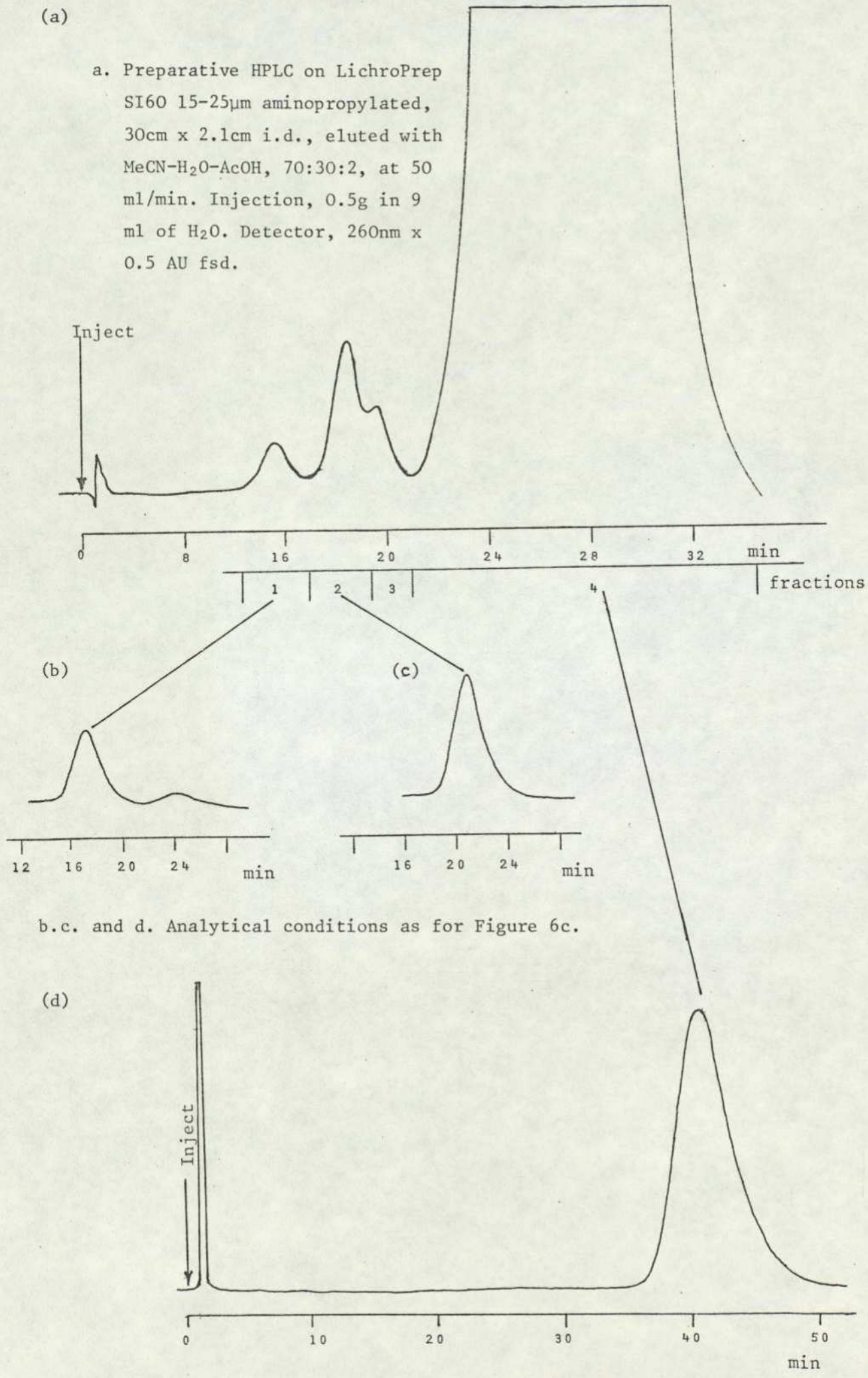


Figure 7 Preparative HPLC of cephacetrile (28).

fully characterised by spectroscopy, which together with HPLC (Figure 7d) confirmed that no hydrolysis had occurred.

Similarly, it does not appear that hydrolysis of the impurities had occurred, since each isolated component still had the same retention time as it showed in the original mixture (Figures 7 b,c). n.m.r and i.r. examination of the isolated impurities showed that none of them was a β-lactam.

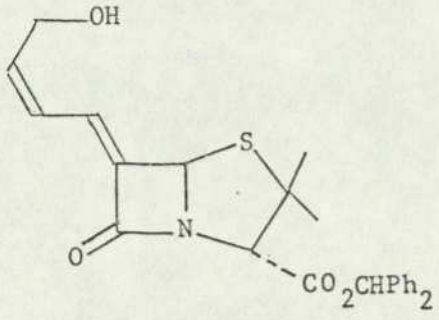
7.5. Purification of Penicillanate Derivatives: Results and Discussion

The laboratory assembled preparative HPLC instrument proved to be an invaluable aid in many aspects of the work with 6-diazopenicillanate esters. In this Section, some important separations are discussed in detail.

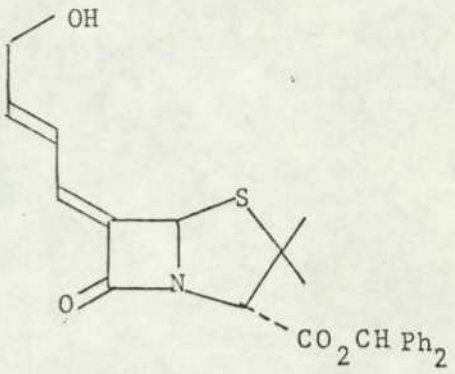
(i) The unbuffered $NaBH_4$ reduction of the dienals obtained by condensation of benzhydryl 6-diazopenicillanate with furan (Chapter 5) led to a mixture of the three dienols (29)-(31) and the two alkenols (32) and (33). The three dienols were also obtained and characterised from separate reduction experiments using buffered conditions, but it was necessary to isolate the two individual alkenols from this reaction mixture. Such separations of 6α- and 6β-epimers have often been difficult in the past and in the present case the separation was impractical by conventional chromatography. However, the problem was very readily solved by preparative HPLC.

The crude reaction mixture resulting from reduction of 450 mg of dienals was first cleaned up by passage through a small, open glass column (14 cm x 1.4 cm o.d.), packed with 10 g of silica gel 100 (70-230 mesh), eluted with 100 ml of MeCN-CH_2Cl_2 (9 : 91) followed by 100 ml of MeCN-CH_2Cl_2 (13 : 81). This small column functions as a guard column, removing any very polar impurities which would seriously contaminate the preparative HPLC column and reduce its life. Evaporation of the eluted solution yielded 330 mg of the crude product mixture, which was then redissolved in CH_2Cl_2 and loaded into a 4 ml sample loop for injection onto the preparative column.

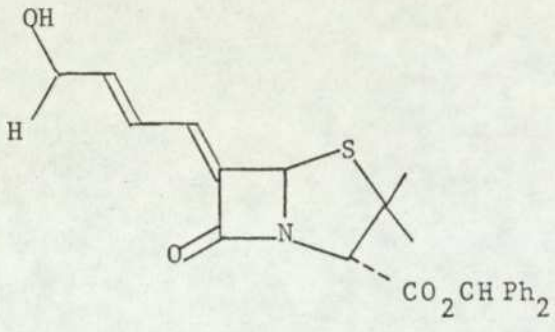
A 30 cm x 1" o.d. column packed with Lichro P rep S.I.60 15-25 μm silica was used for the separation. Isocratic elution with MeCN-CH_2Cl_2 (9 : 91) gave the result shown in Figure 8. The desired fractions were collected separately, evaporated and the compounds identified by spectroscopic analysis. The recoveries of pure compounds were 70 mg of (32), 48 mg of (33), 62 mg of (31) and a mixture of 46 mg of (29) and (30) was also obtained. The separation of (29) and (30) could have been achieved in a single pass by using a less polar solvent system, or by using gradient elution, but this was unnecessary in the present experiment and was not attempted. The entire elution was complete within 20 minutes under the conditions selected, and provided sufficient quantities of the required alkenols as individual, pure compounds for complete characterisation, deprotection of the ester groups and subsequent biological assay of the free acids.



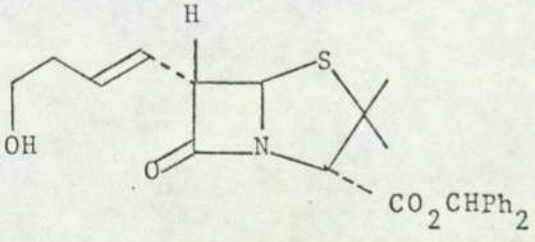
(29) ZE



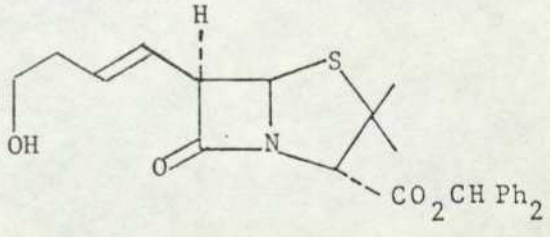
(30) EZ



(31) EE



(32) α



(33) β

LichroPrep SI60, 15-25μm, 30cm x 2.1cm i.d., eluted with MeCN-CH_2Cl_2, 9:91, at 40ml/min. UV 230nm x1 AU fsd. Injection, 400mgm in 4ml CH_2Cl_2.

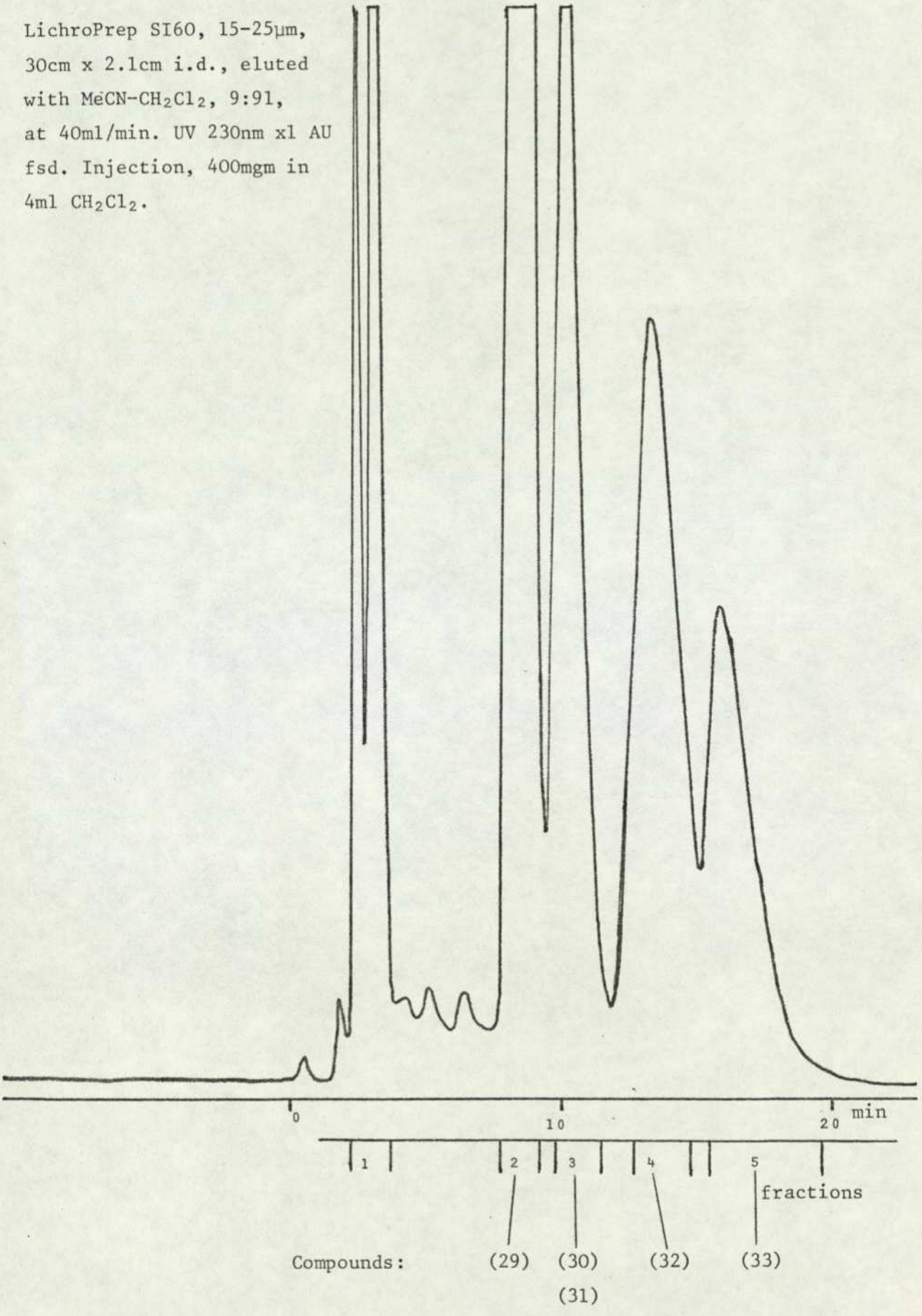
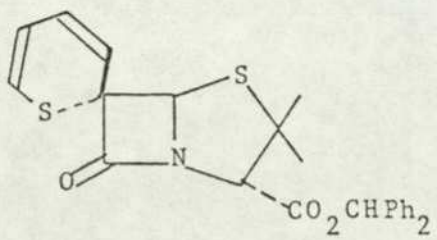


Figure 8 Preparative HPLC of the dienal reduction products (29)-(33).

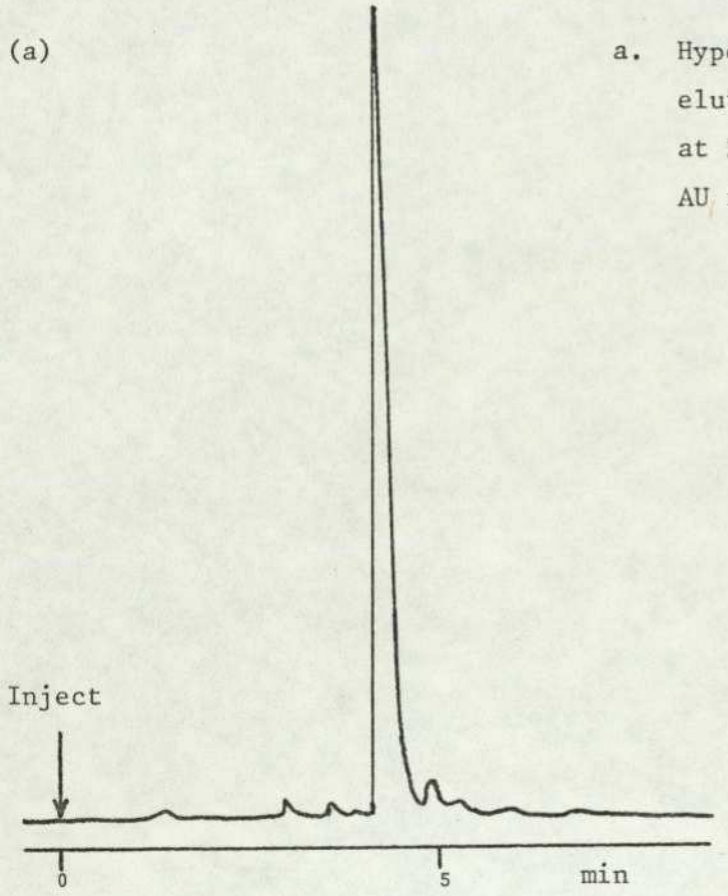
(ii) The examples above are typical of a number of cases in which complex, crude product mixtures containing similar compounds were separated. In a number of other cases, individual components from reaction mixtures could be reasonably well separated by conventional methods such as preparative t.l.c., but the resulting materials were not completely pure. In these circumstances preparative HPLC was very successfully applied to obtain pure material for characterisation and microanalysis.

A typical example is the case of the unusual 2H-thiopyran (34) obtained by carbenoid ring expansion of thiophene (Chapter 3). Preparative t.l.c. of the crude reaction product gave a material which showed significant impurities on HPLC analysis (Figure 9 a) including one close-running component (α = 1.21). A 200 mg sample of (34) was readily purified on the preparative column (Figure 9 b), yielding 120 mg of analytically pure compound.



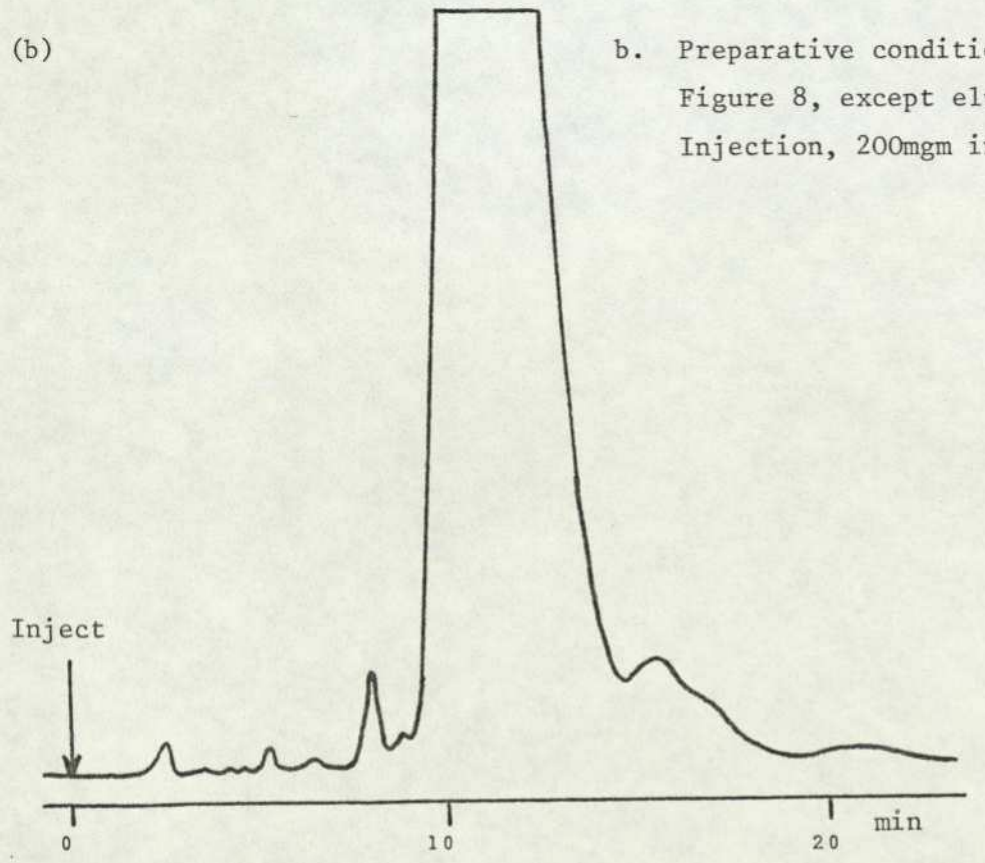
(34)

(a)



a. Hypersil 5μm, 12.5cm x 4.5mm i.d., eluted with MeCN-CH_2Cl_2, 0.3:99.7, at 2.0ml/min. Detector, 232nm x 2 AU fsd. Injection, 5μl in CH_2Cl_2.

(b)



b. Preparative conditions as for Figure 8, except eluent CH_2Cl_2. Injection, 200mgm in 4ml CH_2Cl_2.

Figure 9 HPLC of the 2H-thiopyran derivative (34).

7.6. Experimental

Analytical HPLC was performed with a Waters 6000M pump, Rheodyne 7125 sample injector fitted with a 10 µl loop, Cecil 2012 monitor fitted with an 8 µl flow cell of 10 mm path length, and a 10 mV strip-chart recorder (Linseis or JJ CR 540). Analytical columns (¼" o.d. x 4.5 mm i.d. smooth-bore 316 SS) were terminated with ¼" - 1/16" zero dead volume, modified Swagelok unions fitted with 5 µm porous steel frits.

Preparative HPLC was performed with an El Metripump (Metering pump Ltd) carrying two pump heads, a Rheodyne 7125 injector modified by attachment of 4 ml or 9 ml loops, and a Cecil monitor fitted with a high flow rate, 1 mm path length flow cell. Preparative columns were constructed from 1" o.d. x 21 mm i.d. smooth-bore 316 SS tubing terminated with modified 1" - 1/16" Swagelok unions fitted with 5µm frits.

Preparation of aminopropyl silica samples: The silica was stored over a saturated solution of lithium chloride for 24 h prior to use. 3-Aminopropyl triethoxysilane (5 ml) was added to a slurry of the silica (10 g) in dry xylene (50 ml) and the mixture stirred and refluxed for 30-60 mins. The bonded phase was filtered off, washed with dry benzene, dry dichloromethane, methanol, water, methanol dichloromethane and dried at 40° overnight.

The following loadings were obtained by combustion analysis:

(i) Merck Type H 60 t.l.c. silica gel: 8.3%.

(ii) Merck Type H 60 silica (15 µm mean particle diameter): 10.2%.

(iii) Merck LichroPrep SI 60 (15-25 µm) : 9.7%.

Preparation of large batch of Aminopropyl LichroPrep: The synthesis was carried out as above, using 100 g Lichro Prep S.I. 60 silica and 50 ml 3-aminopropyl triethoxysilane. The reflux was carried out in hexane. The product was found by combustion analysis to have a loading of 9.66% by weight of aminopropyl groups.

Column Packing Procedures [12,13]

(i) Aminopropyl Silica

Analytical: A slurry of packing material (4 g) in water (10 ml), was dispersed by standing in an ultrasonic bath for 10 min., was introduced into a reservoir tube (60 cm x $\frac{3}{8}$" o.d.). To the top of this tube was attached the empty $\frac{1}{4}$" o.d. column, via a $\frac{3}{8}$" - $\frac{1}{4}$" union. Packing was effected by upward displacement, using a 1 : 1 mixture of MeCN-H_2O delivered by a CP III pneumatic column packer (Jones Chromatography) at 6,000 p.s.i. After 200 ml of solvent had been pumped, the tubing assembly was inverted and a further 100 ml pumped through. The air supply for the pneumatic pump was then terminated and the column pressure allowed to decline to zero. The packed column was left for 5 min. before carefully disconnecting it from the packing chamber and fitted with a porous P.T.F.E. frit and a $\frac{1}{4}$" - $^1/_{16}$" ZDV union.

Preparative: A slurry of 3-aminopropyl LichroPrep (70 g) in water (550 ml) was dispersed for 20 min. in an ultrasonic bath and then poured into a reservoir tube (120 cm x 1" o.d.), to the bottom of which was connected to column (30 cm x 1" o.d.) by means of a 1" through union. The end of the column was terminated with a 1" - $^1/_{16}$" modified Swagelok union fitted with a 5 μm steel frit. The slurry was compressed to

a compact bed by downward displacement, using a 1 : 1 mixture of MeCN-H_2O delivered by a Haskell DST 100 pneumatic amplifier pump (Jones Chromatography) at 6000 p.s.i., 200 ml/min. After depressurising the pneumatic pump and allowing the column pressure to fall to zero, the packed column was left for 10 min. before disconnecting it from packing chamber and fitted with a porous P.T.F.E. frit and end union.

(ii) Silica Columns

Analytical and preparative silica columns were packed using the equipment described above and with similar techniques, except that the dispersal and packing solvent used was dichloroethane, and the pressure applied was 11500 p.s.i. Analytical columns were filled with Hypersil 5 μm SiO_2, and preparative columns with LichroPrep S.I. 60 15-25 μm SiO_2.

Preparative HPLC of Cephacetrile Sample: The preparative column (30 cm x 2.1 cm i.d.) was equilibrated by recycling through the mobile phase, MeCN-H_2O-CH_3CO_2H (70 : 30 : 2), for 30 min. at 50 ml/min. A solution of impure cephacetrile (0.5 g) in water (5 ml) was then injected via a 9 ml sample loop and elution continued at 50 ml/min. for 50 min. Fractions were collected manually and the samples recovered by freeze drying. Major component (Fraction 4): 450 mg (95% yield). White solid, m.p. 112-118°, pure by HPLC (Figure 7d), ^1H n.m.r. (D_2O + CD_3CN), τ : 4.30 (1H, d, J = 4.5 Hz, C_7-H), 4.96 (1H, d, J = 4.5 Hz, C_6- H); 4.94 (1H, d, J = 13.2 Hz, C_9- H), 5.24 (1H, d, J = 13.2 Hz, C_9-H), 6.38 (2H, S, C_{15}-H), 6.38 (1H, d,

J = 18.9 Hz, C_2-H), 6.62 (1H, d, J = 18.9 Hz, C_2-H), 8.0 (3H, S, C_{12}-H). ν_{max} (nujol) 3360, 1660 (amide), 1770 (β-lactam), 2260(CN) cm^{-1}. The impurities (Fractions 1, 2 and 3) were isolated as viscous gums (Figure 7) whose ^1H n.m.r. and i.r. spectra showed none of the peaks characteristics of β-lactams.

7.7. References

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APPENDIX

General Experimental Details

^{1}H n.m.r. spectra were recorded on a Jeol 100 MHz continuous wave instrument, as dilute (10%) solutions in $CDCl_3$ with TMS as internal standard, unless otherwise stated.

^{13}C n.m.r. spectra were recorded on a Jeol FX 60 instrument as dilute solution in $CDCl_3$ with TMS as internal standard and D_2O external lock, unless otherwise stated.

Infrared Spectra were recorded on a Perkin-Elmer 157G, 457 or 599 instrument.

Ultraviolet Spectra were recorded on a Perkin-Elmer 402 instrument.

Mass Spectra were recorded on a Kratos MS30 Mass spectrometer.

Melting Points were determined on a Koffler hot-stage apparatus and are uncorrected.

Analytical and Preparative HPLC: Details of the equipment and techniques used are given in Chapter 7.

Solvents: All solvents used for reactions were either purchased as analar grade or were redistilled before use. Special drying procedures for solvents were as follows:

THF Distilled off $LiAlH_4$ and collected at 63-5°/760 mm.

Diethylether, Toluene and Benzene were distilled off sodium-lead alloy and stored over sodium-lead alloy.

Preparation of Preparative t.l.c. Plates

a) Silica

The slurry of silica gel (100g, Silica gel $60GF_{254}$ Merck) in water (180 ml) was spread over 5 x 20cm x 20cm glass plates with a spreader which was adjusted to give a film thickness of 0.9 mm. The plates were left at room temperature overnight before heating in an oven at 120° for 12 hours.

b) Alumina

The slurry of aluminium oxide (150g, 60 GF_{254} Type E Merck, in water (200 ml) was spread over 4 x 20cm x 20cm glass plates which were then treated as above.