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Chemical Modification of the Surface of Wool

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ABSTRACT

The reactions of hypophosphorous acid (H3PO2) and phosphorous acid (H3PO3) with disulphide compounds, particularly those of the cystine residues in wool, are investigated. The observed differences between the reactions of both acids are highlighted. Some of the products of the reaction of H3PO2 and wool cystine are identified. These include the gases hydrogen sulphide (H2S) and sulphur dioxide (SO2) and the reduced form of cystine, the thiol cysteine. Quantification of the amounts of these products formed under given reaction conditions, is achieved by use of H2S and SO2 sensitive meters, and a colorimetric reaction of 2-vinylpyridine with cysteine.

The dyeing properties of wool pretreated with both phosphorus acids alone and a mixture of formaldehyde and both acids are investigated. The H3PO2/formaldehyde mixture displays different dyeing properties to the other pretreatments.

A series of novel aromatic and aliphatic aminoanthryl phosphonous acids are synthesised and their use as fluorescent whitening agents determined. The solution photolysis of some of the compounds of the series is also studied.

Different compounds, namely two commercially available polysulphides, acetic anhydride, diethylpyrocarbonate, isocyanates and their water soluble bisulphite adducts, acid and acid/formaldehyde solutions are applied to the surface of wool. The effects of these compounds on the rate and extent of photoyellowing of wool and on the fluorescence emission and excitation spectra of wool are noted. Acetic anhydride has the most profound effect on both properties.

The possible identity of the compound(s) that contribute to the fluorescence emission spectrum of wool at 450 nm are investigated. A series of aliphatic and aromatic pyruvamides are synthesised and their solution fluorescence emission maxima compared to that of wool.

Finally, the reaction between cysteine and o-phthalaldehyde in the presence of excess primary amine is investigated. An unstable highly fluorescent species may be produced, which though unidentified is most probably an isoindole. Highly coloured products of side reactions also result. The production of the fluorescent species on the surface of wool is undertaken using tributylphosphine and H3PO2 reduced wool as the thiol function in the reaction.

LIST OF ABBREVIATIONS

BT	=	Bityrosine
9-BMA	=	9-Bromomethylacridine
DCCI	=	N,N'-Dicyclohexylcarbodiimide
DEPC	=	Diethylpyrocarbonate
DMF	=	Dimethylformamide
ϕ_f	=	Quantum Yield of Fluorescence
ϕ_f	=	Lifetime of Fluorescence
FABS	=	Fast Atom Bombardment Spectroscopy
FWA	=	Fluorescent Whitening Agent
5-IAF	=	5-Iodoacetamido-fluorescein
IC	=	Internal Conversion
IR	=	Infra-Red
ISC	=	Intersystem Crossing
NFK	=	N-formylkynurenine
NMR	=	Nuclear Magnetic Resonance
1O2	=	Singlet Excited Molecular Oxygen
OPA	=	o-Phthalaldehyde
2-PEC	=	2-Pyridylethyl Cysteine
2-PEW	=	2-Pyridylethyl Wool
TFA	=	Trifluoroacetic Acid
THF	=	Tetrahydrofuran
UV	=	Ultra-Violet
2-VP	=	2-Vinylpyridine

1:1 General Aspects of Photochemistry

A working definition of photochemistry is 'chemical change brought about by light.' Light is normally understood to mean electromagnetic radiation in the visible and ultraviolet range (800 to 200 nm) of the spectrum. Absorption of a photon by a molecule produces an electronically excited state in which the electrons are arranged in a way that is not the lowest energy configuration. This excited state species may then undergo physical and/or chemical change.
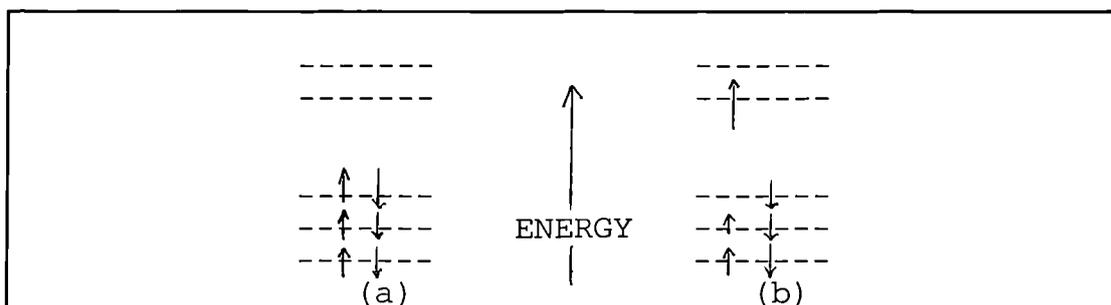
Figure 1:1 Molecular orbital diagram for an organic molecule, showing some of the orbitals and their occupancy in (a) the ground state and in (b) an electronically excited state.

1:1:1 Types of Electronic Transition

It is normally assumed that the inner shell of electrons of the constituent atoms of the molecule remain unaltered in the molecule itself; linear combinations of the remaining valence shell atomic orbitals then provide molecular orbitals that can be used to describe the outer electronic structure of the

molecule. If single atomic orbitals on each of the two

two adjacent atoms are combined, they produce two

molecular orbitals, one of higher energy and one of

lower energy than the separate atomic orbitals.

```
                                    Antibonding
                                        MO
          ↑                         -----------
          |
          |
  ENERGY   Atomic-------        --------
          |     Orbitals
          |                        Bonding
                                     MO
                                  -----------
```
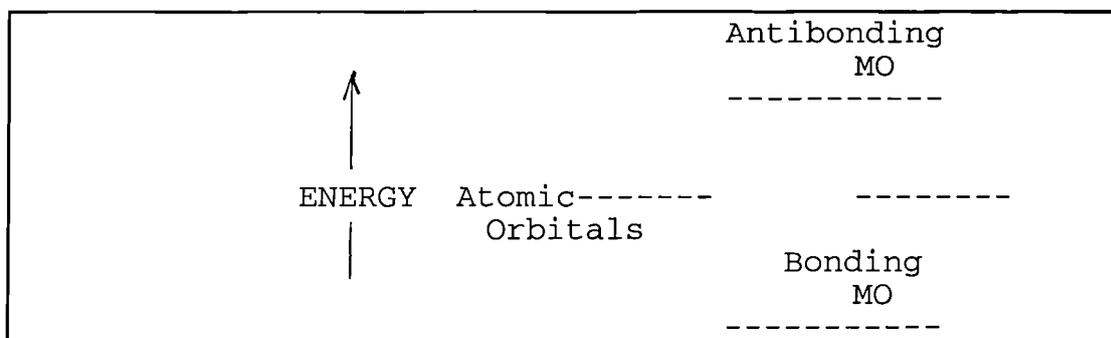
Figure 1:2 Effect on Orbital Energy of a Linear
Combination of Atomic Orbitals

The lower energy orbital is called a bonding orbital,

and where there is a two electron bond between the

atoms, a pair of electrons will occupy the bonding

orbital in the ground state electronic configuration.

The higher energy molecular orbital is called an

antibonding orbital; it is unoccupied in the ground

state, but may be occupied by an electron in the

excited state of the molecule.

Orbitals that are symmetrical about the internuclear

axis are called sigma (σ) or sigma-star (σ^*)

orbitals, depending on whether they are bonding or

antibonding respectively. Orbitals that are

antisymmetric about a plane that includes the

internuclear axis are called pi (π) or pi-star (π^*)

orbitals. The sigma framework of the molecule may be

described in terms of localised electrons, each

covering two nuclei only. Delocalised electrons that cover more than two nuclei appear in the model only for pi-bonding in conjugated molecules. A third type of orbital described is termed an n-orbital. These orbitals are usually non-bonding, and a pair of electrons occupying an n-orbital are a lone pair of electrons on a particular atom. The absorption of radiation in the ultraviolet or visible region of the spectrum causes the transition of electrons present in the molecule to higher energy molecular orbitals. The most common transitions encountered in organic molecules are: $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$, $\sigma \rightarrow \sigma^*$ and less frequently $n \rightarrow \sigma^*$. The resulting states are termed n, π^* state π, π^* state n, σ^* state and σ, σ^* state.
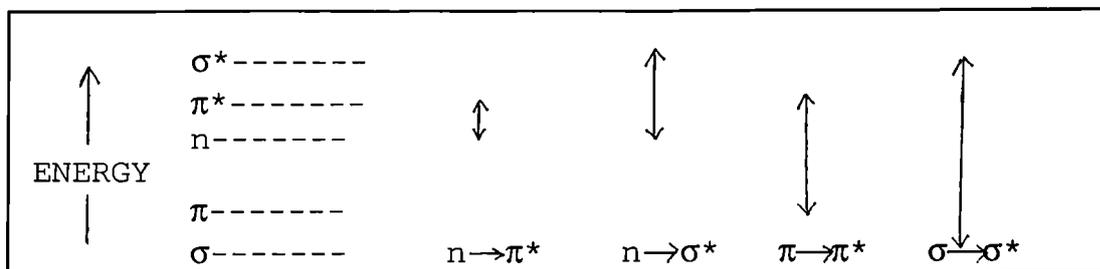
Figure 1:3   Some possible transition types of organic molecules.

1:1:2 The Beer-Lambert Law

The probability of absorption of a photon by a
molecule, and its variation with wavelength, is
reflected in the electronic absorption spectrum of the
compound. At a particular wavelength the bulk
absorption properties of a solution of the compound can
be represented by the following equation;

$$\text{Log}\ \frac{I_o}{I} = A = E * C * L$$

I_o = Intensity of incident radiation
I = Intensity of transmitted radiation
A = Absorbance
C = Concentration of solution (mol/L)
L = Path length (cm)
E = Molar extinction co-efficient (L/mol cm)

The equation is not valid for solutions of
concentrations greater than 0.01 mol/L and for very
high intensity radiation e.g. laser light, and where
molecules aggregate in the ground state.

1:2 Specific Aspects of Photochemistry

1:2:1 Spin Multiplicity of Excited Molecules

An electronically excited state has two unpaired
electrons in different orbitals. The usual situation
involves the unpaired electrons as having opposite spin
so the state has an overall zero spin and is a singlet
state. If the two spins are parallel the state has an
overall non-zero spin and is termed a triplet state.

The singlet and triplet states are of a different

multiplicity and are different chemical species. The

triplet state is of lower energy than the singlet state

due to the repulsive nature of interactions between

electrons of the same spin, but is not readily produced

by absorption of a photon.

1:2:2 Electronically Excited States and Their Deactivation

In the absence of interaction with another chemical

species an electronically excited state can do one of

two things. It can change into a different excited

state of the same compound, or it can change into a

different compound. The first of these processes is a

photophysical one which may be divided into two groups;

radiative processes, in which a photon of ultraviolet

or visible radiation is emitted, and non-radiative

processes, in which no such emission takes place.

1:2:3 Non-Radiative Decay Processes

Non radiative decay processes involve conversion of one

electronic state into another without emission of

light. They can be divided into two categories

according to whether or not an overall change in spin

multiplicity results. If no spin change occurs, the

process is called internal conversion (IC). This is a

very rapid transition that can occur between

isoenergetic vibrational levels. For typical organic

molecules, rate constants are in the order of;

$k\ (\ S_2 \longrightarrow S_1\ ),\quad 10^{11}\ \longrightarrow\ 10^{14}\quad sec^{-1}$

$k\ (\ S_1 \longrightarrow S_0\ ),\quad 10^5\ \longrightarrow\ 10^8\quad sec^{-1}$

The transition is much slower for conversion to the ground state, and this is one of the reasons why negligible emission occurs from the upper excited state but fluorescence may compete effectively with internal conversion to the ground state.

When non-radiative decay involves a change in spin multiplicity it is called intersystem crossing (ISC). This process occurs between isoenergetic levels of different multiplicity, and is continually competing with radiative transitions. Although it is forbidden due to non-conservation of spin, it is the normal route for the population of the triplet state. A third type of non-radiative decay involves loss of excess vibrational energy via molecular collisions to give the electronically excited state in its lowest vibrational level. This process is usually faster than the intermolecular transition between electronic states, with rate constants in the order of $10^{13}\ sec^{-1}$. Therefore most processes involving a change in electronic state take place from the lowest vibrational level.
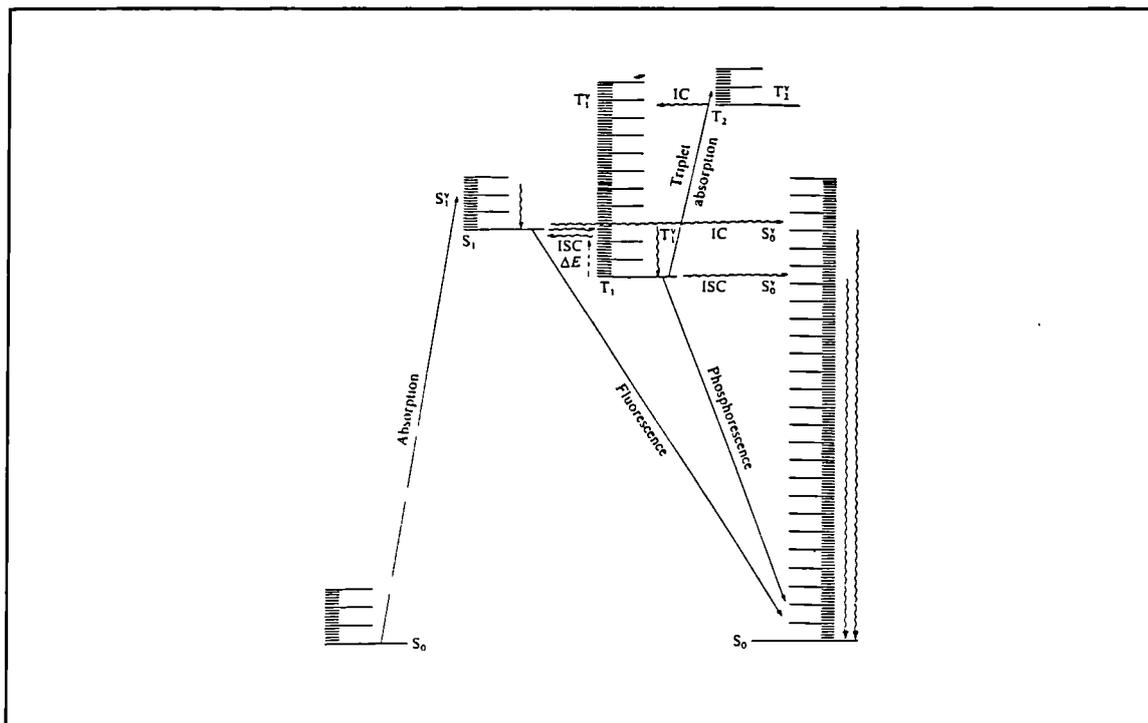
Figure 1:4 A Jablonski Diagram

The various intramolecular processes initiated by light

absorption are illustrated schematically in Figure 1:4.

Such a representation is known as a Jablonski Diagram.

The symbols S_0, S_1 and S_2 represent the ground state

and first and second excited singlet states

respectively, while T_1 and T_2 represent the triplet

states. The multiplicity is given by the expression,

2n + 1, where n = number of unpaired electrons.

Photophysical processes are represented by lines

connecting the states. Straight lines are used for

radiative processes and wavy lines represent

radiationless transitions. The fact that the electronic

states can have varying amounts of vibrational energy

levels is indicated by the series of lighter horizontal

lines for each state.

1:2:4 Radiative Decay Processes

When an excited state emits a photon, the state is normally converted to the ground state which is also singlet. This process in which there is no overall change of spin is ` spin allowed ' and is called fluorescence. (See Figure 1:4)

The most commonly observed transition for compounds in solution, is from the lowest excited singlet state, regardless of which singlet is formed initally by absorption. Because of this a fluorescence spectrum is not normally affected by the wavelength of excitation. The most intense fluorescent compounds are rigid, planar structures. An increase in rigidity decreases the vibrational amplitudes, which in turn reduces the efficiency of intersystem crossing and internal conversion which compete with fluorescence. The frequencies of vibrational excitation in the lowest excited singlet state are within 10-20 % of those of the ground state, which is the factor responsible for the mirror image relationship between absorption and fluorescence spectra. Any deviations from mirror symmetry indicate differences in nuclear configurations in S_0 and S_1.

Fluorescence is affected by; [1],[2]

Solvent Interactions: In most polar aromatic molecules whose lowest singlet states are (π,π^*) the excited state is more polar than the ground state. An increase

in the polarity of the solvent will produce a relatively greater stabilization of the excited state in comparison to the ground state. As the polarity of the solvent increases the absorption and fluorescence spectra of the compound will shift to lower energies. The reverse is true for non-polar solvents.

Solvent pH: The influence of pH upon the fluorescence of an organic compound containing acidic or basic functional groups is often drastically different from that observed for the absorption spectrum. In order for acid-base reactions in the excited state to perturb the fluorescence spectrum, it is necessary for protolytic dissociation reactions to be more rapid than rate constants for fluorescence. In protic solvents , proton transfer processes are known to be rapid. A state of quasi-equilibrium may be attained in the short interval between absorption and fluorescence. The change in the fluorescence spectrum may be measured as a function of pH.

Presence of quenching species may also affect the profile of the fluorescence spectrum. (See 1:2:5)
Two useful parameters are (a) ϕ_f and (b) lifetime. ϕ_f is defined as a measure of the efficiency with which absorbed radiation causes the molecule to undergo a specified change.

$$(\phi_f) = \frac{\text{no. of photons emitted}}{\text{no. of photons absorbed}}$$

The values normally fall in the range 0 to 1.0. ϕ_f is

normally measured relative to that of a standard

compound, excited under identical conditions whose ϕ_f

is known. Lifetime of fluorescence (τ_f) is measured

directly by following the decay of fluorescence

intensity with time, after the excitation radiation has

been interrupted. If the process follows first-order

kinetics described by a rate constant k, the mean

lifetime, τ_f is given by; [3]

$$\tau f = 1/k$$

Phosphorescence is similar to fluorescence in many

ways, except for this process the spin multiplicity of

the species changes. (Fig. 1:4) Phosphorescence occurs

from the lowest excited triplet state to the ground

state singlet. The process is spin forbidden and

therefore has a considerably longer lifetime than

fluorescence for similarly structured molecules. Its

long lifetime makes it susceptible to impurities, and

hence it is normally difficult to detect in solution.

1:2:5 Quenching of Excited Species

An electronically excited molecule M* may lose its

excitation energy and return to the ground state in a

number of ways. A substance which accelerates the decay

of an excited state is described as a quencher. If the

orignal excited state is luminescent, quenching is

observed as a reduction in the quantum yield of light

emission. Photophysical quenching may be divided into
self quenching where the quenching species is M, and
impurity quenching, where the quencher is another
chemical species Q. Impurity quenching may be sub-
divided into (a) energy transfer (b) electron transfer
and (c) heavy atom processes. [4],[5]

1:2:5:1 Self-Quenching

If the excited species M* is quenched by a molecule of
M in the ground state this is termed self quenching. A
decrease in luminescence may be noted e.g. As the
fluorescence of pyrene is quenched, the concomitant
growth of a broad structureless emission band occurs at
460 nm. This corresponds to the production of an
initial complex between the excited species and the
ground state species. [6],[7]

This species is known as an exciplex (or in this case
an excimer, if the species are both of the same
compound). It is defined as a molecular dimer or
stoichiometric complex which is associated in an
excited state and dissociative in its ground state. [7]
Previous work has shown that in contrast to the
behaviour of pyrene, the photodimerisation reaction is
very efficient for anthracene and consequently very
little excimer emission is observed. [8]

$$\text{PYR*}(S_1) \quad + \quad \text{PYR}(S_0) \longrightarrow \quad (\text{PYRPYR})* \quad \longrightarrow$$

$$\text{PYR}(S_0) \quad + \quad \text{PYR}(S_0) \quad + \quad h\nu$$
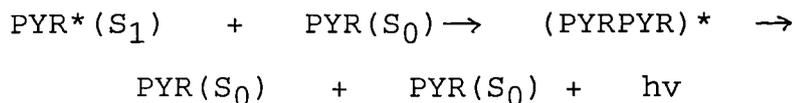
Exciplex emission is observed in solutions of mixed solutes. For example, the fluorescence of anthracene is quenched by tertiary amines e.g. diethylaniline, and a structureless emission from the excited singlet anthracene-diethylaniline complex is observed. [9],[10] Primary and secondary amines can quench the fluorescence of excited states of polyaromatic hydrocarbons but the process is not normally accompanied by exciplex formation. [11] In general fluorescence emission from the majority of excimers is virtually independent of solvent polarity, indicating that little of their stability is due to charge transfer interactions. [6]

1:2:5:2 Quenching via Energy Transfer

An electronically excited molecule in either singlet or triplet state may be quenched by transfer of energy to another molecule having lower lying singlet or triplet energy levels. The excited molecule returns to the ground state whilst the other molecule is elevated to a higher electronic state. The acceptor molecule quenches the excited state of the donor. [4]

$$\text{Donor*} + \text{Acceptor} \longrightarrow \text{Donor (gs)} + \text{Acceptor*}$$

There are four types of energy transfer.

Singlet—Singlet:

The excited singlet state of the acceptor is achieved by energy transfer from the donor excited singlet state. This process may occur over both long and short distances.

e.g. the sensitization of biacetyl fluorescence by energy transfer from the singlet state of acetone in cyclohexane. [12]

$$(CH_3)_2CO \ (S_1) + CH_3(CO)_2CH_3 \ (S_0) \longrightarrow$$
$$(CH_3)_2CO \ (S_0) + CH_3(CO)_2CH_3 \ (S_1)$$
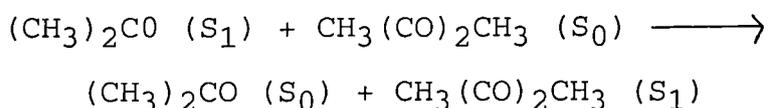
Triplet—Triplet:

An excited donor in its triplet state produces an excited acceptor in its triplet state. As the triplet state lifetime is longer than the singlet state lifetime, triplet-triplet energy transfer is the most common and most important type of energy transfer.

e.g. sensitization of triplet naphthalene by triplet benzophenone in benzene. [13]

$$(C_6H_5)_2CO \ (T_1) + \quad\text{(naphthalene)} \ (S_0) \longrightarrow$$
$$(C_6H_5)_2CO \ (S_0) + \quad\text{(naphthalene)} \ (T_1)$$

Triplet—Singlet:

In solution energy transfer from a triplet donor to an acceptor, to yield the excited singlet state of the acceptor is relatively rare, as the process is spin forbidden. It has been observed in a few notable cases.

e.g. thermolysis of tetramethyl -1-2- dioxetane yields triplet acetone, which undergoes triplet--singlet energy transfer to 9,10-dibromoanthracene to give the singlet excited state of the anthracene derivative. (14)

$$C_6H_{12}O_2 \xrightarrow{\Delta} (CH_3)_2CO \ (T_1) \longrightarrow$$
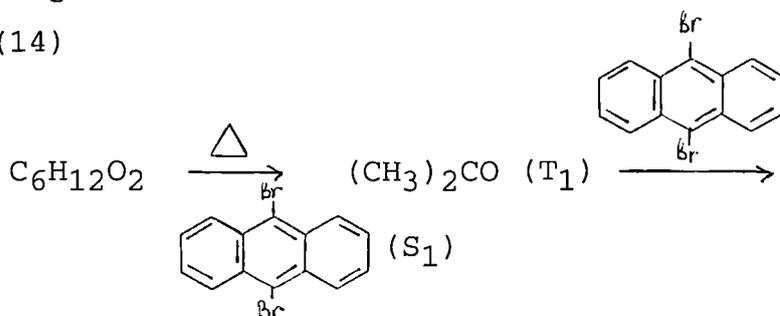
Singlet—triplet:

The rarest of the four energy transfer processes involves an excited singlet donor and a ground state acceptor to produce a triplet excited acceptor.

e.g. the fluorescence of 9,10 dibromoanthracene is quenched by naphthalene in toluene to give triplet naphthalene. [15]
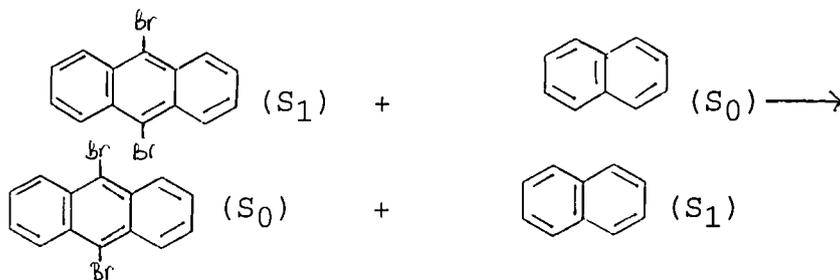
1:2:5:3 Electron Transfer

In photodynamic reactions, triplet states are often responsible for initiating electron transfer reactions: the triplet state, $M(T_1)^*$, reacts with an electron donor B. [2]

$$M(T_1)^* + B \ (\text{other molecule}) \longrightarrow M^{\cdot -} + B^{\cdot +} \longrightarrow M + B$$
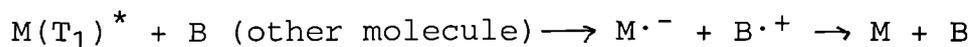
A second type of electron transfer involves the
ejection of an electron from one molecule in an excited
state into the solvent conduction band, followed by
capture by another molecule typically located some
distance away. The process may lead to a photochemical
event if the solvated electron, the photoreduced
molecule or the photo-oxidised molecule are reactive.
The metastable chemical species that result from such
an interaction often revert rapidly to the original
molecules both in their ground states. Electron
transfer with a loss of excitation energy can be a fast
reaction and it represents a deactivation of the
orignal excited state. [5],[16]

1:2:5:4 Heavy Atom Effects

For organic molecules spin-orbit interactions usually
provide the major mechanism for intersystem crossing.
Since the electron is charged and `spinning' it is
expected to have a spin angular momentum as well as a
magnetic moment. The electron inverts its spin i.e.
changes the direction of its magnetic moment in a
singlet to triplet transition. This calls for magnetic
interactions to take place. The required interaction is
provided by the magnetic field produced by the magnetic
moment of the nucleus. The magnetic moment of the
spinning electron becomes coupled to the nucleus's
magnetic field and is termed spin orbit coupling. The
probability of the singlet to triplet transition (ISC)
depends on the energy gap between the states concerned

and upon the size of matrix elements. This quantity increases with increasing atomic number thus giving rise to heavy atom effects.[17],[5]

The probability of electron spin flipping is increased if a heavy atom with a high atomic number e.g. a halogen is present in the system. A heavy atom can enhance intersystem crossing in two ways. If the heavy atom is incorporated into the molecule it can quench the fluorescence intramolecularly, or if it is present in the environment of the molecule i.e. in the solvent, it may quench the fluorescence intermolecularly.

1:3 The Photochemistry of Molecular Oxygen

Photo-oxygenation is an oxygenation reaction in which a substrate A may be transformed, by molecular oxygen into the addition product AO_2 only in the presence of light. It may take place as either a direct process, where light is absorbed by A or O_2 (A), or an indirect (photosensitized) process (B), where light is absorbed by molecules other than A or O_2. Such molecules are known as sensitisers.

$$(A) \qquad A \; + \; O_2 \quad \xrightarrow{\quad hv \quad} \quad AO_2$$

$$(B) \qquad A \; + \; O_2 \quad \xrightarrow{\quad sens \; / \; hv \quad} \quad AO_2$$
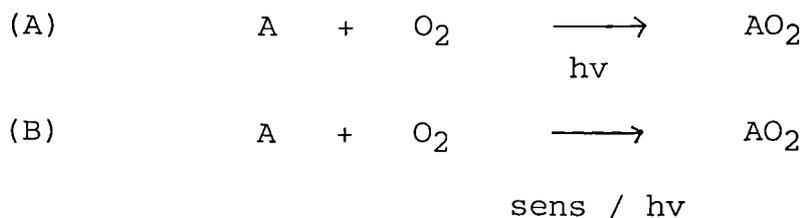
In general photo-oxidation reactions are divided into two types. Type 1 in which free radicals and electronically excited molecules are involved and Type 2 in which only electronically excited molecules occur as intermediates.

1:3:1 Molecular Oxygen: Spin States.

In order to understand the chemistry of molecular oxygen, the distribution of its electrons in the ground and electronically excited state must be considered. Two Lewis structures may serve as a model for discussion.

$\ddot{\underset{\cdot\cdot}{O}} = \ddot{\underset{\cdot\cdot}{O}}$ =   O = O   (i) electrons paired

$\ddot{\underset{\cdot\cdot}{O}} - \ddot{\underset{\cdot\cdot}{O}}$ = $\overset{\cdot}{O} - \overset{\cdot}{O}$ (ii) electrons unpaired

(i) corresponds to the singlet state species and (ii) corresponds to either a singlet or a triplet state depending on whether the spins are paired or unpaired.

Ignoring all but the two highest energy electrons, the electronic ground state configuration for molecular oxygen is given as:

$$O_2, \ldots\ldots\ldots (\pi x^*)^1 (\pi y^*)^1$$
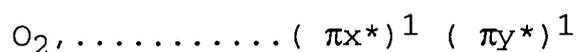
As the orbitals containing the two highest energy electrons are degenerate and molecular oxygen in its ground state has one electron in each orbital, its ground state is of triplet multiplicity. From the

orbital occupancies of the (πx*) (πy*) pair and their corresponding spin states it is possible to derive the higher energy singlet states.

S,   (πx* or πy*, ↑↓)² called ¹△g   -↑↓-   ---

S,   (πx*, ↑)(πy*, ↓)   called E⁺g   -↑- -↓-
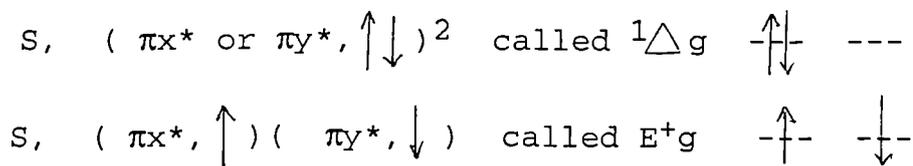
The ¹△g state is commonly referred to as 'singlet oxygen ', (the active oxygenating species) and given the symbol ¹O₂. Ground state molecular oxygen is termed ³O₂. (18),(19)
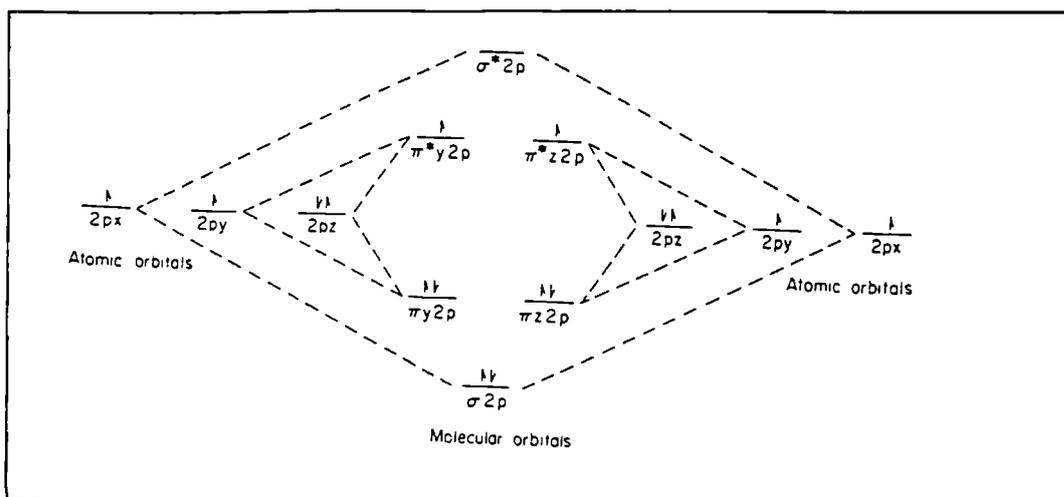
Figure 1:5   Orbital   representation   of   molecular oxygen triplet.

1:3:2 Photochemical Formation of Singlet Oxygen

Although the direct photo-excitation of molecular oxygen is possible, it is not a practical method for the production of singlet oxygen. Photochemical reactions are more commonly performed by the use of sensitisers with which singlet oxygen may be generated. If the energy gap between the excited singlet and triplet states of the sensitiser exceeds 94.5 kJ mol^{-1}, (22) which equals the energy of $^1\Delta$g, singlet oxygen may be formed. Because singlet oxygen has such a low energy of excitation, a vast number of compounds are able to sensitise its production. e.g. dyes such as rose bengal, methylene blue and polyaromatic hydrocarbons such as anthracene . Deactivation of the triplet (or singlet) excited state of the sensitiser by molecular oxygen occurs primarily by energy transfer. The transient precursor of the final quenching products is a collision complex of triplet sensitiser and ground state oxygen that can be formed with singlet, triplet and quintet multiplicity. The following reaction scheme suggests that both singlet and triplet excited states of the sensitiser can transfer their energy to ground state molecular oxygen. However the dissociation of the collision complex to ^{3}Sens$_1$ and ^{1}O$_2$ is the most significant process.

$$^1Sens_0 + hv \longrightarrow {}^1Sens_1$$

$$^1Sens_1 \longrightarrow {}^1Sens_0 + hv \quad (\text{ fluorescence})$$

$$^1Sens_1 \longrightarrow {}^1Sens_0 \quad (\text{ IC })$$

$$^1Sens_1 \longrightarrow {}^3Sens_1$$

$$^3Sens_1 \longrightarrow {}^1Sens_0 \quad (\text{ ISC })$$

$$^1Sens_1 + {}^3O_2 \longrightarrow {}^3Sens_1 + {}^1O_2 \quad (\text{ energy trans.})$$

$$^3Sens_1 + {}^3O_2 \longrightarrow {}^1Sens_0 + {}^1O_2$$
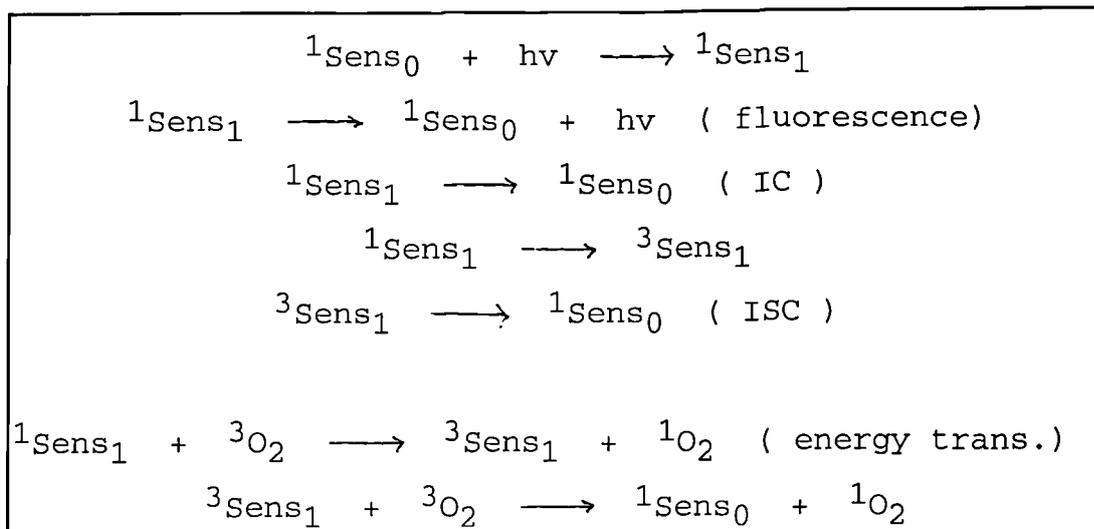
Figure 1:6 Possible transitions involved in the photosensitised production of singlet excited oxygen.

The primary condition in the photosensitized generation of singlet excited oxygen is that the excited state of the sensitiser should be efficiently quenched by oxygen. Quenching of the sensitiser by the substrate may also occur (leading to Type 1 reactions) and it is considered to be the main diversion from singlet oxygen generation.

e.g. free radicals or radical ions may be produced by interaction of the sensitiser triplet with a reducing substrate RH via H atom or electron transfer followed by proton transfer. [19]

$$^3Sens_1 + RH \longrightarrow \cdot SensH + R\cdot$$

$$^3Sens_1 + RH \longrightarrow Sens\cdot^- + RH\cdot^+$$
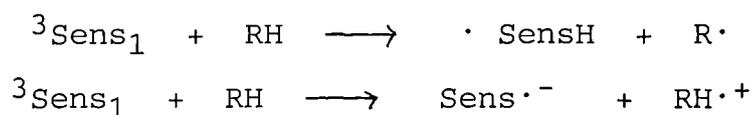
The reduced sensitiser may be oxidised to produce $O_2 \cdot^-$

which may react further. In general competition between

substrate and molecular oxygen for the sensitiser

determines whether Type 1 or Type 2 reactions occur.

1:3:3 Reactions of Singlet Excited Molecular Oxygen

Unlike ground state molecular oxygen which displays a

diradical character, all the electrons in $^1O_2$ are

paired; therefore the type of reactions the excited

molecule undergoes are expected to involve electron

pairs. Functionally $^1O_2$ behaves as the oxygen analogue

of ethylene.

The known photo-oxygenation reactions of singlet

molecular oxygen may be classified into the following

categories,

(a) reactions with olefins and aromatic compounds and

(b) reactions with sulphur-containing compounds (e.g.

sulphides, sulphoxides) and tertiary amines.

(c) reactions with amino acids.

(a) Reactions with Olefins and Aromatic Compounds:

(1) The ` Ene ' Reaction : [18]

This reaction is thought not to go through discrete

radical intermediates formed by hydrogen abstraction,

as invariably a shift of the double bond occurs; it

may be represented as a concerted process as shown, though a more complex mechanism seems likely.
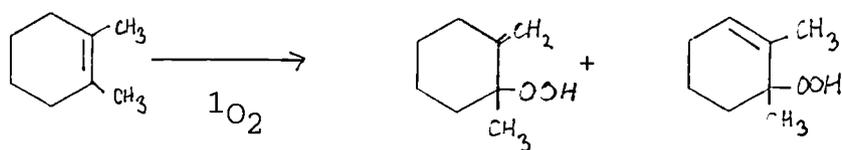
(2) (4+2) Cycloaddition with Endoperoxide Formation:[20]
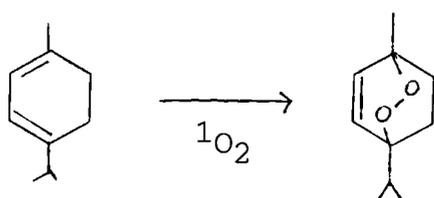
This reaction mechanism is assumed to be of a concerted Diel-Alder type, with a self-sensitised singlet oxygen formation occurring.

(b) Reactions with Sulphides and Tertiary Amines:

(1) Oxidation of Sulphides:[21]

The reaction of sulphides with singlet oxygen is believed to proceed via a zwitterionic intermediate peroxysulphide to produce (depending on the structure of the sulphide and the reaction conditions), sulphoxides and sulphones, or a reversion to sulphide and ground state oxygen producing a net overall quenching.

A mechanism involving nucleophilic reaction of the sulphide with oxygen, rather than charge transfer has been suggested.[18],[19],[22],[23],[24],[25],[26],[27],[28]

$$EtSEt \xrightarrow[{^1O_2}]{} EtS(O)Et$$
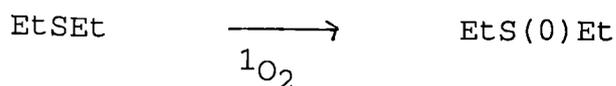
(2) Reaction with Tertiary Amines:

It is believed that tertiary amines quench $^1O_2$ via a charge transfer intermediate. A small spin-orbit coupling between the singlet and triplet states in the charge transfer intermediate may allow a spin flip to occur and hence a facile intersystem crossing from singlet to triplet oxygen may result. e.g. quenching via a series of N,N-dimethylanilines.
[29],[30],[31],[32]

[33]

$$^1O_2 + NR3 \longrightarrow [\; ^1O_2\text{-+}NR3\;] \longrightarrow {}^3O_2 + NR3$$
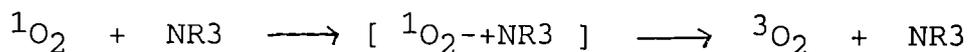
(3) Reaction with amino acids:

The individual amino acids tryptophan ($C_{11}H_{13}N_2O_2$), histidine ($C_6H_9N_3O_2$) and methionine ($C_5H_9SNO_2$) have been found to chemically quench the excited singlet state of molecular oxygen, in methylene blue sensitized photo-oxidation studies.[38],[39] For these amino acids

reaction with ground state O_2 has been reported to be negligible in comparison to that with $^1O_2$.[47]

Tyrosine ($C_9H_{11}NO_3$) has been shown to be much less reactive towards $^1O_2$. A quenching mechanism has been suggested for tyrosine in which a charge separated intermediate breaks down either into an aryloxy radical (ArO·) and the superoxide anion ($O2^-$) or to the phenol (ArOH) and ground state O_2. [52]

The reaction with tryptophan produces N-formyl kynurenine (NFK) which may further sensitize the production of $^1O_2$ through energy transfer from its excited triplet to molecular O_2, or via a Type Two reaction involving electron transfer quenching of NFK by tryptophan. This process gives the NFK radical anion which reacts with molecular oxygen to give the superoxide anion.[40]-[45]

At pH 8.5 the reaction of $^1O_2$ with methionine produces one mole each of methionine sulphoxide and H_2O_2.[41]

The sensitised photooxidation of histidine gives products of cleavage of the imidazole ring; the photodegradation of histidine is extremely complex, inital products have not been isolated but model studies on other imidazoles suggest that the cleavage of the enamine bond is the likely primary pathway, followed by hydrolytic cleavage to give aspartic acid. [49],[50] Other work has suggested that imidazole rings (model for histidine) may undergo ring opening with

the formation of a dicarbonyl compound on reaction with
$^1O_2$. [48] e.g.
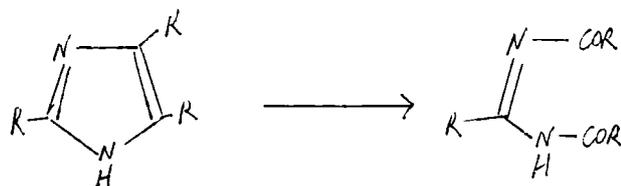
Only the non-ionised ring is photo-oxidisable, while
the unionised form is not. The rate of reaction is
however essentially pH independent. [51]

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2:1 Introduction to the Structure of Wool

2:1:1 Histology of the Wool Fibre

In figure 2:1 below an idealised representation of a wool fibre is shown.
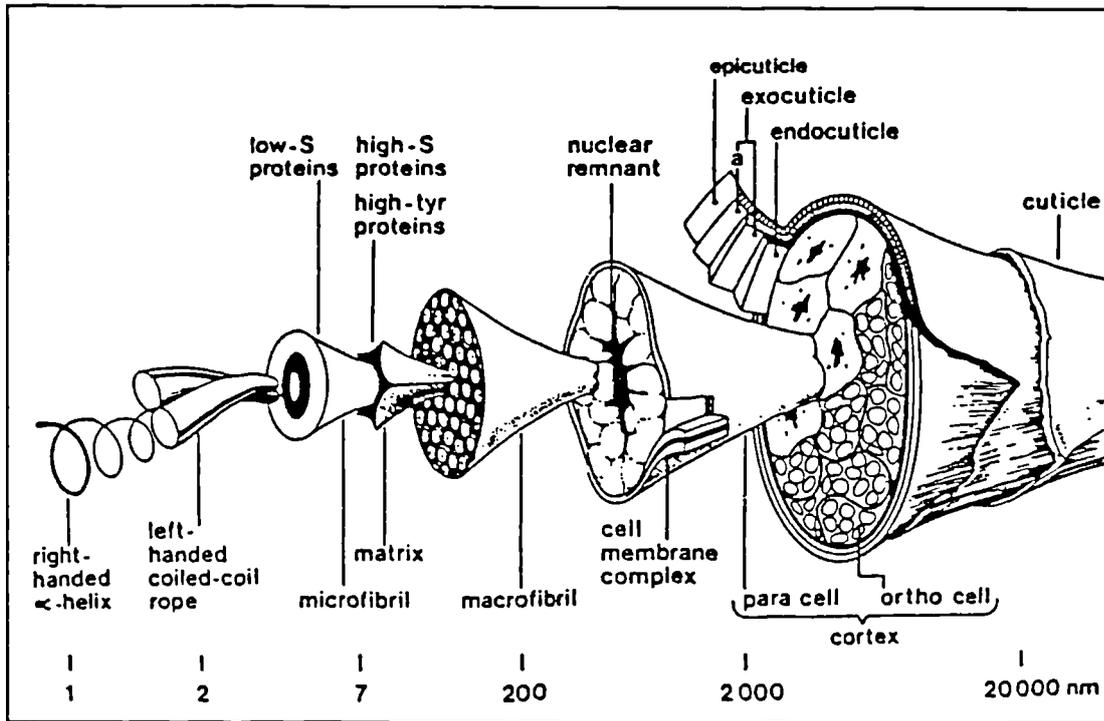
Figure 2:1 The Structure of the Wool Fibre. [1]

A fibre consists of a core of spindle-shaped cortical cells, each approximately 100 μm long and 3-7 μm wide. Surrounding the core of cortical cells is a sheath of flattened overlapping cuticle cells.

Cuticle cells are tilted with respect to the longitudinal fibre axis, and their free edges point towards the tip of the fibre. Generally, the orthocortical cells are covered by only one layer of

slightly overlapping cuticle cells. On the other side
of the fibre the region occupied by the paracortical
cells is covered by layers of overlapped scales which
may be up to three cells thick. [2] Some of the scale
edges do not coincide with true cuticle cell
boundaries. These are called false scale edges and are
thought to be formed by a moulding process during
growth of the fibre. [3]

Individual cuticle cells are approximately 300-700 nm
thick. Internally they consist of two main layers. The
outermost layer exocuticle is composed of highly
crosslinked keratinous protein, which contains
approximately 20 mol% of half cystine. [4]

Along the outer margin of the exocuticle is the A-
layer, the most heavily crosslinked region of the
fibre, containing 33 mol% of half cystine. The
exocuticle extends right around the edges of each scale
cell but it does not appear to be present (or is very
thin) along the underside of each cuticle cell. [5]

The other main component of a cuticle cell is the
endocuticle; it has a low cystine content and
originates largely from non-keratinous material. On the
outer surface of each cuticle cell is the epicuticle.
This is a very thin semipermeable membrane
approximately 3 nm thick. (The F-layer) The
epicuticle is most likely derived from the plasma
membrane that originally surrounded each cell. [6] This

F-layer is believed to be hydrophobic and to contain fatty acid residues. [7],[77]

The β-layers that surround the cuticle and cortical cells are thought to be remnants of lipid components associated with the internal cell membranes. Between the β-layers is the δ-layer or intracellular cement. This layer is irregular and averages approximately eighteen nm in thickness. Its composition appears to consist of proteinaceous material with unusually high proportions of glycine, tyrosine and phenylalanine, and to contain very little cystine. It is presumed therefore to be permeable to dye molecules.

The β- and δ-layers and the resistant membrane constitute the ' cell membrane complex '. Depending on the breed of sheep, three types of cortical cell may be present. These have been designated as orthocortical, mesocortical and paracortical cells.

Cortical cells are composed of macrofibrillar bundles of α-keratin, (see section 2:1:2) microfibrils aligned parallel with the fibre axis and embedded in a matrix of amorphous protein. The basic unit of α-keratin is a polypeptide in the form of a helix. Groups of intermediate filaments are bundled together to form rod-like microfibrils with an apparently annular structure. The microfibrils are closely packed in an amorphous polypeptide matrix.

Ortho, para and meso cortical cells are distinguished by their different arrangements of microfibrils and

macrofibrils. Paracortical cells have been found to be rich in high sulphur proteins while the orthocortex is composed of a high proportion of low sulphur proteins. (8),(9)

2:1:2   Structure of Wool Keratin

Proteins are giant molecules built up by the condensation of comparatively simple α-amino acids. e.g. alanine

$$HOOCCH_2NH_2 \quad + \quad HOOCCH_2NH_2 \quad \longrightarrow$$

$$HOOCCH_2NHOCCH_2NH_2 \quad + \quad H_2O$$
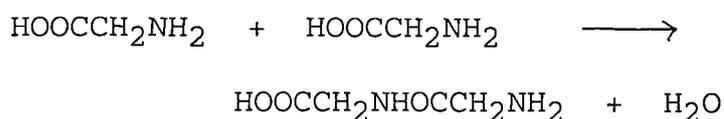
The more complex acids appear as side chains (R) on substitution of one of the hydrogen atoms in the methylene moiety of the main skeleton above.
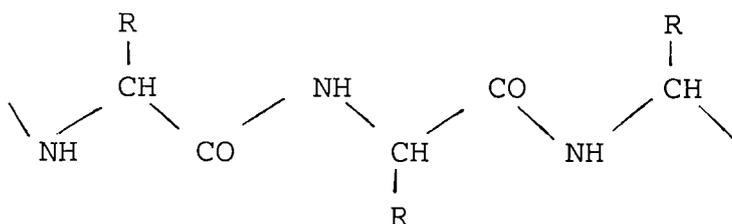
Unstretched and stretched wool fibres show different X-ray diffraction patterns, corresponding to two forms known as α- and β- keratin respectively. Keratin (Greek horn and in) is a generic term applied to structures such as hair, horn, nail and skin. [10] α-keratin consists of an α-helix, in which the

polypeptide backbone is tightly wound around the long
axis of the molecule, while the R groups of the amino
acids protrude outwards from the helical backbone. This
conformation is stabilized via hydrogen bond formation
between every hydrogen atom attached to the
electronegative carbonyl oxygen atom of the fourth
amino acid behind it in the helix. [11] The α-keratins
are rich in amino acids that favour the α-helix
conformation. They are particularly rich in cystine
residues (approximately 500 μmol g-1 in the case of
wool [12]) which can provide inter and intrachenic
crosslinks. The interchenic crosslinks are covalent and
thus are very strong. The covalent crosslinks provided
by the many cystine residues bind adjacent α-helixes
together and give fibres of α-keratin great cohesive
strength. Ionic bonds (salt linkages) may also
originate from interactions between protonated terminal
amine groups and negatively charged carboxyl groups and
those of residues e.g. lysine and glutamic acid. [13]

$$
\begin{array}{c}
\diagup\text{CH(R)}\diagdown \ \diagup\text{CO}\diagdown \ \diagup\text{NH}\diagdown \ \diagup\text{CH(R)}\diagdown \\
\text{NH} \quad \text{CH} \quad \text{CO} \\
(\text{CH}_2)_4 \\
^{+}\text{NH}_3
\end{array}
$$

$$
\begin{array}{c}
^{-}\text{OOC} \\
(\text{CH}_2)_2 \\
\text{NH} \quad \text{CH} \quad \text{CO} \\
\diagdown\text{CH(R)}\diagup \ \diagdown\text{CO}\diagup \ \diagdown\text{NH}\diagup \ \diagdown\text{CH(R)}\diagup
\end{array}
$$

Name	Structure	Per Cent
Alanine	$CH_3CH(NH_2)COOH$	5.5
Arginine	$_2HNCNH(CH_2)_3CH(NH_2)COOH$ $\overset{\|\|}{NH}$	7.1
Aspartic Acid	$HOOCCH_2CH(NH_2)COOH$	6.2
Cystine	$HOOCCH(NH_2)CH_2S-SCH_2CH(NH_2)$ $\overset{\|}{COOH}$	11.0
Cysteine	$HOOCCH(NH_2)CH_2SH$	0.4
Glutamic Acid	$HOOCCH_2CH_2CH(NH_2)COOH$	12.2
Glycine	$CH_2(NH_2)COOH$	5.8
Histidine	$CH_2CH(NH_2)COOH$ (imidazole ring)	0.8
Leucine	$(CH_3)_2CHCH_2CH(NH_2)COOH$	7.6
Lysine	$_2HN(CH_2)_4CH(NH_2)COOH$	2.6
Methionine	$CH_3SCH_2CH_2CH(NH_2)COOH$	0.5
Phenylalanine	(benzene ring)$-CH_2CH(NH_2)COOH$	2.8
Proline	(pyrrolidine ring)$-COOH$	7.5
Serine	$HOCH_2CH(NH_2)COOH$	11.5
Threonine	$CH_3CH(OH)CH(NH_2)COOH$	7.0
Tryptophan	(indole ring)$-CH_2CH(NH_2)COOH$	0.9
Tyrosine	$HO-$(benzene ring)$-CH_2CH(NH_2)COOH$	4.0
Valine	$(CH_3)_2CHCH(NH_2)COOH$	5.6
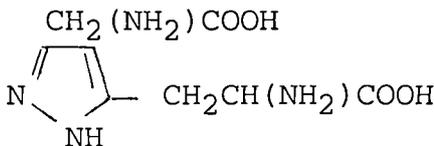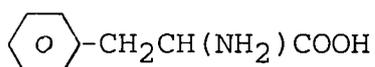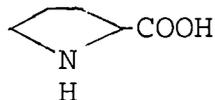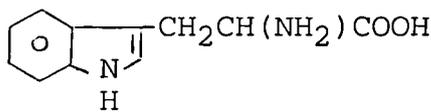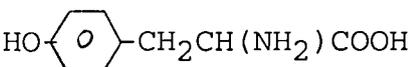
Figure 2:2 Table of the Amino Acid Composition of Wool

On hydrolysis of wool fibre the eighteen amino acids
displayed in figure 2:2 are found. Eight different N-
terminal amino acid residues in wool have been reported
with their total content estimated at < 20 μmol g^{-1}.
[14] Other N-terminal amino acid residues in wool occur
as N-acetyl derivatives e.g. N-acetylalanine
($CH_3CH(NHCOCH_3)COOH$) was identified as the N-terminal
residue of several high sulphur-containing wool
proteins. [15] Approximately 10 μmol g^{-1} of C-terminal
amino acid residues exist.

Chemical treatment of wool may influence the fibre
properties by a) cleaving peptide chains or crosslinks
which bridge them, b) introducing additional
crosslinks, c) altering the properties of ionisable
groups, d) altering the properties of hydrophobic and
hydrophilic groups, e) altering the amount of water
sorbed by the fibre, f) changing the surface properties
of the fibre.

2:2 Introduction to the Fluorescence Emission Properties of Wool Keratin

2:2:1 Fluorescence Emission of Wool

The fluorescence emission spectrum of wool is dependent
on the excitation wavelength, which indicates that one
or more species is responsible for the observed
emission. Emission spectra have been obtained over the
excitation range 265-445 nm, resulting in emission
maxima of approximately 340 nm for 265 nm excitation

and 510 nm for 445 nm excitation respectively. (See
figure 2:3). (16),(78)
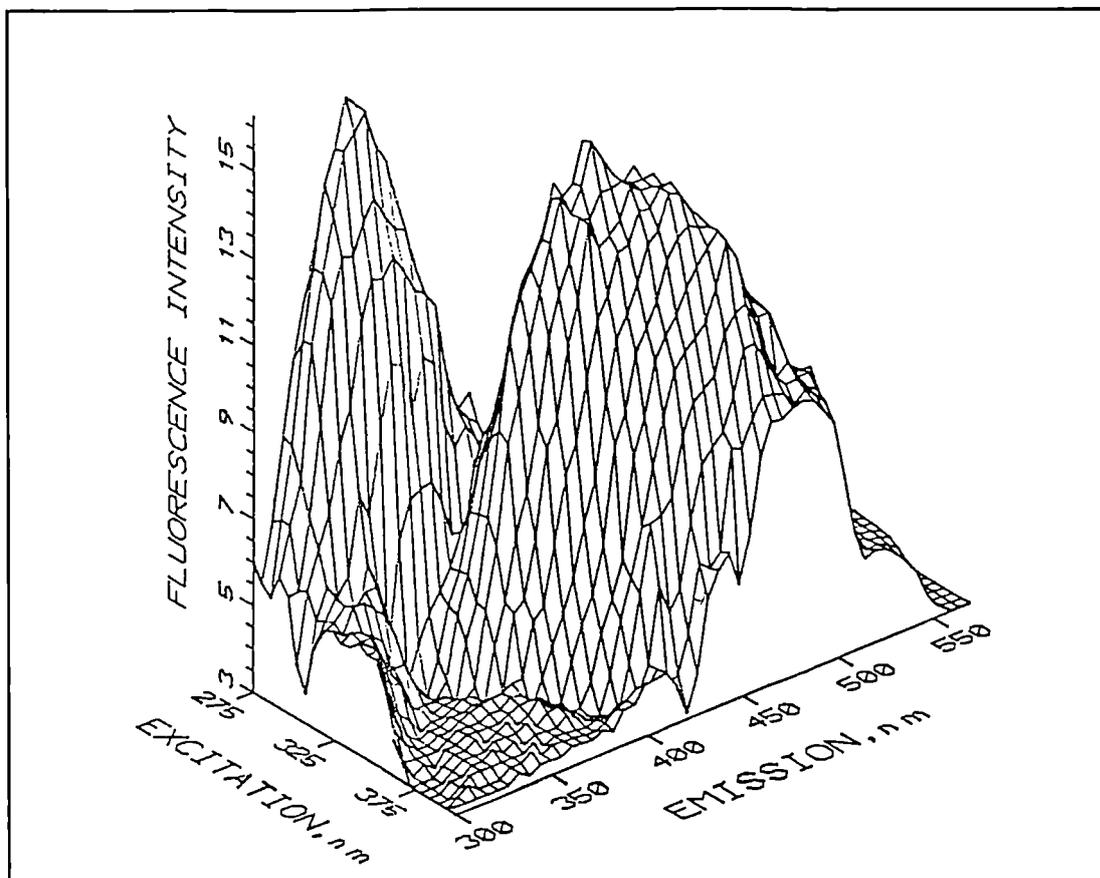
Figure 2:3   3D Plot of Fluorescence Intensity from Wool
in Excitation-Emission Space.

Excitation of wool keratin at 280 nm and 360 nm results

in emission maxima at 335 nm (shoulder at 355 nm) and

450-460 nm respectively. Also apparent is a ridge

extending to longer excitation and emission wavelengths

with a shoulder at an excitation wavelength of 380 nm

and a corresponding emission wavelength of 460 nm.

The fluorophore with a wavelength emission maximum at

335 nm is attributed to the tryptophan residues in

wool. In proteins containing tryptophan and tyrosine

(such as wool), the fluorescence emission spectrum is characteristic of tryptophan to the exclusion of tyrosine, even where it is responsible for most of the absorption at the exciting wavelength. [17] This is due to energy transfer from tyrosine to tryptophan. [18] Reported values of the tryptophan emission maximum have been shown to be different for different proteins. [19] The emission maximum was invariably shifted to shorter wavelengths when tryptophan was incorporated into proteins, as compared to the free amino acid, and previous work has shown that a shift to shorter wavelength occurs with a decrease in solvent polarity. [20],[21] The position of the tryptophan emission maximum at 335 nm in wool is characteristic of emission from a polar environment. [22] Therefore alterations in the environment of the tryptophan residues in a protein would be expected to effect the excitation and emission peaks for this fluorophore. [23]

Different authors have reported that the quantum yield of tryptophan and tyrosine fluorescence is reduced via quenching by the disulphide bond of cystine, and other disulphide-containing compounds. [24]-[29] The nature of the interactions between the disulphide link and the tyrosine and tryptophan singlet levels is not well understood. Cleavage of the cystine disulphide bonds in wool has been reported to cause a marked increase in the ϕ_f for the tryptophan species excited at 280 nm. [25]

The identity of the long wavelength fluorophore(s) in wool keratin is unknown. Previous suggestions have included bityrosine (BT) [30] and photodegradation products of tryptophan, N-formylkynurenine (NFK) [18] and more recently β-carbolines. [16] (See figure 2:4)

Bityrosine has been reported to absorb UV irradiation at a wavelength emission maximum of 350 nm and to emit at 410-430 nm. [30],[31] Previous studies with collagen revealed that bityrosine may be produced on UV irradiation via the combination of transient phenoxy radicals formed by photoelectron ejection from the phenol ring of tyrosine. [32]

N-formylkynurenine is a photo-oxygenation product of tryptophan degradation which has an absorption maxima at approximately 320 nm. Its fluorescence emission properties have been found to differ in micro-environments of different polarity. Previous work by Smith *et al*. [34] has shown that NFK can fluoresce at wavelength emission of 510 nm in non-polar solvents (excitation at 445 nm). The authors concluded that the transition may result from an excited state transfer of the amide hydrogen with the formamido group to the ortho carbonyl group. [34]
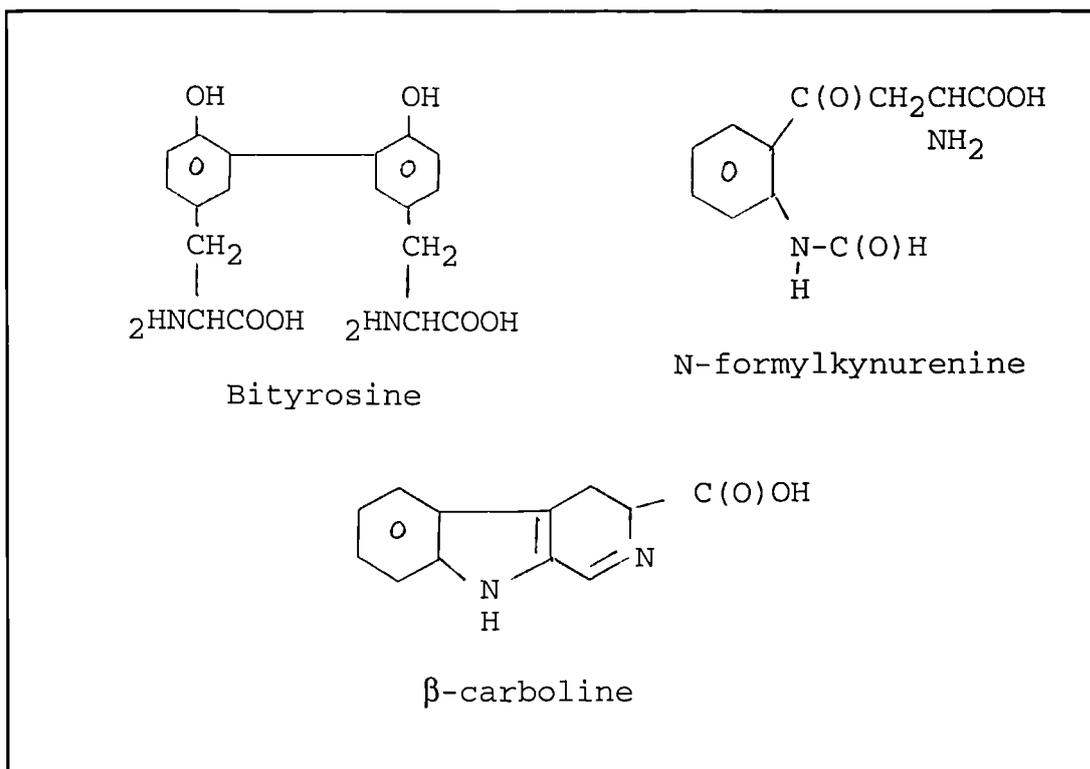
Figure 2:4 Species which have been suggested as responsible for the long wavelength fluorescence of wool.

More recent work by Smith *et al.* [16] has refuted the above claims that either BT or NFK are responsible for the long wavelength fluorescence of wool. They reported that while some of the fluorophores of wool absorbing at 300-350 nm may be tyrosine oligomers they do not contribute to fluorescence excited at longer wavelength. They also observed that wool which had been reduced with sodium borohydride ($NaBH_4$), showed an increase in its fluorescence intensity on excitation at 370 nm. Under this treatment carbonyl groups are converted to alcohols, implying that carbonyl containing-kynurenines are not important contributors to the long wavelength fluorescence of wool. They

suggest that the species responsible may be initally a
3,4-dihydro-β-carboline, which can be formed in some
proteins via anaerobic photolysis of tryptophan. [34]
They are unstable and may be oxidised to the fully
aromatic β-carbolines which fluoresce in the blue
region of the spectrum.

Irradation of wool with UV light (wavelength emission
maximum 300 nm) has been reported to cause a decrease
in the intensity of the tryptophan fluorophore
(excited at 290 nm, emission at 335 nm). Complete
destruction of the fluorophore occurs after
approximately 24 hours of irradiation, in the wet
state. The longwave emitter(s) excited at 360 nm which
emit at a maximum of 430 nm remain unchanged. [35]
Irradiation of wool with longer wavelength blue
radiation results in a decrease in the intensity of
this long wavelength emitter. [16]

2:3 Introduction to the Photoyellowing Properties of Wool Keratin

2:3:1 The Photoyellowing of Wool

Early work has shown that two changes occur on exposure
of wool to direct sunlight; yellowing is produced by
the short wavelength component of light < 320 nm and
bleaching by longer wavelength radiation, with maximum
bleaching occurring at wavelengths of 450-470 nm.
[36],[40] The yellower the wool the greater the

absorption of blue light and the more rapid the
photobleaching action. [41] Factors which accelerate
the photochemical degradation of wool are the
availability of oxygen from the air and the presence of
moisture. If the wool has been chemically bleached or
treated with an F.W.A. the wool is yellowed by a much
wider wavelength band and the rate and extent of
photoyellowing is increased.

Amino acid analysis of wet wool following irradiation
has revealed that destruction of cystyl, tryptophan,
tyrosine and histidine residues accompanies
photoyellowing. [37],[38],[39]

Photochemical reaction with the disulphide bonds of the
cystyl residues appear to be wool's first defence
against photodegradation. Homolytic fission of the
disulphide and carbon-sulphur bonds has been observed
[42]-[47] and thiols and oxidation products including
cysteic acid have been isolated on irradiation in the
presence of air. [48],[49] The photolysis of cystyl
residues occurs not only as a result of energy
absorption by disulphide linkages, but also by
sensitization via the aromatic residues tryptophan and
tyrosine. [50],[51],[52],[53] Flash photolytic studies
of indole and phenol have proved that an electron may
be ejected from excited tryptophan or tyrosine yielding
a hydrated electron which can reduce cystine. [54]-[60]
However, silk which contains only a small amount of
cystyl residues, yellows just as readily as wool during

exposure to UV or sunlight, so it is unlikely that cystyl residues have a role to play in the photoyellowing of wool. [61]

The products of histidine degradation are unknown but these residues may participate in photoyellowing.

The course of photoyellowing closely follows the rate of tryptophan destruction. Previous work by Nicholls and Pailthorpe has shown that in the presence of oxygen the triplet state of tryptophan is rapidly quenched by O_2 to give the excited singlet state of oxygen $^1O_2$. [61] $^1O_2$ then diffuses through the wool keratin to react with tryptophan (to give coloured products) and other amino acids. [62] (See section 1:3:3)

Direct photolysis of tryptophan in the presence of O_2 gives at least nine degradation products. NFK, kynurenine and 3a-hydroperoxypyrrolindole have been isolated. [62]-[69] Nakagawa *et al*. [70] have proposed that the short lived intermediate indolenine hydroperoxide, collapses to form the yellow species NFK via a dioxetane intermediate, according to scheme one.

Tryptophan

Indolenine hydroperoxide

3a-hydroperoxypyrrolindole

3a-hydroxypyrrolindole

NFK

Kynurenine
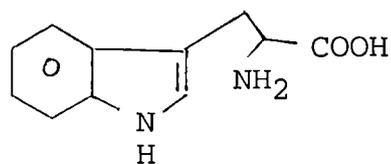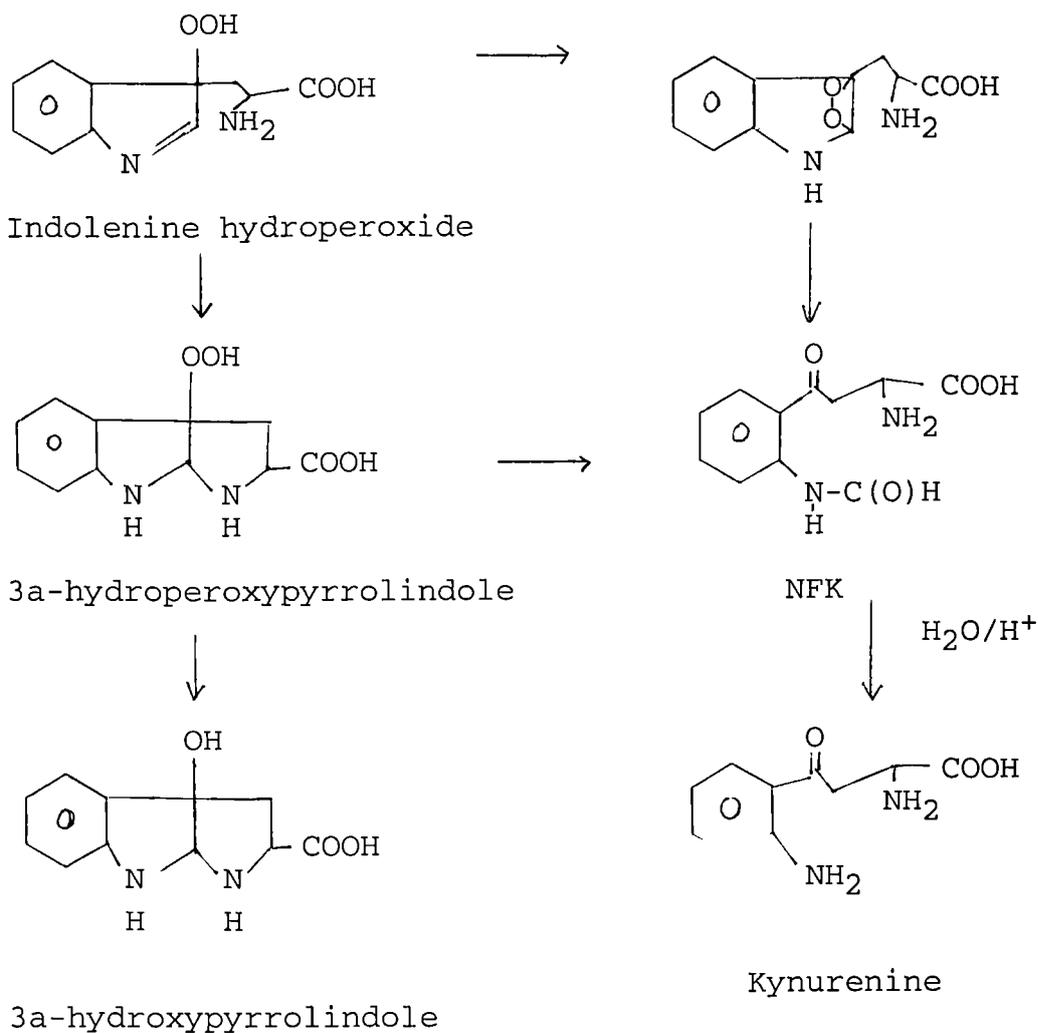
Scheme One

A further source of photoyellowing may be due to the production of melanin (polymers of high molecular weight [9]) in the wool fibre. [71] Melanin may be formed via a tyrosinase catalysed oxidation of side chain tyrosine residues and ring closure to give

dopachrome (2,3-dihydroxy 5,6-di-oxoindole-2-
carboxylic acid). This process is followed by
reduction and decarboxylation to give 5,6-
dihydroxyindole which undergoes oxidative
polymerisation to form melanin, according to scheme
two. [72],[73]
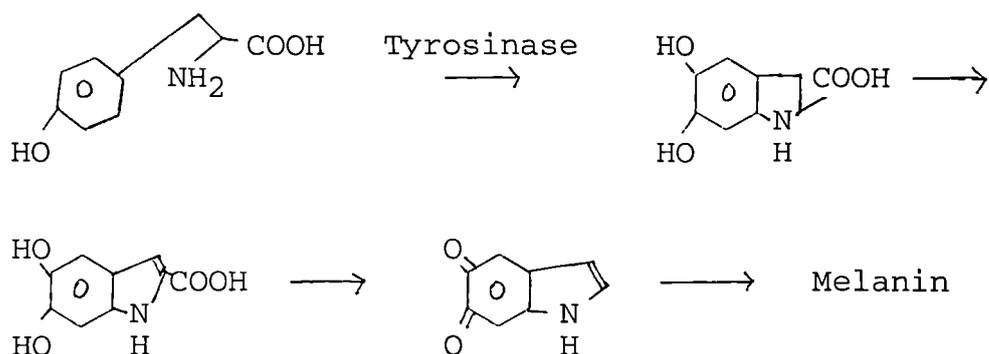
Scheme Two

A third possible cause of photoyellowing may be the
production of highly coloured and reactive quinones via
radical induced phenolic oxidations of tyrosine and its
derivatives such as bityrosine, according to the scheme
below. [74],[75]

o-o'Biphenol

p-quinone
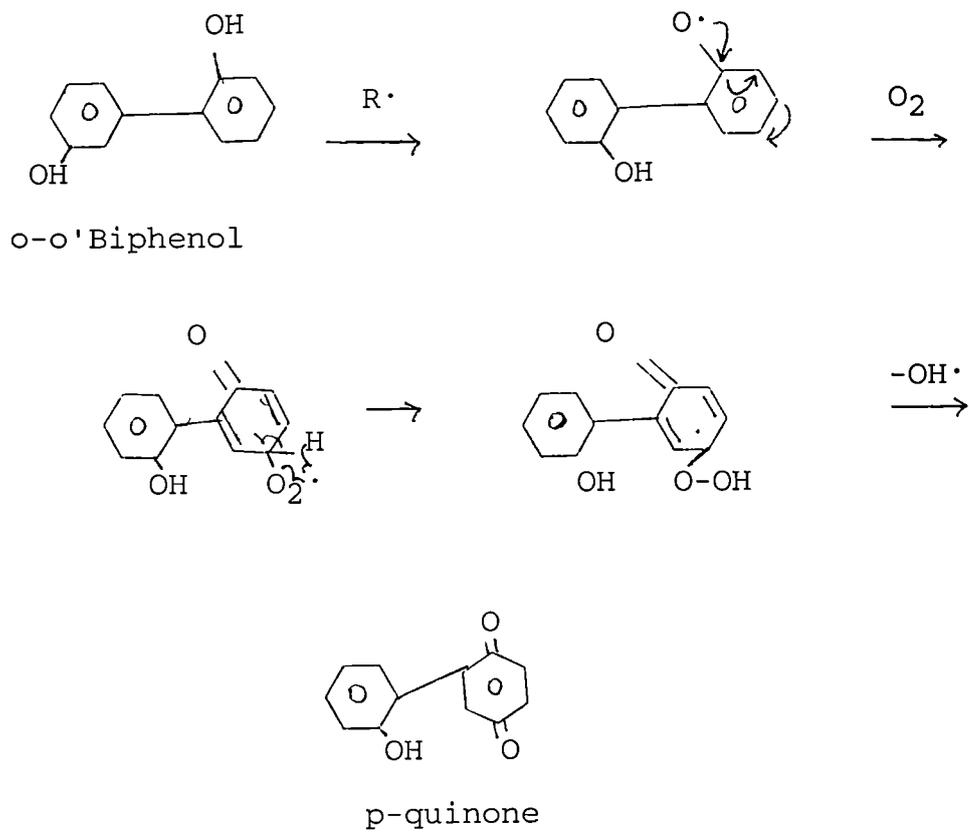
A further suggestion for photoyellowing of wool, namely the production of α-ketoacids by UV initiated radical reactions has been proposed by Meybeck *et al.* [76] (See sec 7:1)

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3:1 Introduction to the Reaction of Tervalent Phosphorus Compounds with Disulphide Bonds.

3:1:1 General Introduction

Phosphorus is a group V element with an electronic structure of [Ne] $3s^2 3p^3$. It may form compounds with valencies of five (PX_5) and three (PX_3) respectively. The bonding in PX_5 compounds is complicated, and it is assumed that with five σ bonds about the central phosphorus atom a fifth atomic orbital must be brought into play. The concept of valence shell expansion i.e. use of a 3d orbital has been proposed, however its inclusion in molecular orbital calculations of PH_5 only had a small effect. (1)

In tervalent PX_3 compounds, phosphorus uses its 3p orbitals for bonding and its non-bonding pair reside in the diffuse and spherically symmetrical 3s orbital. Therefore tervalent phosphorus compounds may behave as both bases and nucleophiles.

Sulphur is a group VI element and has an electronic configuration of [Ne] $3s^2 3p^4$, and like phosphorus is not restricted to an octet of valence electrons. Sulphur can bond to four, five or six atoms.(2) Valence shell expansion theory has been invoked to describe this bonding with the utilisation of 3d orbitals. (3),(4) Bond angles in -S-S- bonds range from 90° to 110° and have been explained by various theories. For some disulphide compounds it has been suggested that an

electron pair on one sulphur atom may be transferred to
a vacant 3d orbital of the sulphur partner, it may be
equally well explained by sp3 orbital hybridisation or
Pauling's suggestion of two unshared electron pairs on
the sulphur atom being assigned to the s and remaining
p orbital. [5]

Phosphorus has a strong affinity for sulphur, as both
elemental and tervalent phosphorus react readily with
sulphur and sulphur-containing compounds. The increase
in size and lower electronegativity of phosphorus leads
to higher nucleophilic reactivity than that of
analogous nitrogen compounds. The size of the
phosphorus atom enables it to utilise the empty 3d
orbital of sulphur more effectively than oxygen or
nitrogen.[6] Sulphur is a 'soft acid' and phosphorus a
'soft base' and a weak adduct may be easily formed
owing to the small inter- electronic repulsion energy.
[1]

In the absence of radical initiators or U.V.
irradiation, the desulphurisation of disulphides by
tervalent phosphorus compounds (phosphines and
phosphites) is generally held to be a bimolecular,
second order, ionic process.

Several acyl and thioacyl disulphides were found to
react with triphenylphosphine ($[C_6H_5]_3P:$) to give the
corresponding monosulphide and triphenylphosphine
sulphide, in non-aqueous solution. [7]

$$R\text{-}S\text{-}S\text{-}R \quad + \quad (C_6H_5)_3P \quad \longrightarrow \quad R\text{-}S\text{-}R \quad + \quad (C_6H_5)_3P\text{=}S$$

The reduction in aqueous methanol of aromatic but not aliphatic disulphides by triphenylphosphine to thiol and triphenylphosphine oxide was first reported by Schonberg in 1949. [8] Challenger and Greenwood later concluded that reduction occurs only in aqueous media and that water must be involved in the reaction. [9]

$$R\text{-}S\text{-}S\text{-}R \quad + \quad (C_6H_5)_3P \quad + \quad H_2O \longrightarrow 2R\text{-}S\text{-}H \quad + \quad (C_6H_5)_3P\text{=}O$$
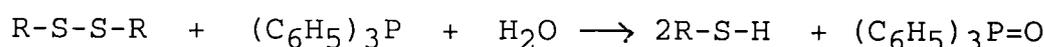
Using phosphine as the tervalent phosphorus compound, the authors Sweetman and McLaren proposed a mechanism for the above reaction. [10] An ionic phosphonium intermediate was suggested, formed by nucleophilic attack at a sulphur atom by the phosphine, involving expansion of the valence shell of sulphur. A further nucleophilic displacement follows to give the expected products.

$$RSSR + PR_3 + H_2O \longrightarrow R\text{-}S\text{-}S\text{-}R + H^+ \longrightarrow 2RS^- + 2H^+ + R_3P\text{=}O$$
$$\overset{+}{P}R_3 \quad {}^-OH$$
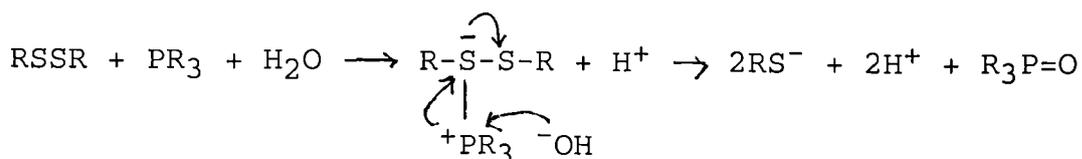
The reaction of sulphur, S_8, with triphenylphosphine has been shown by Bartlett and Meguerian in 1952 [11] and Bartlett, Cox and Davis in 1961, [12] to have a second order rate constant which is increased in ionising solvents and by the presence of electron-withdrawing groups on the phosphine, indicating successive nucleophilic displacements on sulphur. The sulphur ring is opened to a dipolar ion which then

reacts rapidly in a series of follow up reactions with more triphenylphosphine to form triphenylphosphine sulphide.

$$(C_6H_5)_3P + (C_6H_5)_3P^+SSSSSSSS^- \longrightarrow (C_6H_5)_3P^+SSSSSSS^-$$
$$+ \quad (C_6H_5)_3P=S \quad etc,$$
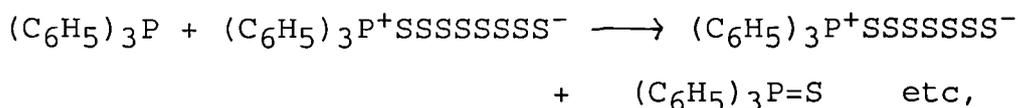
Moore and Trego [13] reported that contrasting with the unreactivity of saturated alkyl disulphides, the ability of triphenylphosphine to desulphurise dialkenyl and alkenyl-alkyl disulphides to the corresponding monosulphide is associated with the observed rearrangement of an alkenyl group during the conversion. The results are consistent with the triphenylphosphine undergoing a prior nucleophilic attack at sulphur to give an ion-pair or polarised complex which then reacts via an S_N1 mechanism as exemplified by the following reaction.

$$Me_2C=CH-CHMe \longrightarrow Me_2C=CH-CHMe$$

$$S-S + :P(C_6H_5)_3 \qquad ^-S....S \quad ^+:P(C_6H_5)_3 \longrightarrow$$

$$Et \qquad\qquad Et$$

$$Me_2C-CH=CHMe + (C_6H_5)_3P=S$$

$$S-Et$$
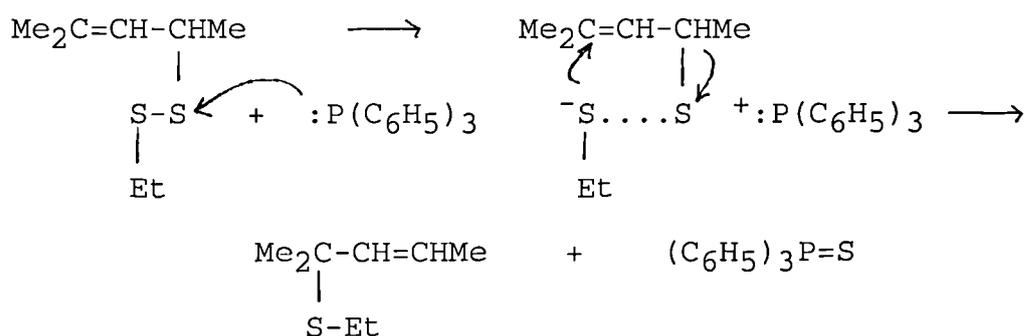
The reaction of tetrakishydroxymethyl-phosphonium chloride with the cystine linkages in wool in aqueous solution to give thiol and the phosphine oxide is also believed to occur via an ionic mechanism. [14],[15] The active tervalent phosphorus-containing species is

reported to be trishydroxymethylphosphine (THP) which
is liberated from tetrakishydroxymethyl-phosphonium
chloride in the following reaction. [16]

$$(HOCH_2)_4P^+Cl^- \longrightarrow CH_2O + HCl + :P(CH_2OH)_3$$
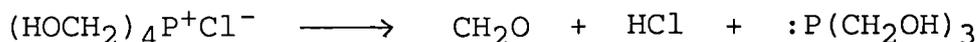
The authors Harrap et al. [19] reported on the smooth
desulphurisation of a wide variety of disulphides and
sulphonates [17] including cystine [18] on reaction
with tervalent tris(diethylamino)phosphine $(Et_2N)_3P$
without rearrangement. e.g.
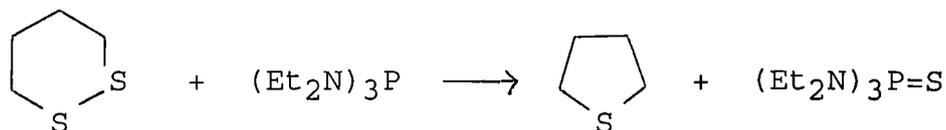
An ionically charged intermediate was proposed for
these reactions. [20] Interestingly, the products of
the reactions of triphenylphosphine and
tris(diethylamino)phosphine with dibenzyltrisulphide
were compared. [21] The triphenylphosphine reaction
extruded the central sulphur atom, in contrast to the
tris(diethylamino)phosphine reaction which extruded
mainly the terminal sulphur atom. The authors concluded
that the mode of desulphurisation is highly dependent
on the type of phosphine used.

In contrast to the reaction of triphenylphosphine with
aliphatic disulphides the reactions of triethyl
phosphite with diethyl disulphide [22] and n-propyl
disulphide [23] were found to produce

triethylmonothiophosphate and the monosulphide in
excellent yield. An ionic mechanism was proposed for
both reactions as indicated by the finding that the
reaction with n-propyl disulphide proceeds with
comparable facility in the presence or absence of
hydroquinone.

$$(EtO)_3P: \ + \ Et-S-S-Et \ \longrightarrow \ EtSP=O(OEt)_2 \ + \ Et-S-Et$$
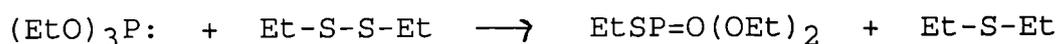
Similar products were reported for the reaction of
trialkylphosphites with dialkyl disulphides at $> 100^{\circ}C$
for 10-40 hours. [24] However, Walling and Rabinowitz
[25] showed that the reaction proceeded at $60^{\circ}C$ by a
free radical mechanism in the presence of free radical
initiators and U.V. irradiation. The following
propagation steps were suggested;

$$RS \cdot \ + \ :P(OEt)_3 \ \longrightarrow \ RS-P \cdot (OEt)_3$$

$$RS-P \cdot (OEt)_3 \ \longrightarrow \ R \cdot \ + \ S=P(OEt)_3$$
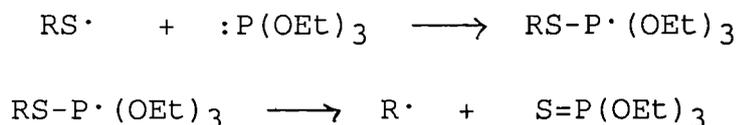
U.V. irradiation does not produce an exclusive free
radical reaction in all cases however, and products of
both ionic and free radical mechanisms were detected on
irradiation of a methanolic solution of methyldiphenyl-
phosphinite containing dibenzyldisulphide. [26]

Purely free radical mechanisms have also been reported.
The addition of sulphur to alkylmethylphosphinate is
inhibited by hydroquinone and it is therefore thought
to react in a predominantly radical manner. [27]

The only species observed on photolysing di-tert-butyl

disulphide in the presence of tri-isopropylphosphine is

the t-butylthiyl radical. [28]

$$[(CH_3)_2CH]_3P \; + \; \cdot SC(CH_3)_3 \longrightarrow [(CH_3)_2CH]_3P=S \; +$$
$$(CH_3)_3C\cdot$$
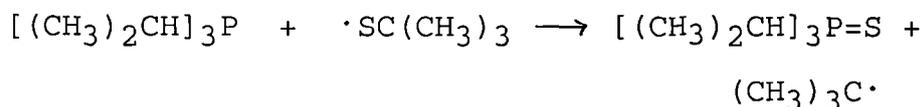
3:1:2 Introduction to the Structures of Hypophosphorous and Phosphorous Acids

According to Walker [29], hypophosphorous acid H_3PO_2

and phosphorous acid H_3PO_3 are monobasic ($pK_1 = 1.1$)

and dibasic ($pK_1 = 1.3$, $pK_2 = 6.7$) acids

respectively. Both acids exist predominantly in the

more stable pentacovalent form (a). e.g.

hypophosphorous acid.

(a)   (b)
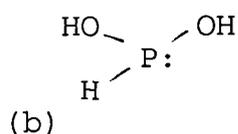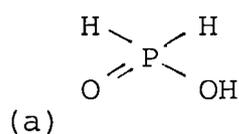
The stability of the pentacovalent form is due to the

high energy of the phosphoryl bond (P=O), which in

turn owes its stability to $p\pi$ - $d\pi$ bonding and

electrostatic factors or both. [30] The equilibrium

constant for the conversion of the pentacovalent into

the tervalent form of hypophosphorous acid has been

estimated from the rates of oxidation to be 10^{-12}. [31]

Their exchange and oxidation mechanisms evidently

proceed by prior enolisation to the tervalent phosphite form. [32]

3:1:2:1 I.R. and [31]P N.M.R. spectra of H3PO2 and H3PO3.

With both phosphorus acids up to six regions of i.r. activity have been correlated with the PO_2H group. [33] Both H_3PO_2 and H_3PO_3 have similar i.r. spectra. [35]

<u>H3PO2:</u> 2650 cm^{-1} v OH, 2410 cm^{-1} v PH_2, 1175 cm^{-1} v P=O, 1160 cm^{-1} v_{as} PO_2^-, 1040 cm^{-1} v_s PO_2^-, 975 cm^{-1} v PO-H, 803 cm^{-1} unassigned.

<u>H3PO3:</u> 2900 cm^{-1} v OH, 2410 cm^{-1} v PH, 1175 cm^{-1} v P=O, 1065 cm^{-1} v_s PO_2^-, 1025 cm^{-1} v_{as} $P(OH)_2$, 935 cm^{-1} v_s $P(OH)_2$.

The band at 803 cm^{-1} in hypophosphorous acid which does not occur in the phosphorous acid spectrum is not assigned in the literature. Present work has indicated that this band disappears on deuteration of the sample and therefore may possibly be due to a PH_2 overtone or a P-OH band.

Phosphorus like hydrogen has a nuclear spin 1/2 and may couple with hydrogen and carbon nuclei. The [31]P N.M.R. spectrum of H_3PO_2 is a triplet (1:2:1), caused by the splitting of the phosphorus atom by two equivalent hydrogens, with chemical shifts 6, 12 and 17 ppm relative to 85% H_3PO_4. [34] The [31]P N.M.R. spectrum of H_3PO_3 is a doublet (1:1), caused by the splitting of the phosphorus atom by one hydrogen, with chemical

shifts of 1 and 9 ppm. Both spectra allow an unambiguous assignment of the pentacovalent form of each acid. [36]

An increase in shielding of the phosphorus nucleus occurs as hydrogens are replaced by hydroxyl groups, the shielding of protons attached to phosphorus is therefore greater in H_3PO_3 than in H_3PO_2. There should be a greater negative charge on the phosphorus atom in H_3PO_3 than in H_3PO_2 making the H_3PO_2 P-H bond less ionic in character. [37]

Present work has shown that on deuteration, the ^{31}P spectra of the acids change considerably. In figure 3:1 the spectrum shows a large 1:2:1 triplet of chemical shift 7, 12 and 18 ppm which has been previously assigned. A doublet of triplets of ratio 1:1:1 is also present. This 1:1:1 triplet is assigned to a P-D coupling, as deuterium has a spin multiplicity of one and will therefore couple with phosphorus in this ratio. This triplet is then split into a doublet by the unexchanged P-H hydrogen. The spectrum infers that only one of the hydrogens directly attached to the phosphorus atom has been exchanged.
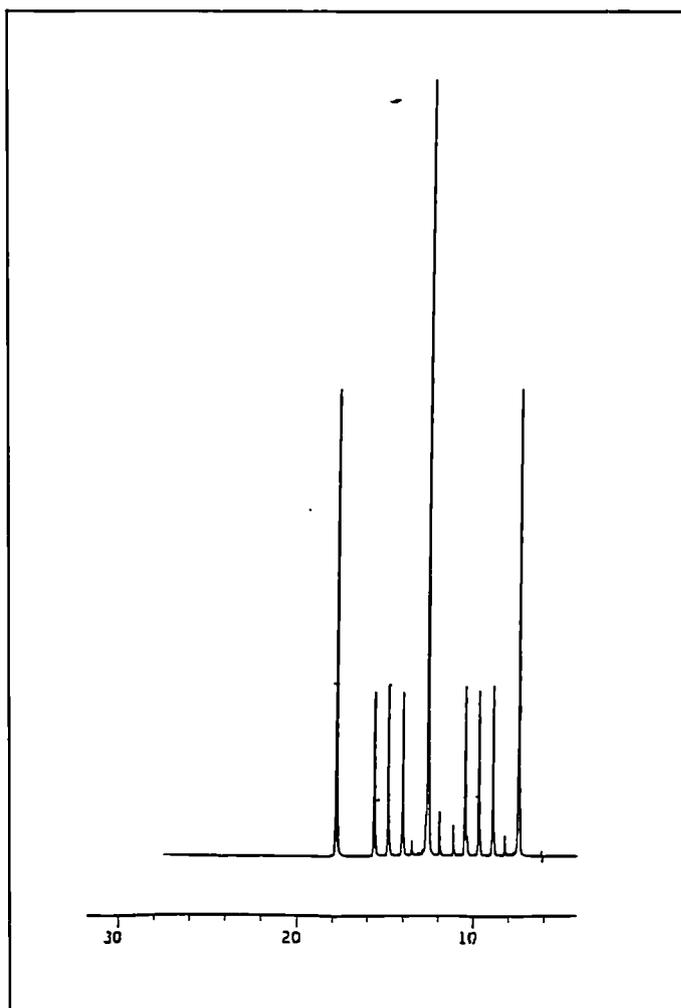
Figure 3:1 ^{31}P N.M.R. spectrum of
H$_3$PO$_2$ in 50% D$_2$O after 24 hours.
Reference 85% H$_3$PO$_4$.

The ^{31}P spectrum of deuterated phosphorous acid in

figure 3:2 shows a 1:1 doublet of chemical shift 1 and

9 ppm previously assigned and one triplet signal of

ratio 1:1:1. The 1:1:1 triplet is assigned to a P-D

coupling which unlike hypophosphorous acid has only one

hydrogen attached to the phosphorus atom and is

therefore not split into a doublet of triplets.
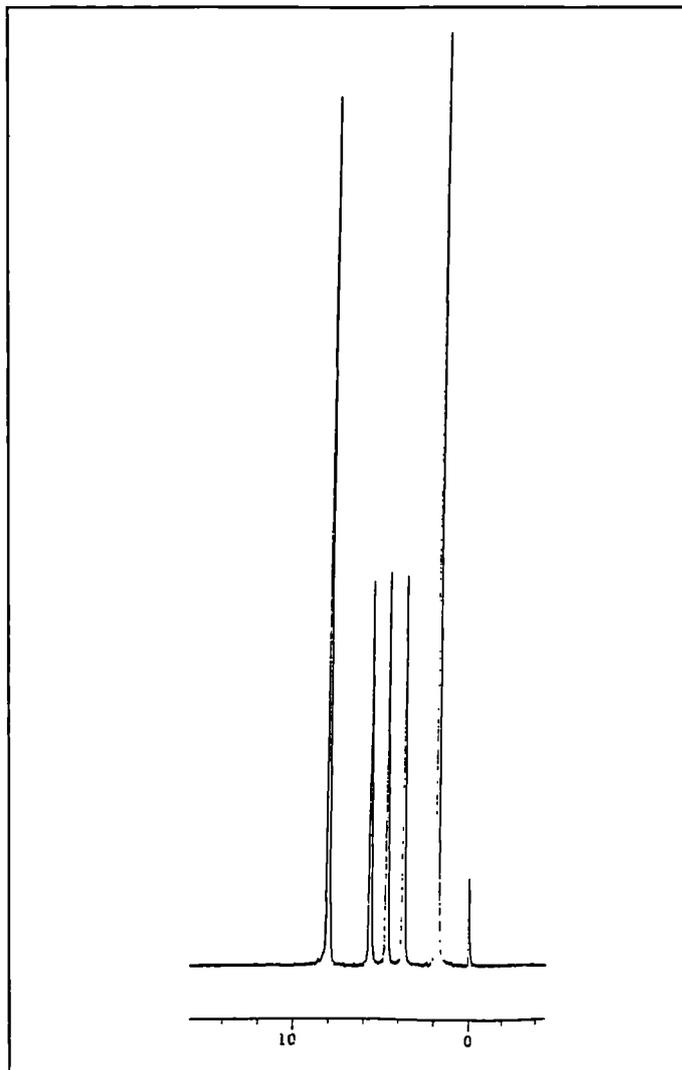
Figure 3:2 ^{31}P N.M.R. spectrum of
H_3PO_3 in D_2O after 24 hours.
Reference 85% H_3PO_4.

The deuterium exchange may be formulated as; (38)
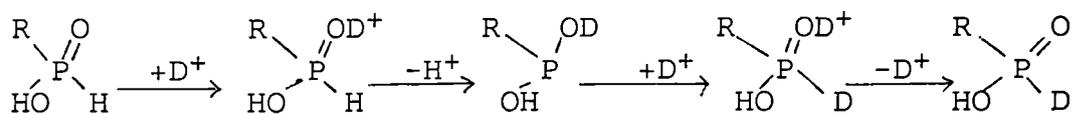
Bailey and Fox suggested that phosphorous and hypophosphorous acid may contain two different types of hydrogen atom with respect to deuterium exchange [39], and also noted that exchange occurred more rapidly with hypophosphorous acid than with phosphorous acid. [40] In fact the rate constant for the abstraction of hydrogen from the two acids has been estimated to be eight times faster for H_3PO_2 relative to H_3PO_3. [71]

A futher difference in reactivities of the two acids was reported by Gunther [49] who found that the reducing ability of H_3PO_2 is greatest in strongly acidic media, and reported that at neutral pH H_3PO_2 is not even oxidised by elemental iodine, in contrast to H_3PO_3 which, under the same conditions gave a quantitative yield of phosphoric acid, H_3PO_4. The reason(s) for this behaviour are unknown.

This view is further supported by Ivanov [61] who reported that both cystine and cysteine were completely desulphurised by H_3PO_3 at pH 8 but that no reaction occurred in acid media.

3:1:3   Introduction to the Reaction of H3PO2 and H3PO3 with Disulphide Bonds in Wool and Model Compounds.

In 1963 Swanepoel and Louw [41], [42] reduced wool and cystine in aqueous solution at elevated temperature (60°C and 85°C) on reaction with unbuffered (0.1 M and 0.5 M) solutions of H_3PO_2 . Disulphide and thiol

(RSH) analyses were then carried out polarographically according to the method of Leach. [43] A stoichiometry of two moles of thiol formed per mole of disulphide reduced was obtained. Further work by Swanepoel and Van Rensburg [44] found that these reactions proceeded at a relatively rapid rate at room temperature if the solutions were subjected to irradiation by U.V. light. Under U.V. irradiation hydrogen sulphide was detected as a reaction product and was determined by the method of Wronski [45] to amount to less than 4% of the total thiol. The authors concluded that as the reaction was promoted by U.V. light, the mechanism was one of free radical scission as outlined below:

$$CySSCy \; + \; h\nu \; \longrightarrow \; 2 \; CyS\cdot$$

$$CyS\cdot \; + \; (H_3O_2)P: \; \longrightarrow \; (H_3O_2)P\dot{S}Cy$$

$$(H_3O_2)P\dot{S}Cy \; + \; CySSCy \; \longrightarrow \; [(H_3O_2)P^+SCyS^-Cy] \; + \; CyS\cdot$$

$$[(H_3O_2)P^+SCyS^-Cy] \; + \; H_2O \; \longrightarrow \; H_3PO_3 \; + \; 2CySH$$
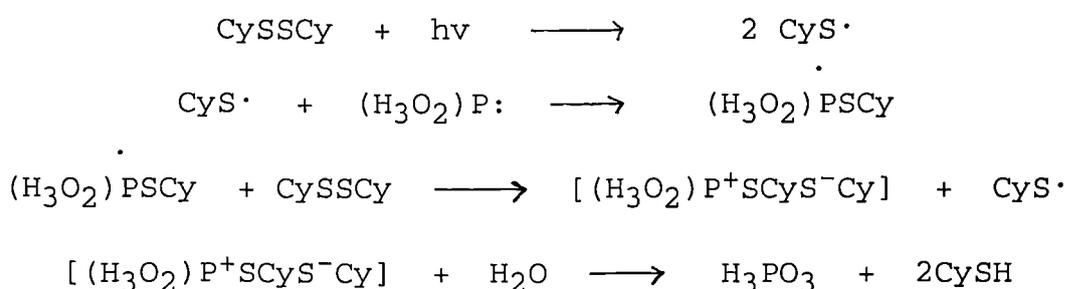
where $Cy = HOOC-CH(NH_2)-CH_2-$ and $(H_3PO_2)P:$ is the enol tautomer of hypophosphorous acid.

In 1969 Swanepoel et al. [46] reported similar findings whereby the disulphide linkages in insulin were partially reduced by irradiation in the presence of hypophosphite.

In the present study the reactions of hypophosphorous acid and phosphorous acid with a number of model disulphides in the absence of U.V. irradiation were investigated. Particular attention was afforded to the production of the gases hydrogen sulphide and sulphur dioxide, and to the total amount of thiol produced by these reactions for the conditions used.

3:2 Investigation into Hydrogen Sulphide (H2S) and Sulphur Dioxide (SO2) Production, on Reaction of H3PO2 and H3PO3 with Wool and Model Compounds.

The chemical change which is first detected when wool is heated in water is the slow liberation of H_2S at $55^{\circ}C$. [47] The maximum rate of production of H_2S and SO_2 is found to occur in acid pH 2-3, (using a mineral acid) and temperature $100^{\circ}C$. At 1 hour the concentrations of H_2S and SO_2 were 22 ppm and 34 ppm respectively. At pH 1-2 and temperature $80^{\circ}C$ the maximum rate of gas production occurs at 20 minutes corresponding to 4 ppm H_2S and 9 ppm SO_2 respectively. The same conditions for an H_2O_2 bleached sample showed a maximum production rate at 30 minutes corresponding to 3 ppm H_2S and 6 ppm SO_2 respectively. [48]

Hydrogen sulphide is also liberated from wool on irradiation with U.V. light. [53] In the presence of air the bulk of H_2S produced is oxidised to sulphuric acid. The rate of evolution of H_2S is increased by the presence of moisture. [54] These reactions are believed

to occur via hydrolysis of the cystinyl disulphide bond.

```
 |              |                    |              |
NH             NH                   NH             NH
 |              |         H2O        |              |
CH-CH2-S-S-CH2-CH    ———→         C=CH2     +    CH-CH2-SSH
 |              |                    |              |
C=O            C=O                  C=O            C=O
 |              |                    |              |
Cystine                  Dehydroalanine    Perthiocysteine
```

```
 |                          |
NH                         NH
 |              H2O         |
CH-CH2-SSH    ———→        CH-CH2-SOH    +   H2S
 |                          |
C=O                        C=O
 |                          |
Perthiocysteine      Alaninesulphenic acid
```
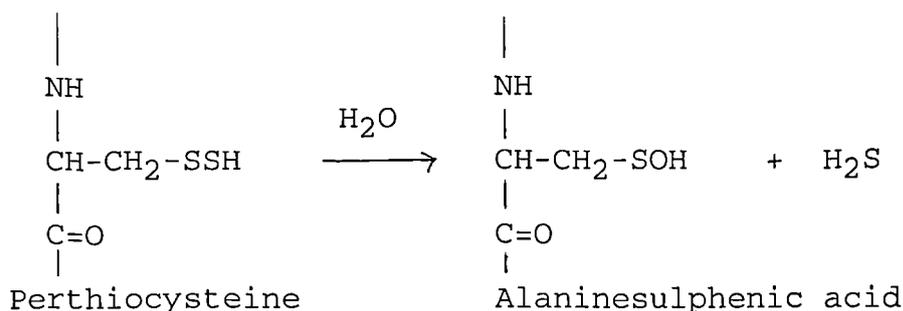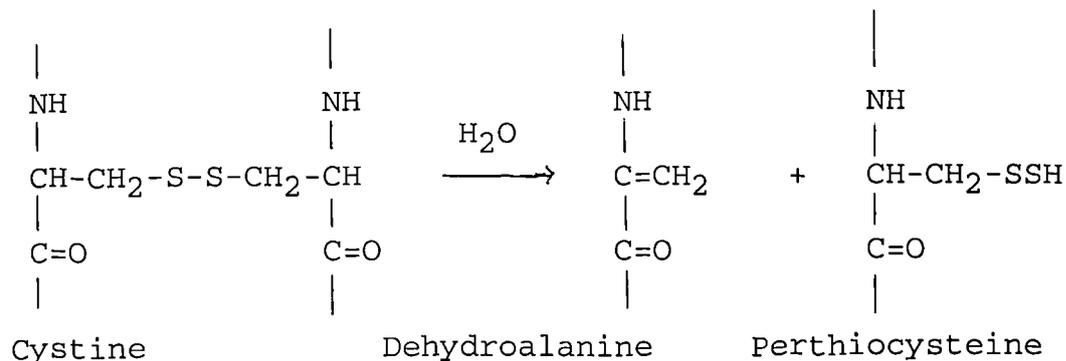
Sulphur dioxide production is generally associated with oxidised cystyl residues in wool, and in acid solution the following reaction is believed to occur.

```
 |                              |
NH                             NH
 |              O     H3O+      |
CH-CH2-S-S-H   ———→          C=CH2   +   SO2   +   H2S
 |            //               |
C=O           O               C=O
 |                             |
```
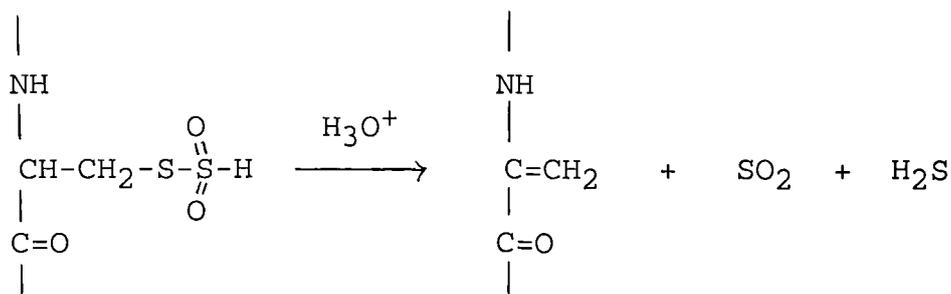
3:2:1 Experimental Conditions

For the reaction of hypophosphorous acid and phosphorous acid with wool, 10g of wool serge was used. The concentration of acid was varied and a temperature of 80°C was used, maintained by a 'Jeffereys' dyeing

machine. All solutions were made up to 250 mls with water. The experiments were carried out under air or under N_2, whose flow rate was maintained at 35-40 mls/min^{-1}.

For the reaction of hypophosphorous acid and phosphorous acid with various model disulphide compounds, $7*10^{-3}$ M of each model compound was used with varying concentration of acid. If the disulphide was not water soluble it was dissolved in the minimum amount of methanol or acetonitrile and this solution was then made up to 250 mls with water. The reactions were carried out under air or N_2 at a temperature of 80^oC.

3:2:2 Experimental System.

A schematic diagram of the system used for determination of H_2S and SO_2 production is shown in figure 3:3. A cylinder of air or N_2 (A) was connected to a 250 ml enclosed test tube (B). This tube contained the reactants and was heated to a constant temperature of 80^oC. The air or N_2 bubbled through the reaction mixture and the gases produced by the reaction were carried to H_2S and SO_2 meters (C). The meters were supplied by City Technology Ltd., a company owned by City University. A soap bubble meter (D) was attached for determination of flow rate.
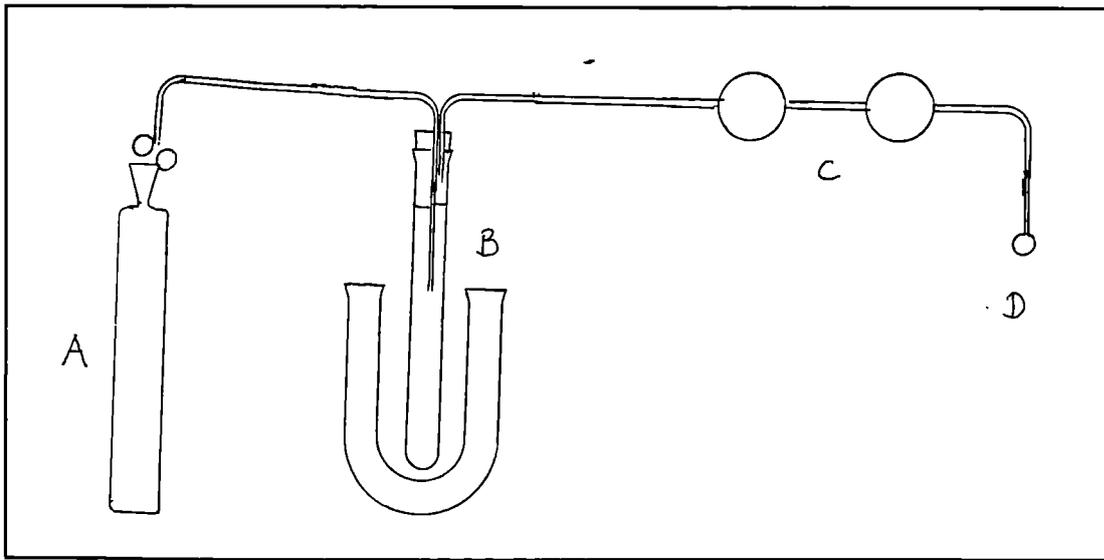
Figure 3:3 A schematic representation of the
experimental system used for H_2S and SO_2 determination.

Reaction No.	Reagents	Conditions	Figure
1	H_3PO_2 + Wool	pH 1, 80°C, Air	3:5
2	H_3PO_3 + Wool	pH 1, 80°C, Air	3:6
3	H_3PO_2 + Cystine	pH 1, 80°C, Air	3:7
4	H_3PO_3 + Cystine	pH 1, 80°C, Air	3:8
5	H_3PO_2 + Wool	pH 1, 80°C, Air, + Hydroquinone	3:9
6	H_3PO_2 + 2-Hydroxy-ethyldisulphide	pH 1, 80°C, Air	3:10
7	H_3PO_2 + Wool	pH 1, 80°C, N_2	3:11
8	H_3PO_2 + Cystine	pH 1, 80°C, N_2	3:12
9	H_3PO_2 + Bleached Wool	pH 1, 80°C, Air	3:13

Figure 3:4 A table of the various reactions of
phosphorus acids and disulphide compounds and the
conditions used.

The table above numbers each reaction, shows the
reagents and conditions used and displays a figure
number which corresponds to one of the following series
of graphs, which depict the amount of H_2S and SO_2
produced by the individual reaction.
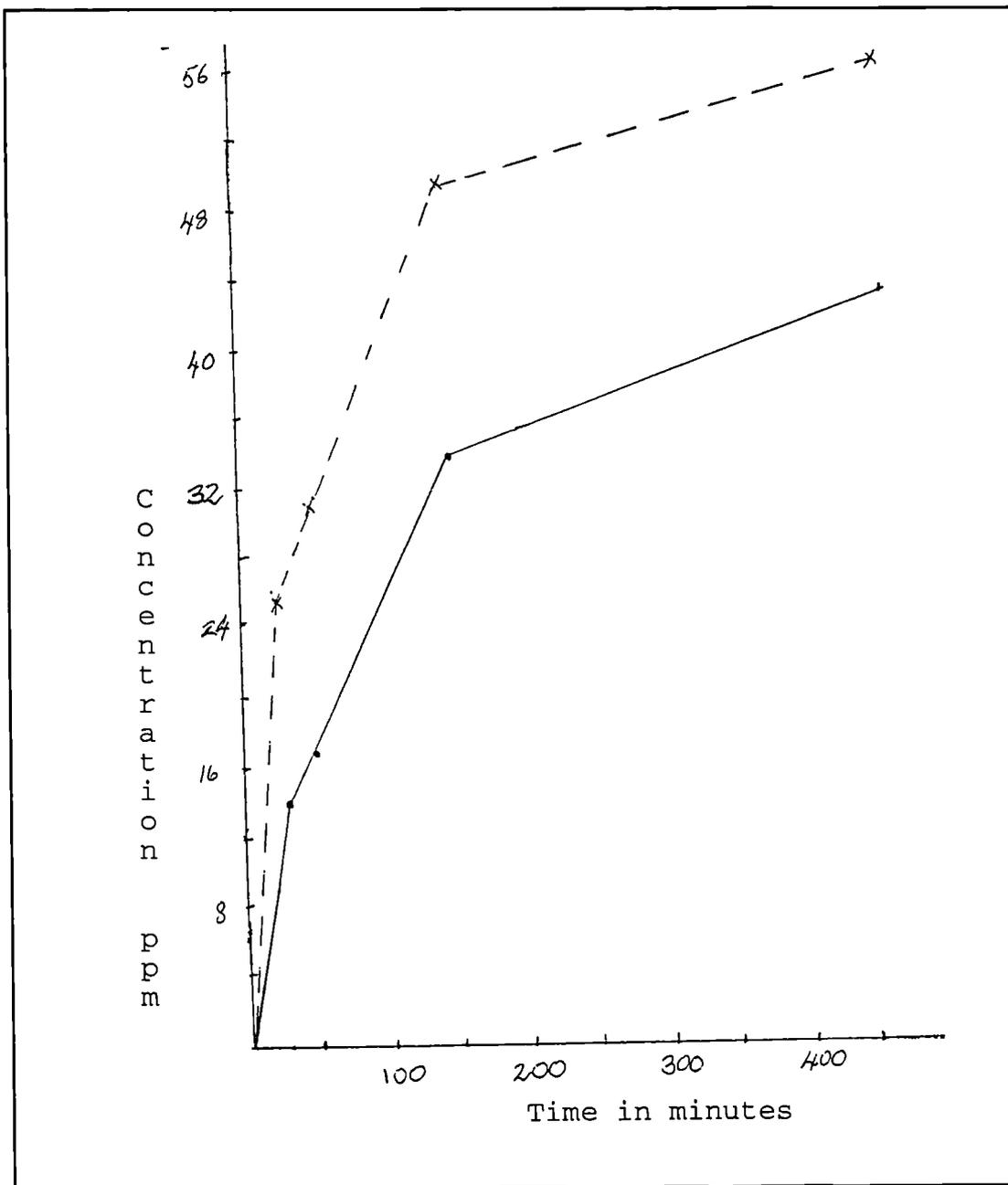
Figure 3:5 Graph of H_2S and SO_2 concentration against time. For the reaction of wool with 2.5 M solution of H_3PO_2, (pH 1 and at 80°C), under air. - - = SO_2 and —— = H_2S.
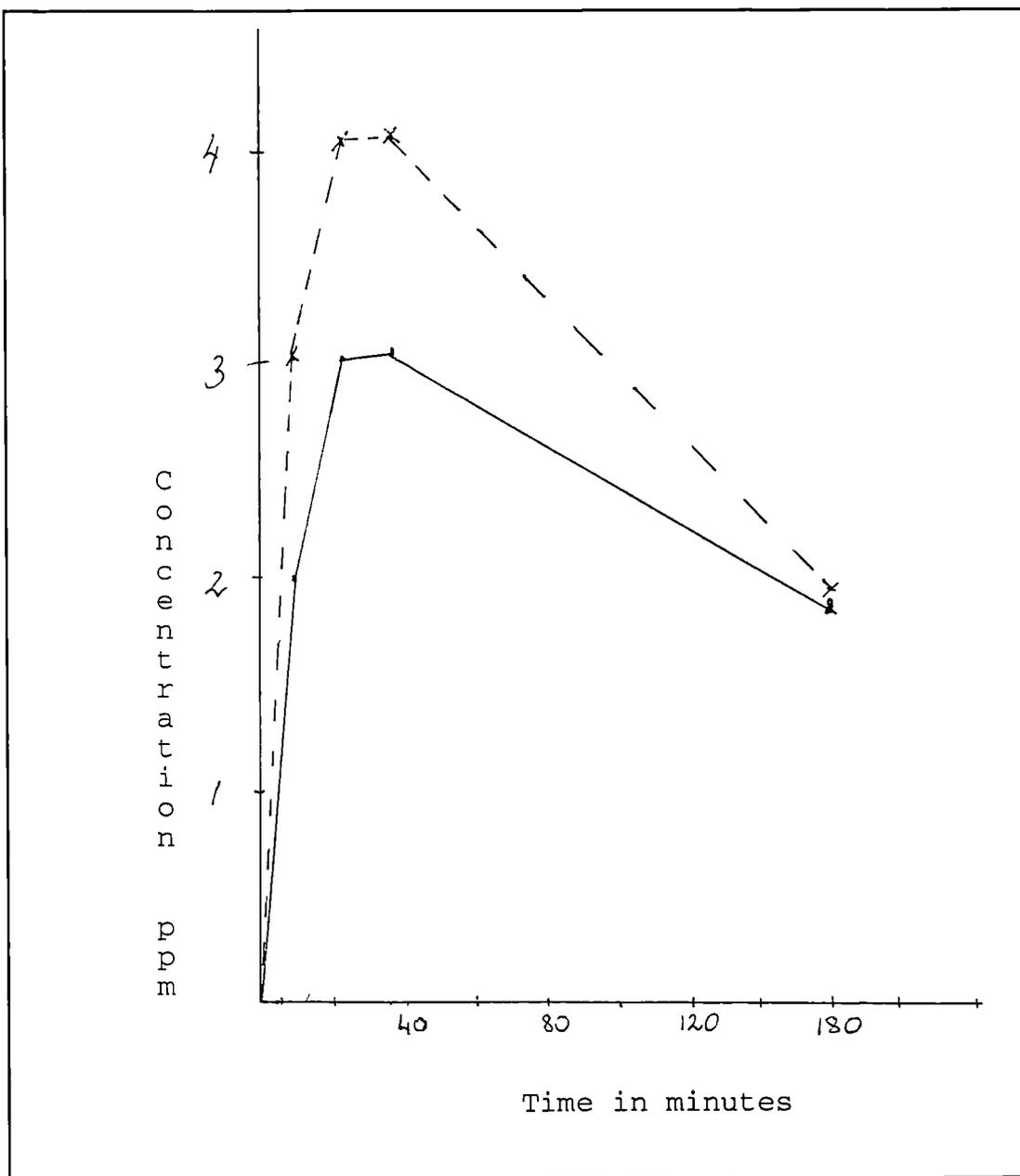
Figure 3:6 Graph of H_2S and SO_2 concentration against time. For the reaction of 2.5 M H_3PO_3 and wool under air.(pH1 and at 80°C).
- - = SO_2 and ——— = H_2S.
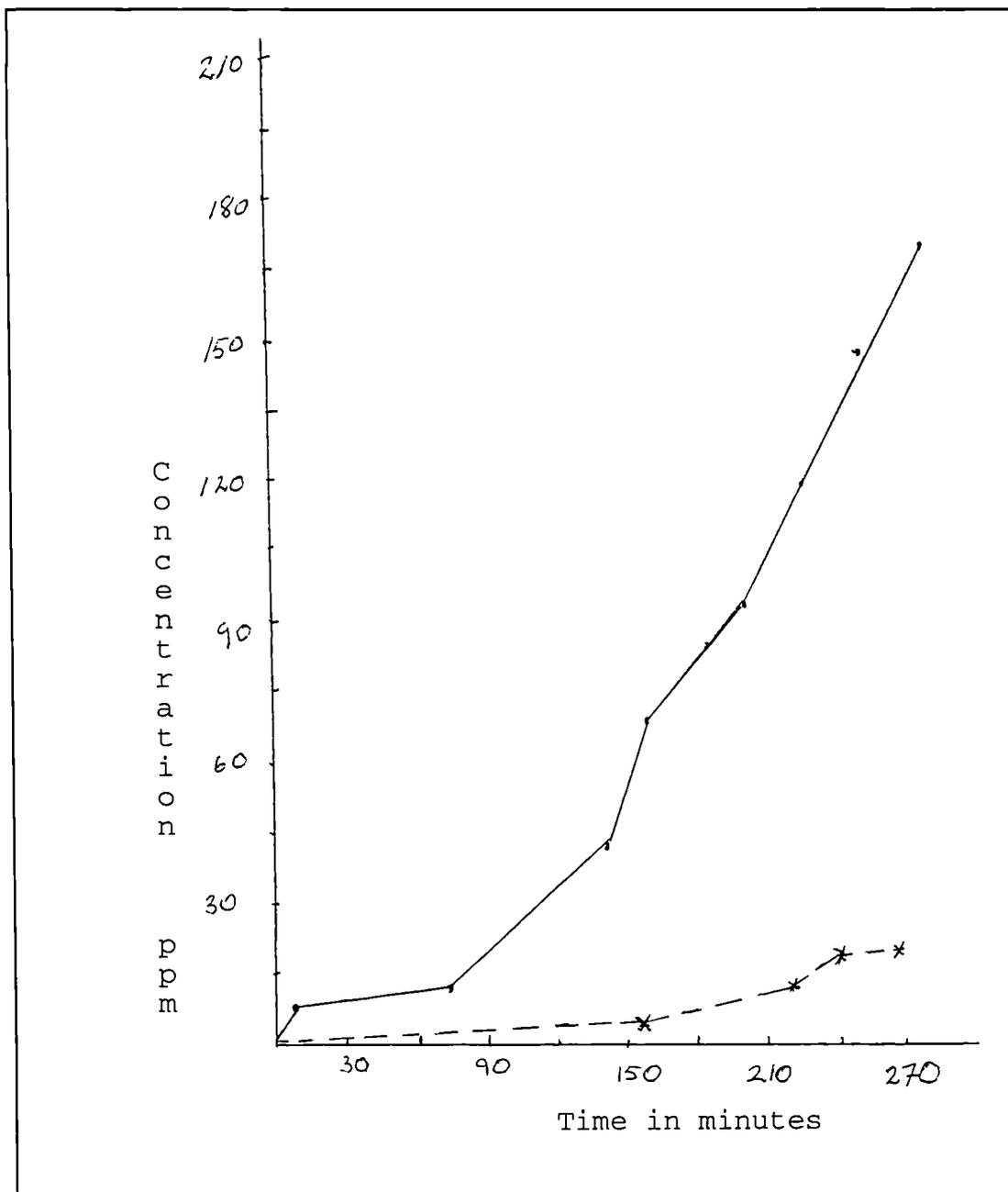
Figure 3:7 Graph of H_2S and SO_2 concentration against time. For the reaction of cystine with 2.5 M H_3PO_2 under air. (pH1 and temperature 80°C).
- - = SO_2 and —— = H_2S.
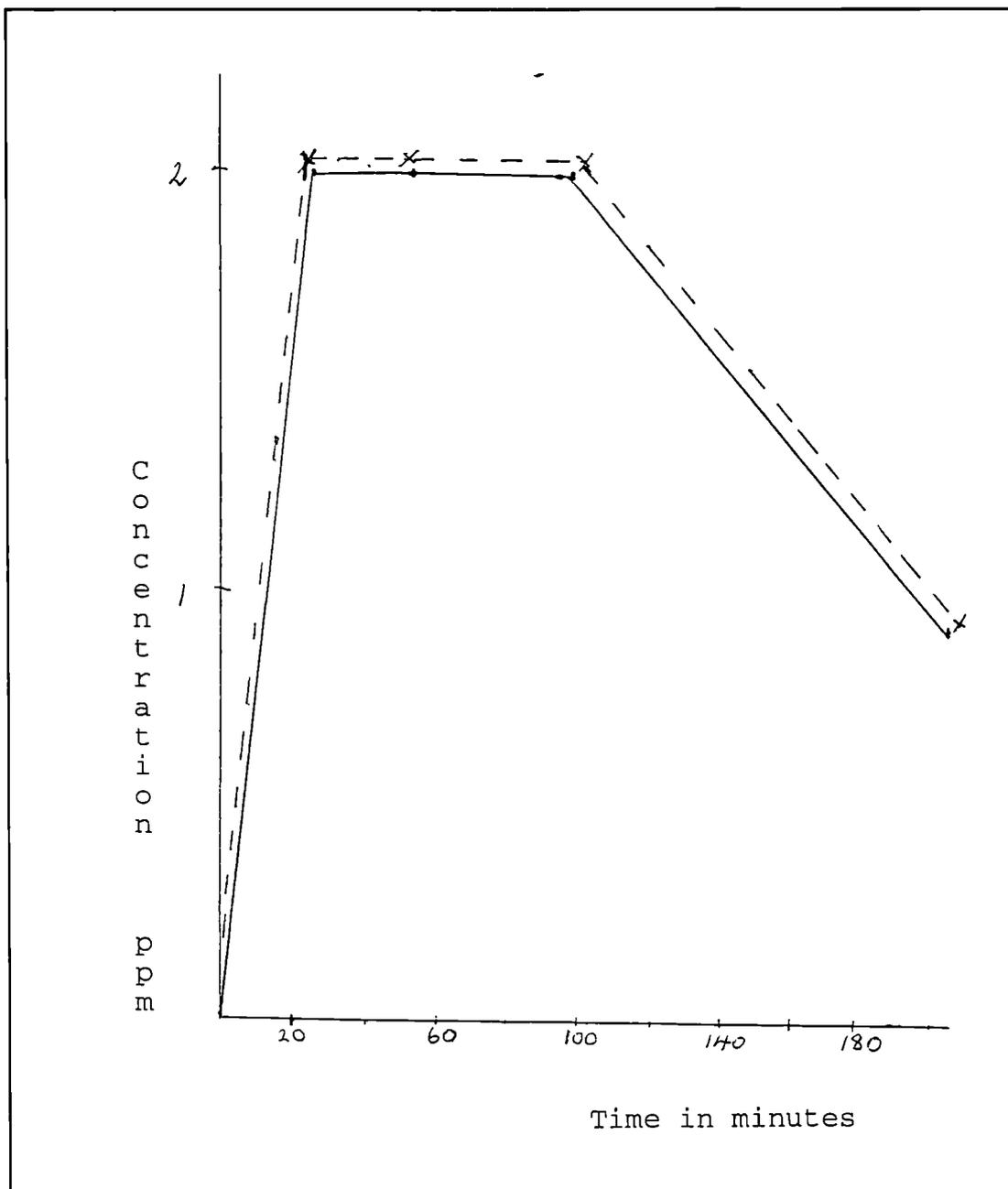
Figure 3:8 Graph of H_2S and SO_2 concentration against time. For the reaction of cystine with 2.5 M H_3PO_3 under air. (pH 1 and temperature $80^{\circ}C$).
- - = SO_2 and —— = H_2S.
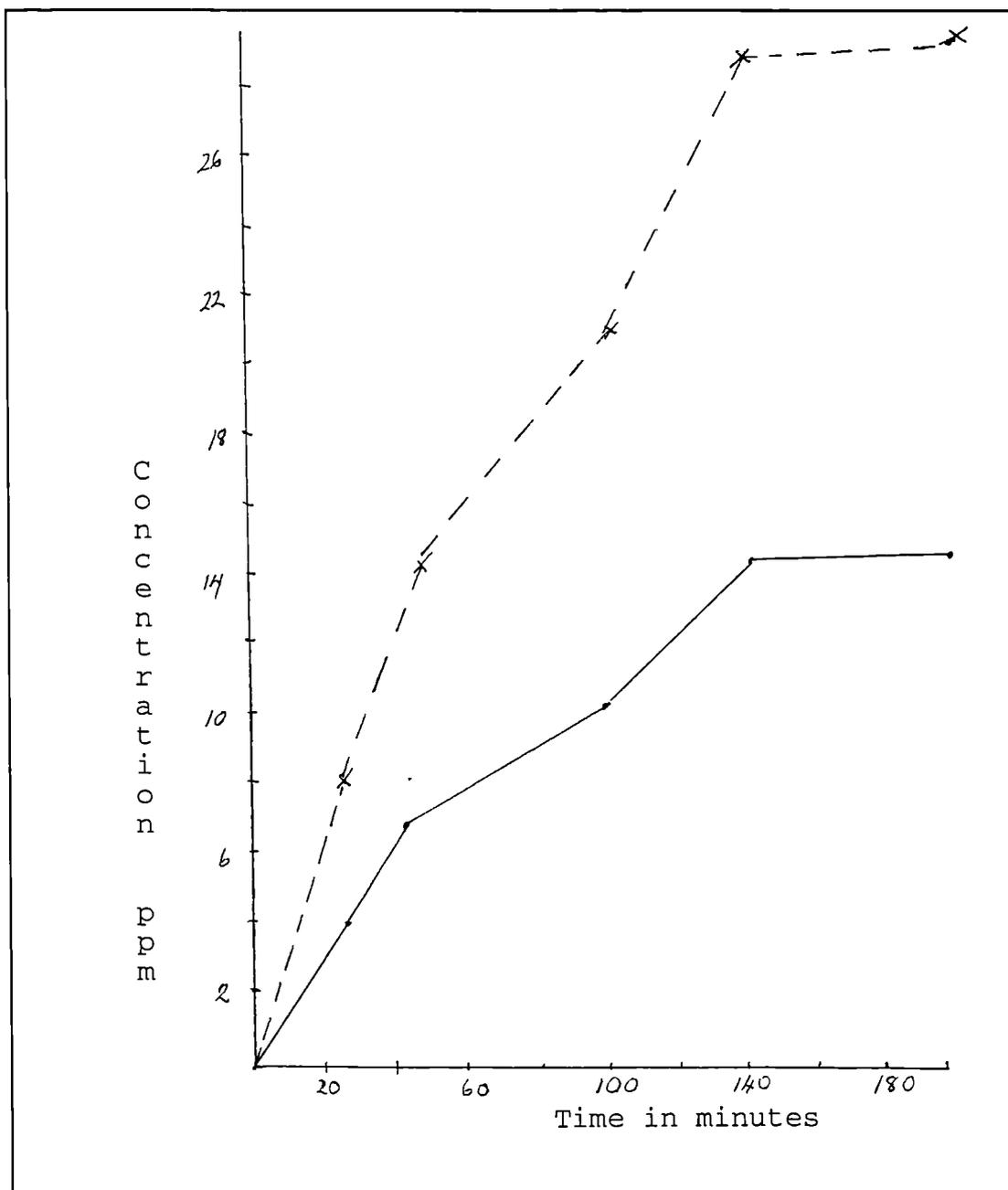
Figure 3:9 Graph of H_2S and SO_2 concentration against time. For the reaction of wool with 2.5 M H_3PO_2 under air in the presence of 0.5 g of hydroquinone. (pH 1 and temperature 80°C).
- - = SO_2 and ── = H_2S.
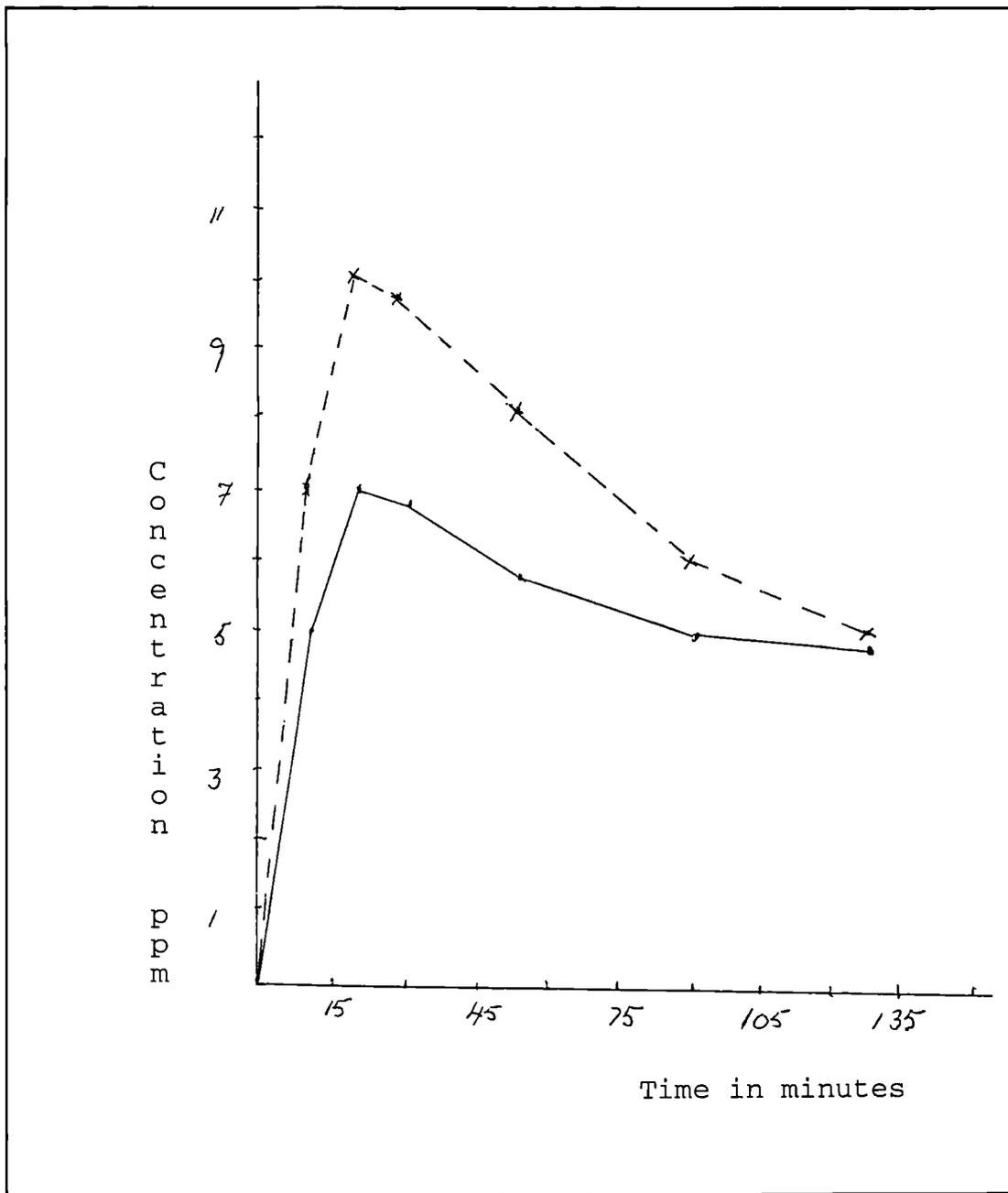
Figure 3:10 Graph of H_2S and SO_2 concentration against time. For the reaction of 2-hydroxyethyl disulphide and 2.5 M H_3PO_2 under air. (pH 1 and temperature 80°C). - - = SO_2 and —— = H_2S.
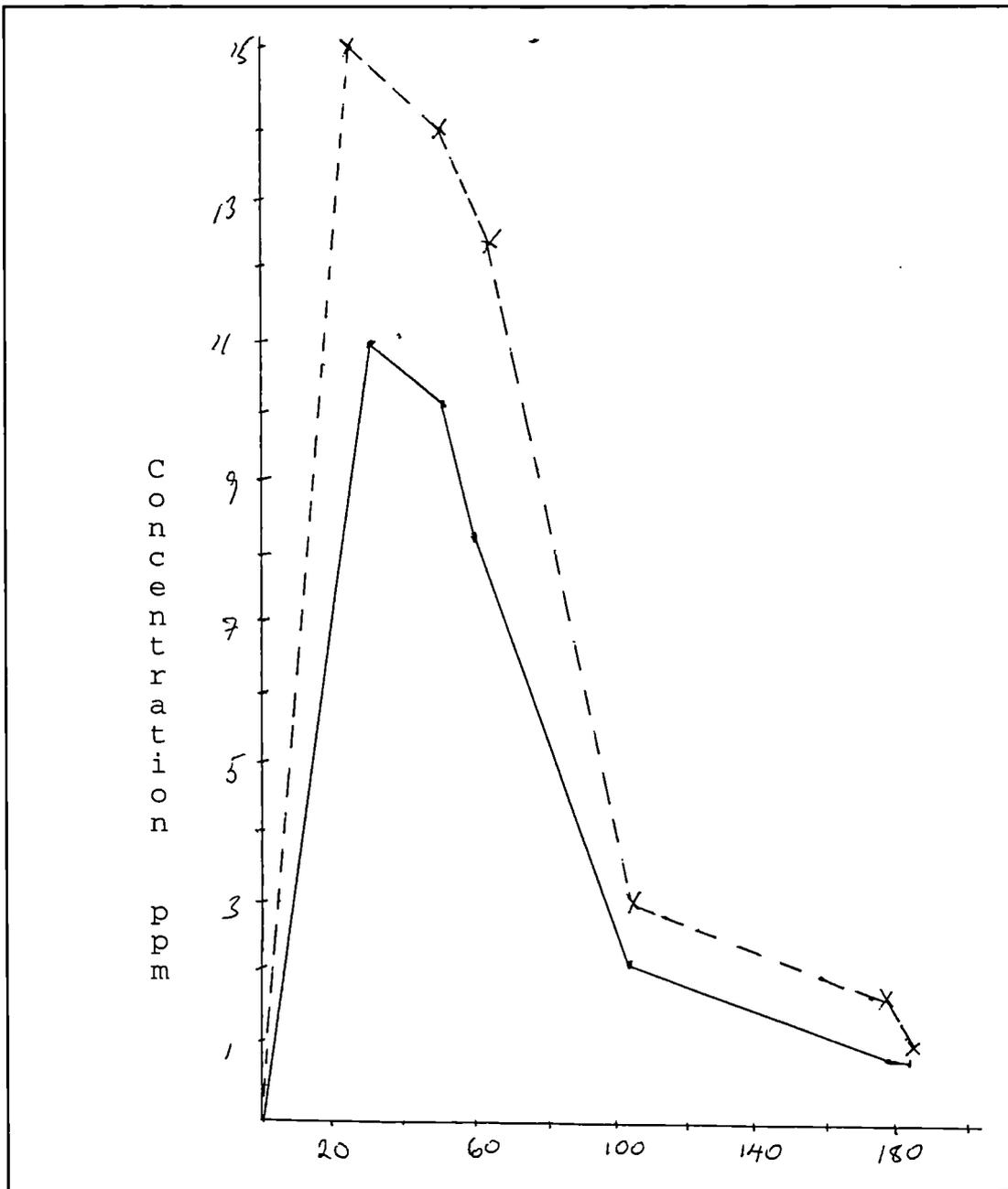
Figure 3:11 Graph of H_2S and SO_2 concentration against time. For the reaction of wool and 2.5 M H_3PO_2 under N_2. (pH 1 and temperature 80°C).
- - = SO_2 and ——— = H_2S.
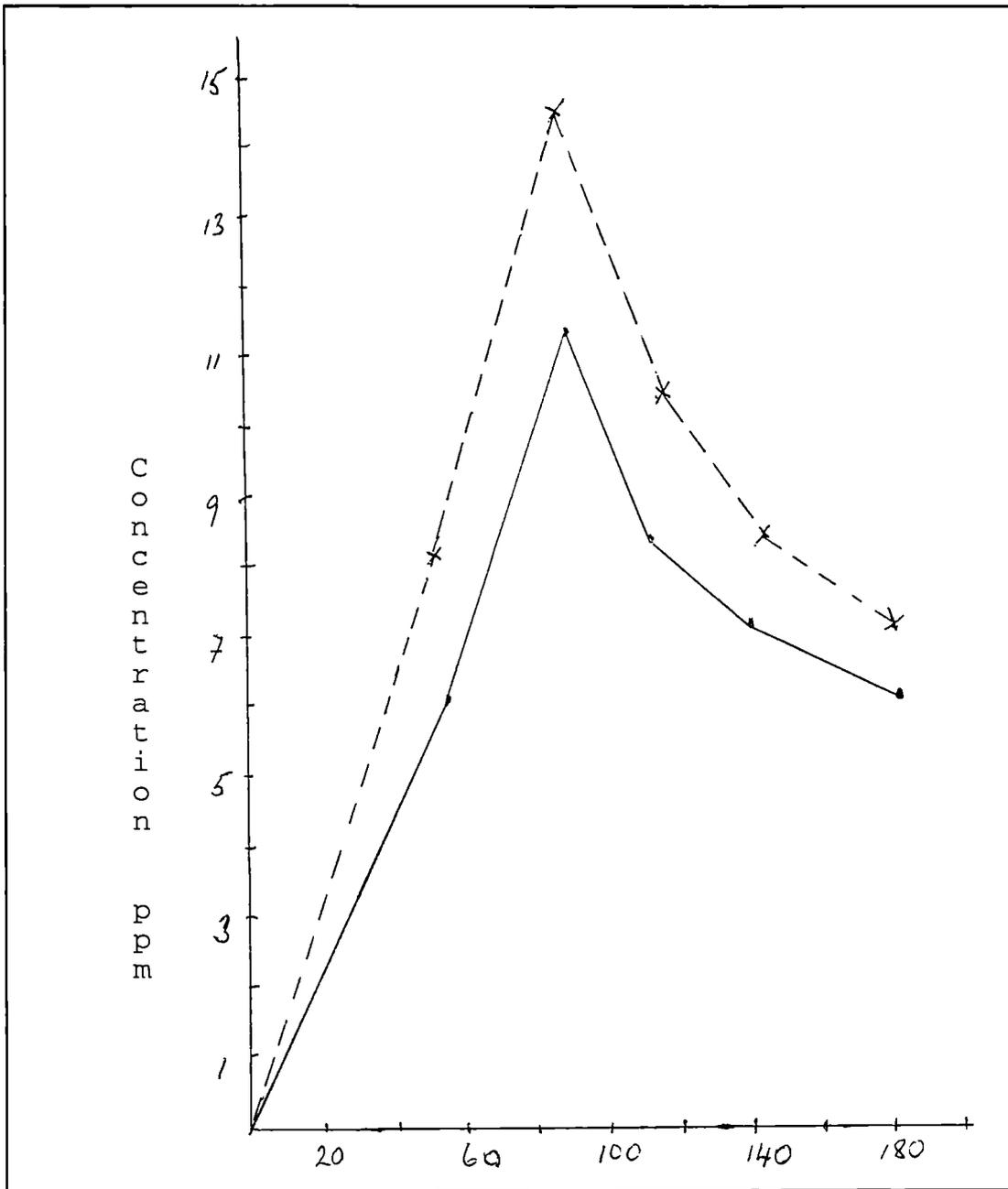
Figure 3:12 Graph of H_2S and SO_2 concentration against time. For the reaction of cystine and 2.5 M H_3PO_2 under N_2. (pH 1 and temperature 80°C).
- - = SO_2 and —— = H_2S.
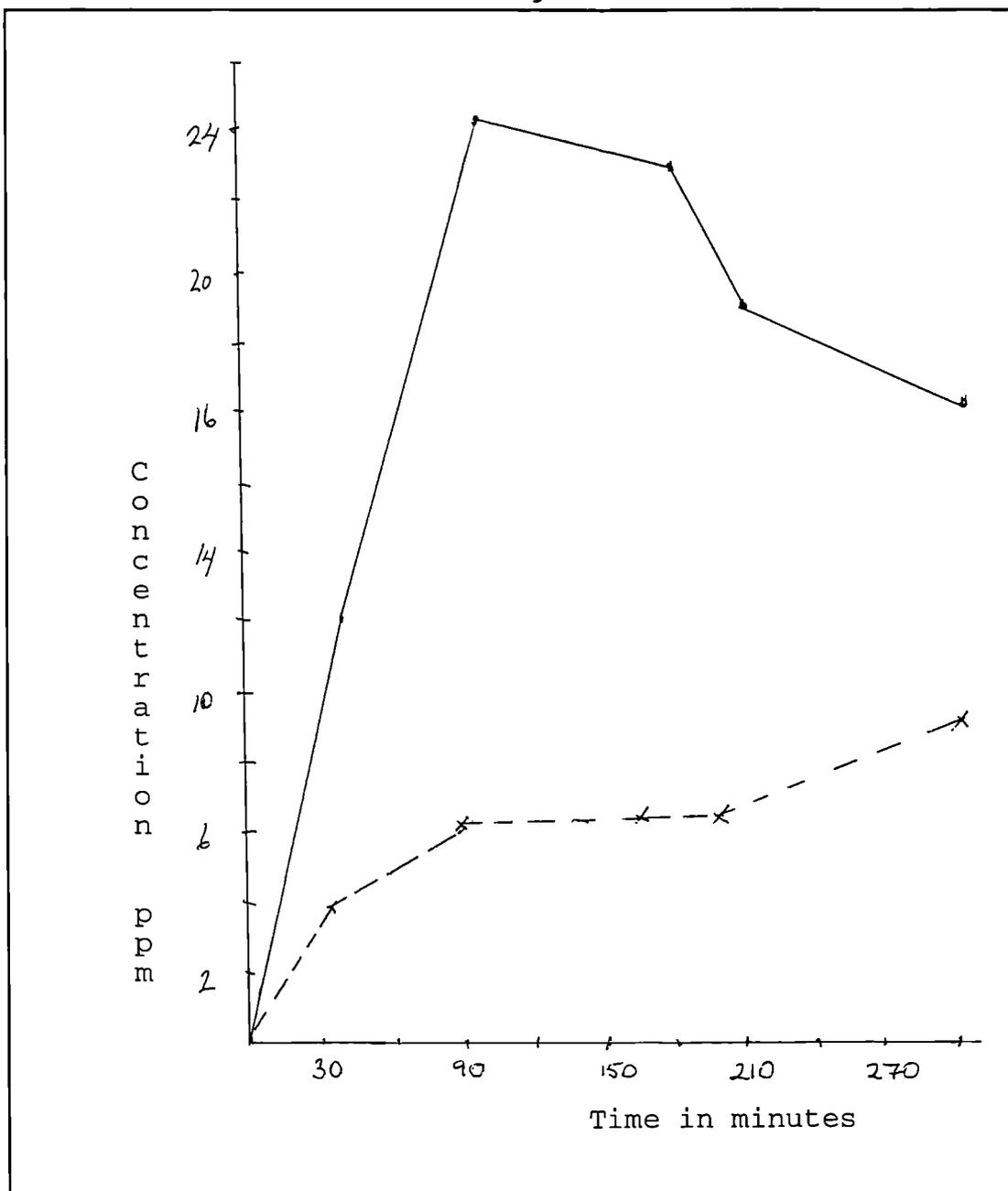
Figure 3:13 Graph of H_2S and SO_2 concentration against time. For reaction of H_2O_2 bleached wool with 2.5 M H_3PO_2 under air. (pH 1 and temperature 80°C).
- - = SO_2 and —— = H_2S.

The evolution of H_2S and SO_2 on reaction of disulphide bonds with H_3PO_2 occurred only in acidic solution (pH 1-2) at a minimum temperature of 80°C and with a minimum concentration of 2-2.5 M solutions of H_3PO_2 for all reactions undertaken. The effect of pH, namely that the reducing ability of H_3PO_2 is greatest in strongly acidic media is in agreement with work done by Gunther [49] in 1966. (See Sec 3:1:2:1)

On reaction of wool serge with a 2.5 M solution of H_3PO_2 at pH 1 under air, (reaction number 1) a much larger quantity of H_2S and SO_2 was produced than for blank wool. (Figure 3:5) Both gases were evolved in similar ratio to one another. The maximum rate of evolution of gas occurred after six hours, (42 ppm H_2S and 58 ppm SO_2) corresponding to $1.26 * 10^{-6}$ M H_2S and to $8.28 * 10^{-6}$ M SO_2 respectively.

The reaction of H_3PO_2 with wool increases the amount of H_2S and SO_2 evolved relative to that reported for a mineral acid (22 ppm H_2S and 34 ppm SO_2).[48]
In reaction 2, a 2.5 M H_3PO_2 solution of acid was reacted with cystine ($[-SCH_2CH(NH_2)CO_2H]_2$) as the model disulphide compound. (Figure 3:7) A large quantity of H_2S was afforded with a maximum rate of production at five hours of 180 ppm H_2S. A small amount of SO_2 was also evolved, whose rate of production also reached a maximum at five hours of 15 ppm. The kinetics of the production of the gases are very different to those of the reaction with wool. These findings are not

in accordance with the authors Swanepoel and Louw [41] who stated that for the same reaction but using a 0.5 M H_3PO_2 solution, two moles of thiol were produced for each mole of disulphide reduced. Their account made no record of SO_2 evolution, and only mentioned H_2S production with reference to the U.V.-catalysed reaction.

The reactions were repeated under similar conditions using phosphorous acid, H_3PO_3 whose reducing strength is known to be significantly lower than hypophosphorous acid at stongly acidic pH. [50],[61] No significant amount of H_2S or SO_2 was afforded for either reaction 3 or 4 with wool or cystine respectively. (Figure 3:5 and Figure 3:7). This result confirms earlier reports that the two acids reduce disulphide links under different pH conditions. [49],[50],[61] Phosphorous acid may not react at all with disulphide linkages under the conditions used or else may react without producing significant amounts of H_2S or SO_2.

A free radical mechanism for the reaction of H_3PO_2 with wool was proposed by Swanepoel and Van Rensburg [44] (See section 3:1:3). In order to test this hypothesis, the reaction of H_3PO_2 with wool was carried out in the presence of 0.5 grams of a radical inhibitor, hydroquinone (C_6H_4-1,4-$(OH)_2$) (reaction 5). If the reaction proceeded exclusively via free radical intermediates, no H_2S or SO_2 should be evolved on addition of hydroquinone. Figure 3:9 shows that H_2S

and SO_2 were produced in similar ratio to the H_3PO_2 and wool reaction without hydroquinone. (figure 3:5) However, the rate of evolution of each gas was diminished. e.g. at 140 mins 48 ppm of SO_2 and 32 ppm of H_2S were produced for the reaction of H_3PO_2 and wool and only 30 ppm SO_2 and 14 ppm H_2S for the reaction in the presence of hydroquinone. The hydroquinone may influence the reaction in other ways however by, e.g. reacting ionically with H_2S, to form a thiol substituted hydroquinone. As the reaction was not fully quenched by addition of the free radical inhibitor it is unlikely that its mechanism is purely free radical in character.

In an attempt to elucidate the mechanism of reaction of H_3PO_2 with the disulphide bond the reaction was repeated using a series of model sulphide and disulphide compounds. 3,3'-Dithiodipropionic acid and benzyl disulphide were found to be reduced to the corresponding thiol on reaction with hypophosphorous acid, by Humphrey et al. [51] No mention of H_2S or SO_2 production was included. However, no reaction occurred (i.e. no evolution of H_2S or SO_2 was noted) for the reaction of H_3PO_2 with cysteine ($HSCH_2CH(NH_2)CO_2H$), cysteic acid ($HO_3SCH_2CH(NH_2)CO_2H$), 3,3'-dithio dipropionic acid ($S_2(CH_2CH_2CO_2H)_2$) and benzyldisulphide ($(C_6H_5CH_2)_2S_2$).

Unlike the other model compounds, a small quantity of H_2S and SO_2 was produced from reaction 6, the reaction of H_3PO_2 with 2-hydroxyethyl disulphide. (figure 3:10) The maximum rate of gas evolution occurred after 20 minutes corresponding to 7 ppm H_2S and 10 ppm SO_2.

On reaction of hydrogen peroxide-bleached wool [48] with H_3PO_2 under air, H_2S and SO_2 were evolved. (Reaction 9, figure 3:13) Their rate and ratio (relative to one another) of production were diminished relative to the non-bleached sample. Maximum rate of H_2S production occurred at 1 hour, 24 ppm and the maximum rate of SO_2 evolution after 4 hours, 7 ppm. These results contrast with the blank H_2O_2-treated wool whose maximum evolution of H_2S occurred after 30 minutes with 3ppm. The amount of SO_2 evolved however was unaltered. The production of SO_2 is generally associated with oxidised species in wool, (sulphoxides, sulphones and cysteic acid) and an increase in the amount and rate of SO_2 production was expected for this reaction. The similarities in rate and amount of SO_2 production between blank and H_3PO_2 treated wool may be due to the conversion of all the cystine residues to cysteic acid within the fibre on reaction with H_2O_2. As noted above no H_2S or SO_2 evolution was observed on reaction of H_3PO_2 with cysteic acid. It has been reported that the sulphoxide that is formed in concentrations of up to 20% of the original disulphide on bleaching, is more stable than

the disulphide itself, and may not be reduced by H_3PO_2 to any significant extent.[58] [59] [70]. This may also account for the observation that H_2S and SO_2 production is decreased relative to that produced by the bleached blank.

The reactions of H_3PO_2 with wool and cystine were re-run under an inert nitrogen atmosphere in order to investigate if the presence of air contributed to SO_2 production in these reactions. (Reactions 7 and 8) Figure 3:11 and figure 3:12 show the results of H_2S and SO_2 evolution for the reaction with wool and with cystine respectively. Both graphs show a substantial decrease in the amount of SO_2 and H_2S in relation to the reactions carried out under air. The maximum rate of H_2S and SO_2 production was the same for each reaction, 15 ppm SO_2 and 11 ppm H_2S. These results are surprising and difficult to explain. The presence or absence of oxygen in the reaction mixture should have no effect on the amount of H_2S afforded by the reactions. It should also have no effect on the amount of SO_2 evolved if SO_2 is produced via hydrolysis of an oxidised disulphide bond. The result implies that intermediates are formed which need atmospheric oxygen to give H_2S and SO_2. Further work must be done to explain this phenomenon.

3:3 Investigation into the Production of Thiol by Reaction of H3PO2 and H3PO3 with Wool.

The amount of thiol produced on reaction of H_3PO_2 with wool serge and cystine has been determined polarographically by Swanepoel and Louw [41] (See Sec. 3:1:3) to be two moles of thiol produced per mole of disulphide reduced. A further investigation into the production of thiol by this reaction under similar conditions for maximum H_2S and SO_2 production was undertaken. (See fig. 3:5) The possibility of thiol production by the reaction of H_3PO_3 with disulphide bonds was also investigated.

In order to detect the presence of thiol, two fluorescent probes 9-bromomethylacridine (9-BMA) and 5-iodoacetamido fluorescein (5-IAF) were used. A quantitative measurement of thiol produced was achieved with a colorimetric method using 2-vinylpyridine. [52]

3:3:1 The Use of Fluorescent Probes for Thiol Determination

A common method for cysteine determination in reduced wool involves the use of fluorescent probes. [67] A chemical species which, when suitably excited, displays fluorescence emission is called a fluorescent probe or fluorophore. The probe should exhibit a large Stokes shift, its fluorescence yield should approach unity and

its extinction co-efficient at its maximum excitation wavelength should be high.

Both probes used in this investigation bonded covalently with thiol groups formed. Under the alkaline conditions of the experiment, the thiol moiety underwent nucleophilic substitution faster than the lysine group in wool or the amine group in cystine. The halogen atom in each probe (bromine in 9-BMA and iodine in 5-IAF) quenched the probe's fluorescence via the heavy atom effect. (See sec 1:2:5:4)   On substitution of the halogen atom with a thiol group the probe regained its fluorescence emission properties and proof of the formation of thiol groups was afforded.

3:3:1:2 Experimental Procedure for Reaction of Fluorescent Probes 9-BMA and 5-IAF with Reduced Wool.

10.0g of wool was reacted at 80°C with a 2.5 M solution of H_3PO_2 or H_3PO_3 for 6 hours. The wool was washed repeatedly with water for 2 minutes and was then stirred in a sodium hydroxide solution of pH 9, for 4 hours. 1 gram of the pretreated wool was then stirred overnight in a 100ml solution of ratio 1:1 of acetonitrile/water, containing $1*10^{-4}$ moles of the fluorescent probe. The pH of the solution was adjusted to pH 9 using TRIS buffer (Tris(hydroxymethyl) aminomethane, $(HOCH_2)_3CNH_2$).

The fluorescence properties of the wool sample were then investigated. Diffuse reflectance U.V. spectra were also obtained for some samples.

3:3:1:3   Results of Reaction with 9-Bromomethylacridine

9-bromomethylacridine (9-BMA) undergoes nucleophilic substitution with a thiol group via the following mechanism;

CH_2Br W-SH CH_2-S-W +   HBr
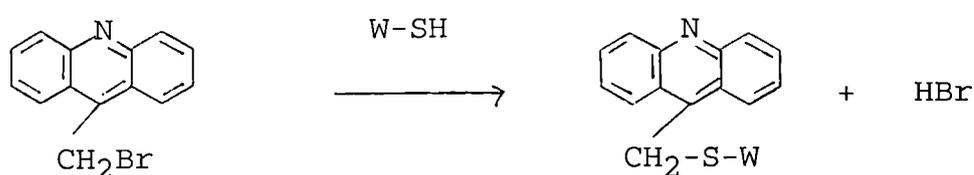
The thiol group may be attached to wool, which can be represented as W in the equation above.

When bound to a protein, 9-bromomethylacridine has an absorption maximum at 380 nm and a fluorescence emission maximum of 475 nm.

Figure 3:14 below, compares the emission spectra of 9-bromomethylacridine on H_3PO_2 treated wool, and untreated wool. The H_3PO_2 pretreated sample has an emission maximum at 470-475 nm. This spectrum corresponds to the thiol-substituted methyl acridine and clearly demonstrates the production of thiol by the reaction of H_3PO_2 with wool fibre. The second spectrum of unreduced wool, shows an emission maximum at 450 nm, corresponding to fluorescent chromophore(s) present in

the wool. No nucleophilic substitution by thiol

occurred with the untreated sample and 9-

bromomethylacridine's fluorescence remained quenched by

bromine.
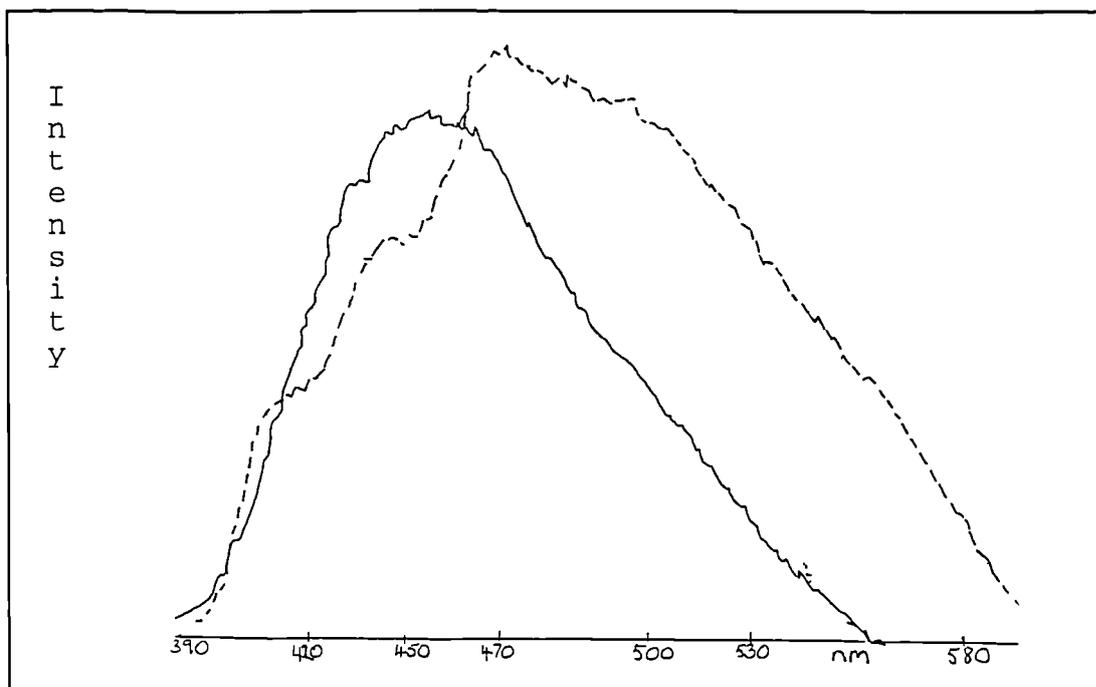
Figure 3:14 Fluorescence emission spectra of H_3PO_2
treated wool and blank alkali wool, both treated with
9-bromomethylacridine. Excitation wavelength at 380 nm
and sensitivity of 100/0.
——— = Wool + BMA, - - - - = H_3PO_2 wool + BMA.

The diffuse reflectance spectrum of hypophosphorous

acid treated wool with 9-bromomethylacridine confirmed

the above results. (Figure 3:15) The spectrum shows

that the H_3PO_2 treated sample had a higher uptake of

9-bromomethylacridine relative to unreduced

9-bromomethylacridine wool. A spectrum of blank wool is

also shown.
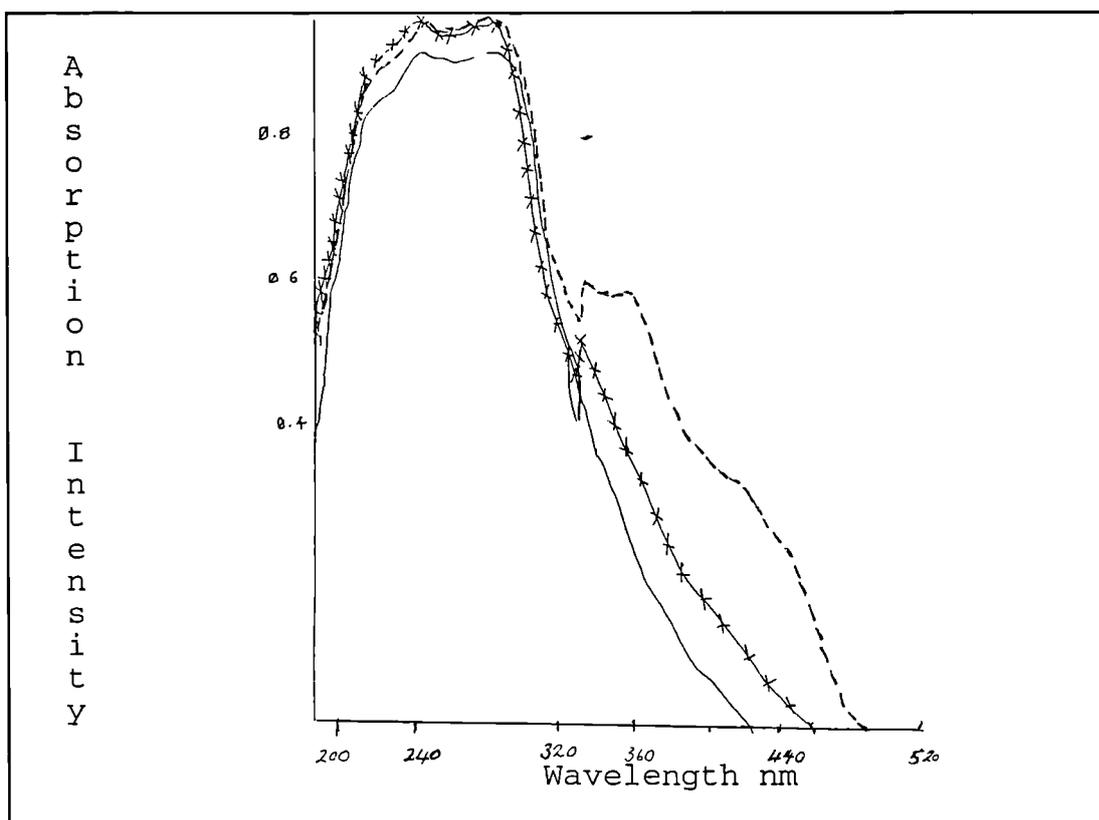
Figure 3:15   Diffuse reflectance spectra of wool, wool
+ 9-BMA and H_3PO_2 pretreated wool   + 9-BMA.

———— = wool, -*-*- = wool + 9-BMA, - - = H_3PO_2 wool +
9-BMA.

The untreated wool sample adsorbed but did not react

with 9-BMA. The concentration of 9-BMA is greatest for

the H_3PO_2 pretreated sample due to (a) presence of

reactive thiol groups on wool surface and (b) the

opening up of the wool structure on removal of

disulphide linkages on reduction.

3:3:1:4   Results of Reaction with 5-Iodoacetamido
fluorescein.

The same experiments were carried out using

5-Iodoacetamido fluorescein (5-IAF), whose absorption

maximum is at 490 nm and emission maximum at 520 nm when bound to a protein.
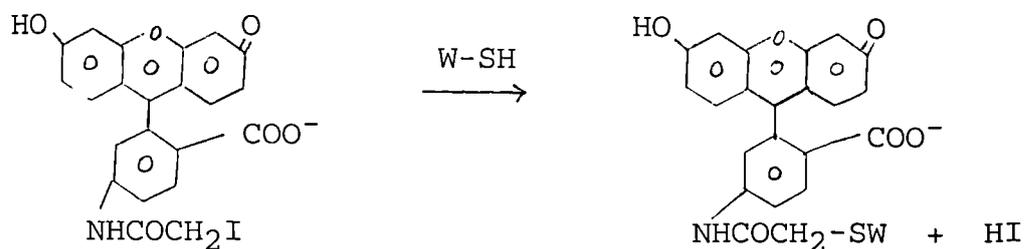
5-iodoacetamido fluorescein like 9-BMA, undergoes nucleophilic substitution by thiol groups at neutral/alkali pH, with the formation of hydriodic acid.

A comparison of the fluorescence spectra of H_3PO_2 , H_3PO_3 and blank pretreated wool on reaction with 5-IAF is shown in figure 3:16. The H_3PO_3 and alkali treated samples show a similar emission maximum of 540-545 nm. The H_3PO_3 /5-IAF spectrum is much broader than that of the untreated sample and is due to the acid treatment of wool itself, and will be further discussed in Sec 3:5. In contrast the H_3PO_2 pretreated sample's emission maximum is observed at 515 nm which corresponds to that of the substituted probe. As with the 9-BMA probe this infers that disulphide bonds in wool have been reduced to thiol on reaction with H_3PO_2. No such reduction had taken place for the reaction with H_3PO_3.

A photograph of H_3PO_2 treated wool reacted with 5-IAF taken with a fluorescence microscope using a visible

filter is displayed in figure 3:17. The emission of the

probe in the yellow/green region (520 nm) is evident.
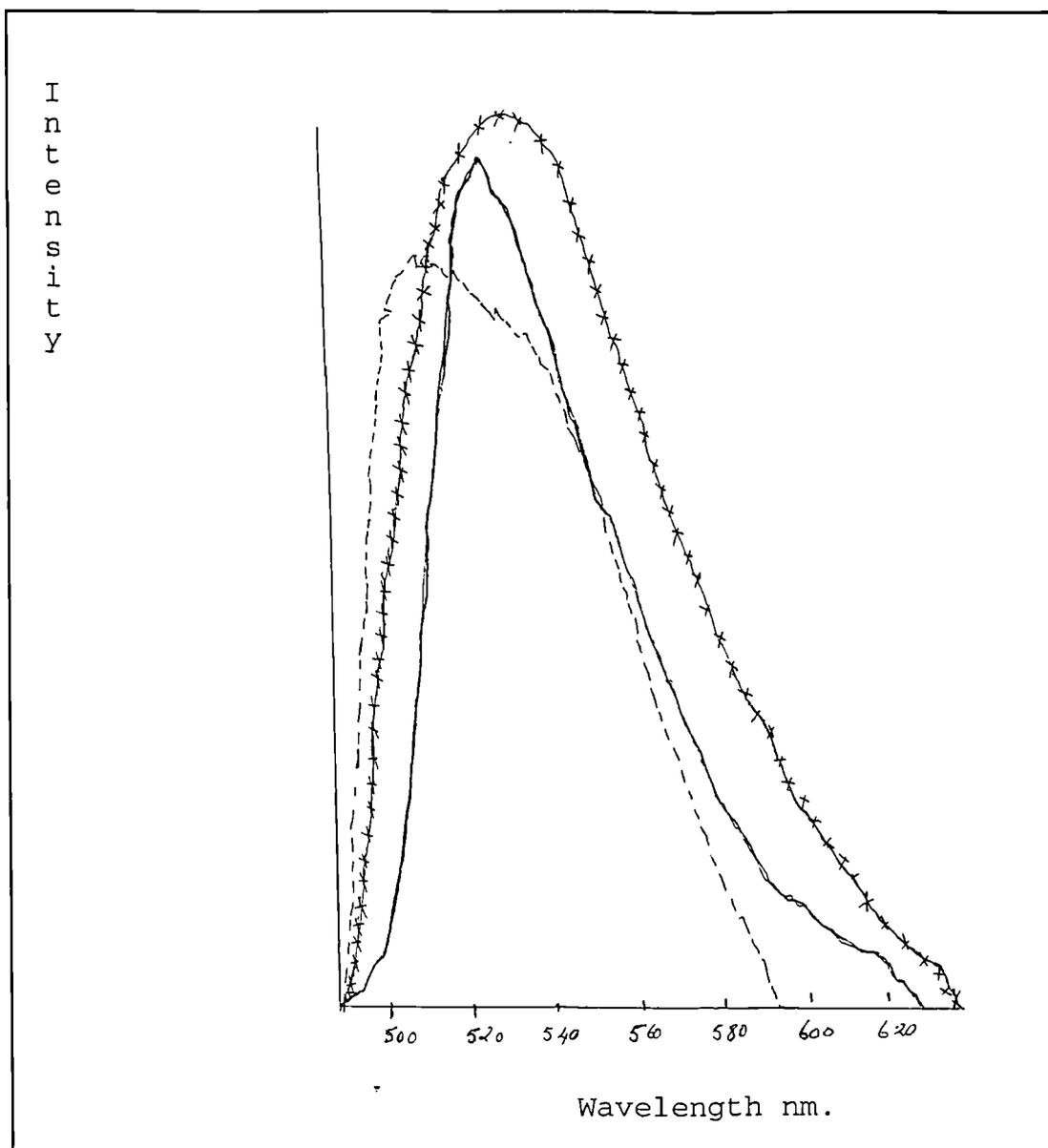
Figure 3:16 Fluorescence emission spectra of alkali
treated wool (blank),H_3PO_2 and H_3PO_3 pretreated
samples on reaction with 5-IAF. At excitation
wavelength 480 nm and sensitivity 3/5.
———— = alkali treated wool, -*-* = H_3PO_3 pretreated
wool and - - - - = H_3PO_2 pretreated wool.

Figure 3:17 A fluorescence microscope photograph of the reaction of 5-IAF with H_3PO_2 pretreated wool, at a magnification of x150.

The above photograph shows that the probe was deposited selectively along the boundary of each cuticle cell, mainly on the surface of the lower scale emerging from each cell junction. This deposition pattern is the same as that reported for the bis-pyrene probe on untreated wool. [70]

A quantitative measurement of thiol production is difficult to obtain using this method. This is due to the fact that wool has its own fluorescence emission which may be altered by changes in pH (See sec 6:2:4) and subsequently interfere with the emission intensity of the probe.

Colorimetric determinations of cysteine concentration in reduced and non-reduced wool using various thiol specific reagents are well known, e.g. fluoro-2,4-dinitrobenzene [68], and 5,5'-dithiobis-(2-nitrobenzoic acid) [69]. In this case, quantification of the amount of thiol produced by the reaction of hypophosphorous acid and wool cystine, the colorimetric method of Friedman and Noma [52] was used.

This method involves the Michael addition of thiol groups with 2-vinylpyridine, in alkali solution. In the case of reduced wool the product formed is, (S)-(2-pyridylethyl) wool or 2-PEW. This compound is then hydrolysed to (S)-(2-pyridylethyl) cysteine or 2-PEC. The pyridine ring of 2-PEC has an absorption maximum at 264 nm and high molar absorptivity in acid media. (See scheme one below) The concentration of 2-PEC and therefore original thiol concentration, may be determined spectroscopically at 264 nm using the Beer-Lambert law. (See sec 1:1:2)

$$Wool-S-S-Wool \quad + \quad H_3PO_2$$

$$W-SH \quad + \quad CH_2=CH-\text{pyridine}$$
$$N$$
(2-vinylpyridine)

$$W-S-CH_2-CH_2-\text{pyridine}$$
$$N$$
(2-PEW)

$$HOOC-\underset{\underset{(\;2-PEC\;)}{\underset{NH_2}{|}}}{CH}-CH_2-S-CH_2-CH_2-\hspace{-0.5em}\text{(pyridyl)}\;N$$
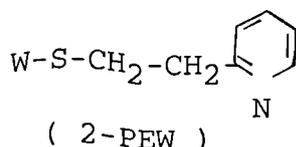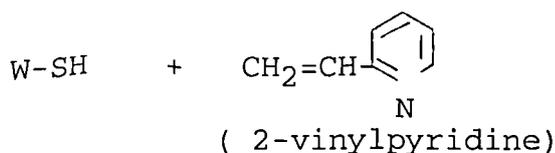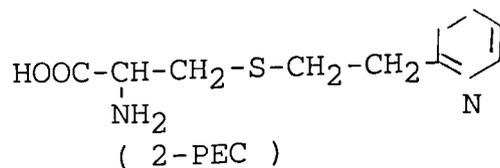
ab.max. 264 nm

Scheme One

3:3:2:1 Experimental Procedure for Reaction of Reduced Disulphide Bonds with 2-Vinylpyridine.

4 grams of wool serge was reduced in aqueous propanol with tri-n-butyl phosphine ($[CH_3(CH_2)_3]_3P$), as described by Sweetman and Maclaren [55]. The authors formulated that two moles of thiol were produced for one mole of disulphide reduced, and assumed that the reduction proceeded to about 95-98% completion.

4 grams of wool was placed in a 500 ml round-bottomed flask, followed by a solution containing 150 mls of n-propanol, 150 mls of 0.1 N Tris buffer, pH 7.6 and 1.6 mls of tri-n-butyl phosphine. Nitrogen was bubbled through the solution for 10 minutes. The stoppered flask was shaken for 48 hours. Then 4 mls of 2-vinylpyridine was added to the reduced wool. The mixture was shaken for an additional 48 hours. The wool was rinsed with water several times and air-dried.

10 grams of wool serge was reacted with the phosphorus acids as before. 2.5 M of each acid was used at pH 1 and at 80°C for 6 hours. The acid treated wools were rinsed with water, and stirred in a sodium hydroxide solution of pH 9 for 4 hours, and thoroughly rinsed again with water. 4 grams of each pretreated wool was

then reacted with 2-vinylpyridine using the same procedure as for the tributylphosphine treated sample.

15 mgs of each pretreated wool sample and an untreated blank, and 15 mls of 6 N HCl solution were placed in individual test tubes which had thick glass walls. The neck of each test tube was heated and narrowed to about one-half of its original diameter. The tube was then degassed using a freeze-thaw vacuum method. The tube was then sealed under vacuum and heated at 110°C in an oven for 24 hours, after which the contents were filtered through sintered glass and diluted to 100 mls with 6 N HCl. The absorbance of each solution at 264 nm was then determined.

3:3:2:2 Results of Reaction of Reduced Wool with 2-Vinylpyridine.

The blank hydrolysed sample of native wool had no significant maximum at 264 nm. The phosphorous acid (H_3PO_3) treated sample showed a similar result indicating that no 2-pyridylethyl cysteine (2-PEC) was formed, confirming that no reduction of disulphide took place with this reagent.

A large peak at 264 nm was observed with an intensity of 0.860 units for the tributylphosphine treated sample. The H_3PO_2 sample clearly showed that some reduction had taken place as an absorption peak with an intensity of 0.291 was observed. On subtraction of the absorption intensity value at 264 nm for the blank

sample (0.261), values of 0.6 units and 0.03 units of absorbance remained for the tributylphosphine and H_3PO_2 treated samples respectively. If the tributylphosphine treated absorbance value is taken to represent 95-98% reduction of cystinyl residues in wool, the H_3PO_2 value must correspond roughly to only 5-10% reduction.
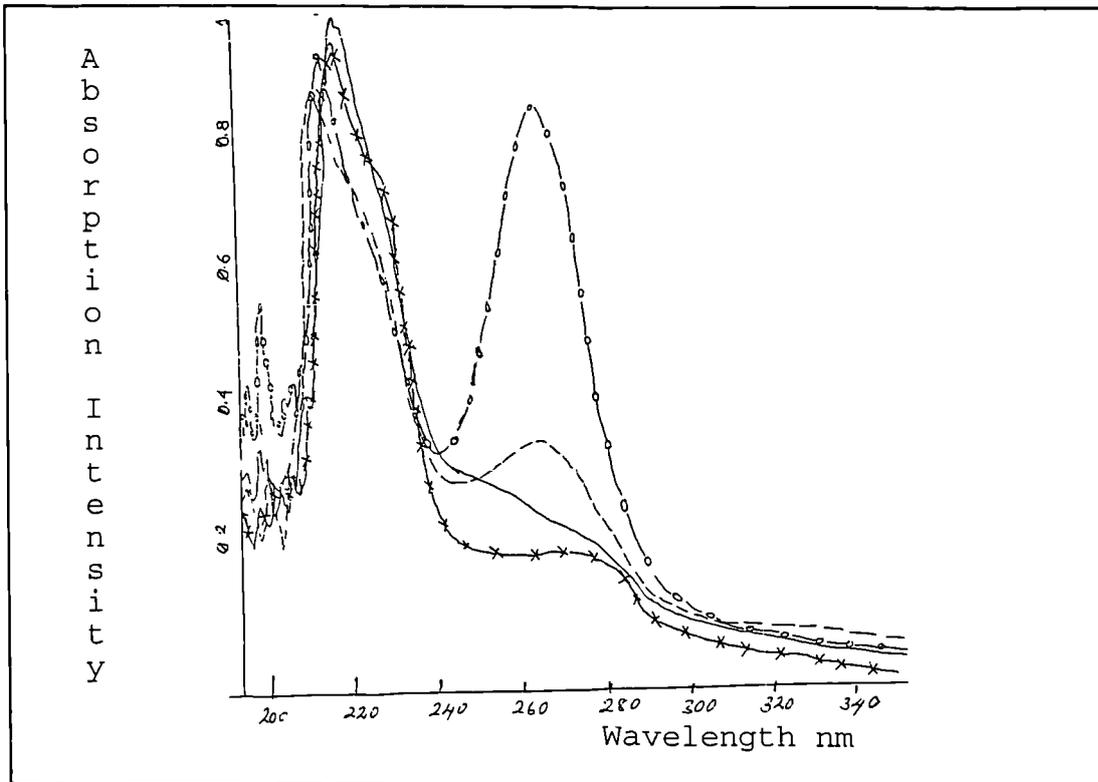
Figure 3:18 Absorption Spectra of 2-PEC. For blank hydrolysed wool, H_3PO_3 treated wool, H_3PO_2 treated wool and tributylphosphine treated wool. Solvent 6 N HCl.
———— = blank, -*-* = H_3PO_3 wool,   - - = H_3PO_2 wool and -o-o- = tributylphosphine wool.

3:3:2:3   Calculation of Results

The absorbance values above correspond to 15 mgs of treated wool. The net absorbance for 100 grams of wool was calculated. Division of this net absorbance by the

molar absorptivity of 2-PEC in 6 N HCl (7000), gives

the number of moles of half-cystine per 100 grams of

wool determined spectrophotometrically.

The following formula may be used to calculate the

half-cystine content of unmodified wool from

spectrophotometrically determined 2-PEC content of

chemically modified wool. [24]

$$\frac{1 * 10^5}{[C_{1/2}]} = \frac{1 * 10^5}{[2\text{-PEC}]} - (M + 1)$$

$C_{1/2}$ = the calculated number of half-cystine

residues in mmoles per 100 grams of dry native wool.

2-PEC is the determined concentration of 2-PEC (mmoles

per 100 grams of dry wool).

M + 1 is the gram formula weight of added pyridylethyl

group (106) per half cystine residue, M being the

molecular weight of 2-vinylpyridine, equal to 105, and

1 being the molecular weight of hydrogen introduced

during the reduction step in the reaction scheme.

Calculation of number of moles of half-cystine per 100

grams of tributylphosphine treated wool:

15 mgs tributylphosphine treated wool absorbance = 0.6

100 grams of tributylphosphine treated wool absorbance

would equal 4000 , on extrapolation of the Beer-Lambert

law.

4000/7000 gives the number of moles of 2-PEC that 4000 absorbance units would correspond to.

$$\frac{4000}{7000} = 0.5714 \text{ M or } 57.14 \text{ mmoles.}$$

Fitting this value into the above formula gives a value for the half-cystine content of tributylphosphine treated wool.

$$\frac{1 * 105}{[C_{1/2}]} \qquad \frac{1 * 105}{57.14} \quad - (106)$$

== $[C_{1/2}]$ = 60.82 mmoles per 100 grams of wool.

Which is equivalent to [cystine] of 30.41 mmoles per 100 grams of wool.

The value of 30.41 mmoles of cystine per 100 grams of wool is low. Most wool samples have cystine concentrations ranging between 40 - 50 mmoles per 100 grams of wool. [56] However it is well established that changes in the diet of a sheep may influence the amino acid composition of wool, the cystine content being especially susceptible to seasonal variation. [57]

The value of $[C_{1/2}]$ for the H_3PO_2 treated sample may be calculated in a similar fashion and yields a value of 3.04 mmoles per 100 grams of wool.

If 95-98% reduction is achieved on reaction of

tributylphosphine with wool, the value of 30.41 mmoles

of cystine per 100 grams may be taken to be the total

amount of cystine per 100 grams of wool.

The % of cysteine formed from cystine in the treatment

of wool with H_3PO_2 can therefore be calculated as;

$$\frac{3.04}{30.41} * 100 = 10\%$$

3:4 Proposed Mechanism for the Reaction of H3PO2 with Disulphide bonds.

The reaction of 10 grams of wool serge with a 2.5 M

solution of H_3PO_2 in water, at pH 1 and at a

temperature of $80^{\circ}C$, produces 0.304 mmoles of cysteine,

1.26 umoles of hydrogen sulphide and 8.28 umoles of

sulphur dioxide. (It is possible that other products

are formed in this reaction but their structures have

not been determined) The reaction of H_3PO_2 for the

production of cysteine is only 5% as efficient as that

of the reaction of wool with tributylphosphine. The

contention of Swanepoel and Louw [41], that the amount

of H_2S produced was less than 4% of the total amount of

thiol produced, is upheld by these findings.

However, the suggestion by the same authors, that the

reaction takes place via a free radical mechanism (See

sec 3:1:3) is not in accordance with the above

results.

The proposed mechanism may hold true for the U.V.
initiated reaction, as U.V. energy could homolytically
cleave the disulphide bond. (In Ph_2S_2 the disulphide
bond strength is 70 kcal). [60] It is unlikely however
that the same bond could be cleaved thermally at 80°C.
Addition of the free radical scavenger, hydroquinone to
the reaction only quenches the rate of H_2S and SO_2
production to a small degree. (See figure 3:9) This
indicates that the reaction cannot be purely free
radical in character.

If the reaction takes place exclusively via a free
radical intermediate, the ammonium salt of the acid
should react in the same manner as the acid itself, and
the pH of the solution should have no bearing on the
products formed. This was found not to be the case. In
fact, no evolution of H_2S or SO_2 occurred on
neutralisation of the reaction mixture with a 5%
aqueous ammonium hydroxide solution.

Both H_3PO_2 and H_3PO_3 would be expected to give the same
products under the same reaction conditions if the
reaction proceeded via a free radical pathway. The
finding that no reaction occurred with H_3PO_3 and wool
or cystine under the same conditions, is incompatible
with this premise.

It is not implied however that free radical
desulphurisations to produce H_2S, SO_2 and cysteine
cannot occur, but in fact do occur on addition of free

radical initatiors and on U.V. irradiation. As the

difference in reactivities of the two acids is pH

dependent and addition of a free radical scavenger did

not halt the reaction, a series of ionic mechanisms for

the reaction of H_3PO_2 with the disulphide bonds of

wool, cystine and 2-hydroxyethyl disulphide are

proposed.

3:4:1 Proposed Mechanisms for the Production of H_2S

(A)

$$\underset{H}{\overset{HO\diagdown \diagup OH}{P:}} + Cy\text{-}S\text{-}S\text{-}Cy \longrightarrow \underset{+\diagdown OH}{Cy\text{-}S\text{-}\overset{H}{\underset{|}{P}}\text{-}OH} + {}^-S\text{-}Cy$$

$$\underset{+\diagdown OH}{Cy\text{-}S\text{-}\overset{H}{\underset{|}{P}}\text{-}OH} + {}^-S\text{-}Cy \longrightarrow \underset{\diagdown OH}{Cy\text{-}S\text{-}P\text{-}OH} + HS\text{-}Cy$$
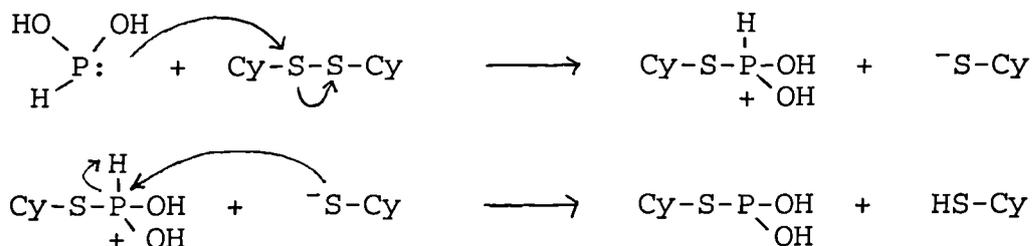
Where Cy = $HOOC\text{-}CH(NH_2)\text{-}CH_2\text{-}$.

In scheme (A) the ionic phosphonium intermediate

produced on reaction of the tervalent tautomer of

hypophosphorous acid with the disulphide bond, loses

its excess hydrogen to the second sulphur molecule

forming cysteine. The cysteine produced is inert to

H_3PO_2 and will not react further to produce H_2S and

SO_2. It is worthy of note that an ionic phosphorous

acid intermediate cannot reduce to cysteine via this

mechanism.

The observation that the reaction of 2-hydroxyethyl

disulphide and hypophosphorous acid produced both

gases, whereas neither gas was produced for the reaction with 3,3'-dithiodipropionic acid (see p.95) may be explained by the following mechanism.

If the charged phosphonium intermediate above, undergoes a proton elimination, two unstable products dehydroalanine and a secondary phosphine sulphide are formed. (B)

(B)

$$
\begin{array}{ccc}
\overset{\displaystyle |}{\underset{\displaystyle |}{NH}} & & \overset{\displaystyle |}{\underset{\displaystyle |}{NH}} \\
H\text{-}C\text{-}CH_2\text{-}S\text{-}P\text{-}OH & \longrightarrow & C=CH_2 \quad + \quad S=P\text{-}OH \\
\end{array}
$$
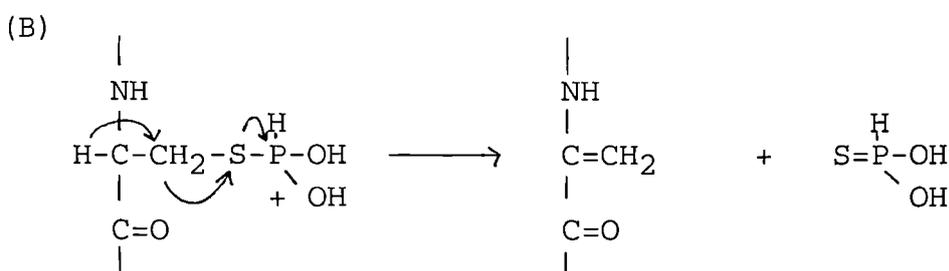
The secondary phosphine sulphide formed may tautomerise to its trivalent state, (See Section 3:1:2) and susequently undergo hydrolysis to form the inert phosphorous acid and hydrogen sulphide. (C) This tautomerisation may lead to an induction time for the production of H_2S as observed for the reaction of H_3PO_2 and cystine.

(C)

$$
S=\overset{H}{P}\text{-}OH \quad \rightleftharpoons \quad H\text{-}S\text{-}P\text{-}OH
$$

$$
H\text{-}S\text{-}P\text{-}OH \quad \overset{H_2O}{\longrightarrow} \quad HO\text{-}P\text{-}OH \quad + \quad H_2S
$$
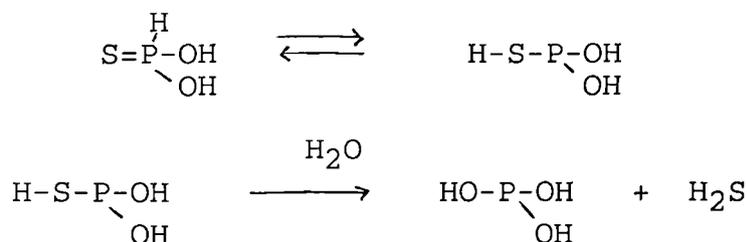
The β- hydrogen in 3,3'-dithiodipropionic acid is much less acidic than that of 2-hydroxyethyl disulphide and for this reason the compound may possibly not undergo a

proton elimination to form hydrogen sulphide.
Benzyldisulphide has no β-hydrogen and therefore as
observed cannot eliminate H_2S via this mechanism.

This elimination reaction cannot occur with cysteine as
no gas evolution was noted for its reaction with H_3PO_2.

Hydrogen sulphide itself is a strong reducing agent,
and may reduce the disulphide bond of cystine to
cysteine and perthiocysteine. [62] (D) Perthiocysteine
may also be formed by hydrolysis of cystine in acid
solution. (See section 3:2).

```
(D)
 |               |                    |                   |
NH             NH           H2S      NH                  NH
 |              |          ───────►   |                   |
CH-CH2-S-S-CH2-CH                    CH-CH2-SH    +     CH-CH2-S-S-H
 |              |                     |                   |
C=O            C=O                   C=O                 C=O
 |              |                     |                   |
Cystine                            Cysteine          Perthiocysteine
```
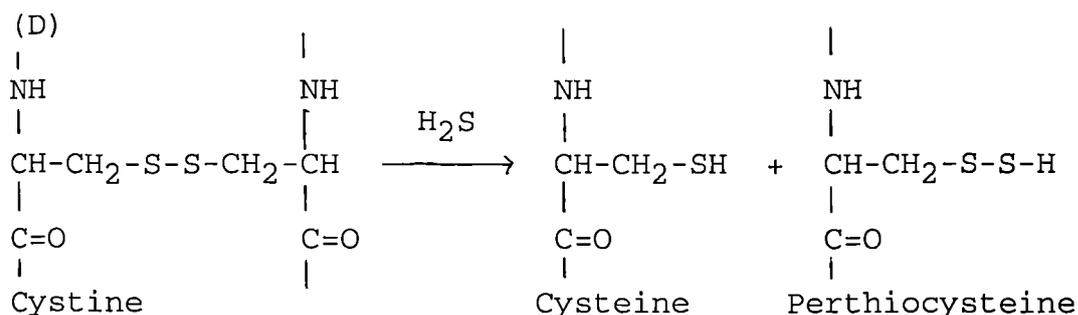
Investigations into the reactivity of hydrodisulphides
namely benzylhydrodisulphide (PhCHSSH), with
phosphines e.g.triphenylphosphine (PhP_3) by Tsurugi
et al. [63]-[65] have shown that H_2S and thiol are
produced by nucleophilic attack on both sulphur atoms.
α-and β-sulphur atoms in the hydrodisulphides (and
perthiocysteine) are designated sulphydryl sulphur and
sulphenyl sulphur atoms respectively, as in $RS(\beta)S(\alpha)H$.
The H_2S arises solely from reaction of the phosphine
with the sulphydryl sulphur atom and thiol solely from
reaction with the sulphenyl sulphur atom. The authors

suggest ionic mechanisms for the reactions. H_3PO_2 may

react in the same manner with perthiocysteine, acting

as a further source for cysteine and H_2S. As the

reaction with H_3PO_2 is undertaken in aqueous solution

the phosphine oxide would be formed in preference to

the phosphine sulphide. (See Sec 3:1)

For thiol production, from perthiocysteine;

$$R-S-S-H + PR'_3 \longrightarrow [\ R-S\ ]^-[\ S-H\ ]^+ \longrightarrow R-SH\ +$$
$$\diagup$$
$$PR'_3 \qquad\qquad S=PR'_3$$
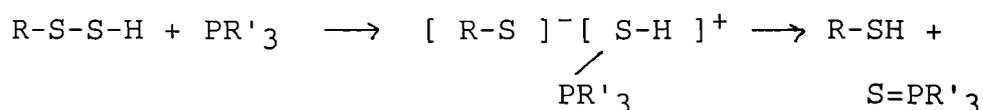
For H_2S production, from perthiocysteine;

$$R-S-S-H + PR'_3 \longrightarrow [\ R\ ]^-[\ \underset{PR'_3}{S-S-H}\ ]^+ \longrightarrow R-H\ +\ R'_3P^+SS^-$$

$$R'_3P^+SS^- + 2R-S-S-H \longrightarrow H_2S + R'_3P=S + R-S-S-S-S-R$$

Or,

$$R-S-S-H + PR'_3 \longrightarrow [\ R-S-PR'_3\ ]^+[\ SH\ ]^-$$

$$HS^- + R-S-S-H \longrightarrow H_2S\ +\ RSS^-$$
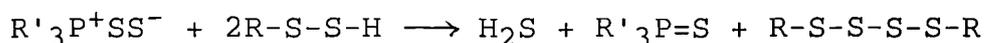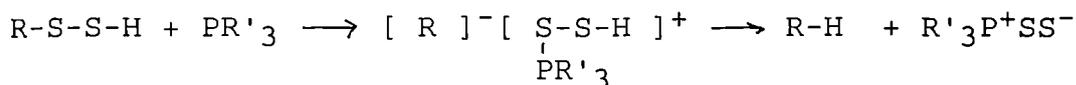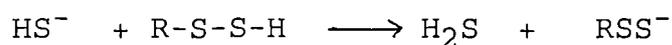
3:4:2 Proposed Mechanisms for the Production of SO$_2$

The production of SO_2 by the reaction of H_3PO_2 with

disulphide linkages is more difficult to explain. As

noted previously in section 3:2:3, no SO_2 evolution was

observed for the reaction of H_3PO_2 with cysteic acid,

and no increase in SO_2 production was found on reaction

with hydrogen peroxide-treated wool. Oxidised

disulphide residues in the wool itself may react via

a proton elimination of a cystine dioxide charged

phosphonium intermediate as before, to produce SO_2,

dehydroalanine, and hypophosphorous acid. (E) The

dioxide moiety is most reactive to nucleophiles.

(Relative to the monoxide and disulphide) [72]

(E)

$$
\begin{array}{l}
\quad\ \ |\\
\quad NH\\
\quad\ |\qquad\ O\\
\quad\ \ \ \ \ \ \ \ \ \ \|\\
HC\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CH \ + \ :P \overset{H\ \diagdown \ OH}{\underset{\diagdown OH}{}}\\
\quad |\qquad\ \|\qquad\quad\ |\\
\quad C{=}O\qquad\ O\qquad\ C{=}O\\
\quad\ |\qquad\qquad\qquad |
\end{array}
$$

$$
\longrightarrow \
\begin{array}{l}
\quad\ \ |\\
\quad NH\\
\quad\ |\qquad\ O\ H\\
\quad\ \ \ \ \ \ \ \ \ \ \|\ |\\
H\text{-}C\text{-}CH_2\text{-}S\text{-}P\text{-}OH \ + \ {}^-SCH_2CH\\
\quad |\qquad\ \|\ \diagdown\\
\quad\ \ \ \ \ \ \ \ \ \ O\ +\ OH\\
\quad C{=}O\qquad\qquad\quad C{=}O\\
\quad\ |\qquad\qquad\qquad\qquad |
\end{array}
$$

$$
\begin{array}{l}
\quad\ \ |\\
\quad NH\\
\quad\ |\qquad\ O\ H\\
\quad\ \ \ \ \ \ \ \ \ \ \|\ |\\
H\text{-}C\text{-}CH_2\text{-}S\text{-}P\text{-}OH \longrightarrow\\
\quad |\qquad\ \|\ \diagdown\\
\quad\ \ \ \ \ \ \ \ \ \ O\ {}^+OH\\
\quad C{=}O\\
\quad\ |
\end{array}
\quad
\begin{array}{l}
\quad\ \ |\\
\quad NH\\
\quad\ |\\
\quad C{=}CH_2 \ + \ SO_2 \ + \ H_3PO_2\\
\quad\ |\\
\quad C{=}O\\
\quad\ |
\end{array}
$$

The oxidised residues needed for SO_2 production may

possibly be formed *in situ* by reaction of the

positively charged phosphonium intermediate formed on

reaction of H_3PO_2 with cystine via nucleophilic attack

with water, initially forming a monoxide and cysteine.

(F)

```
      |                                      |          |                        |
      NH             OH                      NH         NH        O             NH
      |            ⌒ /                        |          |        ‖             |
   H-C-CH2-S-P-OH    +  ⁻SCH2-CH  ⟶  H-C-CH2-S-H  +  HS-CH2CH
      |       ↑ \                            |          |                        |
      C=O    ( + \H                          C=O        C=O                      C=O
      |       O-H                            |          |                        |
              `H                                                  + H3PO2
```

A dioxide may be formed in a similar manner by reaction
of the monoxide charged phosphonium intermediate with a
second molecule of water. The dioxide formed may then
undergo a proton elimination to form sulphur dioxide,
dehydroalanine and the inert phosphorous acid, as
follows. (G) This elimination reaction is believed to
occur in acid media. (See sec. 3:2)

(G)

```
      |                                          ┝
      NH            O        H   OH               NH
      |            ‖          \ /                 |
   H-C-CH2-S-H   +   :P         ⟶   C=CH2   +   SO2 + 2H⁺ + H3PO2
      |            ‖          / \                 |
      |            O        OH                    |
      C=O                                         C=O
      |                                           ¦
```

This process may explain the slow rate of formation of
sulphur dioxide from cystine which unlike wool should
not contain any original oxidised residues.

According to Savige and Maclaren [66] cysteic acid, if
formed, may however react with thiol (produced as
before in a reaction with cystine or perthiocysteine)
to afford cystine, water and sulphur dioxide, which may
be a reaction pathway to SO_2 production in wool. Though
no H_2S or SO_2 was produced from the reaction of H_3PO_2
with pure cysteic acid, this reaction may possibly

proceed in wool keratin where a cysteic acid and a cysteine residue may be in close proximity to one another.

(H)

$$
\begin{array}{c}
| \\
NH \\
| \\
CH-CH_2-S-\overset{\overset{O}{\|}}{\underset{\underset{O}{}}{S}}-OH \\
| \\
C=O \\
|
\end{array}
\;+\;
\begin{array}{c}
| \\
NH \\
| \\
CH-CH_2-S-H \\
| \\
C=O \\
|
\end{array}
\;\longrightarrow\;
\begin{array}{c}
| \\
NH \\
| \\
CH-CH_2-S-S-CH_2-CH \\
| \\
C=O \\
|
\end{array}
\begin{array}{c}
| \\
NH \\
| \\
\\
| \\
C=O \\
|
\end{array}
\;+\; SO_2 \;+H_2O
$$

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4:1 Introduction to the Dyeing of Wool.

When yarn or fabric is immersed in the dye solution,
then the contact between textile material and solution
causes the dye to migrate from solution to fibre until,
finally, a high proportion of the dye is transferred
from outside to the interior of the fibre. In this
process the dye is selectively extracted from the
solution by the fibre; the dye is said to be '
substantive '. [1] The wool fibre has two main
components, the cuticle and the cortex. (See sec 2:1)
The cuticle is an overlapping scalar structure which is
of prime importance in dyeing. The epicuticle or outer
layer of these scales is believed to be composed of
fatty acid residues and is therefore hydrophobic,
providing a barrier to the water and dye molecules. Dye
enters the fibres most readily by diffusion through the
intercellular cement between the scale cells. The
endocuticle and then the exocuticle become coloured as
dye travels through the intercellular cement and
penetrates the cells.

The principal absorption sites for dyes appear to be
within the high-sulphur matrix proteins. It is in these
regions that the fibre provides locations with a
mixture of polar and nonpolar interactions for which
the dyes have an affinity. [38]

A   B

1 Cuticle cell
2 Exocuticle
3 Endocuticle
4 Intercellular cement
5 Orthocortical cell
6 Paracortical cell
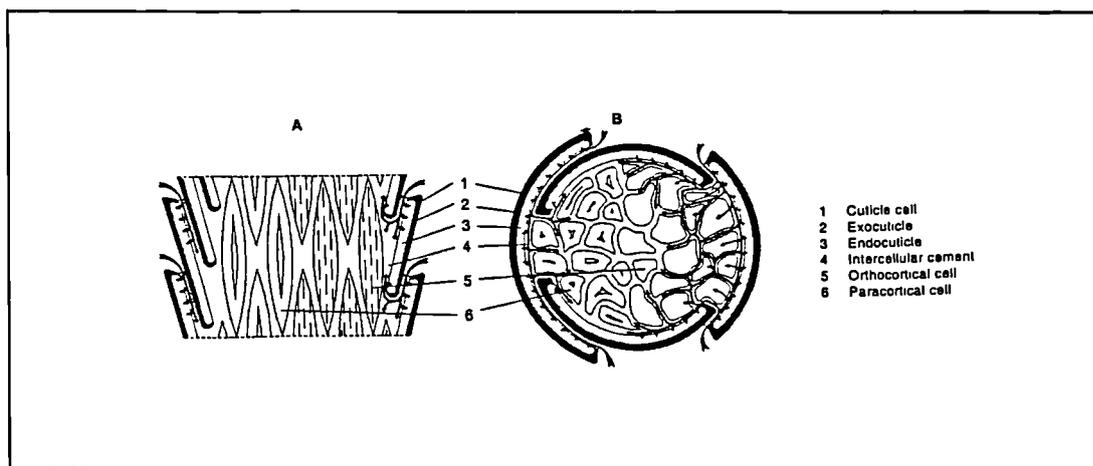
Figure 4:1 Diagram showing the Diffusion Pathways in a Merino Wool Fibre. [39]

The bulky side chain amino acids of the wool keratin (See sec 2:1:2) do not facilitate close approach of adjacent polypeptide chains. Therefore the fibres are predominantly amorphous (80%) and are relatively easily penetrated by the dye molecules. [3]

The acidic and basic side chains are the most important groups in relation to the dyeing of wool. The basic groups arginine, lysine and histidine provide sites for anionic (acid) dyes. N-terminal and C-terminal amino acids are also present but only in relatively small amounts and contribute little to the acidic and basic character of wool. The amine and carboxyl groups of the polyamide backbone may also play a role in wool dyeing.

Four main types of attraction between dye and fibre are known to exist. The first is an ionic electrostatic attraction, which is the predominant contributor to dye adsorption. On immersion in an acid dyebath, the rapidly diffusing protons are adsorbed onto the

carboxyl groups in the fibre, neutralising the charge
on the carboxyl groups and protonating the basic amino
groups. [6],[7] The small molecular size acid anions
(X^-) are adsorbed onto the protonated amino groups. The
fibre is left with a net positive charge and attracts
any anions, e.g. sulphonate or dye anions, which are
present in the solution. Owing to their greater
affinity (due to Van der Waals forces), the larger
molecular size, slower diffusing dye anions (if acid
dye) displace the adsorbed acid anions from the
protonated amino groups, as follows:

$$Wool\text{-}NH_3^+ \ {}^-OOC\text{-}Wool \ + \ H^+X^- \ + \ DyeSO_3^-Na^+ \longrightarrow$$

$$Wool\text{-}NH_3^+ \ HOOC\text{-}Wool \longrightarrow Wool\text{-}NH_3^+ \ HOOC\text{-}Wool$$
$$X^- \qquad\qquad\qquad\qquad DyeSO_3^-$$

Dyed wool.
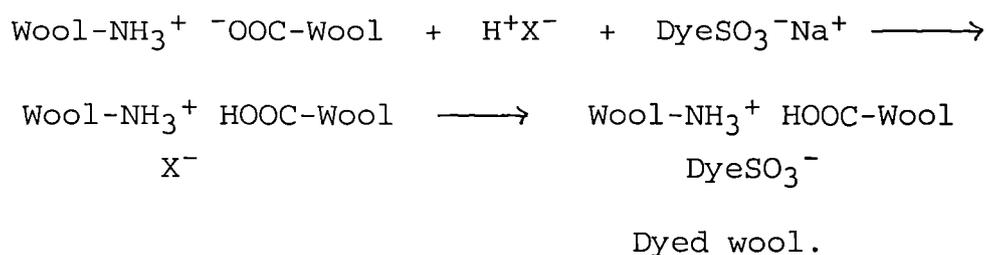
The acid binding capacity of wool has been estimated to
be between 810-860 $molg^{-1}$, and is consistent with the
sum of the aspartic acid, glutamic acid and C-terminal
residue contents of wool.[8] Acid uptake reduces the
cohesion between peptide chains by discharging
carboxylate anions. The acid may also readily form
hydrogen bonds with peptide and other functional groups
in wool, and in this way promote fibre swelling. It has
been reported recently that concentrated formic acid
swells the intercellular cement by as much as 1000%,
which may also account for the observed increase in
rate of dyeing. [39] The quantity of H^+ combined at a

given pH is highly dependent on the nature of the anion; the more complex it is the greater its affinity for the fibre. As the pH decreases the number of H^+ ions adsorbed increases. Dibasic acids have an increased affinity for wool, relative to the corresponding monobasic acid, due to an increase in electrostatic attraction. [8]

Four main reactions occur during treatment of proteins with acid: (a) cleavage of amide side chains, (b) cleavage of peptide bonds, (c) N $\longrightarrow$ O (acyl shift) migration at serine and threonine residues and (d) disulphide interchange.

Complete acid hydrolysis of the wool fibre amide and peptide bonds may occur under prolonged treatments using concentrated acid and temperatures greater than $100^{\circ}C$. [8]

$$R-C(O)-NHR + H_3O^+ \longrightarrow R-\underset{\underset{NHR}{|}}{\overset{\overset{OH}{|}}{C}}-OH_2^+ \longrightarrow R-C(O)-OH + RNH_3^+$$
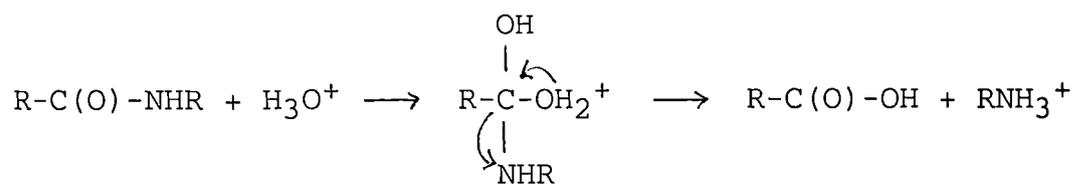
The reaction of sulphuric acid with wool causes an acyl shift at serine (R=H) and threonine ($R=CH_3$) residues. [10],[11]

$$\underset{\displaystyle -C(O)NHCHC(O)-}{\overset{\displaystyle HOCHR}{\overset{|}{}}} \quad \underset{OH^-}{\overset{H^+}{\longrightarrow}} \quad -C\underset{\displaystyle ^+NH-CHC(O)^-}{\overset{\displaystyle O-CHR}{\diagdown\!\!\diagup}} \quad \longrightarrow \quad \underset{\displaystyle NH_3^+}{\overset{\displaystyle C(O)OCHRCHCO-}{}}$$
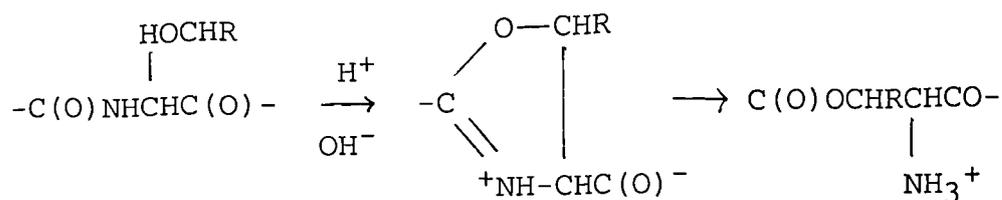
The slow sulphonation of the hydroxyl groups of tyrosine and phenylalanine to form alkyl sulphates ($R-SO_4H$) on treating both the free amino acids and wool with sulphuric acid has been reported. [12], [13]

This ionic force may be augmented by the second attractive force of Van der Waals. Quantum mechanical considerations demonstrate that two chemically inert molecules attract one another with the so called London force which is a type of Van der Waals interaction. The strength of the force is proportional to the area of possible contact and therefore presupposes that larger flatter dye molecules tend to be most firmly held. [4]

It is not known to what extent (if any) hydrogen bonds form a part of the attraction between dyes and wool. They may help dye adsorption by forming a linkage between hydroxyl and amine groups in dyes with the carboxyl group oxygen of the wool's amide bond. e.g.

$$\underset{R'}{\overset{R}{\diagdown}}C=O\ldots.H-\underset{H}{\overset{}{N}}-Dye$$
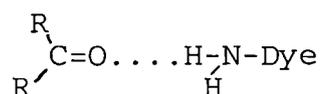
The fourth type of attraction depends on the dye type. Reactive dyes bind covalently to wool fibres. The

resulting bond augments all other binding forces and interactions. These dyes are capable of reacting with functional side chains or terminal amino groups either by nucleophilic displacement (e.g. dichlorotriazine) or by a Michael addition reaction. e.g. with a vinylsulphone dye where X = NH,O or S: [4]

$$\text{Wool-XH} \quad + \quad CH_2{=}CHSO_2\text{-Dye} \quad \longrightarrow \quad \text{Wool-}XCH_2CH_2SO_2\text{-Dye}$$
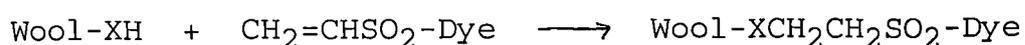
With the exception of disperse dyeing, (where the dye has poor water solubility, and is applied in the form of a fine suspension, which gradually dissolves in the water as dye is absorbed by the fibre)[40] dyeing methods are dependent on sorption processes, preceded by transport phenomena (mainly diffusion). The dyeing process i.e. the distribution of a dye between at least two phases (dyebath and substrate) can be described by dyeing kinetics (transport and reaction phenomena) and dyeing statics (sorption and desorption process in the state of equilibrium, i.e. where adsorption and desorption occur at the same rate with no net transfer of dye from one phase to another). [1]

The rate of dyeing controls the degree of levelness and penetration achieved by the dye. This rate may be described by three separate stages, (a) transfer of the dye from the dyebath to the wool surface (b) adsorption of the dye at the fibre surface and (c) diffusion of the dye from the surface to the interior of the fibre.

The adsorption rate of the dye may be controlled
through its own substantivity for the fibre or through
the desorption rate of another substance, a retarder
such as Glauber's salt. (Sodium sulphate decahydrate
($Na_2SO_4.10H_2O$)). This retarder competes with
sulphonate ions of anionic dyes for the amine sites on
the wool surface. This exerts a levelling action
reducing the speed of dyeing which in effect achieves a
more uniform penetration of the fibre.

4:1:1 Classes of Dye used in Wool Dyeing.

Dyes are classified by chemical structure, the most
important of which for wool dyeing (>50% of dyes
used) are azo dyes.[1] The azo dyes have no natural
counterparts and contain the trans form of the -N=N-
azo group with aromatic groups attached. e.g. Fast
Garnet GBC Base (o-aminoazotoluene, 4'-amino-2,3'-
dimethylazobenzene, C.I. 11160).

Azo and other dyes may be acid (anionic) or basic
(cationic) dyes. If the dye is an acid dye it is
generally used not in the form of free acid but in that
of its sodium salt. The presence of sulphonic acid
groups in the dye ($-SO_3^{-+}Na$) confers water
solubility. Their rate of dyeing is rapid and although
they may be unlevel at the start of dyeing due to rapid
initial strike, they readily migrate from the deeper
dyed parts of the wool to the paler parts via the
dyebath solution. These dyes are very much influenced

by the presence of salts in the dyebath which effect

the equilibrium between the dye present in the dyebath

and the dye on the fibre, favouring migration of dye to

the wool fibre.

Other important classes of dye include (a)

anthraquinone dyes (all important natural red dyes,

and some level dyeing blues e.g. Lissamine Blue B),

(b) triphenylmethane dyes (Patent Blue V), (c)

xanthene dyes (e.g. Fast Acid Violet ARR) and (d)

reactive dyes as before.

4.2 Modification of Wool by Reaction with Acids, Formaldehyde and Acid/Formaldehyde Mixtures.

The dyeing properties of wool that has been pretreated

with solutions of acid, formaldehyde and

acid/formaldehyde are investigated in the following

section. The surface of the pretreated wool samples are

examined using diffuse reflectance spectroscopy. The

subsequent lightfastness and washfastness properties of

the dyed wool samples are also investigated.

4:2:1 Introduction to the Reaction of Wool with Formaldehyde.

The reaction of wool's constituent amino acids with

formaldehyde (CH_2O) under varying pH, concentration

and temperature is extremely complex and to date not

fully understood. Although most evidence is indirect,

it is believed that crosslinks are introduced into both

the matrix and microfibrillar regions of the fibre of both mono and bi-functional type. Formaldehyde may also undergo self-condensation leading to oxymethylene ($-CH_2(OCH_2)_n-$) and methylene ($-CH_2-$) crosslinks. The hypothesis that has received the most general acceptance is that formaldehyde sets up crosslinks by secondary condensation of amino-methylol groups (formed on initial reaction of the primary amine groups on the side chains of the amino acids lysine, arginine and asparagine [14],[15]) and other reactive hydrogen atoms similar to that which occurs in the Mannich reaction. [16],[17]

$$-NHCH_2OH \quad + \quad HR \quad \longrightarrow \quad -NH-CH_2-R \quad + \quad H_2O$$
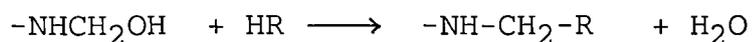
At neutral to mildly acidic pH (6-7), and at both room and elevated temperature, formaldehyde has been reported to react with the aliphatic hydroxyl groups of serine and threonine [14]. However the fact that silk fibroin binds very little CH_2O casts doubt on this, as fibroin is rich in both serine and tyrosine. [18] Tyrosine binds through addition at the 2,6 positions; this compound may subsequently react with lysine forming a new crosslink which is unstable to acid. [19] Thiazolidine-4-carboxylic acid is a product of the reaction of formaldehyde and wool cysteine, other products of the reaction have not been determined, with

approximately half the reacted sulphur unaccounted for. (22),(23)

Under acidic pH (1-3) formaldehyde combines with the primary amide groups which are attached to the residues of glutamic and related acids, to a greater extent than at higher pH. [25],[26] This reaction involves the amino group and results in the formation of methylol and methylene derivatives. The nature of the reactions and the properties of the products formed are modified by the carbonyl group adjacent to the amine.

$$-NH_2COR \quad + \quad CH_2O \quad \longrightarrow \quad HOCH_2NHCOR$$

In acid solution the conversion of formaldehyde derivatives to methylene links is very efficient. [27]

4:2:2 Experimental Procedures

Acid Pretreatment:

100%, 50%, 20% and 10% o.w.f of individual solutions of H_2SO_4, H_3PO_2 and H_3PO_3.

10g of wool, at pH 1, 80°C, 6 hours.

Liquor to wool ratio 60:1.

Formaldehyde Pretreatment:

100%, 50%, 20% and 10% o.w.f., of formaldehyde solution. 10g of wool, at pH 7.4, at 80°C for 6 hours.

Liquor to wool ratio 60:1.

Acid/Formaldehyde Pretreatment:

100%, 50%, 20% and 10% o.w.f., of individual solutions of H_2SO_4, H_3PO_2 and H_3PO_3 in the presence of 100% o.w.f formaldehyde.

10g of wool, at pH 1, at 80°C for 6 hours.

Liquor to wool ratio 60:1.

After treatment all samples were washed thoroughly with water for two minutes and then oven dried at 60°C.

The pretreated wool samples were dyed under various conditions with acid levelling, basic and reactive dyes. Two types of dyeing experiment were carried out;

Method A:

The samples were dyed with a control experiment being run simultaneously using an untreated sample in a separate dyebath.

Method B:

Competative dyeing studies were carried out by having the treated and untreated sample in the same dyebath.

Low temperature dyeings were undertaken in a 'Jefferys' dyeing machine under the following experimental conditions:

Method A

0.5 % o.w.f dye

10g of wool. Liquor: Wool 60:1

Temperature Programme; 40°C for 10 mins.

$$40°C \longrightarrow 60°C \quad 1°min^{-1}$$

60°C for 1 hour

$$60°C \longrightarrow 40°C \quad 1° \, min^{-1}$$

Method B

1 % o.w.f. dye

5g pretreated wool/ 5g blank wool per dyebath

Liquor: Wool   60:1

Temperature Programme; 40°C for 10 mins.

$$40°C \longrightarrow 60°C \quad 1°min^{-1}$$

60°C for 1 hour

$$60°C \longrightarrow 40°C \quad 1° \, min^{-1}$$

Three different acid levelling dyes were used, as
follows;
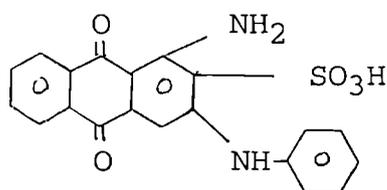
C.I. Acid Blue 62
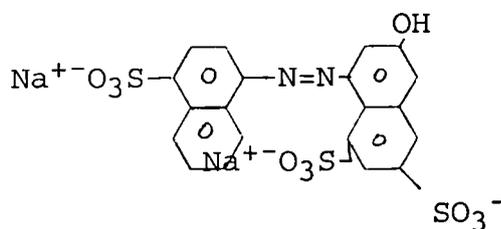
C.I. Acid Red 18
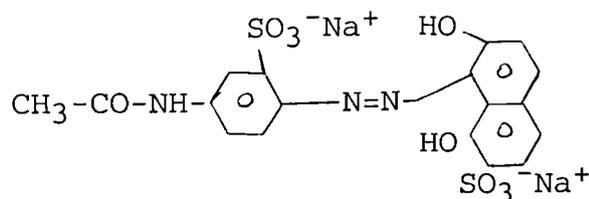
C.I. Acid   Red 37

Two basic (cationic dyes);
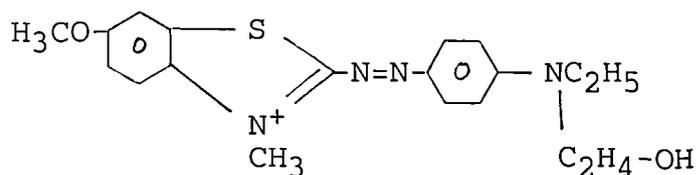
C.I. Maxilon Blue GRL
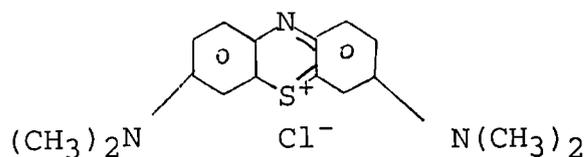
C.I. Methylene Blue

and the reactive dye;
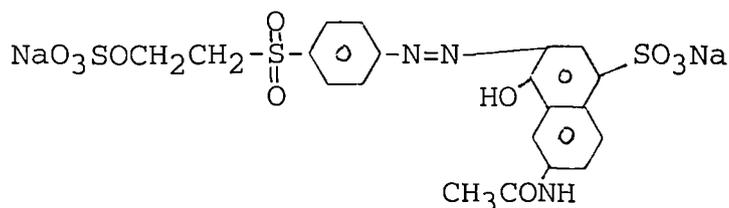
C.I. Reactive Orange 16

4:2:3   The Surface Infra-Red Spectra of Acid, Formaldehyde and Acid/Formaldehyde Pretreated Wool.

Infra-red spectroscopy, which supplies detailed information on the chemical structure of compounds, can be converted into a surface sensitive technique by reduced total internal reflection. The reflection technique succeeds in practice not only because of its simplicity but also multiplicity, as the sample is located on both sides of a thallium iodide (KRS5) crystal, which has a trapezoidal cross section. [28] The absorbed infra-red is reflected internally by the crystal due to its shape and refractive index value.

The wool sample absorbs some of this light to a certain depth (i.e. the surface of the sample), and the difference in the transmittance of the infra-red radiation entering the crystal and that emerging is a measure of the absorbance of the surface of the sample.

The pretreated sample's spectra differ markedly from those of untreated wool. The surface I.R. spectrum of hypophosphorous acid (H_3PO_2) treated wool is very similar to that of a 50% solution of H_3PO_2 on silver chloride plates. (See Sec 3:2:1) IR stretching vibrations at approx. 2400 cm^{-1}, 1170 cm^{-1}, 1040 cm^{-1} and 800 cm^{-1}, correponding to P-H (v), P=O (v), PO_2^- (vas) and possibly P-OH are clearly visible. The spectrum of the reaction of wool with H_3PO_2/CH_2O solution shows a disappearance of the P-H peak at 2400 cm^{-1} and a diminished peak at 800 cm^{-1}, all other peaks are unaltered. (See arrows on fig. 4:2)

Thiol is produced by the reaction of H_3PO_2 with wool (See Sec 3:3), however, the poor dipole moment between the sulphur and hydrogen makes the vibration I.R. inactive and therefore no thiol peak(s) are visible in the spectrum. (See fig. 4:2) The difference in the spectra of H_3PO_2 and H_3PO_2/CH_2O pretreated wool indicates that a different reaction may occur between the two solutions and wool.
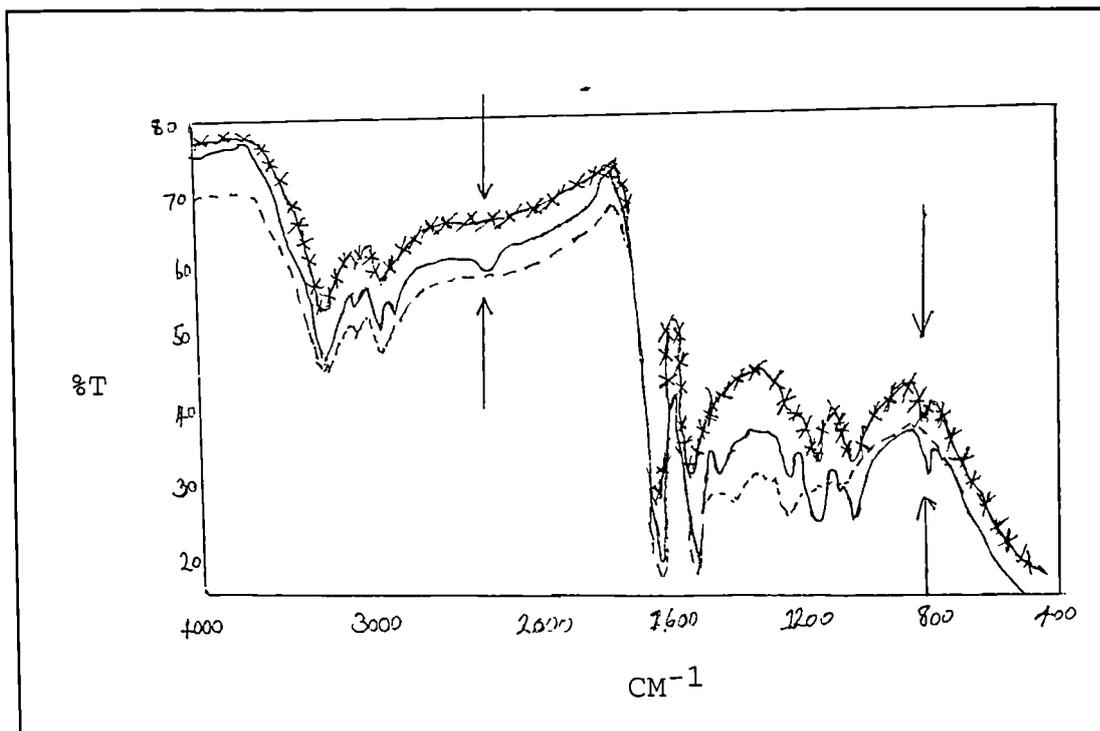
Figure 4:2 Surface I.R. spectra of wool, H_3PO_2 pretreated wool and H_3PO_2/CH_2O pretreated wool.
- - = wool, ——— = H_3PO_2 wool and -*-*- = H_3PO_2/CH_2O wool

The surface I.R. spectra of H_3PO_3 and H_3PO_3/CH_2O pretreated wool are both the same, with peaks at 1170 cm^{-1}, 1060 cm^{-1}, a shoulder at 1020 cm^{-1} and a small peak at approx 950 cm^{-1}; corresponding to P=O (v), PO_2^- (vs), $P(OH)_2$ (vas) and $P(OH)_2$ (vs) respectively. (See fig. 4:3)

The spectra of H_2SO_4 and H_2SO_4/CH_2O pretreated wools are also identical. Peaks are clearly visible at 1180 cm^{-1}, 1040 cm^{-1} and a weak band at 600 cm^{-1}. These bands correspond to sulphonic acid group SO_3H and ionic sulphite SO_3^- respectively. [29] (See fig. 4:4) The pretreatment of wool with formaldehyde on its own shows

no notable difference in its I.R. spectrum to that of
untreated wool.
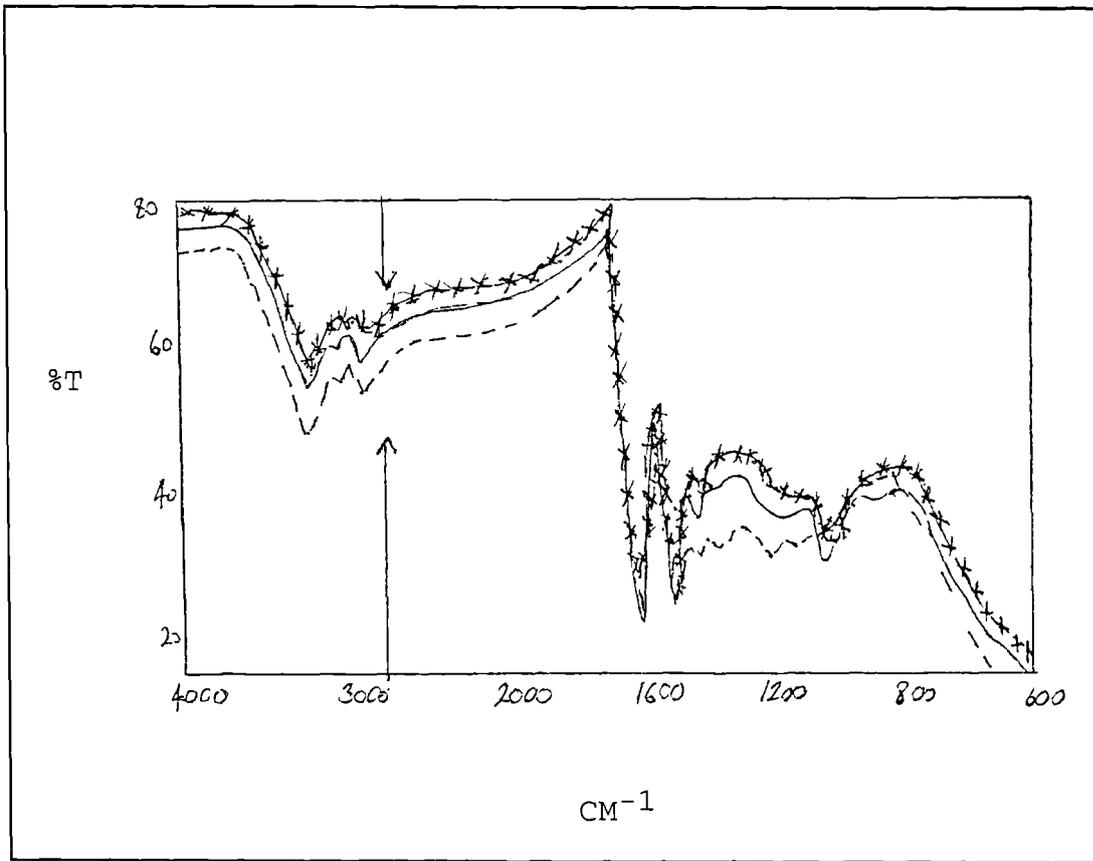
Figure 4:3 Surface I.R. spectra of wool, H_3PO_3
pretreated wool and H_3PO_3/CH_2O pretreated wool.
- - = wool, —— = H_3PO_3 wool and -*-* = H_3PO_3/CH_2O
wool.

Although quantitative measurements are difficult with
this technique, a decrease in the amide content of the
wool (peaks at 1600 cm^{-1}, 1500 cm^{-1} and 1370 cm^{-1})
was noted for all pretreatments, (except that of
formaldehyde) using the aliphatic C-H stretching
frequency at 2950 cm^{-1} as an internal standard.
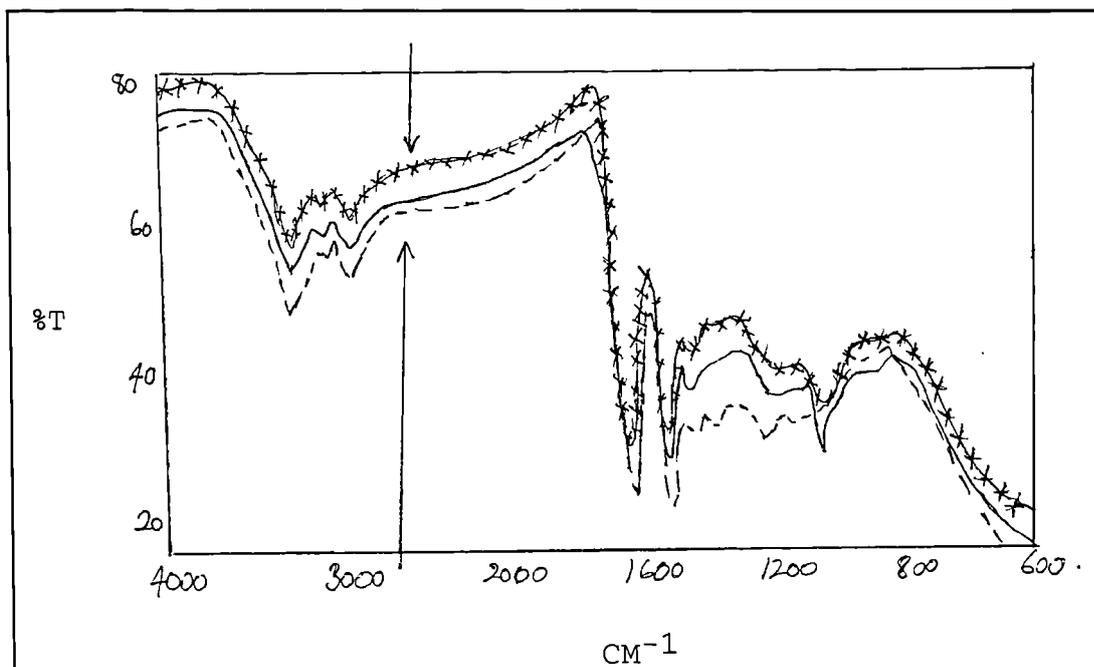
Figure 4:4 The surface I.R. spectra of wool, H_2SO_4 pretreated wool and H_2SO_4/CH_2O pretreated wool.
- - = wool, —— = H_2SO_4 wool and -*-* = H_2SO_4/CH_2O wool.

4:2:4 Results of the Dyeing of Acid, Formaldehyde and Acid/Formaldehyde Pretreated Wool.

	H_3PO_2		H_3PO_3		H_2SO_4	
	No CH2O	CH2O	No CH2O	CH2O	No CH2O	CH2O
Acid dye Uptake	Good	Poor	Good	Good	Good	Good
Basic dye Uptake	Poor	Good	Poor	Poor	Poor	Poor

Figure 4:5 Table of the competitive dyeing results of the uptake of dye using acid and acid/formaldehyde pretreated samples.

The results of this study confirmed reported results (39) showing that all the acid pretreated wool samples

gave an increase in rate of uptake of acid dye relative to an untreated blank, and in competitive dyeing studies preferentially adsorbed the acid dye. A corresponding decrease in the rate of uptake of basic dye and a resistance to its adsorption in competitive studies was noted. It was noted that for acid dyes a 'rapid strike' had taken place as all the acid pretreated samples were very unevenly dyed. The pH of the acid pretreated wool is very low (in fact the acid leaches out of the pretreated samples decreasing the pH of the dyebath to 1-2), resulting in a rapid uptake of the acid dyes by the wool, with no apparent subsequent migration of dye.

The acid pretreated wool's surface I.R. spectra, showed a decrease in amide bond content indicating that a certain amount of hydrolysis of the wool fibre had taken place. (See sec 4:2:3) This hydrolysis opens up the wool structure making it more accessible to dye molecules and may also produce more amine groups which will increase the uptake of acid dye by the sample.
(See Sec 4:1)

The formaldehyde pretreated samples resisted acid dye in both Method A and Method B experiments and preferentially bound basic dye. This is due to the crosslinking of the acid dye binding sites, i.e. the amine groups of wool by formaldehyde, which leads to the formation of methylol groups, which then undergo crosslinking. (See Sec 4:2:1)

The H_3PO_3/CH_2O and H_2SO_4/CH_2O samples behaved in a similar manner to the H_3PO_3 and H_2SO_4 pretreated samples. In competitive studies they preferentially adsorbed acid dye and in rate experiments adsorbed the acid dye faster than an untreated blank. The samples had not undergone a 'rapid strike' however, and were evenly dyed. These dyeing results are similar to those for wool pretreated with tetrakis(hydroxymethyl) phosphonium chloride ($(HOCH_2)_4P^+Cl^-$). It has been suggested that this reaction introduces the positively charged phosphonium ion into the fibre which subsequently increases the uptake of acid dye. (31),(32)

$$W\text{-}SH + (HOCH_2)_4P^+Cl^- \longrightarrow W\text{-}S\text{-}CH_2\text{-}P^+(CH_2OH)_3Cl^- + H_2O$$
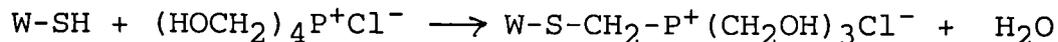
This suggestion has been refuted by other authors who conclude that a zwitterionic intermediate is formed which increases the rate uptake of both acidic and basic dyes. (33)

Treatment of wool by H_3PO_3/CH_2O and H_2SO_4/CH_2O may lead to the ' knitting together ' of hydrolysed wool samples by crosslinking via CH_2O, with a small excess of acidic groups being left on the surface. As a consequence the uptake of acid dyes is less efficient than the samples treated solely with acid. The remaining acid groups on the surface of the wool will repel cationic (basic) dyes thereby causing a resist effect. The surface I.R.

spectra of H_3PO_3/CH_2O, H_2SO_4/CH_2O and their respective acids are similar, which probably indicates that the treatments have not led to substantial crosslinking.

The formaldehyde may be bound reversibly and most of it may be removed by washing after pretreatment. The exchange of any reversibly bound formaldehyde at the active amine binding sites may also be responsible for the even dye uptake.

The dyeing behaviour of H_3PO_2/CH_2O pretreated wool is different to that of the other acid/formaldehyde pretreatments. This pretreatment leads to a faster rate of uptake of acid dye relative to that of an untreated blank, but in competitive dyeing studies the treated wool samples resisted the acid dye and preferentially adsorbed the basic dye. This resistance to acid dye and attraction of basic dye was strongest for the 100% H_3PO_2/100% CH_2O (o.w.f.) pretreatment and least for the 10% H_3PO_2/100% CH_2O. (See fig. 4:6) i.e. an increase in acid dye uptake and a decrease in basic dye uptake is observed with a decrease in the $[H_3PO_2]$: $[CH_2O]$ ratio.
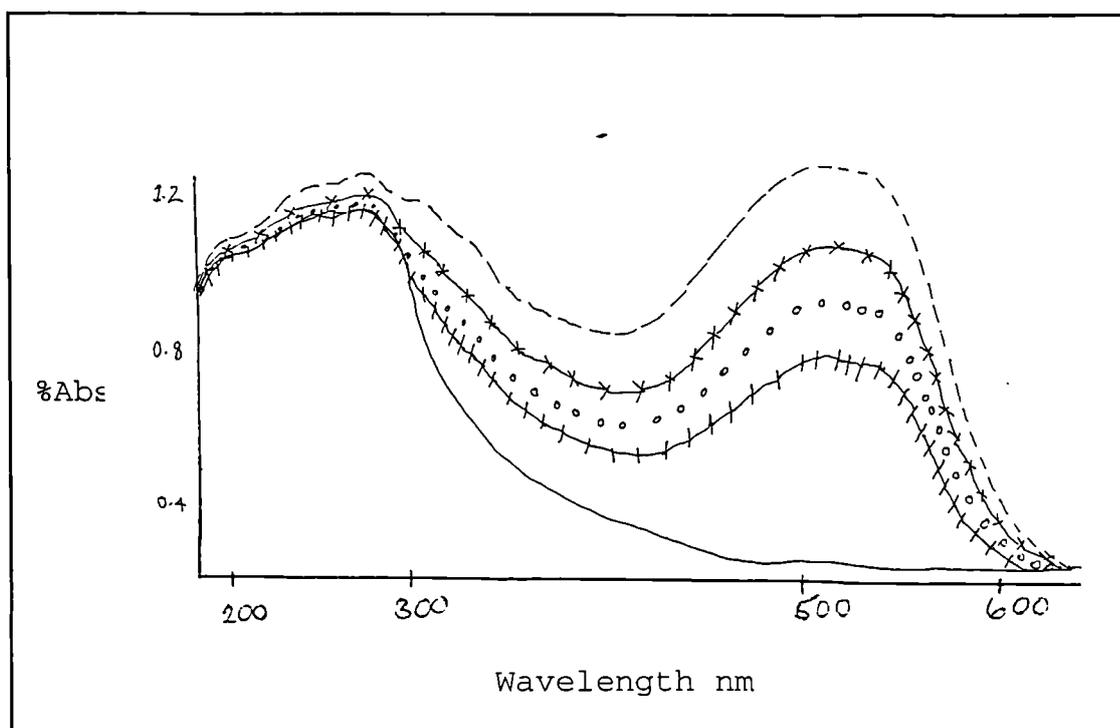
Figure 4:6 Diffuse reflectance spectra of blank wool, competitively dyed with H_3PO_2/CH_2O pretreated wool using Acid Red 37.

—— = blank wool alone, - - = $100\%H_3PO_2$ / $100\%CH_2O$, -*-* = $50\%H_3PO_2$ / $100\%CH_2O$, ... = $20\%H_3PO_2$ / $100\%CH_2O$ and ++++ = $10\%H_3PO_2$ / $100\%CH_2O$.

The fluorescence spectrum of the H_3PO_2/CH_2O pretreated sample indicates that rupture of the disulphide bond of cystine (presumably by reaction with H_3PO_2) occurs under these conditions. (See Sec 3:5) This opening of the wool structure should facilitate dye uptake. It is not known if H_2S and SO_2 are produced on reaction of the disulphide in the presence of formaldehyde. The surface I.R. spectrum shows a disappearance of the P-H bond stretch, suggesting a possible reaction between formaldehyde and hypophosphorous acid. e.g.

$$\text{OH} \diagdown \diagup \text{H}$$
$$P: \quad + \quad \overset{H}{\underset{H}{\diagdown}}C=O \quad \longrightarrow \quad HO-\overset{OH}{\underset{H}{\overset{|}{P^+}}}-CH_2O^-$$
$$\text{OH}\diagup$$

$$HO-\overset{OH}{\underset{H}{\overset{|}{P^+}}}-CH_2O^- \quad \longrightarrow \quad HO-\overset{OH}{\overset{|}{P}}-CH_2OH \quad \overset{H^+}{\longrightarrow} \quad OH-\overset{OH}{\overset{|}{P}}-CH_2^+$$
$$ \qquad\qquad (A)$$

$$OH-\overset{OH}{\overset{\diagdown}{P}}-CH_2^+ \quad \longrightarrow \quad OH-\overset{OH}{\overset{\diagdown}{P^+}}=CH_2$$

$$OH-\overset{OH}{\overset{|}{P^+}}=CH_2 \quad + \quad NH_2-WOOL \quad \overset{-H^+}{\longrightarrow} \quad OH-\overset{OH}{\overset{|}{P}}-CH_2-NH-WOOL$$
$$ \qquad\qquad\qquad (B)$$

$$OH-\overset{OH}{\overset{|}{P^+}}=CH_2 \quad + \quad SH-WOOL \quad \overset{-H^+}{\longrightarrow} \quad OH-\overset{OH}{\overset{|}{P}}-CH_2-S-WOOL$$
$$ \qquad\qquad\qquad (C)$$
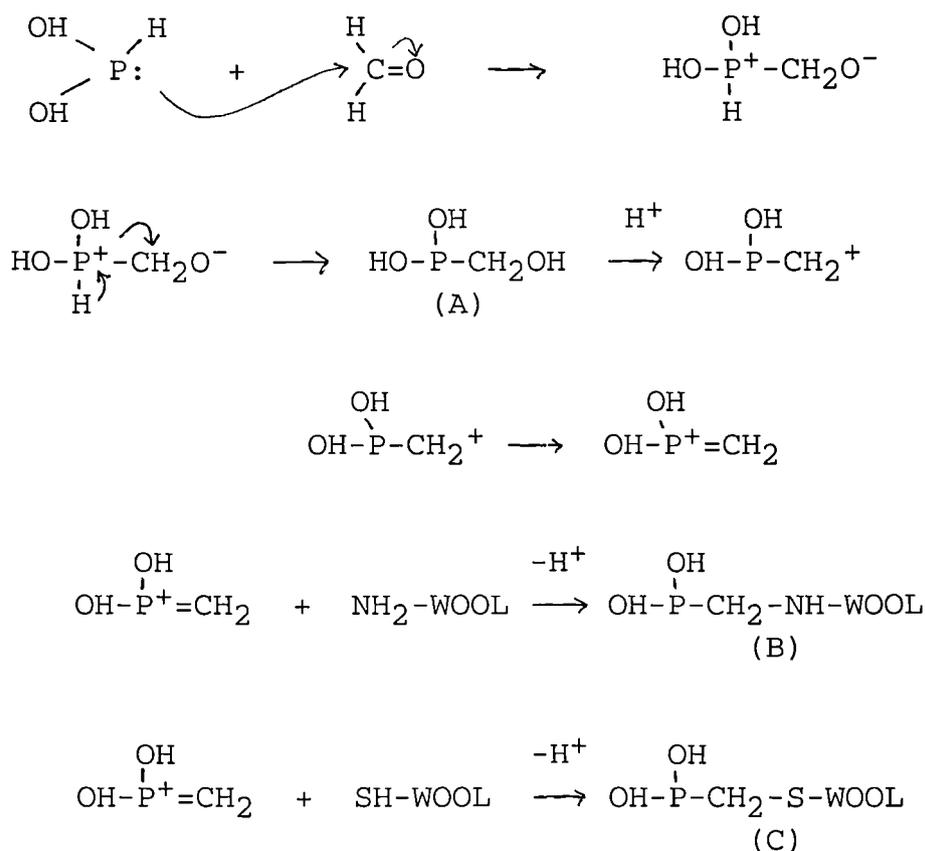
Scheme One

The proposed reaction scheme above involves the intermediate (A), which neither a reaction of H_3PO_3 or of H_2SO_4 and formaldehyde could produce. Many of the intermediates in this scheme are positively charged (pH of solution is 1), and may aid the rate of uptake of acid dye relative to an untreated blank by attracting the negatively charged acid dye anions. At the same time the positively charged basic dye cations are repelled.

The level dyeing result obtained with the H_3PO_2/CH_2O pretreated sample may be due to the decreased amount of acid on the wool surface due to its reaction with

formaldehyde. The acid binding sites of amine and thiol
are however unavailable for reaction with dye molecules
due to the formation of the covalently bonded final
products (B) and (C), and this may account for the
pretreated samples resistance to acid dye in the
competitive study.

The proposed intermediate (A) may undergo a Buckler-
Trippett [35] rearrangement, which is peculiar to
tertiary phosphines containing a hydroxyalkyl
substituent, on heating by itself or in the presence of
an acid catalyst.

$$\underset{\text{OH}}{\text{OH-P-CH}_2\text{OH}} \quad \xrightarrow{\text{H}^+} \quad \underset{\substack{\text{OH}\\ \text{CH}_3}}{\text{OH-P=O}}$$
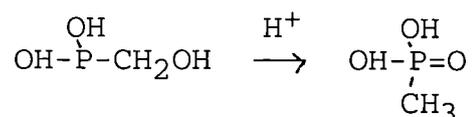
The alkyl phosphine oxide product contains a phosphoryl
bond which may aid the uptake of basic dye if it is
present on the wool surface.

A similar reaction giving identical products to Scheme
One may occur by reaction of the wool amine or thiol
with formaldehyde. Loss of water by (A) follows in acid
media and the tertiary H_3PO_2 may attack this species to
give products (B) and (C). [34]

$$\text{WOOL-NH}_2 + \text{CH}_2\text{O} \xrightarrow{\quad} \underset{\text{(A)}}{\text{WOOL-NHCH}_2\text{OH}} \xrightarrow{\text{H}^+} \overset{+}{\underset{\text{H}}{\text{WOOL-NHCH}_2\text{OH}}}$$

$$\text{WOOL-NHCH}_2\overset{+}{\underset{\underset{H}{|}}{\text{OH}}} \longrightarrow \text{WOOL-NH=CH}_2{}^+ \longrightarrow \text{WOOL-}\overset{+}{\text{NH=CH}}_2$$

$$\text{WOOL-}\overset{+}{\text{NH=CH}}_2 + \ :\overset{\underset{H}{|}}{\underset{}{\text{P}}}\text{-OH} \xrightarrow{-H^+} \text{WOOL-NH-CH}_2\text{-}\overset{\overset{OH}{|}}{\underset{}{\text{P}}}\text{-OH}$$
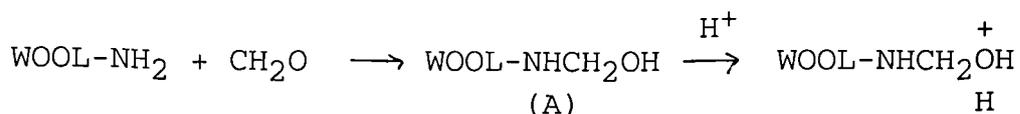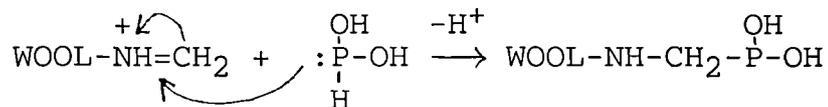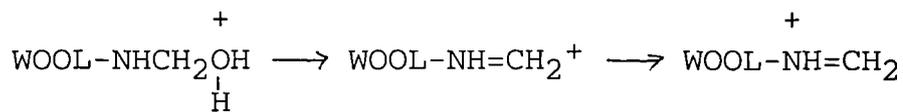
Scheme Two

Similar dyeing results to that of the H_3PO_2/CH_2O

pretreatment have been reported recently for wool that

has had acid-phosphate groups introduced to its

surface, via reaction with phosphorus trichloride

(PCl_3), according to the following mechanism. [42]

$$\text{WOOL-XH} \xrightarrow{PCl_3} \text{WOOL-X-P(}Cl_2\text{)} \xrightarrow{H_2O} \text{WOOL-P(O)(OH)}_2$$
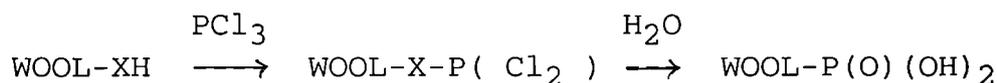
4:3 Lightfastness and Washfastness of Dyed, Pretreated Wool.

It is a fundamental requirement that dyed textiles

withstand the conditions encountered during processes

following dyeing and during their subsequent useful

life. Fastness is defined as the resistance of the

material to change when subjected to a particular set

of conditions (e.g. water or light).

Colour fastness with respect to change is based on

assessing the visual contrast between two coloured

areas, one being the original material and the other a

tested specimen. The contrast is specified as a number

on a grey scale, ranging between 5 (no contrast, and

therefore expressing maximum resistance to change) to 1 (large contrast, expressing little or no resistance to change).

Treatment	Acid Red 18		Reactive Orange 16	
	Wash	Light	Wash	Light
BLANK	1	2-3	2.5	3
H_3PO_2	1	5	2.5	3.5
H_3PO_3	/	/	2.5	3.5
H_2SO_4	/	/	2	3.5
CH_2O	/	/	4	3
H_3PO_2/CH_2O	1	4	1.5	3
H_3PO_3/CH_2O	1-2	4	2	3
H_2SO_4/CH_2O	2-3	4	2	3

Figure 4:7 Table of results of lightfastness and washfastness properties of acid, formaldehyde and acid/formaldehyde pretreated wool, dyed with Acid Red 18 and Reactive Orange 16.

For fastness to light, exposed samples are compared with the scale of eight standard blue wool cloths, which have been exposed simultaneously. They range from 1 (little or no lightfastness) to 8 (maximum achievable light fastness), on the scale used. [1] Both procedures were carried out at the I.W.S., using washfastness test IWS TM-193 (the minimum fastness

requirement for machine washable wool using this test is 3-4 [38]) and lightfastness test IWS TM 5 to Std 8.

The tests were carried out on pretreated wool samples that had been dyed with the acid levelling azo dye Acid Red 18 and and the reactive dye Reactive Orange 16. In general the washfastness of acid levelling dyes on wool leaves much to be desired, being of the order of 1-2 units. [2] With reference to figure 4:7 above, an improved washfastness to that of untreated wool is noted for the H_3PO_3/CH_2O and H_2SO_4/CH_2O but not H_3PO_2/CH_2O pretreated samples. [2] It is suggested in section 4:2:3 that H_3PO_2 and CH_2O react with each other in solution to give various positively charged intermediates bound to the wool surface. These intermediates may attract the azo dye anions but may also provide a barrier preventing further diffusion of the dye into the fibre. Consequently, the dye may be more easily removed by washing than the other acid/formaldehyde pretreatments.

A very high degree of washfastness is achieved using reactive dyes as these are covalently bound to the fibre and therefore differ from all other classes of dye. A large increase in the washfastness of wool pretreated with CH_2O is observed. The result is surprising in that many of the available sites of reaction on the wool surface amine, thiol groups, etc will have reacted with formaldehyde in the pretreatment step,(See Sec 4:2:2) and not be available for

reaction with the reactive dye. A similar decrease in the washfastness of the reactive dye on H_3PO_2/CH_2O pretreated sample as that of the azo dye is observed. This may be due to the reaction of the dye with the proposed intermediate $(OH)_2$-P-(CH_2OH), to form compound(s) which are not bonded directly onto the fibre surface and are subsequently easily removed by washing.

Surprisingly no increase in washfastness is observed for the H_3PO_2 pretreated sample. The thiol that is produced by reaction of H_3PO_2 and wool keratin (See sec 3:3), should act as an extra binding site for the dye molecule and further improve its stability to washing.

The lightfastness of azo acid dyes range from 3-5 units depending on the strucure of the dye. [2] The method of photodegradation of the dyes is not well understood, but photoxidation may play a part. It is generally recognised that an increase in the moisture content of dyed textiles reduces the lightfastness of the dye. One explanation for this phenomenon proposes that the swelling of the fibres by the water allows increased diffusion of oxygen and other substances that play a role in the fading process. [37] The H_3PO_2 pretreated wool sample showed an increase in lightfastness for both dyes.

The presence of the P-H bond on the wool surface may help minimise photoxidation, and therefore increase lightfastness. The crosslinking of the keratin by formaldehyde may improve both dyes lightfastness by leading to less diffusion of substances responsible for the photofading processes.

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5:1 Introduction to the Use of Aminoanthrylphosphonous Acids as Fluorescent Whitening Agents.

Fluorescent whitening agents (F.W.A.) are colourless compounds which when applied to wool improve the whiteness of the final product. [1] They absorb in the UV and emit in the blue region of the spectrum. This emission makes the fabric appear whiter because it neutralises the effect of the yellowness of the material and increases the total amount of visible radiation coming from it. [1] However, a major drawback with their use is that the F.W.A.s act as sensitisers, increasing the rate of photoyellowing of wool. In addition, they also accelerate the photodecomposition of tryptophan residues in wool, a process which always accompanies photoyellowing. [2] (See Sec 2:3:1) It has been shown that F.W.A.s act as sensitisers for the production of singlet oxygen ($^1O_2$) (see Sec 1:3), which may then react react with tryptophan residues located some distance away. [3]

A new approach to stabilizing F.W.A.s is to incorporate a reducing agent into their structure. Therefore a series of anthrylamino phosphonous acids were synthesised which contain a P-H bond. This functional group may alleviate photoyellowing by reducing damaging peroxides. The tervalent phosphorus moiety may also reduce photoyellowing by deactivating singlet $^1O_2$.

5:1:1 Results of the Synthesis of a Series of α-Amino-Anthrylphosphonous Acids.

The addition of tervalent hypophosphorous acid (H_3PO_2) to the electropositive carbon of an imine (C=N) to give N-substituted α-aminophosphonous acids is well documented. (Method A) [5]-[10] Alternatively the condensation of the hypophosphite salt of the amine and hypophosphorous acid with an aldehyde or ketone in ethanol or preferably dioxane gives the product. (Method B) [11]-[13]

A third method (Method C) involves the addition of hypophosphorous acid to oximes. [7] This reaction mechanism has not been elucidated; it is thought however that the intermediate hydroxylaminoacid is reduced by excess hyphosphorous acid.

Method A which involves the production and isolation of imine intermediates was chosen as according to Jungermann et al., [11] the subsequent reaction of imine with hypophosphorous acid gave a higher yield and purer final product than the corresponding hypophosphite salt reaction (method B). (See Sec 5:3:3).

Method A:

$$\underset{R}{\overset{R}{\diagdown}}C=N-\underset{\diagdown Ph}{\overset{\diagup Ph}{CH}} \quad + \quad H_3PO_2 \quad \longrightarrow$$

$$\underset{R}{\overset{R}{\diagdown}}\underset{\underset{NHCH(Ph)_2}{|}}{\overset{\diagup O}{C}}\underset{OH}{\overset{\|}{P}}-H$$

Method B:

$$\underset{Ph}{\overset{Ph}{\diagdown}}HC-NH_3{}^+H_2PO_2{}^- \quad + \quad \underset{R}{\overset{R}{\diagdown}}C=O \quad \nearrow$$

Method C:

$$\underset{R}{\overset{R}{\diagdown}}C=NOH \quad + \quad H_3PO_2 \quad \longrightarrow \quad \underset{R}{\overset{R}{\diagdown}}\underset{\underset{NH_2}{|}}{\overset{\diagup O}{C}}\underset{OH}{\overset{\|}{P}}-H$$
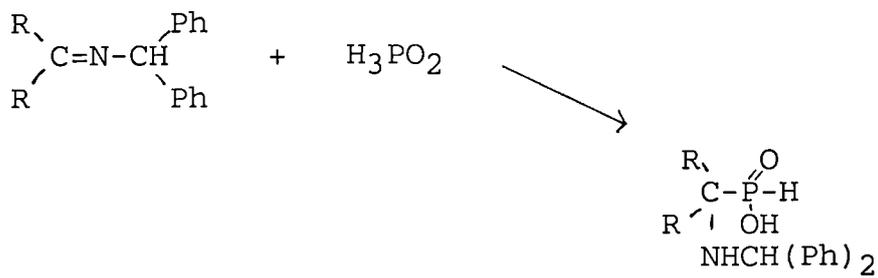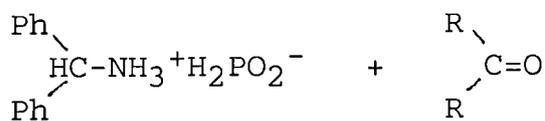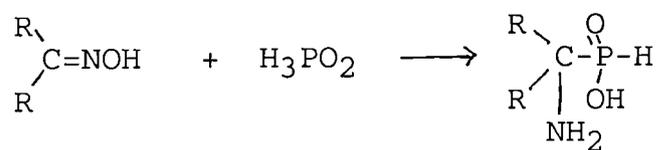
Results:

Imine No.	Structure	Starting Materials	% Yield
A	Ph-N=CH (benzene ring)	Aniline ($PhNH_2$) + Benzaldehyde (PhCHO)	74%
B	Ph-N=CH (anthracene ring)	Aniline ($PhNH_2$) + 9-Anthraldehyde ($C_{14}H_9CHO$)	70%
C	Ph-N=CH (pyrene ring)	Aniline ($PhNH_2$) + 1-Pyrenecarboxaldehyde ($C_{16}H_9CHO$)	95%
D	CH_3 / CH_3 CH-N=CH (naphthalene ring)	Isopropylamine (C_3H_9N) + 2-Naphthaldehyde (C_7H_7CHO)	80%
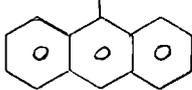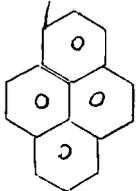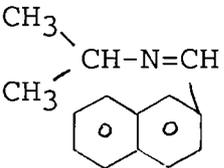
Figure 5:1 Table of imine intermediates synthesised.

This method was successful in one case only. The
reaction of imine A (N-benzylidineaniline) with the
acid gave a white fluffy product with the correct I.R.
stretching frequencies. Analysis showed the compound to
be low on carbon however, and resisted all further
attempts at purification. The other imines gave no

products and returned high yields of starting material. Jungermann et al. [11] reported the success of this method for the production of aromatic phosphonous acids based on imines derived from substituted benzaldehyde and substituted aniline ring systems. None of his compounds contained any conjugated ring systems.

Possible reasons for the failure of this method may be:

a) Steric hindrance at the electropositive carbon by bulky ring systems attached and/or
b) Conjugation of the imine moiety and aromatic rings, leading to a more stable imine, which is not successfully attacked by the tervalent phosphorus atom in hypophosphorous acid.

Method B was then tried (See Sec 5:3:3) , whereby the hypophosphite salt of the amine used is condensed with an aldehyde or ketone.

.

Results:

Product No.	Structure	Starting Materials	Yield
1	$$Ph-NH-CH-\overset{\overset{O}{\|\|}}{P}H$$ OEt (anthracene ring)	Hypophosphite salt of aniline ($PhNH_3{}^+H_2PO_2{}^-$) + 9-Anthraldehyde ($C_{14}H_9CHO$)	22%
2	$$CH_3-C_6H_4-NH-CH-\overset{\overset{O}{\|\|}}{P}H$$ OH (anthracene ring)	Hypophosphite salt of p-toluidine ($CH_3-C_6H_4NH_3{}^+H_2PO_2{}^-$) + 9-Anthraldehyde ($C_{14}H_9CHO$)	66%
3	$$Ph-NH-CH-\overset{\overset{O}{\|\|}}{P}H$$ OH (phenanthrene ring)	Hypophosphite salt of aniline ($PhNH_3{}^+H_2PO_2{}^-$) + Phenanthrene-9-carboxaldehyde ($C_{14}H_9CHO$)	32%
4	(anthracene ring)$NH-CH-\overset{\overset{O}{\|\|}}{P}H$ Ph OH	Hypophosphite salt of 2-Aminoanthracene ($C_{14}H_9NH_3{}^+H_2PO_2{}^-$) + Benzaldehyde (PhCHO)	15%
5	(anthracene ring)$NH-CH-\overset{\overset{O}{\|\|}}{P}H$ OH (naphthalene ring)	Hypophosphite salt of 2-Aminoanthracene ($C_{14}H_9NH_3{}^+H_2PO_2{}^-$) + 2-Naphthaldehyde ($C_{10}H_7CHO$)	20%
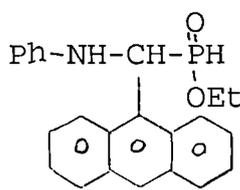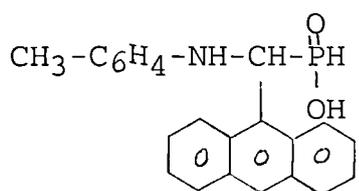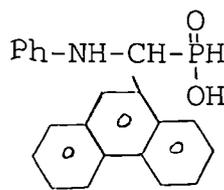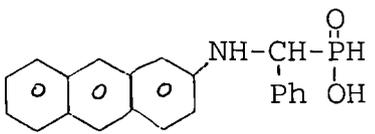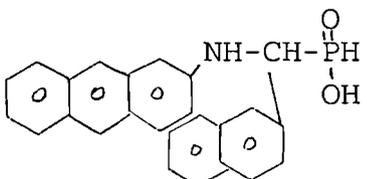
Figure 5:2 Table of synthesised α-Aminoanthrylphosphonous Acids derived from aromatic amines.

Product No.	Structure	Starting Materials	Yield
6	$CH_3(CH_2)_3NH-CH-\overset{\overset{O}{\|}}{P}H$ $\|\ \ OH$ $(CH_3)_2$	Hypophosphite salt of n-butylamine ($CH_3(CH_2)NH_3{}^+H_2PO_2{}^-$) + 9-Anthraldehyde ($C_{14}H_9CHO$) Solvent acetone	92%
7	$CH_3(CH_2)_3NH-CH-\overset{\overset{O}{\|}}{P}H$ OH	Hypophosphite salt of n-butylamine ($CH_3(CH_2)NH_3{}^+H_2PO_2{}^-$) + 9-Anthraldehyde ($C_{14}H_9CHO$)	27%
8	$CH_3(CH_2)_3NH-CH-\overset{\overset{O}{\|}}{P}H$ OH	Hypophosphite salt of n-butylamine ($CH_3(CH_2)NH_3{}^+H_2PO_2{}^-$) + 1-Pyrenecarboxaldehyde ($C_{16}H_9CHO$)	29%
9	$CH_3(CH_2)_3NH-CH-\overset{\overset{O}{\|}}{P}H$ OH	Hypophosphite salt of n-butylamine ($CH_3(CH_2)NH_3{}^+H_2PO_2{}^-$) + Phenanthrene-9-carboxaldehyde ($C_{14}H_9CHO$)	41%
10	$O\!\!\diagdown\!\!N-CH-\overset{\overset{O}{\|}}{P}H$ $\|\ \ OH$ $C=O$	Hypophosphorous acid (H_3PO_2) + Phenylglyoxal monohydrate ($PhCOCHO.H_2O$) + Morpholine (C_4H_9NO)	85%
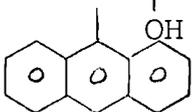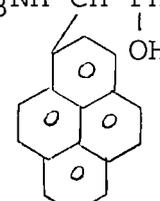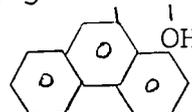
Figure 5:3   Table of synthesised α-Amino-anthrylphosphonous Acids derived from aliphatic amines.

5:1:2 Ultra-Violet Spectral Data of α-Aminoanthryl phosphonous acids.

Most of the phosphonous acids synthesised showed the characteristic bands of the aromatic chromophore present in the molecule in their respective U.V. spectra. e.g. anthracene [17],[18] in figure 5:5. In order to characterise the type of transition observed, the spectra were run in polar (methanol) and non-polar (toluene) solvents. The U.V. spectra of some compounds showed a pH dependence and consequently the spectra were recorded for the compound in strong acid and strong base.
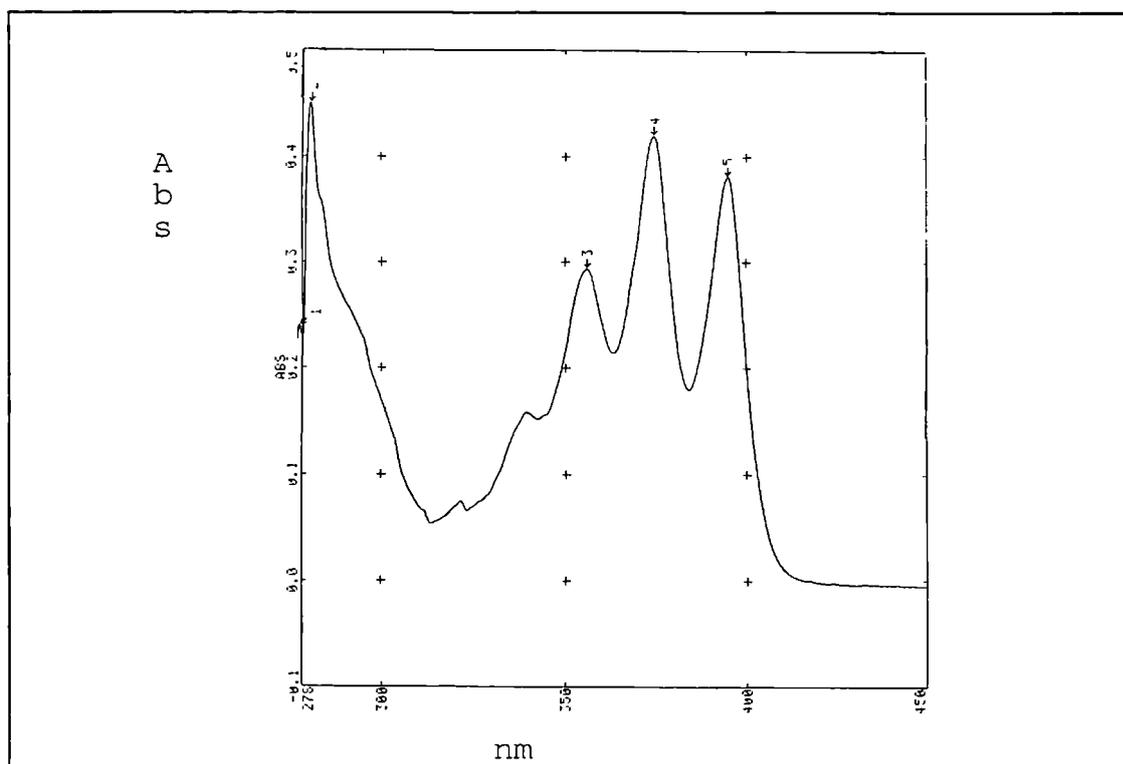
Figure 5:4 U.V. spectrum of product (α-phenylamino anthryl phosphonous acid), solvent toluene.

The U.V. data for the compounds synthesised are tabulated in the following figure 5:5.

Product No.	Solvent	Absorption (Ext. Co-efficient) nm mol-1lcm-1
1	Methanol	279 nm (16,600), 352 nm (2842), 371 nm (4184), 391 nm (3796).
1	Toluene	281 nm, 355 nm, 374 nm, 395 nm.
2	Toluene	255 nm, 357 nm, 376 nm, 396 nm.
3	Methanol	254 nm, 271 nm, 289 nm, 300 nm.
3	Toluene	259 nm, 292 nm, 303 nm.
4	Methanol	272 nm (39,542), 319 nm (8294), 336 nm (10,300), 354 nm (5133), 397 nm (3192).
4	Toluene	278 nm, 321 nm, 339 nm, 355 nm, 376 nm, 399 nm.
5	Methanol	277 nm (17,878), 319 nm (5741), 335 nm (12,830), 353 nm (4890), 394 nm (1158).
5	Toluene	282 nm, 321 nm, 339 nm, 346 nm, 400 nm.
7	Toluene	256 nm, 357 nm, 376 nm, 396 nm,
8	Methanol	255 nm (72,800), 265 nm (69,641), 276 nm (30,608), 314 nm (8166), 328 nm (19,000), 344 nm (27,866).
8	Toluene	282 nm, 333 nm, 350 nm, 395 nm,
9	Methanol	271 nm (22,810), 289 nm (8704), 300 nm (9328).
9	Toluene	282 nm, 292 nm, 304 nm.

Figure 5:5   U.V. data of synthesised α-anthrylamino phosphonous acids.

All of the phosphonous acids displayed an absorption

maximum shift towards lower wavelength in the polar

medium and are therefore exclusively due to $\pi \rightarrow \pi^*$

transitions. (See sec 1:1:1) In general the electron

charge distribution of a $\pi \rightarrow \pi^*$ excited state is more

extended than the ground state and is therefore more

polarisable. A decrease in energy of the excited state

in a polar solvent is slightly greater than that of the

ground state and absorption maxima shift to lower

wavelength.

The U.V. spectra of products 4 and 5 (unlike all other

phosphonous acids synthesised) showed none of the

expected characteristic anthracene transitions, instead

both compounds showed a broad band with maximum

absorption at 400 nm present in each. The U.V. spectra

of 2-aminoanthracene (a parent compound to products 4

and 5) revealed the presence of a charge transfer band

with an absorption maximum at 411 nm , which on

addition of strong acid (T.F.A.) completely

disappeared. (See fig. 5:6) This suggests that the

charge transfer transition is due to donation of one of

the electrons of the lone pair in the p-orbital of the

nitrogen to the lowest unoccupied orbital of

anthracene. In acid solution the NH_3^+ group is formed

and no charge transfer can take place. The broad

absorption band centred at 400 nm observed in products

4 and 5 also disappeared in acid solution inferring

that the transition may be the same as that described

for 2-aminoanthracene.
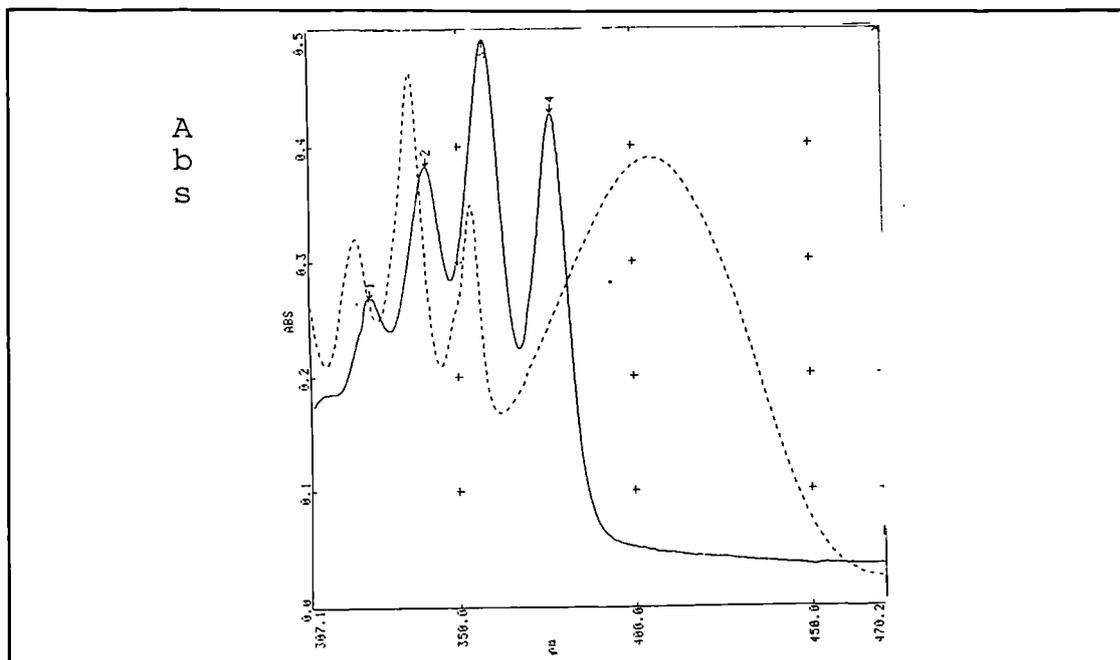
Figure 5:6 The U.V. spectra of 2-aminoanthracene (a)
- - - in alkali (NaOH) and (b) ——— in acid
(trifluoroacetic acid) solvent methanol.

5:1:3 The Fluorescence Spectral Data of α-Aminoanthryl phosphonous acids.

Fluorescence quantum yield is a measure of the

efficiency with which absorbed radiation causes a

molecule to fluoresce. (See sec 1:2:4) Values

normally fall in the range of 0 to 1.0. As emission is

in all directions and a polychromatic detector response

can vary with wavelength a common practise is to

measure quantum yields relative to that of a standard

compound whose quantum yield is known.   (For method

see sec 5:3:6)

Results:

Product No.	Fluorescence Quantum Yield
1	0.018
2	0.015
3	0.020
4	0.080
5	0.120
8	0.060
9	0.012

Figure 5:7 ϕ_f for α-Aminoanthryl phosphonous acids synthesised, solvent ethanol.

These ϕ_f values are very low for this series of products and make them unsuitable compounds for use as F.W.A 's. The reason(s) for the decrease in ϕ_f of the phosphonous acids series relative to that of their parent aromatic ring systems is investigated with reference to the compounds' individual fluorescence spectra. The spectra were run in polar (methanol) and non-polar (toluene) solvents, acid and basic media and excited at various wavelengths. The fluorescence spectral data for the parent aromatic ring compounds, anthracene, phenanthrene and pyrene are also given.

Results:

Product No.	Solvent	Fluorescence Emission Maxima nm	Excitation nm
Anthracene	methanol	380, 400, 422, 450.	350
Pyrene	methanol	380, 390, 415, *460*.	350
Phenanthrene	methanol	378, 402, 425, 455.	350.
1	methanol	402, 420, 440.	370
1	methanol	432, *520*.	400
2	methanol	410, 465, *495*.	395
2	methanol	410, 465, *495*.	400
2	toluene	435, 465, *495* (sh).	400
3	toluene	380, 400, 425, 455.	360
4	methanol	420, 453, *480*.	370
4	methanol	*490*.	400
5	toluene	405, 440, *485*.	380
5	toluene	440, 465.	400
5	methanol	460 (sh), *500*.	420
7	methanol	402, 422, 450, *500*.	365
8	methanol	376, 396, 417, 445 (sh).	340
9	methanol	400, 420, 445, *505*.	360

Figure 5:8   Fluorescence spectral data for α-amino anthryl phosphonous acids, run in polar and non-polar media, solution concentrations $1*10^{-3}$ M, slit width 4.

All suspected excited state complexes are shown in italics.

In neutral solution product 2 has the same excited

complex emission maximum in methanol and in toluene

inferring that emission is possibly from an exciplex.

The structureless bands at longer wavelengths present

in the α-aminoanthryl phosphonous acid series (See

fig. 5:8) may be due to (a) the formation of an

intermolecular excimer or (b) the formation of an

intermolecular exciplex between the secondary amine and

the aromatic hydrocarbon moiety. (See sec. 1:2:5:1)

If the structureless bands are due to (b), they should

disappear on protonation with acid, if due to (a) the

spectra should remain the same. Some of the

fluorescence spectra were re-run in strongly acidic

solution using trifluoroacetic acid. (See fig. 5:9 and

fig 5:10)

Results:

Product No.	Solvent	Fluorescence Emission Maxima nm	Excitation nm
1	acid	432, *505*.	400
4	acid	376, 398, *520*.	320
5	acid	430, *540*.	390
8	acid	385, 397, 415, 440 (sh)	340
9	acid	360, 370, 380, 390, *460*	330

Figure 5:9 Fluorescence spectral data for α-amino
anthryl phosphonous acid series in T.F.A. methanol.
Solution concentrations $1*10^{-3}$ M, slit width 4.

All suspected excited state complexes are shown in
italics.

Excited state emission is observed at longer
wavelengths in acid solution for the α-aminoanthryl
phosphonous acid series. This would indicate that the
emitting species are not secondary amine/aromatic
hydrocarbon exciplexes but more probably an excimer.
The relative orientation of the two groups forming the
excited state species is of far greater importance for
excimer formation than it is for exciplex formation.
The products are amphoteric in character and may exist
as zwitterions, due to NH and OH moieties. Hence, it
appears that stacking due to H bonding occurs in
solution, leading to the correct conformation for
exciplex formation. e.g.
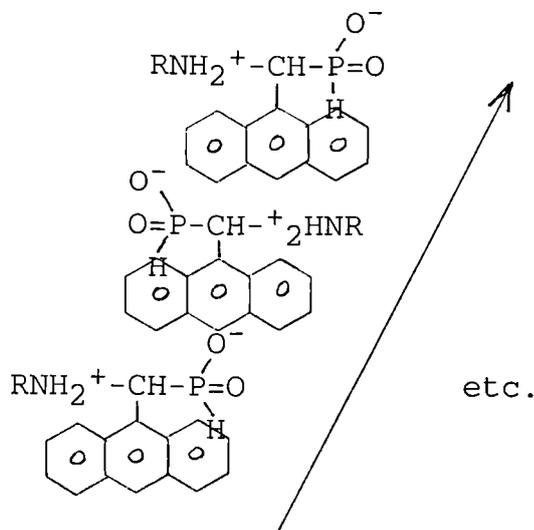
The observation that in acid solution the wavelength of
maximum emission of the excited species has shifted to
longer wavelength (and therefore lower energy) for
products 1 and 9 and to shorter wavelength for products

4 and 5 is difficult to explain and further work must
be done to explain the observation.

5:1:4   The Results of α-Aminoanthryl phosphonous acids as F.W.A.'s for Wool.

From the analysis of the phosphonous acids synthesised
it is clear that most are unsuitable for use as
fluorescent whitening agents. All of the products have
very poor quantum yields of fluorescence, and all
except 3, 8 and 9 (See sec 5:1:3) are yellow or have
a distinct yellow tinge. Products 4 and 5 fluoresce in
the green region of the spectrum.

F.W.A.'s are normally applied to wool after or during a
chemical bleaching process. Bleaching is necessary as
residual yellowness in wool may cause quenching of the
whitener by providing molecules which absorb energy at
wavelengths of fluorescent emission or may have an
absorption tail in the U.V. region of the spectrum
which competes with the whitener for irradiating
energy. [21] Products 3 (phenylamino-phenanthryl
phosphonous acid), 8 (n-butylamino-pyrenyl
phosphonous acid) and 9 (n-butylamino phenanthryl
phosphonous acid) are not soluble in water or in
solutions of very high or low pH. As peroxide bleaching
is normally carried out at pH 9-10 [19] a bi-functional
exhaustive process involving whitener and chemical
bleach was not possible. Therefore a pad-batch method
of application was chosen. (See sec 5:3:7)

5:1:4:1 Yellowness Index (YI) Values

The yellowness of wool was determined by the following equation.

$$YI = \frac{100(1.316X - 1.164Z)}{Y}$$

where X, Y, and Z are the C.I.E. (Commission International de l'Eclairage) tristimulus values obtained from a Macbeth Micromatch reflectance spectrometer. The lower the index value, the whiter the wool colour. [4]

Wool	Whiteness (C.I.E.)	YI (E313)
Blank	6.21	20.57
1% owf, product 3	1.27	21.13
1% owf, product 8	6.31	20.37
1% owf, product 9	1.96	20.88

Figure 5:10 The YI values for wool treated with α-aminoanthryl phosphonous acids.

From the results in figure 5:10 it is clear that no improvement in the whiteness of wool was achieved on application of the α-aminoanthryl phosphonous acids. In fact products 3 and 9 yellowed the wool to a small extent.

Accelerated weathering tests of the treated fibres were undertaken to see if this type of compound could

minimise the photoyellowing process of the wool. The
yellowing was followed by diffuse reflectance
spectroscopy. (See Sec 5:3:8)

5:1:4:2 Photoyellowing Results of Irradiated Wool Pretreated with F.W.A.

The graph of figure 5:11 shows clearly that no
amelioration in photoyellowing was achieved using the
aminoanthryl phosphonous acids as F.W.A.s. The
phosphonous acids photoyellowed the wool to the same
extent as the commercial stilbene FWA,' Photine-HV '
(HWL). However, the stilbene had a very high
fluorescent quantum yield and unlike the phosphonous
acid series, whitened the wool to a considerable
extent. (Photine-HV, YI (E313) value is 10.34).
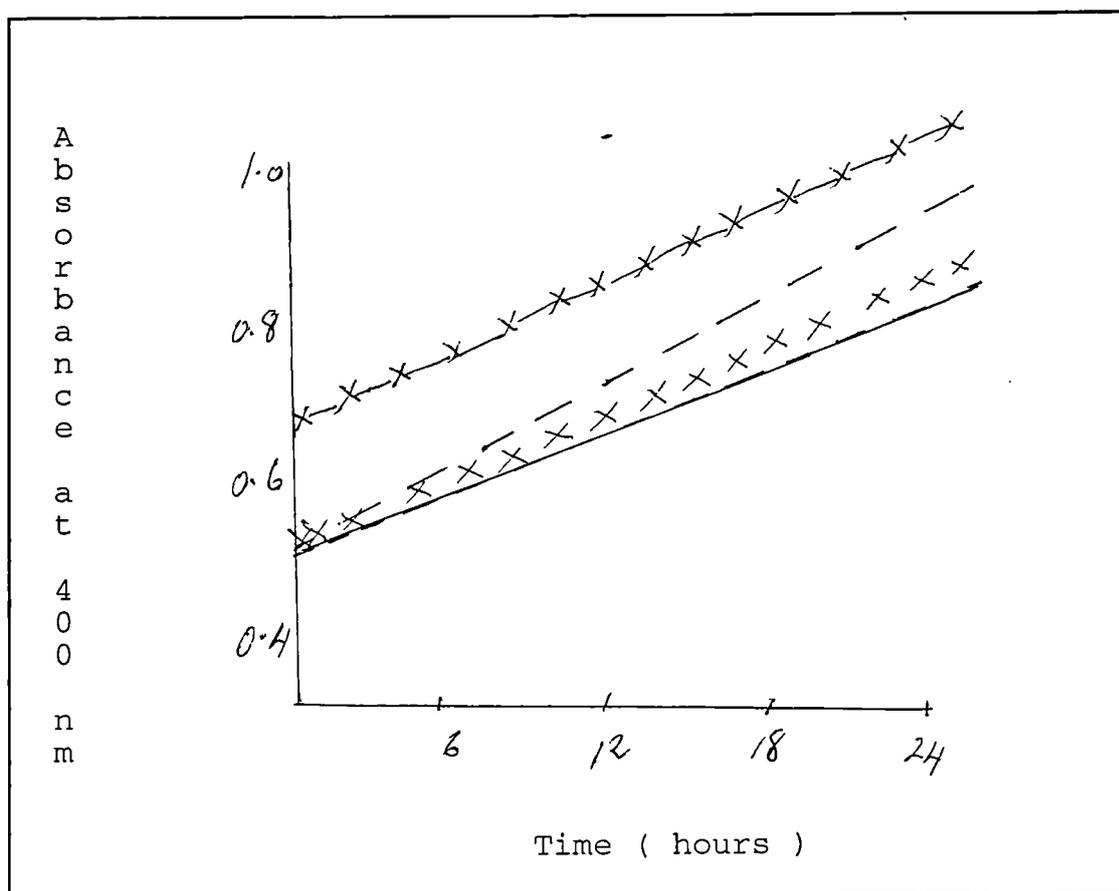
Figure 5:11 Graph of absorbance at 400 nm against irradiation time, for α-aminoanthryl phosphonous acid pretreated wool. —— = Bleached blank, - - = Photine-HV, *-* = Product 8 and *** = Product 9.

As the phosphonous acid series gave no protection against photoyellowing, had very low quantum yields of fluorescence, and therefore imparted minimal whiteness to the wool fibre, it is concluded that these compounds are of no use as fluorescent whitening agents.

5:2 Photolysis of α-Aminoanthryl phosphonous acids in Solution

In an attempt to elucidate the possible process and products of the photoreaction of the α-aminoanthryl

phosphonous acids on wool, the photolysis of the
compounds in solution was studied.

When a solution containing a polynuclear aromatic
hydrocarbon is oxygenated and irradiated at a pertinent
wavelength, a type two (see sec 1:3) 4 + 2
cycloaddition reaction may occur between
photochemically generated singlet oxygen ($^1O_2$) and
the cisoid 1,3-diene system to yield a 1,4-endoperoxide
with a double bond at the 2,3-position. (See sec
1:3:3) Previous work has shown that anthracene yields
the 9,10-endoperoxide, which on heating oxidises to
anthraquinone. [29]-[31]
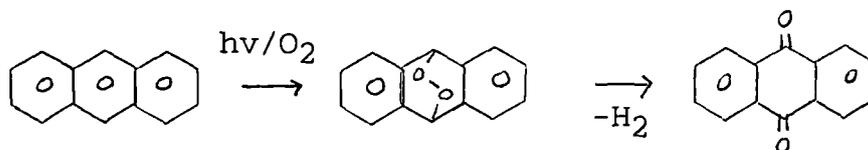
The 1,4-cycloaddition of $^1O_2$ to a wide variety of
substituted anthracenes has been reported. Willemart
[27] reported the photo-oxidation of 9-methyl, 9-ethyl
and 9,10-dimethyl anthracene to give the corresponding
9,10-endoperoxide. Lepage [28] showed that 9-methoxy
anthracene is photo-oxidised to the 9,10-endoperoxide
which then rapidly decomposes to anthraquinone. The
outcome of an anthracenyl photo-oxidation is influenced
by the nature of the substituents on the anthracene
nucleus. The presence of electron-donating groups has
been found to increase the reactivity of the 1,3 π-
systems, while conversely electron-withdrawing groups

decrease the reactivity of the diene systems. In addition to photo-oxidation anthracene is also known to photodimerise on irradiation [29] and therefore competition between photo-oxidation and photodimerisation may occur.

Anthracene compounds show a complex U.V. spectrum (see sec 5:1:2:1) which frequently includes a structured emission centred between 320 and 400 nm. [17],[18] e.g. 9-methylanthracene (See fig. 5:12) As a photo-reaction occurs, so the conjugation within the anthracene nucleus is altered, giving rise to a corresponding change in its absorption properties. On photo-oxidation (or photodimerisation) this change results in the loss of the structure from the spectrum. As the reaction proceeds the bands at 334, 349, 366, and 386 nm disappear with the concomitant formation of a broad band centred at 290 nm and another at 270 nm. The reaction is complete when the structure has completely disappeared from the spectrum.

The increase of the band centred at 270 nm, may be correlated with the formation of 9,10-anthraquinone, as this band is present in the 9,10-anthraquinone UV spectrum. The chromophore responsible for the increase in the absorption at 290 nm is unidentified and may be due to dimerisation. A similar increase, though more pronounced at 290 nm is observed for the photolysis of product 1 (benzylamino anthryl phosphonous acid).

(See fig. 5:13) From the observation that the
reaction of 9-methylanthracene is much faster than that
for product 1 and the fact that no 9,10-anthraquinone
is observed in the UV spectrum or mass spectrum for
this product, it is suggested that product 1 and 9-
methylanthracene form different compounds on
irradiation.
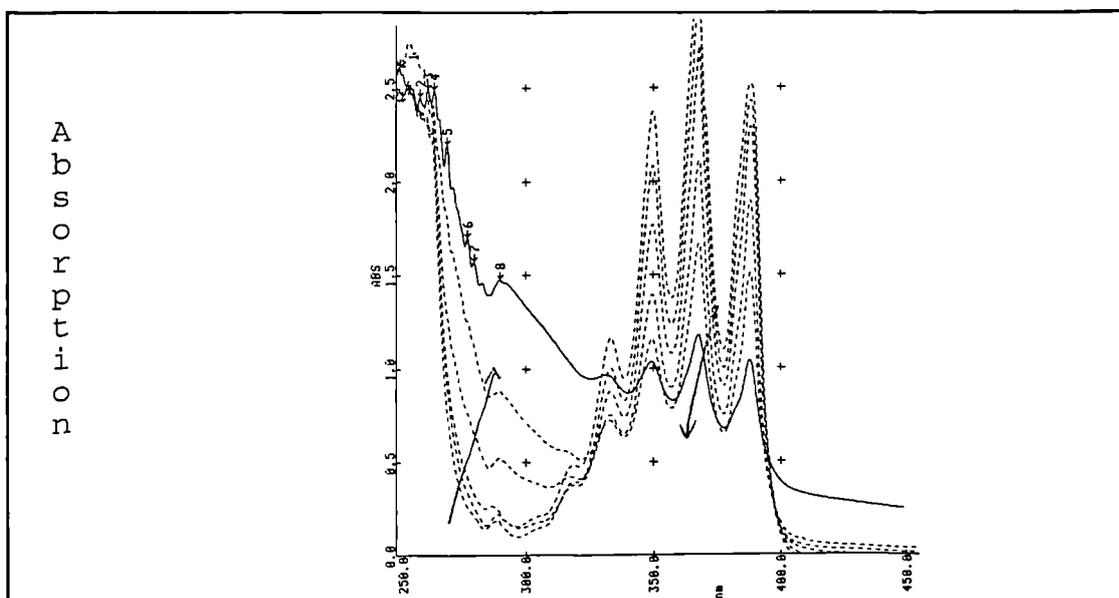
Figure 5:12 The UV spectrum of a $1*10^{-3}$ M solution of
9-methylanthracene in THF. On irradiation with black
light at time intervals (mins) = 0, 5, 15, 30, 45,
75.

Previous work by Davidson et al. [30] on the direct
photo-oxidation of oxygenated solutions of anthryl
phosphate compounds showed that irradiation resulted in
the fragmentation of the photo-chemically formed
endoperoxide to give 9,10-anthraquinone. At the same
time cleavage of the phosphorus moiety from the
anthracene nucleus occurred. Cleavage of the

- 177 -

amine/phosphorus moiety may occur in a similar fashion with product 1 without the formation of anthraquinone. A stable endoperoxide or dimer may be liberated along with $PhNHCH_2P(O)(OH)(OEt)$, which like aniline may have an absorption maximum at 296 nm. [31]
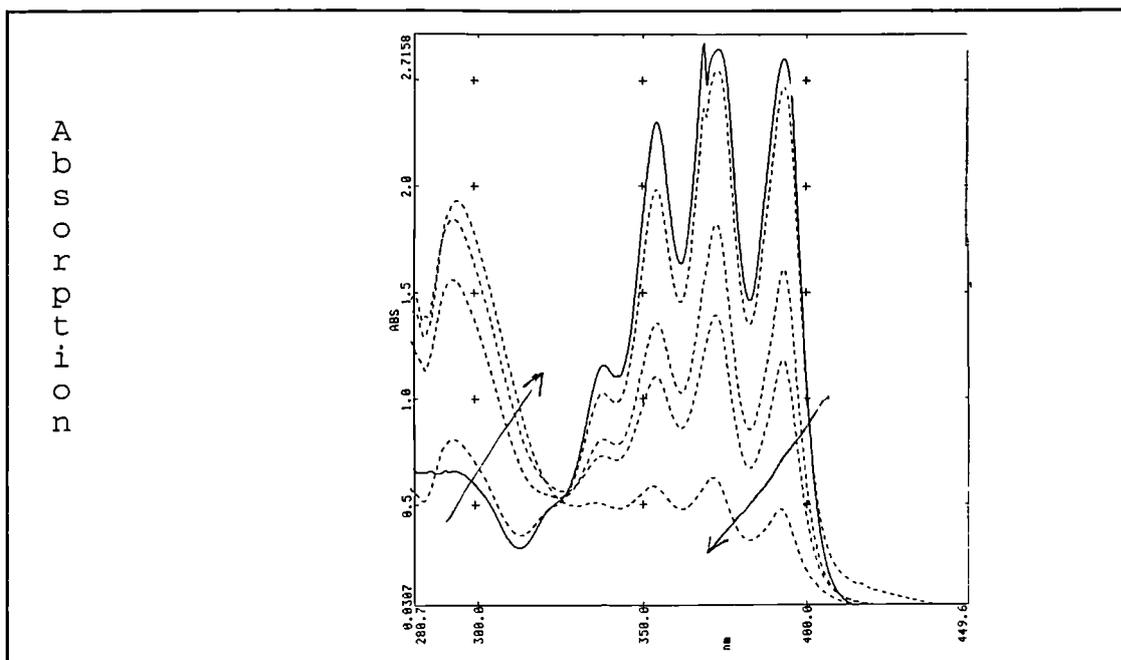
Figure 5:13 The UV spectrum of a $1*10^{-3}$ M solution of product 1 (benzylamino anthryl phosphonous acid) in THF. On irradiation with black light at time intervals (mins) = 0, 25, 105, 135, 160.

5:3 Experimental Methods

5:3:1 Synthesis of α-Aminoanthrylphosphonous Acids using method A.

Method of synthesis of imine intermediates:

All imine intermediates were synthesised in a similar way. Equimolar amounts of primary amine and an aromatic aldehyde were refluxed in an inert solvent (toluene) with azeotropic removal of the water of reaction using

a Dean-Stark trap. The reaction mixture was refluxed
for three hours and the solvent was removed under
vacuum. The product was isolated in high yield and
recrystallised from ethanol.

Mechanism of reaction:

$$R_1 \diagdown C=O + 2HNR_3 \longrightarrow \begin{matrix} R_1 \\ \diagdown \\ \diagup \\ R_2 \end{matrix} \begin{matrix} O^- \\ | \\ C-N^+ \\ \diagup \\ R_3 \end{matrix} \begin{matrix} H_2 \\ \diagup \\ \diagdown \end{matrix} \longrightarrow \begin{matrix} R_1 \\ \diagdown \\ \diagup \\ R_2 \end{matrix} \begin{matrix} OH \\ | \\ C-N \\ \diagdown \\ R_3 \end{matrix} \begin{matrix} H \\ \diagup \\ \diagdown \end{matrix} \longrightarrow \begin{matrix} R_1 \\ \diagdown \\ \diagup \\ R_2 \end{matrix} C=NR_3$$
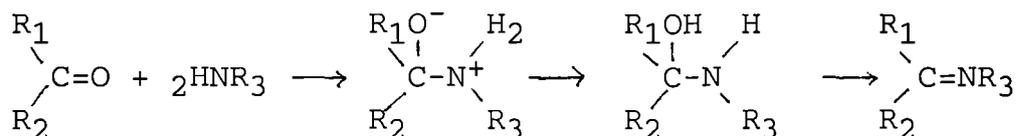
Nucleophilic attack on the carbonyl carbon results in
the formation of a zwitterion which undergoes internal
neutralisation to give a tetrahedral carbinolamine
intermediate. The equilibrium for the reaction lies to
the left, therefore the azeotropic removal of water is
essential for a high yield of imine.

Method of reaction of imine intermediates with H_3PO_2:

The imine intermediates were then reacted individually
with 95% H_3PO_2 using the method A according to
Jungermann et al. [11] Commercially available H_3PO_2 is
a 50% solution in water. 100 mls of this solution was
distilled under vacuum until 50 mls of H_3PO_2 had
distilled over and an approximately 95-100% solution of
H_3PO_2 was achieved.

To a 0.01M solution of imine in 75mls of ethanol heated
to 65°C, was added 0.7g (0.01M) of 95% H_3PO_2. the
reaction mixture was refluxed for three hours . The

solution was cooled and 1.6g (0.02M)of 50% NaOH was

added dropwise. The mixture was evaporated to dryness

under vacuum. The product was then dissolved in warm

water and extracted with ether until the ether layer

was clear. The product was then precipitated out on

addition of the water layer to a stirring solution of

3M HCl. The product was filtered and recrystallised

from an ethanol/water mixture.

5:3:2 Analytical Data for Imine Intermediates

Imine A: N-Benzylidineanaline

Creamy white shiny crystals.

Melting Point: 52-54°C, (lit. 52-54°C) [32]

Elemental Analysis:
For $C_{13}H_{11}N$ Theor: %C 86.15   %H 6.11   %N 7.72
                    Found: %C 86.10   %H 6.18   %N 7.76

Infra-Red: (KBr)
3400 (cm^{-1}) aromatic CH, 1620 (cm^{-1})
imine C=N, 1400-1480 (cm^{-1}) aromatic CH.

Mass Spectrum :
180.01 (m/e) 100% molecular ion, 104.02
(m/e) 16.9%, 75.06 (m/e) 83.5%.

^{1}H N.M.R. : (100MHz, $CDCl_3$)
  8.2   (1H, s, lone H on imine C)
  7.8   (2H, s, 2 aromatic protons adjacent to
carbonyl C).*
  6.8-7.4 (8H, m, other aromatic protons)

* As on comparison with two reference compounds N.M.R.

data, benzylidene methylamine and N-

dichloromethyleneaniline, it was observed that the

protons adjacent to the carbon in the first compound
were downfield w.r.t. all others.

Imine B: N-Anthrylidineaniline

Bright yellow needles.

Melting Point: 104-105°C, (lit. 104°C)

Elemental Analysis:
For $C_{21}H_{15}N$ Theor: %C 89.64   %H 5.37   %N 4.97

                           Found: %C 89.90   %H 5.37   %N 4.89

Infra-Red: (KBr)
3400 (cm^{-1}) aromatic CH, 1620 (cm^{-1})
imine C=N, 1400-1480 (cm^{-1}) aromatic CH.

Mass Spectrum :
281.12 (m/e) 79% molecular ion, 280.1
(m/e) 100%, 177.01 (m/e) 12.9%.

^{1}H N.M.R. : (100MHz, $CDCl_3$)
 9.7   (1H, s, lone H on imine C)
 8.5-8.7   (4H, m, 4 aromatic protons adjacent to imine
C).
 8.0-8.3   (2H, m, aromatic protons)
 7.2-7.65 (8H, m, aromatic protons)

U.V. Spectrum:
261.5 nm , sharp strong band.
400.4 nm, broad band, π—π* in anthracene moiety.

The five bands of fine structure associated with
anthracene have disappeared on conjugation of the
electrons with the imine moiety.

Imine C: N-Pyrylidineaniline

Bright yellow powder.

Melting Point: exp. 114°C

Elemental Analysis:
For $C_{23}H_{15}N$ Theor: %C 90.46   %H 4.95   %N 4.59

                           Found: %C 90.34   %H 4.94   %N 4.41

Infra-Red: (KBr)
3400 (cm^{-1}) aromatic CH, 1611 (cm^{-1})
imine C=N, 1400-1480 (cm^{-1}) aromatic CH.

Mass Spectrum :
305.1 (m/e) 6% molecular ion, 304.0
(m/e) 7.5%, no 100% base peak was present.

^{1}H N.M.R. : (100MHz, CDCl$_3$)
 9.6   (1H, s, lone H on imine C)
 8.5-8.7   (4H, m, 4 aromatic protons adjacent to imine
 C).
 8.0-8.3   (2H, m, aromatic protons)
 7.2-7.65 (8H, m, aromatic protons)

U.V. Spectrum: (Toluene)
261.5 nm , sharp strong band.
400.4 nm, broad shoulder, $\pi \rightarrow \pi^*$ in anthracene moiety.

Imine D: N-Naphthylidine-isopropylamine

Deep brown oil, which was purified by distillation.

Elemental Analysis:
For C$_{14}$H$_{15}$N   Theor: %C 85.23   %H 7.66   %N 7.09
                           Found: %C 85.86   %H 7.64   %N 6.79

Infra-Red: (NaCl plates)
3400 (cm^{-1}) aromatic CH, 1620 (cm^{-1})
imine C=N, 1400-1480 (cm^{-1}) aromatic CH.

Mass Spectrum :
197.1 (m/e) 100% molecular ion, 196.0
(m/e) 70%, 182.0 (m/e) 83.5%, 154.0 (m/e) 70%.

^{1}H N.M.R. : (100MHz, CDCl$_3$)
 9.0   (1H, s, lone H on imine C)
 8.0-7.3   (7H, m, 4 aromatic protons).
 3.6   (1H, sept, hydrogen on amine C split by 6
 equivalent methyl protons).
 1.5-1.3   (6H, d, 2 methyl groups split into a doublet
 by single hydrogen attached to C).

5:3:3 Synthesis of α-Aminoanthrylphosphonous Acids Using Method B

General Method: (based on a reaction by Schmidt [5])

To a stirring solution of equimolar amounts of primary amine and hypophosphorous acid (95%) in a minimum amount of inert solvent, the same molar concentration of aromatic aldehyde was added. The solution was refluxed overnight and the solvent removed under vacuum. The resulting liquid/oil was left in the freezer overnight. The solid product that resulted was recrystallised from an alcohol/water mixture.

If no solid product was realised some water was added to the mixture and the contents were then left to stand overnight at room temperature. A solid product was realised which was then recrystallised from an alcohol/water mixture.

5:3:4 Analytical Data on Phosphonous Acid Series Derived from Aromatic Amines

Product 1: Phenylamino-anthrylphosphonous acid

It was found that the product contained an ethoxyl group and this was probably introduced via an acid catalysed esterification reaction involving the solvent and the hydroxyl group. e.g.

$$R-P\overset{O}{\underset{H}{\diagdown}}O-H \xrightarrow{H^+} R-P\overset{OH^+}{\underset{H}{\diagdown}}O-H + \overset{:O-H}{\underset{C_2H_5}{\diagup}} \longrightarrow R-P\overset{O-H}{\underset{\overset{+O}{\diagdown}H}{\diagup}}O-H \longrightarrow$$

$$\underset{C_2H_5}{\diagup} \quad H \quad :H_2PO_2^-$$

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$$\begin{array}{c} H \quad OH \\ \diagdown \diagup \\ R-P-O-H \\ | \quad \searrow H-H_2PO_2 \\ O \\ | \\ C_2H_5 \end{array} \longrightarrow \begin{array}{c} H \quad O-H \\ \diagdown \diagup \\ R-P-O^+-H \\ | \quad \diagdown \\ O \quad H \\ | \\ C_2H_5 \end{array} \longrightarrow \begin{array}{c} O \\ \diagup\diagup \\ R-P-OC_2H_5 \\ \diagdown \\ H \end{array}$$
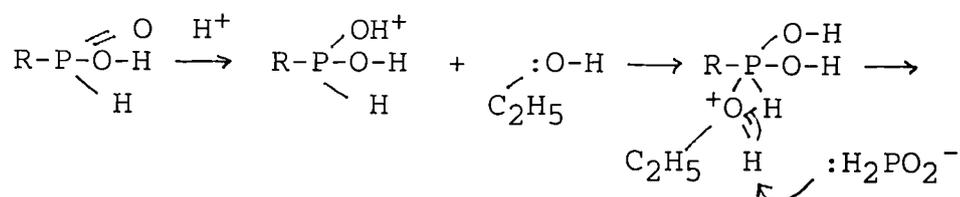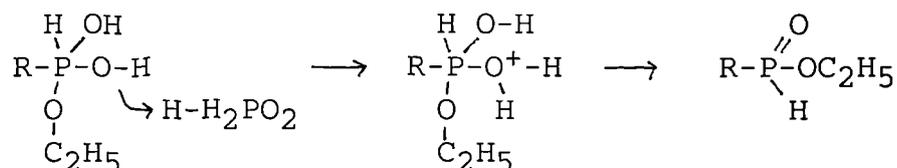
For this reason, reagent grade ethanol which contains a small amount of water was used in subsequent reactions.

Light yellow powder.

Melting Point: exp. 140°C

Elemental Analysis:
For $C_{23}H_{22}NO_2P$ Theor: %C 73.59   %H 5.91   %N 3.73
                     Found: %C 73.32   %H 5.84   %N 3.78

Infra-Red: (KBr)
3347 (cm^{-1}) amine NH, 2319 (cm^{-1}) P-H,
1400-1480 (cm^{-1}) aromatic CH, 1174 (cm^{-1}) P=O, 1030 (cm^{-1}) P-O$^-$.

Mass Spectrum :

All the phosphonous acids synthesised showed a simliar

fragmentation pattern in their mass spectra. No

molecular ions were observed as cleavage occurred

between the carbon and the hypophosphorous acid moiety

in each case.

283.1 (m/e) 23% , 282.1 (m/e) 100% base peak, 281.1
(m/e) 67%, 280 (m/e) 93%, 204.6 (m/e) 11%, 177 (m/e)
22%, 104 (m/e) 17%.

[1]H N.M.R. : (100MHz, $CDCl_3$)
12.2   (1/2H, s, P-H doublet chem shift = 7.5)
8.6-6.0 (15H, m, aromatic protons)
3.8   (3H, t, methyl protons)
2.8   (1/2H, s, P-H doublet J_{P-H} 560 Hz.)
1.3-0.9 (2H, m, CH_2 protons)

Phosphorus like hydrogen has a nuclear spin of 1/2 and

may couple with hydrogen and carbon nuclei. Coupling

constants for the P-H bond in similar compounds have
been reported and as above are of the order of 540-570
Hz. (14),(15)

^{13}C N.M.R.:(CDCl$_3$)

The presence of an ethyl group in the molecule was
proven by running a decoupled C13 N.M.R. spectrum.
(The coupled spectrum was too difficult to analyse due
to C-H and C-P coupling). The C13 spectra of 9-methyl-
anthracene and aniline were used as references. (16)
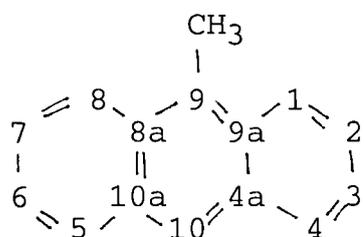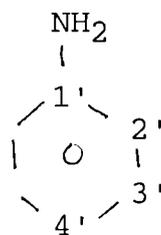
9-Methylanthracene   Aniline

147.5 and 146.8   (C1' , d, split by phosphorus)
131.9 and 131.7   (C10a, C4a)
131.0 and 130.6   (C3', C5)
129.8 and 129.4   (C9a, C8a)
126.4 and 126.7   (C4, C5)
125.2 and 125.7   (C2, C7)
130.4 (C9)
124.4 and 124.6   (C1, C8)
123.7 (C10)
118.8 (C4')
113.8 (C2)
63.4 and 62.9 (OCH$_2$ methoxy moiety, d, split by
phosphorus)
58.7 and 52.0 (CH, d, split by phosphorus. Its
chemical shift 6.7 therefore the coupling constant of
J$_{C-P}$ is 101 MHz.)
16.2 and 15.8 (CH$_3$, d, split by phosphorus)

D$_2$O Exchange Proton N.M.R. Spectra: (See fig. 5:14)

The C13 spectrum shows that the peak for N-CH-PH moiety's carbon in the molecule is a doublet of doublets of doublets.(J$_{C-H}$ = 140 Hz and J$_{C-P}$ = 101 Hz). This indicates the presence of a coupling other than J$_{C-P}$ or J$_{C-H}$, which may be due to either NH or PH hydrogens. In order to elucidate which coupling was present a D$_2$O exchange experiment was undertaken. The more labile NH proton should exchange faster than the PH proton to form ND and if the extra coupling is due to the NH proton it should disappear from the spectrum leaving a doublet of doublets. On the other hand, if the coupling is due to the PH proton the proton should not exchange and a doublet of doublets of doublets should remain. D$_2$O was added to a solution of the sample and the proton N.M.R. spectrum was run after seven hours. A decrease in the intensity of PH peaks was noted as exchange occurred forming PD. It was therefore impossible to designate the correct extra coupling (via this method).
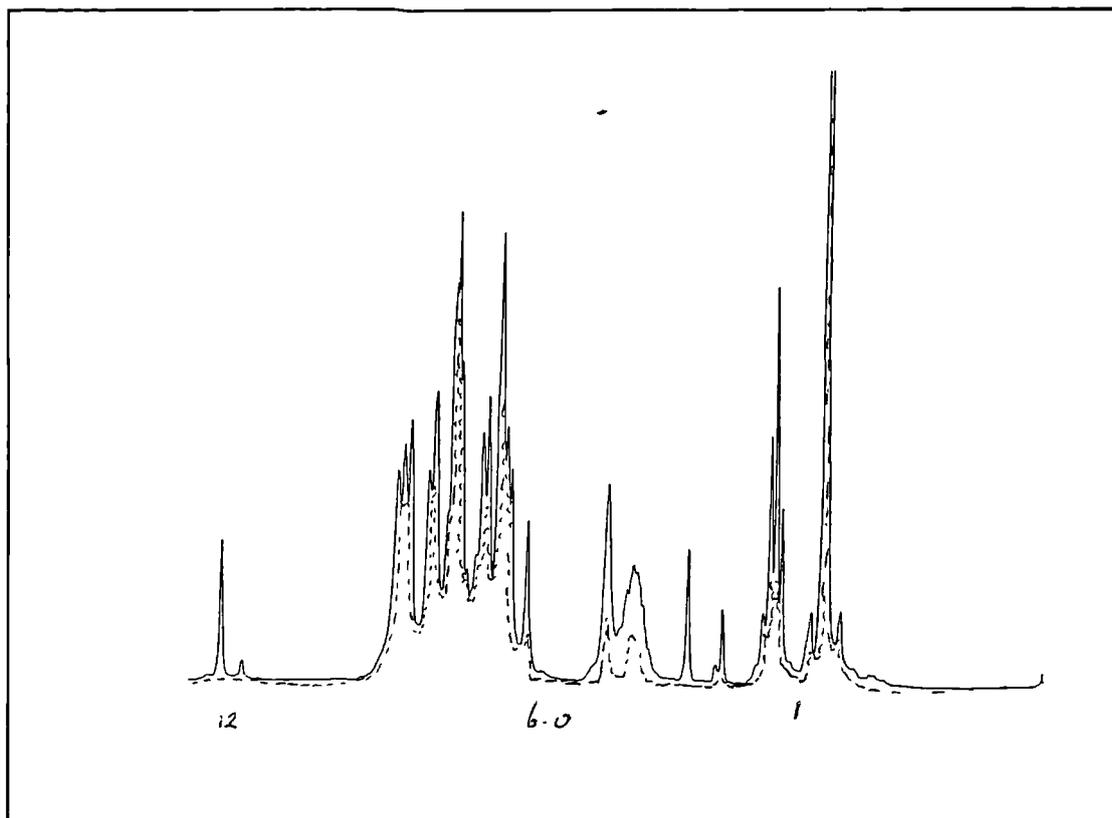
Figure 5:14 Proton N.M.R. spectra of (a) ——— product 1 and (b) - - - product 1 with D_2O after 24 hrs. Solvent $CDCl^3$, reference T.M.S.

F.A.B.S. (Fast Atom Bombardment Spectroscopy):

376 (m/e) = [M + 1]

This base peak in the F.A.B.S. mass spectrum confirms that the product formed contained an ethoxy instead of the expected hydroxyl group on the phosphorus atom.

Product 2: p-Toluidine-anthrylphosphonous acid.

Light yellow powder.

Melting Point: exp. 138°C

Elemental Analysis:
For $C_{22}H_{20}NO_2P$ Theor: %C 73.12   %H 5.58   %N 3.88
                           Found: %C 72.57   %H 5.27   %N 3.39

Infra-Red: (KBr)
3321 (cm^{-1}) amine NH, 2359 (cm^{-1}) P-H,
1400-1480 (cm^{-1}) aromatic CH, 1185 (cm^{-1}) P=O, 1039
(cm^{-1}) P-O⁻.

Mass Spectrum :
 296.4 (m/e) 23%, 295.1 (m/e) 56%, 294.1 (m/e) 63%,
 191.1 (m/e) 40%, 107.0 (m/e) 78%, 106.0 (m/e) 97%.

^{1}H N.M.R. : (100MHz, d-DMSO)
 12.3   (1/2H, s, P-H doublet chem shift = 7.25)
 9.0-6.8 (15H, m, aromatic protons)
 2.2   (1/2H, s, P-H doublet J_{P-H} 567 Hz.)
 2.0   (3H, m, CH_3 group attached to aniline ring.)

Product 3: Phenylamino-phenanthrylphosphonous acid.

Very white powder.

Elemental Analysis:
For $C_{21}H_{18}NO_2P$ Theor: %C 72.62 %H 5.22 %N 4.03
                           Found: %C 72.68 %H 5.01 %N 3.84

Infra-Red: (KBr)
3321 (cm^{-1}) amine NH, 2359 (cm^{-1}) P-H,
1400-1480 (cm^{-1}) aromatic CH, 1190 (cm^{-1}) P=O, 1039
(cm^{-1}) P-O⁻.

Mass Spectrum :
 281.1 (m/e) 21%, 280.1 (m/e) 29%, 93.0 (m/e) 30%, 77.0
 (m/e) 23%, 64.9 (m/e) 100% base peak.

^{1}H N.M.R. : (100MHz, d-DMSO)
 12.3   (1/2H, s, P-H doublet chem shift = 7.3)
 9.0-6.2 (18H, m, aromatic protons)
 2.8   (1/2H, s, P-H doublet J_{P-H} 570 Hz.)

Product 4: Anthra-amino-benzylphosphonous acid.

Deep yellow needles.

Elemental Analysis:
For $C_{21}H_{18}NO_2P$ Theor: %C 72.62 %H 5.22 %N 4.03
                           Found: %C 71.71 %H 5.27 %N 3.68

Infra-Red: (KBr)
3352 (cm^{-1}) amine NH, 2331 (cm^{-1}) P-H,
1400-1480 (cm^{-1}) aromatic CH, 1181 (cm^{-1}) P=O, 1057
(cm^{-1}) P-O⁻.

Mass Spectrum :
282.1 (m/e) 13%, 281.1 (m/e) 13%, 177.0 (m/e) 6%.

[1]H N.M.R. : (100MHz, CDCl$_3$)
  12.2   (1/2H, s, P-H doublet chem shift = 7.25)
  8.95-7.1 (14H, m, aromatic protons)
  4.1   (1H, d, CH proton in NH-CH-PH moiety)
  2.35   (1/2H, s, P-H doublet J$_{P-H}$ 578 Hz.)

[13]C N.M.R. (CDCl$_3$)

This spectrum was too complex to analyse due to the

presence of C-P couplings. All aromatic carbons were

present and the anthracene moiety gave similar shifts

to those of product 1.

F.A.B.S. (Fast Atom Bombardment Spectroscopy):

347 (m/e) = [M]

This base peak in the F.A.B.S. mass spectrum confirms

that the product formed has a molecular weight of 347

a.m.u.

Product 5: Anthra-amino-naphthylphosphonous acid.

Deep yellow powder.

Elemental Analysis:
For C$_{25}$H$_{20}$NO$_2$P   Theor: %C 75.56   %H 5.07   %N 3.52
                          Found: %C 75.19   %H 5.17   %N 3.35

Infra-Red: (KBr)
3348 (cm^{-1}) amine NH, 2358 (cm^{-1}) P-H,
1400-1480 (cm^{-1}) aromatic CH, 1181 (cm^{-1}) P=O, 1035
(cm^{-1}) P-O$^-$.

Mass Spectrum :
332.1 (m/e) 54%, 331.1 (m/e) 100% base peak, 329.0 (m/e)
43%, 193.0 (m/e) 19%, 177.0 (m/e) 37%, 141.0 (m/e) 19%.

^{1}H N.M.R. : (100MHz, CDCl$_3$)
  11.8   (1/2H, s, P-H doublet chem shift = 7.5)
  8.2-6.6   (16H, m, aromatic protons)
  5.5   (1H, m, CH proton in NH-CH-PH moiety or
  possible impurity.)
  3.2   (1/2H, s, P-H doublet.)

^{13}C N.M.R. (d-DMSO/CDCl$_3$)

This spectrum was too complex to analyse due to the

presence of C-P couplings. All aromatic carbons were

present and the naphthacene moiety gave similar shifts

to the anthracene moiety of product 1.

5:3:5 Analytical Data on Phosphonous Acid Series Derived
from Aliphatic Amines

Product 6: n-Butylamino-bismethylenephosphonous acid.

This product was synthesised unintentionally. Using

acetone as the solvent for the reaction (as

recommended by Schmidt [5]) the hypophosphite salt

reacted with the sterically less hindered carbonyl

group of acetone than with that of 9-anthraldehyde. For

this reason acetone was not used as a solvent in

subsequent reactions.

Clean white crystals.

Melting Point: 215°C (lit. 214°C)[10]

Elemental Analysis:
For C$_7$H$_{19}$NO$_2$P   Theor: %C 46.92   %H 10.05   %N 7.82
                Found: %C 47.15   %H 10.08   %N 7.62

Infra-Red: (KBr)
3352 (cm^{-1}) amine NH, 2331 (cm^{-1}) P-H,
1181 (cm^{-1}) P=O, 1057 (cm^{-1}) P-O$^-$.

^{1}H N.M.R.: (100MHz, d-DMSO)

    9.8   (1/2H, s, P-H doublet chem shift = 7.1)
    4.4.   (1/2H, s, P-H doublet J_{P-H} 550 Hz.)
    3.3-3.1   (3H, t, methyl CH$_3$ protons split by CH$_2$.)
    1.7-1.4   (13H, m, other CH$_2$ and CH^3 motieties.)

Product 7: n-Butylamino-anthryl phosphonous acid.

Fine creamy white powder.

Elemental Analysis:
For C$_{19}$H$_{22}$NO$_2$P   Theor: %C 69.71   %H 6.77   %N 4.28
                               Found: %C 69.47   %H 6.54   %N 4.31

Infra-Red: (KBr)
3352 (cm^{-1}) amine NH, 2335 (cm^{-1}) P-H,
1400-1480 (cm^{-1}) aromatic CH, 1195 (cm^{-1}) P=O, 1074
(cm^{-1}) P-O$^-$.

Mass Spectrum :
262.1 (m/e) 31%, 261.1 (m/e) 91%, 191.0 (m/e) 35%,
189.0 (m/e) 21%, 177.0 (m/e) 24.5%.

^{1}H N.M.R.: (100MHz, CF$_3$CO$_2$H (TFA))

The product dissolved in TFA with no apparent reaction.

An unsatisfactory proton N.M.R. spectrum was obtained.

The

spectrum did however contain broad peaks indicating the

presence of both aromatic and aliphatic

protons.

^{13}C N.M.R. (TFA)

The spectrum showed peaks at 11.17, 18.52, 57.09 and

57.96 corresponding CH$_2$,CH$_3$ and CH respectively. The CH

carbon that is closest to the phosphorus group in the

molecule is clearly split into a doublet. At least

eight non-equivalent aromatic carbons are present in

the molecule and can be seen downfield at 110-135 ppm.

Most of these signals are split by the phosphorus

nucleus making the spectrum too difficult to analyse.

All aromatic carbons were present however and the

anthracene moiety gave similar shifts to those of

product 1.

Product 8: n-Butylamino-pyryenyl phosphonous acid.

Creamy white powder.

Melting Point: exp. 222°C

Elemental Analysis:
For $C_{21}H_{22}NO_2P$ Theor: %C 71.78   %H 6.31   %N 3.99
                   Found: %C 71.24   %H 6.25   %N 3.77

Infra-Red: (KBr)
3352 (cm^{-1}) amine NH, 2359 (cm^{-1}) P-H,
1400-1480 (cm^{-1}) aromatic CH, 1172 (cm^{-1}) P=O, 1052
(cm^{-1}) P-O⁻.

Mass Spectrum :
285.1 (m/e) 79%, 284.0 (m/e) 40%, 242.1 (m/e) 100% base
peak, 228.1 (m/e) 37%, 227.0 (m/e) 22%, 215.0 (m/e) 78%,
201.1 (m/e) 23.5%, 121.0 (m/e) 10.5%.

^{1}H N.M.R.: (100MHz, $CDCl_3$)

The product dissolved in TFA with no apparent reaction.

An unsatisfactory proton N.M.R. spectrum was obtained.

The spectrum did however contain broad peaks indicating

the presence of both aromatic and aliphatic

protons.

^{13}C N.M.R. ($CDCl_3$)

Like product 7 the spectrum showed peaks at 11.31,

18.53, 57.13 and 57.96 corresponding CH_2,CH_3 and CH

respectively. The CH carbon that is closest to the

phosphorus group in the molecule is clearly split into
a doublet. At least eight non-equivalent aromatic
carbons are present in the molecule and can be seen
downfield at 110-135 ppm. Most of these signals are
split by the phosphorus nucleus making the spectrum too
difficult to analyse.

F.A.B.S. (Fast Atom Bombardment Spectroscopy):

352 (m/e) = [M]

This base peak in the F.A.B.S. mass spectrum confirms
that the product formed has a molecular weight of 352
a.m.u.

Product 9: n-Butylamino-phenanthryl phosphonous acid.

Very white powder.

Melting Point: exp. 128-130°C

Elemental Analysis:
For $C_{19}H_{22}NO_2P$ Theor: %C 69.71   %H 6.77   %N 4.28
                         Found: %C 69.97   %H 6.73   %N 4.21

Infra-Red: (KBr)
3352 (cm^{-1}) amine NH, 2330 (cm^{-1}) P-H,
1400-1480 (cm^{-1}) aromatic CH, 1203 (cm^{-1}) P=O, 1066
(cm^{-1}) P-O⁻.

Mass Spectrum :
261.1 (m/e) 2%, 261.1 (m/e) 5%, 191.0 (m/e) 62%.

^{1}H N.M.R.: (100MHz, $CDCl_3$)
  11.1   (1/2H, s, P-H doublet chem shift = 7.2)
  8.8-7.1   (9H, m, aromatic protons)
  3.4.   (1/2H, s, P-H doublet J_{P-H} 562 Hz.)
  1.4   (1H, m, CH proton.)
  1.3   (6H, m, CH_2 protons.)
  1.1   (3H, m, CH3 protons of n-butyl moiety.)

Product 10: n-Morpholino-phenylglyoxyl phosphonous acid.

This product was synthesised for use as a photoinitiator in U.V. curing of various monomers via a method described by Schmidt [5] for the preparation of piperidinemethene phosphonous acid. Equimolar amounts of hypophosphorous acid and phenylglycoxal monohydrate were mixed together in 50 mls of methanol. The solution was cooled to 5°C and the same molar amount of morpholine was added dropwise. The solution was stirred for 5 hours at room temperature. The solvent was removed under vacuum and a deep brown oil remained. No solid product was obtained from the oil after 24 hours refrigeration in the freezer compartment.

The oil was purified by distillation.

Deep brown oil.

Elemental Analysis:
For $C_{11}H_{17}NO_3P$ Theor: %C 53.33   %H 5.92   %N 5.18
                          Found: %C 52.63   %H 5.32   %N 5.68

Infra-Red: (NaCl plates)
3338 (cm^{-1}) amine NH, 2331 (cm^{-1}) P-H, 1595 (cm-1) C=O, 1400-1480 (cm^{-1}) aromatic CH, 1187 (cm^{-1}) P=O, 1071 (cm^{-1}) P-O⁻.

Mass Spectrum :
215.0 (m/e) 2%, 105.0 (m/e) 100% base peak, 100.0 (m/e) 75%, 87.1 (m/e) 51%, 64.9 (m/e) 50%.

^{1}H N.M.R.: (100MHz, $CDCl_3$)
   9.6   (1/2H, s, P-H doublet chem shift = 7.3)
   6.9-8.0   (6H, m, aromatic protons)
   4.6.   (1/2H, s, P-H doublet J_{P-H} 510 Hz.)
   2.6-4.1   (9H, m, CH protons on morpholine moiety and
                lone CH proton.)

5:3:6 Method of Sample Preparation for Fluorescent Quantum Yield Measurements

The samples were made up to an optical density of 0.1 at the chosen excitation wavelength and were flushed with nitrogen for 3 mins. against a standard solution of anthracene whose ϕ_f in ethanol is 0.27. [19]

Degassing removes atmospheric oxygen which may quench the excited singlet state of the samples, thus reducing their fluorescence quantum yield.

5:3:7 Pad-Batch Procedure for Application of Phosphonous Acids onto Wool

0.1 g of F.W.A. was dissolved in 10 mls of D.M.F. (dimethylformamide) which is known to swell the wool fibre [22] and therefore help with the uptake of the products. 90 mls of water containing a commercial emulsfying agent Igepal and 0.05g of the wetting agent Lissapol N ($C_9H_{19}PhO(CH_2CH_2O)_{10}H$)) was added, and the solution stirred for 10 mins. 10g of bleached wool was immersed in the solution and 100% uptake of F.W.A. by weight was achieved, to yield a concentration of 1% F.W.A. owf (on weight of fibre).

5:3:8 Procedure for U.V. Irradiation of Wool Samples.

The samples (20*5 cm supported on rectangular glass plates of similar dimensions) were exposed to 3*20 W long-wavelength U.V. fluorescent tubes with a maximum output at 350 nm. The samples were irradiated in a

flat, open, stainless steel tray which contained 4 cm
of water (height). Each sample was divided into two
halves. One half was exposed to U.V. light. Samples of
wool were taken at regular intervals and were rinsed in
cold water and dried at room temperature. [4]

5:3:9 Method of Photolysis of Phosphonous Acids in Solution:

A $1*10^{-3}$ M solution of substrate in THF (tetrahydro
furan) was irradiated with a circular array of 12
black light fluorescent lamps of emission maximum 350
nm. A 10 ml cuvette was used and the irradiation
proceeded until UV spectroscopy indicated that no
starting material remained.

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6:1 Introduction to the Compounds Used to Confer Protection against Photoyellowing

Previous work has shown that the inclusion of reducing agents such as thioglycollic acid ($HSCH_2CO_2H$) [1] and triarylphosphines (R_3P) leads to some stabilisation of wool (untreated and fluorescently whitened) toward photoyellowing. [2],[3] Such species may act by transforming any damaging radical species or their derivatives e.g. peroxides, hydroperoxides etc., which are produced in the wool keratin on irradiation with U.V. light in the presence of O_2, [4]-[6] to colourless unreactive products. Alkyl sulphides have been shown to reduce peroxy radicals in this manner.[7] The reducing agent should also protect tyrosyl residues and related species such as dityrosine by preventing the radical-induced oxidations of these phenols that lead to quinones. [8]

In an attempt to find alternative way(s) of stabilization of wool toward photoyellowing, we have studied the effect of applying a number of different compounds to the wool surface: polysulphides, diethylpyrocarbonate and acetic anhydride, two isocyanates and their bisulphite adducts, acids, formaldehyde and acid/formaldehyde mixtures. The pretreated samples were subsequently irradiated under the conditions described in section 5:3:8.

As sulphides have been reported to act as antioxidants,
[7] two commercially available polysulphides LP3 and
Z-LP3 were investigated.

Diethylpyrocarbonate (DEPC) ($(C_2H_5OCO)_2O$) has been
reported to carbethoxylate a variety of nucleophilic
residues which can occur in proteins, including,
sulfhydryl, tyrosyl, arginyl, tryptophyl, α- and ε-amino
groups, and most readily with histidyl residues. [12]-
[21] At high concentrations of DEPC the
dicarbethoxyhistidyl residue is formed according to the
following equation. (No mechanism is given) [13]

$$\underset{\substack{N \diagdown NH}}{\overset{R}{\diagup\diagdown}} \quad \xrightarrow[\text{Excess}]{(C_2H_5OCO)_2O} \quad \underset{\substack{O \quad C \quad O \\ O}}{C_2H_5O\overset{R}{C}-N \diagdown N-\overset{}{C}OC_2H_5}$$
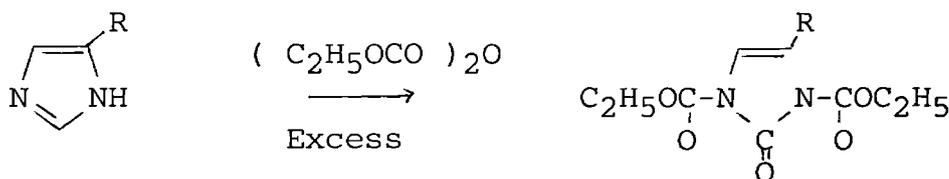
The extent of modification of histidyl residues may be
monitored by measuring the increase in absorbance at
240-242 nm. However, o-carbethoxylation of tyrosyl
residues results in a difference spectrum which shows a
minimum at 278 nm and a major decrease in intensity at
wavelengths below 240 nm. Therefore simultaneous
modification of histidyl and tyrosyl residues results
in an anomalously small change in the difference
absorption spectra at 240 nm. [14]

The N-carbethoxylation of the indole nucleus by DEPC
after 3 days of reaction has been demonstrated. This
leads to a decrease in its fluorescence emission
intensity, [14] and a shift in its absorbance spectrum
from 280 nm to 240 nm, indicating that a new
chromophore has been formed. [12] However, Mulhrad et
al. reported that no reaction of tryptophyl, tyrosyl or
cysteinyl occurred with a 10% solution of DEPC
occurred. [22]

The ε-amino and tyrosyl groups of wool have been
reported to undergo rapid acylation with acetic
anhydride at room temperature. An acetyl content of
1000 μmol gram-1 has been recorded. [23] The products
of the reaction of wool with boiling acetic anhydride
have not been identified but the process leads to the
wool acquiring an orange-brown colour. [24] A decrease
in the uptake of acid dye is reported for both
pretreatments, confirming the acetylation of lysine
side chains. (See Sec 4:1). [25],[26]

Early work by Frankel-Conrat et al. reported that under
mild reaction conditions lysyl, tyrosyl and cysteinyl
residues in wool, reacted with phenyl isocyanate. [28]
e.g. lysyl

$$WOOL-NH_2 \quad + \quad Ph-N=C=O \quad \longrightarrow \quad WOOL-NH-C(O)-NH-Ph$$

Farnworth [29] reported a value of 3500 umol gram-1 of
phenylcarbamoyl content after treatment in pyridine

solution at 70°C for 3 days. Four amino acid
derivatives arising from reaction of phenyl isocyanate
with side chains of lysine, threonine, serine and
glutamic acid were found in the enzymatic hydrolysates
of pretreated wool. [30]

The covalent binding of thioureido groups (via
reaction of the lysyl residues with methyl
isothiocyanate ((CH_3NCS)) has been reported to
significantly retard the yellowing of wool during
exposure to simulated sunlight. It would appear that
sulphur has a beneficial effect as the simple
acetylation of amino groups with N-acetoxysuccinimide
yellows faster than untreated fabric. [31] Because
isocyanates react rapidly with water, they must be
applied to wool from anhydrous aprotic solvents and
this is a disadvantage for them in technological
application. The ' masked ' water soluble bisulphite
adducts of ethyl and phenyl isocyanate, which react
with wool in a similar manner as the ' unmasked '
isocyanates, (synthesised via method of Guise et al.
[32], see section 6:3:4) have also been applied to
wool. [33]

Simpson reported that wool fabrics exhibit reversible
changes in their reflectance spectra according to the
pH value to which they are conditioned and are markedly
brighter when acidified. [35] Similar results for wool
were reported by Kronman et al. [36] Bhatnagar and
Gruen [37] examined the longwave emission fluorescence

characteristics of a number of acid pretreated wools. They found that dilute acid enhanced the emission intensity but in contrast to the above results prolonged treatment, increased acid concentration or higher temperatures all led to reduced fluorescence. Pretreatment with acid has been reported to accelerate the photochemical degradation of wool. The reasons for this phenomenon remain unclear. [38]

Partial protection of wool against photoyellowing was conferred via pretreatment with formaldehyde at 105°C. [35] This effect may be due to the reported concomitant decrease in the production of thiyl radicals ($RCH_2S\cdot$) on pretreatment with formaldehyde. This decrease is most probably due to reaction of formaldehyde with thiol groups to form methylol derivatives thereby reducing the possibilty of thiyl radical production.

The photoyellowing and emission properties of H_3PO_2, H_3PO_3 and H_2SO_4 acid treated wool are investigated, along with a CH_2O pretreated sample. A solution of each acid in 100% owf CH_2O was also applied (see sec 4:2:1)

6:2   Results of Fluorescence and Photoyellowing Properties of Wool Pretreated with Various Additives

6:2:1   Wool Pretreated with Polysulphides

(a) LP3 and (b) Z-LP3 were applied to wool. (See sec 6:3:1). LP3 is a mercaptan terminated polymer of

diethyleneoxymethane with Sx linkages and Z-LP3 is an

epoxy-terminated polysulphide-polymer. [10],[11]

(Suppliers Morton Chemicals)

(a) $HS-(C_2H_4-O-CH_2-O-C_2H_4-S_x)_6-C_2H_4-O-CH_2-C_2H_4-SH$

(b) $H_2C-CH-(R-CH-CH_2-S-R)_n-S-CH_2-CH-R-CH-CH_2$
         $\diagdown O \diagup \quad\quad OH \quad\quad\quad\quad\quad\quad\quad OH \quad\quad \diagdown O \diagup$
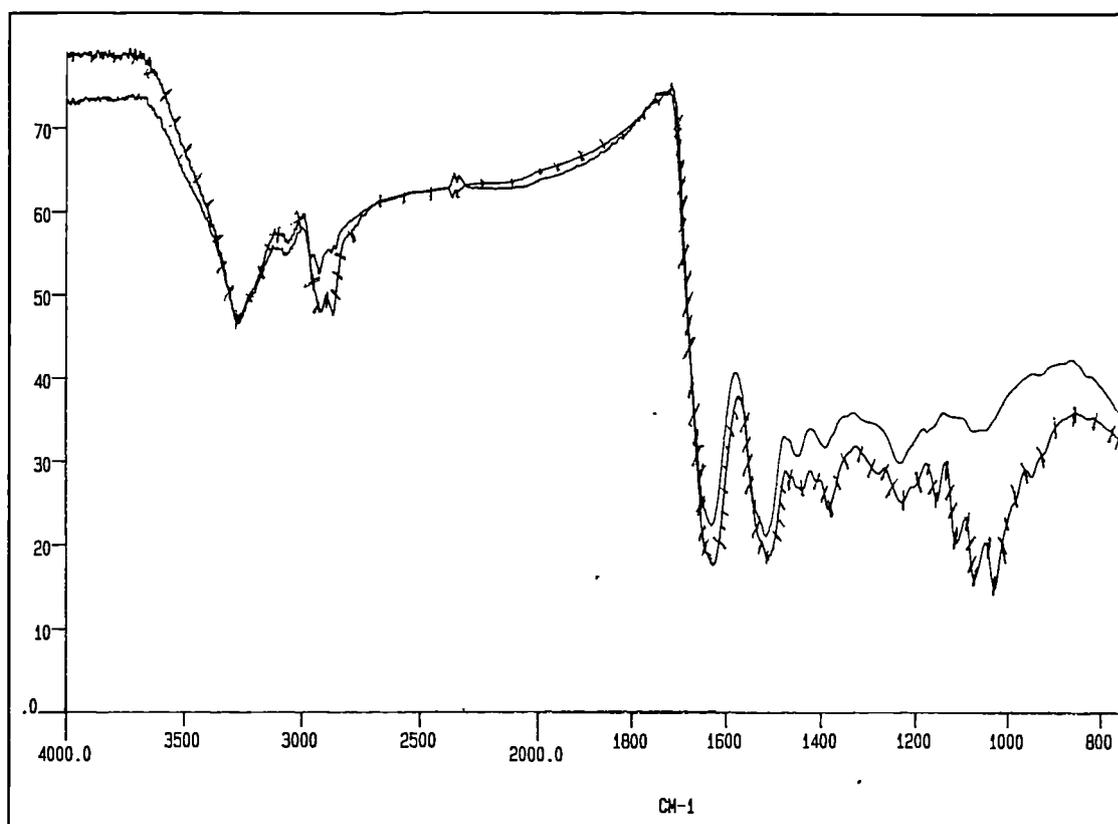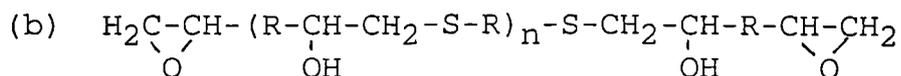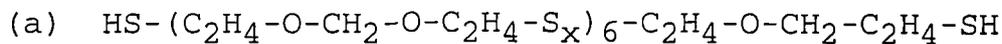
Figure 6:1 Surface I.R. spectrum of (a) ──➔ blank wool
and (b) ++++ LP3 pretreated wool.

Polymer (a) may react with wool via the cystyl

disulphide bridges forming free thiol groups, and

polymer (b) may react like epichlorohydrin [9] with the

carboxyl and amine groups in wool to form ester and

amide links respectively. Both polymers are

bifunctional and may form crosslinks with wool. [39]

		Excite 280 nm	Excite 380 nm
(1)	Untreated Wool	1.0	1.0
(2)	Untreated Wool irr. 24 hrs.	0.0	0.8
(3)	LP3 Wool	0.8	1.0
(4)	LP3 Wool irr. 24 hrs.	0.25	1.1
(5)	Z-LP3 Wool	0.9	1.0
(6)	Z-LP3 Wool irr. 24 hrs.	0.4	1.1

Figure 6:2 Table of fluorescence emission intensity of
LP3 and Z-LP3 treated wool. Where the intensity for
each excitation maximum of the untreated, non-
irradiated blank is arbitrarily set at unity.

No change is observed in the position of the wavelength

of maximum emission in the fluorescence spectra of

polysulphide pretreated wool samples. Excitation at 280

nm and 380 nm results in emission values of 335 nm for

tryptophan and 460 nm for the long wavelength emitter

respectively. Identical values are observed for

untreated wool. (See sec 2:1:1) The emission

intensity of the tryptophan fluorophore is slightly

reduced however, most probably via quenching by

polysulphide bonds. (See sec 6:1:2). This proposal

may be supported by the observation that the tryptophan

emission intensity is quenched to a greater extent in

the LP3 pretreated sample, as this polymer contains the

higher number of polysulphide linkages. (See figure 6:2)

After 24 hours of irradiation, (See sec 5:3:8) complete destruction of the tryptophan fluorophore (irradiated at 280 nm) is observed for the untreated blank (2), as the fluorescence intensity decreases to zero. However, emission from the tryptophan fluorophore is still present (albeit diminished) after 24 hours, (4),(6) in both the polysulphide pretreated samples. The polysulphide treatments protect the tryptophan moiety by slowing down its rate of photodestruction. This may be achieved by reaction of the sulphide groups with $^1O_2$ or peroxy species.

The unknown species X excited at 380 nm, but unlike the tryptophan fluorophore it is not quenched on application of the polysulphides. (3),(5) The emission intensity of this moiety does decrease slightly on irradiation in the untreated blank sample. Like tryptophan, the polysulphide pretreatments protect this species from destruction as after 24 hours of irradiation its fluorescence emission has actually increased slightly.   (4),(6)

From the table below it is evident that the application of both polysulphides to wool gave no protection against photoyellowing. In fact the pretreated samples photoyellowed to a greater extent than the untreated blank. (5),(6) As the rate of photodegradation of tryptophan residues (which form coloured products) is

diminished on application of both polysulphides, it is
probable that the increased photoyellowing is due to
photodegradation of the polysulphides themselves, to
form yellow photoproducts.

	Wool	Whiteness (Berger)	YI (E313)
(1)	Blank	42.06	13.20
(2)	5% owf, LP3	39.35	14.28
(3)	5% owf, Z-LP3	38.87	14.38
(4)	Blank irr. 24 hrs.	29.85	18.30
(5)	5% owf, LP3 irr. 24 hrs.	23.49	20.63
(6)	5% owf, Z-LP3 irr. 24 hrs.	22.22	21.09

Figure 6:3 Table of whiteness and yellowness values of
polysulphide pretreated wool.

Indeed, irradiation of both polysulphides in solution
using a quartz cuvette and lamps described in section
5:3:8, gave a large increase in absorption in the
visible part of the spectrum. (See fig 6:4) The
photoproducts formed have not been identified, but may
be tri-, tetra-, polysulphides formed by radical-
induced photoreactions, or sulphoxides or sulphones
formed by reaction with $^1O_2$.
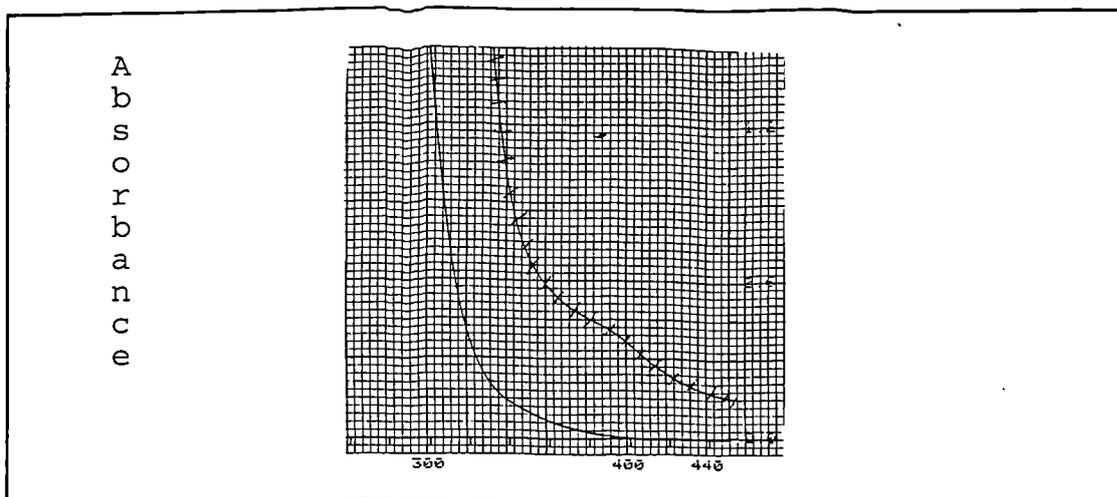
Figure 6:4 UV spectrum of (a) ——— solution of Z-LP3 in DMF, and (b) ++++ solution of Z-LP3 in DMF, irradiated for 24 hours.

6:2:2 Wool Pretreated with Anhydrides

6:2:2:1 Wool Pretreated with Diethylpyrocarbonate

Wool was pretreated with diethylpyrocarbonate (DEPC) (See sec 6:3:2). No alterations in the fluorescence emission spectra were observed on application, and the rates of tryptophan and species X destruction were the same as for the untreated blank. This result implies initially that no reaction between tryptophan residues and DEPC occurred under the conditions of the experiment.

The DEPC pretreatment did however stabilize the wool towards photoyellowing. On application of DEPC, the wool brightened slightly. The most significant effect of the pretreatment occurred on irradiation. After 2.5 hours of irradiation the DEPC sample was photobleached to a greater extent (2 units whiter) than the blank sample, though it was photoyellowed to a greater extent

also. (5),(6) After 24 hours it remained 2 units whiter than the untreated blank, and had photoyellowed to a similar extent. (7),(8)   (See figure 6:5)

	Wool	Whiteness (Berger)	YI (E313)
(1)	Blank	42.06	13.20
(2)	DEPC Soaked	42.53	12.97
(3)	Blank irr. 1 hr.	41.23	13.59
(4)	DEPC Soaked irr. 1 hr.	41.73	13.86
(5)	Blank irr. 2.5 hrs.	39.21	14.16
(6)	DEPC Soaked irr. 2.5 hrs.	41.82	14.00
(7)	Blank irr. 24 hrs.	29.85	17.52
(8)	DEPC Soaked irr. 24 hrs.	32.58	17.34

Figure 6:5 Table of whiteness and yellowness values of DEPC pretreated wool.

Unlike the diffuse reflectance spectrum of the untreated blank, which on irradiation for up to 3 hours shows a steady increase in absorbance from 340-450 nm, the DEPC pretreated sample showed a steady decrease in the absorbance from 365-450 nm with an isobestic point at 358 nm. (See fig. 6:6) The presence of an isobestic point indicates that a clean reaction is occurring. DEPC may react with the unknown species which absorbs at wavelengths > than 360 nm in wool. On irradiation this species is progressively destroyed

with concomitant formation of a species with a maximum

at 335 nm. The destruction of this longwave chromophore

may be responsible for photobleaching the wool.
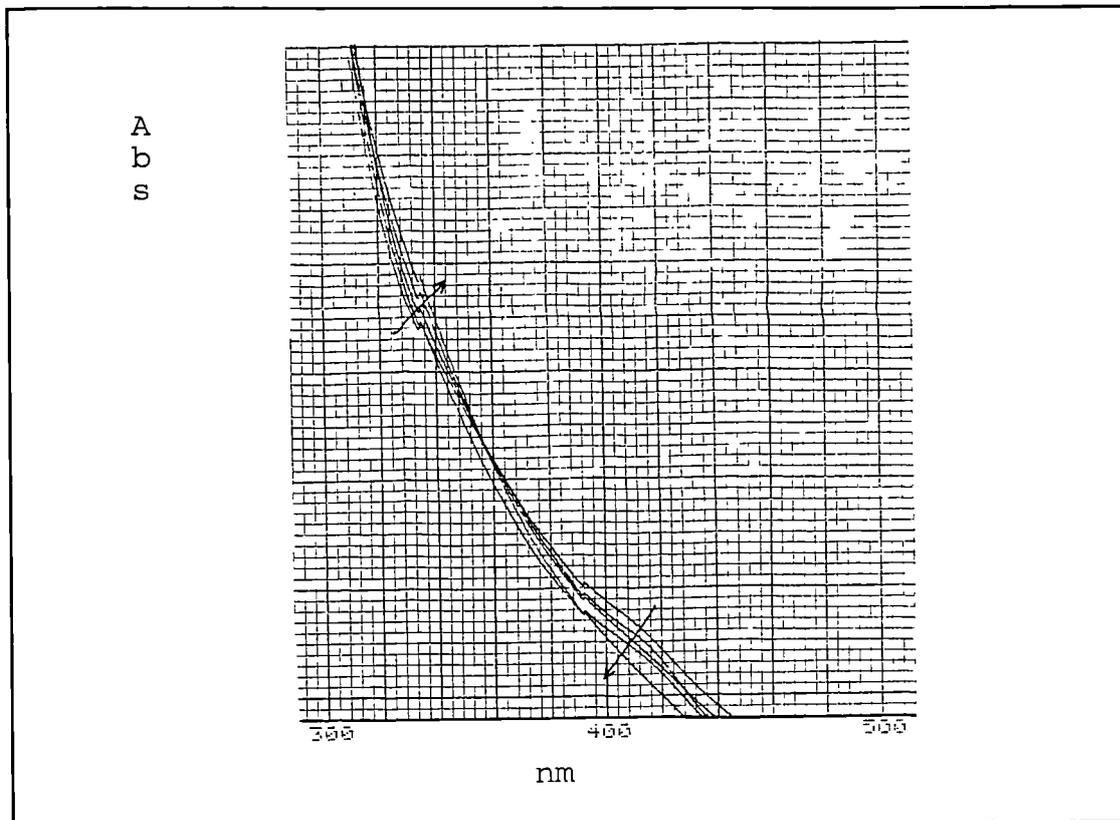
Figure 6:6 Diffuse reflectance spectrum of the irradiation of DEPC pretreated wool. Time 0, 30, 60, 90, 120 mins.

The identity of the longwave absorber is unknown,

however it seems likely that it contains a nucleophilic

moiety which may react with the carbonyl group(s) in

DEPC. This reaction may also block paths by which the

species may react to from photoproducts of absorbance >

400 nm. Though histidine does not absorb at wavelengths

> 250 nm, the fact that DEPC reacts most readily with

this residue implies that it may play a part in the

photoreactions of wool, possibly via crosslinking,

and/or via reactions of the products of its photodegradation. This proposal is supported by Girotti's [40] observation that the photo-oxidation of histidine is accompanied by a decrease in the concentration of primary amine groups. Some type of coupling between the amine moiety and inital photoproducts of the imidazole ring has been suggested. [51] Blockage of both sites of this reaction by DEPC may occur.

DEPC may also react with with other intermediates or final products in the photoyellowing process, leading to non-coloured products. e.g. reaction with the amine group of indoleine hydroperoxide may block the formation of 3a-hydroperoxypyrrolindole, or reaction with the hydroxyl group of tyrosine may block the formation of melanin. [27] Ethoxyformation of the N atom of the indole ring of tryptophan via reaction with DEPC would be expected to prevent the formation of the neutral radical which is formed by deprotonation after electron ejection. [42],[43] If the electron ejection process is also diminished by this reaction then a slower rate of destruction of cystine residues will ensue. The rate of the destruction of the wool's lightfastness will therefore decrease.

No change in the fluorescence spectra (both emission and excitation) was observed on either application or

irradiation of DEPC pretreated wool relative to that of
blank wool.

6:2:2:2 Wool Pretreated with Acetic Anhydride

Acetic anhydride is a simpler anhydride than DEPC. This
compound was applied to wool in the same manner as
DEPC. (See sec 6:3:2)

		Excite 280 nm	Excite 380 nm
(1)	Untreated Wool	1.0	1.0
(2)	Untreated Wool irr. 24 hrs.	0.0	0.8
(3)	Acetic Anhydride pretreated Wool	0.0	1.0
(4)	Acetic Anhydride pretreated Wool irr. for 24 hrs	0.0	0.8

Figure 6:7 Table of fluorescence emission intensity of
acetic anhydride pretreated wool. Where the intensity
for each excitation maximum of the untreated, non-
irradiated blank is arbitrarily set at unity.

The acetic anhydride treatment results in a significant
alteration in the fluorescence emission intensity of
wool. On application, the emission for tryptophan is
reduced to zero. (3) The longwave emission is unchanged
and after 24 hours of irradiation the pretreated and
untreated samples result in identical emission spectra.
(2),(4) The decrease in tryptophan fluorescence may be
due to (a) reaction of acetic anhydride with the indole
moiety at the N position, and/or other site in the

molecule (similar reaction reported for DEPC, see sec

6:1:3), or (b) acylation of the amino group of the

residue. The ϕ_f for N-acetyl tryptophan has been

reported to be diminished relative to that for

tryptophan and a concomitant decrease in photoyellowing

was also reported.[27]
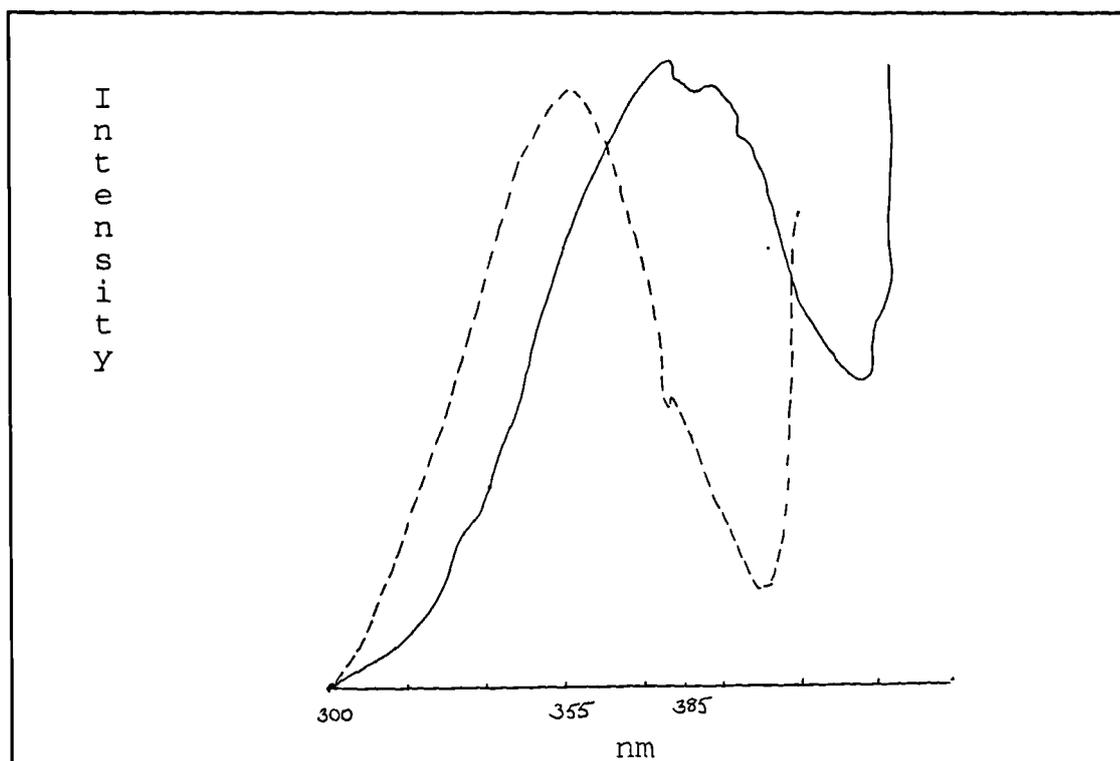
Figure 6:8 Fluorescence excitation spectrum of (a) - -
acetic anhydride pretreated wool and (b) —— untreated
blank, excited at 450 nm.

The excitation spectrum at 450 nm of acetic anhydride

treated wool shows a large shift in its emission

maximum relative to the untreated sample, from a

chromophore which absorbs at 385 nm in untreated wool

to one at 355 nm in the acetic anhydride sample. (See

fig. 6:8)

The shift of this long wavelength chromophore and the concomitant decrease in the tryptophan emission implies that the long wavelength absorber may have some indolic character and/or that it is in conjugation with tryptophan residues.

	Wool	Whiteness (Berger)	YI (E313)
(1)	Blank	42.06	13.20
(2)	Acetic Anhydride Soaked	42.50	13.02
(3)	Blank irr. 2.5 hrs.	39.21	14.16
(4)	Acetic Anyhdride Soaked irr. 2.5 hrs.	42.33	12.93
(5)	Blank irr. 24 hrs.	29.85	17.52
(6)	Acetic Anhydride Soaked irr. 24 hrs.	30.07	16.73

Figure 6:9 Table of whiteness and yellowness values of acetic anhydride pretreated wool.

Like the DEPC pretreated sample the acetic anhydride pretreated sample brightens the wool on application, and after 2-3 hours of irradiation displayed greater photobleaching than the blank. (2),(4) After 24 hours irradiation the acetic anhydride sample remained whiter than the untreated blank. (6) The acetic anhydride pretreatment appears to increase photobleaching in the same manner as DEPC. The chromophore that absorbs at

maximum 385 nm is removed (presumably by reaction with the anhydride) and shifted to a maximum at 355 nm. The removal of the 385 nm absorber bleaches the wool. The reaction of wool with acetic anhydride appears to be more efficient than that with DEPC, as this pretreatment photobleaches the wool to a greater extent. Similar reactions for acetic anhydride as those for DEPC are proposed. (See Sec 6:2:2:1)

6:2:3 Wool Pretreated with Isocyanates

Wool was pretreated separately with ethyl and phenyl isocyanates, (see sec 6:3:5). The water soluble bisulphite adducts of these compounds were synthesised (32),(33) (see sec 6:3:4) and applied to wool from aqueous solution.

On application, the fluorescence emission spectra of all the isocyanate pretreated samples displayed huge increases in intensity for both the tryptophan and unknown X fluorophores. [2]-[6] The bisulphite adducts showed the most marked effect. [7]-[10] The reason(s) for this are unclear. The marked change in the intensity and shape of the band emitting at 355 nm relative to that at 335 nm, (See fig. 6:11) in the fluorescence emission spectrum of tryptophan, is indicative of an alteration in the polarity of the residue's environment. (See sec 6:1:1)

		Excite 280 nm	Excite 380 nm
(1)	Untreated Wool	1.0	1.0
(2)	Untreated Wool irr. 24 hrs.	0.0	0.8
(3)	Ethyl isocyanate pretreated Wool	1.75	2.5
(4)	Ethyl isocyanate pretreated Wool irr. for 24 hrs	6.5	5.5
(5)	Phenyl isocyanate pretreated Wool	3.0	3.0
(6)	Phenyl isocyanate pretreated Wool irr. for 24 hrs	5.0	3.00
(7)	Ethyl isocyanate bisulphite adduct pretreated Wool	10.0	10.0
(8)	Ethyl isocyanate bisulphite adduct pretreated Wool irr. for 24 hrs	10.0	10.0
(9)	Phenyl isocyanate bisulphite adduct pretreated Wool	10.0	10.0
(10)	Phenyl isocyanate bisulphite adduct pretreated Wool irr. for 24 hrs	10.0	10.0

Figure 6:10 Table of fluorescence emission intensity of isocyanate and isocyanate bisulphite adduct pretreated wool. Where the intensity for each excitation maximum of the untreated, non-irradiated blank is arbitrarily set at unity.

This effect is most likely due due to both the modification of hydrophilic groups and to the physical exclusion of water by the bulky carbamoyl groups. (44),(45) The tyrosyl groups may also play a part as

it has been reported that the principal effect of their acetylation is to increase the ϕ_f of tryptophan residues in proteins which contain both amino acids. [52]

A third possible explanation is the removal of a species which quenches both the fluorophores via reaction with the isocyanate moiety, e.g. cysteinyl residues. However, reaction of isocyanates with thiols has been reported to be sluggish. [34]

The observation that on irradiation the fluorescence intensity for both excitation wavelengths in the isocyanate pretreated samples increases to an even greater extent, is difficult to rationalise. [4]-[7] It may be due to progressive removal, on irradiation, of a species which quenches both fluorophores on reaction with isocyanate. No change in the emission intensity for either excitation wavelength on irradiation is observed for the bisulphite adduct pretreated samples, indicating that some protection against photodegradation is conferred by the pretreatment.

No shift in the fluorescence emission maximum peak at 385 nm (excited at 440 nm) to a lower wavelength as for the anhydride pretreatments (see sec 6:2:2:2) is observed. This implies that the reaction of isocyanate with wool is different to that of anhydride and wool.
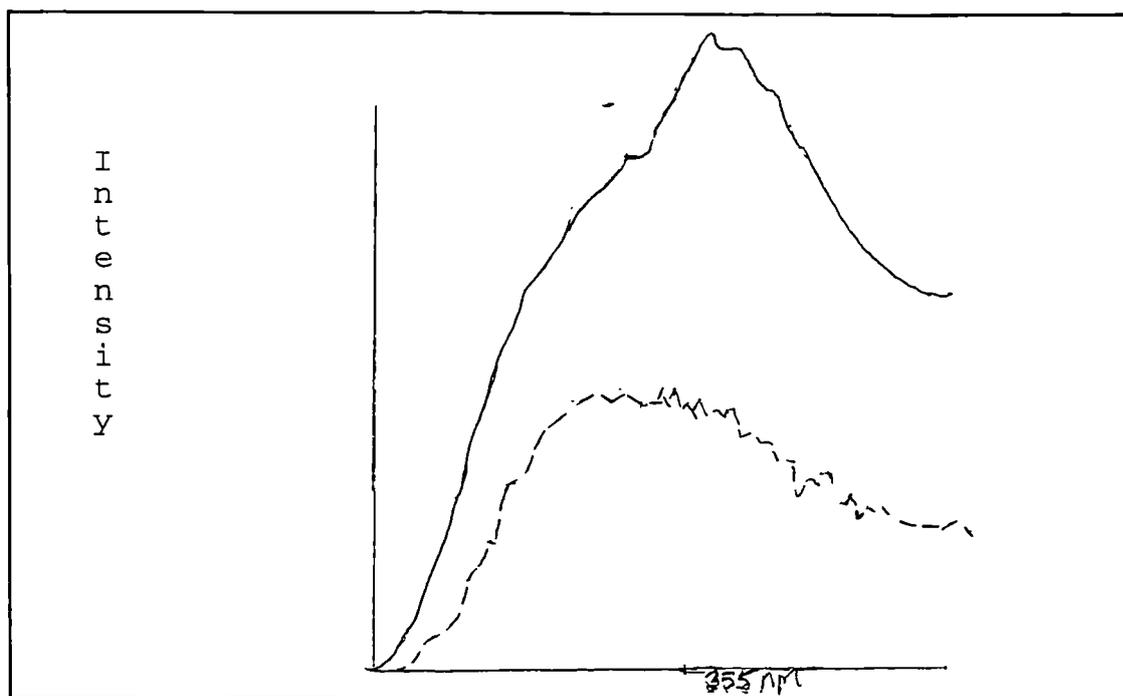
Figure 6:11 Fluorescence emission intensity of (a) --- untreated wool and (b) ———— phenyl isocyanate pretreated wool, excited at 280 nm. Sensitivity of (a) 100, of (b) 30.2. Slit width 4.

Unlike the reaction of wool with isothiocyanates [31] it is clear from figure 6:12 that both the isocyanate pretreated samples yellowed the wool to a greater extent than the untreated blank. Their bisulphite adducts however did confer some protection, with the wool yellowing to a slightly lesser extent than the blank. This is to be expected, as no decrease in the fluorescence emission intensity for the tryptophan residue is observed on irradiation. The concomitant photoyellowing and increase in tryptophan emission intensity for the ethyl isocyanate and phenyl isocyanate pretreated samples, infers that yellow compounds are formed by degradation of other residues or by photoreactions of the carbamoyl group itself. It is puzzling as to why only the bisulphite adducts

confer protection, and the sulphonate moiety must play

a role. Indeed sodium sulphite and sodium bisulphite

have been reported to improve the lightfastness of

fluorescently whitened wool. It has been suggested that

this is due to reduction of coloured products of

tryptophan photodegradation to non-coloured

products.[46]
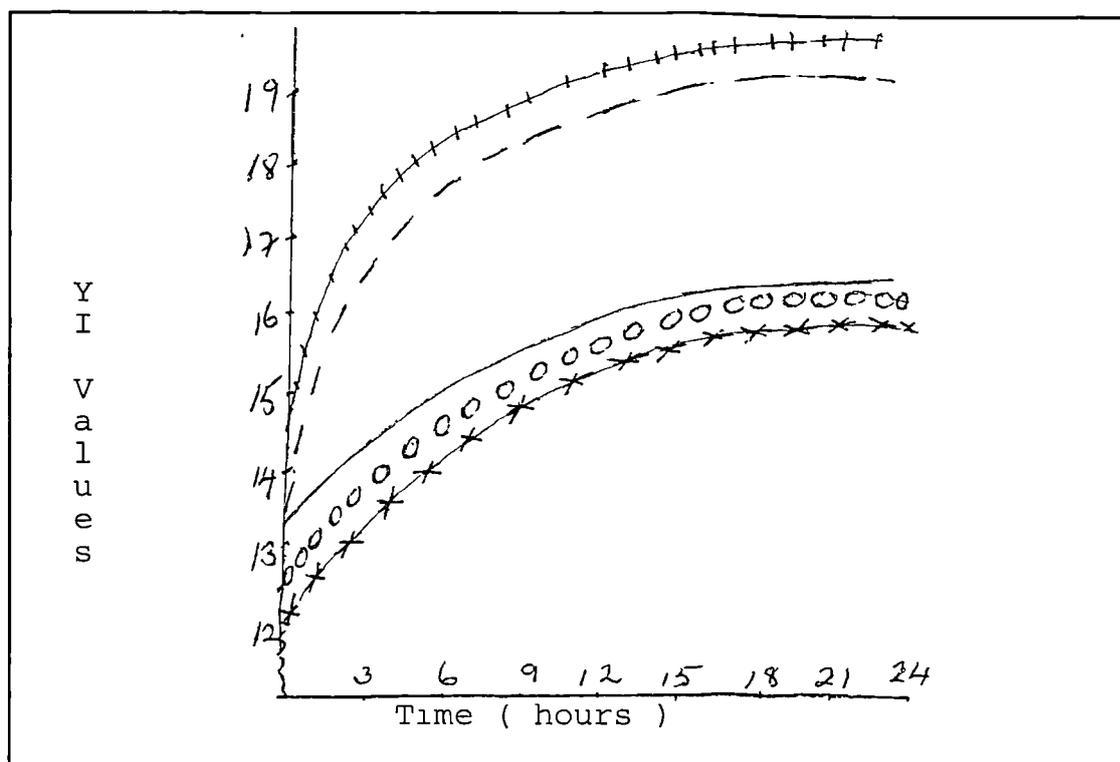
Figure 6:12 Graph of YI (E3I3) values against time
(hours) of irradiation. For (a) —— wool, (b) ++++
Phenyl isocyanate pretreated wool, (c) - - - - Ethyl
isocyante pretreated wool, (d) o o o Phenyl isocyanate
bisulphite pretreated wool, (e) * * Ethyl isocyanate
pretreated wool.

6:2:4   Wool Pretreated with Solutions of Acid and Acid/Formaldehyde.

The reaction of wool with hypophosphorous acid (H_3PO_2), phosphorous acid (H_3PO_3), the mineral acid sulphuric acid (H_2SO_4) and a mixture of H_3PO_2 and formaldehyde (CH_2O) under the experimental conditions described in Sec 4:2:3, caused a large increase in the fluorescence emission intensity of the unidentified X species excited at 380 nm. (See Fig 6:13) A doubling of emission intensity of the tryptophan species, excited at 280 nm, is observed only on reaction with hypophosphorous acid (H_3PO_2) and the H_3PO_2/CH_2O mixture. (6),(2)

	Excite 280 nm	Excite 380 nm
(1) Untreated Wool	1.0	1.0
(2) H_3PO_2 Wool	2.0	6.8
(3) H_3PO_3 Wool	1.0	3.3
(4) H_2SO_4 Wool	1.0	8.0
(5) CH_2O Wool	1.0	7.0
(6) H_3PO_2/CH_2O Wool	2.0	8.0
(7) H_3PO_3/CH_2O Wool	1.0	1.0
(8) H_2SO_4/CH_2O Wool	1.0	1.0

Figure 6:13   Table of fluorescence emission intensity of acid and acid/ formaldehyde treated wool. Where the intensity for each excitation maximum of the untreated blank sample is arbitrarily set at unity. Sensitivity is 100.

Interestingly, no emission enhancement for either wavelength was observed on reaction of H_3PO_3/CH_2O and

H_2SO_4/CH_2O mixtures with wool. (7),(8) The cause(s) of these phenomenena remain unclear.

The physical structure of the wool may be opened up by reaction with strong acid. This occurs in two ways: (a) via hydrolysis of the polypeptide backbone and (b) via disruption of the salt links which hold neighbouring chains together. (See Sec 4:2:1) This opening of the wool structure may increase the distance between a quencher molecule (if indeed one exists) and chromophore X, which causes an increase in the emission intensity of X.

Cowgill has reported that the peptide bond itself acts as a quencher [53],[54], and this ability was ascribed to an inductive electronegativity effect of the carbonyl moiety. The hydrolysis of the peptide bond would render it useless as a quencher and consequently an increase in emission may be observed at 450-460 nm. However, it has been shown [48] that the quantum yield of fluorescence of tyrosine and tryptophan derivatives and peptides in P.V.A. film are the same as those of the free amino acids, and is therefore unlikely that the peptide bond causes significant fluorescence quenching in wool keratin.

The doubling of the emission intensity of the tryptophan species at 280 nm on reaction of wool with H_3PO_2 and H_3PO_2/CH_2O mixture further proves the acid's reducing ability. The reaction cleaves the disulphide

bond forming thiol and other products (See Sec 3:2:3
), and gives an increase in the emission intensity of
the species excited at 280 nm. The inability of H_3PO_3
and H_2SO_4 to do the same is also noted.

From these observations it may be concluded that the
quencher of the X species in wool is not, or is not
exclusively, the disulphide bond, as species X's
emission intensity increased after reaction with
compounds that are known not to cleave the disulphide
bond.

The reaction of wool with formaldehyde gives numerous
products, but reacts primarily with amine groups to
form methylol links, which in turn form crosslinks.
(See Sec 4:2:2) The reaction of wool with CH_2O/H_3PO_2
is clearly different to that of formaldehyde and the
other acids. The formation of a phosphorylated product
by reaction of thiol with a hypophosphorous acid/
formaldehyde intermediate is suggested in sec 4:3:1. A
10% reduction of disulphide bonds to thiol occurs on
reaction with H_3PO_2 alone. A similar reaction appears
also to occur with H_3PO_2/CH_2O (as the fluorescence
intensity of the tryptophan moiety is observed to
increase). Subsequent crosslinking of the thiol moiety
with formaldehyde may then occur, forming $R-S-CH_2-S-R$
crosslinks. The most potent quencher, the disulphide
bond is removed. The increase in the intensity of
fluorescence of the X species may be due to the

reaction of unidentified species in wool with CH_2O or acid.

Wool	Whiteness (Berger)		YI (E313)	
	Time of irr.		Time of irr.	
	0 hrs.	24 hrs.	0 hrs.	24 hrs.
Blank	42.06	29.85	13.20	17.52
H_3PO_2 Wool	34.66	22.04	18.95	24.79
H_3PO_3 Wool	35.57	16.25	18.25	27.79
H_2SO_4 Wool	23.88	7.31	23.95	33.08
CH_2O Wool	38.31	32.04	16.30	19.39
H_3PO_2/CH_2O Wool	43.42	22.78	14.58	24.48
H_3PO_3/CH_2O Wool	39.92	14.48	16.33	29.22
H_2SO_4/CH_2O Wool	3.61	4.90	35.41	36.67

Figure 6:14 Table of whiteness and yellowness values of acid, formaldehyde and acid/formaldehyde pretreated wool.

In comparison, the observation that H_3PO_3/CH_2O and H_2SO_4/CH_2O pretreatments have no effect on the wool's fluorescence at either of the emitting wavelengths suggests that no such reduction/crosslinking reaction occurs for these compounds, and therefore no increase in the emission intensity of the tryptophan moiety is observed. As reaction with each acid and CH_2O solution alone increases the intensity of emission of the longwave emitter and no such increase is observed for

the H_3PO_3/CH_2O and H_2SO_4/CH_2O mixtures, it implies that the presence of both species in solution cancels the effect of the other out.

In agreement with other authors (see sec 2:3), all acid pretreatments (with or without formaldehyde) increase the rate and extent of the photoyellowing of wool. The reason(s) for this are unclear. The opening of the wool structure by acid would lead to an increase in oxidative degradation by easing the access of oxygen to the reactive sites. It has also been reported that the protonated amino acid group shows an increase in reactivity toward a solvated electron, $e_{(aq)}$ (produced on irradiation of aromatic residues in wool) compared to the unprotonated NH_2 group. [50] This may lead to a faster and more extensive rate of photodestruction and photoyellowing than the untreated blank.

Pretreatment with formaldehyde alone conferred some protection against photoyellowing. The irradiated sample of formaldehyde pretreated wool is whiter after 24 hours than the blank. Formaldehyde may block the 2-position of the indole ring of tryptophan to form 2-methylol indole. This compound may subsequently form a cyclic product via condensation with the amide linkage or a crosslink via condensation with a primary amine group. (See reaction below) [50] The photoreaction with $^1O_2$ may therefore be hindered, reducing the amount of yellow photoproducts formed.

R_1
CH_2OH + $2 HN-R_2$ $\longrightarrow$ R_1
CH_2-NH-R_2

(left structure: bicyclic ring system with O and N–H; right structure: same with CH_2-NH-R_2)
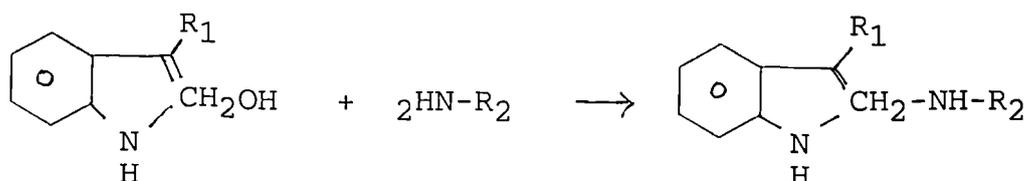
6:3 Experimental Methods

6:3:1 Procedure for Application of Polysulphides onto Wool.

0.5 g of each polysulphide (LP3 and ELP3) was dissolved in 10 mls of D.M.F. (dimethylformamide). 90 mls of water containing a commercial emulsifying agent Igepal and 0.05g of the wetting agent Lissapol N ($C_9H_{19}PhO(CH_2CH_2O)_{10}H$)) was added, and the solution stirred for 10 mins. 10g of wool was immersed in the solution and 100% uptake of polymer by weight was achieved, to yield a concentration of 5% polymer owf (on weight of fibre).

6:3:2 Procedure for Application of Diethylpyrocarbonate and Acetic Anhydride onto Wool.

5 grams of wool was placed in a round-bottomed flask which contained 5 mls of pure anhydride. The contents were stirred for 1 hour ensuring that the wool was evenly covered with anhydride, to yield a concentration of 100% owf of anhydride. Excess anhydride was removed by patting the wool with white tissue. The wool was then air dried.

6:3:3 Method of Synthesis and Analysis of Bisulphite Adduct of Ethyl Isocyanate

0.38 mls (5mmol) of ethyl isocyanate (C_2H_5NCO) was added to a solution of potassium metabisulphite ($K_2S_2O_5$) 0.64 grams (2.75 mmol) in 2.5 mls of water. The stoppered flask was shaken for 1 hr. at R.T. and then the water was evaporated at 40°C under vacuum, to give N-ethylcarbamylsulphonate ($C_2H_5NHCOSO_3K$) as a colourless solid. [30]

The infra-red spectrum (KBr) of the product $C_2H_5NHCOSO_3K$ displayed a carbonyl peak (C=O) at 1680 cm-1, and a wavelength maximum of 240 nm in its UV spectrum. (Solvent H_2O)

6:3:4 Method of Synthesis and Analysis of Bisulphite Adduct of Phenyl Isocyanate

0.24 grams (49.5 mmol) of sodium sulphite (Na_2SO_3) was dissolved in dioxan (16 mls) and acetic acid (3 mls, 52.5 mmol). The solution was cooled in an ice-bath and stirred. 5.0 mls (45.8 mmol) of phenyl isocyanate (C_6H_5NCO) was added dropwise over 30 mins. Stirring in the ice-bath continued for a further 3 hrs. The reaction mixture was filtered and the residue was stirred with iced water (100 mls) and filtered again. Both filtered solutions were evaporated at 40°C under vacuum. The residues were then purified by dissolving in a mimimum volume of cold water and immediately adding 2 volumes of saturated brine to precipitate out

the sulphonate salt. The product $C_6H_5NHCOSO_3Na$ was recrystallised by preparing a near saturated solution in water at R.T., and cooling at 0^oC for several hours. (32)

In agreement with the literature, (32) the UV spectrum of the product showed a UV peak at 242 nm.

6:3:5   Procedure for Application of Isocyanates onto Wool.

6:3:5:1   Procedure for Application of Ethyl and Phenyl Isocyanates onto Wool.

5 grams of wool was placed in a round bottomed flask which contained 25 mls of a 10% (w/v) solution of isocyanate in pyridine. The contents were stirred for 3 days at room temperature, ensuring that the wool was evenly covered with isocyanate, to yield a concentration of 50% isocyanate owf. The wool was patted dry, and then dried at room temperature.

6:3:5:2   Procedure for Application of the Bisulphite Adducts of Ethyl and Phenyl Isocyanates onto Wool.

5 grams of wool was placed in a round bottomed flask which contained 20 mls of a 10% (w/v) solution of the isocyanate bisulphite adduct. The solution was brought to pH 4.0 with addition of sulphuric acid (to release isocyanate). The contents of the flask were stirred and heated to 60^oC for 2 hours. To yield a

concentration of 40% adduct owf. The wool was patted

dry, and then dried at room temperature.

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7:1 Introduction to Pyruvyl Groups and Their Production in Wool.

Exposure of wool to sunlight in the presence of oxygen causes both side chain and main chain photo-oxidation. (1),(2) The identification of small amounts of pyruvic and glyoxylic acids in the protein hydrolysate indicate according to Meybeck and Meybeck, (3),(4) main chain breakdown at glycine and alanine residues, with photochemical conversion of glycine and alanine residues to glyoxylyl and pyruvyl peptides, according to the following scheme.

$$-CO-NH-CHR-CO-NH-$$
$$\downarrow hv$$
$$-CO-NH-\overset{\bullet}{C}R-CO-NH- + \overset{\bullet}{H}$$

$$O_2 \swarrow \qquad \searrow$$

$$-CO-NH-\underset{O-O\cdot}{\overset{|}{C}}R-CO-NH- \qquad\qquad -CON=CRCO-$$

$$\downarrow R'H \qquad\qquad\qquad \downarrow H_2O$$

$$-CO-NH-\underset{OOH}{\overset{|}{C}}R-CO-NH- \quad + R'\cdot \qquad -CO-NH_2 + RCO-CO-NH-$$

$$\searrow \qquad\qquad \nearrow$$

$$-CO-NH-\underset{OH}{\overset{|}{C}H}-CO-$$
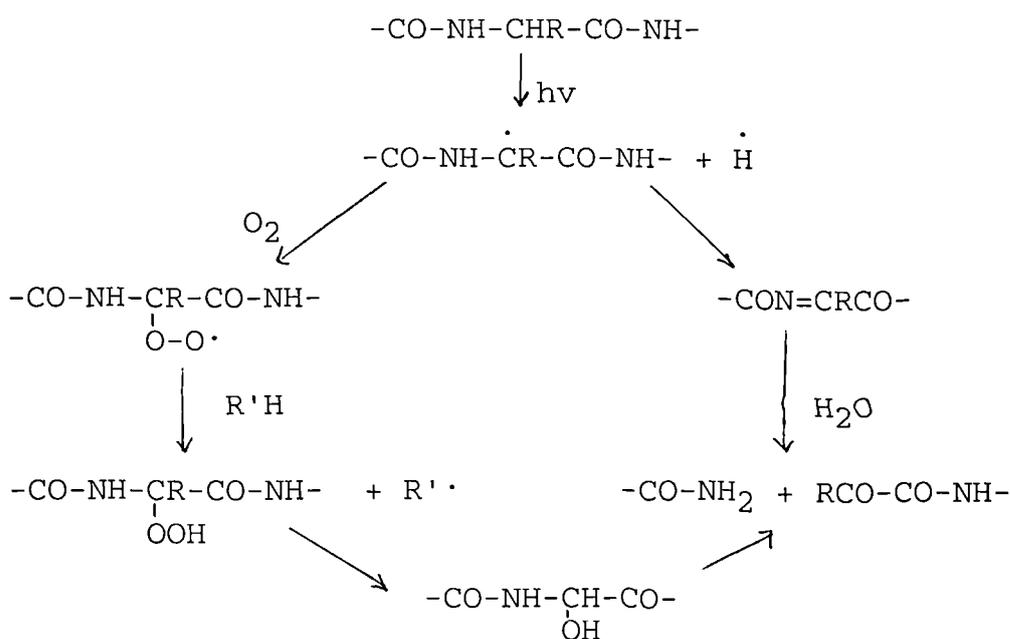
Pyruvyl peptides absorb maximally at 310-337 nm, close to the absorption maximum of 320-330 nm displayed by photoyellowed wool. The photoyellowing may not however be exclusively due to the production of pyruvyl groups, as the rate and extent of production of carbonyl groups

is the same for bleached, unbleached and fluorescently whitened wool. Yet these fabrics photoyellow at markedly different rates. [2] (See section 6:1)

It is proposed here that pyruvyl residues may occur in wool by non-photochemical processes and may play an important part in the alkali yellowing of wool. This yellowing process is not well understood but it has been shown that the cystine residue plays a role, as wool that has been modified to form the more stable -S-CH_2-CH_2-S- crosslink (via reduction followed by reaction with 1,2-dibromo-ethane) yellowed to about half the extent of untreated wool in alkaline solution. [5] The disulphide bonds in wool, in the presence of alkali may be cleaved by removal of a proton from the β-carbon atom to give the corresponding anion, followed by β-hydride elimination to produce a perthiocysteine residue and a dehydroalanine residue, according to the following mechanism: [6]

```
 -NH              CO-          -NH              CO-
  |                |            |                \
-CH-CH2-S-S-CH2-C⁻   ⟶    -CH-CH2-SS⁻  +    H2C=C-
  |                |            |                /
 -CO              NH-          -CO              NH-
```

                    Perthiocysteine   Dehydroalanine
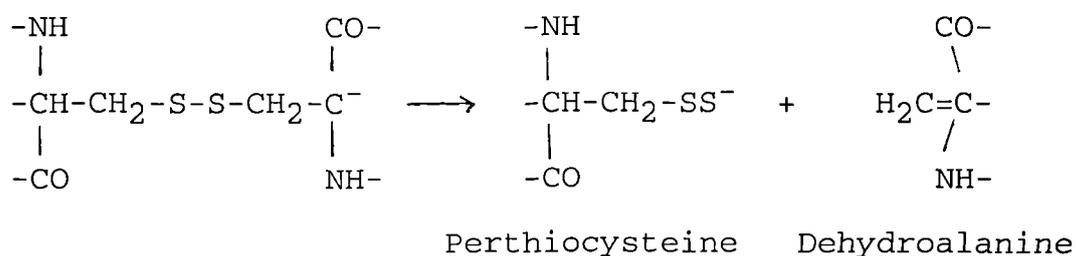
The dehydroalanine residue may tautomerise to form the imine species, which on reaction with water may form a pyruvamide and a primary amide as in scheme one.

$$-NH-CO-\underset{\underset{CH_3}{|}}{C}=N-CO- \quad \xrightarrow{H_2O} \quad -NH-CO-\underset{\underset{CH_3}{|}}{\overset{\overset{+}{OH_2}}{C}}-N^- -CO- \quad \longrightarrow$$

$$-NH-CO-\underset{\underset{CH_3}{|}}{\overset{\overset{OH}{|}}{C}}-NH-CO \quad \longrightarrow \quad -NH-CO-CO-CH_3 \quad + \quad NH_2-CO-$$
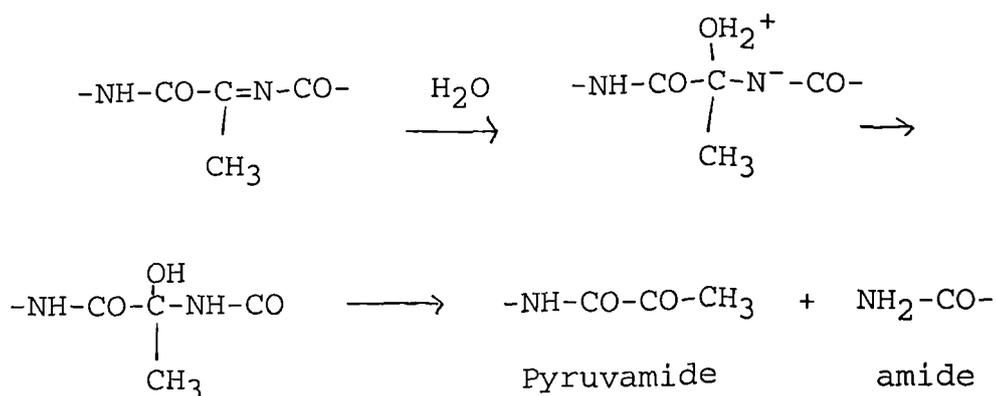
Pyruvamide   amide

The pyruvyl group is fluorescent and may be in part responsible for the long wavelength fluorescence of wool keratin after irradiation. A series of pyruvamides (aliphatic and aromatic) are synthesised and their fluorescence spectra are investigated.

7:2 Results of Fluorescence and Yellowness Values of Pyruvate and Pyruvamide Pretreated Wool.

	Excite at 350 nm	
Pyruvic Acid	440 nm	maximum
t-Butylpyruvate	430 nm	maximum
Ethylpyruvate	430 nm	maximum
n-Butylpyruvamide	425 nm	maximum
n-Propylpyruvamide	430 nm	maximum
Anilinepyruvamide	455 nm	maximum
p-Toluidinepyruvamide	450 nm	maximum

Figure 7:1 Table of fluorescence emission maxima of pyruvate and pyruvamide compounds in acetone, excited at 350 nm. Solutions are 1*10-4 M.

The emission spectrum of wool (see figure 6:1) displays fluorescence emission (depending on excitation wavelength) up to 550 nm. The solution emission spectra are very broad for all pyruvyl species synthesised with emission tapering off at 580 nm, so it is possible that these species play a part in the emission of wool keratin. It is interesting to note that both aromatic pyruvamides have emission maxima at longer wavelengths than their aliphatic counterparts.

The proposal that pyruvamide groups may contribute to the yellow colour of UV irradiated (or alkali) treated wool is supported by the following yellowness/whiteness results of pyruvamide pretreated wool samples, irradiated by UV.

Wool	Whiteness (Berger)		Yellowness (E313)	
	Time of irr.		Time of irr.	
	0 hrs.	24 hrs.	0 hrs.	24 hrs.
Blank	42.05	29.85	13.20	17.52
n-Butyl Pyruvamide	25.94	16.06	21.90	23.93
Aniline Pyruvamide	25.30	15.89	22.80	23.45
p-Toluidine Pyruvamide	23.08	14.30	23.63	25.48
n-Propyl Pyruvamide	24.90	15.01	23.90	24.67

Figure 7:2 Whiteness and Yellowness Values for Pyruvamide Pretreated Wools, irradiated for 24 hrs.

The results in figure 7:2 show that all pyruvamide pretreatments yellow wool on application. Further yellowing is observed on irradiation of samples with UV light. This phenomenon may be due to the production of active peroxy radical species in the pretreated samples via reaction of the pyruvamides with oxygen. i.e. the pyruvamide groups may react in a similar fashion to that reported for peroxy acids. [15]

$$CH_3-CO-CO-NHR \xrightarrow{\ h\nu\ } CH_3-CO-CO-NHR$$

$$O_2$$

$$\begin{array}{c} OO\cdot \\ | \\ CH_3-C-CO-NHR \\ | \\ O\cdot \end{array} \qquad CH_3-CO\cdot \ + \ \cdot CO-NHR$$

$$O_2$$

$$CH_3-CO-OO\cdot \ + \ \cdot CO-NHR$$
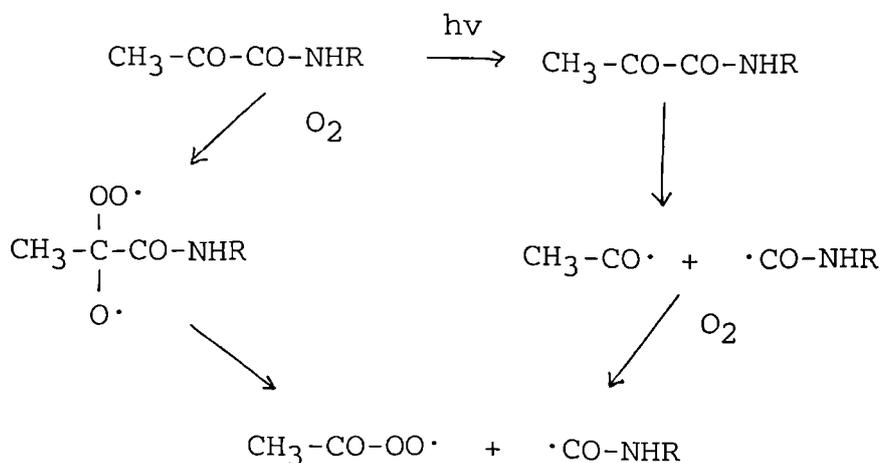
The observation that wool treated with DCCI and pyruvic acid (see section 7:3) to form pyruvamide is yellower than wool pretreated with pyruvic acid alone, (see figure 7:3) further supports the view that pyruvamide species may be responsible in part for the yellowness of irradiated or alkali treated wool. This reaction assumes that coupling with the terminal amino and lysine ε-amino groups of the wool surface and pyruvic acid occurs.

	Whiteness (Berger)	YI (E313)
Wool	42.06	13.20
Pyruvic Acid Wool	38.42	15.73
Pyruvic Acid DCCI Wool	37.35	16.91

Figure 7:3 The whiteness/yellowness values of pyruvic acid, pyruvic acid DCCI pretreated wool.

7:3   Experimental Methods

7:3:1   Synthesis of t-Butylpyruvate

The acid chloride of pyruvic acid pyruvoyl chloride, was prepared using the method of Offenheijm and De Man. (7)

$$CH_3COCOOH + Cl_2CHOCH_3 \longrightarrow CH_3COCOCl + HCOOCH_3 +$$
$$HCl$$
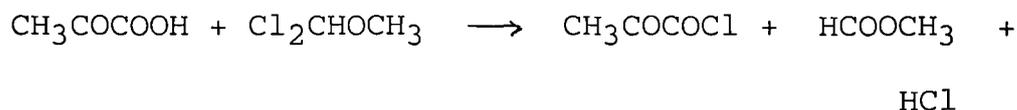
α–α dichloromethyl methylether (0.06 mole) was added dropwise with stirring to a solution of pyruvic acid (0.06 mole), under N_2, at RT with the evolution of HCl gas. The mixture was heated in an oil-bath for 30 mins, at 50°C. The formation of pyruvoyl chloride was confirmed by NMR and no further purification was undertaken. A yield of 50% (0.03 mole) was assumed. (It was not necessary to distil the pyruvoyl chloride provided two equivalents of pyridine were used in the esterification step.)

t-butyl pyruvate was prepared using the method
described by Binkley as outlined below. [8]

$$RCOCOCl \ + \ R'OH \ \longrightarrow \ RCOCOOR'$$
$$< 10^{\circ}C$$
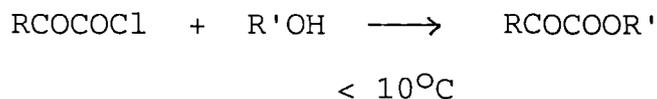
t-butyl alcohol (0.03 mole) and dry pyridine (0.06
mole) were dissolved in anhydrous benzene (100 ml).
The prepared pyruvoyl chloride (0.03 mole) in
anhydrous benzene (50 ml) was added dropwise with
stirring under N_2. Precipitation of pyridinium
hydrochloride was immediate. The reaction was cooled in
an ice-bath to keep the temperature below $10^{\circ}C$. When
the addition of the acid chloride was complete, the
mixture was allowed to reach RT and stirring continued
for a further 30 mins. The pyridinium hydrochloride was
removed by filtration and the benzene solution
distilled in vacuo, to yield t-butylpyruvate
contaminated with pyridinium hydrochloride. The
contaminant was removed by shaking the mixture in CCl_4
(50 ml) and allowing it to stand overnight and then
filtering off the insoluble material. The CCl_4 was
removed in vacuo leaving the pure t-butylpyruvate oil.

7:3:2 Analysis of t-Butylpyruvate

Yellow/brown oil.

Boiling Point: 45-46°C, 24 mm (lit. 35°C, 12 mm) [8]

Elemental Analysis:
For $C_6H_{12}O_3$ Theor: %C 54.54   %H 9.09
                  Found: %C 53.14   %H 9.89

Infra-Red: (Neat film)
1786 (cm^{-1}), 1750 (cm-1) carbonyl (C=O), 1372
(cm^{-1}) , 1317 (cm^{-1}) CH_3, 1263-1223 (cm^{-1}) $(CH_3)_3$.

^{1}H N.M.R.: (100MHz, $CDCl_3$)
 2.45   (3H, s, CH_3)
  1.58   (9H, s, $(CH_3)_3$)

UV Spectrum: (DMF)
Peak maximum at 263 nm.

7:3:3 Synthesis of Pyruvamides

Acylamino acids and amino components are coupled after

adding an equimolar quantity of N,N'-dicyclohexyl

carbodiimide (DCCI) in solution at 0°C. [9]-[11] The

first step involves the addition of the acid group to

the diimide DCCI. This produces an o-acyllactim

derivative which reacts on addition of amine to give

the amide product and N,N'-dicyclohexylurea. N,N'-

dicyclohexylurea has a very low solubility in most

organic solvents and is easily separated. [12]

$$CH_3COCOOH + Cy-N=C=N-Cy \longrightarrow CH_3COCOO$$

$$/$$

$$Cy-N=C-N-Cy$$
$$H$$

         Pyruvic Acid   DCCI

$$CH_3COCOO \quad + \quad RNH_2 \longrightarrow CH_3COCO-NHR \quad + \quad CyN-CO-NCy$$
$$|$$
$$Cy-N=C-N-Cy$$
$$H$$

                 Amine   Pyruvamide   N,N'-di
                                            cyclohexylurea
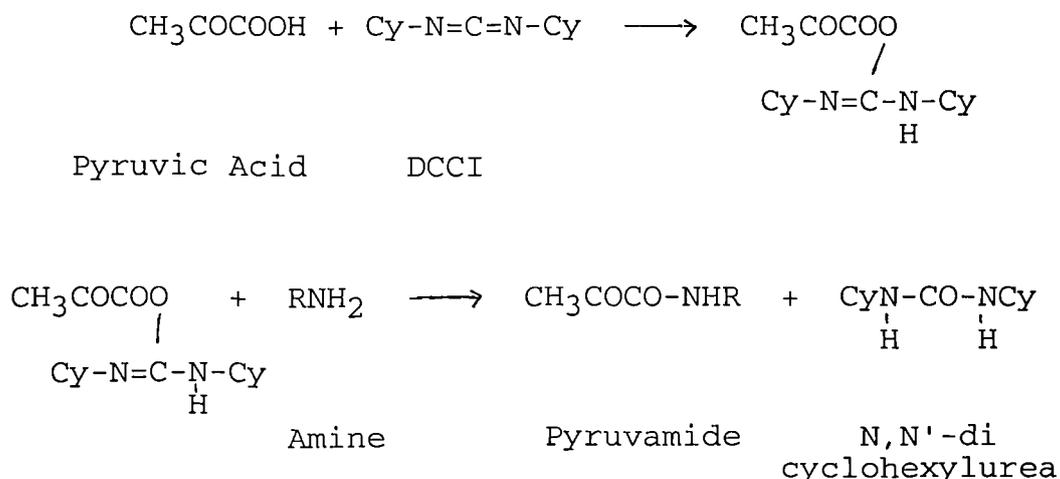
Where Cy = Cyclohexyl , and R = phenyl, n-butyl, n-

propyl.

0.02 moles of pyruvic acid was added to a stirred

solution of 0.02 moles of dicyclohexylcarbodiimide in

50 mls of THF. (The DCCI had been heated in its

container in an oven at 40°C for 30 minutes or until

enough of the compound was in liquid form.) On

addition an immediate colour change occurred with the

solution turning red-umber. Stirring was continued for

30 minutes. Addition of 0.02 moles of amine followed

over approximately 15 minutes, with the precipitation

of N,N'-dicyclohexylurea. The solution was stirred at

room temperature overnight. The solution was then

filtered to remove the solid urea and the excess

solvent was removed in vacuo. All pyruvamides

synthesised were purified by column chromatography on

silica using acetone as eluent. In all cases sticky

brown/yellow oils were realised.

7:3:4   Analysis of Pyruvamides

n-Butylpyruvamide

Deep yellow sticky oil.

Molecular formula: $C_7H_{13}NO_2$

Infra-Red: (Thin Film)
1767 and 1709 (cm^{-1}) carbonyl stretches, 1635 (cm^{-1})
secondary amide stretch, [14] 624 (cm^{-1}) amide C-N
vibration mixed with NH.

^{1}H N.M.R.: (100MHz, $CDCl_3$)
  3.2   (1H, broad s, N-H)
  2.1   (3H, s, CH_3)
  0.9-1.9   (9H, m, C_4H_9)

UV Spectrum: (DMF)
Sharp peak with maximum at 322 nm.

n-Propylpyruvamide

Deep yellow sticky oil.

Molecular formula: $C_6H_{11}NO_2$

Infra-Red: (Thin Film)
1767 and 1701 (cm^{-1}) carbonyl stretches, 1638 (cm^{-1})
secondary amide stretch, 624 (cm^{-1}) amide C-N
vibration mixed with NH.

^{1}H N.M.R.: (100MHz, $CDCl_3$)
  3.1   (1H, broad s, N-H)
  2.0   (3H, s, CH_3)
  0.9-1.9   (9H, m, C_3H_7)

UV Spectrum: (DMF)
Peak with maximum at 270 nm, and shoulder at 340 nm.

Aniline pyruvamide

Deep yellow/brown sticky oil.

Molecular formula: $C_9H_9NO_2$

Infra-Red: (Thin Film)
3100 (cm-1) aromatic C-H stretches, 1765 and 1709
(cm^{-1}) carbonyl stretches, 1638 (cm^{-1})
secondary amide stretch, 624 (cm^{-1}) amide C-N
vibration mixed with NH.

^{1}H N.M.R.: (100MHz, $CDCl_3$)
  6.5-7.8   (5H, m, aromatic protons)
  4.8   (1H, broad s, N-H)
  2.0   (3H, s, CH_3)
  0.9-1.9   (9H, m, C_3H_7)

UV Spectrum: (DMF)
Sharp peak with maximum at 340 nm.

p-Toluidine pyruvamide

Deep yellow/brown sticky oil.

Molecular formula: $C_{10}H_{12}NO_2$

Infra-Red: (Thin Film)
3090 (cm-1) aromatic stretches, 1765 and 1709 (cm^{-1}) carbonyl stretches, 624 (cm^{-1}) amide C-N vibration mixed with NH.

^{1}H N.M.R.: (100MHz, $CDCl_3$)
6.5-7.8   (5H, m, aromatic protons)
4.8   (1H, broad s, N-H)
3.8   (3H, m, CH_3 on aromatic ring)
2.0   (3H, s, CH_3)
0.9-1.9   (9H, m, C_3H_7)

UV Spectrum: (DMF)
Sharp peak with maximum at 332 nm.

7:3:5 Method of Application of Pyruvates and Pyruvamides on Wool

0.05 grams of each pyruvamide, t-butylpyruvate and commercial ethyl pyruvate was added to 50 mls of methanol. 1g of wool fabric was added and the solution was stirred overnight. The wool was then patted dry with tissue paper and air dried at room temperature. Assuming 100% uptake, the method gave a concentration of 5% owf, for each sample.

7:3:6 Method of Application of Pyruvic Acid and N,N'-Dicyclohexylcarbodiimide on Wool

5g of wool fabric was added to A) 50 mls of a 1% (w/v) solution of pyruvic acid in methanol and to B) 50 mls of a solution which was 1% (w/v) of pyruvic acid and 1%

(w/v) of N,N'-dicyclohexylcarbodiimide in methanol. Both solutions were shaken on a mechanical shaker for 3 days. Assuming 100% uptake, this method gave a concentration of 10% owf for each solution. Treatment of wool with N,N'-dicyclohexylcarbodiimide alone via this method is known to modify approximately 20% of the amino groups in wool. [13]

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It has been reported that most common amino acids with the exception of cysteine, proline and hydroxyproline, react with o-phthalaldehyde (OPA) in the presence of 2-mercaptoethanol (or a similar thiol) to produce a highly fluorescent species with an emission maximum at 450 nm. The fluorophore formed is unstable to light and a slow decrease in fluorescence intensity is observed after 25 mins in air and light. [1]-[5] The identity of the fluorophore is reported to be the bicyclic heterocycle 1, alkylthio-2-alkyl-substituted isoindole. (6)
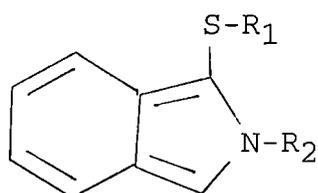
R_1 = alkyl or aryl

R_2 = alkyl

The proposed mechanism for its formation is as follows;

o-phthalaldehyde
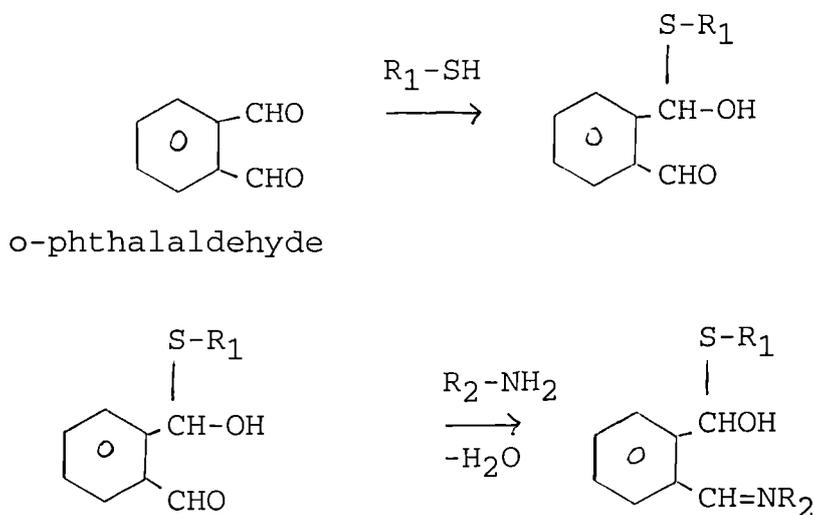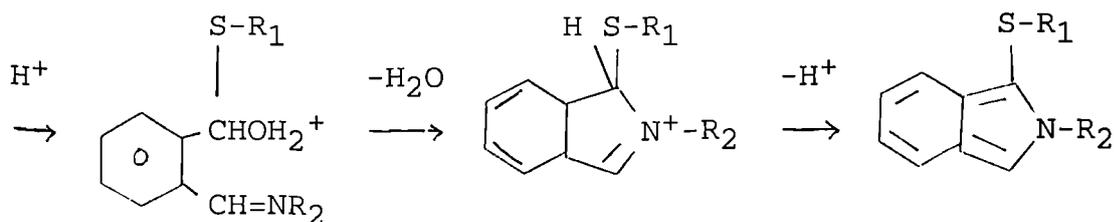
Scheme One

A similar reaction takes place with glutathione
($HO_2CCH(NH_2)(CH_2)_3CONHCH(CH_2SH)CONHCH_2CO_2H$) and OPA

alone to produce a fluorophore with an emission maximum

at 420 nm. The glutathione species presumably behaves

simultaneously as an amino compound and by virtue of

its thiol group as a reducing agent, sharing the two

functions otherwise assumed by distinct compounds. [3]

The identity of this fluorescent species is however

unknown.

The reasons for the failure of cysteine to produce

isoindole on reaction with OPA in the presence of

excess amine are poorly understood. Chen et al. [8]

observed the production of a weakly fluorescent

unstable fluorophore on reaction of cysteine

($HSCH_2CH(NH_2)CO_2H$) with OPA. An unidentified pink/red

product was also noted. This reaction is investigated

and an attempt to produce the highly fluorescent

isoindole using different reaction conditions is

undertaken in solution and on the surface of reduced

wool.

Many studies have shown that application of fluorescent

whitening agents to the wool surface accelerates the

rate and wavelength range responsible for

photoyellowing. [9]-[17] The reason for this is

believed to be the photosensitization of the tryptophan

residues of wool by the brightener. [18],[19] In order

to avoid the use of commonly employed FWA's which are

known to be photochemically unstable, the reaction of

reduced wool with OPA in the presence of excess amine

was undertaken, in an attempt to produce the highly

fluorescent isoindole species covalently bonded onto

the wool surface.

8:2 Results of Reaction of o-Phthalaldehyde with Thiol in the Presence of Excess Primary Amine

It was proposed that the reaction of OPA and cysteine

may produce a fluorescent isoindole if instead of

excess thiol an excess of amine was employed. The

isoindole would then be produced via reaction of the

thiol group of cysteine and not as before via that of

the added thiol. The addition of an excess of various

primary amines to equimolar solutions of OPA and

cysteine was undertaken to investigate if this could be

achieved. (See sec 8:4:1)

From figure 8:1 below it is evident that for all amines

employed, a highly fluorescent species with an emission

maximum the same as that for the reaction with

glutathione at 420-430 nm is produced. As the species

has the same emission maximum for all amines employed,

it infers that the same fluorophore is formed in each

case. This species may be the isoindole shown in Scheme
One or possibly an unidentified fluorophore formed from
the sole reaction of cysteine with OPA.

Amine Used	UV (maxima) (in water)	Fluorescence Emission (excited at 350 nm)
Ethanolamine	234 nm, 335 nm	420-430 nm
n-Butylamine	234 nm, 335 nm	420-430 nm
n-Propylamine	234 nm, 335 nm	420-430 nm
Furfurylamine	234 nm, 335 nm	420-430 nm
Dodecylamine	234 nm, 335 nm (sh), 525 nm, 621 nm, 667 nm	420-430 nm

Figure 8:1 Table of UV and fluorescence spectral data
for the product(s) of reaction of OPA and cysteine in
neutral solution with an excess of primary amines.

If a mechanism similar to Scheme One is followed for
this reaction the imine product below may result. This
species may or may not be the fluorescent species that
emits at 420 nm.
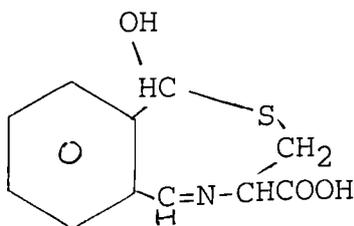
The fluorescence intensity of all the solutions reached
a maximum after 30 mins and then faded to zero after 24
hours.

The reaction of OPA, cysteine and dodecylamine also
produces a compound that absorbs strongly in the blue
region of the spectrum. (See figure 8:2) The identity
of this chromophore is unknown but a charge transfer
complex seems unlikely as the solution displays a
structured UV spectrum.
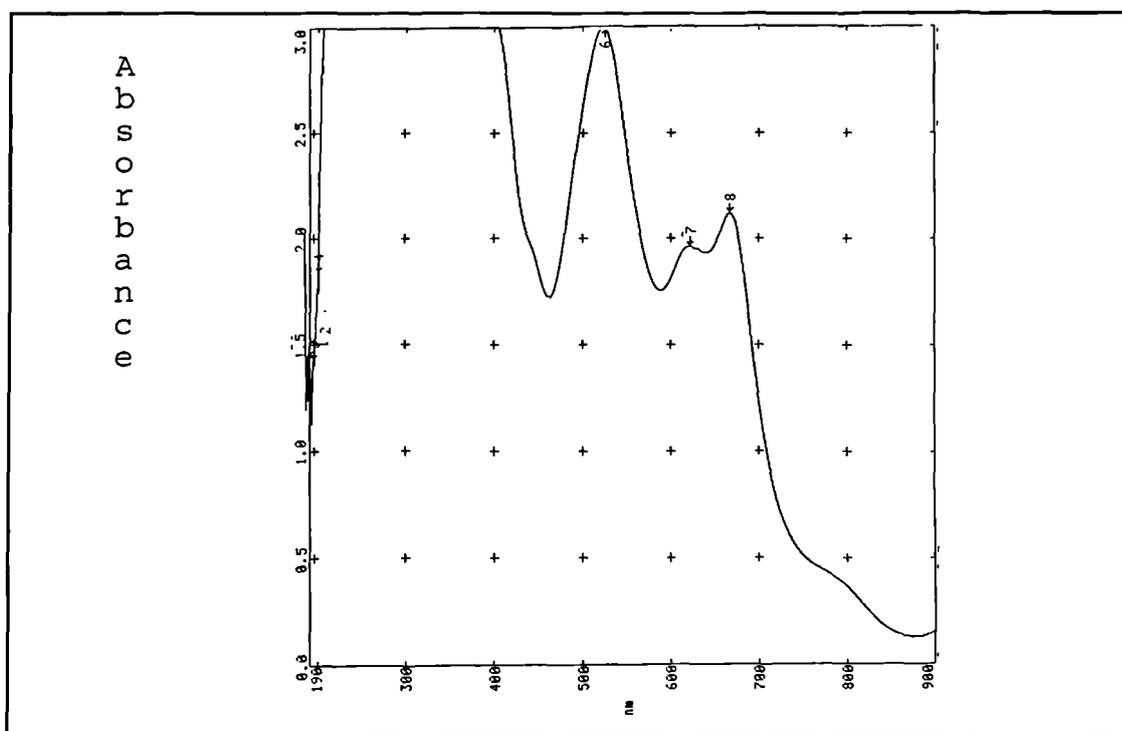
Figure 8:2   UV spectrum of compound formed after
addition of a molar excess of dodecylamine to an
equimolar solution of OPA and cysteine.

Decomposition of isoindole formed from an amine and 2-
mercaptoethanol may occur via intramolecular attack, to
give 2,3-dihydro-isoindol-1-amine and the insoluble
solid polyethylene sulphide. [6] If an isoindole is

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formed a similar type of reaction may occur in solution

to produce the unidentified blue product e.g.

S-CH$_2$CHCOOH(NH$_2$)

[isoindole structure with N-(CH$_2$)$_{11}$CH$_3$] $\longrightarrow$ [cyclic structure with S, COOH, -NH$_2^+$, N-(CH$_2$)$_{11}$CH$_3$]

NH$_2$

N-(CH$_2$)$_{11}$CH$_3$ +   -(CH-CH$_2$-S-)$_n$
                                    COOH
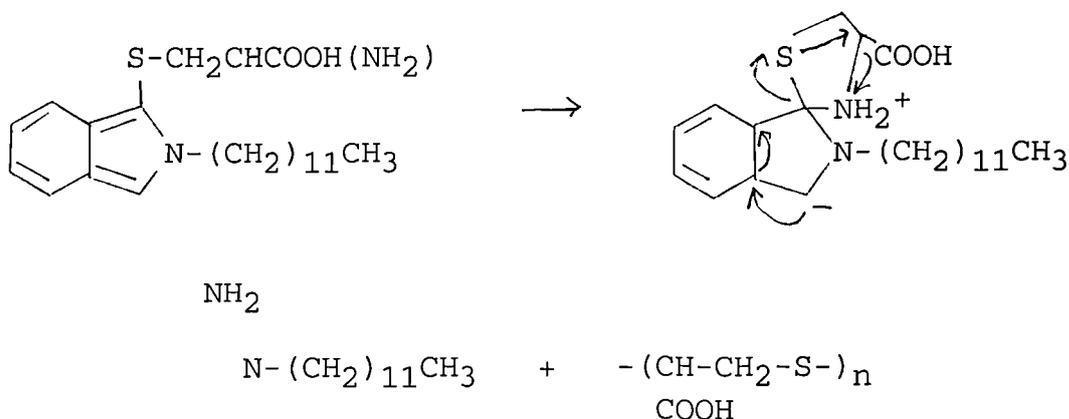
The isoindol-1-amine may exist in two forms.

NH$_2$

[isoindole structure N-(CH$_2$)$_{11}$CH$_3$] $\rightleftharpoons$ NH [isoindoline structure N-(CH$_2$)$_{11}$CH$_3$]
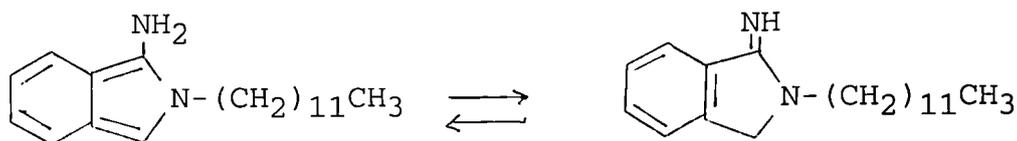
Investigations into the effect of pH on the reaction

showed that different final products are achieved on

alteration of the pH. Subsequent reduction of the

samples with sodium borohydride (NaBH$_4$) gave further

products. Unfortunately none of the products or

intermediates formed could be isolated due to their

instability in solution and instability in the solid

form. A summary of the reaction colour changes and

fluorescence properties of the solution formed via

reaction of a) equimolar amounts of OPA and cysteine

and b) equimolar amounts of OPA and cysteine in the

presence of excess ethanolamine ($_2$HNCH$_2$CH$_2$OH) is as

follows.

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	pH	Time (hrs)	Colour\Fluorescence	NaBH$_4$
OPA + CySH	4-5	0	None\None	
		1	None\None	
		24	None\None	No Rxn
OPA + CySH + EA	4-5	0	Clear\Intense Blue	Blue Fluor.
OPA + CySH	7	0	None\None	
		1	Red\None	
		24	Red\None	Yellow
OPA + CySH + EA	7	0	Yellow\Blue Fl.	Yellow
OPA + CySH	8-9	0	Yellow\Blue Fl.	
		1	Yellow\Blue Fl.	
		24	Yellow\None	Yellow
OPA + CySH + EA	8-9	0	Clear\None	No Reaction

Figure 8:3 Table of colour changes and fluorescence properties of solutions of o-phthalaldehyde and cysteine and o-phthalaldehyde/cysteine and ethanolamine solutions where o-phthalaldehyde is represented by OPA, cysteine by CySH and ethanolamine by EA.

From the table above it is evident that no reaction occurs with OPA and cysteine in acid solution. Though the identity of the OPA/cysteine adduct is unknown, presumably the reaction is hindered by protonation of the thiol group of cysteine. Surprisingly addition of ethanolamine to this solution produced the characteristic blue fluorescence of the isoindole, which was not subsequently reduced by reaction with NaBH$_4$. This is supported by the suggestion of Simons *et*

al. [6] that a concerted reaction occurs when the three components of the reaction are mixed together.

A red solution (wavelength absorption maximum 518 nm) is produced on reaction of OPA and cysteine in neutral solution. The formation of a red-purple compound has been observed by Bonnett *et al.* [4]. The solid isoindole formed by these authors rapidly darkened to a red-purple compound at RT. Again the identity of the red species is unknown, however the colour of the solution changes dramatically to a deep yellow on addition of $NaBH_4$. Reduction by $NaBH_4$ implied that the red solution contained an imine (see following equation) and analysis by IR proved this to be the case. (See fig. 8:4)

$$-C=N- \ + \ NaBH_4 \ \longrightarrow \ -CH-NH-$$

The IR spectrum of a), displayed in figure 8:4 below, shows a strong peak at 1669 cm^{-1} which corresponds to a -C=N- vibration. This peak completely disappears on addition of $NaBH_4$ as evidenced by spectrum b).
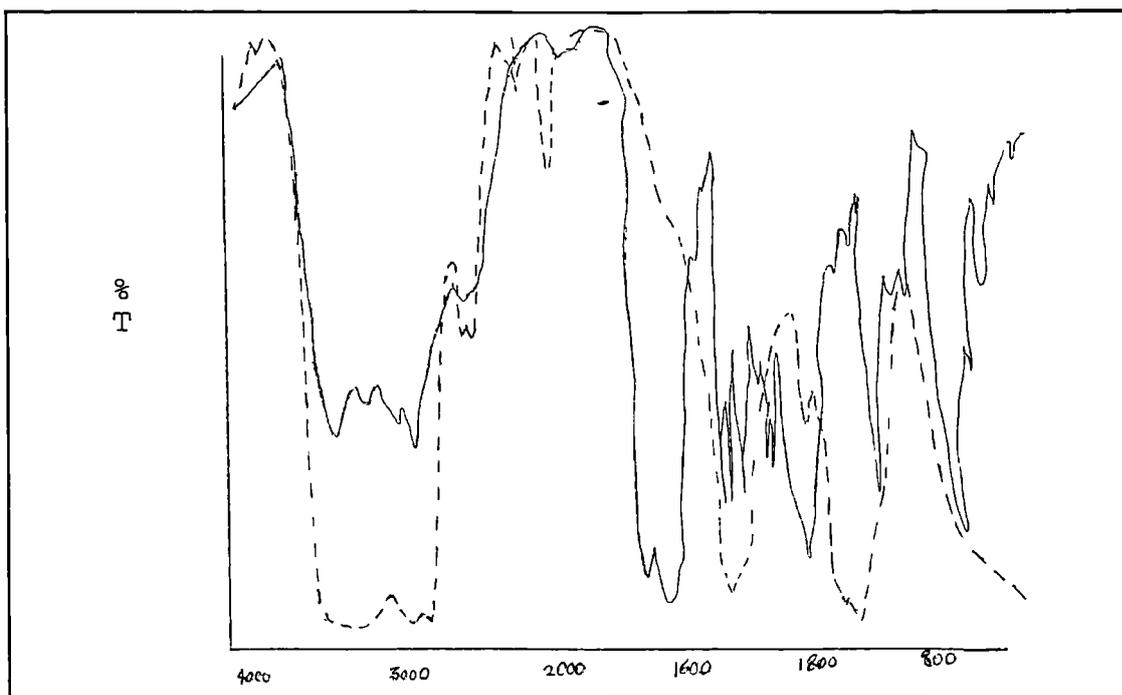
Figure 8:4 IR spectrum (liquid in H_2O) of a) ——— OPA and cysteine red solution and b) - - - same solution reacted with $NaBH_4$.

The identities of the red imine and yellow reduced species are unknown but they may correspond to the following compounds.

(A)
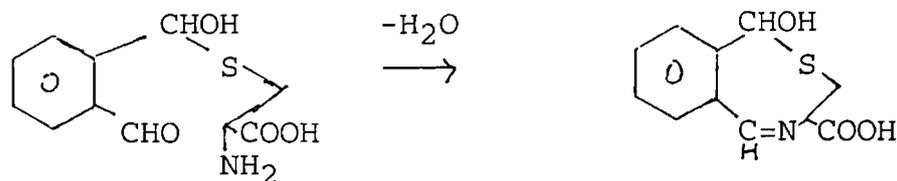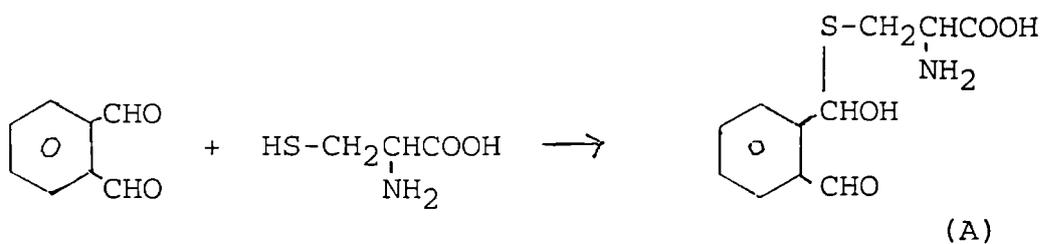
A second molecule of cysteine may react with intermediate (A) to form the imine (B).

S-CH$_2$CHCOOH
         |
         NH$_2$
CHOH   + HSCH$_2$CHCOOH ⟶   S-CH$_2$CHCOOH
                        | | NH$_2$
                        NH$_2$ CHOH
CHO

C=N-CHCH$_2$SH
H |
     COOH
                                              (B)
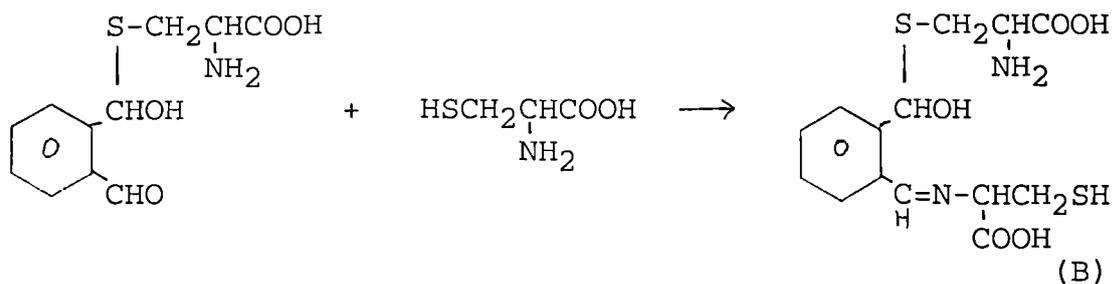
The deep yellow species produced on reaction with NaBH$_4$

may correspond to the reduced imine species (C) and/or

(D).

CHOH
    S   CHOH
                    NaBH$_4$ S
                    ⟶
C=N COOH   CH$_2$-NH COOH

                                    (C)

S-CH$_2$CHCOOH   S-CH$_2$CHCOOH
        | |
        NH$_2$ NaBH$_4$ NH$_2$
CHOH   ⟶   CHOH

C=N-CHCH$_2$SH   CH$_2$-NH-CHCH$_2$SH
H | |
     COOH   COOH

                                            (D)
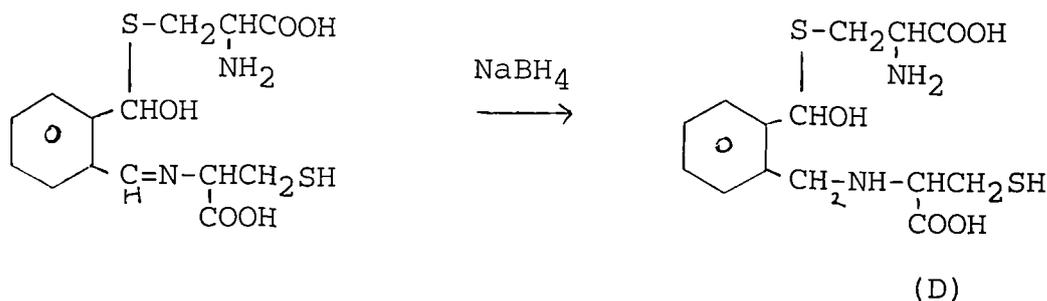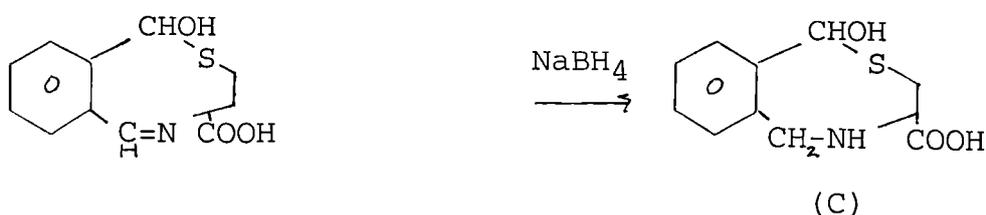
The reaction of OPA and cysteine alone in alkali

produces a fluorescent species. Presuming the

fluorophore has isoindole character, the cysteine

moiety must assume a dual role, reacting at both its

thiol and amine sites. The unidentified yellow species

is also formed and on reduction of the solution with

NaBH$_4$ no change is noted. (The intensity of the

fluorescent species decreases to zero after four

hours.) The presence of both the yellow and blue

fluorescent species initally in solution implies that

both species may be interconvertible with the final

equilibrium lying in favour of the unidentified yellow species.

The addition of ethanolamine to the alkaline solution of OPA and cysteine gave no notable products.

8:3 Results of Reaction of Reduced Wool with o-Phthalaldehyde in the Presence of Excess Primary Amine

H_3PO_2 reduced wool, tributylphosphine reduced wool and blank wool were treated with OPA and ethanolamine in alkali solution. (See section 8:4:3) It was presumed that unlike in solution where the fluorophore was produced by reaction of cysteine and OPA alone, added primary amine was needed, as the concentration of primary amine groups available on the wool surface to form the fluorophore would be minimal. The fluorescence emission spectra in figure 8:5 shows that a fluorescent species is formed on the wool surface as excitation at 350 nm for both reduced samples yields emission maxima at 410-420 nm. The blank wool sample shows no such peak. If it is assumed that the tributylphosphine pretreated wool is reduced with 100% efficiency then previous results (see sec 3:3:2:3) indicate that 10% reduction is achieved with the H_3PO_2 treatment. A somewhat similar result is observed with this treatment as the intensity of emission (and therefore concentration) of the isoindole species formed on the surface of the tributylphosphine sample is approximately three times that of the H_3PO_2 sample.
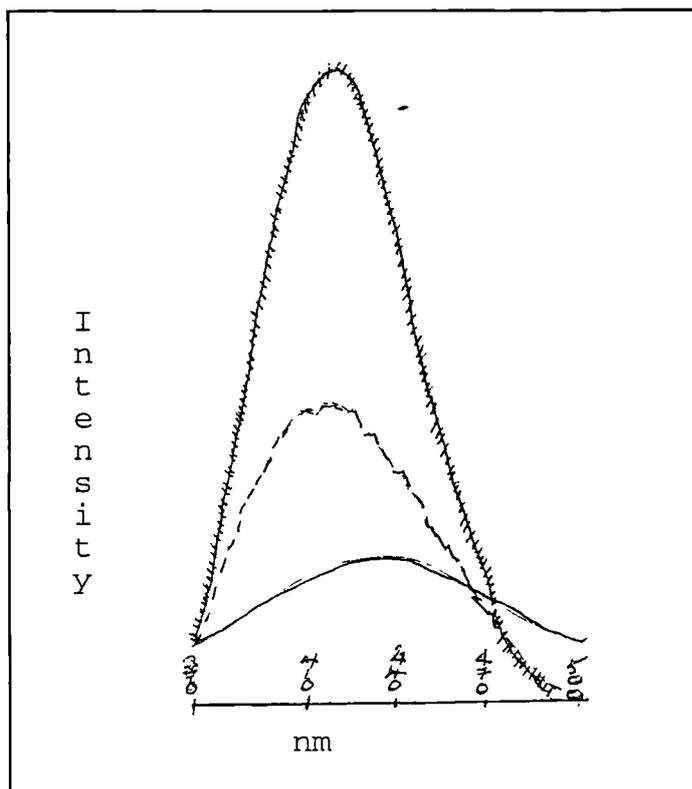
Figure 8:5 The fluorescence emission spectrum of a) —
blank wool treated with OPA and EA, b) - - - H3PO2
reduced wool treated with OPA and EA, and c) ++++
tributylphosphine reduced wool treated with OPA and EA.
Excited at 350 nm, slit width 4, sensitivity 30/0.

Unfortunately both reduced wool samples were yellowed

severely by this treatment to a degree that is

unacceptable for use as an FWA.

8:4   Experimental Methods

8:4:1   Method of Reaction of o-Phthalaldehyde with Cysteine in the Presence of Excess Amine.

At Neutral pH

50 mls of an aqueous 1*10-4 M solution of o-

phthaldehyde was added to 50 mls of an aqueous 1*10-4 M

sol of cysteine and stirred at RT for 5 mins. The

colour change (if any) and fluorescence properties of

the sample were noted. 50 mls of a 1*10-3 M solution of primary amine in methanol or other suitable solvent was added. Amines used included n-butyl, n-propyl, furfuryl, dodecyl and ethanolamine. The colour change (if any) and fluorescence properties of the resulting solution were noted.

At Alkaline pH

The same procedure as for neutral pH was followed except that the aqueous solution of OPA and cysteine was buffered to pH 9-10 via addition of 10% solution of aqueous NaOH.

At Acid pH

The same procedure as for neutral pH was followed except that the aqueous solution of OPA and cysteine was buffered to pH 4-5 via addition of aqueous HCl.

8:4:2 Procedure for Reaction of Sodium Borohydride with the Products and Intermediates of the OPA and Cysteine Reaction.

10 mls of each solution, at each stage described in section 8:4:1 was removed and placed in an unstoppered phial. Approximately 0.05g of the reducing agent sodium borohydride ($NaBH_4$) was added to each phial. Care was taken as a vigorous reaction occurs with $NaBH_4$ and water. If no visible reaction occurred the phials were heated in a water bath until reaction ensued. The phials were then removed to a fume cupboard where they

remained until reaction was complete. Colour changes
and fluorescence properties of the treated solutions
were then noted.

8:4:3 Procedure for the Reaction of Reduced Wool with OPA in the Presence of Excess Amine

5g of wool serge that had been reduced via a) reaction
with 50% owf H3PO2 (see sec 3:2:1) and b) reaction
with tributylphosphine (see sec 3:3:2:1) were placed
separately in 100 mls of solution (50:50 water:n-
propanol) containing 2.68g (2*10-3 moles) of OPA.
The pH of the solution was adjusted to 9-10 via
addition of aqueous NaOH. A molar excess of
ethanolamine (approx 2g) was added to the solution.
The solutions were shaken on a mechanical shaker for 24
hours. The wool was then air-dried and the fluorescence
spectra read.

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INSTRUMENTATION

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Mass Spectra- KRATOS MS 30. Electron Impact Instrument, linked to a KRATOS DS 50 data system.

Microanalysis- CARLO ERBA Model 1106 Elemental Analyser

UV- PERKIN ELMER Lambda 5 , double beam UV/VIS Spectrometer. A Diffuse Reflectance Attachment for surface spectra also used.

Fluorimetry- PERKIN ELMER MPF-4. For solid samples a special holder was used which was designed at City University.

Dyeing- JEFFEREYS DYEBATH system.

Nuclear Magnetic Resonance- JEOL PMX -100

Differences in reaction of H_3PO_2 and H_3PO_3 with the

disulphide bonds of wool cystine and other model

compounds were observed. Under the given reaction

conditions (pH 1 , 80°C), H_3PO_2 reduced 10% of the

cystine residues to cysteine and produced umolar

quantities of H_2S and SO_2. A series of ionic

mechanisms were proposed for the reaction of H_3PO_2

with disulphide bonds. Reaction with oxidised

disulphides (monosulphoxide and disulphoxides) was

proposed for the production of SO_2. Other products

may be formed in this reaction and have not yet been

identified. It has not been established which area

of the wool fibre reacts with H_3PO_2. Is it a

specific part of the fibre (cuticle, matrix etc)

that reacts to give these products?

No reaction was noted for H_3PO_3 under the same

conditions. A comprehensive explanation for the

behaviour of H_3PO_3 has not been given and further

work must be done on this problem.

The amount of cysteine produced by the reaction of

H_3PO_2 and wool cystine may be quantified by reaction

of the reduced sample with o-phthalaldehyde in the

presence of excess amine. A fluorescent species that

emits at 420 nm was produced. The wool was dyed a

deep yellow by this treatment and the identities of

both the fluorescent and yellow species are unknown. The production of a fluorescent isoindole was suggested but not proven.

A similar reaction of cysteine, o-phthalaldehyde and dodecylamine in aqueous solution gave (in seconds) an unidentified deep inky blue product which was water soluble and light stable. Further work must be done to identify this structured chromophore.

The dyeing properties of wool pretreated with various acid/formaldehyde solutions were investigated. In competitive dyeing studies the H_3PO_2/CHOH pretreated sample (unlike all other acid/formaldehyde pretreatments) resisted acid dye (anionic dye) and preferentially adsorbed basic dye (cationic dye).

The reasons for this extraordinary behaviour were explained by a Mannich reaction of the acid with the amino-methylol groups initially formed by reaction of side chain amine groups with formaldehyde. Structures of the form Wool NH CH_2-PH(O)OH were suggested as products. The NH and PH groups may then react further forming a crosslinked system on the surface of the wool.

The acid dye binding sites i.e. primary amine, thiol groups etc have been blocked by this reaction and the sample resists acid dye. The preferential uptake

of basic dye was explained by the increase in concentration of P=O groups which like C=O groups aid the uptake of basic dye.

Future work using the surface IR technique may prove that these reactions are indeed occurring for the H_3PO_2/CHOH system alone and that under the conditions of the experiment that the H_3PO_3/CHOH system may not react in a similar manner.

In order to investigate the possibility of diminishing the photoyellowing of fluorescently whitened wool a series of amino-anthryl phosphonous acids which contained the potential reducing P-H bond were synthesized. Their structures were R_1-NH-CHR_2-PH(O)(OH), where R_2 = a large aromatic ring system. The compounds were very weakly fluorescent and it was suggested that this was due to the formation of an excimer. Further work must be done to prove that this is so. Wool that had been pretreated with these FWAs photoyellowed to a greater extent than untreated wool and further work must be done to identify the photoproducts of this reaction, both in solution and on the surface of wool.

Application of a series of different compounds to the wool surface was undertaken in order to investigate if an improvement in photoyellowing could be achieved. The reaction of wool with two

anhydrides, acetic anhydride and diethyl-
pyrocarbonate, resulted in an increase in
photobleaching and a subsequent decrease in
photoyellowing for both compounds. The fluorescence
emission intensity of tryptophan was reduced to zero
and its fluorescence excitation spectrum was shifted
to a lower wavelength on reaction with acetic
anhyride. Further work must be done to explain why
both these observations occur. Work in solution with
individual amino-acids and/or di,tri-peptides on
reaction with both anhydrides may prove useful.

The identity of the yellow longwave fluorescent
emitter(s) was investigated. A series of pyruvamides
of structure $R-NH-C(O)-C(O)-CH_3$ were synthesized and
their fluorescent and photoyellowing properties were
noted. It was suggested that these compounds may be
implicated in alkali yellowing of wool. They may
possibly be produced via tautomerisation of
dehydroalanine (formed after hydride elimination of
cystine) to form an imine which may then react with
water to give a pyruvamide species. More extensive
photoyellowing was observed for wool samples that
were pretreated with pyruvamide than blank wool and
further work must be done to identify these species.