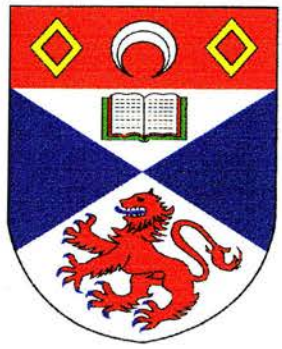

Chemistry and Biology of Radicals Centred on Nitrogen and Oxygen

A thesis presented by M. Afzal to the University of St. Andrews in application for the degree of Doctor of Philosophy

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June 1998

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Introduction

Results and Discussion

Declarations

I, Mohammad Afzal hereby certify that this thesis has been written by myself, it is a record of my own work and that it has not been accepted in partial or complete fulfilment for any other degrees or professional qualifications.

Signed----------

Date----------

I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No. 12 on the 1st October, 1994 and as a candidate for the degree of PhD on the 1st October, 1995.

Signed----------

Date----------

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the Degree of PhD.

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"An explanation should be simple and no simpler."

Albert Einstein

To my mother and father with immense gratitude

ABSTRACT

Model cellular compounds, capable of forming resonance-stabilised carbon-centred radicals, have been reacted, in the presence and absence of air, with nitric oxide. The extent of delocalisation in these molecules increases from allyl acetate, through the β-ionyl derivatives to retinyl acetate, whilst 1,1-diphenylethylene provides benzylic stabilisation for the intermediates. The fate of any derivatives formed was monitored by EPR spectroscopy. Low temperature work was attempted to identify the earliest radicals in the reaction sequence. Results indicate that nitric oxide, in the absence of an initiator such as nitrogen dioxide, shows little tendency to react with these conjugated systems. However, in the presence of oxygen, nitrogen dioxide-derived radicals react with nitric oxide to give nitroso compounds. These latter molecules, upon combination with initially generated radicals, lead to the accumulation of stable di-alkyl and alkyl-acyl nitroxides which were detected by EPR spectroscopy.

Product analysis with GC-MS showed the presence of alkylated aromatics and a series of carbonyl compounds. Polyunsaturated lipids, components of cell membranes, upon activation with suitable initiators, may become susceptible to attack by nitric oxide and so undergo transformations along the pathways outlined above. The ensuing damage to these important cellular structures could be one of the routes via which nitric oxide exerts its cytotoxic effect.

In addition to reacting with a large number of reagents to give an impressive array of products, nitroso compounds are strongly implicated in the storage and transport of nitric oxide in living systems. Their biological role is paradoxical in that they are endogenous carcinogens yet, as nitrosoureas, they are anti-cancer agents. The ease with which alkyl radicals combine reversibly with nitric oxide, has made the resulting nitroso compounds serious candidates as nitric oxide-donor drugs.

In view of their multitude of biofunctions, the importance of synthetic routes to nitroso compounds cannot be underestimated. The present study aims to develop the

synthetic potential of nitric oxide by a novel approach to extending the Barton reaction. Nitric oxide was reacted, in the absence of oxygen, with carbon-centred radicals of varying thermodynamic stabilities, generated from the photo-decomposition of diacyl peroxides. The resulting C-nitroso products, contained α–hydrogen and, as such, they were expected to undergo tautomeric change to the corresponding oximes which upon *in situ* hydrolysis gave carbonyl compounds at low levels. The poor solubility of the reagent in the reaction media and/or insufficient photolysis may account for the disappointing yields. Other products, derived from the combinations between the solvent and the initially generated radicals from photolysis of the peroxides, were also detected.

It was anticipated that carbon-centred radicals immediately adjacent to a spiroepoxide would be prone to undergo a cascade rearrangement (chapter 4, section 4.5, scheme 12). The precursor, α–halomethylepoxides, for generating such systems, cannot be readily accessed. Thus, a protocol for the synthesis of cycloalkylepoxy bromides from cycloalkanones was developed.

Except for the cyclobutyl derivative, all compounds were obtained in excellent yields. Simple spiroepoxy bromides, possessing no substituents in their cycloalkyl rings, were photo-reduced with stannanes in aromatic solvents under different conditions of temperature and reagent concentrations.

Product analysis showed the presence of ring-expanded cycloalkanones in the reaction mixtures and this confirmed the propensity of the starting materials to undergo rearrangement through the expected series of sequential reactions. Kinetic studies, involving the measurement of product proportions, indicated cyclisation to be the rate-controlling step with rate constant values comparable to those in the literature for analogous systems. EPR work detected the transient intermediate radicals and thereby provided further evidence to support the proposed mechanism for this three-stage cascade.

CHAPTER 1

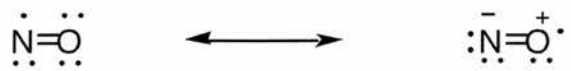
Model Cellular Targets for Nitric Oxide: Implications for its Cytotoxicity

INTRODUCTION

1.1 Physicochemical Aspects of Nitric Oxide

Nitric oxide is a colourless, monomeric paramagnetic gas at room temperature with bp -151.8°C and mp -163.6°C. Its solubility in water at 25°C and 1 atmosphere pressure is approx. $1x10^{-2}$ mol dm^{-3} within the pH range 2-13.

It is the simplest thermally stable odd-electron molecule at ordinary temperatures but, at elevated temperatures (1100-1200°C), it decomposes into its elements. Despite being a free radical, it is insensitive to EPR (Electron Paramagnetic Resonance) spectroscopic detection in the gas and aqueous states and, surprisingly, its tendency to dimerise is low. This is in direct contrast to the readiness with which its close relative nitrogen dioxide (NO_2) forms the dimeric species (N_2O_4) below room temperature. The molecular orbital description of its bonding is similar to that in dinitrogen with an extra electron in one of its antibonding π orbitals. The bond order for the molecule is, therefore, 2.5 and the N-O distance (115 pm) is intermediate between that in the triple bonded NO^+ and values typical of doubly bonded NO species. In valence bond terms, its structure is best shown as a resonance hybrid of several canonical forms. Two of the more important ones are shown below.

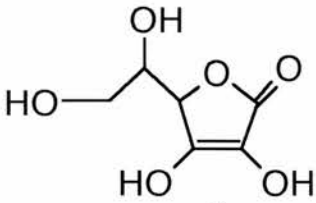


1.2 Preparation of Nitric Oxide

On the commercial scale, nitric oxide is an intermediate in the production of nitric acid which is prepared by catalytic oxidation of ammonia (**Equation 1**).

$$4NH_3 + 5NO_2 \xrightarrow[900^0C]{Rh\text{-}Pt} 4NO + 6H_2O \quad \mathbf{1}$$

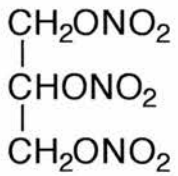
In the laboratory it is readily obtained from:



1

$[Fe(CN)_5NO]^{2-}$

3



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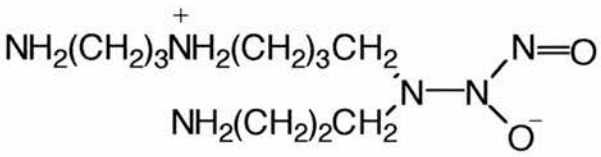

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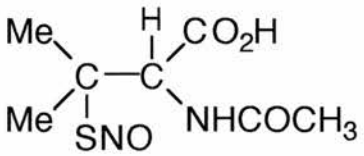

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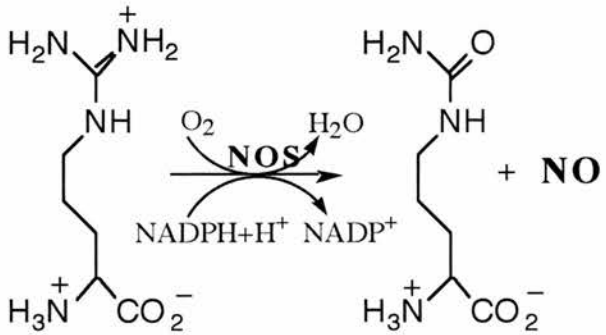
Scheme 1: Structures of compounds used for the generation of nitric oxide.

- Reduction of nitrites e.g. sodium nitrite[1] with ascorbic acid **1**
- Decomposition of organic nitrates e.g. glyceryl trinitrate **2**
- Photo-decomposition of an iron-nitrosyl complex e.g.nitroprusside anion **3**
- Catalytic or photo-decomposition of an S-nitrosothiol[2] e.g. S-nitroso-N-acetylpenicillamine **4**
- Spontaneous oxidation of Drago complexes[3] e.g. spermine NONOate **5**

The *in vivo* synthesis of nitric oxide occurs when a family of enzymes, nitric oxide synthases (NOS), catalyse the aerobic oxidation of an amino acid, L-arginine, to citrulline (**Scheme 2**).



Scheme 2: Biosynthesis of nitric oxide.

The origin of this biosynthesis of nitric oxide was discovered by Moncada[4] in a series of elegant experiments involving analysis of the extracts obtained from endothelial cells, stimulated with bradykinin, a primary messenger molecule, grown on L-arginine labelled with ^{15}N at its terminal position. ^{15}NO was a product indicating that nitric oxide's cellular precursor is L-arginine. Reports from other research groups fully established the L-arginine-nitric oxide pathway for its endogenous synthesis[5].

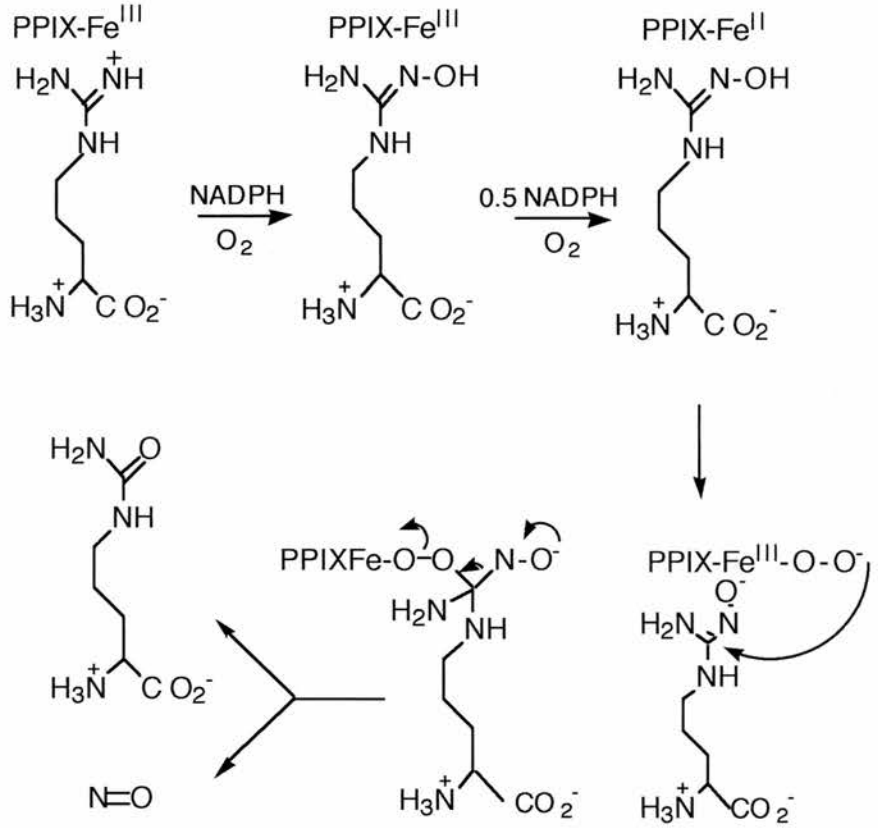
Extensive study of nitric oxide synthase has revealed that there are several isoforms of this enzyme which is related to the P-450 reductase. The endothelial enzyme is of a constitutive variety present in the cytosol at all times and able to respond quickly to a stimulus. Whereas, the inducible form of it, found in macrophages, is absent from these cells until they are activated by a cytokine, a messenger molecule released by the immune system. The main features of the two types are summarised below.

Constitutive	**Inducible**
Dioxygenase	Dioxygenase
NADPH dependent	NADPH dependent
Ca^{2+} / Calmodulin dependent	Ca^{2+} / Calmodulin independent
Picomols NO released	Nanomols NO released
Short-lasting release	Long-lasting release
Inhibited by L-arginine analogues	Inhibited by L-arginine analogues

Structural analysis of the two enzymes has shown a great deal of homology in their amino acid sequence leading to the belief that a single gene is responsible for the

production of the various isoforms[6]. More recently, the iNOS has been shown to incorporate an iron containing prosthetic group, iron protoporphyrin IX (FePPIX)[7] and both types seem to require a protein, tetrahydrobiopterin (H_4B) as a cofactor[8] in addition to possessing a binding site for FAD and FMN.

Isotopic labelling experiments indicate that L-arginine provides NO_2^- and NO_3^- and that the oxygen atom in citrulline is derived from aerobic oxygen. The proposed mechanism for the mode of action of nitric oxide synthase involves an initial N-hydroxylation requiring both NADPH and O_2 with one-electron oxidation of the guanidino nitrogen, followed by the elimination of nitric oxide from the complex[9,10].



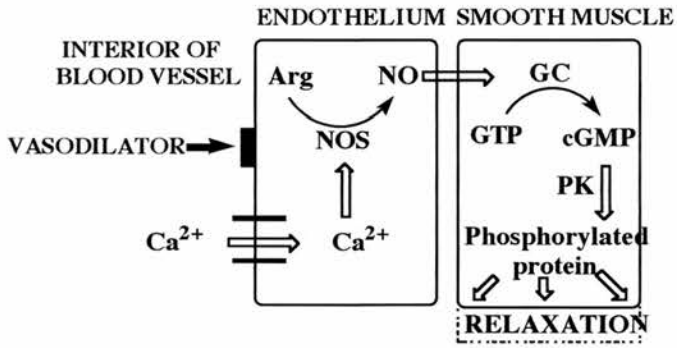
Scheme 3: Mechanism for the action of nitric oxide synthase.

The independent discovery of its critical role in mammalian physiology in 1987, by Moncada[11], Ignarro[12] and confirmation of this[13], has led to a surge of interest in the biological chemistry of nitric oxide. The main aspects of its biology are given below.

1.3 Beneficial Physiological Actions of Nitric Oxide

1.3.1 Smooth muscle relaxation

The inside of a mammalian blood vessel is lined with specialised cells referred to as the endothelium. Nitric oxide, produced here, diffuses to the underlying smooth muscle and activates an enzyme, guanylate cyclase (GC), which increases the intracellular cyclic guanosine monophosphate (cGMP)[14]. The latter, via a cascade system involving protein kinases (PK), brings about the phosphorylation of important structural proteins of the muscle cells. The net effect of these biochemical changes is that the muscles relax thereby enlarging the internal diameter of the blood vessel[15]. Thus, nitric oxide exerts an influence on blood pressure by regulating the state of contractility of the muscles that control the inner size of blood vessels.



Scheme 3: Mechanism for the activation of guanylate cyclase[16].

Disorders related to the excessive constriction of vascular smooth muscle are treated effectively through therapies based on the vasodilatory effect of nitric oxide. For example, *angina pectoris* is characterised by narrowing of the arteries in the heart itself which, therefore, functions abnormally. Glyceryl trinitrate[17], a nitric oxide-donor, has proved as an efficient therapeutic agent for relieving the symptoms of this condition. *Pulmonary oedema* results from severely contracted blood vessels in the lungs and this causes leakage of fluid from the blood into the surrounding tissue. Nitric oxide gas is used, in intensive care units, for treating this ailment[18].

1.3.2 Immunity against invading microbes

Cells called macrophages, found in all body tissues, under the influence of messenger molecules, cytokines, which in turn are generated by the immune system in response to the presence of foreign micro-organisms in the body, are involved in the body's defence mechanism. These protective cells inject toxic substances into the invading microbes. The *killer* molecules function as anti-microbial agents by delivering lethal dosage of nitric oxide to their targets[19]. The way in which nitric oxide causes toxicity towards bacteria is uncertain but there is some support for the view that it combines with an Fe-S centre of an enyzme essential for their metabolic activity[20,21]. The Fe-S cluster nitrosyl thus formed renders the enzyme inactive. Under special circumstances, the concentration of these destructive chemicals reach unusually high levels that can lead to potentially hazardous side-effects. Glutathione, an endogenous biological scavenger of free radicals, can be introduced into the bloodstream in order to overcome the tendency for vasodilation and its consequential adverse effects.

In a healthy body, there is a balance between the rate at which the inefficient cells are removed from the system and replaced with new ones. Some defective cells, however, can accumulate and, under in these situations, they may become the precursors for tumorous tissue. The reactive oxygen intermediates (ROIs), formed from secondary interactions of nitric oxide, with *in vivo* free radicals, act as tumouricidal agents, and thereby help control the spread of cancer[22].

1.3.3 Inhibition of the aggregation and adhesion of platelets

Upon injury to the blood vessels, cell fragments, in the circulatory system, known as platelets *clump together* to form blood clots. In this capacity, these particles prevent excessive bleeding by forming a plug. Biosynthesis of nitric oxide, known to be a potent inhibitor of platelet aggregation[23], is also triggered off during this process as a result of damage to the endothelial tissue of the blood vessel. This co-production of nitric oxide is an instance of negative feedback, a sort of fail-safe phenomenon commonly encountered in biological systems. If abnormal clotting occurs, the body could suffer detrimental effects. The physiological significance of this mechanism is that

it ensures that any such damage is limited. For example, the risk of *heart attack* from impaired blood flow through the cardiac tissue, caused by clots on the inner surface of the coronary artery, is reduced by nitric oxide.

1.3.4 Neurotransmission in the central and peripheral nervous system

The amino acid glutamate is an endogenous neurotransmitter found in the gaps between nerve cells located in the brain and the spinal chord. It has been discovered that a related compound, N-methyl-D-aspartate, mimics its action and brings about the release of nitric oxide from these cells[24] (**Figure 1**). Further work in this field has led to the isolation of neuronal nitric oxide synthase[25], unambiguous demonstration of nitric oxide as an excitatory neurotransmitter[26] and the confirmation for the existence of the L-arginine-NO pathway in the central nervous system[27]. The nitric oxide produced in the brain can then diffuse in all directions and exert an effect on the entire tissue. The presence of nitric oxide in the brain, as a neurotransmitter[28], may help solve a few medical mysteries. For example, it is proposed that the establishment of memory could occur through the communicative ability of this biomolecule. Recently, nitric oxide has also been identified as a chemical messenger molecule in the peripheral nervous system[29].

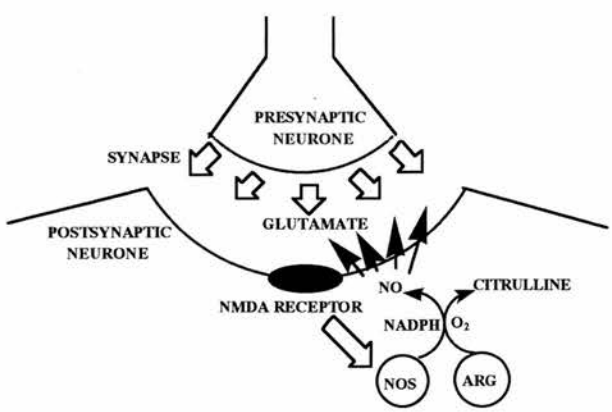


Figure 1: A schematic diagram showing the production of nitric oxide in the brain.

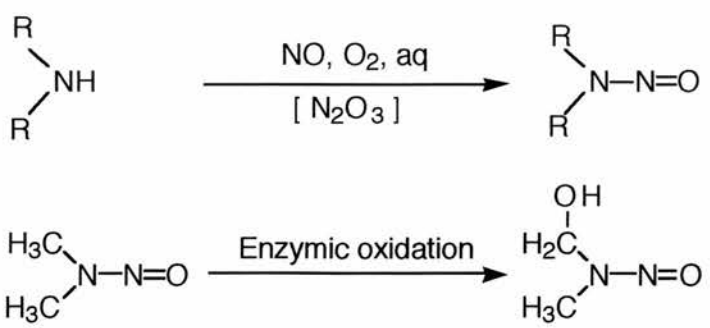
1.4 Adverse Physiological Effects of Nitric Oxide

1.4.1 Carcinogenesis

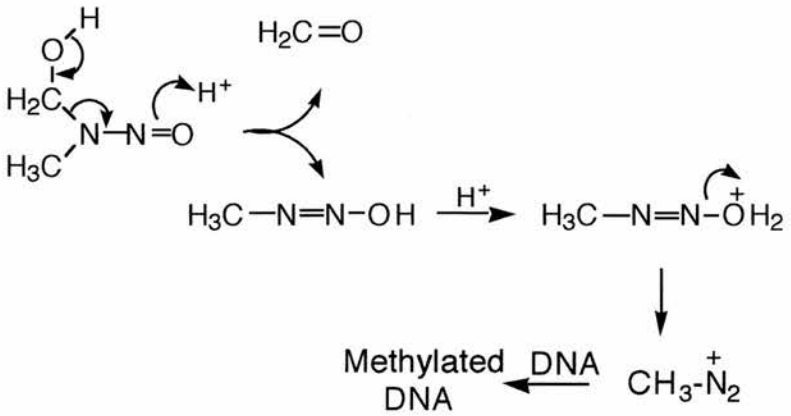
Some forms of cancer are believed to originate from damage to the genetic material. Research involving exposure of nucleic acid bases, the building blocks of genes, to nitric oxide has shown that these molecules undergo chemical change, with a consequential disruption of the coded information in the intact genetic material[30]. The identity of the active species is, as yet, unknown but N_2O_3 is strongly implicated in this process which involves three major routes:

- Formation of endogenous N-nitrosamines (known carcinogens) from secondary amines
- Nitrosative deamination of the primary amine functionalities on nucleic acid bases
- Generation of reactive oxygen intermediates from endogenous oxygen-centred radicals e.g. superoxide anion

The reaction of N_2O_3, a product of NO/O_2 interaction, with a secondary amine is the *in vivo* source of N-nitrosamines which are further metabolised to strongly alkylating electrophiles that act upon DNA at several nucleophilic sites[31] (**Schemes 4** and **5**).

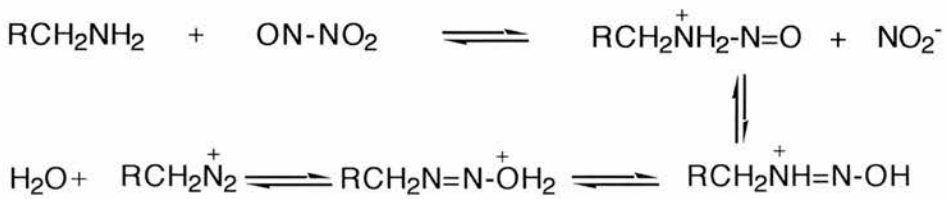


Scheme 4: Endogenous formation of an N-nitrosamine and its subsequent metabolism.



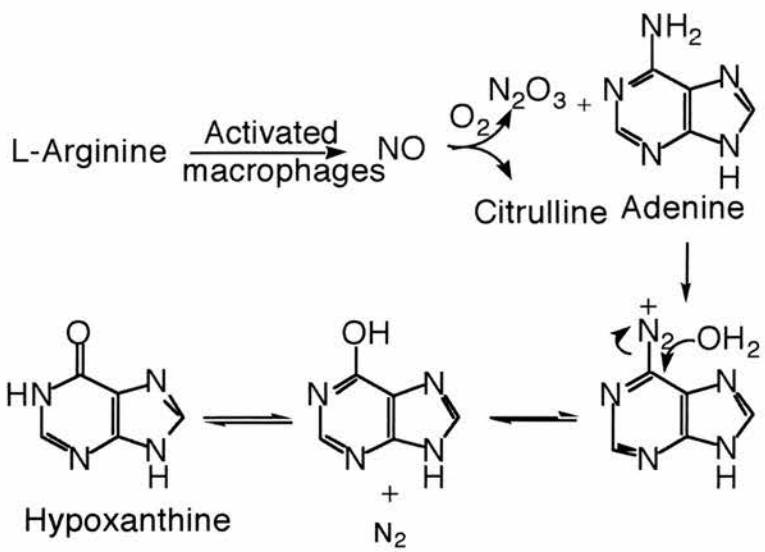
Scheme 5: Methylation of DNA by metabolites of an N-nitrosamine.

Nitrosative deamination via diazonium ions or diazohydroxides (**Scheme 6**) with N_2O_3 is a well established consequence of the reaction of a primary amine with an acidic nitrite[32].



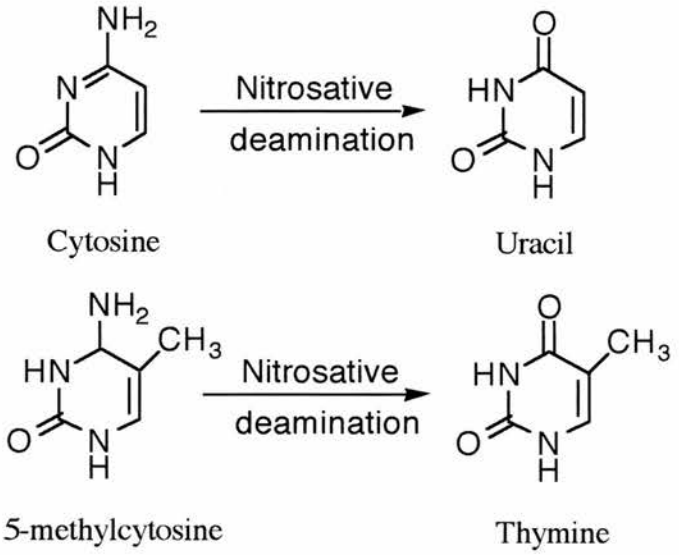
Scheme 6: Nitrosative deamination of a primary amine.

In principle the above reaction is possible with most purines and pyrimidines, the heterocyclic bases in DNA. An exocyclic amino group is the main structural requirement for this to happen (**Scheme 7**).



Scheme 7: Nitric oxide mediated deamination of adenine to hypoxanthine.

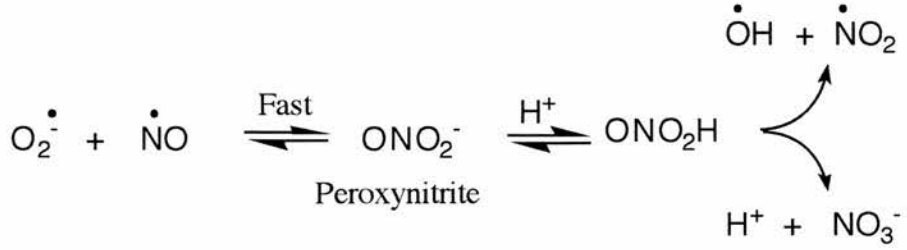
The overall transformations for cytosine and 5-methylcytosine are summarised in **Figure 8**.



Scheme 8: Deamination products of representative nucleic acid bases.

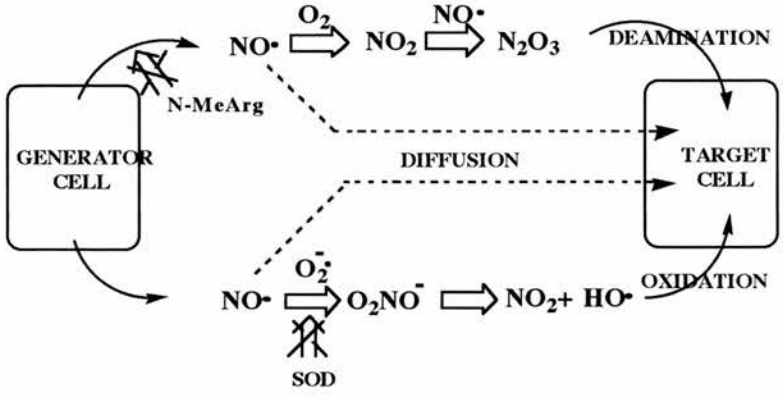
The potential consequences of these reactions depend on the identity of the nucleoside undergoing the transformation. Deamination of guanine to xanthine, for example, may result in the loss of basic sites in the nucleic acid and consequently the single stranded DNA breaks or misrepairs. Methylation of cytosine to 5-methylcytosine and the latter's subsequent deamination to thymine could result in a G-C → A-T transition. This change in the base pairing sequence of DNA alters the encoded genetic information and hence leads to mutagenesis[33].

Although the specific mechanisms of nitric oxide-mediated radical reactions with the genetic material are incompletely understood, there is good evidence for the involvement of superoxide anion[34] which is thought to form damaging secondary products. There is increasing evidence that the generation of hydroxyl radicals[35] occurs by decomposition of peroxynitrite which itself accumulates from the direct combination of superoxide anion and nitric oxide (**Scheme 9**).



Scheme 9: Reaction of superoxide with nitric oxide.

The implication of this destructive mutagenic potential of nitric oxide is far reaching. A proposed mode of its action is summarised in **Scheme 10**. Inhibitors of nitric oxide synthase, e.g. N-methyl arginine, and the enzyme, superoxide dismutase (SOD), limit the damage caused. Clearly, it could represent a new breakthrough in our understanding of the processes leading up to carcinogenesis and open up novel strategies for its treatment .



Scheme 9: Pathways of damage to the genetic material by nitric oxide.

1.4.2 Septic shock

In the elderly, a severe microbial infection can lead to very high levels of nitric oxide in the blood system. A fatal condition called *septic shock* may arise if the vasodilation becomes so acute that the associated lowering in blood pressure reaches dangerously low levels. It is successfully treated with a suitable nitric oxide synthase inhibitor.

1.4.3 Degenerative disorders of the brain

A popular explanation for the origin of degenerative disorders e.g. *senile dementia* and *Alzheimer's disease*, in the brain, is being linked with the excessive production of nitric oxide which causes gradual destruction of the brain tissue.

1.4.4 Thrombosis

Pre-eclampsia (a condition characterised by high blood pressure amongst other symptoms) in pregnant women can lead to thrombosis owing to the build up of plaques in the blood. The latter situation can cause serious complications for the unborn baby and it may arise from a deficiency in nitric oxide. It is counteracted with a drug, S-nitrosoglutathione which restores the endogeneous levels of nitric oxide[36].

1.4.5 Brain damage

A stroke in the brain arises from the blockage of an artery in it. This reduces the oxygenation of the tissue. In an attempt to increase the flow of blood, the body produces the inducible form of nitric oxide synthase. Upon reoxygenation, there is a sudden burst in nitric oxide levels as the enzyme catalyses the the oxidation of L-arginine. This dramatic increase in the concentration of a free radical causes irreversible brain damage[37].

1.5 Chemistry of Nitric Oxide

1.5.1 As a free radical

Reaction with molecular oxygen

It reacts rapidly with dioxygen to give a brown gas nitrogen dioxide, a normal product of reactions that release nitric oxide if these are performed in air. This oxidation, in the gas phase, is unusual in following third-order kinetics and in having a negative temperature coefficient.

$$\text{Rate} = k\,[NO]^2\,[O_2]^1$$

These observations have been accounted for by proposing a mechanism that involves the initial equilibrium formation of a dimer which subsequently reacts with dioxygen (**Equation 2**).

$$2NO \rightleftharpoons N_2O_2 \xrightarrow{O_2} 2NO_2 \qquad 2$$

Aqueous solutions of nitric oxide also undergo aerobic oxidation and nitrite ions are the only products of this reaction (**Equation 3**). No nitrate ions have been detected[38]

$$4NO + O_2 + 2H_2O \longrightarrow 4NO_2^- + 4H^+ \qquad 3$$

and the rate law[39] for the reaction in water is the same as that in the gas phase (k= 8-9x10^6 $M^{-2}s^{-1}$ at 25°C)[40]. Even though the intermediate formation of N_2O_3, anhydride of nitrous acid, has not been unambiguously demonstrated, the generally accepted mechanism is given below (**Equations 4-6**)[41].

$$NO + 1/2\ O_2 \longrightarrow NO_2 \qquad 4$$
$$NO_2 + NO \longrightarrow N_2O_3 \qquad 5$$
$$N_2O_3 + H_2O \longrightarrow 2HNO_2 \qquad 6$$

Hydrolysis of nitrogen dioxide would give an equimolar mixture of NO_2^- and NO_3^- (**Equation 7**).

$$2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3 \qquad 7$$

In view of the absence of nitrates from the products when nitric oxide oxidises in aqueous solutions, the reaction of NO with NO_2 is much faster than the hydrolysis of NO_2.

Reaction with reactive oxygen intermediates

It is well known that in deaerated aqueous alkaline solutions (pH 12-13), superoxide radical reacts with nitric oxide to form the comparatively stable peroxynitrite anion[42-44]. Under neutral conditions, the peroxynitrite anion is a potent oxidant and partially protonated to give the highly unstable peroxynitrous acid (**Equations 8** and **9**).

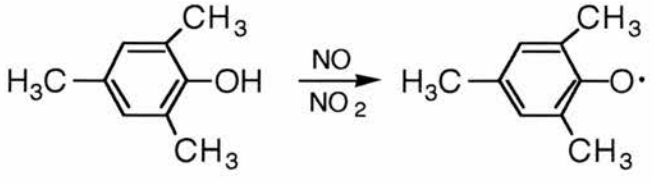
$$O_2^{\bullet -} + NO \longrightarrow ONOO^- \qquad 8$$
$$ONOO^- + H^+ \longrightarrow ONOOH \qquad 9$$

Treatment of nitric oxide with hydrogen peroxide, another oxygen derived species, results in the formation of singlet oxygen which, upon return to the ground state, emits energy as light and so exhibits the phenomenon of chemiluminescence[45] (**Equation 10**).

$$2NO + H_2O_2 \longrightarrow {}^1O_2 + H_2O + N_2O \qquad \textbf{10}$$

Hydrogen atom abstraction

Phenols are facile hydrogen atom donors and, as such, their reaction with hydroxyl radicals is well established. Nitric oxide does not perform hydrogen abstraction from neutral molecules as efficiently. The reported case of this chemistry in the literature[46,47] is its reaction with α-tocopherol and other biological phenolic compounds which get converted to phenoxyl radicals. There is ample corroborative evidence, including our own findings, to suggest that the actual abstraction of hydrogen in these reactions, is performed by small quantities of nitrogen dioxide which is often present from oxidation of nitric oxide unless its absence is experimentally demonstrated.



Radical coupling

Nitric oxide is able to combine with a variety of organic radicals. The most documented examples are for its reaction with alkyl, thiyl and alkoxyl radicals. With carbon-centred radicals it forms oximes via the intermediate nitroso compounds but a second radical may be trapped leading to the production of a nitroxide. Thiyl radicals couple with it to give nitrosothiols and alkoxyl radicals lead to alkylnitrite compounds (**Equations 11-13**). It is difficult to gauge the biological significance of these reactions but short-lived radicals, from degradative processes, do exist *in vivo* and an enzyme ribonucleotide reductase has been shown to carry a stable tyrosyl radical, essential for its activity[48], that may be important in a regulatory mechanism.

$$R\cdot + NO \longrightarrow \underset{\text{Nitroso}}{R{-}N{=}O} \xrightarrow{\cdot R} \underset{\text{Nitroxide}}{R{-}\overset{\displaystyle R}{\overset{|}{N}}{-}O\cdot} \qquad \textbf{11}$$

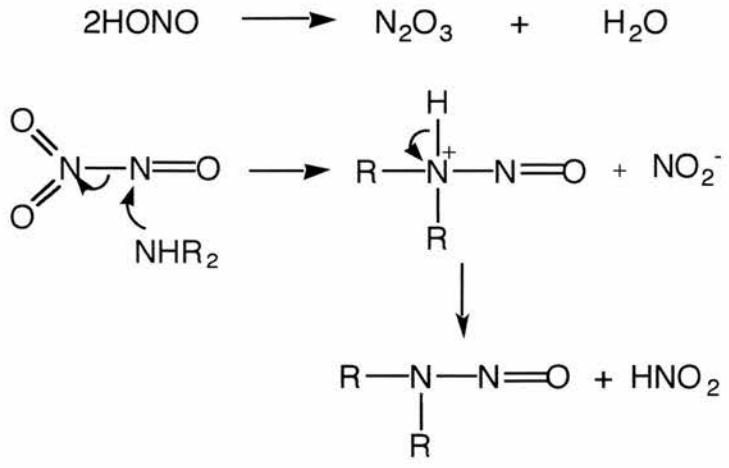
$$RS\cdot + NO \longrightarrow \underset{\text{S-nitrosothiol}}{RS{-}N{=}O} \qquad \textbf{12}$$

$$RO\cdot + NO \longrightarrow \underset{\text{Alkylnitrite}}{RO{-}N{=}O} \qquad \textbf{13}$$

1.5.2 As an electrophile

Nitrosation

When oxygen is rigorously excluded from the reaction mixture, nitric oxide does not act as a nitrosating species. However, N-nitrosamines are quickly formed from secondary amines in the presence of air[49] (**Scheme 11**).



Scheme 11: Nitrosation of a secondary amine by an aqueous solution of N_2O_3.

The NO/O_2 interaction in aqueous solution must supply the electrophile. NO^+ and NO_2 could be suitable candidates for this role but experimental evidence suggests that these are not the key intermediates responsible for the nitrosating properties of the NO/O_2 system[39]. Solutions of N_2O_3, in aqueous alkali, have been used to effect the nitrosative substitution reactions of NO^+ with a range of nucleophiles, bases and aromatic compounds which include: thiols, alcohols, amines and arenes.

Formation of 'Drago' complexes

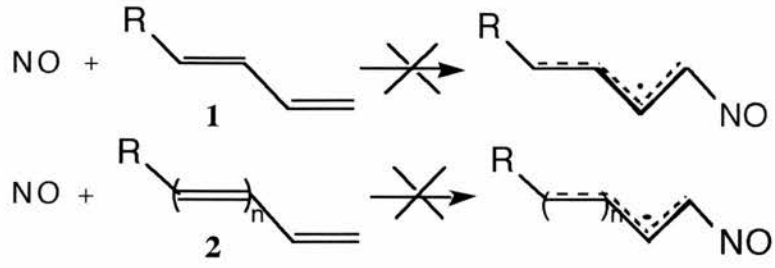
In anaerobic medium, nitric oxide reacts with secondary amines and polyamines to form salts called Drago complexes or NONOates[50] (**Equation 14**).

$$2NO + NHR_2 \longrightarrow R_2N[N(O)NO]^- + H^+ \qquad \mathbf{14}$$

Upon aerobic oxidation these complexes give rise to N-nitrosamine products[51]. They also decompose slowly in aqueous solutions to liberate nitric oxide and therefore have the potential to be used as therapeutic agents.

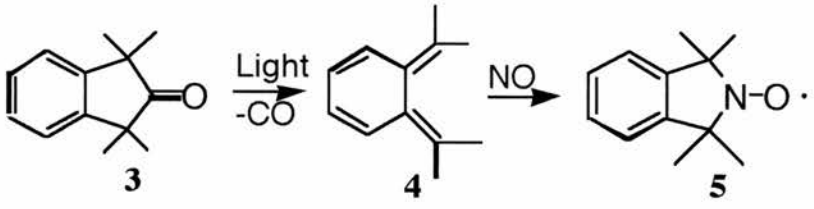
Addition reactions

Work with simple dienes **1** and conjugated unsaturated molecules **2**, in the absence of oxygen, has shown that nitric oxide is surprisingly inert towards these reactants[52] (**Scheme 11**).



Scheme 11: Reaction of nitric oxide with a diene and a polyene.

One interesting example in which nitric oxide acts as an electrophilic species is its addition to ortho quinodimethanes to yield relatively long-lived nitroxide radicals. This chemistry has been exploited by Ingold[53] with a suitable diene molecule 1,2-bis(exopropylidene)cyclohexa-3,5-diene **4**, obtained by photolysis of 1,1,3,3-tetramethylindan-2-one **3**, to trap nitric oxide and give a nitroxide 1,1,3,3-tetramethylisoindolin-2-oxyl **5**, capable of being studied with highly sensitive EPR spectroscopy. This method of analysis would be advantageous for investigative work in living systems, where the concentration of nitric oxide tends to be at a sub-micromolar level (**Scheme 12**).



Scheme 12: A diene spin trap for nitric oxide.

1.5.3 Complexation with transition metals

Complexation of nitric oxide with a range of transition metals occurs readily to generate highly coloured nitrosyl complexes. The red or green iron-nitrosyl complexes,

containing a strong metal-NO bond, are particularly well characterised. These compounds are useful because of their ability to:

- Act as electrophilic nitrosating species[54] e.g. donors of nitosonium ion (NO^+).

Complexes of ruthenium have also been used to nitrosate a variety of organic compounds[54]

$$M—N{=}O + R_2—NH \longrightarrow R_2N—N{=}O + H^+ + M \qquad \textbf{15}$$

- React with a range of nucleophiles e.g OH^-, RS^-, NH_3 etc.

- Release nitric oxide e.g. nitroprusside anion[55] $[Fe(CN)_5NO]^{2-}$

$$[Fe(CN)_5NO]^{2-} \xrightarrow{h\nu} Fe(CN)_5^{2-} + NO \qquad \textbf{16}$$

- Trap nitric oxide giving paramagnetic species that are detectable by EPR spectroscopy[56] e.g. N,N-diethyldithiocarbamate-Fe complex

Most of these nitrosyl complexes are synthesised by the action of nitric oxide or a nitrosonium salt on solutions of transition metal compounds[57].

The ability of nitric oxide to bind to free coordination sites of iron complexes has important implications in biology. The reversible formation of nitrosyl complexes[58] may function to activate/inhibit enzymatic systems containing iron centres. For example, the activation of the enzyme guanylate cyclase, effector of vascular muscle relaxation, depends on the formation of a nitrosyl complex between the haem moiety of the protein and nitric oxide. Although the molecular details of this process have not been determined, Traylor[59,60] has shown that the binding of nitric oxide to iron-porphyrins provokes the removal of the proximal ligand by weakening the haem-histidine bond (**Figure 2**).

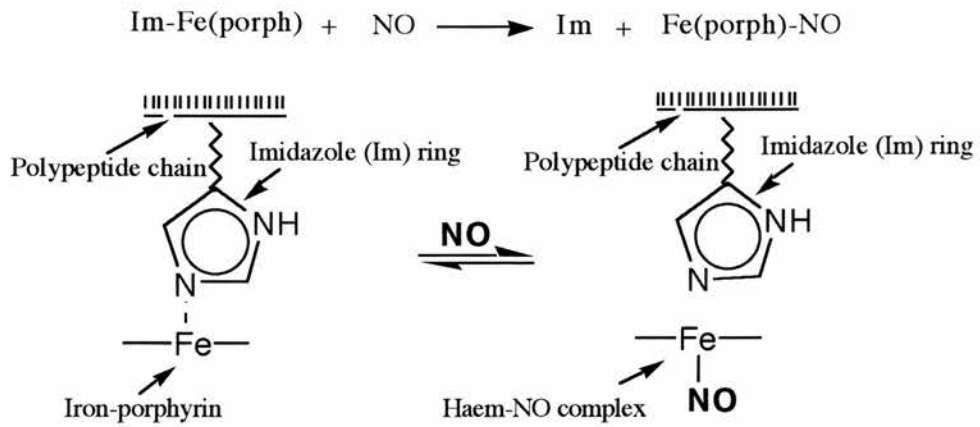


Figure 2: Proposed mechanism for the activation of guanylate cyclase.

An oxygen-carrier haemprotein, haemoglobin, combines with nitric oxide with a much greater affinity than it does with its normal substrate. The nitrosyl-haemoglobin has a distinctive EPR spectrum that has been analysed in some detail[61]. In the presence of oxygen, the iron centre of the nitrosyl-haemoglobin is oxidised and methaemoglobin is formed together with nitrite and nitrate (**Equations 17** and **18**).

$$HbFe^{II} + NO \longrightarrow HbFe^{II}\text{-}NO \qquad \mathbf{17}$$

$$HbFe^{II}\text{-}NO + O_2 \longrightarrow HbFe^{III} \qquad \mathbf{18}$$

The binding of nitric oxide to non-haem iron is of a different character and it contrasts with its reaction with haem iron which is reversible. There is considerable evidence that nitric oxide coordinates to mitochondrial enzymes at their Fe-S clusters. This has been demonstrated by examination of a coculture of cytotoxic activated macrophages (CAM) and tumour cells (which lack nitric oxide synthase) with EPR spectroscopy[62]. The spectra observed were typical of those obtained from synthetic Fe-S-NO complexes[63]. In the case of mitochondrial aconitase, nitric oxide converts the active [4Fe-4S] cluster into the inactive [3Fe-4S] form by binding the labile iron and removing it from the cluster[21]. As mentioned earlier, this could be one of the mechanisms by which nitric oxide is cytotoxic[20].

The mechanistic details of nitric oxide's toxicity are, at present, speculative. However, current knowledge suggests that a combination of three major processes

operate to bring about this action. As a radical species, nitric oxide itself or derivatives from its secondary reactions such as hydroxyl radicals[64] (highly reactive), impair cellular function through inhibition of DNA synthesis[65-68] (tyrosyl radical on ribonucleotide reductase is scavenged by nitric oxide) and cause peroxidative damage to important biological structures respectively. Results from this study lend support to this view and imply that, in non aqueous environments, under oxidative stress, nitric oxide could trigger off degradation of suitably activated conjugated biomolecules[69]. Activated macrophages, with high levels of peroxynitrite, have been shown to display bactericidal properties[70-72]. The situation is related to the elevated levels of this oxidant that would exist under pathological conditions within living systems. The irreversible destruction of the Fe-S clusters, contained in non-haem proteins, and the consequential disruption to the catalytic sites of key enzymes is another route by which nitric oxide inhibits life processes.

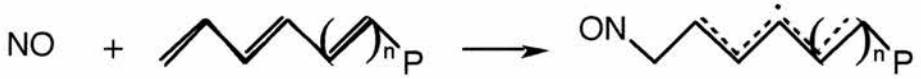
1.6 Objectives of this study

The principal aims of this work were:

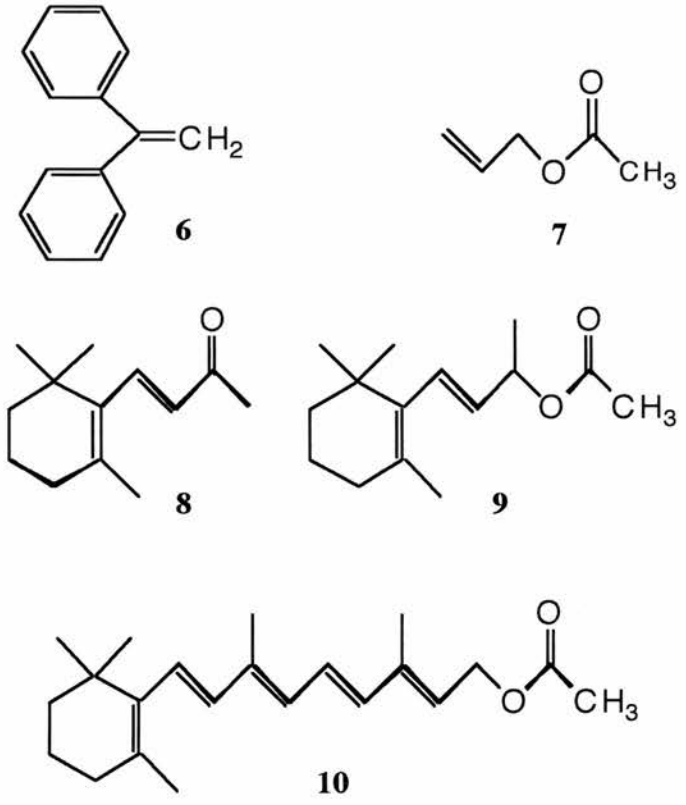
- To investigate the chemical reactions of nitric oxide with a range of model conjugated systems
- To identify any product(s) resulting from the above reactions using modern analytical techniques
- To propose plausible reaction mechanisms in view of the information obtained from analysis of the product(s)
- To assess the potential of nitric oxide for cytotoxicity in its capacity as a mediator of peroxidative degradation of materials that possess structural features closely related to the important cellular constituents

RESULTS AND DISCUSSION

Reactions of nitric oxide with model conjugated systems, structures **6-10**, were investigated. These compounds were chosen for two reasons. Work with simple alkenes and nitric oxide had indicated that this reagent is inert towards such starting materials[52]. It seemed likely, however, that if there could be sufficient delocalisation of the unpaired electron in the initial adduct radical, nitric oxide-addition might occur at an appreciable rate to such a substrate.



The majority of the chosen molecules can form delocalised radical systems with a suitable initiator. Secondly, the structures of these molecules show a close resemblance to vitamin A, β-carotene and unsaturated lipids species that are widely distributed *in vivo*.

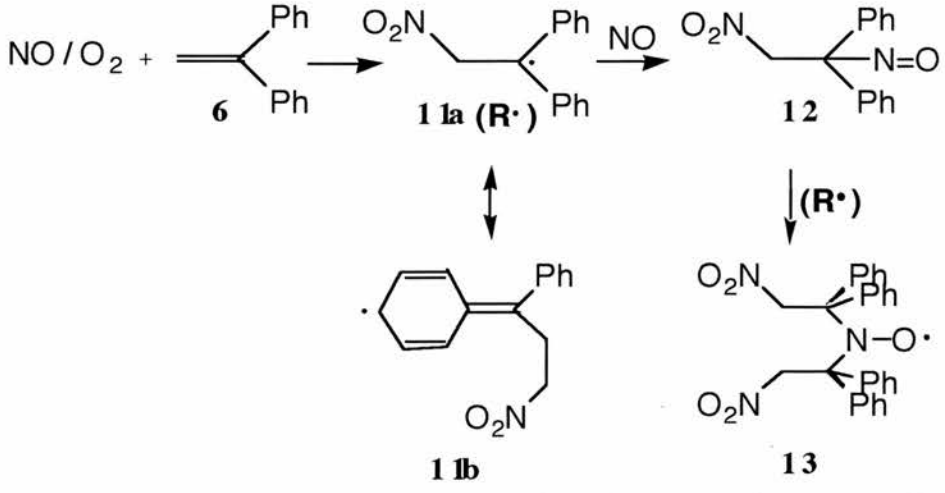


Scheme 13: Structures of model cellular compounds.

2.7 Large-scale Reactions of Nitric Oxide/Nitrogen Dioxide with Model Cellular Compounds

2.7.1 1,1-Diphenylethylene

An EPR spectrum, of the sample withdrawn from the reaction mixture when nitric oxide had been passed into substrate **6** for 3h, was an extremely weak triplet signal. This implied the presence of a di-tertiary nitroxide which could have arisen from the series of reactions depicted in **Scheme 14**.

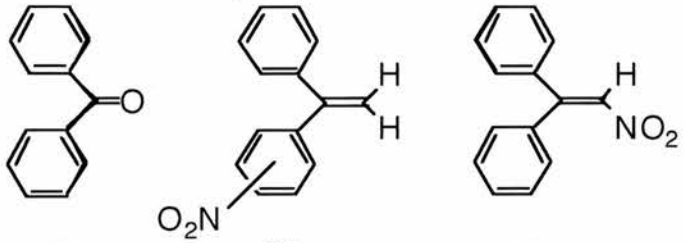


Scheme 14: Reaction of nitric oxide, in limited amount of oxygen, with 1,1-diphenylethylene to give a di-tertiary nitroxide.

Although air was excluded from the system while it was being saturated with nitric oxide, it is unlikely that a completely air-free atmosphere was maintained for the entire duration of the reaction because slow release of air often occurs from internal surfaces. Nitrogen dioxide, an oxidation product of nitric oxide, added to the alkene **6** and produced a transient, resonance-stabilised, benzylic radical **11** which combined with nitric oxide to give an intermediate nitroso compound **12**. The latter, upon addition of **11**, gave the persistent nitroxide **13**.

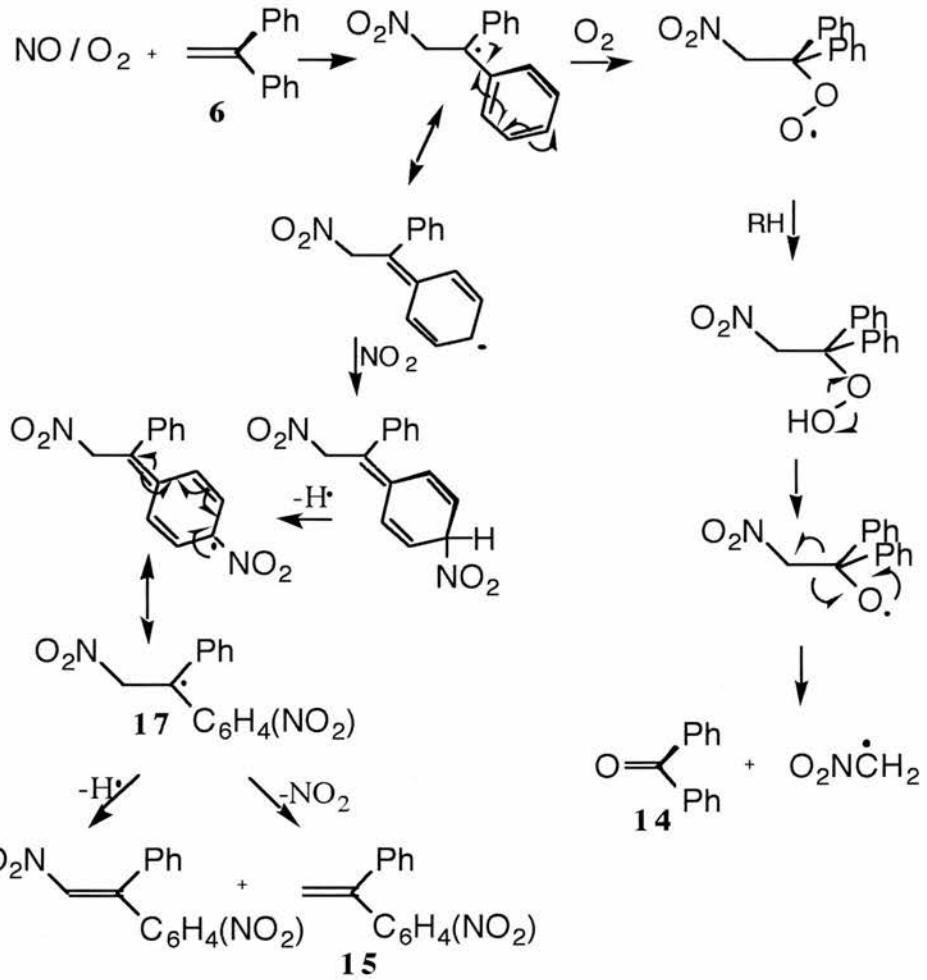
After admitting a restricted volume of air into the system, NMR spectroscopy and GC-MS revealed no substantial quantities of new substances. However, 21h after

allowing excess air into the reaction vessel, two additional singlets, in the vinylic region of the NMR spectrum were observed. This indicated a substantial formation of nitro compounds, δ_H 5.08 ppm and 5.75 ppm, **15** or **16** respectively. GC-MS analysis of the reaction mixture, at this stage, detected the presence of the ketone **14** as well as confirming the presence of nitro-diarylethylenes.



14 **15** **16**

The existence of two of the above products can be explained by the sequence of reactions shown in **Scheme 15**.

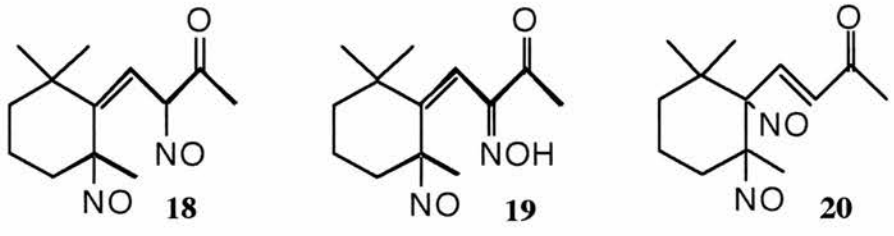


Scheme 15: Reactions between nitrogen dioxide/oxygen and 1,1-diphenylethylene.

The formation of benzophenone **14** occurs through the peroxidative degradation of the initially formed adduct between substrate **6** and the initiator nitrogen dioxide. The pathway leading to the production of $Ph_2C=O$ also gives a highly reactive $\cdot CH_2NO_2$ which, in the presence of a hydrogen atom donor and oxygen, is likely to give CH_3NO_2 and $CH_2=O$ respectively. However, neither of these materials were registered by NMR spectroscopy and GC-MS probably because the former is highly volatile and prone to further reaction and the latter is liable to undergo polymerisation. The diarylethylene **15** results from the elimination of nitrogen dioxide from the intermediate species **17**.

2.7.2 β-Ionone

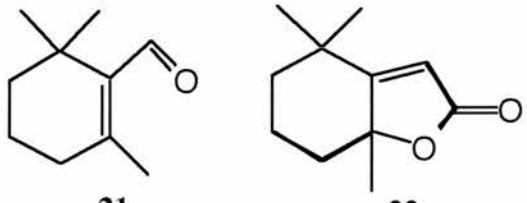
The unsaturated ketone **8** was expected, upon reaction with nitric oxide, to give a mixture of products the structures of which are indicated in **Scheme 16**. NMR and GC-MS analysis showed that the substrate was essentially unchanged after exposure to nitric oxide for 4h.



Scheme 16: A likely mixture of products from the nitric oxide/β–ionone reaction.

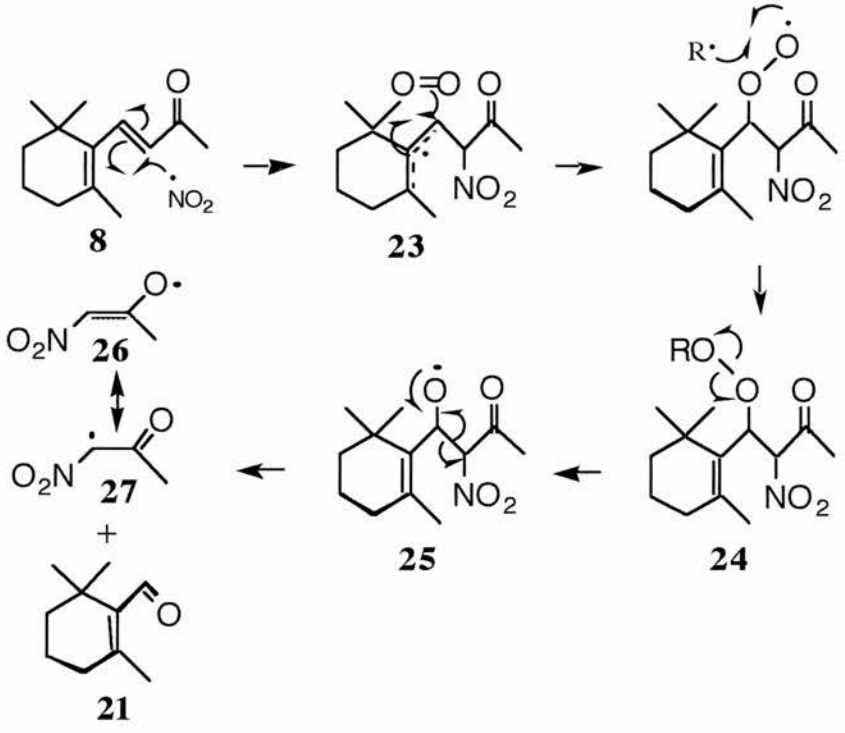
In the presence of nitrogen dioxide, generated from nitric oxide when air is present, it was anticipated that β–ionone would produce relatively stable spin-trapped compounds readily detectable by EPR spectroscopy. The reaction was monitored with this technique by analysing a sample of the reaction mixture after treatment of the enone with nitrogen dioxide for 4h. The spectrum consisted of an unexpected broad doublet a(1H)=9.5 G. This result suggested that the observed radical was a stabilised advanced-stage material of some earlier, more unstable, precursor molecules. An experiment designed to analyse samples of the reaction mixture, withdrawn at earlier stages of the reaction, failed to identify the radical. ^{1}H NMR spectroscopy indicated that all of the

substrate **8** had been consumed, during the course of this experiment and a complex mixture of products existed. Peaks in the spectrum corresponded to the presence of a number of methyl groups on six-membered rings. The pattern of signals was consistent with at least three such groups being adjacent to a carbonyl functionality in addition to resonances located in the olefinic and aromatic regions. GC-MS successfully identified two main substances **21** and **22** amongst an array of several other unidentified molecules.



21 **22**

Nitrogen dioxide seems to initiate a radical-chain autoxidation of β-ionone to give β-cyclocitral **21**, a molecule previously detected in β-carotene oxidation. A plausible mechanism for the reaction of nitrogen dioxide with β-ionone is given in **Scheme 17**.

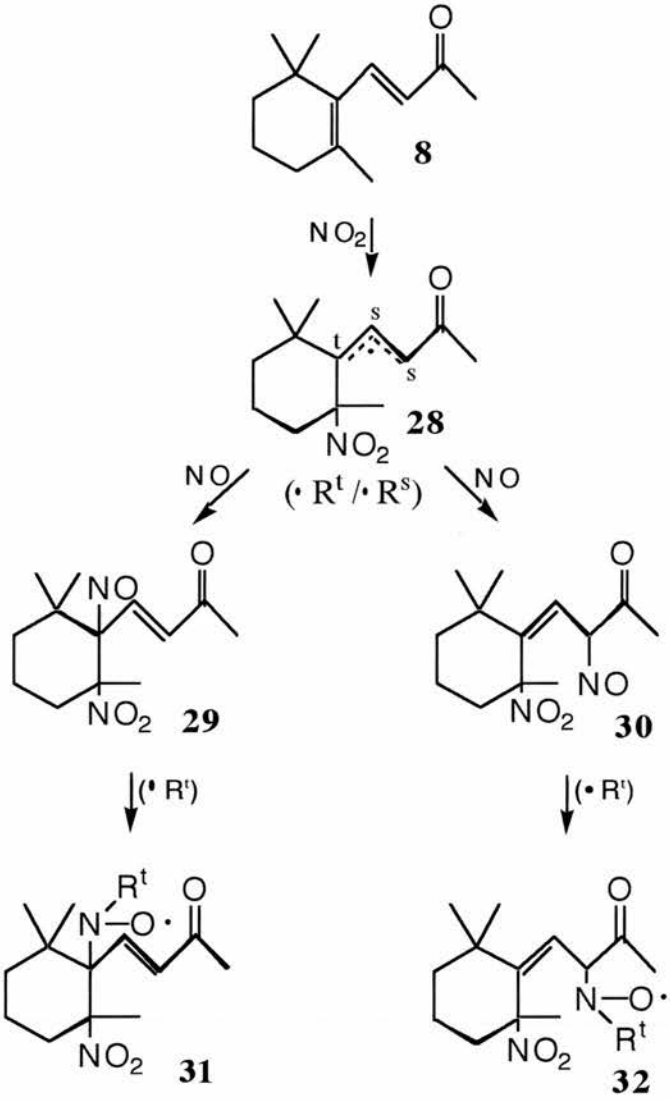


Scheme 17: Nitrogen dioxide initiated autoxidation of β-ionone.

Nitrogen dioxide will add at the least hindered end of the conjugated system because this affords radical **23** which will have greatest resonance-stabilisation. Attack

by oxygen on this yields the alkoxyl radical **25** from the intermediate peroxy species **24**. β–Scission of the nitro derivative **25** leads to the formation of β–cyclocitral **21** and reactive species **26** and **27** which are probably too volatile for detection.

Introduction of air into the system containing a sample of β–ionone saturated with nitric oxide, gave an EPR spectrum, after 15 mins., which was that of a di-tertiary alkyl nitroxide ($\mathbf{R^t\text{-}N(O\cdot)\text{-}R^t}$), a(N)=13.7 G, and this was still detectable after 2h. Additional signals, from other radicals with different hfs owing to the presence of hydrogen(s) adjacent to the unpaired electron, were observed 30.5h into the reaction. The nature of these radicals could not be deduced owing to the poor resolution of the spectrum that did not permit unambiguous interpretation. Some of the observations made by EPR spectroscopy can be accounted for by the mechanism shown in **Scheme 18**.



Scheme 18: Products of a prolonged reaction between nitrogen dioxide and β–ionone.

The initially-formed nitrogen dioxide adduct radical **28**, containing both secondary and tertiary sites, undergoes combination with nitric oxide to give the nitroso compounds **29** and **30**. These latter intermediates can combine with radical **28**, in a variety of ways, furnishing di-alkyl nitroxides. A di-tertiary alkyl nitroxide **31**, thermodynamically the most stable and, the species observable throughout the experiment, results from the interaction of a tertiary site in **28** and the nitroso functionality of **29**. In a comparable manner di-secondary alkyl and secondary-tertiary alkyl nitroxides can also build up in the reaction mixture. Since the latter two types of nitroxides are relatively short-lived, their concentrations, during the earlier stages of the reaction, are undetectably low. Upon prolonged reaction, the quantities of such species reach sufficient levels for them to be registered. However, even 30.5h of reaction, led to no substantial conversion of the reactant which was shown to be present in the reaction mixture, at this stage, in large excess by both NMR spectroscopy and GC-MS.

In an experiment analogous to the one described above, the nitric oxide-saturated enone **8** exposed to excess air, in a single aliquot, resulted in no detectable reaction. NMR and GC-MS study of the reaction mixture taken after 52.5h, revealed the presence of only the unchanged reactant.

2.7.3 Retinyl acetate

Commercial retinyl acetate was shown, by ^{1}H NMR spectroscopy, to be unfit for direct use. In addition to the expected resonances, peaks corresponding to impurities, at both upfield (0.90 ppm, m) and downfield (2.33 ppm, t; 5.35 ppm, t) ends of the spectrum were also present. Complete absence of these signals from the purified material indicated the total effectiveness of solvent precipitation in removing the unwanted components.

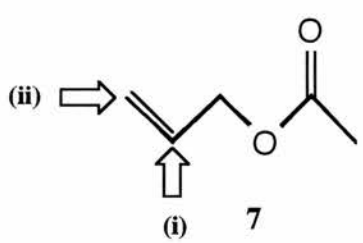
The EPR trace, from a sample in which nitric oxide had been in contact with retinyl acetate for 4h, was a triplet of doublets having two different hfs values. This suggested the presence of two nitroxides: the more intense was a di-tertiary alkyl radical, a(N)=13.8 G, and the less abundant one was a secondary-tertiary radical, a(N)=13.0 G, a(H)=13.2 G. After 7.5h, the initially observed EPR signal became stronger indicating

accumulation of these two radicals in the reaction mixture. Although steps were taken to exclude air from the reaction vessel, some leakage of it from the internal surfaces may have contributed to the presence of small but significant concentration of nitrogen dioxide. The latter acting in concert with nitric oxide, added to the conjugated chain of the acetate **10** producing a collection of delocalised radicals highly receptive towards nitric oxide which, upon addition to such systems, led to the intermediate nitroso compounds. Further addition of nitrogen dioxide-derived radicals to the nitroso compounds, generated a range of nitroxides of varying stabilities. NMR spectroscopy and GC-MS carried out on the material saturated with nitric oxide for 4h, showed no products and the substrate was detected virtually unchanged. The reaction mixtures from the more advanced stages, were shown to contain several new compounds.

2.8 Small-scale Reactions of Nitric Oxide/Nitrogen Dioxide with Model Cellular Compounds

2.8.1 Allyl acetate

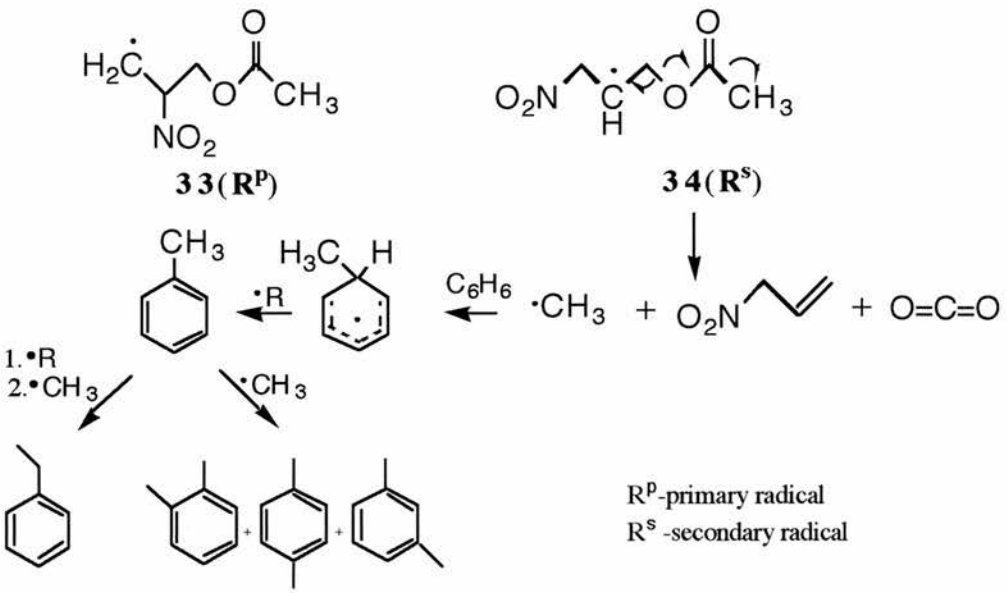
An EPR spectrum taken 10 mins. after admitting air into the system, consisted of a weak triplet, a(N)=7.2 G, which remained unchanged for up to 3.5h. The main products, identified in this reaction mixture by GC-MS, included a variety of alkylated aromatics amongst a host of other, unidentified compounds. Possible initial reaction sites on this substrate, by an initiator such as nitrogen dioxide, are indicated in **Scheme 19** below.



Scheme 19: Alternative sites for nitrogen dioxide attack on allyl acetate.

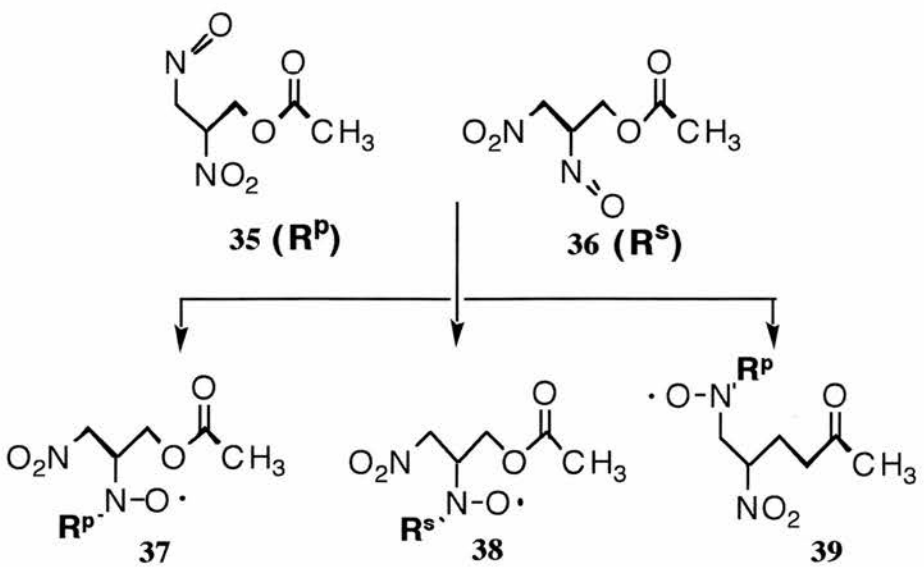
Nitrogen dioxide addition to allyl acetate at both site **(i)** and **(ii)** produces a mixture of primary **33** and secondary **34** radicals respectively. Initial attack of nitrogen

dioxide at site **(ii)** only, followed by elimination explains the formation of a mixture of alkylated aromatic compounds in the manner indicated by **Scheme 20**.



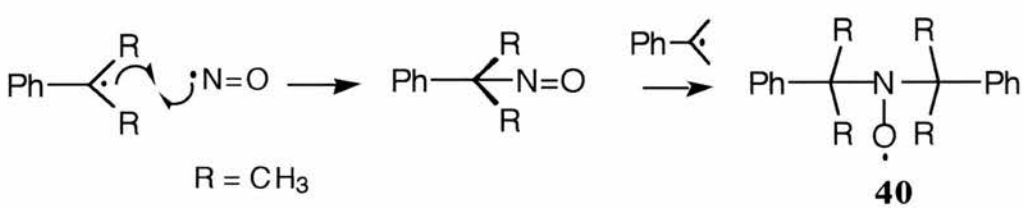
Scheme 20: A mechanism that accounts for the alkylated aromatics when nitric oxide/nitrogen dioxide react with allyl acetate.

With nitric oxide both of these radicals **33** and **34** can form a mixture of nitroso compounds, R^p-N=O **35** and R^s-N=O **36** respectively each of which can, in turn, react further with R^p or R^s to give characteristic nitroxides **37-39** as suggested by **Scheme 21**.



Scheme 21: Reaction of nitroso compounds , derived from allyl acetate , with R^p and R^s radicals to give various types of nitroxides.

Nitroxides **37**, **38** and **39** all contain multiple β–hydrogens and are expected therefore to be short-lived due to easy disproportionation. The simple triplet observed in the EPR spectrum was unexpected because such a signal normally corresponds to a di-tertiary nitroxide which is not a direct product in this reaction mixture. A possible explanation is that further oxidative degradation leads to the removal of the β–hydrogens from the initially formed nitroxides in the reaction mixture. The di-*t*-nitroxides which result are the only ones detected because of their much greater stability. Clearly, in the absence of the above mentioned process, a di-secondary alkyl nitroxide **38** should persist the longest, owing to its greater relative stability and, therefore, a basic three-line signal that is split by the two secondary hydrogens (12 lines in total) might be expected. In practice **38** was too short-lived under the prevailing experimental conditions. Alternatively, the tertiary radicals, generated from the alkyl aromatics, might have been trapped by the nitroso compounds to give a di-tertiary alkyl nitroxide **40** in the way shown below.



Scheme 22: A di-tertiary alkyl nitroxide derived from secondary products in the allyl acetate/nitric oxide reaction.

A study of allyl acetate and nitric oxide was carried out, at 250 K, in the presence of a limited amount of air. This experiment gave an EPR spectrum that consisted of the background noise only. Further lowering of the temperature to 225 K, and the introduction of air, produced no significant change in the appearance of the EPR trace. The reason for carrying out this low temperature work was to identify the site of initial attack on the substrate by nitrogen dioxide. If the attack occurred at site (**i**), (**Scheme 19**) then a primary carbon-centred radical would be generated. In this case, a triplet of doublets would be expected. In contrast, nitrogen dioxide addition at site (**ii**), would

lead to a secondary carbon-centred radical which gives rise to a doublet of triplets. Since neither of these observations were made in practice, a likely explanation is that even at these low temperatures such radicals have far too short a life-span for detection.

2.8.2 Retinyl acetate

A degassed solution of retinyl acetate, saturated with nitric oxide for 1h, gave the expected EPR signal that consisted of the background noise only (**Figure 3a**). Over the next 2.5h, first a 4 line signal appeared (**Figure 3b**) this passed through a maximum and was gradually replaced by a 6 line spectrum (**Figure 3c**). The 6 line signal itself decayed over the next few hours and was replaced by a strong 1: 1: 1 triplet (**Figure 3d**). By 42h the 1: 1: 1 triplet intensity had diminished and a new triplet with a narrower hyperfine splitting (hfs) was beginning to appear (**Figure 3e**).

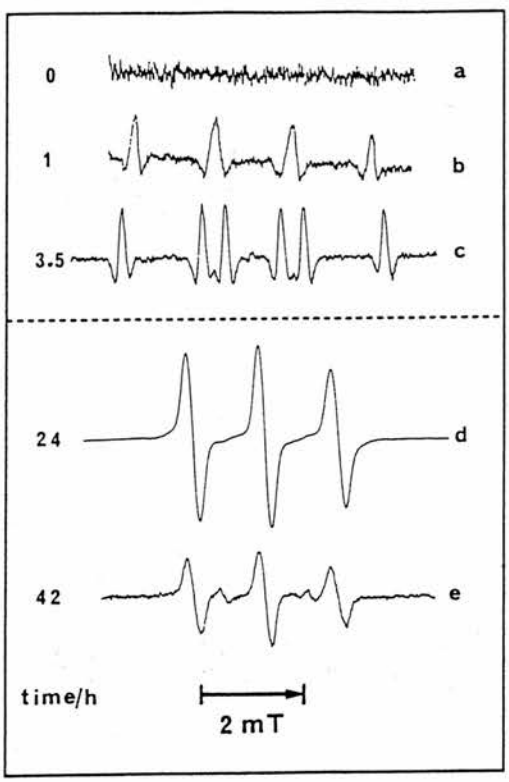
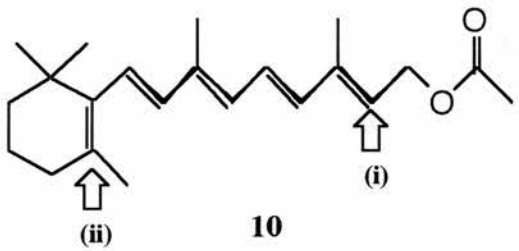


Figure 3: 9.1GHz spectra of radicals generated from retinyl acetate in benzene solution by passing nitric oxide gas through it at 293K.

Upper box: second derivative spectra. Lower box: first derivative spectra.

Note that the inner two lines in **Figure 3b** are broader than the outer lines due to overlap of spectral components; in **Figure 3c** the inner lines are fully resolved.

These spectral changes may be interpreted as follows: pure nitric oxide did not react with retinyl acatate and hence no signals were observed during the first hour. The subsequent development of spectra from nitroxide radicals results from the formation of an initiator radical (**X**·) which adds to it producing a delocalised radical that combined with nitric oxide to give nitroso compounds.

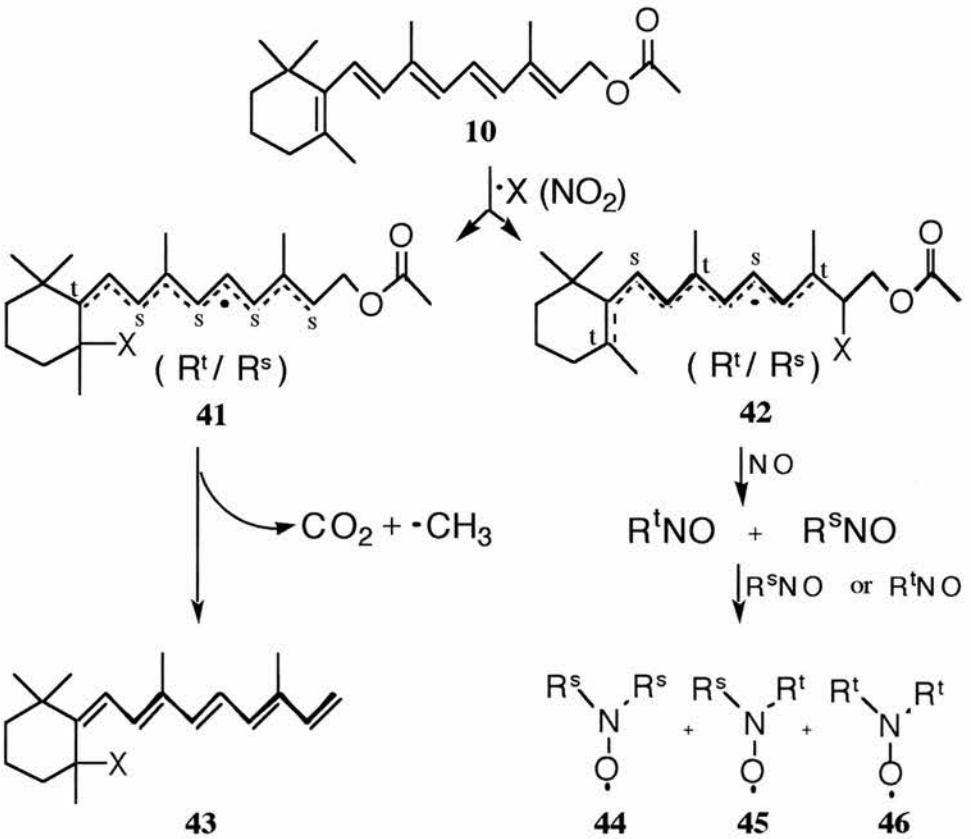


10

Scheme 23: Alternative sites for initial attack by **X**· on retinyl acetate.

The EPR hfs of the first species (**Figure 3b**) and the second species (**Figure 3c**), determined by computer simulations, were: a(N)=15.1 G, a(1H)=14.3 G and a(N)=14.6 G, a(1H)=18.8 G respectively. These parameters indicated that both species were dialkyl nitroxides containing one tertiary and one secondary alkyl group of the type $R^1R^2R^3CN(O\cdot)CHR^4R^5$. The magnitude of the hfs from the β–hydrogen in such radicals depends strongly on the conformation about the N-C_β bond and so the observed diffence between the a(1H) values points to the fact that the two species have different substituents R^4 and R^5. The nitrogen hfs of the strong triplet (**Figure 3d**) (13.3 G) indicated formation of di-*t*-alkyl nitroxides, the relatively large linewidth suggested that several similar radicals of this type overlapped to give the observed signal. The final weak triplet had a(N)=7.7 G which is indicative of a *t*-alkylacylnitroxide $R^tN(O\cdot)C(O)R$.

A rationale for the process, developed from a previously proposed mechanism for the reaction of nitric oxide with dienes[73], is outlined in **Scheme 23**. The identity of the initiator **X**· is uncertain but it is probably nitrogen dioxide resulting from nitric oxide/oxygen interaction (**Equation 19**).



Scheme 23: Explanation for the observed EPR spectra from the reaction of nitric oxide/nitrogen dioxide with retinyl acetate.

Although air was excluded from the system, it is concievable that, after 1h, minute quantities of oxygen had built up by degassing from internal surfaces or by seepage. This conclusion was supported by the observation that when small amounts of air were deliberately introduced into the system using a syringe, leading to the formation of nitrogen dioxide via reaction indicated in **Equation 19**, a similar spectral series ensued without the induction period.

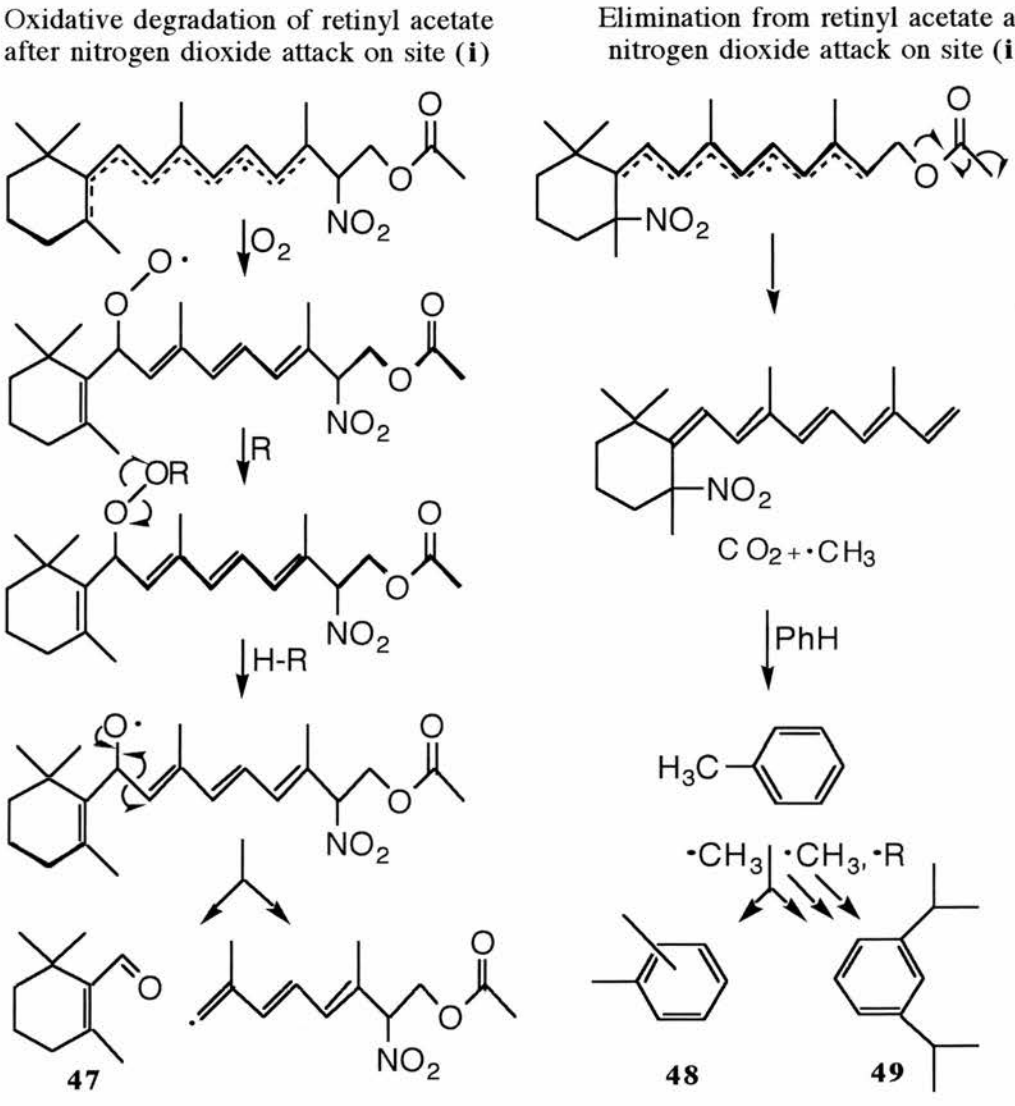
$$2NO + O_2 \longrightarrow 2NO_2 \quad \mathbf{19}$$

Under these conditions the strong triplet (**Figure 3d**) dominated from an earlier stage. Addition of nitrogen dioxide will occur preferentially at the two ends of the conjugated system of the acetate **10** because this leads to the adduct radicals **41** and **42** which have maximum resonance stabilisation. Product analyses (See below) suggested that **41** underwent β–scission to give a methyl radical together with the conjugated polyene **43** and carbon dioxide. Acetoxy group migration in related β–

acetoxyalkyl radicals is well documented and whether the mechanism is elimination followed by readdition, or concerted, has been vigorously debated[74]. The resonance stabilisation of radical **41** would disfavour acetoxy migration and therefore elimination may predominate because this produces a conjugated polyene **43**. Fragmentation of the released acetoxy radical to the methyl radical and carbon dioxide is known to be rapid[75]. The spin density of the delocalised unpaired electron in radical **42** will lead to the formation of 5 different nitroso compounds, 2 secondary and 3 tertiary. Both types of nitroso compounds will pick up a second radical **42** at either s or t site producing a mixture of three sorts of dialkylnitroxides viz **44-46** in **Scheme 23**. The di-secondary nitroxides ($R^sN(O\cdot)R^s$) will be too short-lived for EPR detection because they decay rapidly by disproportionation. The s, t -nitroxides will be longer lived and two of these are well enough resolved for detection (**Figure 3b**, **3c**). The di-tertiaryalkyl nitroxides have much longer lifetimes because disproportionation is not possible and therefore they eventually dominate. Clearly, several different di-*t*-alkyl nitroxides can be formed and they overlap to give the rather broad lines of **Figure 3d**. Further oxidation of the starting material **10**, the polyene **43** and the nitroxides gave carbonyl compounds from which the final carbonyl nitroxide (**Figure 3e**) was derived.

The products of the reaction were examined at each stage by GC-MS (EI and CI) which confirmed the absence of reaction in the first hour and showed the subsequent steady emergence of: (i) a string of alkyl aromatics (e.g. xylenes **48**, diisopropylbenzenes **49** and other polyalkylbenzenes and (ii) a series of carbonyl compounds (e.g. β–ionylidene acetaldehyde, β–ionone, β–cyclocitral **47**, 2,2,6-trimethylcyclohexanone, dihydroactinidiolide and others). The alkylbenzenes probably resulted from addition of the methyl radicals to the solvent giving, after oxidation, toluene which experienced further addition reactions to produce xylenes; attack at the benzylic hydrogens would eventually afford other polyalkylbenzenes (**Scheme 24**). The carbonyl compounds are similar to those identified from oxidation of other retinoids and carotenoids[76,77]. In this context, adduct radicals **41** and **42** must acquire oxygen at s and t sites and undergo oxidative scission of each of the double bonds, via an established mechanism[76,77]

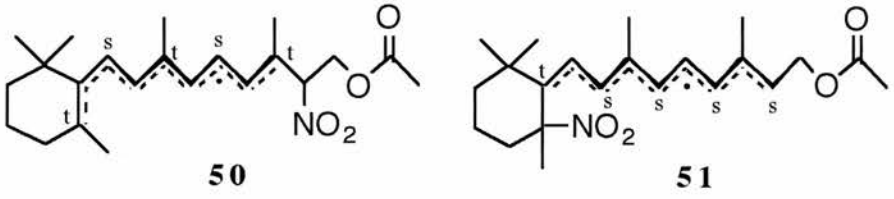
(**Scheme 24**). The entire sequence of events culminates in the observed products. In the later stages of oxidation the nitroxides will themselves undergo oxidative degradation affording the same carbonyl compounds.



Scheme 24: Mechanism for the formation of carbonyl compounds and alkylated aromatics from retinyl acetate and nitrogen dioxide.

A nitric oxide-treated sample of retinyl acetate, maintained at 250 K, gave an EPR signal consisting of background noise only. This situation persisted after allowing the temperature of the microwave cavity of the spectrometer to increase to 270 K over a period of 40 min. At 290 K, no observable change in the spectrum occurred. This latter result is not unexpected in view of the apparent reluctance of the reagent, in the absence of oxygen, to react even with the highly conjugated system such as the one present. As

with allyl acetate **7**, this low temperature study was undertaken in an attempt to identify the site of preferential attack on this molecule by nitrogen dioxide. Following the initial nitrogen dioxide addition to acetate **10** at sites **(i)** and **(ii)** (**Scheme 22**), radicals **50** and **51** are formed.



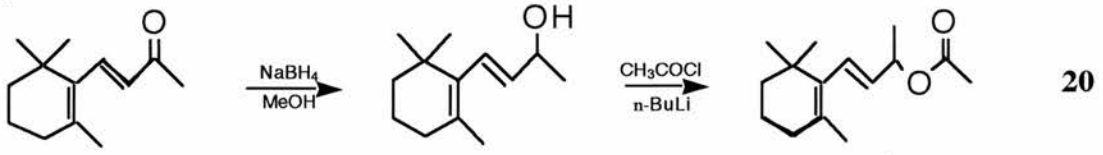
50 **51**

The EPR spectra of these two radicals would be different thereby permitting their identifcation. Since no spectra were observed, it is likely that both of the above species are highly reactive, even at the lowest temperatures practicable, probably undergoing rapid combination and other processes.

2.8.3 β–Ionyl acetate

Reduction of β–ionone

β–Ionone **8** was successfully reduced to β–ionol **52** via the reaction indicated by **Equation 20**.



8 **52** **9** **20**

Esterification of β–ionol

The conversion of alcohol **52** to the corresponding ester **9** was effected by standard methodology in respectable yield as shown by **Equation 20**. A deaerated solution of β–ionyl acetate **9** was exposed to nitric oxide, and it gave no EPR signal, which indicated the absence of a reaction with the reagent. However, 30 mins. after introduction of air into the system, a very broad EPR trace was produced. The broadness of this signal prevented its unambiguous interpretation. The large amount of air present helps explain the nature of this spectrum that took 23h to decay away. In the prolonged presence of oxygen, peroxidative degradation of the starting material may have occurred

to give a variety of radicals the signals from which overlap to give a poorly resolved broad spectrum.

CONCLUSIONS

- Nitric oxide, in the absence of an initiator, shows remarkable stability towards simple organic molecules

- Pure nitric oxide is also surprisingly reluctant to undergo direct addition reactions with more conjugated compounds

- The initial nitrogen dioxide-derived resonance-stabilised radicals of some compounds trap nitric oxide in a secondary reaction to furnish nitroso compounds which subsequently combine with the earlier radicals to give longer-lived di-alkyl nitroxides

- In the presence of excess oxygen, the nitrogen dioxide adducts, from suitable substrates, combine with oxygen to give the intermediate peroxyl radicals which, upon oxidative degradation, form carbonyl compounds

- Some of the nitrogen dioxide activated species flow down an alternative pathway which involves elimination of carbon dioxide from these β–acetoxy species along with the generation of a polyene and a methyl radical. The action of the latter on aromatic solvents, leads to alkylated aromatic compounds

- Polyunsaturated lipids, prevalent in a cellular environment, having comparable structure to the model systems used in this study, have the potential to form delocalised radicals from the addition of initiator radicals (probably nitrogen dioxide). As such, upon rapid combination with nitric oxide these intermediates are likely to undergo oxidative degradation via the nitroso compounds

- The relative contribution of this oxidative stress to the overall cytotoxicity of nitric oxide will depend critically on the rate of nitric oxide's conversion to nitrogen dioxide

(**Equation 19**). Although this is slow in aqueous media, the present study points towards this pathway being either enhanced in lipid phase or that other initiator radicals may act in conjunction with nitric oxide causing an abnormal turnover of unsaturated lipids to carbonyl compounds[69]

EXPERIMENTAL

3.9 Generation of Nitric Oxide

An aqueous solution (20 cm^3) of L-ascorbic acid (3.52 g, 0.02 mol) was placed in a three-necked flask. An aqueous solution (25 cm^3) of sodium nitrite (3.45 g, 0.05 mol) was added to it, dropwise, from a dropping funnel. The nitric oxide was dried and led to the reaction vessel in the manner described in section **3.11**. This method, first employed by Nocek and Kurtz[1], provides a cheap source of nitric oxide that is free from contaminants. Typically, this procedure of generating nitric oxide was utilised in investigating the reactions of this reagent with a range of model cellular compounds. Reacted in the fashion described above, the solutions of ascorbic acid and sodium nitrite released a steady stream of nitric oxide for up to 4h.

3.10 Reaction Conditions for Work with Nitric Oxide/ Nitrogen Dioxide and Model Cellular Compounds

Usually the reactions of nitric oxide/nitrogen dioxide were performed using either benzene or *t*-butylbenzene as the solvents. The solutions of the substrate were not prepared volumetrically and their approximate concentration was 0.13 M.

Routinely, before allowing any contact of the reagent, either nitric oxide or nitrogen dioxide, with a potential reactant, all solutions and the entire system were degassed with nitrogen gas, from a cylinder, for about 1h. This procedure ensured the removal of all dioxygen, present as a dissolved species in the solutions or contained in the air occupying the whole system.

Generally, the amounts of nitric oxide-generating materials used allowed a continuous supply of the gas to be passed through a solution of the reactant, at a steady rate, for approximately 4h. Experience showed this to be the normal reaction time with a variety of reactants. Further passage of the reagent into the reaction mixture caused no observable changes in the latter.

For the experiments conducted at room temperature, outside the microwave cavity of the EPR spectrometer, continuous stirring of the reaction mixture was maintained. Whereas no such treatment was possible for a number of other studies that were carried out, under analogous conditions, within the cavity of the spectrometer. Investigations at lower temperatures, were also done in the microwave cavity of an EPR machine. In such cases, the cavity was cooled by blowing cold nitrogen gas through it using a commercially available apparatus that vaporised liquid nitrogen from a Dewar by means of controlled electrical heating of the latter. The temperature of the microwave cavity and hence the reaction vessel being recorded by an instrument incorporating a thermocouple.

Periodically, air was injected into the system and, for the reactions done in the spectrometer cavity, the contents of the reaction vessel were concurrently subjected to an EPR analysis. The product identification, after prolonged exposure of the starting materials to nitric oxide/nitrogen dioxide mixture, was established by the use of ^{1}H NMR and/or GC-MS.

Reactions involving larger quantities of materials were carried out in 100 cm^3 three-necked flasks and these were constantly monitored by withdrawing small samples which were then examined by a combination of EPR, NMR and GC-MS techniques.

In order to assess the chemical integrity of the starting material and to ascertain its presence or otherwise during the course of the reaction, a ^{1}H NMR spectrum of it was recorded at the beginning of a reaction and, periodically, during the reaction and it was compared with that published in the literature if available.

^{1}H NMR spectra were obtained on a Varian Gemini 200 MHz spectrometer. All samples were dissolved in deuterochloroform and tetramethylsilane was used as an internal standard. EPR spectra were recorded in the 9.1-9.3 GHz range with a Bruker ER 200D instrument employing 100 kHz modulation. Samples were prepared in quartz tubes with benzene or *t*-butylbenzene as the solvents and degassed by bubbling nitrogen through them for 30 min. GC-MS work was undertaken on a Finnigan Incos 50 quadrupole mass spectrometer interfaced with a Hewlett-Packard HP 5890 capillary gas chromatograph fitted with a column coated with methylsilicone as the stationary phase.

Samples for analysis by this technique were prepared either in dichloromethane or benzene.

3.11 Experimental Arrangement for Reaction of Nitric Oxide/ Nitrogen Dioxide with a Substrate

In the microwave cavity of an EPR spectrometer

The reaction vessel for this experiment consisted of an NMR tube filled with a small volume (≈1 cm^3) of the reaction mixture. A plastic capillary tube, carrying the reagent gas, was dipped into the solution of the potential substrate through a rubber septum that acted as a cap for the tube. A steel-syringe needle was also placed through the septum until its tip just entered the tube. This needle was threaded with a piece of plastic capillary tubing one end of which formed an air-lock (**Figure 4**).

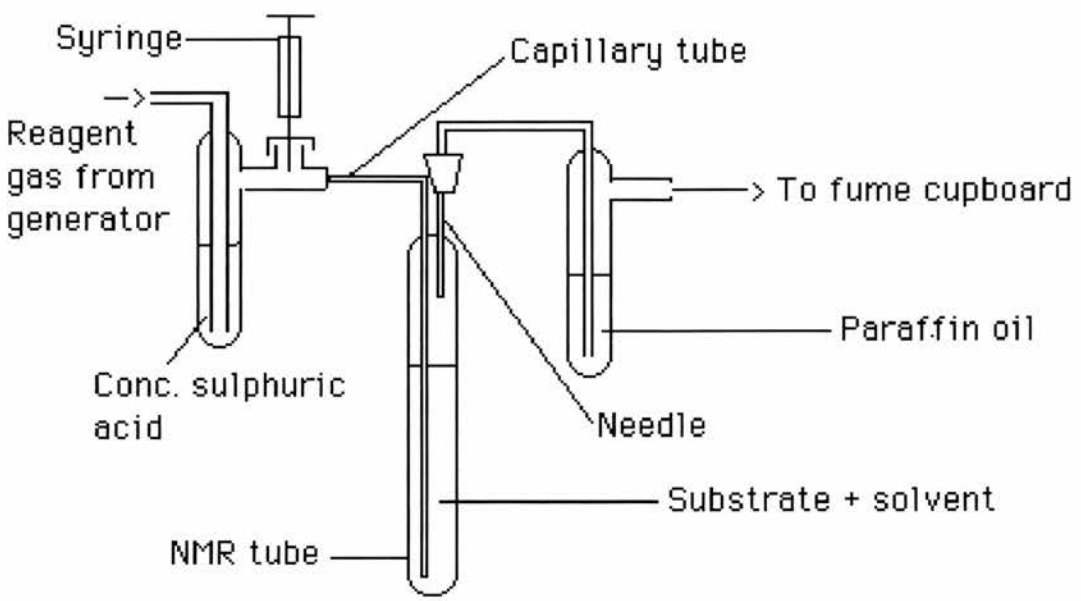


Figure 4: Apparatus for performing the reaction of NO/NO_2 with the solution of a reactant in the cavity of EPR spectrometer.

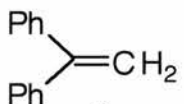
In a vessel permitting regular withdrawal of a sample of the reaction mixture

A 100 cm^3 three-necked round-bottomed flask made a convenient vessel which not only allowed larger volumes of the substrates to be studied but also enabled periodic

removal of small samples of the reaction mixture for analysis. One of the necks of the reaction vessel was connected to the supply of purified, dried nitric oxide gas while another was attached to an air-lock in order to prevent influx of air into the system. The central neck of the flask, covered by a rubber septum, thus offered easy access to its contents via a syringe.

3.7 Large-scale Reactions of Nitric Oxide/Nitrogen Dioxide with Model Cellular Compounds

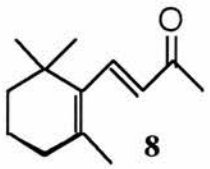
3.7.1 1,1-Diphenylethylene



6

1,1-Diphenylethylene **6** (0.47 g, 2.61 mmol) was dissolved in benzene (20 cm^3) and this mixture was stirred continuously in a 100 cm^3 three-necked flask. The system was deaerated by passing nitrogen gas through it for approx. 1h and then nitric oxide was slowly admitted into the reaction vessel over a period of 3h. Under nitrogen gas, a sample (1 cm^3) of the reaction mixture was withdrawn and subjected to EPR analysis. Air (2 cm^3) was injected into the stirred contents of the reaction vessel at half-hourly intervals and, simultaneously, samples (2 cm^3) of the reaction mixture were taken out of it for study using NMR and GC-MS. After removal of four half-hourly portions of the reaction mixture, air (10 cm^3) was introduced into the system and the contents of the reaction flask were stirred for a further 21h. At this stage the reaction mixture was again analysed by NMR and GC-MS. EPR (after NO saturation) triplet, a(N)=14.7 G; δ_H(200MHz, $CDCl_3$, after 4x2 cm^3 air) 5.43(2H, s, CH_2), 7.35(10H, m, 2xPh); δ_H(200MHz, $CDCl_3$, after 1x10 cm^3 air) 5.08(2H, s, $\underline{CH_2}Ar(NO_2)$), 5.75(1H, s, $\underline{CH}NO_2$), 7.22-7.45(9H or 10H, m, 2xPh); GC-MS peak no. 1205, retention time 22.4 min., 1,1´-(1-nitro-1,2-ethanediyl)bisbenzene **15**, or 1-nitro-2,2-diphenylethene **16**, m/z(relative intensity %) 225(M^+, 1), 178(100), 165(19), 152(27), 141(14), 76(29), 51(42), 39(17); GC-MS peak no. 1143, retention time 21.1 min., benzophenone **14**, m/z(relative intensity %) 182(M^+, 29), 105 (64), 77(100), 51(34).

3.7.2 β–Ionone



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β–Ionone (0.50 g, 2.60 mmol) was dissolved in benzene (20 cm^3) and nitric oxide was allowed to bubble through the nitrogen-degassed substrate for 4h. A sample (2 cm^3) of the reaction mixture, withdrawn under air-free conditions, was examined by NMR and GC-MS. δ_H(200MHz, $CDCl_3$) 1.02(6H, s, 2xCH_3), 1.40-1.48(2H, m, CH_2), 1.60(2H, m, CH_2), 1.72(3H, s, C=C($\underline{CH_3}$)), 2.04(2H, t, $\underline{CH_2}(CH_3)C=C$), 2.28(3H, s, $\underline{CH_3}CO$), 6.08(1H, d, CH), 7.24(1H, d, $\underline{CH}CO$); GC-MS peak no. 750, retention time 13.5 min., β–ionone **8**, m/z(relative intensity %) 192(M^+, 4), 177(100), 135(16), 91(16), 77(17), 43(73), 29(9).

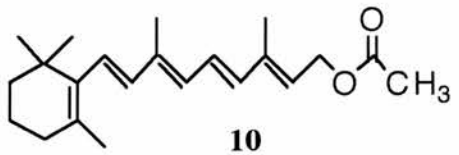
β–Ionone (0.50 g, 2.60 mmol) in benzene (20 cm^3), was treated with nitric oxide for 4h without any of the preliminary degassing of the system with nitrogen gas. The air-lock at the end of the apparatus described in section **3.11** was omitted in this experiment. The products of the reaction were identified by EPR, NMR spectroscopies and by GC-MS. EPR (after NO saturation) broad doublet, a(H)=9.5 G; δ_H(200MHz, $CDCl_3$) 1.12-1.31(6H, m), 1.46-1.65(4H, m), 1.80(3H, m), 2.33(3H, m); GC-MS peak no. 501, retention time 9.3 min., β–cyclocitral **21** m/z(relative intensity %) 152(M^+, 62), 137(89), 123(70), 109(63), 77(63), 67(100), 39(80), 29(46); GC-MS peak no. 770, retention time 13.9 min., 5,6,7,7A-tetrahydro-4,4,7A-trimethyl-2(4H)-benzofuranone **22**, m/z(relative intensity %) 180(M^+, 16), 137(26), 111(99), 67(74), 44(100), 42(70), 28(21).

β–Ionone (20.0 cm^3 of a 0.26 M solution) was degassed with nitrogen gas for approx. 1h, and then saturated with nitric oxide for 30 min. At 15 min. intervals, air (2 cm^3) was injected into the reaction vessel and, concurrently, a sample (1 cm^3) was removed from it for testing by EPR and NMR spectroscopies and by GC-MS. Eight aliquots of air were introduced into the system and the reaction was allowed to proceed for a total of 30.5h at which stage a final portion of of the reaction mixture was subjected

to further anlysis. EPR (after 15 min.) triplet, a(N)=13.7 G; EPR (after 120 mins.) triplet, a(N)=13.7 G along with other extremely weak signals; EPR (after 30.5h) triplet, a(N)=13.7 G and other signals, poorly resolved, with hfs owing to hydrogen(s), showing increased intensity; δ_H(200MHz, $CDCl_3$) 1.02(6H, s, 2xCH_3), 1.40-1.48(2H, m, CH_2), 1.60(2H, m, CH_2), 1.72(3H, s, C=C$\underline{CH_3}$), 2.04(2H, t, $\underline{CH_2}(CH_3)C=C$), 2.28(3H, s, $\underline{CH_3}CO$), 6.08(1H, d, CH), 7.24 9(1H, d, $\underline{CH}CHO$); GC-MS peak no. 734, retention time 13.6 min, β–ionone **8**.

β–Ionone (1.02 g, 5.30 mmol) was mixed with benzene (20 cm^3) and the solution was stirred and freed of air by passing a continuous stream of nitrogen gas through it for approx. 1h. Having passed nitric oxide through it for 30 min., air (10 cm^3) was admitted into the reaction vessel. Samples (7x2 cm^3) of the reaction mixture were withdrawn, periodically, over 52.5h for analyses. ^{1}H NMR spectrum and the GC-MS data obtained were the same as stated above indicating the presence of β–ionone **8**.

3.7.3 Retinyl acetate



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Purification of the commercial compound

Retinyl acetate obtained commercially is an oily concentrate and so it was in need of purification before use, as was indicated by the presence of additional signals in the ^{1}H NMR spectrum of the material. The removal of this oil and stabilisers (BHT) from the impure substance was achieved by solvent precipitation using freshly distilled petroleum ether (bp 40-60°C). Contaminated retinyl acetate (10.0 g) had small volumes (10 cm^3) of petroleum ether added to it, with continuous stirring, until the supernatant ethereal layer had acquired a distinct yellow colour and a white solid had settled at the bottom of the container. The solid was isolated by filtration under suction and washed with petroleum ether (3x20 cm^3). The last traces of the solvent were removed from the solid under high vacuum for 30 mins. and this gave a sample of the pure dry acetate **10**. δ_H(200MHz, $CDCl_3$) 1.02(6H, s, $CH_2C(\underline{CH_3})_2$), 1.42-1.50(2H, m, CH_2), 1.61(2H, m, $CH_2\underline{CH_2}CH_2$), 1.70(3H, s, $CH_2(\underline{CH_3})C=C$), 1.89(3H, s, $CH(\underline{CH_3})C=CH$), 1.95(3H,

s, $\underline{(CH_3)}CHCH_2O$), 2.02(2H, t, $CH_2\underline{CH_2}C(CH_3)$), 2.07(3H, s, $\underline{CH_3}CO$), 4.72(2H, d, $\underline{CH_2}O$), 5.60(1H, t, $(CH_3)C=\underline{CH}CH_2$), 6.13(2H, d, $(CH_3)C=\underline{CH}CH=\underline{CH}C(CH_3)$), 6.65(1H, dd, $\underline{CH}=CHC(CH_3)$), 6.27(2H, d, $\underline{CH}=\underline{CH}$).

The purified retinyl acetate (0.86 g, 2.60 mmol) was added to benzene (20 cm^3). This mixture was degassed with nitrogen gas for 1h and then saturated with nitric oxide for 4h. A sample (2 cm^3) of the reaction mixture was withdrawn for analysis by EPR, NMR spectroscopies and GC-MS. Subsequently, air (10 cm^3) was added to the reaction flask at regular intervals and, simultaneously, samples (2 cm^3) were removed for study by NMR and GC-MS. The total time the reactant was in contact with nitrogen dioxide/oxygen was 45h and during this period 60 cm^3 air had been allowed into the system. EPR (after NO saturation) triplet with two components: major signal a(N)=13.8 G and a minor signal a(N)=13.0 G, a(H)=13.2 G; EPR (7.5h after admitting air) increased intensity of the two-component spectrum; ^{1}H NMR showed unchanged **10** with the same spectrum as above. GC-MS peak no. 1022, retention time 20.1min., retinyl acetate **10,** m/z(relative intensity %) 269(5), 145(100), 119(52), 105(51), 91(34), 77(27), 69(9), 54(19), 41(35), 29(7).

3.8 Small-scale Reactions of Nitric Oxide/Nitrogen Dioxide with Model Cellular Compounds

3.8.1 1,1-Dipenylethylene

Using the apparatus shown in **Figure 4** approx. 1 cm^3 of a 0.13 M solution of the alkene **6** in benzene was degassed with nitrogen gas for 1h. At this stage, a 'blank' EPR spectrum of the solution was recorded. Nitric oxide was passed through the contents of the NMR tube and EPR traces of the reaction mixture were obtained after it had been in contact with the reagent for 3 min. and for 10 mins. EPR (degassed substrate) extremely broad triplet, a(N)=15.8 G; EPR (10 mins. after NO) spectrum remained unchanged.

3.8.2 Allyl acetate

After degassing all solutions for 1h with nitrogen gas, approx. 1 cm^3 of allyl acetate (0.14 g, 1.40 mmol) solution in benzene (10 cm^3), had nitric oxide passed through it for 3.5h. Following the recording of an initial EPR spectrum immediately after degassing, air (10 cm^3) was admitted into the nitric oxide stream after it had been bubbled through the reaction mixture for 30 min. EPR spectra were obtained at 10 min. and at 3.5h intervals following this treatment. Once the nitric oxide supply was exhausted, a GC-MS analysis was also performed on the reaction mixture. EPR (degassed solution) signal showed only background noise; EPR (10 min. after NO) weak triplet, a(N)=7.2 G; EPR (3.5h after NO) same weak triplet with very little change in intensity; GC-MS peak no. 180, retention time 3.3 min., ethylbenzene (**Scheme 20**), m/z(relative intensity %) 106(M^+, 28) 91(100), 78(18), 65(8), 51(11), 39(7); peak no. 207, retention time 3.7 min., 1,2-dimethylbenzene (**Scheme 20**), m/z(relative intensity %) 106(M^+, 39), 91(100), 78(31), 65(16), 51(16) 39(13); peak no. 241, retention time 4.4 min., 1-methylethylbenzene or phenylethanone, m/z(relative intensity %) 120(M^+, 22), 105(92), 78(100), 50(30), 39(16), 28(12).

Allyl acetate (0.14 g, 1.40 mmol) was added to *t*-butylbenzene (10 cm^3) and approx. 1 cm^3 of this solution contained in an NMR tube, placed in the microwave cavity of an EPR spectrometer, was degassed with nitrogen gas for 1h. A 'blank' EPR spectrum of the starting material, at 250 K, was recorded. The reaction mixture was then saturated with nitric oxide and another EPR trace was obtained. Air (2 cm^3) was admitted into the nitric oxide stream and immediately a further EPR signal was recorded. The temperature of the cavity was lowered to 225 K and, following the introduction of an additional sample of air (10 cm^3) a further EPR trace of the reaction mixture was obtained. EPR spectroscopy showed only the background noise under all conditions.

3.8.3 Retinyl acetate

Purified retinyl acetate (0.21 g, 0.64 mmol) was added to benzene (20 cm^3) to give a 0.13 M solution. Approx. 1 cm^3 of this solution, placed in an NMR tube, was degassed with nitrogen gas for 1h. Nitric oxide was passed through the tube, placed in

the microwave cavity of an EPR spectrometer, for 3.5h. Immediately after this treatment of the substrate, EPR spectra were recorded at regular intervals. Air (2 cm^3) was injected into the nitric oxide-saturated reaction mixture and concurrently, EPR spectra of the substrate were also recorded periodically up to a total of 42h after the initial admission of air into the system. A sample corresponding to 24h of reaction time with nitrogen dioxide was analysed by GC-MS in order to identifiy the products. EPR (1h after NO addition) weak signal, a(N)=15.1 G, a(H)=14.3 G **Figure 3b**; EPR (3.5h after NO addition) first signal reached its maximum intensity , a second signal appeared, a(N)=14.6 G, a(H)=18.8 G **Figure 3c**; EPR (24h after addition of air) first and second signals not observable, a new three-line trace present, a(N)=13.3 G **Figure 3d**; EPR (42h after addition of air) third signal present at very low intensity a(N)=7.7 G **Figure 3e**; GC-MS peak no. 194, retention time 3.6 min., 1,2-dimethylbenzene (**Scheme 20**); GC-MS peak no. 610, retention time 11.3 min., 1,3-bis(methylethyl)benzene **46**, m/z(relative intensity %) 162(M^+, 33), 147(72), 119(75), 105(100), 91(99), 77(41), 55(32).

Purified retinyl acetate (0.21 g, 0.64 mmol) was dissolved in *t*-butylbenzene (5.0 cm^3) to give a 0.13 M solution. 1 cm^3 of this solution, maintained at 250 K, was degassed with nitrogen gas. After saturation with nitric oxide, an EPR spectrum of the reaction mixture was registered. The temperature of the microwave cavity of the spectrometer was increased to 270 K over a period of 40 min. and another EPR trace was obtained. Finally the reaction was allowed to attain room temperature and a further EPR analysis was performed on it under these conditions. EPR (under all reaction conditions) spectra corresponding to background noise only were observed.

3.8.4 β–Ionyl acetate

β–Ionone **8** (10.0 g, 0.05 mol) was dissolved in methanol (100 cm^3). Sodium borohydride (2.65 g, 0.07 mol) was added slowly, over 15 min., to the magnetically stirred solution of the enone. Once the addition of the hydride was complete, the reaction mixture was stirred for a further 2h at room temperature. The bulk of the solvent was removed under reduced pressure and the residue was carefully poured into water (100 cm^3) contained in a separating funnel. The product was extracted with diethyl ether

(3x50 cm^3) and the combined ethereal extracts were dried over anhydrous sodium sulphate. The alcohol β–ionol **52** (8.69 g, 86%) was isolated after removal of the solvent using a rotatory evaporator. δ_H(200MHz, $CDCl_3$) 0.96(6H, s, $CH_2C(\underline{CH_3})_2$), 1.30(3H, d, $CH(OH)\underline{CH_3}$), 1.40-1.48(2H, m, $\underline{CH_2}C(CH_3)_2$), 1.60(2H, m, $CH_2\underline{CH_2}CH_2$), 1.67(3H, s, $CH_2(\underline{CH_3})C=C$), 1.98(2H, t, $CH_2\underline{CH_2}(CH_3)C=C$), 4.35(1H, q, $\underline{CH}(OH)CH_3$), 5.46(1H, dd, $CH=\underline{CH}CH(OH)$), 6.02(1H, d, $\underline{CH}=CHCH(OH)$).

Esterification of β–ionol

Attempts to esterify β–ionol using neat acetyl chloride in the presence of a base, N,N-dimethylaniline, gave poor results. A more successful reaction employed β–ionol (2.0 g, 0.96 mol) in a round-bottomed three-necked flask containing dry THF (15 cm^3), equipped with a magnetic stirrer, a dropping funnel, a reflux condenser and a nitrogen inlet. Under an atmosphere of nitrogen gas, n-butyllithium (0.96 g, 0.015 mol) was added to the continuously stirred contents of the flask and the resulting mixture was stirred for a further 30 min. at room temperature. Acetyl chloride (1.20 g, 0.015 mol), diluted with THF (15 cm^3), was added slowly to the reaction vessel from the dropping funnel. The solution obtained was refluxed for 1h, cooled to 0°C and hydrolysed by addition of water (30 cm^3). The aqueous layer was separated and extracted with diethyl ether (5x15 cm^3). The combined ethereal extracts were dried over anhydrous magnesium sulphate and concentrated at a rotatory evaporator. The crude product was purified by distillation using a Vigreux column to yield β–ionyl acetate **9**, (1.22 g, 52%) bp 60-70°C / 1.5 mm Hg. δ_H(200MHz, $CDCl_3$) 0.96(6H, s, $CH_2C(\underline{CH_3})_2$), 1.35(3H, d, $CH_3CO_2CH(\underline{CH_3})$), 1.38-1.46(2H, m, $\underline{CH_2}C(CH_3)$), 1.60(2H, m, $CH_2\underline{CH_2}CH_2$), 1.64(3H, s, $CH_2(\underline{CH_3})C=C$), 1.95(2H, t, $CH_2\underline{CH_2}(CH_3)C=O$), 2.02(3H, s, $\underline{CH_3}CO$), 5.30(1H, m, $CH_3CO_2\underline{CH}(CH_3)$), 5.40(1H, dd, $CH=\underline{CH}CH_2(CH_3)O_2CCH_3$), 6.09(1H, d, $\underline{CH}=CHCH(CH_3)O_2CCH_3$).

β–Ionyl acetate (0.15 g, 0.65 mol) was dissolved in benzene (15 cm^3). Approx. 1 cm^3 of this solution was deaerated with nitrogen gas and its EPR spectrum was recorded. 15 min. after exposure of this solution to nitric oxide, another EPR trace was

registered. Air (20 cm^3) was injected into the nitric oxide, passed through the substrate for a total of 3.5h, which was swept through the reaction mixture with nitrogen gas. At this stage, corresponding to 30 min. reaction time, a further EPR signal was recorded. A final spectrum of the reaction mixture was also obtained after 23h. EPR (after NO saturation) spectrum of background noise observed; EPR (30 min. after air injection) very broad signal noticeable; EPR (23h after air injection) previously observed signal had decayed away to background noise level.

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CHAPTER 2

Diacyl Peroxides as Precursors for Carbonyl Compounds: A Nitric Oxide Mediated Transformation

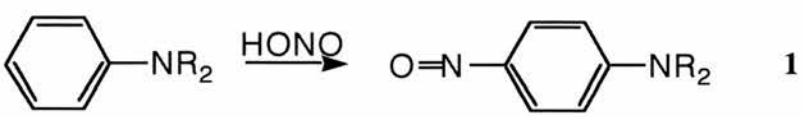
INTRODUCTION

C-Nitroso compounds have a valuable role to play in organic chemistry. The readiness with which they can be obtained from a variety of starting materials and through the use of a range of reactions, makes them the key precursors for nitrogen-containing compounds of synthetic and analytical interest. Their syntheses can be accomplished using a number of well established routes.

1.1 Synthesis of C-Nitroso Compounds

1.1.1 Nitrosation of activated aromatic rings

Nitrosation of activated aromatic rings of tertiary amines and phenols has been achieved with nitrous acid[1] (**Equation 1**).



1

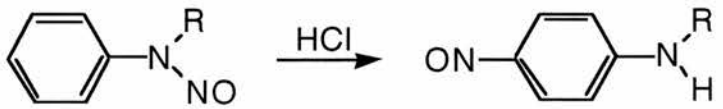
Primary aromatic amines yield diazonium salts[2] while secondary amines give N-nitroso-compounds (also called nitrosamines)[3] (**Equations 2** and **3**). The reaction with secondary amines occurs with dialkyl-, diaryl-, alkylarylamines and even with mono-N-substituted amides[4]. The analogous aliphatic diazonium salts are extremely unstable owing to the absence of the resonance interaction between the diazonium group and the aromatic ring.

2

3

Despite the above mentioned limitation, secondary aromatic amines have been C-nitrosated in two ways. In the first of these strategies, the initially obtained N-nitroso compound can be isomerised to the desired product in a reaction known as the Fischer-Hepp rearrangement[5] by treatment with aqueous hydrochloric acid. The process involves

the migration of the nitroso group to the *para-* position in the aryl ring, a transformation normally not accessible by direct C-nitrosation of a secondary aromatic amine (**Equation 4**).



4

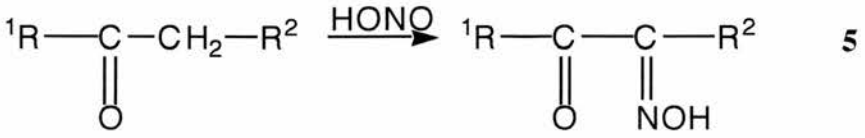
The mechanism of this rearrangement is not fully understood. It is known[6] that the reaction is facilitated in a large excess of urea, a fact that indicates that the migration must occur via an intramolecular complex[7] because any free electrophilic nitrosating species would be captured by the nucleophilic urea. Secondly, on treatment of the initial nitrosamine with another mole of nitrous acid, an N,C-dinitroso compound can be formed.

Much less is known about the nitrosation of aromatic nuclei compared with the corresponding nitration[8]. The attacking electrophile is the nitrosonium ion (NO^+) or a carrier of this species (e.g. NOCl, NOBr and N_2O_3 etc.). Nitration is performed by the nitronium ion (NO_2^+), an electrophile 10^{14} times more reactive than its counterpart involved in nitrosation[9] and hence the latter's requirement for the ring-activated substrates. Work with phenols has indicated that the first site of attack on the substrate, is the hydroxyl group and this leads to the formation of an intermediate nitrite ester which then rearranges to the C-nitroso compound[10].

The ring nitrosation of activated aromatic nuclei produces C-nitroso compounds which are stable because the α–carbon atoms in these molecules lack hydrogen, thus eliminating any likelihood of a further tautomeric change resulting in an oxime.

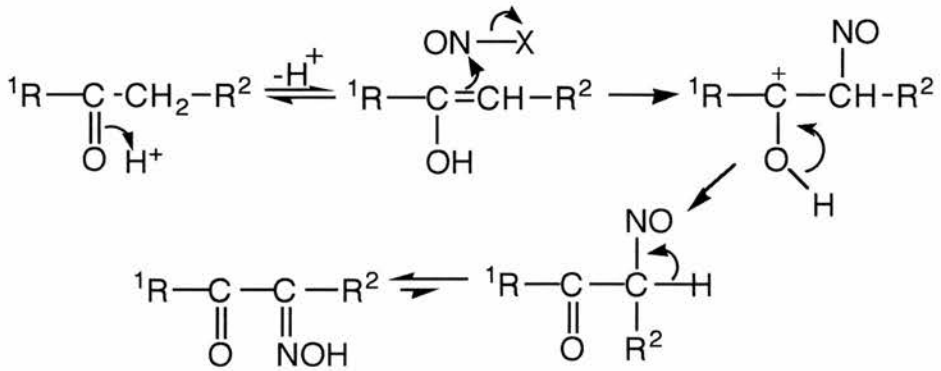
1.1.2 Nitrosation of a carbon with active hydrogen

Another method of effecting C-nitrosation is to use nitrous acid or alkyl nitrites for displacement of active hydrogens on a carbon adjacent to an electron withdrawing group[11]. In the presence of tautomerisable hydrogens on the α–carbon atom of the initially formed C-nitroso compound, the stable product isolated in this reaction is an oxime (**Equation 5**).



5

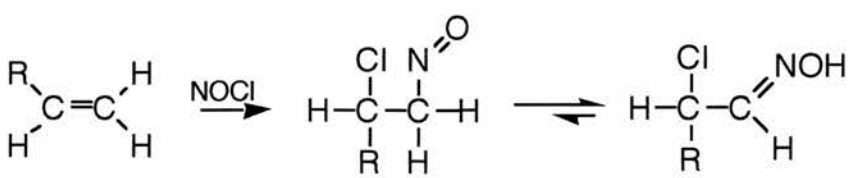
The mechanism of this process involves the nitrosonium ion, or a carrier of it e.g. NOCl, and if the activating group is an acyl group, then the reaction proceeds via an enol as shown in the generalised **scheme 1**.



Scheme 1: Mechanism for nitrosation at a site activated by a carbonyl group.

1.1.3 Addition to an alkene

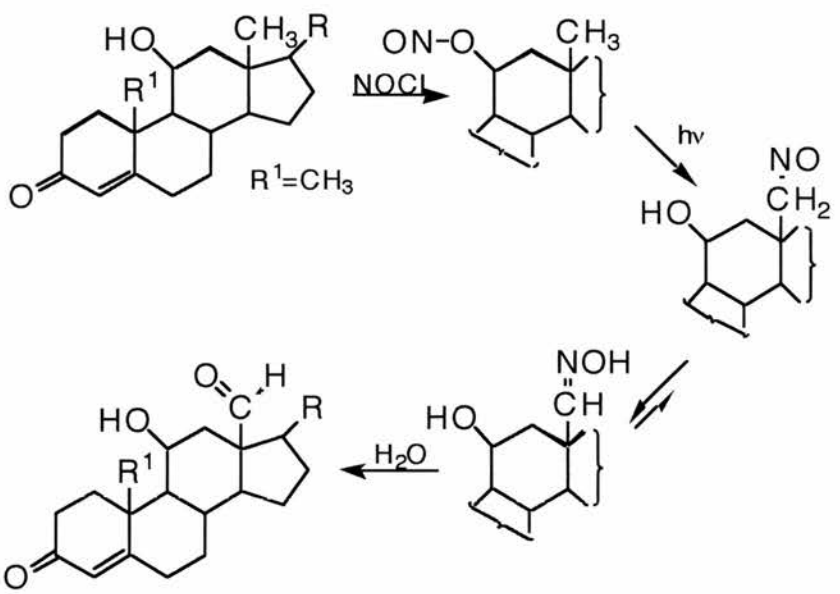
A versatile method for forming C-nitroso compounds is the addition of nitrosyl chloride to an alkene to give a β–chloronitroso adduct[12]. This product is isolable only if the carbon bearing the nitrogen has no hydrogen. Furthermore, with some alkenes, the initial β–halo nitroso compound is susceptible to oxidation by the reagent and a β–halo nitro compound is likely to be the product. The process occurs via electrophilic addition in the *anti* fashion. Markovnikov's regiochemistry is followed and the electrophile (NO^+) attacks the less substituted carbon atom of the alkene (**Scheme 2**).



Scheme 2: Electrophilic addition of nitrosyl chloride to an alkene.

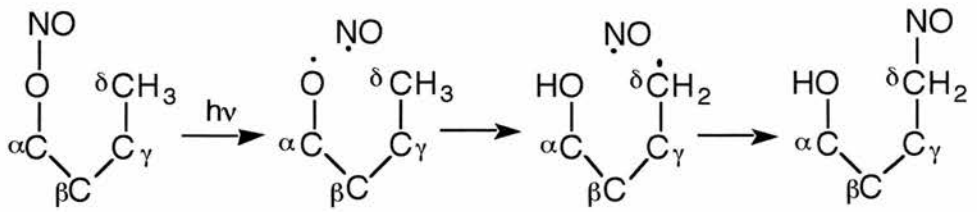
1.1.4 Photolysis of nitrite esters

The Barton reaction[13] remains a well publicised procedure for introduction of functionality at a position remote from the functional groups already present in a molecule. The reaction oxidises a methyl group, in the δ–position relative to a hydroxyl group of the starting material, to an aldehyde. The alcohol is converted to a nitrite ester by its reaction with nitrosyl chloride. Photolysis of this ester produces an alkoxyl radical which abstracts a hydrogen atom from the δ–carbon generating a carbon-centred radical that traps nitric oxide forming an intermediate nitroso compound. The oxime tautomer formed from the latter is hydrolysed to the aldehyde[14] (**Scheme 3**).



Scheme 3: Oxidation of a methyl group to an aldehyde in the Barton reaction.

The reaction occurs only when the methyl group has a favourable steric position[15] and the mechanism involves a six-membered transition state[16] (**scheme 4**).

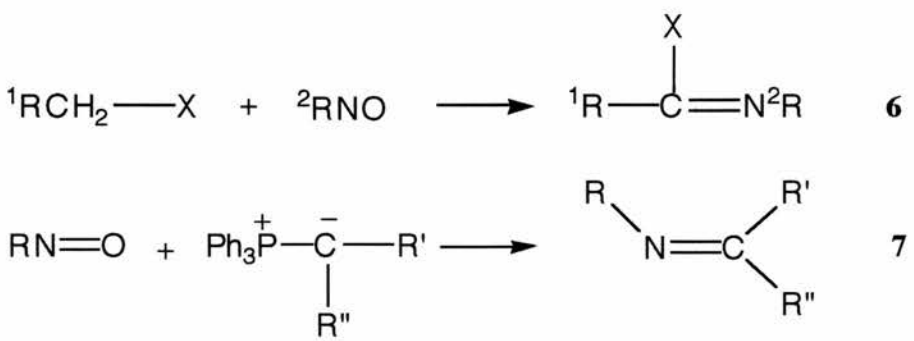


Scheme 4: Hydrogen abstraction via the six-membered transition in the Barton reaction.

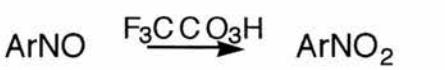
1.2 Chemistry of C-Nitroso Compounds

C-Nitroso compounds occupy an important place in organic chemistry because of the multitude of transformations which can be achieved through the manipulation of this functional group. A rich variety of compounds that can be synthesised include:

- *Imines* by condensation of a C-nitroso compound with a substrate possessing activated hydrogen (**Equation 6**) and by the Wittig reaction of a phosphorane with the -NO group of a suitable nitroso compound[17] (**Equation 7**).



- *Nitro compounds* by oxidation of aromatic nitroso compounds using peroxytrifluoroacetic acid and many other oxidants[18] (**Equation 8**).



- *Azo compounds* from coupling of an aromatic nitroso derivative and a primary aryl amine in glacial acetic acid[19] (**Equation 9**).

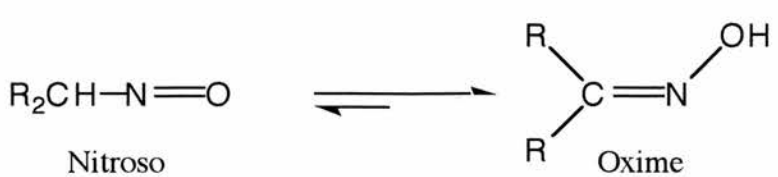
• *Amines* by reduction of the nitroso compound with a metal and an acid[20] (**Equation 10**).

$$RN{=}O \xrightarrow{Zn/HCl} RNH_2 \qquad \mathbf{10}$$

Electron paramagnetic resonance spectroscopy is the method commonly used to detect species containing one or more unpaired electrons. Failure to observe an EPR spectrum does not necessarily mean the absence of radicals, because the concentration of any such intermediates might be too low for direct observation. In these situations, the *spin trapping*[21] technique is used to prolong the lifetimes of the most unstable radicals. The procedure involves the addition of a compound to the material being analysed which combines with it to produce more persistent radicals capable of ready detection. Nitroso compounds are an important class of spin-traps. They react with carbon-centred radicals to give stable nitroxide radicals[22] that are easily identifiable from their characteristic splitting pattern (**Equation 11**).

$$^1RN{=}O + {}^2R\cdot \longrightarrow {}^1R^2RN{-}O\cdot \qquad \mathbf{11}$$

Perhaps the most significant aspect of the bonding in the C-nitroso compounds is that they consist of two structurally distinct types of molecules that are in a dynamic equilibrium and which correspond to the same molecular formula. The phenomenon[23] arises from a proton shift from the α–carbon atom of a molecule to the oxygen atom of the nitroso group of that molecule. The position of the equilibrium lies well in the favour of the resulting oxime[24] and, as a general rule, nitroso compounds are stable only when there is no α–hydrogen.



Nitroso Oxime

The significance of this isomerism in nitroso compounds is that the isolable products of many nitrosations are oximes which can act as starting materials for a large number of potentially useful conversions because of their high chemical versatility.

1.3 Biological Action of Nitroso Compounds

Nitrosation at other types of atoms produces nitroso compounds that have been demonstarted to play an important role in mammalian physiology. In this context, the most prominent have been the nitroso compounds derived from: thiols (thionitrites), amines (nitrosamines) and alcohols (alkyl nitrites). S-Nitrosothiols (thionitrites) have been frequently cited in connection with the bioactivity of nitric oxide. N-Nitroso derivatives (nitrosoamines) possess carcinogenic properties and the reversible formation of the alkyl nitrites of some enzyme systems has been proposed as a means of bioregulation for some critical cellular functions[25].

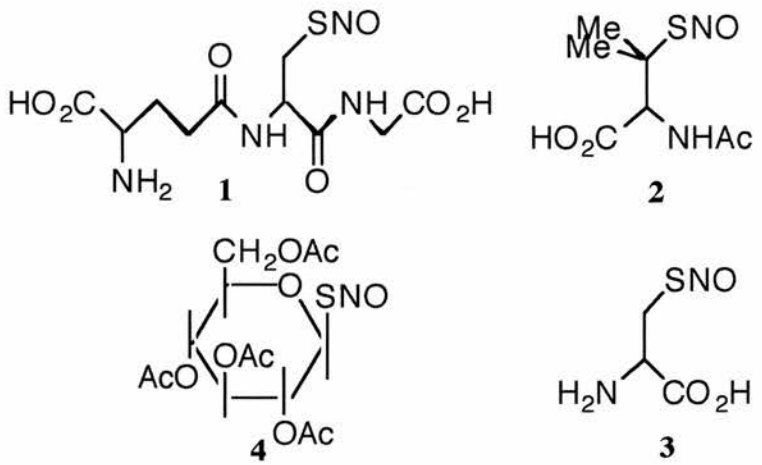
1.3.1 Nitrosothiol and nitrosamine synthesis

Nitrosothiols can be readily prepared by electrophilic nitrosation of thiols with a reagent that can act as a carrier of the nitrosonium ion (NO^+). If the thiol is water-soluble, then it may be treated with sodium nitrite in aqueous acidic solution whereas synthesis of thiols with poor water solubility, can be conveniently achieved with *tert*-butyl nitrite in acetone[26]. Species responsible for generating the nitrosating agent[11] include: the hydrated form of nitric oxide ($H_2NO_2^+$), nitrosyl halides (NOX), nitrous acid anhydride (N_2O_3), dimeric nitrogen dioxide (N_2O_4), alkyl nitrites (RONO) and nitrosamines (R_2NNO).

Nitrosamines can be obtained from the nitrosative substitution action of N_2O_3, present in aerobic aqueous solutions containing nitrous acid, on the nucleophilic secondary amines.

1.3.2 Biochemical behaviour of nitrosothiols and nitrosamines

Until quite recently, only a handful of examples of nitrosothiols were known owing to their inherent instability. However, with increased interest in their potential as nitric oxide-donor drugs, a number of them have been synthesised and characterised (**Scheme 5**).

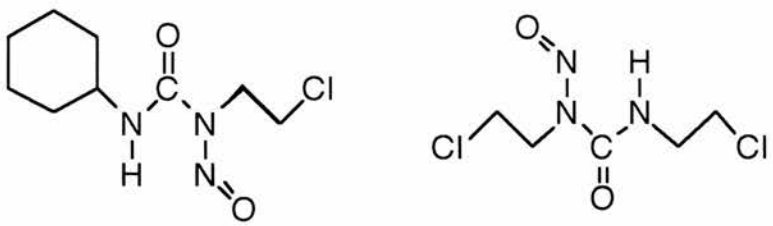


Scheme 5: Structures of some synthetic nitrosothiols. (**3** stable in solution only)

S-Nitroso-N-acetylpenicillamine **2** (SNAP), in *ex vivo* experiments undergoes Cu^{2+} catalysed rapid decomposition releasing nitric oxide. Recently, Cu^{+} has been identified as the true catalyst[27] with the use of a chelator that is specific for this ion[28]. Under physiological testing, nitrosothiols display the bioactivity of nitric oxide in causing vasodilation[26,29] and prevention of platelet adhesion and aggregation[30]. The discovery of endogenous nitrosothiols in blood plasma as the nitrosothiols of human serum albumin[31], glutathione (GSNO)[32] **1**, and and sulphur containing proteins[33] lends some support for the view that these compounds may be involved in the storage and transport of nitric oxide *in vivo* but this needs experimental verification.

Nitrosamines must be metabolised in order to elicit their mutagenic properties. While the mode of carcinogenic biochemical activation is not known for all nitrosamines, many of them are activated through the process of α–hydroxylation. Their detrimental effect stems from the ease with which the hydroxylated derivatives decompose to the diazonium ions which are aggressive alkylating agents. Endogenous nitrosamines have been shown to form from the action of nitrous acid, generated in the stomach following the dietary ingestion of nitrate and its reduction in the oral cavity, on nitrogen compounds[34,35]. Bacteria are also capable of mediating the nitrosation of amines[36]. While the deleterious effects of many N-nitroso compounds has been the central theme

for much research in the last few years, several N-nitrosoureas are receiving clinical attention for their properties as antitumour agents.



1-Chloroethyl-3-cyclohexyl-1-nitrosourea

Bischloroethyl nitrosourea

Scheme 6: Nitrosoureas used as anti-cancer drugs.

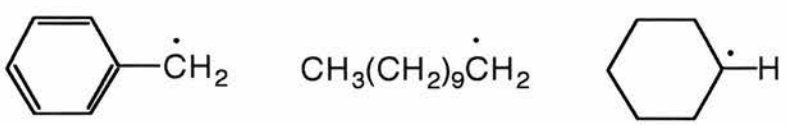
1.4 C-Nitrosation from Radical Coupling

Nitric oxide bearing an unpaired electron offers some scope for use as a radical species in coupling reactions with carbon-centred radicals thereby yielding either C-nitroso derivatives, or the their tautomers, the corresponding oximes. Nitric oxide has been frequently used in this capacity to carry out spin-trapping studies. The methodology has then been applied to the detection of the gas at very low levels by EPR spectroscopy. The principle of this approach is based on the readiness with which the nitroso compounds themselves combine with carbon-centred radicals to give the more stable EPR-active nitroxides. Under normal circumstances, therefore, substantial quantities of the nitroso product are unlikely to be obtained. However, with appropriate manipulation of the reaction conditions, it seems feasible to use nitric oxide as a synthetic reagent and to achieve conversion of various types of carbon-centred radicals into potentially useful nitroso compounds (with tertiary radicals) or oximes (with primary and secondary radicals).

The interest in developing this strategy stemmed from a closer look at the Barton reaction and from the desire to extend the existing methods of synthesis for C-nitroso compounds and oximes. In view of their diverse chemistry, a likely involvement in

bioregulation, a proven key role in carcinogenesis and a rich promise as therapeutic agents, nitroso compounds are vitally important in synthetic organic chemistry, in clinical medicine and in research.

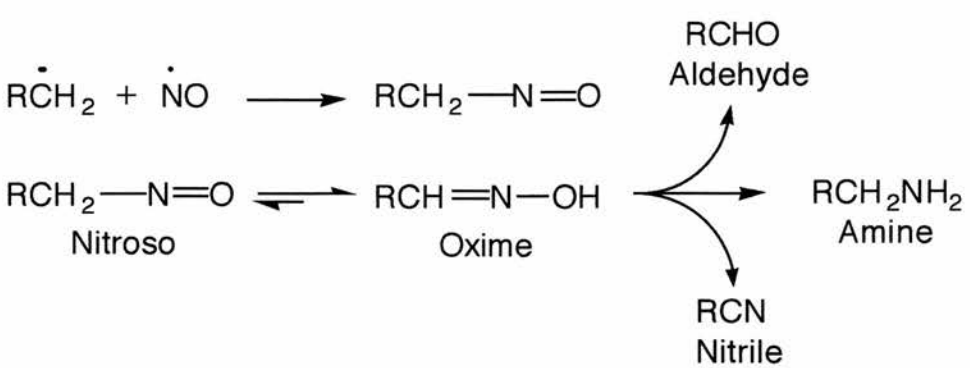
The reaction of photochemically formed carbon-centred radicals with externally added excess nitric oxide, under anaerobic conditions, represents a novel route to nitrogen compounds. The extent of coupling of these radicals, in the reaction mixture, with the reagent gas was expected to be dependent on the thermodynamic stability of the former. With this in mind, a primary, a secondary and a benzyl radical were investigated. Benzyl radical **5**, with its extra resonance stabilisation, the undecyl radical **6** and the cyclohexyl radical **7** were chosen (**Scheme 7**).



5 **6** **7**

Scheme 7: Structure of radicals used to react with nitric oxide.

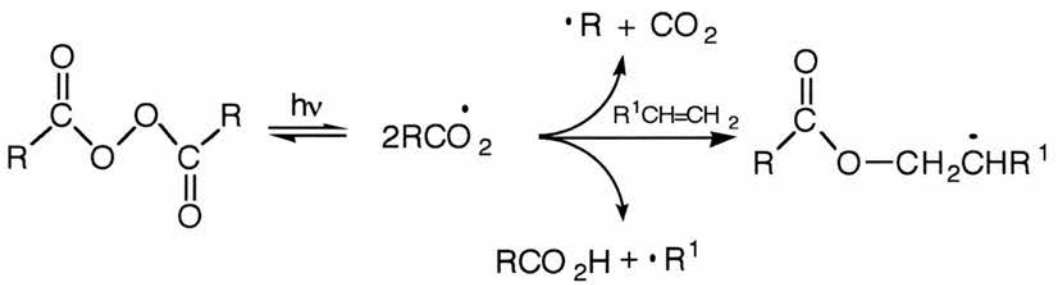
All of the above radicals contain α–hydrogens, therefore oximes were expected to be the main products from their reaction with nitric oxide owing to the nitroso-oxime equilibrium being well in the favour of the latter. If the initial results proved successful, tertiary carbon-centred radicals would then be tested with a view to forming their nitroso derivatives. The resulting oximes were intended to be converted to other products. For example, hydrolysis of an oxime would yield an aldehyde, its dehydration leads to a nitrile and its reduction gives a primary amine (**Scheme 8**).



Scheme 8: Potential transformations of an oxime.

1.5 Use of Diacyl Peroxides

In order to test whether the oxime formation proposed in scheme **8** occurred in practice, organic diacyl peroxides were used as a convenient source of carbon-centred radicals[37]. These compounds undergo thermal and photo decomposition to furnish acyloxyl radicals which have been characterised with laser flash photolysis (LFP) of peroxide/tetrachloromethane solutions and time-resolved EPR spectroscopy[38,39]. The EPR parameters of these radicals have later been confirmed by Ingold and co-workers[40-44]. The acyloxyl radicals are capable of undergoing three types of reactions: decarboxylation, hydrogen abstraction and addition to unsaturated centres in other molecules (**Scheme 9**).



Scheme 9: Reactions of acyloxyl radicals from photolysis of diacyl peroxide.

The kinetics of decarboxylation of acyloxyl radicals have been directly determined from the first-order decay curve of the visible region absorbance of a number of these radicals generated by LFP of the corresponding diacyl peroxides in tetrachloromethane. The first-order rate constant (k_β) for decarboxylation of benzoyloxyl radicals at 297K in tetrachloromethane is (2.0×10^6 s^{-1})[42]. The strong affinity of the acyloxyl radicals for hydrogen atom donors such as alkanes has also been demonstrated by the measurement of their second-order rate constants for the hydrogen abstraction from a range of substrates. For example, the rate constant (k_{abs}) of the benzoyloxyl radical for its hydrogen abstraction from cyclohexane at 297K in tetrachloromethane is (1.4×10^6 $mol^{-1}dm^3$ s^{-1})[42]. Acyloxyl radicals readily take part in addition reactions with

unsaturated molecules. Kinetic data indicates that compared with the *tert*-butoxyl radical, benzoyloxyl radicals have similar reactivity in hydrogen atom abstractions but it is much more reactive in addition to multiple bonds[41]. The extremely high reported reactivity of benzoyloxyl radicals towards phenol and nitroxide radicals[41] has been explained by a single electron transfer from these substrates to the benzoyloxyl radical.

1.6 Objectives of this Study

The main aims of this study were:

- To make diacyl peroxides using standard methods and to use photo-decomposition of these peroxy compounds as a source of carbon-centred radicals which would have different inherent thermodynamic stabilities

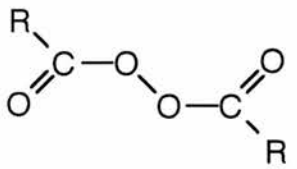
- To perform the reaction of nitric oxide, in the absence of oxygen, with photolytically produced carbon-centred radicals as a novel extension to the Barton reaction

- To effect further functionalisation of the oximes formed from tautomerism of the intermediate C-nitroso compounds containing α– hydrogens

- To attempt to develop this methodology in synthesising C-nitroso derivatives from the coupling of nitric oxide with tertiary carbon-centred radicals

RESULTS AND DISCUSSION

To explore the viability of the strategy outlined in the introduction, symmetrical diacyl peroxides were used as a convenient source of carbon-centred radicals (**Scheme 10**).



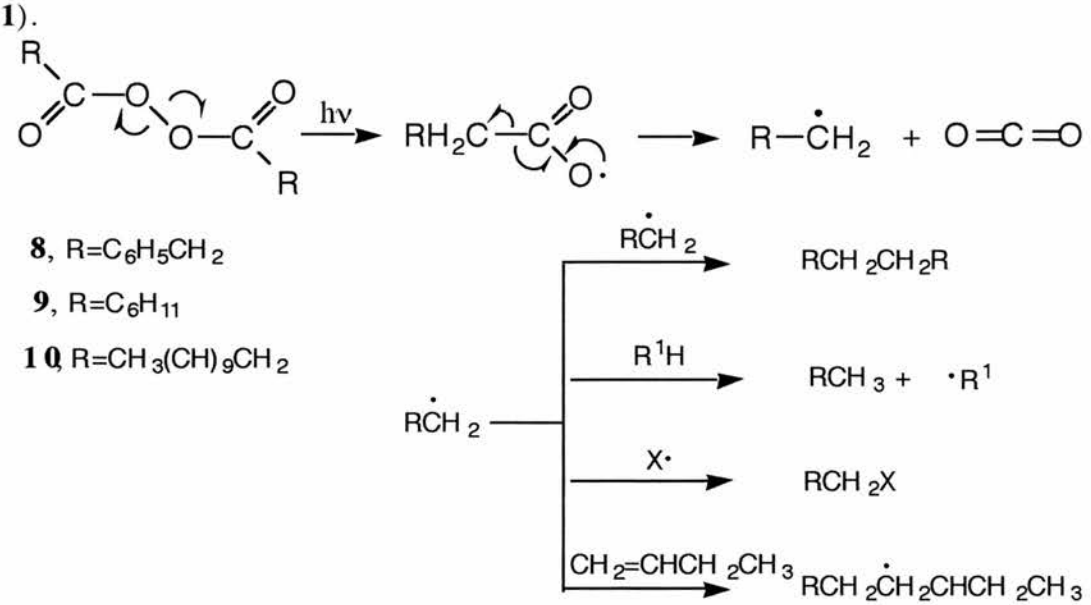
8, R=$C_6H_5CH_2$

9, R=C_6H_{11}

10, R=$CH_3(CH)_9CH_2$

Scheme 10: Diacyl peroxides used as a source of carbon-centred radicals.

Diphenylacetyl peroxide **8** and lauroyl peroxide **10**, a commercially available compound which produce benzyl and primary radicals respectively whereas dicyclohexylcarbonyl peroxide **9**, upon suitable treatment, furnishes secondary radicals. Photolysis of the peroxy bond in these compounds and the attendant decarboxylation is one of the most widely employed methods for creating reactive intermediates[37] (**Scheme 11**).



8, R=$C_6H_5CH_2$

9, R=C_6H_{11}

10, R=$CH_3(CH)_9CH_2$

Scheme 11: Photo-induced generation of a carbon-centred radical from an acyl peroxide and its subsequent reactions.

Depending on the thermodynamic stability of the ensuing radicals , they can participate in a variety of elementary reactions with species already present in the reaction mixture or with those that are introduced externally. Hydrogen atom abstraction from a suitable donor , self-combination , addition to unsaturated centres and combination with other molecules are some of the common reactions that can occur in a medium containing free radicals (**Scheme 13**).

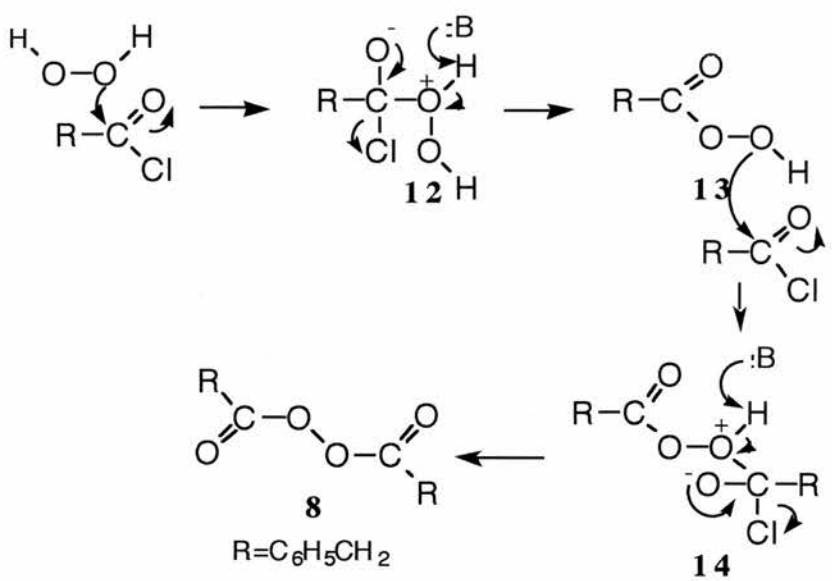
2.1 Preparation of Organic Acyl Peroxides

2.1.1 Diphenylacetyl peroxide

Diphenylacetyl peroxide **8**, was made in a moderate yield (54%) by modified pyridine acylation of hydrogen peroxide[45] from phenylacetyl chloride **11** in the presence of the base as shown in **Equation 12**.

$$\underset{\mathbf{11}}{2C_6H_5CH_2COCl} + H_2O_2 \xrightarrow{C_5H_5N} \underset{\mathbf{8}}{(C_6H_5CH_2COO)_2} + 2HCl \qquad \mathbf{12}$$

The role of the base involves the abstraction of a proton from the intermediates **12** and **14**, formed from the nucleophilic attack of a peroxy oxygen on the electrophilic carbonyl carbon of the acid chloride, as illustrated in **Scheme 12**.



Scheme 12: Action of the base in preparation of a diacyl peroxide with hydrogen peroxide and an acylating agent.

A more general method uses sodium peroxide in combination with acylating agents e.g. acid chlorides and acid anhydrides **Equation 13**.

$$2RCOX + Na_2O_2 \longrightarrow (RCOO)_2 + 2NaX \qquad \mathbf{13}$$

This latter approach dispenses with the use of a base which is necessary in conjunction with the hydrogen peroxide.

Owing to the tendency for thermal decomposition of a number of these peroxides and because of the need to minimise explosive hazards associated with these compounds, they were not isolated. Instead, their safety and storage-life are both increased by using them as dilute solutions. The NMR data for the peroxide **8** showed it to have been synthesised in sufficient purity and so it was used as a starting material for subsequent reactions.

2.1.2 Dicyclohexanecarbonyl peroxide

Dicyclohexanecarbonyl peroxide **9** was made in a modest (45%) yield by the strategy described for the peroxide **8** in section 2.1.1. The starting acid chloride in this case was cyclohexanecarbonyl chloride **15** (**Equation 14**).

$$\underset{\mathbf{15}}{2C_6H_{11}COCl} + H_2O_2 \xrightarrow{C_5H_5N} \underset{\mathbf{9}}{(C_6H_{11}COO)_2} + 2HCl \qquad \mathbf{14}$$

The NMR analysis on the product peroxide **9** showed it to be fit for use in further chemical manipulations. All the peaks from the two cyclohexyl ring hydrogens were in the anticipated regions of the spectrum.

The efficiency of the synthesis of peroxides using this route depends on a number of factors but the effectiveness with which the temperature is controlled below 10°C and the choice of solvent are the two of the most important features[45]. Diethyl ether has been shown to be the best for concentrating aqueous solutions of hydrogen peroxide[46] and it is therefore the ideal medium for development of a homogeneous reaction with this reagent. In this study, the use of benzene as the solvent explains the modest amounts of the products obtained. However, its choice was governed by the fact that the peroxides were to be used for the production of carbon-centred radicals which, in a hydrogen-donor solvent such as diethyl ether, may well have led to undesirable side reactions.

The instability of peroxides can be attributed to their ability for triggering-off uncontrolled chain-reactions via the initially generated acyloxyl radicals from their thermolysis[47]. The condensation of carboxylic acids with hydrogen peroxide in the presence of dicyclohexylcarbodiimide (DCC) is a useful alternative for obtaining diacyl peroxides on a laboratory scale. The preparation of phthaloyl peroxide **17**, using phthalic anhydride[48] **16**, is summarised by **Equation 15**.

$$\underset{\mathbf{16}}{C_6H_4(CO)_2O} \xrightarrow[H_2O_2]{DCC} \underset{\mathbf{17}}{C_6H_4(CO_2)_2} + (C_6H_{11}NH)_2CO \qquad \mathbf{15}$$

The direct use of acids without their prior conversion into acid chlorides and acid anhydrides, the use of a single organic solvent without any need for an acid or a base catalysis and high yields of clean products are some of the advantages offered by this second method.

2.2 Reactions of Nitric Oxide with Photolysed Peroxides

2.2.1 Diphenylacetyl peroxide

Upon treatment with nitric oxide for 4h at room temperature, under photolytic conditions, the benzene solution of diphenylacetyl peroxide **8** produced no evidence of substantial reaction. The ^1H spectrum essentially consisted of unreacted peroxide. However, study of the reaction mixture with GC-MS, a more sensitive technique, revealed the low-level presence of a range of compounds, the structures of which are given in **Table 1**.

Compound	R_t/min.	Name	Structure
17	3.0	Toluene	$C_6H_5CH_3$
18	7.0	Benzaldehyde	C_6H_5CHO
19	9.2	Phenylmethanol	$C_6H_5CH_2OH$
20	11.0	2-Nitrophenol	$(NO_2)C_6H_4(OH)$
21	14.1	Phenylacetic acid	$C_6H_5CH_2CO_2H$
22	15.8	Diphenylmethane	$C_6H_5CH_2C_6H_5$
23	17.0	1,2-diphenylethane	$C_6H_5(CH_2)_2C_6H_5$
24	20.0	Phenylmethyl (phenyl)ethanoate	$C_6H_5CH_2CO_2Bz$

Table 1: The main products from photolysis of diphenylacetyl peroxide and nitric oxide.

In view of the relative concentration of the peroxide **8** and nitric oxide in the reaction mixture, the principal reaction anticipated was that between the initially formed benzyl radical **25** (**Equation 16**) and the reagent to give the nitroso compound **26** which tautomerises to the oxime **27** with the equilibrium lying well in favour of the latter[24] (**Equation 17**).

$$[C_6H_5CH_2CO_2]_2 \xrightarrow[-CO_2]{h\nu} 2C_6H_5\dot{C}H_2 \qquad \mathbf{16}$$

25

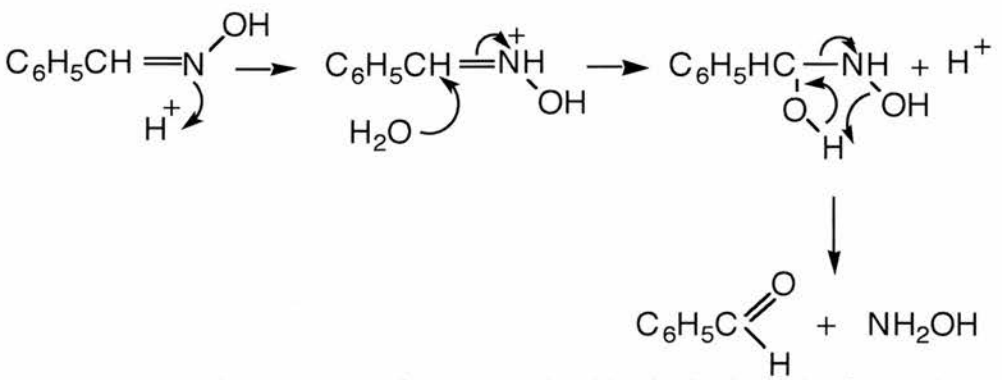
$$C_6H_5\dot{C}H_2 \xrightarrow[\dot{N}O]{h\nu} \underset{\mathbf{26}}{C_6H_5CH_2NO} \rightleftharpoons \underset{\mathbf{27}}{C_6H_5CH{=}NOH} \qquad \mathbf{17}$$

The oxime **27**, however, is highly susceptible to hydrolysis yielding benzaldehyde **18** as indicated in **Equation 18** below.

$$C_6H_5CH{=}NOH + H_2O \longrightarrow \underset{\mathbf{18}}{C_6H_5CHO} + NH_2OH \qquad \mathbf{18}$$

The ready conversion of these primary products **26** and **27**, formed at low levels, to aldehyde **18** helps explain the absence of any substantial quantities of either the nitroso intermediate **26** or its isomeric counterpart **27**. The reaction mixture when analysed by NMR spectroscopy also failed to show the presence of the aldehyde because of its relatively weak concentration.

The mechanistic steps for the hydrolysis of an oxime are well understood[49]. The general sequence involves initial addition of water to the carbon-nitrogen double bond followed by elimination of the nitrogen moiety as shown in **Scheme 13**.



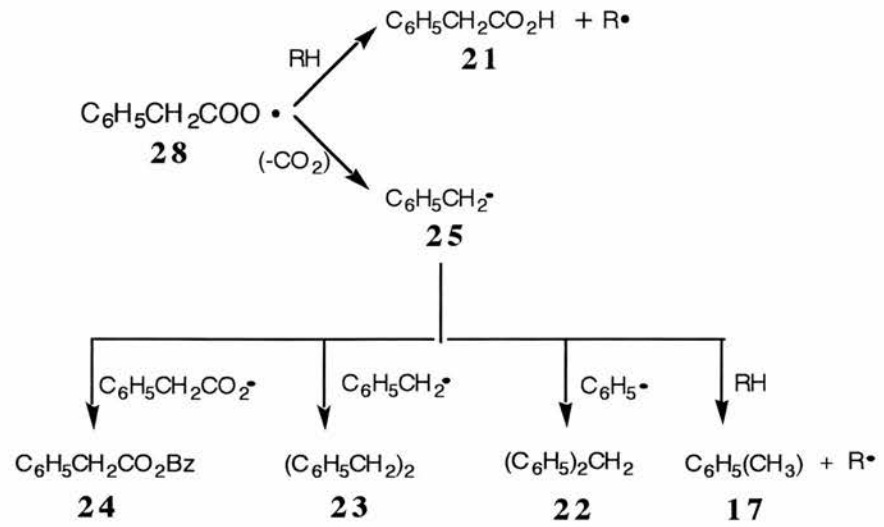
Scheme 13: The sequence of steps involved in the hydrolysis of an oxime.

The initial, relatively unstable, acyloxyl radical **28** is formed from the photolysis of diphenylacetyl peroxide **8** (**Equation 19**).

$$[C_6H_5CH_2CO]_2 \longrightarrow 2C_6H_5CH_2COO\cdot \qquad \mathbf{19}$$

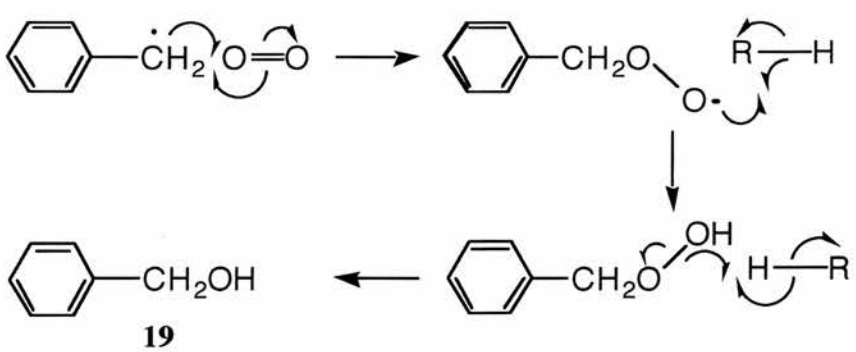
28

Radical **28** can participate in a number of reactions which lead to the array of products detected by GC-MS. These reactions can occur at three levels: unimolecular decarboxylation to give the resonance-stabilised benzyl radical **25**. Secondly, hydrogen-abstraction from the solvent or other suitable donors with the production of phenylacetic acid **21**. Thirdly, the benzyl radicals themselves undergo several reactions e.g. trapping of nitric oxide to furnish the short-lived nitroso compound **26**, abstraction of hydrogen atom, from a suitable donor, to form toluene **17** and the bimolecular combinations with other radicals in the reaction mixture forming diphenylmethane **22**, 1,2-diphenylethane **23** and phenylmethyl (phenyl)ethanoate **24** as depicted in **Scheme 14** below.



Scheme 14: Reactions undergone by initially formed phenylacetoxyl radicals.

The existence of phenylmethanol **19** and 2-nitrophenol **20** as two of the prominent products in the reaction mixture is unexpected. However, nitric oxide was passed into the reaction vessel with a stream of nitrogen gas during the photolysis of the starting peroxide **8**. It is possible that, even under these essentially anaerobic conditions, a trace amount of air entered the system and caused the oxidation of benzyl radical **25** to phenol. **Scheme 15** outlines the steps for the analogous transformation of the benzyl radicals to phenylmethanol **19**. Under these conditions, nitric oxide is oxidised to nitrogen dioxide which, in the presence of moisture, shows a tendency to form nitrous acid and nitric acid. The ready electrophilic nitration of the activated aromatic ring in a molecule such as phenol could account for the presence of 2-nitrophenol **20**.



Scheme 15: Formation of phenylmethanol from photolysed diphenylacetyl peroxide.

The absence of any nitrogen-containing products and a large number of radical combination products implies that nitric oxide either reacts slowly with carbon-centred radicals or that it is present in insufficient concentration. The latter seems more likely because the saturation concentration of nitric oxide in benzene is (*ca* 1.2×10^{-2} mol dm^{-3})[50].

2.2.2 Dicyclohexanecarbonyl peroxide

When a deaerated benzene solution of dicyclohexanecarbonyl peroxide was treated with nitric oxide, under photolytic conditions, NMR spectroscopy on a sample of the reaction mixture indicated little or no conversion of the starting material. However, GC-MS performed on the sample showed that a small fraction of the peroxide **9** had been converted to a mixture of products which are reported in **Table 2** below. Several additional trace (minor) compounds were also present.

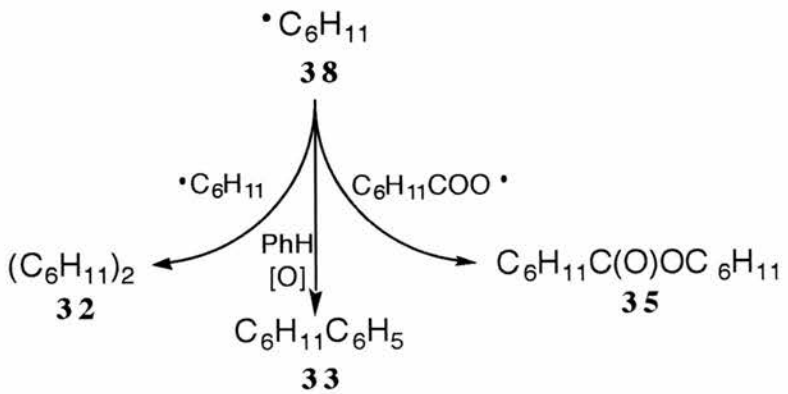
Compound	R_t/min.	Name	Structure
29	4.1	Cyclohexanone	$C_6H_{10}O$
30	5.1	Cyclohexanol	$C_6H_{11}OH$
31	12.6	Cyclohexanecarboxylic acid	$C_6H_{11}CO_2H$
32	13.8	Bicyclohexyl	$(C_6H_{11})_2$
33	14.0	Cyclohexylbenzene	$C_6H_{11}(C_6H_5)$
34	14.7	2-Methyl-1,1-bicyclohexyl	$C_6H_{11}[C_6H_{10}(CH_3)]$
35	17.6	Cyclohexyl cyclohexane-carboxylate	$(C_6H_{11})_2CO_2$

Table 2: Products of reaction between dicyclohexanecarbonyl peroxide and nitric oxide.

The products represented in **Table 2** can be categorised according to their origins in this reaction. Cyclohexanone **29** is the only compound derived indirectly from the interaction of the reagent gas, nitric oxide, and the starting peroxide **9** in the manner summarised by **Equation 20**.

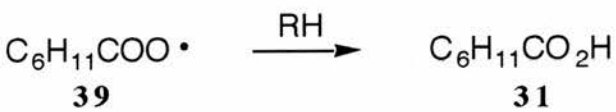
$$(C_6H_{11}CO)_2O_2 \xrightarrow[NO]{h\nu} \underset{\mathbf{36}}{C_6H_{11}NO} \rightleftharpoons \underset{\mathbf{37}}{C_6H_{11}NOH} \xrightarrow{H_2O} C_6H_{10}O \qquad \mathbf{20}$$

In a comparable sequence of changes to those discussed for the reaction of phenylacetyl peroxide and nitric oxide, the initially formed nitroso derivative **36** gives the more stable ketone **29** via the intermediate oxime **37**. **Scheme 16** shows the formation of the second class of products *viz* those obtained from the combination between the most prevalent, and therefore the most stable, secondary radical **38**, from the photolysis of the peroxide **9**, and other radical species.

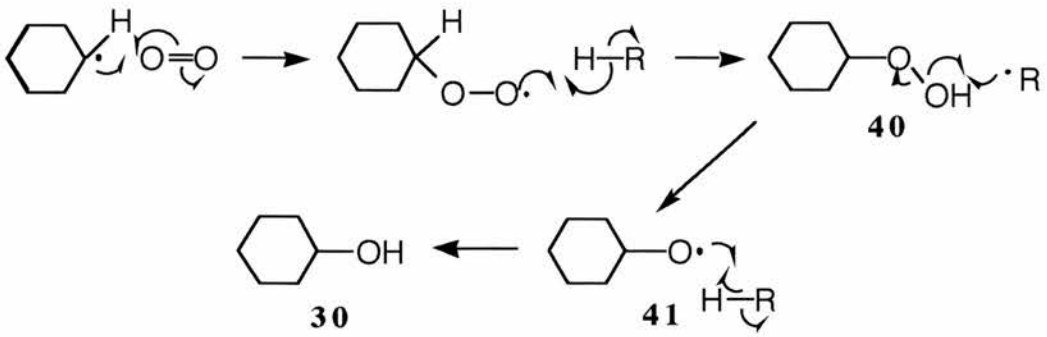


Scheme 16: Reactions of cyclohexyl radical with carbon and oxygen-centred radicals.

Although prone to ready fragmentation and concomitant release of carbon dioxide, the acyloxyl radical **39** has a sufficiently long life-span to act as a precursor for more stable products by either direct combination or by hydrogen atom abstraction from a suitable donor as shown below.



The final group of substances result from the presence of trace quantities of air and cyclohexanecarbonyl chloride in the reaction mixture. The latter was used in the preparation of the peroxide **9** and so it must have remained as a minute contaminant in the former. **Scheme 17** indicates the formation of cyclohexanol **30** from the oxidation of cyclohexyl radical **38**.



Scheme 17: Oxidation of cyclohexyl radical in the formation of cyclohexanol.

Normally, compounds derived from the oxygen-centred radical **41**, in **Scheme 17** above, could be anticipated. However, the complete absence of the peroxide **42** and the ether **43** points to the fact that the former is unstable under the reaction conditions and reverts quickly to the parent oxygen-centred radical and that the rate of hydrogen abstraction by these radicals is significantly faster than their speed of combination with cyclohexyl radicals **38**.

42 **43**

Absence of nitrogen containing species is also striking, possibly, owing to the low dissolved concentration of the reagent gas[50].

2.2.3 Lauroyl peroxide

A deoxygenated solution of lauroyl peroxide **10** in benzene, saturated with nitric oxide, upon photolysis gave a reaction mixture which by ^{1}H NMR spectroscopy showed no evidence of new substances and the major peaks in the spectrum agreed with those expected from peroxide starting material only. GC-MS analysis on a sample of the reaction mixture indicated a small-scale reaction between the peroxide and nitric oxide leading to a complex mixture of products which are shown in **Table 3**.

Compound	R_t/min.	Name	Structure
44	10.4	Undec-1-ene	$CH_3(CH_2)_8CH=CH_2$
45	10.7	Undecane	$CH_3(CH_2)_9CH_3$
46	14.0	Undecanal	$CH_3(CH_2)_9CHO$
47	15.1	Undecanol	$CH_3(CH_2)_9CH_2OH$
48	17.8	Dodecanoic acid	$CH_3(CH_2)_{10}CO_2H$
49	20.0	Undecylbenzene	$C_6H_5(CH_2)_{10}CH_3$

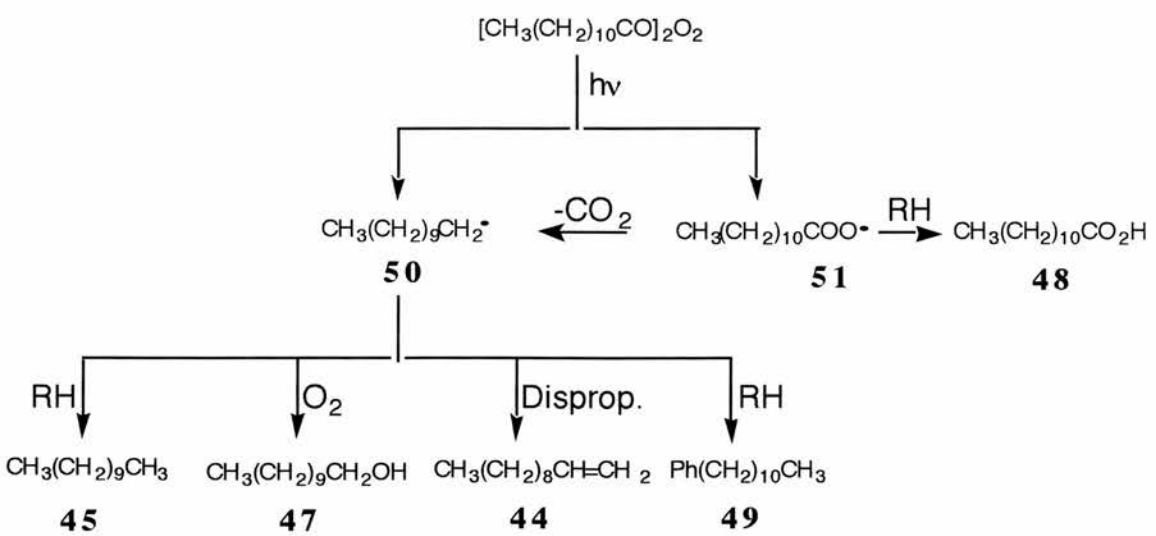
Table 3: Products from the reaction of lauroyl peroxide and nitric oxide.

In common with the reaction of diphenylacetyl peroxide with nitric oxide, lauroyl peroxide gives an aldehyde **46** as the only product resulting from the photochemical reaction between the peroxide and nitric oxide in reactions represented by **Equations 21** and **22.**

$$\underset{\mathbf{10}}{[CH_3(CH_2)_{10}CO]_2O_2} \xrightarrow[NO]{h\nu} 2CH_3(CH_2)_9CH_2N{=}O \qquad \mathbf{21}$$

$$CH_3(CH_2)_9CH_2N{=}O \rightleftharpoons CH_3(CH_2)_9CH{=}NOH \xrightarrow{H_2O} \underset{\mathbf{46}}{CH_3(CH_2)_9CHO} \qquad \mathbf{22}$$

The compounds detected in the reaction, stem from two key intermediates **50** and **51** obtained from the photolysis of the peroxide **10**. **Scheme 18** outlines the various routes these intermediates follow that eventually lead to the observed products.



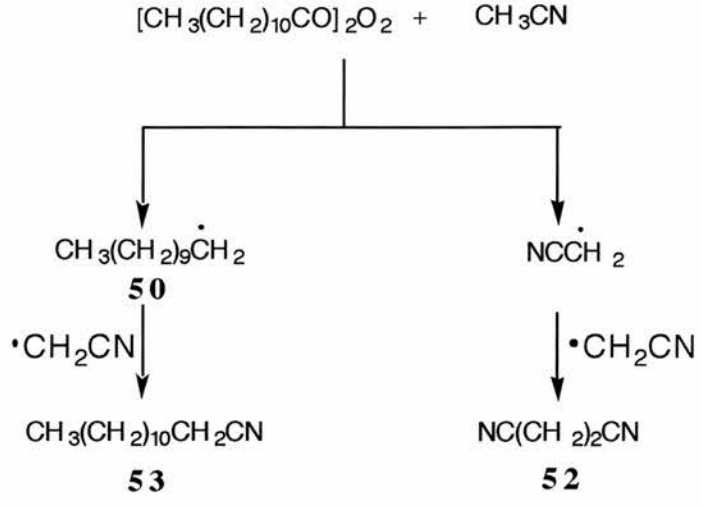
Scheme 18: Products from photolysis of lauroyl peroxide and nitric oxide in benzene.

Benzene was replaced by acetonitrile as a solvent so as to minimise the influence of the reaction medium in restricting other pathways open to the initially formed intermediates from photolysis of the peroxide **10**. Under similar conditions, GC-MS of the photolysed material gave a wider than the expected range of products which are listed in **Table 4**.

Compound	R_t/min.	Name	Structure
52	7.3	Butanedinitrile	$NC(CH_2)_2CN$
44	10.4	Undec-1-ene	$CH_3(CH_2)_8CH=CH_2$
45	10.7	Undecane	$CH_3(CH_2)_9CH_3$
53	13.5	Dodecanenitrile	$CH_3(CH_2)_{10}CH_2CN$
46	14.0	Undecanal	$CH_3(CH_2)_9CHO$
47	15.1	Undecanol	$CH_3(CH_2)_9CH_2OH$
54	16.7	Undecanone	$CH_3(CH_2)_8COCH_3$
55	17.7	Tridecanenitrile	$CH_3(CH_2)_{11}CH_2CN$
48	18.1	Dodecanoic acid	$CH_3(CH_2)_{10}CO_2H$

Table 4: Products from the reaction of lauroyl peroxide with nitric oxide in acetonitrile.

As can be seen from **Table 4**, majority of the additional products, in the presence of acetonitrile, result from the solvent's participation in the reaction in a number of ways indicated by **Scheme 19**.

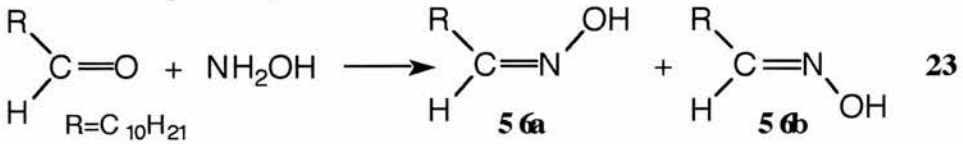


Scheme 19: Solvent participation in the photolytic reaction of nitric oxide and lauroyl peroxide in acetonitrile.

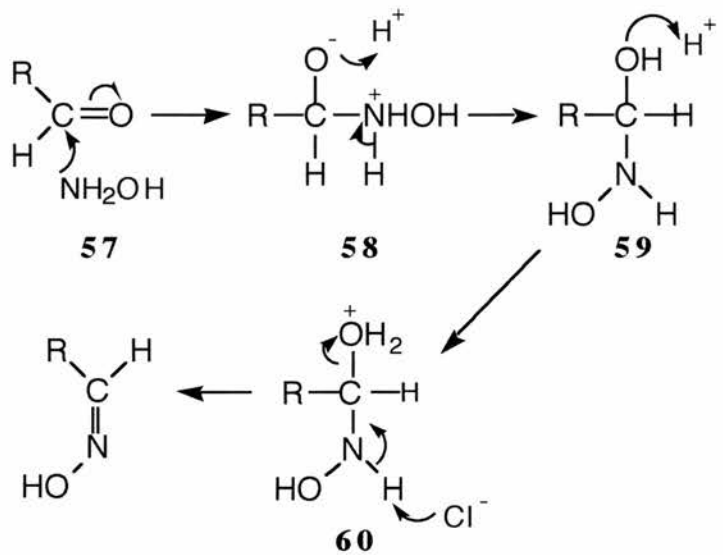
2.3 Undecanal Oxime

2.3.1 Preparation of undecanal oxime

Undecanal oxime was made in order to photolyse it in the presence of nitric oxide and show that this compound does not form carbon-centred radicals and, as such, should have little or no affinity for the reagent gas. Undecanal **46** was oximated using a modified method suggested by Doyle and Mungall[51] in an efficient reaction, **Equation 23**, giving 92% of the product as an isomeric mixture of *syn* **56a** and *anti* **56b** in an approx. 2:1 ratio respectively.



Steric considerations dictate that the *anti* isomer should be formed in a higher quantity. This departure from the expected result may be explained by examining the final mechanistic step, in **Scheme 20**, that leads to the product.



Scheme 20: Mechanism for oximation of an aldehyde.

It involves deprotonation and loss of water from intermediate **60**. If the proton was in close proximity of the bulky R group, which would be the case if this intermediate adopted an *anti*-type conformation, the approach of a suitable acceptor would be hindered thereby reducing the rate of deprotonation compared to the converse situation.

2.3.2 Photolysis of undecanal oxime in the presence of nitric oxide

Undecanal oxime **56**, saturated with nitric oxide, was photolysed in order to confirm that the only product resulting from this starting material is the aldehyde undecanal **46**. The products from a similar treatment of the peroxides owe their origin to either the initially formed radicals from the decomposition of these peroxy compounds or to their subsequent reactions leading to the intermediates that ultimately give stable species.

^{1}H NMR spectroscopy on the reaction mixture containing the oxime **56** and nitric oxide showed the presence of *syn* and *anti* isomers of the oxime and, as such, indicated only a low-level conversion of the reactant under these conditions. GC-MS on the photolysed sample, as expected, detected one product only *viz* undecanal which, as explained earlier, forms upon hydrolysis of the oxime and not owing to any photochemical process.

2.3.3 Hydrolysis of undecanal oxime derived from photolysis of lauroyl peroxide

The concentrated reaction mixture from photolysis of lauroyl peroxide **10**, containing amongst other products undecanal **46**, was hydrolysed using nitrous acid in the fashion carried out by Barton[14a]. The same range of products were identified by GC-MS as those resulting from the reaction of lauroyl peroxide with nitric oxide in benzene. Significantly, there was a substantial increase in the concentration of the aldehyde **46**. A plausible explanation for this observation is that under the relatively high injector temperature of the GC (200°C) the oxime does not survive and undergoes ready hydrolysis to give the aldehyde. However, when an already hydrolysed sample of a solution containing the oxime was injected into the GC-MS, no change occurred and the aldehyde is detected at elevated levels.

CONCLUSIONS

- Photodecomposition of a diacyl peroxide with 250-350nm light in an inert solvent is a well established method for generating alkyl radicals[37]. In the current study, the profile of the products achieved in the photolytic reaction of each of the peroxides, in the presence of nitric oxide, suggests that such carbon-centred radicals are indeed the initially formed species.

- The stabilities of the carbon-centred radicals obtained from the three peroxides were expected to decrease in the order indicated below.

$$\underset{\mathbf{25}}{C_6H_5\dot{C}H_2} > \underset{\mathbf{38}}{\dot{C}_6H_{11}} > \underset{\mathbf{50}}{CH_3(CH_2)_9\dot{C}H_2}$$

This asertion is supported by the increase in the values for the rate of decarboxylation with greater radical-stabilizing ability of the alkyl radical formed (aryl < primary alkyl < secondary alkyl ≈ benzyl < tertiary alkyl)[42]. This trend is the same as for decarbonylation of the acyl radical which, however, is 10^6 times more sensitive to the structural effect than the decarboxylation of acyloxyl radicals. This data reflects the higher extrusibility of CO_2 than that of CO[52-54].

All three peroxides yielded carbonyl compounds. These were the stable products derived from the interaction between the carbon-centred radicals and the most prevalent species, other than the solvent, the reagent gas nitric oxide.

- The extent of the reaction between the radicals (benzyl radical **25**, the cyclohexyl radical **38** and the undecyl radical **50**) and nitric oxide was disappointingly low and this does not permit a meaningful comparison, based on product analysis, in the verification of the different reactivities of these radicals towards nitric oxide. The low solubility of nitric oxide[50] in the reaction media used and/or insufficient photolysis could account for these observations.

- In all cases, a significant array of the products in the reaction mixture resulted from the combinations between the initially formed photodecomposition radicals, orginating from the peroxides, and those derived from the solvent.

- Apart from a trace amount of 2-nitrophenol, no other substance was detected which could be regarded as a derivative of nitric oxide's action on the starting peroxides.

- The formation of the intermediate oxime was confirmed in the case of lauroyl peroxide **10** by the fact that there was a sharp increase in the amount of undecanal **46** when a previously hydrolysed sample of the photolysed reaction mixture was injected into the GC-MS instrument

- Use of a more inert reaction medium e.g. pentane may have reduced the solvent-derived products thereby giving a greater scope for the initially generated carbon-centred radicals to trap nitric oxide. However, solubility problems connected with nitric oxide discouraged this approach. The peroxides were found to be only sparingly soluble in benzene and even less so in acetonitrile and other similar solvents.

- Solubility of nitric oxide in benzene and acetonitrile is known[50] and, therefore, the peroxides were used in quantities that ensured several fold excess of the reagent gas.

- Although reasonable steps were taken to set up an air-free system for the reaction of the peroxides with nitric oxide, because of the prolonged reaction times, it is quite likely that a small quantity of air may have entered the system, through seepage, leading to the formation of oxidation products.

EXPERIMENTAL

2.4 Preparation of Organic Peroxides

Unless commercially available, the required peroxides, diphenylacetyl peroxide and dicyclohexanecarbonyl peroxide, were prepared by established procedures which acylate corresponding acid chlorides using hydrogen peroxide in the presence of an equimolar amount of base in an aprotic solvent. Owing to the thermal instability of some peroxides, the synthesised compounds were not isolated from their respective reaction mixtures except on a small scale for NMR purposes. Instead, the work-up procedures ensured complete removal of the excess reagents and the products, dissolved in the appropriate solvents, were employed in subsequent reactions.

2.5 Reactions of Nitric Oxide with Photolysed Peroxides

Usually the reactions of nitric oxide were performed using benzene as the solvent. Before allowing any contact of the reagent gas with the peroxides, all solutions and the entire system used for carrying out the reactions were degassed with nitrogen for approx. 1h. This procedure ensured the removal of all oxygen, present as dissolved species in solutions or contained in the air occupying the whole system. The amounts of reactants used for the generation of pure nitric oxide allowed continous supply of it, at a steady rate, for approx. 4h.

The reactions were carried out, under high dilution, in a two-necked cylindrical vessel especially adapted for performing photochemistry. One of the necks of the reaction container was connected to the supply of dry nitric oxide via a capillary tube which bubbled it through the reaction mixture under continuous stirring. The second neck of the reaction vessel led the excess reagent gas into an air-lock consisting of paraffin oil. Before commencing the photolysis , the reaction mixture was saturated with nitric oxide which continued to be passed through the reactants for the duration of the

experiment. The photolysis was carried out using medium pressure 125 Watt UV lamp, housed in a quartz cavity within the reaction vessel, surrounded by a jacket of continuously circulating cold water so as to minimise the heating effect from it. At the end of the reaction, residual nitric oxide was swept out of the system by nitrogen gas. The product identification was done by GC-MS and/or NMR spectrocopy.

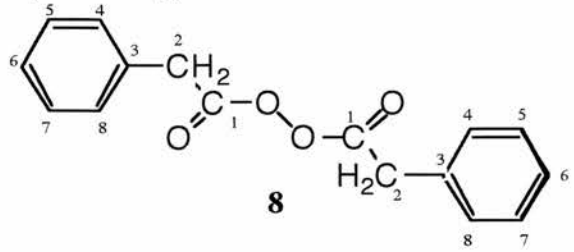
^{1}H NMR spectra were obtained on Varian (Gemini 200MHz) and/or Bruker (300MHz) spectrometers. All samples were dissolved in deuteriochloroform and tetramethylsilane was used as an internal standard. ^{13}C NMR were recorded on the Bruker (75MHz) instrument. GC-MS analysis was carried out with a Finnigan Incos 50 quadrupole mass spectrometer interfaced with a Hewlett-Packard HP 5890 capillary gas chromatograph fitted with a column coated with methylsilicone as the stationary phase. Samples for analysis by this technique were injected as concentrated reaction mixtures.

2.1 Preparation of Organic Peroxides

2.1.1 Diphenylacetyl peroxide

Phenylacetyl chloride **11** (1.55 g, 0.01 mol) was weighed into a two-necked round-bottomed flask containing a magnetic stirrer and fitted with a dropping funnel and a thermometer. Benzene (100 cm^3) was introduced to the flask with continuous stirring and cooling of the reaction mixture to ~5°C. Hydrogen peroxide (0.76 g, 27.5% solution, 0.006 mol), pyridine (0.83g, 0.011 mol), diluted with benzene (5 cm^3), were added to the reaction vessel, over a period of 15 mins., while maintaining the temperature in the 5-10°C range. Once the addition of the oxidant and the base, to the acid chloride, was complete the reaction mixture was allowed to reach ambient temperature and stirring was continued for 1.5h. The benzene solution was first washed with 2M hydrochloric acid (3x25 cm^3) then with 5% aqueous potassium bicarbonate (3x25 cm^3) and finally with water (3x25 cm^3). The solution containing the product was dried over anhydrous sodium sulphate. The dessicant was removed by suction filtration and the volume of the resulting solution was measured. A sample (10 cm^3) of this solution was evaporated to

dryness for determination of the yield of diphenylacetyl peroxide **8** (0.73 g, 54%) which was analysed by NMR spectroscopy.

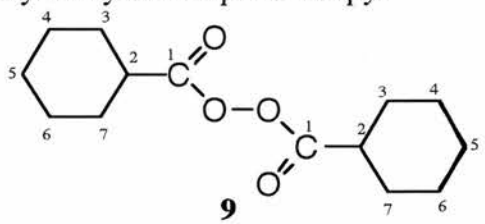


8

δ_H(300MHz, $CDCl_3$) 3.70(4H, s, 2xCH_2-2), 7.20(4H, m, 2xCH-4, 2xCH-8), 7.32(6H, m, 2xCH-5, 2xCH-7, 2xCH-6); δ_C(75MHz, $CDCl_3$) 42.0(2xC-2), 127.1(2xC-3), 127.3(2xC-6), 127.6(2xC-5, 2xC-7), 129.4(2xC-4, 2xC-8), 167.0(2xC-1).

2.1.2 Dicyclohexanecarbonyl peroxide

Cyclohexanecarbonyl chloride **15** (1.47 g, 0.01 mol) was added to benzene (100 cm^3) contained in a two-necked flask fitted with a thermometer and a dropping funnel. Hydrogen peroxide (0.76 g, 27.5% solution, 0.006 mol), pyridine (0.83 g, 0.011 mol), diluted with benzene (5 cm^3), was dropped into the reaction flask, over 15mins., with continuous stirring and controlling the temperature of the reaction between 5-10°C. The contents of the flask were allowed to warm up to room temperature and stirred for 1.5h. The isolation of the product involved sequential washings of the benzene solution with 2M hydrochloric acid (3x25 cm^3), 5% aqueous potassium bicarbonate (3x25 cm^3) and water (3x25 cm^3). The final solution was treated with anhydrous sodium sulphate which was filtered off and the total volume of this solution was determined. Dicyclohexanecarbonyl peroxide **9** (0.57 g, 45%) was obtained in the manner described for the peroxide **8** and analysed by NMR spectroscopy.



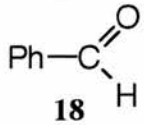
9

δ_H(300MHz, $CDCl_3$) 1.30(4H, m, 2xCH_2-5), 1.45(8H, m, 2xCH_2-4, 2xCH_2-6), 1.80(8H, m, 2xCH_2-3, 2xCH_2-7), 2.30(2H, m, 2xCH-2); δ_C(75MHz, $CDCl_3$) 23.7(2xC-5), 25.4(2xC-4 , 2xC-6), 28.8(2xC-3, 2xC-7), 31.6(2xC-2), 178.3(2xC-1).

2.2 Reactions of Nitric Oxide with Photolysed Peroxides

2.2.1 Diphenylacetyl peroxide

Diphenylacetyl peroxide **8** (0.30 g, 1.11 mmol), contained in the apparatus described in section 2.5, was diluted with benzene (200 cm^3). Following the deaeration of its stirred mixture with nitrogen gas for 1h, nitric oxide (1h) was passed through it. It was photolysed for 4h while a mixture of nitrogen/nitric oxide was passed through the reactants. Upon termination of the reaction nitrogen gas alone was passed through the system in order to remove the unreacted reagent. The resulting solution was partially concentrated and examined by GC-MS. A mixture of the authentic samples of some of the products was subjected to GC-MS and the retention time and mass spectrum of each component were compared with the equivalent data for each compound from the reaction mixture. The solvent was completely removed from a small sample of the final solution and the residue was studied by NMR spectroscopy.



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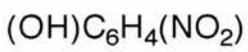

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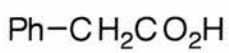

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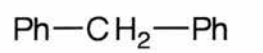

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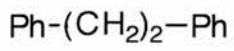

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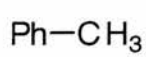

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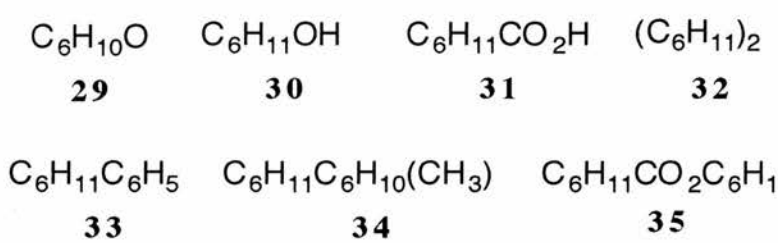
24

GC-MS peak no. 165, R_t=3.0 min., toluene **17**, m/z(relative intensity %) 92(M^+, 92), 91(100), 78(34), 65(16), 63(15), 51(20), 39(26), 27(6); peak no. 378, R_t=7.0 min., benzaldehyde **18**, m/z(relative intensity %) 106(M^+, 68), 105(77), 77(100), 51(67), 39(15), 29(11); peak no. 498, R_t=9.2 min., phenylmethanol **19**, m/z(relative intensity %) 108(M^+, 58), 107(43), 79(100), 91(17), 77(78), 51(42), 39(28), 28(29); peak no. 591, R_t=11.0 min., 2-nitrophenol **20** m/z(relative intensity %) 139(34), 109(19), 81(26), 65(41), 63(36), 53(20), 51(20), 39(65), 30(17), 28(42); peak no. 759, R_t=14.1 min., phenylacetic acid **21**, m/z(relative intensity %) 136(M^+, 17), 92(20), 91(100),

65(23), 39(18), 28(27); peak no. 854, R_t=15.8 min., diphenylmethane **22**, m/z(relative intensity %) 168(M^+, 88), 167(100), 165(40), 152(32), 115(15), 91(69), 83(26), 77(15), 65(44), 51(39), 39(47); peak no. 919, R_t=17.0 min., 1,2-diphenylethane **23**, m/z(relative intensity %) 182(M^+, 8), 91(100), 65(22), 39(40); peak no. 1100, R_t=20.0 min., phenylmethyl (phenyl)ethanoate **24**, m/z(relative intensity %) 226(M^+, 1), 91(100), 77(4), 65(21), 39(10).

2.2.2 Dicyclohexanecarbonyl peroxide

Dicyclohexanecarbonyl peroxide **9** (0.25 g, 1.0 mmol) was dissolved in benzene (250 cm^3). This mixture, contained in the apparatus described in section 2.5, was degassed with nitrogen gas for 1h. After saturation with nitric oxide for 1h, the continuously stirred contents of the reaction vessel were photolysed for 4h while a stream of nitric oxide was passed through them. At the end of the reaction, the unreacted nitric oxide was flushed out of the system with nitrogen gas and the resulting solution was concentrated and analysed by GC-MS. A sample was completely evaporated and the residue was subjected to NMR spectroscopy.



$C_6H_{10}O$ **29** $C_6H_{11}OH$ **30** $C_6H_{11}CO_2H$ **31** $(C_6H_{11})_2$ **32**

$C_6H_{11}C_6H_5$ **33** $C_6H_{11}C_6H_{10}(CH_3)$ **34** $C_6H_{11}CO_2C_6H_{11}$ **35**

GC-MS peak no. 258, R_t=4.1 min. cyclohexanone **29**, m/z(relative intensity %) 98(M^+, 24), 69(24), 55(92), 42(100), 41(54), 39(47), 27(47); peak no. 281, R_t=5.1 min., cyclohexanol **30**, m/z(relative intensity %) 100(M^+, 2), 82(34), 67(27), 57(100), 44(39), 41(48), 39(42), 29(49), 27(44); peak no. 683, R_t=12.6 min., cyclohexanecarboxylic acid **31**, m/z(relative intensity %) 128(M^+, 12), 110(10), 99(12), 83(30), 70(51), 68(27), 55(100), 41(81), 39(62), 29(29), 27(49); peak no. 754, R_t=13.8 min., bicyclohexyl **32**, m/z(relative intensity %) 166(M^+, 9), 82(100), 67(76), 55(97), 41(89), 29(26); peak no. 759, R_t=14.0 min., Cyclohexylbenzene **33**, m/z(relative intensity %) 160(M^+, 31), 117(62), 104(100), 91(77), 78(22), 65(16),

55(18), 41(29), 39(35), 27(21); peak no. 796, R_t=14.7 min, 2-methyl-1,1-bicyclohexyl **34**, m/z(relative intensity %) 180(M^+, 7), 97(37), 82(42), 76(41), 55(100), 41(65), 29(20); peak no. 958, R_r=17.6 min., cyclohexyl cyclohexanecarboxylate **35**, m/z(relative intensity %) 129(53), 83(77), 67(34), 55(100), 41(96), 29(31); in addition, several unidentified components were observed.

2.2.3 Lauroyl peroxide

Lauroyl peroxide **10** (0.64 g, 1.60 mmol) in benzene (280 cm^3), stirred in the cylindrical vessel described in section 2.5, had air removed from the system by flushing it out with nitrogen gas. The reaction mixture was saturated with nitric oxide (1h) before photolysing it for 4h. The final solution was partially evaporated and subjected to GC-MS analysis. Another sample of it was completely evaporated and assessed by NMR spectroscopy. In order to confirm the presence of undecanal, undecanol and dodecanoic acid in the reaction mixture, a mixture of authentic samples of these compounds was analysed with GC-MS under identical conditions. The retention times and the mass spectra of these were compared with the equivalent data from the compounds in the reaction mixture.

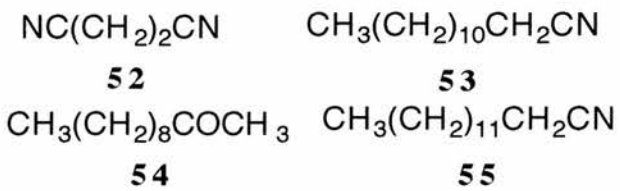
$CH_3(CH_2)_8CH=CH_2$ **44** $CH_3(CH_2)_9CH_3$ **45** $CH_3(CH_2)_9CHO$ **46**

$CH_3(CH_2)_9CH_2OH$ **47** $CH_3(CH_2)_{10}CO_2H$ **48** $C_6H_5(CH_2)_{10}CH_3$ **49**

GC-MS peak no. 567, R_t=10.4 min., undec-1-ene **44**, m/z(relative intensity %) 154(M^+, 2), 97(16), 83(28), 69(50), 55(75), 43(81), 41(100), 29(52), 27(40); peak no. 597, R_t=10.7 min., undecane **45**, m/z(relative intensity %) 156(M^+, 2), 85(18), 71(37), 57(89), 43(100), 41(51), 29(35), 27(23); peak no. 758, R_t=14.0 min., undecanal **46**, m/z(relative intensity %) 96(15), 82(30), 71(27), 69(29), 57(63), 55(53), 43(57), 41(100), 39(26), 29(67), 27(43); peak no. 817, R_t=15.1 min., undecanol **47**, m/z(relative intensity %) 111(8), 97(23), 83(33), 69(54), 55(80), 43(91), 41(100), 29(61), 27(38); peak no. 967, R_t=17.8 min., dodecanoic acid **48**, m/z(relative intensity %) 200(M^+, 3), 129(15), 85(18), 73(63), 69(20), 60(70), 55(53), 43(68), 41(65),

29(43); peak no. 1085, R_t=20.0 min., undecylbenzene **49**, m/z(relative intensity %) 232(M^+, 4), 133(6), 106(9), 92(100), 91(76), 57(12), 43(26), 41(25), 28(17).

The above experiment was repeated in acetonitrile in an attempt to restrict the participation of benzene-derived species in the reaction. Lauroyl peroxide **10** (0.76 g, 1.90 mmol) was stirred in acetonitrile (500 cm^3) maintained at 30°C in order to achieve complete dissolution of the peroxide. Having deaerated the entire apparatus with nitrogen gas (1h), and introduced the nitric oxide, the reaction mixture was photolysed for 4h ensuring that the temperature did not rise abve 30°C. Following the removal of the residual nitric oxide by flushing the system with nitrogen gas (30 mins.), the reaction mixture was concentrated in order to use GC-MS for product identification.



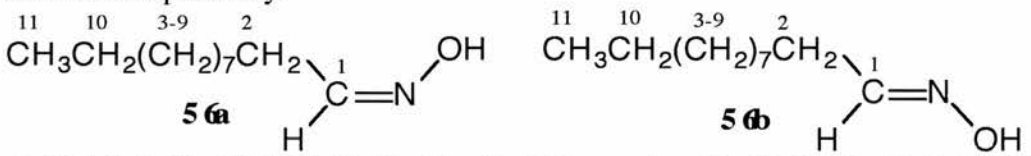
GC-MS peak no. 390, R_t=7.3 min., butanedinitrile **52**, m/z(relative intensity %) 80(M^+,13), 79(35), 53(100), 40(71), 28(28), 26(32); peak no. 567, R_t=10.4 min., undec-1-ene **44**, m/z(relative intensity %) 154(M^+, 1), 97(11), 83(22), 70(39), 55(68), 43(73), 41(100), 39(35); peak no. 579, R_t=10.7 min., undecane **45**, m/z(relative intensity %) 156(M^+, 1), 85(16), 71(33), 57(83), 43(100), 41(62), 29(40), 27(25); peak no. 732, R_t=13.5 min., dodecanenitrile **53**, m/z(relative intensity %) 110(9), 96(12), 82(16), 69(14), 57(16), 55(19), 43(40), 41(47), 39(16), 28(39); peak no. 757, R_t=14.0 min., undecanal **46**, m/z(relative intensity %) 96(13), 82(42), 71(24), 69(32), 57(60), 55(57), 43(95), 41(100), 39(28), 29(81), 27(49); peak no. 817, R_t=15.1 min., undecanol **47**, m/z(relative intensity %) 111(4), 97(14), 83(24), 69(40), 55(69), 43(81), 41(100), 39(21), 29(59), 27(34); peak no. 901, R_t=16.7 min., undecanone **54**, m/z(relative intensity %) 110(8), 96(15), 82(33), 71(24), 58(60), 43(100), 41(70), 29(42); peak no. 962, R_t=17.7 min., tridecanenitrile **55**, m/z(relative intensity %) 124(10), 110(20), 97(27), 82(25), 69(22), 55(37), 43(66), 41(100), 29(54); peak no.

983, R_t=18.1 min., dodecanoic acid **48**, m/z(relative intensity %) 200(M^+, 3), 129(16), 85(19), 73(78), 69(24), 60(87), 55(58), 43(88), 41(100), 29(64).

2.3 Preparation of an Oxime

2.3.1 Undecanal oxime

Undecanal **46** (5.10 g, 0.03 mol) was stirred with water (15 cm^3). This solution was treated with aqueous sodium hydroxide (10 cm^3, 10% w/v). Hydroxylamine hydrochloride (2.43 g, 0.035 mol) was added to the reaction flask followed by gradual introduction of ethanol (20 cm^3) which achieved the complete dissolution of the aldehyde. The reaction mixture was heated under reflux for 30 min. and upon cooling, initially, to room temperature and subsequently to 0°C gave a white solid which was filtered off under suction and washed with ice-cold water (3x10 cm^3). The solid was purified by recrystallisation from dry diethyl ether and characterised using NMR spectroscopy, microanalysis, MS and GC-MS. The undecanal oxime **56** was obtained as a white crystalline solid (5.11 g, 92%) mp 59.5-64.5°C in a 2:1 *syn* **56a** :*anti* **56b** isomeric ratio respectively.



56a **56b**

Found: C, 71.6; H, 13.0; N, 7.4%; $C_{11}H_{23}NO$ requires C, 71.3; H, 12.5; N, 7.6%; δ_H(300MHz, $CDCl_3$) 0.91(3H, t, J=6.7, CH_3-11, *syn/anti*), 1.28(14H, m, 2x$(CH_2)_7$-3-9, *syn/anti*), 1.51(2H, m, J=6.8, CH_2-10, *syn/anti*), 2.20(2H, q, CH_2-2, *anti*), 2.40(2H, q, CH_2-2, *syn*), 6.75(1H, t, CH-1, *anti*), 7.43(1H, t, CH-1, *syn*); δ_C(75MHz, $CDCl_3$) 14.1(CH_3, C-11, *syn/anti*), 22.7(CH_2, C-10, *syn/anti*), 25.0(CH_2, C-2, *anti*), 26.1(CH_2, C-4, *syn/anti)*, 26.6(CH_2, C-3, *syn/anti*), 29.1(CH_2, C-9, *syn/anti*) 29.3(CH_2, C-8, *syn/anti*) 29.4(CH_2, C-7, *syn/anti*) 29.5(CH_2, C-6, *syn/anti)*, 29.6(CH_2, C-5, *syn/anti)*, 31.9(C-2, *syn*), 152.4(C-1, *anti*), 153.0(C-1, *syn*); GC-MS peak no. 821, R_t=15.1 min., either *syn*- or *anti*- undecanal oxime **56**, m/z(relative

intensity %) 124(11), 110(19), 96(29), 82(31), 69(24), 55(34), 41(100), 29(59), 43(52), 27(49); peak no. 916, R_t=17.0 min., either *syn*- or *anti*- undecanal oxime **56**, m/z(relative intensity %) 124(7), 110(10), 96(16), 82(20), 69(20), 59(100), 55(37), 41(94), 29(64), 43(91), 27(46); HRMS; found M^+, 185.1776; $C_{11}H_{23}NO$ requires M^+, 185.1779.

2.3 Photolysis of an Oxime in the Presence of Nitric oxide

2.3.2 Reaction of undecanal oxime with nitric oxide

Undecanal oxime **56** (185.0 mg, 1.0 mmol) in benzene (250 cm^3) was strirred in the photolysis setup described in section 2.5. After removal of air from the entire system with nitrogen gas (1h), nitric oxide was passed through the reaction vessel for 30 min. Photolysis was performed for 4h while the reagent gas was slowly purged through the reaction mixture. Upon conclusion of the reaction, excess nitric oxide was removed from the system with nitrogen gas and the resulting solution was concentrated for study with GC-MS and a sample of it was completely freed from the solvent for NMR spectroscopic analysis. The ^1H NMR showed only oxime **56** with the same spectrum as reported above. GC-MS peak no. 760, R_t=14.3 min., undecanal **46**; peak no. 819, R_t=15.1 min., either *syn*- or *anti*- undecanal oxime **56**; peak no. 915, R_t=17.0 min.,.either *syn*- or *anti*- undecanal oxime **56**.

2.3.3 Hydrolysis of undecanal oxime derived from photolysis of lauroyl peroxide

Sodium nitrite (3 cm^3) of 5% w/v solution was mixed with 2M acetic acid (6 cm^3) and this mixture was stirred and maintained below 10°C. The crude oxime **56** (0.34 g, 1.84 mmol), obtained as a concentrated solution from the photolysis of lauroyl peroxide, was added to the reaction flask. After 48h, the acid was neutralised with 1M aqueous sodium bicarbonate (10 cm^3) and the products of hydrolysis were extracted with dichloromethane (3x10 cm^3). This solution was partially evaporated and GC-MS was

used to identify its constituents. GC-MS peak no. 581, R_t=10.7 min., undec-1-ene **44**; peak no. 594, R_t=10.9 min., undecane **45**; peak no. 771, R_t=14.2 min., undecanal **46**; peak no. 830, R_t=15.3 min., undecanol **48**; peak no. 987, R_t=18.2 min., dodecanoic acid **48**; peak no. 1100, R_t=20.2 min., undecylbenzene **49**.

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CHAPTER 3

Synthesis of Spiroepoxy Bromides and Spiroaziridines from Cycloalkanones

INTRODUCTION

3.1 Epoxides as Intermediates in Synthesis

Epoxides are highly regarded intermediates in organic synthesis. This is a direct result of the special reactivity shown by this functional group. The introduction of an epoxide moiety into a polyfunctional compound is normally a straightforward procedure because this functionality is easily accessible from a number of precursors that are discussed below.

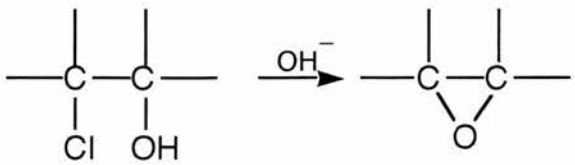
They undergo an impressive array of reactions in which the epoxy ring cleavage is initiated by a wide variety of nucleophiles. Some noteworthy examples are given below.

- Alcohols lead to β–hydroxy ethers some of which are useful solvents[1]
- Ammonia/amines give β–hydroxyamines[2]
- *t*-Butyldimethylsilyl iodide and several other reagents to form allylic alcohols[3]
- Bisulphite yields β–hydroxy sulphonic acids[4]
- N,N-Dimethylthiocarbamate forms β–hydroxy thiols[5]
- Sodium azides furnish β–azido alcohols[6] which are easily converted to aziridines

3.2 Formation of Epoxides

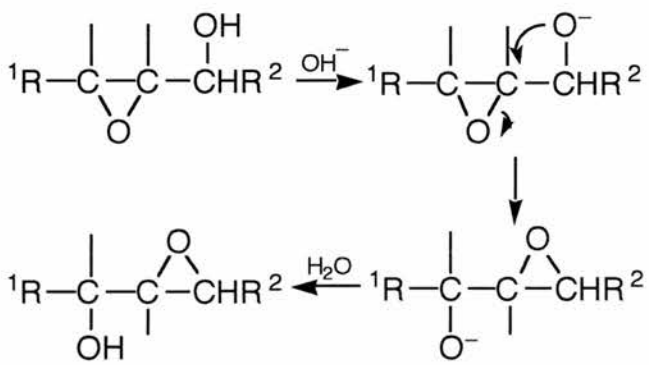
3.2.1 Cyclisation of halohydrins

This is a special case of the Williamson reaction, a method of preparing ethers[7]. A base removes the proton from the hydroxy group, and the nucleophilic oxygen thus generated, attacks in an internal S_N2 fashion[8]. The method can also be used to make larger cyclic ethers.



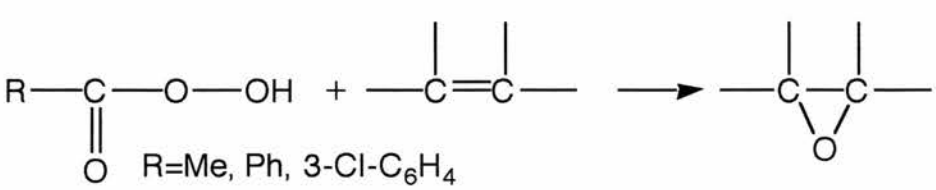
3.2.2 Payne rearrangement

In the Payne rearrangement, a 2, 3-epoxy alcohol is converted to an isomer by its treatment with a base[9]. Inversion of configuration occurs at C-2 and the product can revert to the starting material by the same pathway and so a mixture of epoxy alcohols is obtained.



3.2.3 Epoxidation of alkenes

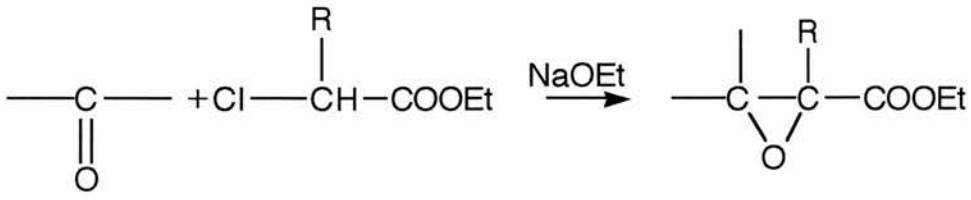
Alkenes can be readily epoxidised by any number of peroxyacids[10]. The most frequently used has been *m*-chloroperoxybenzoic acid. This reaction, called the Prilezhaev reaction, has wide application and so alkyl, aryl, hydroxy, ester and many other groups are tolerated in the starting material. The conditions for this transformation are mild and the yields are high. The proposed mechanism consists of a single step[11].



3.2.4 Darzens condensation

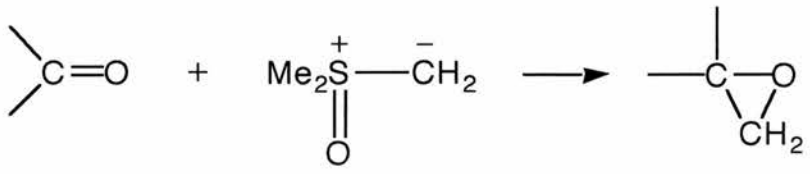
In this reaction aldehydes and ketones condense with α–halo esters in the presence of bases to give α,β–epoxy esters[12]. Sodium ethoxide is the commonly used base and both aromatic and aliphatic carbonyl compounds have been shown to give good yields (~80%) of the glycidic esters[13] which are easily converted to adehydes. The reaction mechanism

consists of deprotonation of the halo ester, addition of this to the carbonyl compound followed by an internal S_N2 reaction in the adduct.



3.2.5 Addition of sulphur ylides to aldehydes and ketones

Aldehydes and ketones can be converted to epoxides in good yields with sulphur ylides e.g. dimethyloxosulphonium methylide[14]. The generally accepted mechanism for this reaction is similar to that of the reaction of sulphur ylide with carbon-carbon double bonds.

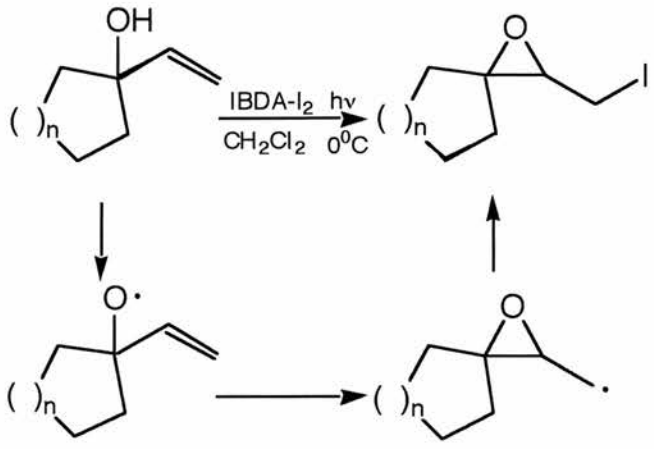


3.3 Synthesis of Spiroepoxides

In order to examine the viability of the proposed cascade rearrangement of carbon-centred radicals immediately adjacent to an epoxide (chapter 4, section 4.5, scheme 12), an efficient preparative methodology for cycloalkylepoxy bromides needed to be established. These α–halomethylepoxides cannot be readily accessed from simple precursors and reports of their synthesis in the literature are limited[15]. One of the more successful strategies for making these compounds is outlined below.

3.3.1 Photochemical reaction of tertiary cycloalkyl allylic alcohols

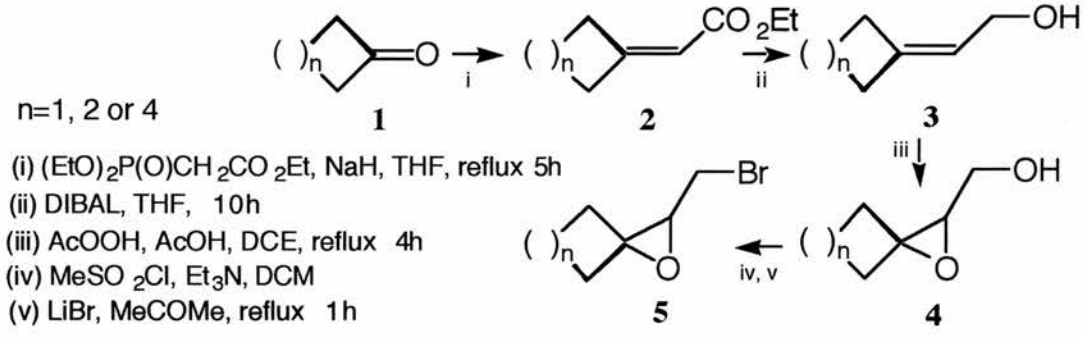
Treatment of tertiary allylic alcohols, readily available from addition of vinylmagnesium bromide to the corresponding cycloketones[16], with iodobenzene diacetate-iodine under photochemical conditions results in the formation of the corresponding cycloalkylepoxy iodides in respectable yields[17,18] (**Scheme 1**).



Scheme 1: Synthesis of cycloalkylepoxy iodides.

3.3.2 Proposed synthesis of 2-bromomethyl-1-oxa-spiro[2.n]alkanes from cycloalkanones via allylic alcohols

In our study, a protocol for the synthesis of the desired spirobromides was developed by using cycloketones as the starting materials. Exposure of the cycloalkanones **1** to triethylphosphonoacetate under Wadsworth-Emmons-Horner conditions afforded unsaturated esters **2**. Selective reduction of these latter compounds with DIBAL and other reducing agents furnished allylic alcohols **3** which were efficiently oxidised with peroxyacetic acid to epoxy-alcohols **4**. A two-stage reaction of these cycloalkylepoxy alcohols with methanesulphonyl chloride and lithium bromide gave the required spirobromides **5** which were subsequently converted to spiroaziridines[19].



Scheme 2: Synthesis of spirobromides from cyloalkanones.

3.4 Objectives of this Study

The main aims of this work were:

- To explore the viability of synthesising cycloalkylepoxy bromides from cycloalkanones using cyclopentanone as a model starting ketone

- To examine the efficiency of obtaining the intermediate cyclopentyl allylic alcohol *en route* to the target molecule by two different strategies. One approach involving the Wittig reaction and the other utilising the Wadsworth-Emmons-Horner reaction.

- To assess the relative merits of tetrahydropyran and of *t*-butyldimethylsilyl chloride as protecting agents for the hydroxyl group

- To establish optimum conditions for each step in the multi-stage conversion, outlined in section 3.3.2, of the cyclopentyl derivative to the corresponding spirobromide

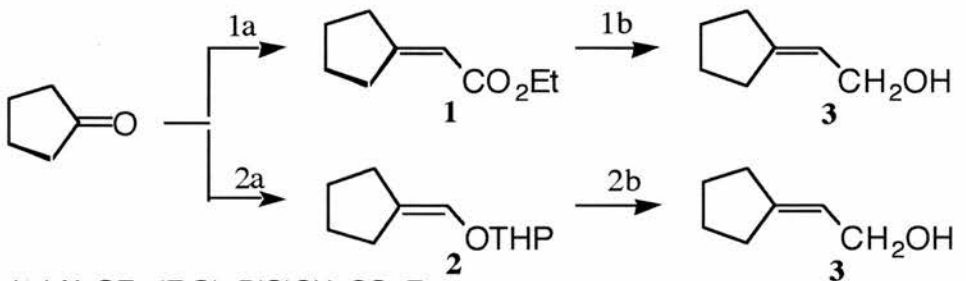
- To apply the above methodology effectively for the conversion of cyclobutanone and cycloheptanone to their respective spirobromides

- To convert the cyclopentyl allylic alcohol and its ethyl ester into the corresponding aziridine derivatives by their photolytic reaction with ethylazidoformate.

- To ascertain the economics of preparing the precursor cyclobutanone, required for the formation of cyclobutylepoxy bromide, compared with commencing the synthesis with the commercially purchased material

RESULTS AND DISCUSSION

The synthesis of the allylic alcohol **3** was considered to be of pivotal importance for the success of the synthetic strategy in **Scheme 2** leading eventually to the desired compounds, the spiroepoxides and the spiroaziridines. Consequently, two routes, were adopted using the five-membered ring ketone as a model starting material (**Scheme 3**).



(1a) NaOEt, $(EtO)_2P(O)CH_2CO_2Et$

(1b) Red-Al or DIBAL or $LiAlH_3(OEt)$

(2a) nBuLi, $Br^-Ph_3\overset{+}{P}CH_2CH_2OTHP$, EtOEt

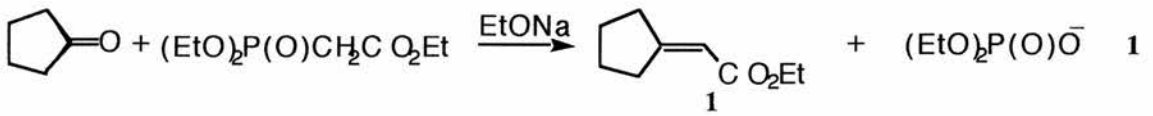
(2b) PPTS, EtOH

Scheme 3: Routes to the allylic cyclopentyl alcohol from cyclopentanone.

3.1 Synthesis of α-(Hydroxymethyl)methylenecyclopentane Using the Wadsworth-Emmons-Horner Reaction

3.1.1 Formation of α-(ethoxycarbonyl)methylenecyclopentane 1

Wadsworth-Emmons-Horner reaction[20] was used to make ester **1** from triethylphosphonoacetate and sodium ethoxide as the base (**Equation 1**).



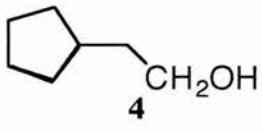
1

This well established method employs the base to deprotonate the triethylphosphonoacetate and the anion generated acts as the nucleophilic species in attacking the cycloketone. Elimination from the intermediate forms the required product

in a respectable quantity (75%). ^{1}H NMR spectroscopy of the material revealed it to be of high degree of purity. All the characteristic signals were intact: the downfield vinylic proton (δ=5.80 ppm), the methylene protons of the ethyl group (δ=4.25 ppm), the upfield methyl protons of the ethyl group (δ=1.28 ppm) and the peaks for the methylene groups of the cyclopentyl ring were the prominent features of the spectrum. ^{13}C NMR analysis of the ester also gave the nine peaks expected from the compound. A single peak (R_t=11.17 min.), observed by GC-MS, further confimed the ester's integrity while the MS analysis (M_r=154) led to the fragmentation pattern in line with the expected result.

3.1.2 Reduction of α–(ethoxycarbonyl)methylenecyclopentane 1

Initial exploratory experiments were carried out to achieve the selective reduction of ester **1**, using lithium aluminium hydride, under a variety of conditions, during its attempted conversion to the desired product. In each case, the required cyclopentyl enol **3** was made in disappointing amounts. The major problem with the reagent was its tendency, even at low temperatures, to give a reaction mixture consisting of the required allylic alcohol as well as the fully reduced alcohol **4** and the saturated ester **5**.



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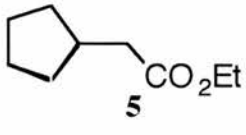
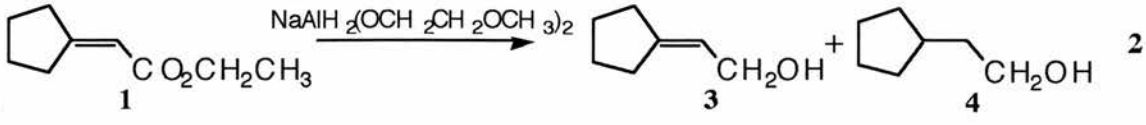


5

In view of the limited success in this reaction, the use of more chemoselective reagents was attempted in order to effect this transformation.

3.1.3 Reduction with sodium-bis-(2-methoxyethoxy) aluminium hydride [Red-Al]

This reduction was carried out using the procedure of Bazant[21] but it yielded equal amounts of the undesirable fully reduced alcohol **4** and the required partially reduced alcohol **3** (**Equation 2**).



1 **3** **4** **2**

Despite a satisfactory overall efficiency (75%), the quantity of alcohol **3**, determined by NMR spectroscopy, formed in the reaction was unacceptably low. The presence of the distinctive downfield peaks viz the doublet at δ=4.12 ppm for the allylic alcohol **3** and the triplet at δ=3.72 ppm for the 2-cyclopentylethanol **4** helped determine their relative proportions in the reaction mixture. Red-Al demonstrated high reactivity towards the starting material as was indicated by the latter's complete absence from the material isolated at the end. Under the conditions used, the reagent displayed limited chemoselectivity in reducing ester **1** in the requisite way.

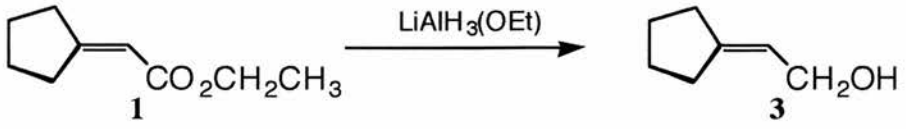
3.1.4 Reduction with diisobutylaluminium hydride [DIBAL]

This reducing agent, used according to a modified procedure suggested by Miller[22], turned out to be a promising method for achieving the required transformation. The distillate obtained represented a respectable yield (73%) and it consisted of a 6:1 molar ratio of the cyclopentyl allylic alcohol **3** and 2-cyclopentylethanol **4** respectively (**Equation 2**).

The relative composition of the isolated material was determined by ^{1}H NMR spectroscopy. The expected resonance for the -CH_2OH group in the alcohol **3** was, again, observed as a doublet at δ=4.12 ppm while the corresponding signal for the same group in alcohol **4** was a triplet at δ=3.72 ppm. Although this reagent's chemoselectivity was superior to that of Red-Al, the reaction mixture still needed to be chromatographed in order to obtain contaminant-free alcohol **3**. Owing to the inherent loss of material in using this purification technique , a more selective reagent with respect to the formation of the allylic alcohol **3** was sought.

3.1.5 Reduction with lithium aluminium ethoxyhydride [$LiAlH_3(OEt)$]

This reagent, not available commercially , was generated *in situ* in the manner described by Davidson[23]. The reaction showed total efficiency in accomplishing the selective reduction of the unsaturated ester **1** and in forming exclusively the allylic alcohol **3** (**Equation 3**).

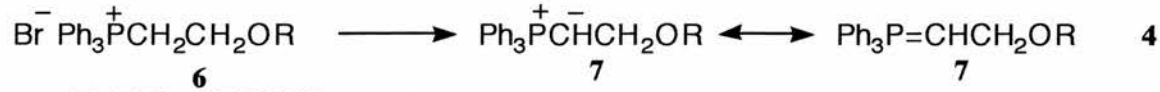


1 3 3

The yield of the product (95%) was excellent and its analysis indicated a degree of purity unmatched by any of the methods described earlier. ^{1}H NMR spectroscopy gave a spectrum with resonances for two of the four cyclopentyl ring methylenes at δ=1.65 ppm and the remaining two at δ=2.28 ppm. The primary alcohol group produced a doublet at δ=4.12 ppm and the distinctive vinylic proton resulted in a multiplet at δ=5.49 ppm. The ^{13}C NMR spectrum confirmed the presence of seven carbon atoms in the molecule. High resolution mass spectrometry used for accurate mass determination (M_r=112.0883) for the molecular ion corresponded with the molecular formula of $C_7H_{12}O$. The fragmentation of this species, in the EI mode of the instrument, gave a pattern entirely consistent with that expected from such a molecule.

3.2 Synthesis of α-(Hydroxymethyl)methylenecyclopentane Using the Wittig Reaction

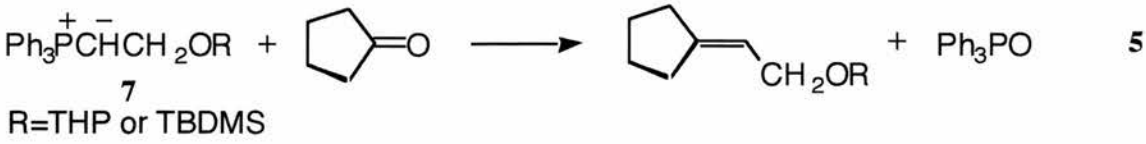
In the Wittig reaction[24], a base, n-butyllithium, deprotonates the phosphonium salt to generate an ylide (**Equation 4**) which acts as the nucleophilic species in attacking the cycloketone.



6 7 7 4

R=THP or TBDMS

The resulting betaine intermediate then undergoes elimination of triphenylphosphine oxide in forming the alcohol **3** (**Equation 5**).



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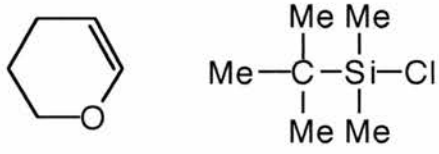
Preparation of the allylic alcohol **3** utilising this ylide chemistry involves 2-bromoethanol as one of the starting materials. In order to enhance the overall efficiency of the reaction, it is necessary to protect the the hydroxyl group in the bromohydrin **10** prior to its treatment with a base. Otherwise, instead of forming the ylide, deprotonation of the hydroxyl may well predominate leading to undesirable byproducts in the final reaction mixture. The effectiveness of two protecting groups: dihydropyran **8** and *t* -butyldimethylsilyl chloride **9** was studied in this connection. Clearly, there are two

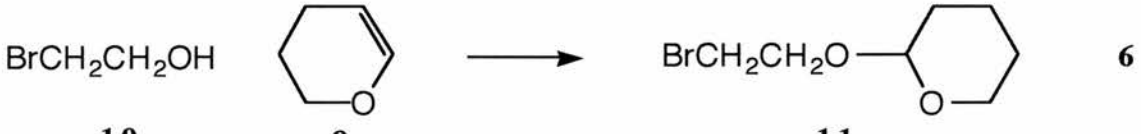


8 **9**

strategies for converting the bromohydrin **10** to the required ylide **7**. First, the hydroxyl group in the 2-bromoethanol **10** could be protected before its treatment with triphenylphosphine in order to generate the phosphonium salt. Secondly, the phosphonium salt could be formed from 2-bromoethanol **10** and this could then be treated with the protecting agent. Both of these approaches were examined.

3.2.1 Preparation of THP-protected phosphonium salt

Protection of the hydroxyl group in 2-bromoethanol as THP-ether

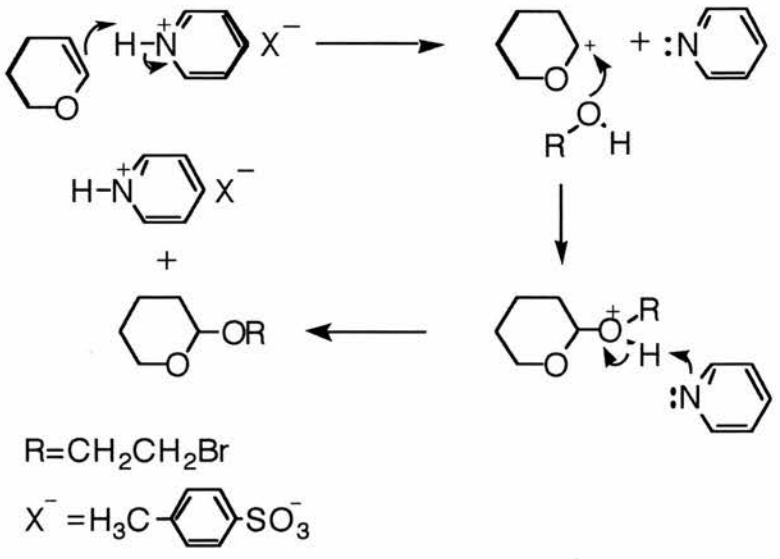


10 **8** **11**

6

This protection was achieved highly effectively by following the procedure indicated by Miyashita[25]. The tetrahydropyranylated ether **11** was prepared in good yield (87%) and ^{1}H NMR spectroscopy showed it to be free of any contaminants. The most striking signal in the spectrum corresponded to the single proton in the tetrahydropyran ring at δ=4.63 ppm. The role of the catalyst, pyridinium-*p*-toluene

sulphonate, is to protonate the unsaturated centre of dihydropyran **8** thus forming a carbocation from the latter. A nucleophilic attack by the alcohol and concurrent protonation of pyridine helps to regenerate the catalyst in the manner shown by **Scheme 4**.



Scheme 4: Formation of tetrahydropyranylated ether from 2-bromoethanol.

Formation of phosphonium salt from THP-protected ether

$$\underset{\mathbf{11}}{BrCH_2CH_2OTHP} + Ph_3P \longrightarrow \underset{\mathbf{12}}{Br^- Ph_3P^+CH_2CH_2OTHP} \qquad \mathbf{7}$$

Despite the prolonged reaction conditions specified by Pommer[26], a disappointing amount (42%), of the corresponding salt was obtained and significant quantities of the starting materials were recovered from the reaction mixture at the termination of the reaction. It is likely that the extended stirring of the reaction mixture (24h) allowed moisture into the system, through seepage, that caused hydrolysis of the ester upon refluxing. However, reducing the contact time between the reagents did not result in significant improvement in the performance of the reaction although the product was obtained in high purity as evidenced by ^1H NMR spectroscopy. The characteristic resonance for the triphenylphosphine (δ=7.52-7.82 ppm) as well as the relevant peaks for

the rest of the molecule, were observed in the THP-protected bromohydrin **11**, albeit at slightly different chemical shift values.

Formation of phosphonium salt from unprotected 2-bromoethanol

$$\underset{\mathbf{10}}{BrCH_2CH_2OH} + Ph_3P \longrightarrow \underset{\mathbf{13}}{Br^- \, Ph_3\overset{+}{P}CH_2CH_2OH} \qquad \mathbf{8}$$

The phosphonium salt **13** was prepared in moderate yield (60%) by a slightly modified method to the one described in section 3.2.1 earlier. The initial crude product, obtained after refluxing, was contaminated with unreacted starting materials. The purification of it was achieved through solvent precipitation. The impure solid was dissolved in a polar solvent, chloroform, the polarity of which was gradually decreased by dropwise addition to it of a less polar solvent, diethyl ether, until no further precipitation of the pure material occurred. A clean ^1H NMR spectrum consisted of signals at δ=3.70, 3.97 ppm for the two methylene groups and at δ=7.53-7.80 ppm for the three phenyl groups in the molecule.

THP-protection of the hydroxyl group in the phosphonium salt

$$\underset{\mathbf{13}}{Br^- \, Ph_3\overset{+}{P}CH_2CH_2OH} + THP \xrightarrow{PPTS} \underset{\mathbf{12}}{Br^- \, Ph_3\overset{+}{P}CH_2CH_2OTHP} \qquad \mathbf{9}$$

This protection of the hydroxyl group in the phosphonium salt **13** was carried out in the fashion described earlier. The tetrahydropyranylation occurred quantitatively (99%) and the ^1H NMR spectrum, as expected, was identical with the final product obtained when strategy 1 was followed. It is apparent that the initial transformation of the bromohydrin **10** into its phosphonium salt **13** followed by tetrahydropyranylation of its hydroxyl group to give the THP-protected phosphonium salt **12** is a superior route for making this salt.

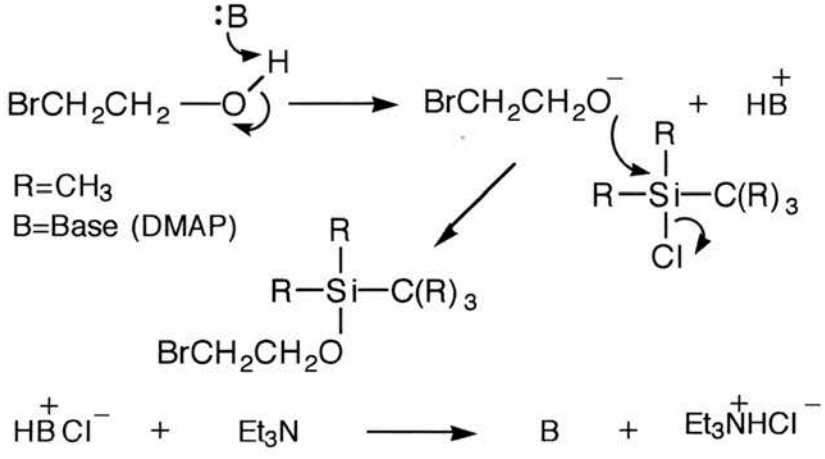
3.2.2 Preparation of TDBMS-protected phosphonium salt

Protection of the hydroxyl group in 2-bromoethanol as TBDMS-ether

$$\underset{\mathbf{10}}{BrCH_2CH_2OH} + TBDMSCl \longrightarrow \underset{\mathbf{14}}{BrCH_2CH_2OTBDMS} + HCl \qquad \mathbf{10}$$

The Hernandez[27] method of protecting the hydroxyl group in the bromohydrin **10** proceeded with good facility giving a high yield (83%) of the corresponding ether **14**. In the ^{1}H NMR spectrum of the product, the dimethyl group (δ=0 ppm), the *tert*-butyl group (δ=0.80 ppm) and the two methylenes (δ=3.30, 3.80 ppm) were readily identifiable.

This protection of hydroxyl groups as silyl ethers is widely adopted and it is catalysed by dimethylaminopyridine. The non-nucleophilic base, DMAP, deprotonates the alcohol generating the nucleophilic alkoxide anion which displaces the chlorine from the reagent. Basic triethylamine, present in excess, helps to maintain acid-free conditions by forming the ammonium salt and thereby increasing the stability of the TBDMS-ether towards hydrolysis. **Scheme 5** outlines the sequence of reactions involved.



Scheme 5: Formation of *t*-butyldimethylsiyl ether from 2-bromoethanol.

Formation of phosphonium salt from TBDMS-protected ether

$$\underset{\mathbf{14}}{BrCH_2CH_2OTBDMS} + Ph_3P \longrightarrow \underset{\mathbf{15}}{Br^- \; Ph_3\overset{+}{P}CH_2CH_2OTBDMS} \qquad \mathbf{11}$$

Despite encouraging reports in the literature[28] for the effectiveness of this reaction, the TBDMS-protected ether **15** was made in a very poor yield (4.83 g, 32%). Product analysis, by ^{1}H NMR, on the solid, showed all the expected resonances including the downfield signal (δ=7.22 ppm) corresponding to the triphenylphosphine

group in the salt. A possible explanation for the poor performance of this reaction is that, upon refluxing the reaction mixture, some of the starting triphenylphosphine may have been oxidised to triphenylphosphine oxide which, owing to its higher solubility compared to the product, remains in the aromatic solvent thereby not appearing as a contaminant in the TBDMS-ether.

In view of the poor performance of TBDMSCl when employed as a protecting group in carrying out the sequence of reactions in line with strategy 1, it seemed unlikely that its effectiveness would be any greater if strategy 2 was followed with this reagent and so its further use was abandoned.

3.3 Synthesis of Spiroepoxides

3.3.2 Synthesis of 2-bromomethyl-1-oxa-spiro[2.n]alkanes from cyclo-alkanones via allylic alcohols

In view of the poor efficiency of the Wittig method in forming the intermediate cyclopentyl allylic alcohol, this route to such a compound was abandoned in favour of the more effective approach involving no protection-deprotection steps of the reacting species (bromohydrin **10**). Preliminary work with cyclopentanone, as the starting material, had established a protocol for effecting its excellent two-stage conversion via the initial formation of the ester **1** (75%) and the subsequent reduction of the latter to the unsaturated alcohol **3** (95%). Selective reduction of the ester proved troublesome owing to the co-production, with a range of reducing agents, of some of the saturated analogue **4**. Highest quantities (cycloheptyl allylic alcohol **25**; 82%) of the desired product were obtained with the hindered hydride, lithium aluminium ethoxyhydride, which was generated by slow addition of a molar equivalent of ethanol to a dry ethereal solution of lithium aluminium hydride. The resulting mixture was then added, gradually, to the reaction mixture. Even this reagent was ineffective with the unsaturated ester **20** which contained the highly strained cyclobutyl ring. This reduction was accomplished with DIBAL and the product **21** was isolated, in a modest yield (36%) by column

chromatography of the distillate from the reaction mixture. In all cases, the instantaneous concentration of the reductant seemed a critical factor in determining the course of the reaction. Low concentration of the reagent, and therefore its gradual addition to the substrate, favoured the reduction of the ester functionality only whereas excessive amounts of it, resulted in both the ester grouping and the unsaturated centre in the starting molecule being attacked. In the Wadsworth-Emmons-Horner conversion of cycloalkanones to the unsaturated esters, sodium hydride performed better as a base compared to sodium ethoxide and yields of the products in excess of 80% were obtained with the former in each case.

Epoxidation of the allylic cyclopentyl alcohol **3** was initially attempted with *m*-chloroperoxybenzoic acid, a well established reagent for such conversions. However, in this particular case, the procedure was only moderately successful (65% in a 2:1 ratio of spiroepoxy alcohol **17** and 3-chlorobenzoic acid **18**) owing to the difficulties connected with complete removal of the byproduct, 3-chlorobenzoic acid, from the reaction mixture. A more efficient formation of the cyclopentylepoxy alcohol **17** (91%) was achieved with peroxyacetic acid in the presence of sodium carbonate, the latter assisting in the removal of the byproduct, acetic acid. This methodology was used to obtain cyclobutylepoxy alcohol **22** (86%) and cycloheptylepoxy alcohol **26** (87%).

Once the mild conditions for bromination of the cyclopentylepoxy alcohol **17** had been developed, it was converted to the bromide **19** in a respectable amount (63%) involving a two-step process in which the alcohol was mesylated and this was followed by displacement of the mesylate with a bromide. Corresponding analogues containing cyclobutyl ring **23** (76%) and cycloheptyl ring **27** (87%) were made in identical fashion. Rigorous exclusion of moisture and a strict control of temperature below 60°C were crucial to the success of this reaction. The former prevents hydrolysis of the intermediate mesylate while the latter helps minimise the decomposition of highly temperature-sensitive bromides.

Aziridines are commonly prepared from compounds containing carbon-carbon double bond by photolysis or thermolysis of a mixture of the substrate and an azide[29].

The reaction has been performed with a variety of other functionalities being present in the azide e.g. CN, Ar, EtO_2C etc. Attempts were made to convert the unsaturated cyclopentyl ester **1** and the alcohol **17** to the corresponding spiroaziridines required for analytical work, by their photolytic reaction with ethylazidoformate which forms a triazoline with the substrate that eventually extrudes nitrogen to give the required products. With the cyclopentyl allylic alcohol as the starting material, the corresponding aziridine **28** was obtained in a poor quantity as off-white solid (21%). Its purification was attempted, from the reaction mixture, by chromatography on an alumina column. However its recovery from the column, even with a highly polar solvent such as methanol, proved difficult and substantial loss of the product occurred. Recrystallisation, undertaken with methanol/dichloromethane, of the solid was also inefficient owing to its very poor solubility in this mixture and in a number of other polar solvents. The attempted conversion of ester **1** to the corresponding aziridine, in an analogous manner, did not succeed as evidenced by the total recovery of the starting material at the end of the reaction. It is possible that, in this case, the presence of the bulky ester group in the substrate may hinder a close approach of the reagent to its unsaturated centre.

3.4 Synthesis of Cyclobutanone

Although commercially available, cyclobutanone is an expensive material. In the interests of economy, its four-step synthesis, from 1-bromo-3-chloropropane, using literature methods, was undertaken. The Truce[30] procedure converted the dihalo compound to 3-phenylthiyl-1-chloropropane in excellent yield (81%) and the formation of the cyclopropylphenylsulphide proceeded with equal facility (85%). Use of Trost[30] methodology achieved further functionalisation of the cyclopropyl ring to give 1-hydroxymethyl-1-phenylthiocyclopropane (82%). The final step in the synthsis was achieved with disappointing yield (52%). In view of the poor efficacy of the last step and the toxicity associated with the use of mercury(II)chloride in order to accomplish this transformation, it was decided to use the commercially available cyclobutanone as the starting compound for the synthesis of the desired cyclobutylepoxy bromides.

CONCLUSIONS

- The intermediate cyclopentyl allylic alcohol, from cyclopentanone, was obtained in greater overall efficiency by adopting the Wadsworth-Emmons-Horner route (60%) than by carrying out this conversion using the Wittig reaction (37%)

- The formation of the THP-protected phosphonium salt of 2-bromoethanol gave the product in a higher yield than the corresponding conversion with *t*-butyldimethylsilyl chloride

- Except for the selective reduction of the unsaturated ester **1** (n=1), all stages of the synthetic scheme proposed in the introduction (section 3.3.2.) concerning the 4-step conversion of cycloalkanones to the cycloalkylepoxy bromides, gave good yields of the spirobromides

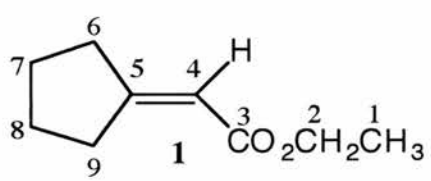
- The ethyl ester **2** (n=2, section 3.3.2) did not undergo photolytic reaction with ethylazidoformate to give the corresponding aziridine. Analytical techniques showed a low-level formation of the aziridine from the allylic alcohol **3** (n=2) under comparable conditions. However, isolation of the product proved extremely difficult owing to very poor solubility in a number of solvents

- The synthesis of cylobutanone, using literature methods, was accomplished satisfactorily but it offered no economic advantage over using the commercially available compound as the starting material for obtaining the corresponding spirobromide

EXPERIMENTAL

Formation α-(ethoxycarbonyl)methylenecyclopentane 1

Sodium (2.76 g, 0.12 mol) was added, in small amounts, to absolute ethanol (80 cm^3) which was placed in a three-necked flask fitted with a thermometer and a dropping funnel. Once the metal had dissolved, triethylphosphonoacetate (21.50 g, 0.096 mol) was added to the cooled solution of the ethoxide. The resulting mixture was stirred at 0°C for 2h and cyclopentanone (10.08 g, 0.12 mol) was added to it at such a rate so as to maintain the temperature of the reaction mixture below 10°C. The ice-bath was removed and the contents of the flask were stirred for an additional 14h. Dilution with brine (200 cm^3) was followed by extraction of the product with hexane (6x50 cm^3). The combined extracts were dried over anhydrous magnesium sulphate and concentrated at the rotatory evaporator. Distillation, using a Vigreux column, gave the unsaturated ester **1** as a colourless liquid (11.03 g, 75%); (bp 38°C / 0.7 mm Hg).

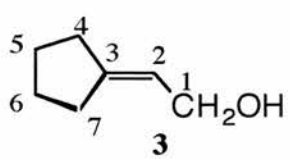


δ_H(300MHz, $CDCl_3$) 1.28(3H, t, CH_3-1), 1.72(4H, m, CH_2-7,8), 2.44(2H, t, CH_2-6), 2.78(2H, t, CH_2-9), 4.14(2H, q, CH_2-2), 5.80(1H, m, CH-4); δ_C(75MHz, $CDCl_3$), 14.4(CH_3, C-1), 26.6(CH_2, C-7), 27.7(CH_2, C-8), 32.5(CH_2, C-6), 36.6(CH_2, C-9), 59.4(CH_2, C-2), 111.8(CH-4), 166.8(1C, C-5), 168.8(1C, C-3); GC-MS peak no. 607, R_t=11.17 min., α-(ethoxycarbonyl)methylenecyclopentane ***1***, m/z(relative intensity %) 154(M^+, 41), 125(11), 108(36), 81(100), 80(85), 79(99), 67(27), 53(21), 41(20), 29(24).

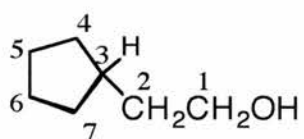
Reduction of α-(ethoxycarbonyl)methylenecyclopentane 1

Reagent: Sodium bis-(2-methoxyethoxy) aluminium hydride [Red-Al]

α-(Ethoxycarbonyl)methylenecyclopentane **1** (4.65 g, 0.03 mol) was added to dry benzene (100 cm^3) contained in a three-necked flask fitted with a nitrogen gas inlet and a dropping funnel. To the continuously stirred solution, under nitrogen, Red-Al (7.47 g, 0.024 mol), diluted with benzene and placed in the dropping funnel, was added at such a rate that the temperature was controlled below 0°C. After allowing the mixture to stir for 45 min. at room temperature, the excess reductant was decomposed by slow addition of moist diethyl ether (35 cm^3). The alcohol was released from its alkoxide by dropwise addition of dil. sulphuric acid (35 cm^3) while maintaining the temperature of the flask around 20°C. The organic layer was separated, treated with water (25 cm^3), dried over anhydrous sodium sulphate and the solvents were removed under reduced pressure. The product (2.03 g, 75%) was obtained as an equimolar mixture† of α-(hydroxymethyl)methylenecyclopentane **3** and of 2-cyclopentylethanol **4** by distillation using a Vigreux column; (bp 70-74°C / 0.9 mm Hg).



3



4

δ_H(300MHz, $CDCl_3$), 1.56-1.75(4H, m, CH_2-5,6 of **3**, 4H, m, CH_2-5,6 of **4**), 1.90(1H, m, CH-3 of **4**), 2.20-2.32(4H, m, CH_2-4,7 of **3**, 4H, m, CH_2-4,7 of **4**), 2.38(2H, m , CH_2-2 of **4**), 3.72(2H, t, CH_2-1 of **4**), 4.12(2H, d, CH_2-1 of **3**), 5.48(1H, m, CH-2 of **3**).

Reagent: Diisobutylaluminium hydride [DIBAL]

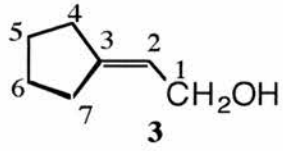
DIBAL (18.03 g, 0.127 mol) was added under nitrogen gas in 10 equal aliquots, over 10h, to a stirred solution of α-(ethoxycarbonyl)methylene cyclopentane **1** (4.62 g, 0.03 mol) in dry tetrahydrofuran (25 cm^3) contained in a three-necked flask fitted with a

thermometer and a nitrogen inlet. The temperature was maintained around 30°C for the duration of the addition of the reductant. After allowing the reaction mixture to stir at room temperature for 1h, the excess reagent was decomposed by slow addition of methanol/THF (11 cm^3:22 cm^3 resp. v/v) mixture and subsequently with water (10 cm^3). The precipitated aluminium salts were filtered under suction, washed with methanol (5x20 cm^3) and the washings were added to the filtrate which was concentrated at the rotatory evaporator. The product (2.45 g, 73%), a colourless liquid, was isolated as an 85% mixture† of α–(hydroxymethyl)methylenecyclopentane **3** and of 2-cyclopentylethanol **4** resp. by distillation using a Vigreux column; (bp 36-38°C / 0.4 mm Hg). δ_H(300MHz, $CDCl_3$) 1.58-1.76(4H, m, CH_2-5,6 of **3**, 4H, m, CH_2-5,6 of **4**), 1.90(1H, m, CH-3 of **4**), 2.20-2.34(4H, m, CH_2-4,7 of **3**, 4H, m, CH_2-4,7 of **4**), 2.38(2H, m, CH_2-2 of **4**) , 3.72(2H, t, CH_2-1 of **4**), 4.12(2H, d, CH_2 of **3**), 5.48(1H, m, CH-2 of **3**). †Determined by NMR spectroscopy.

Reagent: Lithium aluminium ethoxyhydride [$LiAlH_3(OEt)$]

Preweighed lithium aluminium hydride (5.29 g, 0.135 mol) was quickly added, under nitrogen gas, to stirred diethyl ether contained in a three-necked flask which had been equipped with a nitrogen inlet and a dropping funnel. A separate flask was charged with dry diethyl ether (250 cm^3) under an atmosphere of nitrogen gas. The solvent in this flask was magnetically stirred and α–(ethoxycarbonyl)methylenecyclopentane **1** (10.0 g, 0.065 mol) was added to it. Ethanol (4.99 g, 0.108 mol), diluted with dry diethyl ether (10 cm^3), was placed in the dropping funnel fitted to the flask containing the hydride suspension. The alcohol was added dropwise to the contents of the flask. After allowing the resulting lithium aluminium ethoxyhydride to stir for 30 min., aliquots (10.0 cm^3, 2.70 mmol) of it were added, hourly, to the flask containing the ester. The progress of the reaction was monitored by TLC which, after 17h, showed the reaction to be complete. The reagent (170.0 cm^3, 0.046 mol) had been consumed in achieving a complete reaction which was terminated by decomposing the excess reducing agent initially with wet diethyl

ether (2x50 cm^3) and subsequently with water (50 cm^3) and with periodic cooling of the reaction vessel. The precipitated aluminium salts were filtered under suction, washed with water (3x50 cm^3) and then with diethyl ether (3x50 cm^3). The aqueous layer in the combined filtrates was separated and extracted with diethyl ether (3x50 cm^3). These ethereal extracts were combined with the organic layer obtained previously and the resulting solution was dried over anhydrous sodium sulphate. The desiccant was filtered off and the product, α–(hydroxymethyl)methylenecyclopentane **3**, (7.87 g, 95%), was obtained as a colourless, viscous liquid after removal of solvent from it under reduced pressure.

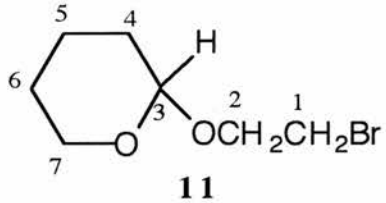


3

δ_H(300MHz, $CDCl_3$) 1.65(4H, m, CH_2-5,6), 1.90(OH), 2.28(4H, m, $^4J_{(CH)}$=2.3, $^4J_{(CH_2OH)}$=1.2, CH_2-4,7), 4.12(2H, dq, $^3J_{(CH)}$=7.0, $^4J_{((2xCH_2)}$=1.2, CH_2-1), 5.49(1H, m, $^3J_{(CH_2OH)}$=7.0, $^4J_{(2xCH_2)}$=2.3, CH-2); δ_C(75MHz, $CDCl_3$), 26.0(CH_2, C-5), 26.3(CH_2, C-6), 28.6(CH_2, C4), 33.7(CH_2, C-7), 60.9(CH_2OH, C-1), 119.1(CH, C-2), 147.6(1C, C-3); HRMS (EI) found M^+, 112.0883; $C_7H_{12}O$ requires M^+, 112.0888; MS m/z(relative intensity %) 112(M^+, 32), 94(48), 83(58), 79(100), 67(61), 55(46), 41(52).

Protection of hydroxyl group in 2-bromoethanol as a THP-ether

2-Bromoethanol (30.0 g 0.24 mol) was added to dry dichloromethane (1.6 l), dihydropyran (30.30 g, 0.36 mol) and the catalyst pyridinium-*p*-toluene sulphonate (6.30 g, 0.025 mol). This mixture was stirred for 4h and then diluted with diethyl ether (200 cm^3). The catalyst was decomposed by the use of half-saturated brine (200 cm^3) and, after separation, the solvents were removed at the rotatory evaporator. The crude THP-ether **11** (43.40 g, 87%) was purified by distillation under reduced pressure giving a colourless liquid (bp 80-84°C / 1.2 mm Hg).

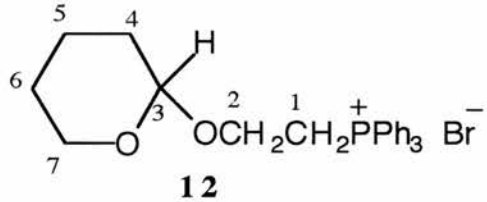


11

δ_H(200MHz, $CDCl_3$) 1.44-1.82(4H, m, CH_2-5,6), 3.48(4H, m, CH_2-4,7), 3.83(4H, m, CH_2-1,2), 4.63(1H, s, CH-3).

Formation of the phosphonium salt from THP-ether of 2-bromoethanol

Triphenylphosphine (16.24 g, 0.062 mol) was dissolved in dry diethyl ether (100 cm^3) which was contained in a flask fitted with a nitrogen inlet. The flask was cooled in an ice-bath and the THP-protected bromohydrin (17.24 g, 0.082 mol) was slowly added to the reaction mixture with stirring. Once this addition was complete, the contents of the flask were allowed to reach room temperature and stirred continuously, under these conditions, for further 24h. Finally, the flask was heated under reflux for 5h and, upon cooling, the precipitated phosphonium salt was filtered and washed with dry diethyl ether (2x20 cm^3). The product, phosphonium salt **12**, (10.90 g, 37%) was obtained as a white crystalline material after drying it under high vacuum for 2h.



12

δ_H(200MHz, $CDCl_3$) 1.20-1.42(4H, m, CH_2-5,6), 3.34(2H, dt, CH_2-4), 3.42(2H, m, CH_2-1), 3.78(2H, t, CH_2-2), 4.04(2H, m, CH_2-7), 4.22(1H, m, CH-3), 7.52-7.82(15H, m, 3xPh).

Formation of the phosphonium salt from the unprotected 2-bromoethanol

2-Bromoethanol (18.80 g, 0.15 mol) and triphenylphosphine (26.60 g, 0.10 mol) were placed in a flask containing dry diethyl ether (30 cm^3). The mixture was stirred until all of the latter had dissolved. The reaction mixture was then refluxed for 6h. The precipitated phosphonium salt was isolated by suction filtration and the crude solid was purified by dissolving it in chloroform and then reprecipitating it from this solution with dropwise addition of diethyl ether. Reduced pressure filtration of the suspension gave the required product, the phosphonium salt **13**, as a white solid that was dried under high vacuum (22.90 g, 60%).

$$Br^- \; Ph_3\overset{+}{P}CH_2CH_2OH$$

13

δ_H(200MHz, $CDCl_3$) 3.70(2H, dt, CH_2-2), 3.97(2H, dt, CH_2-1), 4.70(OH), 7.53-7.80(15H, m, 3xPh).

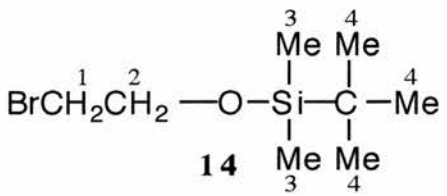
THP-protection of the hydroxyl group in the phosphonium salt

The phosphonium salt (4.40 g, 0.01 mol) was added to dry dichloromethane (85 cm^3), dihydropyran (1.26 g, 0.015 mol), and the catalyst pyridinium-*p*-toluene sulphonate (0.31 g, 1.22 mmol). This mixture was stirred at room temperature for 4h and then diluted with dry diethyl ether (90 cm^3). The catalyst was decomposed by use of half-saturated brine (15 cm^3) and, after separation, the organic layer, upon removal of solvents, afforded a viscous oily liquid which, when washed with dry diethyl ether (5x15 cm^3), gave the product, the THP-protected phosphonium salt **12**, (4.70 g, 99%), as a white solid that was dried overnight under high vacuum. ^1H NMR spectrum was the same as given above.

Protection of hydroxyl group in 2-bromoethanol

t-Butyldimethylsilyl chloride (10.10 g, 0.06 mol) was weighed into a flask fitted with a nitrogen inlet. DMAP (0.30 g, 2.50 mmol), triethylamine (7.40 g, 0.07 mol) and 2-bromoethanol (9.76g, 0.06 mol) were also added to the flask. After dry

dichloromethane (125 cm^3) addition, the reaction mixture was stirred for 20h. The work-up of the reaction involved addition of water (200 cm^3), separation of the organic layer followed by treatment with saturated aqueous ammonium chloride (100 cm^3). The dichloromethane portion was separated and dried over anhydrous sodium sulphate. After removal of the solvent, the silyl ether **14** was obtained by distillation on a Kugelrohr under high vacuum as a colourless liquid (11.90 g, 83%); (bp 82-90°C / 1.3 mm Hg).

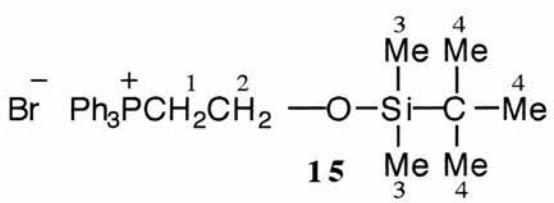


14

δ_H(200MHz, $CDCl_3$) 0(6H, s, 2xCH_3-3), 0.80(9H, s, 3xCH_3-4), 3.30(2H, t, CH_2-1), 3.80(2H, t, CH_2-2).

Conversion of TBDMS-ether to its phosphonium salt

Triphenyl phosphine (8.12 g, 0.03 mol) was weighed into a flask containing dry benzene (50 cm^3) and fitted with a thermometer and a dropping funnel. After cooling this solution with an ice-bath, TBDMS-protected 2-bromoethanol (10.0 g, 0.04 mol), dissolved in benzene (5 cm^3), was slowly added to the reaction mixture from the dropping funnel, over 15 mins. The resulting solution was stirred for a further 48h and it was then refluxed for 5h. The precipitated product, TBDMS-protected phosphonium salt **15**, (4.83 g, 32%) was isolated as a white solid.



15

δ_H(200MHz , $CDCl_3$) 0(6H, s, 2xCH_3-3), 0.80(9H, s, 3xCH_3-4), 3.30(2H, t, CH_2-1), 3.80(2H, t, CH_2-2), 7.22(15H, s, 3xPh), 7.52-7.82(15H, m, 3xPh) unreacted Ph_3P.

Formation of the phosphonium salt from unprotected 2-bromoethanol

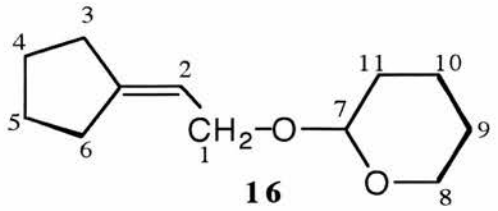
The procedure followed has been described earlier.

TBDMS-protection of hydroxyl group in the phosphonium salt

t-Butyldimethylsilyl chloride (5.0 g, 0.033 mol) was weighed into a flask fitted with a nitrogen inlet. DMAP (0.15 g, 1.18 mmol), triethylamine (3.34 g, 0.033 mol) and the phosphonium salt of 2-bromoethanol (3.31 g, 0.025 mol) were also added to the flask. After dry dichloromethane (100 cm^3) addition, the reaction mixture was stirred for 24h and treated with diethyl ether. Further stirring for 30 mins. gave a precipitate which was filtered under suction and washed with dry diethyl ether (3x100 cm^3). The white solid, TBDMS-protected phosphonium salt **15**, (4.80 g, 38%) was finally dried under high vacuum for 2h. ^1H NMR spectrum was the same as described above.

Preparation of the THP-protected α-(hydroxymethyl)methylene-cyclopentane 3

Dry diethyl ether (50 cm^3) was placed into a three-necked flask fitted with a reflux condenser, a nitrogen inlet and a dropping funnel. n-Butyllithium (12.5 cm^3) was added to the stirred solvent along with the THP-protected phosphonium salt of 2-bromoethanol **12** (7.10 g, 0.015 mol) over a 10 min. period. The resulting suspension was stirred for 4h. Cyclopentanone (1.26 g, 0.015 mol) was added via the dropping funnel to the reaction vessel and, after allowing its contents to stir for 1h, refluxing was performed for a further 12h. The precipitated triphenylphosphine oxide was removed by filtration. The residue was washed with dry diethyl ether (3x50 cm^3) and these washings were combined with the filtrate from the earlier stage. The resulting solution was treated with portions of water (25 cm^3) until the latter was neutral. After removal of the solvent, the product, THP-protected alcohol **16** (1.10 g, 37%), a colourless liquid, was obtained by distillation under reduced pressure using a Kugelrohr (bp 95-105°C / 1.3 mm Hg).



16

δ_H(200MHz, $CDCl_3$) 0.89(2H, m, CH_2-10), 1.28(2H, m, CH_2-11) , 1.69(4H, m, CH_2-4,5), 1.94(2H, q, CH_2-9), 2.30(4H, m, CH_2-3,6), 2.55(2H, m , CH_2-8), 2.78(2H, m, CH_2-1), 3.64(1H, t, CH-7), 5.38(1H, m, CH-2).

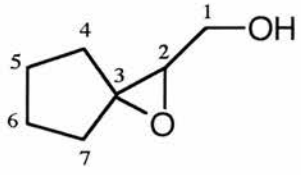
Deprotection of the THP-protected α-(hydroxymethyl)methylene-cyclopentane 16

THP-protected α-(hydroxymethyl)methylenecyclopentane (0.40 g, 2.04 mmol) was placed into a single necked round-bottomed flask fitted with a condenser. Ethanol (20 cm^3) and pyridinium-*p*-toluene sulphonate (0.05 g, 0.21 mmol) were also added to the flask. The reaction mixture was stirred for 3h at 55°C. At the end of this period, the catalyst was decomposed with half-saturated brine (10 cm^3) and the product was extracted with dry diethyl ether (3x20 cm^3). These ethereal extracts were combined and dried over anhydrous sodium sulphate. After removing the solvent, the residue was subjected to distillation under reduced pressure using a Vigreux column and the allylic alcohol **3** (0.12 g, 52%) was collected as a colourless, viscous liquid; (bp 80-85°C / 1.7 mm Hg). ^1H NMR was the same as that given on p120.

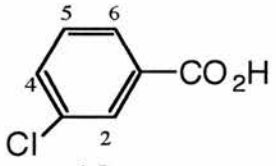
Epoxidation of α-(hydroxymethyl)methylenecyclopentane 3

α-(Hydroxymethyl)methylenecyclopentane **3** (0.21 g, 0.019 mol) was weighed into a three-necked flask containing dichloromethane (35 cm^3) and it was fitted with a thermometer, a condenser and a dropping funnel. The mixture was stirred and *m*-chloroperoxybenzoic acid (4.31 g, 0.025 mol) dissolved in dichloromethane (50 cm^3), was added to it slowly through the dropping funnel over 15 mins. The reaction was allowed to proceed for a further 30 mins. and the excess peroxy acid was decomposed by addition of sodium sulphite solution (10%) until the starch-iodide paper gave a negative

result. The organic layer was separated and washed with 5% sodium bicarbonate solution to extract the byproduct *m*-chlorobenzoic acid. This was followed by washing it with water (50 cm^3) and finally with saturated sodium chloride solution (50 cm^3). The separated dichloromethane layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The high-vacuum removal of the solvent gave the required spiroepoxy alcohol **17** (1.57 g, 65%) as a very pale yellow viscous liquid in a 2:1 ratio with *m*-chlorobenzoic acid **18** respectively.



17



18

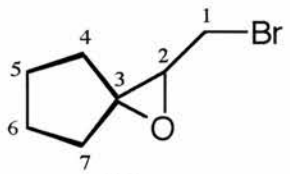
δ_H(200MHz, $CDCl_3$) 1.62(4H, m, CH_2-5,6), 1.80(4H, m, CH_2-4,7), 2.32(OH), 3.20(1H, dd, CH-2), 3.59(1H, dd, CH_2-1), 3.82(1H, dd, CH_2-1); Acid **18** 7.48(1H, t, CH-5), 7.68(1H, d, CH-6), 7.98(1H, d, CH-4), 8.06(1H, s, CH-2).

Bromination of 2-hydroxymethyl-1-oxa-spiro[2.4]heptane 17

The cyclopentylepoxy alcohol **17** (6.50 g, 0.05 mol) was added to a flask fitted with a nitrogen inlet and containing a magnetic stirrer. Under nitrogen gas, triethylamine (6.36 g, 0.06 mol) and dry dichloromethane (175 cm^3) were added to the reaction vessel which was maintained around -5°C. Methanesulphonyl chloride (5.38 g , 0.047 mol), contained in a dropping funnel, was slowly introduced into the reaction mixture that was continuously stirred during this addition and subsequently for 30 mins. The reaction was terminated by addition of water (145 cm^3) and the organic layer was separated and washed successively with 2M hydrochloric acid (200 cm^3), 5% brine (100 cm^3) and saturated sodium bicarbonate (200 cm^3). The resulting solution was dried over anhydrous sodium sulphate and concentrated at room temperature.

Lithium bromide (10.44 g, 0.120 mol) , dried under high vacuum with gentle periodic heating for 1h , was quickly added to continuously stirred acetone (125 cm^3) contained in a two-necked flask fitted with a nitrogen inlet. The cyclopentylepoxy

mesylate, diluted with dry acetone (10 cm^3), was gradually added to the reaction mixture with aid of a dropping funnel over 15 mins. The contents of the reaction vessel were stirred until no further precipitate appeared. The white solid was filtered off under suction and the filtrate was concentrated at a rotatory evaporator operated at room temperature. The residue was treated with water (90 cm^3) and the aqueous mixture was extracted with pentane (3x50 cm^3) which was completely driven off leaving a very pale yellow oily material. The latter was purifed under high vacuum distillation using a Kugelrohr over several hours to yield a pale yellow oily product, the 2-bromomethyl-1-oxa-spiro[2.4]heptane **19** (2.75 g, 31%); (bp 30°C / 0.10 mm Hg).



19

δ_H(300MHz, $CDCl_3$) 1.70(4H, q, CH_2-5,6), 1.95(4H, q, CH_2-4,7), 3.26(1H, dd, CH-2), 3.33(1H, dd, CH_2-1), 3.58(1H, dd, CH_2-1). The ^1H NMR and mass spectra showed the presence of unidentified impurities. GC-MS peak no. 637, retention time 11.69 min., spiroepoxy bromide **19**, m/z(%intensity) 190(1), 97(24), 83(39), 67(30), 55(100), 53(25), 41(89), 39(63), 29(37), 28(27), 27(99).

Conversion of Cyclopentanone to α-(ethoxycarbonyl)methylene-cyclopentane 1

Sodium hydride (7.87 g, 0.197 mol) dispersion in mineral oil (60% w/w) was rapidly added, under nitrogen gas to dry THF (350 cm^3). The resulting suspension was stirred and a concentrated solution of triethylphosphonoacetate (40.09 g, 0.179 mol) in dry THF (15 cm^3) was added to it at such a rate so as to regulate the temperature of the reaction around 30°C. Once the liberation of hydrogen had ceased (~2h) cyclopentanone (15.0 g, 0.179 mol) was introduced into the reaction flask, controlling the rate of its addition so that the temperature was again maintained at around 30°C. The progress of the reaction was followed with TLC on a half-hourly basis. After 2h, the reaction was

arrested by pouring the contents of the flask slowly and cautiously into a slurry of ice and water. Once the ice had melted, the aqueous layer was separated and extracted with diethyl ether (5x50 cm^3). These ethereal extracts were combined with the previously obtained organic layer and the entire solution was dried over anhydrous sodium sulphate and the solvents were stripped off on rotatory evaporator. The purified product, ethyl ester **1**, was obtained from the residue by distillation under reduced pressure using a Vigreux column, as a colourless liquid; (22.81 g, 83%); (bp 42-44°C / 0.3 mm Hg). δ_H(300MHz, $CDCl_3$) 1.25(3H, t, ^3J=7.1, CH_3-1), 1.66(2H, dq, ^2J=13.6, ^3J=7.0, CH_2-8), 1.75(2H, dq, ^2J=13.5, ^3J=6.9, CH_2-7), 2.44(2H, t, ^3J=7.1, CH_2-6), 2.76(2H, t, ^3J=7.0, CH_2-9), 4.14(2H, q, ^3J=7.1, CH_2-2), 5.78(1H, m, CH-4); δ_C(75MHz, $CDCl_3$) 14.4(CH_3, C-1), 25.5(CH_2, C-7), 26.5(CH_2, C-8), 32.7(CH_2, C-3), 35.9(CH_2, C-9), 59.4(CH_2, C-2), 111.7(CH, C-4), 166.9(1C, C-5), 169.0(CO_2, C-3); HRMS (EI) found M^+, 154.0997; $C_9H_{14}O_2$ requires M^+, 154.0994; MS m/z(relative intensity %) 154(M^+, 100), 126(65), 109(88), 79(65), 67(49), 53(27).

Selective reduction of α-(ethoxycarbonyl)methylenecyclopentane 1

α-(Hydroxymethyl)methylenecyclopentane **3** (13.88 g, 95%) was prepared as a colourless liquid in exactly the manner as described previously. The product conformed to the same analytical standards as reported earlier.

Epoxidation of α-(hydroxymethyl)methylenecyclopentane 3

Anhydrous sodium carbonate (13.36 g, 0.126 mol) was weighed into a three-necked flask and anhydrous dichloromethane (300 cm^3), dispensed under nitrogen gas, was added to the solid. The allylic cyclopentyl alcohol **3** (6.50 g, 0.058 mol) was introduced into this suspension which was continuously stirred. Peroxyacetic acid (12.61 g, 0.063 mol), diluted with dichloromethane (10 cm^3), was added dropwise into the reaction mixture over a period of 1h. After stirring the contents of the reaction flask for a further 1h, heating under reflux was commenced and the progress of the reaction was monitored by TLC on an hourly basis. After 1h of heating the reaction had gone to

completion and a gelatinous white precipitate had appeared. The reaction mixture was allowed to cool down to room temperature and the precipitated white solid was removed by suction filtration and washed with dichloromethane (3x50 cm^3). The filtrate was initially concentrated at the rotatory evaporator and then the residual solvent was removed under high vacuum. The product, 2-hydroxymethyl-1-oxa-spiro[2.4]heptane **17**, was obtained as a colourless, oily liquid; (6.75 g, 91%). δ_H(300MHz, $CDCl_3$) 1.62(4H, m, CH_2-5,6), 1.82(2H, m, CH_2-4), 1.92(2H, m, CH_2-7), 2.91(OH), 3.21(1H, dd, CH-2), 3.60(1H, dd, CH_2-1), 3.83(1H, dd, CH_2-1); δ_C(75MHz, $CDCl_3$) 25.4(CH_2, C-5), 25.8(CH_2, C-6), 29.8(CH_2, C-7), 34.4(CH_2, C-4), 62.2(CH, C-2), 63.0(CH_2OH, C-1), 70.1(1C, C-3); HRMS(EI) found M^+, 128.0840; $C_7H_{12}O_2$ requires M^+, 128.0837; MS m/z(relative intensity %) 128(M^+, 4), 111(20), 97(39), 85(100), 83(26), 67(98), 57(22), 55(40), 43(24), 41(56).

Bromination of 2-hydroxymethyl-1-oxa-spiro[2.4]heptane 17

Cyclopentylepoxy alcohol **17** (2.0 g, 0.016 mol), under nitrogen, was added to a flask containing a stirred mixture of triethylamine (1.97 g, 0.02 mol) and dry dichloromethane (55 cm^3). Having cooled the reaction vessel to around -5°C, methanesulphonyl chloride (1.72 g, 0.015 mol) was slowly added to it. The resulting solution was stirred for a further 30 min. before terminating the reaction by the addition of water (45 cm^3). The organic layer was separated and sequentially washed with 2M hydrochloric acid (65 cm^3), 5% brine (35 cm^3) and saturated sodium bicarbonate (65 cm^3). The solution obtained was dried over anhydrous sodium sulphate and freed of the solvent, at room temperature, under reduced pressure.

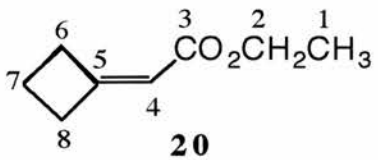
Dried lithium bromide (3.31 g, 0.038 mol), under nitrogen, was quickly added to stirred dry acetone (40 cm^3). The cyclopentylepoxy mesylate, diluted with dry acetone (5 cm^3) was gradually (15 min.) introduced into the reaction flask. This solution was refluxed under gentle heating until no more precipitate formed. After suitable cooling of the reaction mixture, the white solid was filtered off and the filtrate was concentrated, at room temperature, using a rotatory evaporator. The residue was treated with water

(30 cm^3) and this aqueous mixture was extracted with pentane (3x20 cm^3). The extracted solution was initially concentrated under reduced pressure and then completely freed of the solvent under high vacuum. The ensuing crude material, a pale yellow viscous liquid, was purified by slow micro-distillation at room temperature, under high vacuum to give a colourless, oily product 2-bromomethyl-1-oxa-spiro[2.4]heptane **19** (1.80 g, 63%). δ_H(300MHz, $CDCl_3$) 1.67(4H, q, CH_2-5,6), 1.92(4H, q, CH_2-4,7), 3.21(1H, dd, CH-2), 3.31(1H, dd, CH_2-1), 3.51(1H, dd, CH_2-1); δ_C(75MHz, $CDCl_3$) 25.0(2xCH_2, C-5,6), 28.5(CH_2, C-4), 31.1(CH_2, C-7), 33.4(CH_2, C-1), 60.0(CH, C-2), 71.6(1C, C-3); HRMS (CI) found M^++1, 191.0081; $C_7H_{12}O^{79}Br$ requires M^++1, 191.0073; MS m/z(relative intensity %) 193(98), 191(M^++1, 100), 175(77), 173(76), 129(15), 111(49), 93(36), 67(16), 61(35).

Conversion of cyclobutanone to α-(ethoxycarbonyl)methylene cyclobutane 20

A three-necked flask, equipped with a magnetic stirrer, a calcium chloride drying tube and a dropping funnel was charged with dry THF (300 cm^3). Sodium hydride (6.28 g, 0.157 mol) was rapidly added to the reaction vessel. The resulting suspension was stirred and a solution of triethylphosphonoacetate (32.03 g, 0.143 mol) in dry THF (15 cm^3) was added dropwise to this suspension over 45 min. period. The rate of addition was regulated such that the temperature of the stirred mixture did not rise above 30°C. After this addition was complete, the reaction mixture was stirred until hydrogen liberation had stopped. Cyclobutanone (10.0 g, 0.143 mol), diluted with THF (15 cm^3) was added to the flask while maintaining the temperature of the reaction mixture around 30°C. Immediately after the addition of the ketone, a sample of the solution was analysed by TLC and the progress of the reaction was subsequently monitored every 30 min. After 2h the reaction was arrested by pouring the contents of the flask slowly into a slurry of ice and water. Once the ice had melted the aqueous layer was separated and extracted with diethyl ether (3x50 cm^3). The ethereal extracts were combined the organic layer and this combined mixture was dried over anhydrous sodium sulphate and the solvents were

removed from it under reduced pressure. The purified product, α–(ethoxycarbonyl)methylene cyclobutane **20**, was obtained as a colourless liquid from the residual oil by distillation under vacuum using a Vigreux column (16.48 g, 82%); bp 48-50 °C / 1.0 mm Hg.



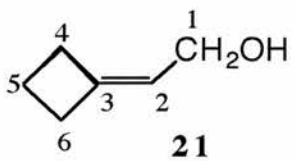
20

δ_H(300MHz, $CDCl_3$) 1.25(3H, t, ^3J=7.4, CH_3-1), 2.08(2H, q, ^3J=8.0, CH_2-7), 2.83(2H, t, ^3J=8.0, CH_2-6 or CH_2-8), 3.12(2H, t, ^3J=8.0, CH_2-6 or CH_2-8), 4.13(2H, q, ^3J=7.4, CH_2-2), 5.57(1H, m, CH-4); δ_C(75MHz, $CDCl_3$) 12.5(CH_2, C-7), 15.7(CH_3, C-1), 31.2(CH_2, C-6 or C-8), 37.1(CH_2, C-6 or C-8), 57.2(CH_2, C-2), 59.6(CH, C-4), 80.1(C, C-5), 172.5(CO_2, C-3); HRMS (EI) found M^+, 140.0843; $C_8H_{12}O_2$ requires M^+, 140.0837; MS m/z(relative intensity %) 140(M^+, 46), 112(80), 95(100), 84(23), 67(86), 55(31), 41(74).

α–(Hydroxymethyl)methylene cyclobutane 21

Ester **20** (15.0 g, 0.107 mol) was weighed into a flask fitted with a thermometer, a nitrogen inlet and a magnetic stirrer. Under nitrogen gas, dry THF (120 cm^3) was added to the ester and the mixture was stirred. DIBAL (45.58 g, 0.321 mol) was added to the reaction flask, at hourly intervals, in small aliquots (30 cm^3) and the progress of the reaction was monitored by TLC. After the last addition of the reducing agent, the reaction mixture was stirred for another 60 min. Finally, the excess reagent was decomposed by the slow addition of methanol-THF (40 cm^3/80 cm^3 resp.) to the reaction mixture. This hydrolysis was fully completed by the dropwise addition of water (25 cm^3), with cooling, to the reaction vessel. The precipitated aluminium salts were filtered off under suction and washed with methanol (3x50 cm^3). All washings were combined with the initial filtrate. After drying the methanolic solution over anhydrous sodium sulphate, it was concentrated at the rotatory evaporator and the remaining solvents were removed by

distillation at atmospheric pressure. The residue was subjected to vacuum-distillation using a Vigreux column and a colourless liquid was collected within the boiling range 39-48°C / 0.9 mm Hg. The product, α-(hydroxymethyl)methylene cyclobutane **21**, (5.4 g, 36%), a colourless liquid, was isolated from the distillate by column chromatography on silica and using EtOAc/petroleum ether mixture (2:8, v/v resp.) as the eluent.

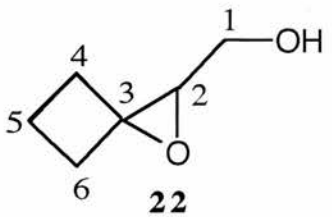


21

δ_H(300MHz, $CDCl_3$) 1.99(2H, q, ^3J=7.8, CH_2-5), 2.10(OH), 2.70(4H, m, ^3J=7.8, CH_2-4,6), 4.01(2H, d, CH_2OH-1), 5.32(1H, m, CH-2); δ_C(75MHz, $CDCl_3$) 17.2(CH_2, C-5), 29.3(CH_2, C-4 or C-6), 31.1(CH_2, C-4 or C-6), 59.3(CH_2OH, C-1), 119.3(CH, C-2), 145.1(1C, C-3); HRMS (EI) found M^+, 98.0734; $C_6H_{10}O$ requires M^+, 98.0732; MS m/z(relative intensity %) 98(M^+, 10), 83(24), 79(43), 70(100), 69(45), 67(20), 55(35), 53(24), 41(72).

2-Hydroxymethyl-1-oxa-spiro[2.3]hexane 22

Continuously stirred anhydrous dichloromethane (100 cm^3), contained in a three-necked flask, had anhydrous sodium carbonate (0.98 g, 0.01 mol) added to it. After the addition of the allylic cyclobutyl alcohol **21** (1.20 g, 0.012 mol), peroxyacetic acid (1.07 g, 0.014 mol) was added dropwise to the reaction flask over a period of 15 min. The resulting mixture was stirred for 1.5h after which period it was refluxed. TLC analysis performed on the reaction mixture after 5.5h of heating indicated the complete absence of the starting material. After cooling, the crude material was concentrated under reduced pressure followed by the removal of the residual solvent and/or byproduct under high vacuum. This treatment gave the product, 2-hydroxymethyl-1-oxa-spiro[2.3]hexane **22** as a pale yellow viscous liquid; (1.18 g, 86%).



22

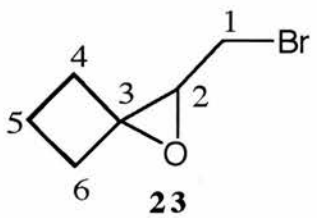
δ_H(300MHz, $CDCl_3$) 1.85(2H, m, CH_2-5), 2.32(2H, m, CH_2-4), 2.50(2H, m, CH_2-6), 2.86(OH), 3.07(1H, m, ^3J=6.1, ^3J=3.7, CH-2), 3.53(1H, dd, ^2J=12.2, ^3J=6.1, CH_2-1), 3.82(1H, dd, ^2J=12.2, ^3J=3.7, CH_2-1); δ_C(75MHz, $CDCl_3$) 13.2(CH_2, C-5), 28.8(CH_2, C-4), 31.2(CH_2, C-6), 61.0(CH, C-2), 62.1(CH_2OH, C-1), 63.9(1C, C-3); HRMS (CI) found M^++1, 115.0764; $C_6H_{11}O_2$ requires M^++1, 115.0759; MS m/z(relative intensity %) 115(M^++1, 54), 99(23), 97(100).

2-Bromomethyl-1-oxa-spiro[2.3]hexane 23

Cyclobutylepoxy alcohol **22** (1.01 g, 8.86 mmol), was added to a three-necked flask. Under nitrogen gas, triethylamine (1.09 g, 11.0 mmol) and dry dichloromethane (30 cm^3) were also placed in the reaction vessel and the mixture was continuously stirred while being surrounded by an ice-salt bath. Methanesulphonyl chloride (0.93 g, 8.14 mmol) was added slowly to the reaction mixture which was stirred for a further 30 min. Following the addition of water (25 cm^3), the dichloromethane layer was separated and washed successively with 2M hydrochloric acid (35 cm^3), 5% brine (20 cm^3) and saturated sodium bicarbonate solution (35 cm^3). In order to obtain the cyclobutylepoxy mesylate, the resulting solution was dried over anhydrous sodium sulphate and the dichloromethane was stripped off, at room temperature, on a rotatory evaporator.

The mesylate was placed in a two-necked flask containing dry acetone (25 cm^3). The mixture was continuously stirred and dried lithium bromide (1.81 g, 20.80 mmol) was introduced into the flask the contents of which were refluxed until all the lithium mesylate had precipitated out of the solution as a white solid. The latter was filtered off and the acetone was removed from the filtrate under reduced pressure, at room temperature, using a rotatory evaporator. The residue was treated with water (15 cm^3) and this aqueous mixture was extracted with pentane (3x15 cm^3). The pentane was

initially evaporated and residual amounts were removed under high vacuum. The very pale yellow liquid was further purified by high vacuum distilation, at room temperature, using a specially designed micro-distillation apparatus. The 2-bromomethyl-1-oxa-spiro[2.3]hexane **23** was obtained as a colourless liquid; (1.09, 76%).

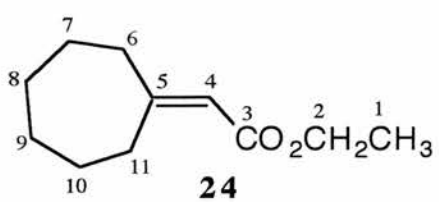


23

δ_H(300MHz, $CDCl_3$) 1.90(2H, m, CH_2-5), 2.44(1H, t, CH_2-4), 2.55(1H, t, CH_2-6), 3.05(1H, dd, ^3J=10.0, ^3J=7.5, CH-2), 3.17(1H, dd, ^3J=7.5, ^2J=5.4, CH_2-1), 3.45(1H, dd, ^3J=10.0, ^2J=5.4, CH_2-1); δ_C(75MHz, $CDCl_3$) 12.9(CH_2, C-5), 28.0(CH_2, C-1), 30.7(CH_2, C-4), 31.0(CH_2, C-6), 59.2(CH, C-2), 65.7(C, C-3); HRMS (CI) found M^++1, 176.9921; $C_6H_{10}O^{79}Br$ requires M^++1, 176.9915; MS m/z(relative intensity %) 179(10), 177(M^++1, 11), 137(5), 113(12), 112(17), 111(10), 99(32), 97(100).

Conversion of Cycloheptanone to Ester 24

Ester **24** was prepared as a colourless liquid; (27.3 g, 91%); (bp 42-46°C / 0.02 mm Hg); from cycloheptanone (18.47 g, 0.165 mol) in exactly the same way as described for the preparation of ester **20**.



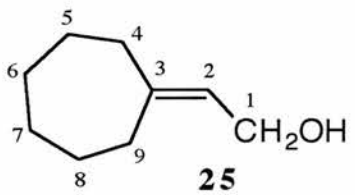
24

δ_H(300MHz, $CDCl_3$) 1.25(3H, t, ^3J=7.1, CH_3-1), 1.54(4H, m, CH_2-8,9), 1.68(4H, m, CH_2-7,10), 2.38(2H, t, ^4J=1.2, ^3J=6.0, CH_2-6), 2.86(2H, t, ^4J=1.2, ^3J=6.0, CH_2-11), 4.14(2H, q, ^3J=7.1, CH_2-2), 5.67(1H, m, ^4J=1.2, CH-4); δ_C(75MHz, $CDCl_3$) 14.4(CH_3, C-1), 26.6(CH_2, C-8), 28.0(CH_2, C-9), 29.0(CH_2, C-7), 29.8(CH, C-10), 32.1(CH_2, C-6), 39.0(CH_2, C-11), 59.3(CH_2, C-2), 115.6(CH, C-4), 130.2(1C, C-5), 166.7(CO_2, C-3); HRMS (CI) found M^++1, 183.1392; $C_{11}H_{19}O_2$ requires M^++1,

183.1385; MS m/z(relative intensity %) 183(M^++1, 98), 113(4), 137(5), 112(4), 99(4), 97(6).

α-(Hydroxymethyl)methylene cycloheptane 25

The methodology described for **21** was applied to selectively reduce the unsaturated ester **24** (51.68 g, 0.284 mol) to yield a viscous, colourless liquid α-(hydroxymethyl)-methylenecycloheptane **25** (32.6 g, 82%).

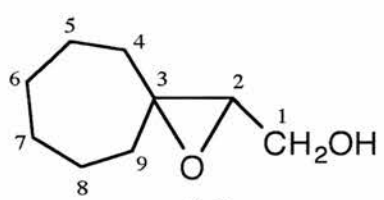


25

δ_H(300MHz, $CDCl_3$) 1.56(8H, m, CH_2-5,6,7,8), 2.26(4H, m, CH_2-4,9), 4.14(2H, d, CH_2-1), 5.40(1H, t, CH-2); δ_C(75MHz, $CDCl_3$) 28.0(CH_2, C-6), 29.5(CH_2, C-7), 29.6(CH_2, C-5), 30.4(CH_2, C-8), 30.6(CH_2, C-4), 38.4(CH_2, C-9), 59.8(CH_2, C-1), 124.5(CH, C-2), 146.3(C, C-3); HRMS (EI) found M^+, 140.1206; $C_9H_{16}O$ requires M^+, 140.1201; MS m/z(relative intensity %) 140(M^+, 14), 122(86), 107(31), 96(60), 83(54), 81(100), 70(64), 67(92), 57(56), 55(82), 41(92).

2-Hydroxymethyl-2-oxa-spiro[2.6]nonane 26

This transformation was accomplished in the same manner as described for **22** to give the 2-hydroxymethyl-2-oxa-spiro[2.6]nonane **26** (9.23 g, 87%) as a colourless oily liquid.



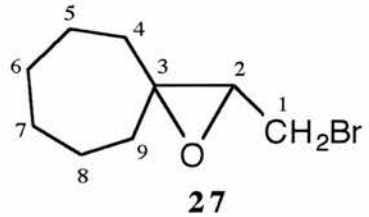
26

δ_H(300MHz, $CDCl_3$) 1.55(8H, m, CH_2-5,6,7,8), 1.70(4H, m, CH_2-4,9), 2.70(OH), 2.97(1H, dd, CH-2), 3.65(1H, dd, CH_2-1), 3.83(1H, dd, CH_2-1); δ_C(75MHz, $CDCl_3$) 25.0(CH_2, C-6), 25.3(CH_2, C-7), 29.4(CH_2, C-5), 29.5(CH_2, C-8), 32.1(CH_2, C-4),

38.1(CH_2, C-9), 61.9(CH_2, C-1), 65.4(CH, C-2), 65.5(1C, C-3),; HRMS (CI) found M^++1, 157.1231; $C_9H_{17}O_2$ requires M^++1, 157.1229; MS m/z(relative intensity %) 157(M^++1, 8), 139(20), 121(26), 113(100), 95(62), 81(37), 67(46), 55(46), 43(38), 41(46).

2-Bromomethyl-1-oxa-spiro[2.6]nonane 27

This reaction was accomplished using the procedure described for the analogous transformation of the cylobutylepoxy alcohol to the corresponding bromide **23**. The 2-bromomethyl-1-oxa-spiro[2.6]nonane **27** after purification, was obtained as a colouless liquid; (1.13 g, 87%).



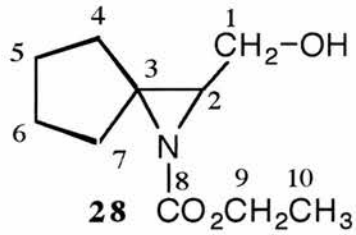
27

δ_H(300MHz, $CDCl_3$) 1.59(8H, m, CH_2-5,6,7,8), 1.74(4H, m, CH_2-4,9), 3.06(1H, dd, ^2J=6.0, ^3J=7.6, CH_2-1), 3.26(1H, dd, ^3J=7.6, ^3J=10.4, CH_2-2), 3.52(1H, dd, ^2J=6.0, ^3J=10.4, CH_2-1); δ_C(75MHz, $CDCl_3$) 24.3(CH_2, C-6), 24.7(CH_2, C-7), 28.9(CH_2, C-5), 29.0(CH_2, C-8), 29.9(CH_2, C-4), 30.9(CH_2, C-9), 37.0(CH_2, C-1), 63.1(CH, C-2), 66.6(1C, C-3); HRMS (EI) found M^+, 218.0300; $C_9H_{15}O^{79}Br$ requires M^+, 218.0306; MS m/z(relative intensity %) 218(M^+, 6), 125(100), 121(33), 107(17), 95(37), 81(74), 67(29), 55(41), 41(26).

Conversion of α-(hydroxymethyl)methylenecyclopentane 3 to the corresponding aziridine

Cyclopentyl allylic alcohol **3** (0.50 g, 4.46 mmol) was weighed into a 1 cm diameter tube made from quartz. Ethyl azidoformate (1.60 g, 13.89 mmol) was introduced into the tube. The reaction mixture was photolysed with a 400 W medium pressure mercury arc lamp. The contents of the tube were continuously stirred during its exposure to UV light. The reaction was terminated when the evolution of nitrogen gas from the solution had stopped (24 h). The reaction mixture was chromatographed on an

alumina column and eluted with diethyl ether. However, ^{1}H NMR analysis showed the product to be absent from any of the collected fractions which consisted of byproducts from the reagent and the unreacted starting material. The column was treated with methanol (200 cm^3) and the solvent was removed from the collected fractions to obtain a white solid. This product was recrystallised from methanol. Because of the unsatisfactory purification, a repeat recystallisation of the solid was attempted from methanol with dropwise addition of dichloromethane upon cooling of the hot methanolic solution. The product, spiroaziridine **28** was finally obtained as an off-white solid; (0.11 g, 21%).



28

δ_H(200MHz, $CDCl_3$) 1.25(3H, t, CH_3-10), 1.59(4H, m, CH_2-5,6), 1.70(4H, m, CH_2-4,7), 3.92(2H, q, CH_2-9), 4.05-4.25(3H, d/dd, CH_2/CH-1,2); GC-MS peak no. 989, retention time 18.08 min., spiroaziridine **28**, m/z(relative intensity %) 153(49), 125(34), 95(28), 94(100), 80(42), 67(67), 53(23), 41(78), 39(73), 28(56).

Attempted conversion of ester 1 to the corresponding aziridine

Ester **1** (0.50 g, 3.25 mmol) dissolved in ethyl azidoformate (1.16 g, 10.12 mmol) was photolysed in an identical manner to the one described in section 3.3.1 for 36h. Separation on an alumina column with diethyl ether as the eluent followed by analysis of the fractions, showed the presence of the unreacted starting ester and the byproducts from the photochemical reactions of the reagent.

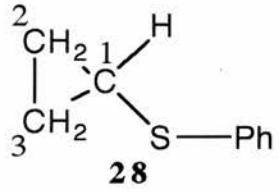
Formation of 3-phenylthiyl-1-chloropropane

To a mechanically stirred solution of sodium hydroxide (80.0 g, 2.0 mol), successively, 1-bromo-3-chloropropane (314.8 g, 2.0 mol) and thiophenol (220.4 g, 2.0 mol) were added gradually. The reaction mixture was refluxed for 6h, cooled to room

temperature, and extracted with diethyl ether (3x150 cm^3). The combined ethereal extracts were washed with water (3x100 cm^3) and dried over anhydrous sodium sulphate. After removal of the solvent, the residue was subjected to distillation under reduced pressure. The product, 3-phenylthiyl-1-chloropropane (304.2 g, 81%) was isolated as a colourless liquid; (bp 90-96°C / 0.1 mm Hg, lit. 110-111°C / 1.6 mm Hg[30]); δ_H(200MHz, $CDCl_3$) 2.20(2H, q, $CH_2\underline{CH_2}CH_2$), 3.09(2H, t, $Cl\underline{CH_2}CH_2$), 3.69(2H, t, $\underline{CH_2}SPh$), 7.18-7.44(5H, m, Ph).

Cyclisation of the phenylthiyl derivative of chloropropane

Potassium (17.4 g, 0.45 mol) was added in small pieces to a rapidly stirred solution of hydrated iron(III)nitrate (1.0 g, 0.003 mol) in liquid ammonia (500 cm^3) placed in a flask equipped with a mechanical stirrer, a dropping funnel and a drying tube of anhydrous calcium chloride. The phenylthiyl derivative (38.0 g, 0.21 mol) mixed with dry diethyl ether (500 cm^3) was slowly added to the reaction flask. Ammonia was allowed to evaporate from the reaction mixture which was then refluxed for 3h, cooled to room temperature, hydrolysed with wet diethyl ether (3x50 cm^3) followed by water (3x50 cm^3). The precipitated iron salts were filtered and washed with diethyl ether (50 cm^3). The initial filtrate was combined with this washing and the organic layer was separated from the aqueous layer which was further extracted with diethyl ether (3x100 cm^3). The ether solutions were combined and washed with water (100 cm^3), separated, dried over anhydrous calcium chloride and concentrated under reduced pressure. The residue was distilled under vacuum to give the cyclised product cyclopropylphenyl sulphide **28** (26.9 g, 85%) as a colourless liquid; (bp 60-66°C / 0.9 mm Hg, lit. 62-63°C / 1.0 mm Hg[30]).

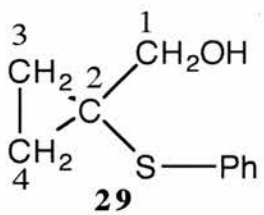


28

δ_H(200MHz, $CDCl_3$) 0.75(2H, m, CH_2-2), 1.14(2H, m, CH_2-3), 2.23(1H, m, CH-1), 7.17-7.50(5H, m, Ph).

Formation of 1-hydroxymethyl-1-phenylthiocyclopropane

Cyclopropylphenyl sulphide **28** (25.0 g, 0.17 mol) was added to a three-necked flask fitted with a thermometer, a nitrogen inlet and a pressure equalising funnel. Dry tetrahydrofuran (100 cm^3) was also placed in the flask and the resulting solution was stirred at 0°C. n-Butyllithium (133 cm^3, 0.20 mol) in hexane was added to this solution over 1h period and the resulting mixture was stirred for 2h at 0°C and then allowed to warm up to room temperature for a further 2h. Paraformaldehyde (5.80 g, 0.19 mol) was added, in small quantities, over 30 min. After stirring this solution for 1h, the reaction was quenched with saturated aqueous ammonium chloride (50 cm^3). The aqueous layer was separated and extracted with ethyl acetate (2x50 cm^3). The combined organic portions were washed with saturated aqueous sodium chloride (50 cm^3) and dried over anhydrous sodium sulphate. The solvents were removed under reduced pressure and the crude residue was purified by distillation using a Vigreux column. The hydroxy product **29** was obtained as a viscous pale yellow liquid (19.8 g, 82%); (bp 131-136°C / 0.6 mm Hg, lit., 98-102°C / 0.2 mm Hg[30]).



29

δ_H(200MHz, $CDCl_3$) 1.04(4H, m, CH_2-3,4), 2.75(OH), 3.53(2H, s, CH_2-1), 7.18-7.46(5H, m, Ph).

Ring expansion of 1-hydroxymethyl-1-phenylthiocyclopropane

1-Hydroxymethyl-1-phenylthiocyclopropane **29** (37.0 g, 0.21 mol), tetralin (52 cm^3), mercury(II) chloride (32.5 g, 0.12 mol), water (4.30 g, 0.20 mol) and *p*-toluenesulphonic acid (3.90 g, 0.38 mol) were added to a three-necked flask equipped with a nitrogen inlet, a Vigreux column and a magnetic stirrer. The stirred suspension was heated to 70°C for 1.5h and the temperature of the reaction mixture was raised to 120°C and a two-layered distillate was collected over a 2h period. The aqueous layer was separated and extracted with dichloromethane (3x5 cm^3). The combined organic portions

were dried over anhydrous sodium sulphate and the residue was distilled at atmospheric pressure through a Vigreux column. Cyclobutanone **30** was obtained as a colourless liquid (7.64 g, 52%); (bp 97-100°C, lit. 97-99°C[31]). δ_H(200MHz, $CDCl_3$) 1.95(2H, q, $\underline{CH_2}(CH_2)_2$), 3.10(4H, t, $(\underline{CH_2})_2CO$).

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CHAPTER 4

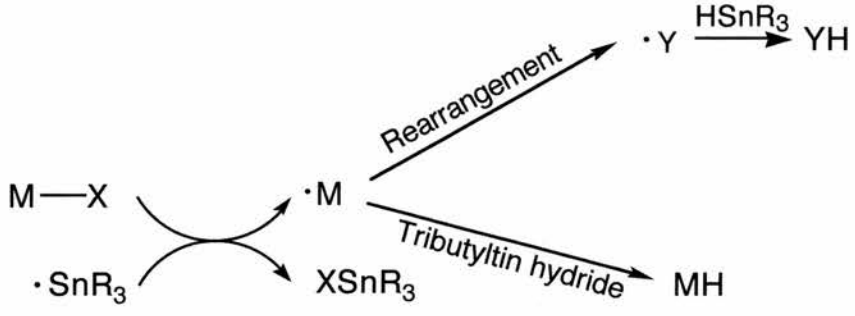
Ring Expansion Via Spiroepoxycarbinyl Radicals: A Cascade Rearrangement Yielding Cycloalkanones

INTRODUCTION

4.1 Free Radical Rearrangement Reactions

Modern methods of generating carbon-centred radicals, in a controlled way, have led to a number of useful approaches in employing these radicals in rearrangement reactions to form a variety of valuable organic compounds[1]. One of the advantages of their use in this context is the fact that, unlike some of their polar counterparts, carbocations and carbanions, they do not participate in alkyl shifts or display nucleophilicity respectively.

The outcome of these reactions is strongly dependent on the relative concentration of the reagents because, generally, a range of competing processes is available to individual radicals. Use of a stannane is commonplace for forming carbon-centred radicals. If the direct reduction product is the aim of the reaction, then the appropriate procedure is to add the substrate to a neat or concentrated solution of a reactive reagent e.g. triphenyltin hydride. Alternatively, if the rearranged product is the object of the exercise, then slow addition of tributyltin hydride to a dilute solution of the starting material is the favoured method (**Scheme 1**). Other methods of directing the reactants along the required pathways have involved the use of silicon[2] and germanium[3] hydrides in which the metal-hydrogen bonds are stronger than the tin-hydrogen bonds in stannanes.



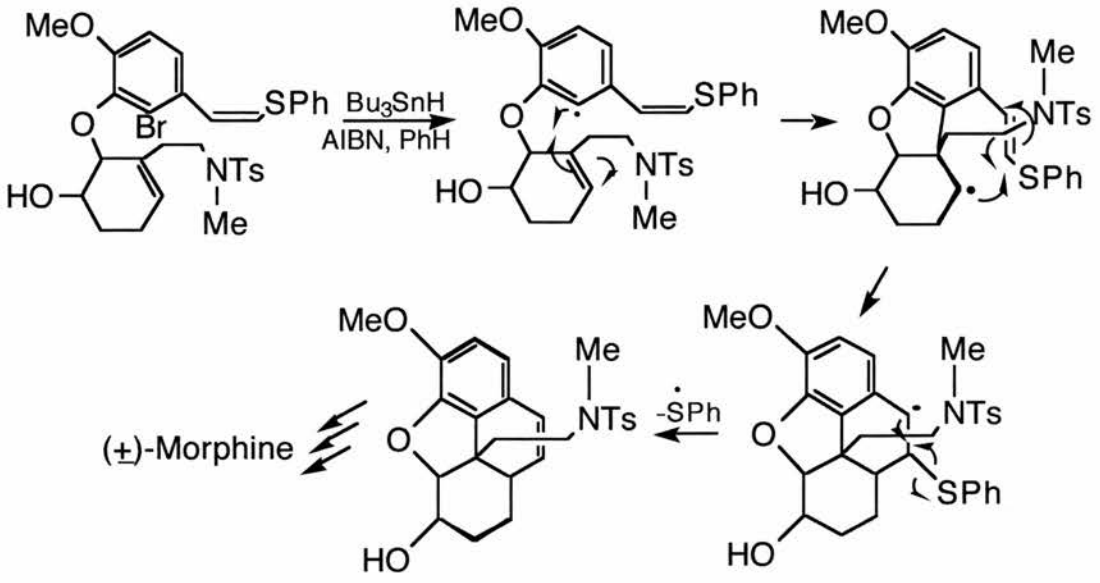
Scheme 1: Alternative pathways for stannane-generated carbon-centred radicals.

4.2 Radical-mediated Cascades

A cascade or a tandem reaction results from initially formed intermediates that are capable of undergoing sequential transformations involving elementary steps either intermolecularly or intramolecularly. These reactions are commonly classified according to the nature of the primary step while their kinetics can be unimolecular and/or bimolecular. Cascades initiated with radicals are characterised by a combination of cyclisation, 1,2-aryl migration, 1,5-hydrogen transfer and β–scission as the elementary steps. These occur in a given order which is specified by the molecular structure and the spatial disposition of the functional groups in the intermediates. The synthetic potential of such reactions has been exploited in establishing carbon-carbon bonds. Thus, multiple cyclisation sequences for the assembly of polycyclic compounds have received the greatest attention in this field of chemistry[4-9]. In contrast, cascades involving several β–scissions usually lead to a complete reorganisation of the molecular structure of the starting material. This has been well illustrated by the comprehensive degradation of the cage-like cubylcarbinyl radical to the bis-cyclobutenyl structure by a series of three β–scissions[10-12].

The initial radical precursors are usually formed from a halide in conjunction with a stannane and azobis-isobutyronitrile (AIBN) or a peroxide in aromatic solvents. Radical-mediated tandem reactions enjoy a particularly high profile in the chemical literature because of the distinctive advantages they offer over ionic methods of effecting one-pot sequential syntheses. For example, the initial radicals are formed under mild conditions, the gentle reaction conditions tolerate many functionalities in the substrates, the use of protecting groups is avoided and the radicals are able to add to unactivated double/triple bonds as well as to those with polarising groups. The initial stages in a multi-step synthesis of the commercially important pharmaceutical agent, morphine, involve a radical-mediated cascade rearrangement in which two cyclisations are followed

by a β–scission to form the precursor that is subsequently converted to the desired product[13] (**Scheme 2**).



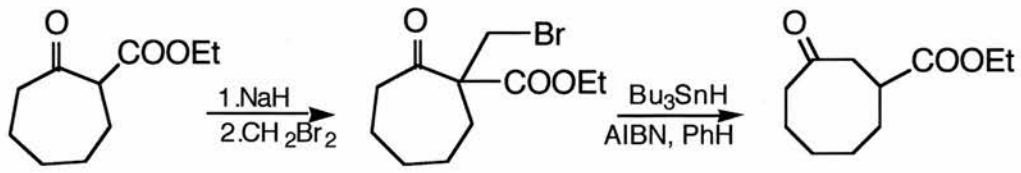
Scheme 2: Radical-initiated intramolecular cascade leading to the synthesis of morphine.

4.3 Radical-initiated Ring Expansions

Ring enlargements via ionic methods are of importance in the synthesis of medium-sized rings and the topic has been reviewed recently by Hesse[14]. Advances in new methodologies encompassing both chain insertions and ring annulations, have made radical-initiated ring expansions increasingly significant. Well established methods will be briefly considered below.

4.3.1 Ring expansion of β–keto esters

This method takes advantage of the fact that β–keto esters are readily available from Dieckmann condensations and that they are easily alkylated. Treatment of the bromomethyl derivatives with tributyltin hydride in refluxing benzene leads to one-carbon ring expansion[15,16] (**Scheme 3**).



Scheme 3: Ring expansion of a β–keto ester by one carbon atom.

The presence of the ester group is important because it directs β–scission of the intermediate cyclopropyloxyl radical towards the ring expansion mode.

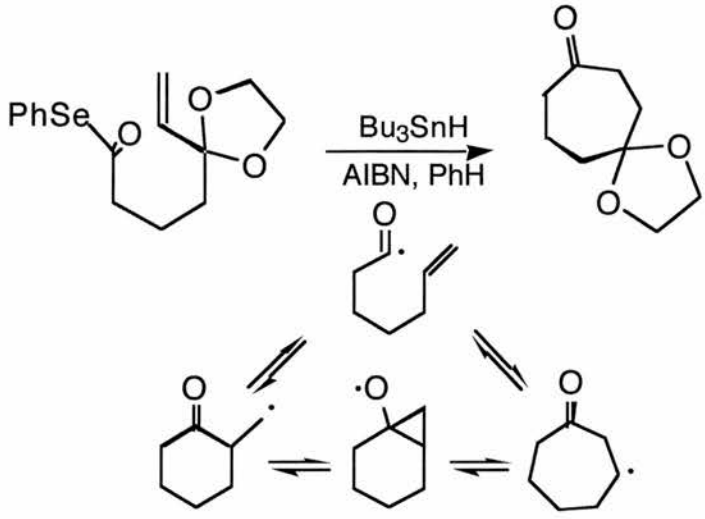
4.3.2 Rearrangement of fused cyclobutanones

Radical-based ring expansion of cyclobutanones has been achieved through the intramolecular attack of a side chain radical[17] (**Scheme 4**).

Scheme 4: Ring expansion from the rearrangement of a fused cyclobutanone.

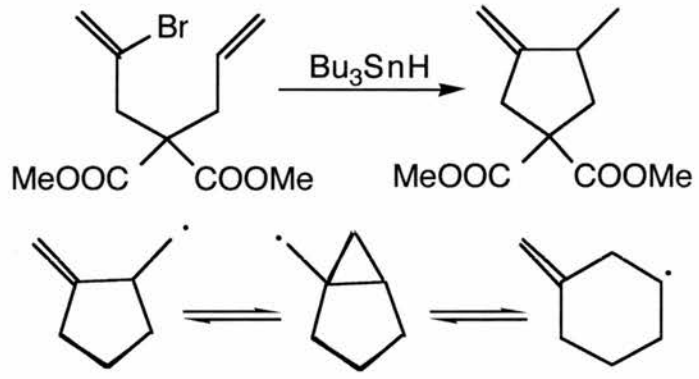
4.3.3 Cyclisation of acyl and vinyl radicals

Acyl radicals undergo intramolecular addition to terminal alkenes in the *exo*-mode[18]. Reported cases of *endo*-cyclisation may be explained by the one-carbon ring expansion that follows the initial *exo*-cyclisation[19] (**Scheme 5**).



Scheme 5: *Exo*-cyclisation of acyl radicals.

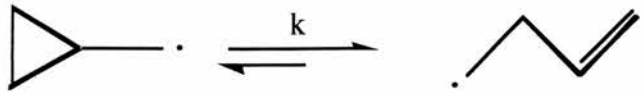
Beckwith[20] has shown that vinyl radicals react in a comparable way. At high concentration of stannane, 5-*exo* is the only product whereas at low hydride concentration the 5-*exo* cyclised radical rearranges by one-carbon ring expansion to the thermodynamically more favourable 6-*endo* product[21] (**Scheme 6**).



Scheme 6: Cyclisation of vinyl radicals.

4.3.4 Fragmentation of cyclopropylcarbinyl radicals

Cyclopropylcarbinyl radicals are easily ring-cleaved and this has proved to be a useful way to enlarge carbocyclic compounds[22]. Work on bicyclo radicals has revealed that there is a preference for stereoelectronic control on the ring opening[23] (**Equation 1**).



1

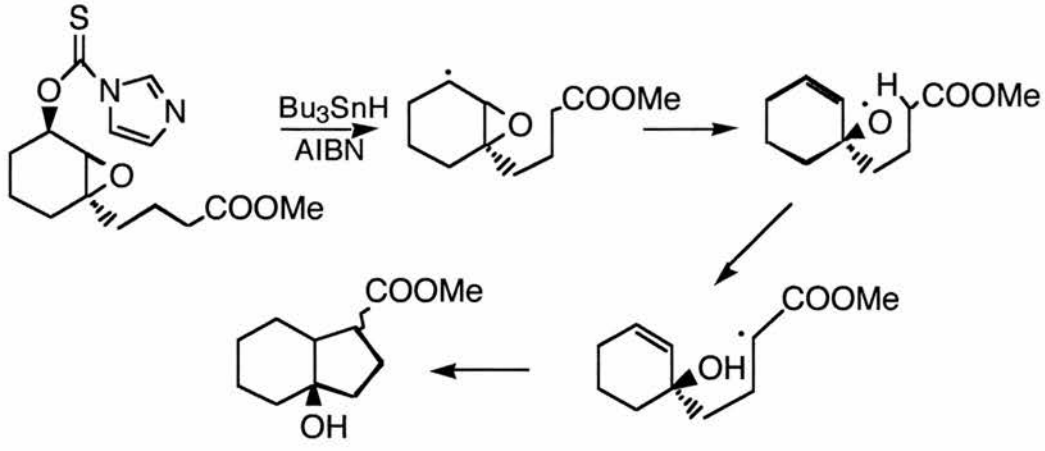
$k = 1.3 \times 10^8\ s^{-1}$ at 298 K

4.3.5 Fragmentation of cyclobutylcarbinyl radicals

Walton and Ingold[24] have demonstrated the rapid ring opening of cyclobutylcarbinyl radicals and this has been exploited to provide another approach to ring expansion[25].

4.3.6 1,5-Hydrogen transfer promoted ring expansion

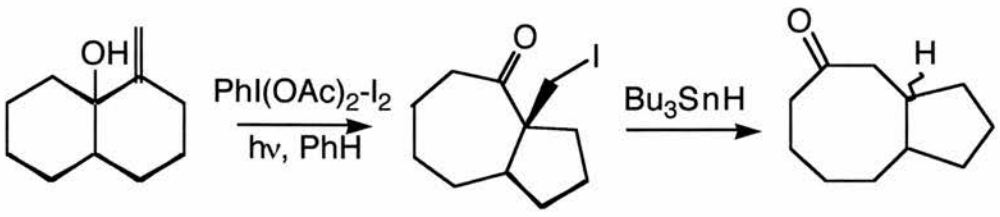
Bicyclic alcohols have been formed from combining an intramolecular hydrogen transfer with an epoxide fragmentation. Intermediate radicals are generated which then add to a suitably disposed unsaturated centre in them[26](**Scheme 7**).



Scheme 7: Formation of a bicyclic alcohol promoted by 1,5-hydrogen shift.

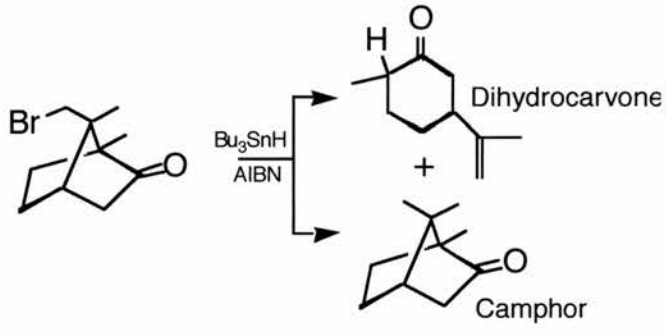
4.3.7 β–Scission of alkoxyl radicals

Numerous workers have made use of the high reactivity of alkoxyl radicals, available by a variety of methods, to accomplish tandem fragmentation-transannular addition to produce polycyclic structures. Pattenden has applied this methodology particularly effectively to form polycyclic ketones[27] (**Scheme 8**).



Scheme 8: Alkoxyl radical fragmentation to effect ring expansion.

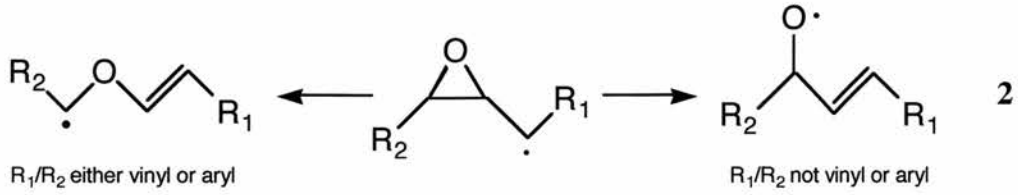
Normally a combination of strain-relief together with the formation of a particularly stabilised radical are the requirements for carbon-carbon bond cleavage adjacent to a carbon-centred radical. Radical-fragmentation of 8-bromobornan-2-one to give dihydrocarvone, under low stannane concentration, serves to illustrate these considerations[28] (**Scheme 9**). At high concentration of the tin hydride the main product is camphor.



Scheme 9: Radical-initiated rearrangement of 8-bromobornan-2-one.

4.4 Ring Opening of Epoxides

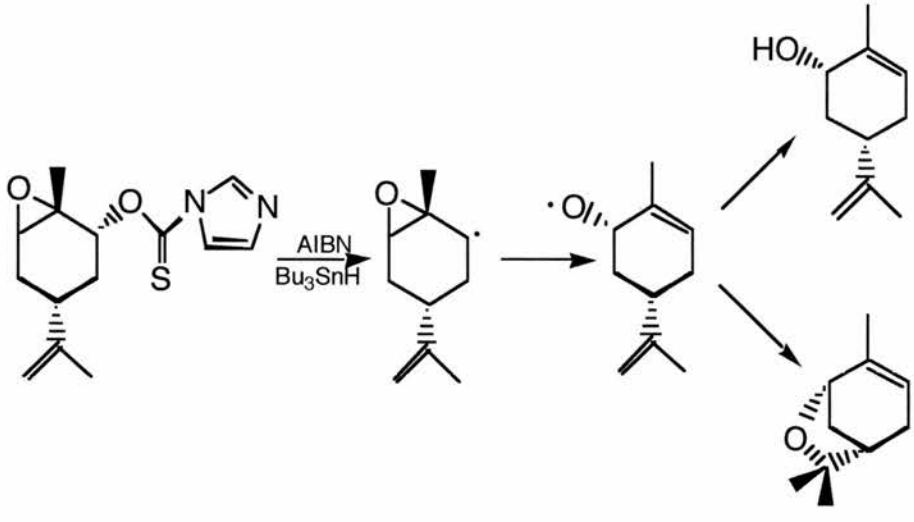
Ring opening of an epoxide by an adjacent carbon-centred radical is the equivalent process to the fragmentation of cyclopropylcarbinyl radical. Both are extremely fast but the former differs in that either carbon-oxygen or carbon-carbon bond cleavage may be observed (**Equation 2**).



2

Carbon-oxygen bond cleavage leads to the formation of an alkoxyl radical which is capable of undergoing further rearrangement by cyclisation, β–scission or hydrogen transfer. Generally, in the absence of substituent effects which form particularly stabilised carbon-centred radicals, cleavage of the weaker carbon-oxygen bond predominates. Hence, carbon-centred radicals adjacent to an epoxide may be considered as precursors of allylic alkoxyl radicals[29].

Barton has utilised the high propensity of an alkoxyl radical to effect its intramolecular cyclisation and form a strained tetrahydrofuranyl derivative[30] (**Scheme 10**).

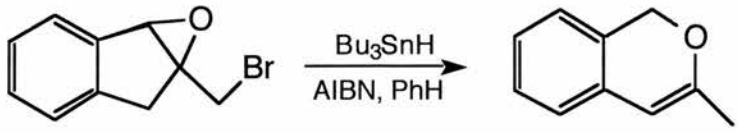


Scheme 10: Formation of a strained pinol from an oxiranylcarbinyl radical.

Similarly, Murphy has extended this method in a tandem cyclisation from open chain precursors to produce trisubstituted tetrahydrofurans with promising diastereoselectivity[31].

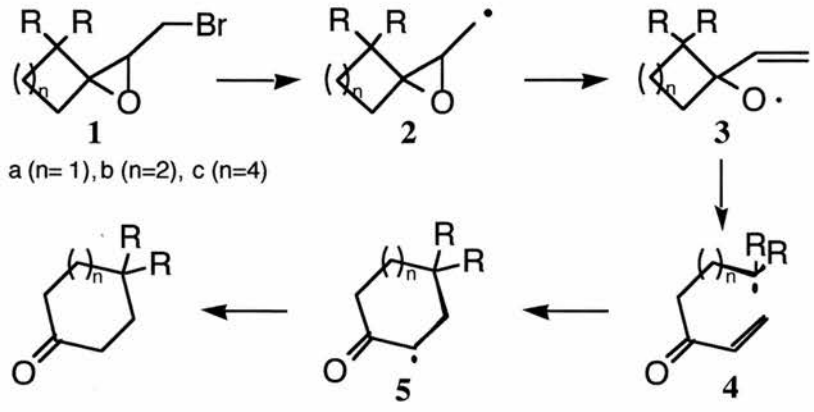
In complete contrast to the behaviour of acyl substituted epoxides, carbon-carbon bond fission has been shown to occur in cases where the final carbon-centred radical is

resonance stabilised[32]. A recent example of ring expansion involving this type of fragmentation from a ring-fused epoxide is shown below[33].



4.5 Cascade Rearrangement of Spiroepoxides

Certain β–scissions, notably those of alkoxyl radicals which yield ketones, do not require excessive ring strain as the driving force[34]. In principle, therefore, this fragmentation could be combined with a number of elementary steps forming a cascade rearrangement leading to a potentially valuable structural reorganisation in a suitable starting material. Spiroepoxides of type **1** could act as precursors to simple epoxycarbinyl radicals **2** which are known to undergo very rapid β–scission[35,36] of the carbon-oxygen bond[37] to yield 1-vinylcycloalkoxyl radical **3**. This intermediate is expected to rearrange selectively furnishing the more stabilised 3-oxoalkenyl radical **4**, in preference to the scission of either of the other two β–bonds. Cyclisation of radical **4** can occur in two ways. The *exo*-mode ring closure gives 2-oxocycloalkanylcarbinyl radical whereas the *endo*-ring closure leads to the formation of 2-oxocycloalkyl radical **5** (**Scheme 11**). In order to test the viability of this intriguing cascade, spiroepoxy bromides **1a-c** were subjected to photo-reduction with stannanes in aromatic solvents under different conditions of temperature and reagent concentrations.



Scheme 11: Projected series of reactions from fragmentation of spiroepoxy bromides.

4.6 Objectives of this Study

The main aims of this work were:

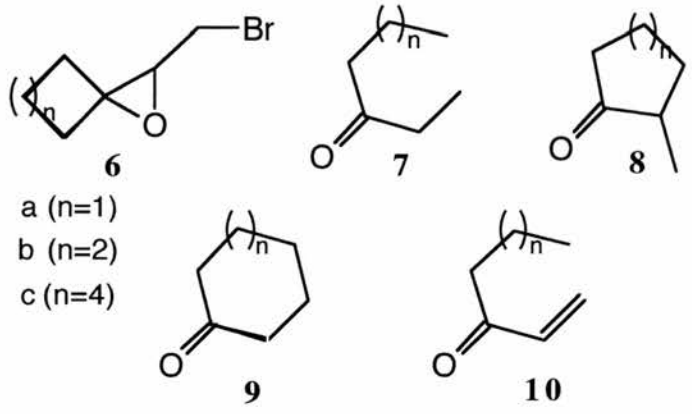
- To generate archetype epoxycarbinyl radicals from photo-induced fragmentation of spiroepoxy bromides lacking any substituents in their cycloalkyl rings
- To assess the type of β–bond cleaved in the oxiranyl ring
- To incorporate this β–scission into an intramolecular rearrangement
- To demonstrate the propensity of the cycloalkylepoxycarbinyl radicals to undergo a 3-stage cascade consisting two β–scissions and a cyclisation
- To obtain experimental evidence for the cascade by determining the rate-limiting step from kinetic studies involving measurement of product proportions and from EPR characterisation of the transient radicals
- To assess the mechanism for the cascade
- To evaluate the viability of the cascade as a two-carbon ring expansion method

RESULTS AND DISCUSSION

4.7 Photo-induced Reaction of Spiroepoxy Bromides with Organotin Reagents

4.7.1 Temperature sensitivity of Bu_3SnH-mediated reduction of spiroepoxy bromides

The three 2-oxaspiro[2.n]alkyl bromides, (cycloalkylepoxy bromides) with structures **6a-c** inclusive, on reduction, in the presence of the organotin reagent, under different temperature conditions, gave a mixture of straight-chain alkanones (**7a-c**), and ring-expanded cycloalkanones either by 1-carbon atom (**8a-c**), or by 2-carbon atoms (**9a-c**).



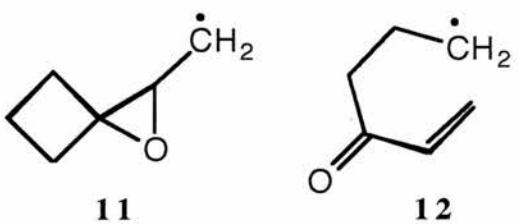
Scheme 12: Products from the tributyltin hydride reduction of spiroepoxy bromides.

The relative proportions of the products obtained with 2-oxaspiro[2.3]hexyl bromide (cyclobutylepoxy bromide) **6a** as a starting material and a fourfold excess of the reducing agent are indicated in **Table 1**.

Product	n	% Yield at stated temp./°C			
		5	50	120	185
7a	1	58	66	63	59
8a	1	10	7	9	11
9a	1	13	8	8	10

Table 1: Product yields (mol%) from the reduction of bromide **6a** with fourfold excess of tributyltin hydride.

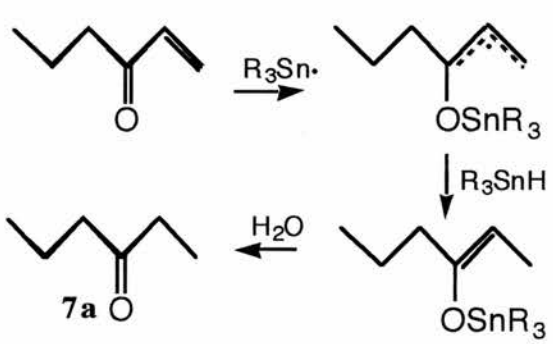
Tributyltin hydride reduction is known to proceed with an efficiency ≥ 80%[38]. The data in **Table 1** can be explained in terms of the following: when the starting spiroepoxy bromide is **6a**, the strained cyclobutyl ring contributes to the facile fragmentation of the initially formed radical **11** to yield intermediate **12** via a vinylcycloalkoxyl radical.



11 **12**

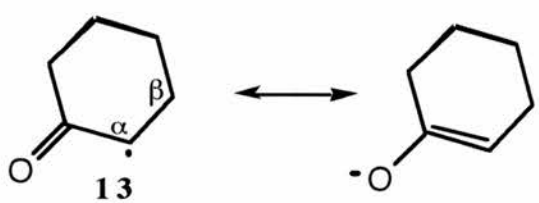
Scheme 13: Structures of the radicals from the tribuyltin hydride reduction of cyclobutylepoxy bromide.

The latter, after hydrogen atom abstraction, gives hex-1-en-3-one **10a**. This enone, upon attack by stannyl radicals, forms the saturated ketone **7a** probably in the manner shown by **Scheme 14** in the presence of excess organotin hydride.



Scheme 14: Organotin mediated reduction of an enone.

The oxo-hexenyl radical **12** can undergo ring closure either in the *exo*-mode to give 2-methylcyclopentanone **8a** or in the *endo*-mode to make cyclohexanone **9a**. At all temperatures studied with a fourfold organotin hydride excess, hexan-3-one predominated. This showed that at high organotin concentrations, irrespective of the reaction temperature, the rate of cyclisation was considerably slower than that for hydrogen abstraction from the reagent. This is in accordance with expectation because of the known tendency of the oxohex-5-enyl radical **12** to undergo almost instantaneous reaction. At lower temperatures, the rate of cyclisation to the six-membered ring is favoured compared with the corresponding conversion to the five-membered ring. Lower. At lower temperatures, the enhanced thermodynamic stability of the cyclised radical **13** becomes an important factor and so, under these conditions, it is formed at a higher rate.



13

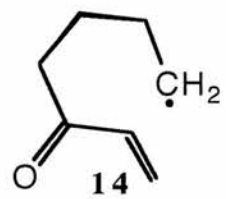
At 185°C, the ratio of the six-membered ring ketone to the five-membered ring compound is approx. 1:1 whereas this ratio, at 5°C, is more in the favour of cyclohexanone formation.

The relative quantities of the products resulting from treatment of 2-oxaspiro[2.4]heptyl bromide **6b** (cyclopentylepoxy bromide) and with a molar equivalent of tributyltin hydride are stated in **Table 2**.

Product	**n**	**% Yield at stated temp./°C**			
		30	**80**	**150**	**180**
10b	2	12	5	6	5
8b	2	34	50	40	47
9b	2	34	25	34	28

Table 2: Yields (mol%) from the bromide **6b** with a molar equivalent of Bu_3SnH.

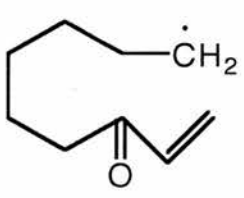
The product ratios were completely altered on gradual addition of the reducing agent to the reaction mixture containing bromide **6b** as the substrate. With the exception of the reaction at 30°C, the product profile was almost exclusively the cyclised ketones **8b** and **9b**. Thus, under restricted concentration of the reductant, the process was driven along the alternative pathway.



14

The rapidly formed precursor radical, 5-oxo-heptenyl **14**, with only a limited access to the hydrogen donor, forms a small amount of hept-1-en-3-one **10b** but the rest cyclises in both *exo*-and *endo*-modes to give 2-methylhexanone **8b** and cycloheptanone **9b** respectively. The *exo*-ring closure, and hence the formation of a thermodynamically stable six-membered cycloketone, is dominant over the alternative route. The latter would be expected to assume greater importance with increase in temperature when thermodynamic control takes over. Surprisingly, this behaviour was not observed in the accessible temperature range. At 30°C, a smaller proportion of the cyclised products was detected but they were present in an equimolar ratio.

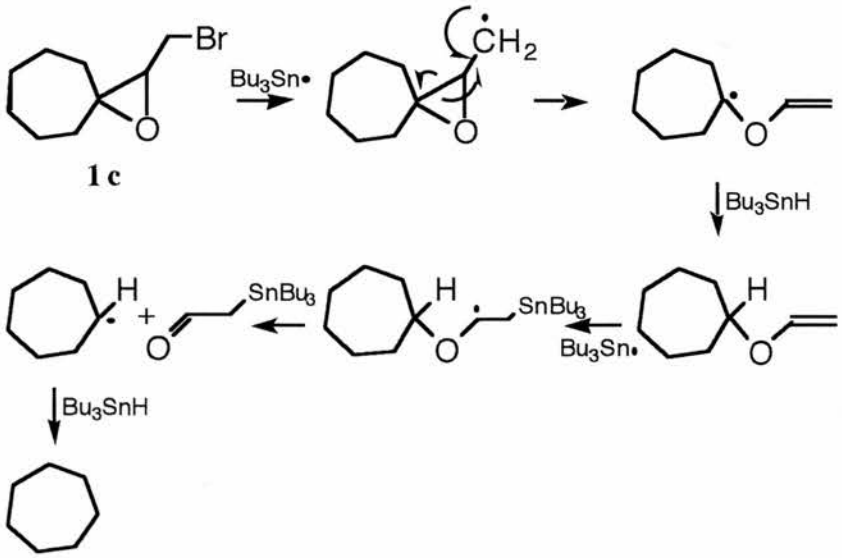
Performing the reaction at 75°C with 2-oxaspiro[2.6]nonanyl bromide **6c** (cycloheptylepoxy bromide), gave a low yielding mixture of both the open-chain and the cyclic ketones along with a number of unidentified products. Nonan-3-one **7c** and non-1-en-3-one **10c** both result from the reduction of the intermediate radical **15**.



15

The reluctance of this radical to form cyclononanone **9c** and 2-methylcyclooctanone **8c** is not unexpected. There is only a limited barrier to the conformational mobility of the oxo-nonenyl radical **15** and the unpaired electron is far removed from the unsaturated functionality. At 150°C, cycloheptane was the only product derived from the starting

material. The latter was completely absent from the reaction mixture. It would seem that the cycloheptylepoxy bromide **6c** must simply fragment to give the cycloheptyl radical which, after hydrogen abstraction, leads to the corresponding cycloalkane possibly in the manner described in **Scheme 15**.



Scheme 15: Reactions leading to the formation of cycloheptane from the photolysis of cycloheptylepoxy bromide.

4.7.2 Effect of Bu_3SnH concentration on the reduction of spiroepoxy bromides

The reaction of cyclobutylepoxy bromide **6a** with a molar equivalent of tributyltin hydride, at 120°C, gave hex-1-en-3-one **10a** and the cyclised ketones **8a** and **9a**. Results from the reduction with a fourfold excess of the organotin reagent were in direct contrast to the findings in this experiment when no hexan-3-one **7a** was detected. The implication of these observations is that, at a lower instantaneous concentrations of the reductant, the addition of hydrogen to the enone **10a** is markedly suppressed and that the oxo-hexenyl radical **12** is diverted down the cyclisation route.

Photolysis of cyclopentylepoxy bromide **6b** with a molar equivalent of the hydride, with faster addition, gave three products: hept-1-en-3-one **10b**, 2-methylcyclohexanone **8b** and cycloheptanone **9b**. The substantial accumulation of the

unsaturated ketone was as expected. Under these conditions, a significant yield of the saturated heptan-3-one **10b** was not anticipated. There are three competing reactions tending to deplete 5-oxo-heptenyl radical **14** which are: cyclisation, hydrogen abstraction and addition. In light of these observations, the rate of addition to the unsaturated ketone must be the slowest of these processes. The faster removal of hydrogen from the reagent by the oxoalkenyl radical **14** reduces the concentration of the hydrogen-donor in the reaction mixture.

When the reduction was performed on cycloheptylepoxy bromide **6c** at 75°C, the only substrate-derived products that were identified included nonan-3-one **7c** and 2-methylcyclooctanone **8c.** In contrast to this, when the reagent was added to the substrate gradually both the open-chain and the cyclic ketones were amongst the products. With excess hydride, the oxoalkenyl radical **15** underwent both hydrogen abstraction and addition to give high levels of the saturated ketone **7c**. The tendency for the *endo*-mode ring closure of the alkenyl radical to give the nine-membered ring cycloalkanone is not expected to be substantial. The formation of the smaller ring-sized methylcycloalkanone is more facile[39]. This explains the existence of 2-methylcyclooctanone **8c** in the reaction mixture in preference to cyclononanone **9c**.

4.7.3 Temperature dependence of Ph_3SnH-initiated reduction of spiroepoxy bromides

The radical 4-oxo-hex-5-enyl **12**, generated from cyclobutylepoxy bromide **6a**, underwent ready cyclisation to the corresponding six-membered cycloalkanone at 80°C in the presence of triphenyltin hydride in the reaction mixture. The use of this reagent at higher temperatures tended to produce tin-derived solid residues in the reaction mixtures. These were difficult to remove completely from the solutions and so the analytical data were inconclusive. The comparative performance of the triaryltin reagent, for enhancing cyclisation, was better than that of the trialkyltin reagent (80% cyclisation compared with 59%-see **Table 3**). These results reflect the greater tendency for the resonance-stabilised tin-centred radicals to form on photolysis of the triaryltin hydride. The stannyl radicals

will generate correspondingly larger amount of the intermediate oxohexenyl radical **12**. Since such reactions were carried out in molar equivalent quantities, a significant proportion of the aforementioned radical will tend to cyclise rather than undergo hydrogen abstraction from the reagent.

Product		% Yield	
		Ph_3SnH	Bu_3SnH
Hexan-3-one	**7a**	-	14
Hex-1-en-3-one	**10a**	17	28
Cyclohexanone	**9a**	58	45
2-Methylcyclopentanone	**8a**	22	14

Table 3: Performance of organotin reagents in reducing bromide **6a** at 80°C.

Analysis of the products with ^1H NMR spectroscopy proved very difficult following the reaction of cyclopentylepoxy bromide **6b** using triphenyltin hydride. The spectral peaks overlapped severely and so the proportions of the individual components could not be deduced. GC-MS, however, resulted in an efficient separation of the reaction mixture which consisted of: hept-1-en-3-one **10b**, 2-methylcyclohexanone **8b** and cycloheptanone **9b**. This product profile compares well with that obtained from tributyltin hydride. Thus, the two reagents behave similarly towards the oxoheptenyl radical **14**.

Photolysis of cycloheptylepoxy bromide **6c** at 20°C gave, amongst a number of unidentified products, non-1-en-3-one **10c**, nonan-3-one **7c** and cyclononanone **9c**. A similar reaction at 75°C, carried out on a larger scale, yielded nonan-3-one and cyclononanone after column chromatography of the reaction mixture. At higher temperatures the 7-oxonon-8-enyl radical **15** tended to cyclise. The starting material simply fragments with tributyltin hydride at higher temperature (150°C) and gives cycloheptane. The efficiency of the triaryl reagent is very similar to the trialkyl reagent as is suggested by the observation that both these compounds fail to give the cyclised ketones at low temperatures. **Table 4** gives the yields of the products when cycloheptylepoxy bromide was treated with triphenyltin hydride at different temperatures.

Product	n	%Yield at stated temp./°C	
		20	75
9c	4	20	36
7c and **10c**	4	11	-

Table 4: Product-yields from photolysis of cycloheptylepoxy bromide **6c** with triphenyltin hydride.

4.7.4 Extent of reduction of spiroepoxy bromides and Ph_3SnH concentration

The reaction of cycloheptylepoxy bromide **6c** at 75°C with slow addition of triphenyltin hydride gave cycloheptane, non-1-en-3-one **10c**, nonan-3-one **7c**, 2-methylcyclooctanone **8c** and cyclononanone **9c**. The product-profile was substantially altered when this spirobromide was treated for 10h with 2 molar equivalents of the reagent added to the reaction mixture as a single aliquot. Under these conditions, the only products identified by isolation were nonan-3-one **7c** and cyclononanone **9c**. The higher concentration of the reagent and a more prolonged reaction seem to have driven the process towards the formation of the larger nine-membered ring. The absence of the unsaturated open-chain ketone from the products is an expected result since the excess reducing agent is more likely to give the corresponding saturated product.

4.8 EPR Characterisation of Transient Radicals from Photolysis of Spiroepoxides

4.8.1 Detection of radicals from photolysis of the spiroepoxides with di-*tert*-butyl peroxide

Oxaspiro[2.3]hexane derivatives

Spectra recorded in the microwave cavity of an EPR spectrometer during photolysis of cyclobutylepoxy bromide **6a** and a mixture of di-*tert*-butyl peroxide and triethylsilane in the temperature range 150-160 K, gave a 9 line signal (**Figure 1**).

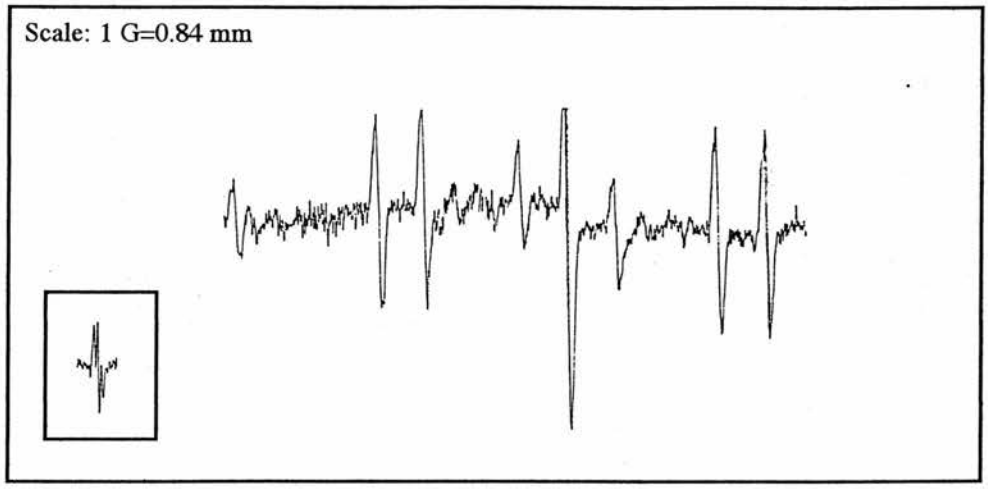
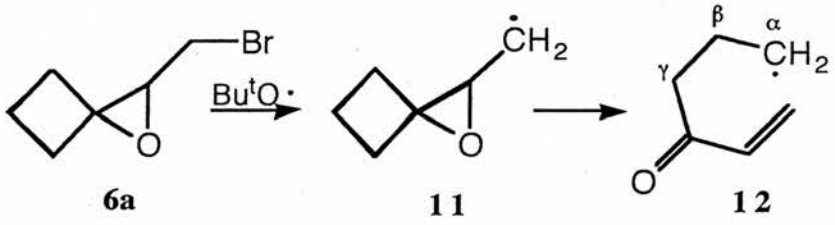


Figure 1: 9.1 GHz EPR spectrum, in cyclopropane at 155 K, of the radical obtained from photolysis of cyclobutylepoxy bromide with di-*tert*-butyl peroxide. (Note that the high field end of the spectrum is omitted).

This corresponded to the primary carbon-centred radical **12** which results from successive fragmentations of the initially formed cyclobutylepoxycarbinyl radical **11**.



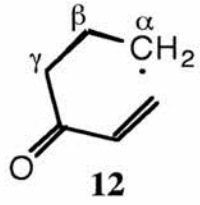
6a **11** **12**

The observed splitting pattern arises from the unpaired electron coupling with the two hydrogen nuclei on each of the α and β carbons. The spectrum shows two triplets, the γ–hydrogens are not resolved in the main spectrum of **Figure 1**; however, under higher resolution a further triplet splitting could be resolved (see inset in **Fig. 1**). The concentration of this radical fell away to virtually zero at 200 K in line with the known high reactivity of a primary radical of this type. A minor radical was also present in the reaction mixture but it was too weak and/or extensively overlapped for analysis. Radical **11** is known to have an extremely short life-span[36] and so the minor spectrum was not expected to have originated from this source. Information from the spectra of the main radical **12**, at each temperature, is summarised in **Table 5**.

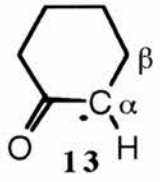
Temperature/K	$a(2H_\alpha)$	$a(2H_\beta)$	$a(2H_\gamma)^c$
150	22.2	29.6	-
155	22.3	29.2	0.55
160	22.3	28.8	-

Table 5: EPR parameters[a,b] for 4-oxo-hex-5-enyl radical **12**.
[a]g factor 2.003±0.001, [b]hfs in G (1 mT=10 G); [c]At 150 K and 160 K, resolution of the spectrum was too poor to determine $a(2H_\gamma)$.

In a comparative series of experiments, the photolysis of spirobromides **6a-c** was performed with hexamethyldistannane instead of triethylsilane. EPR spectra from **6a** showed, at low temperature (200 K), the presence of the primary radical **12** which was also detected in the earlier work. However, at higher temperature (210-235 K) the *endo*-cyclised secondary radical **13** was also identified. This was not detected in the reaction with the peroxide and triethylsilane.



12



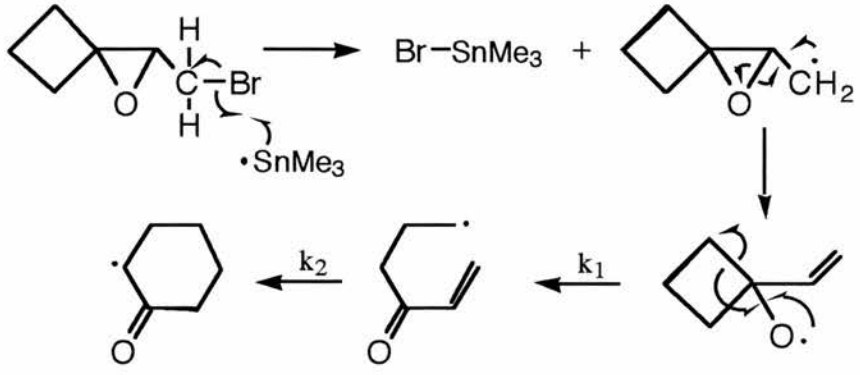
13

It is conceivable that the concentration of the triethylsilyl radical never reached high enough proportion so that it could produce sufficiently high levels of the primary radical **12** for observation above 200 K. The EPR parameters of the two radicals are presented in **Table 6** and the sequence of reactions leading to the formation of the cyclised radical **13** is indicated in **Scheme 16**.

Radical	T/K	hfs[a]
12	200	$(2H_{\alpha})$=22.2; $(2H_{\beta})$=28.0
13	210	$(1H_{\alpha})$=18.0; $(1H_{\beta})^{b}$=24.0; $(1H_{\beta})^{b}$=43.0
	235	$(1H_{\alpha})$=18.0; $(2H_{\beta})^{c}$=34.2

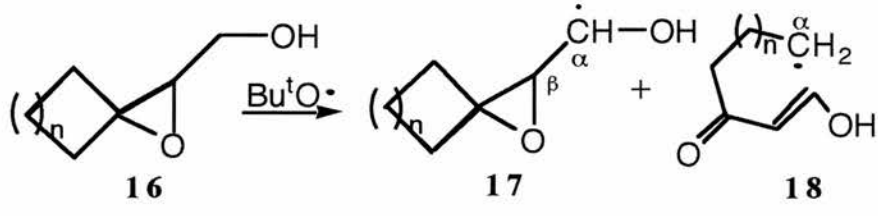
Table 6: EPR parameters for the radicals 4-oxo-hex-5-enyl **12** and cyclohexanonyl **13** from the photolysis of cyclobutylepoxy bromide at 200-235 K with hexamethylditin. [a]hfs in G. [b]hfs of the non-equivalent β–hydrogens at lower temperature. [c]hfs for the equivalent β–hydrogens at higher temperature owing to exchange broadening.

The EPR parameters of radical **13** were essentially the same as those given in the literature[40,41].



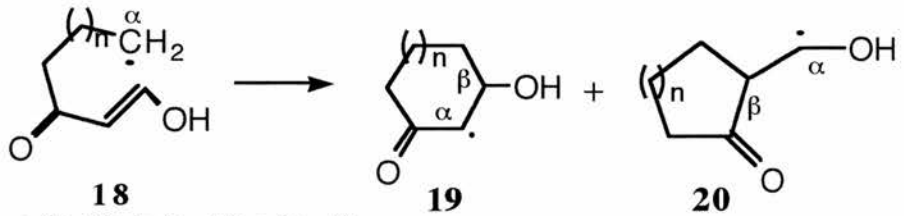
Scheme 16: Reactions leading to the *endo*-cyclisation of cyclobutylepoxy bromide with hexamethyldistannane.

The spiroepoxy alcohol **16** contains an exocyclic methylene group which is flanked by a highly strained epoxy ring and an electron-withdrawing hydroxy group.



a (n=1); b (n=2); c (n=4)

It seemed feasible that, under suitable conditions, such a methylene group should be susceptible to the loss of hydrogen atom and form the secondary radical **17** which, in turn, could fragment to give the oxoalkenol radical **18** that can ring-close in two ways to furnish the cyclised radicals **19** and **20**.



18
a (n=1); b (n=2); c (n=4)

19 **20**

In order to assess the validity of this projected sequence of changes, experiments were carried out with cycloalkylepoxy alcohols **16a-c** in di-*tert*-butyl peroxide in the temperature range 145-340 K.

Spiroalcohol **16a** gave a mixture of two EPR-active species. A 9 line trace and a doublet were observed. The concentrations of both radicals were maximal at 150 K and the primary radical was not observable beyond 300 K whereas the doublet was not observable above about 190 K. The 9-line triplet of triplets can be attributed to the ring-opened primary radical **18a**. The identity of the radical responsible for the doublet in the spectrum is unclear. It might be the spiroepoxymethyl radical **17a** in which coupling of the unpaired electron to H_β and the -OH group are unresolved. This type of radical undergoes very rapid β–scission but the presence of the hydroxy group at the radical centre might increase its stability sufficiently for it to be observed up to about 190 K. **Figure 2** shows the observed spectral changes with increase in temperature of the reaction mixture containing radicals **17a** and **18a**.

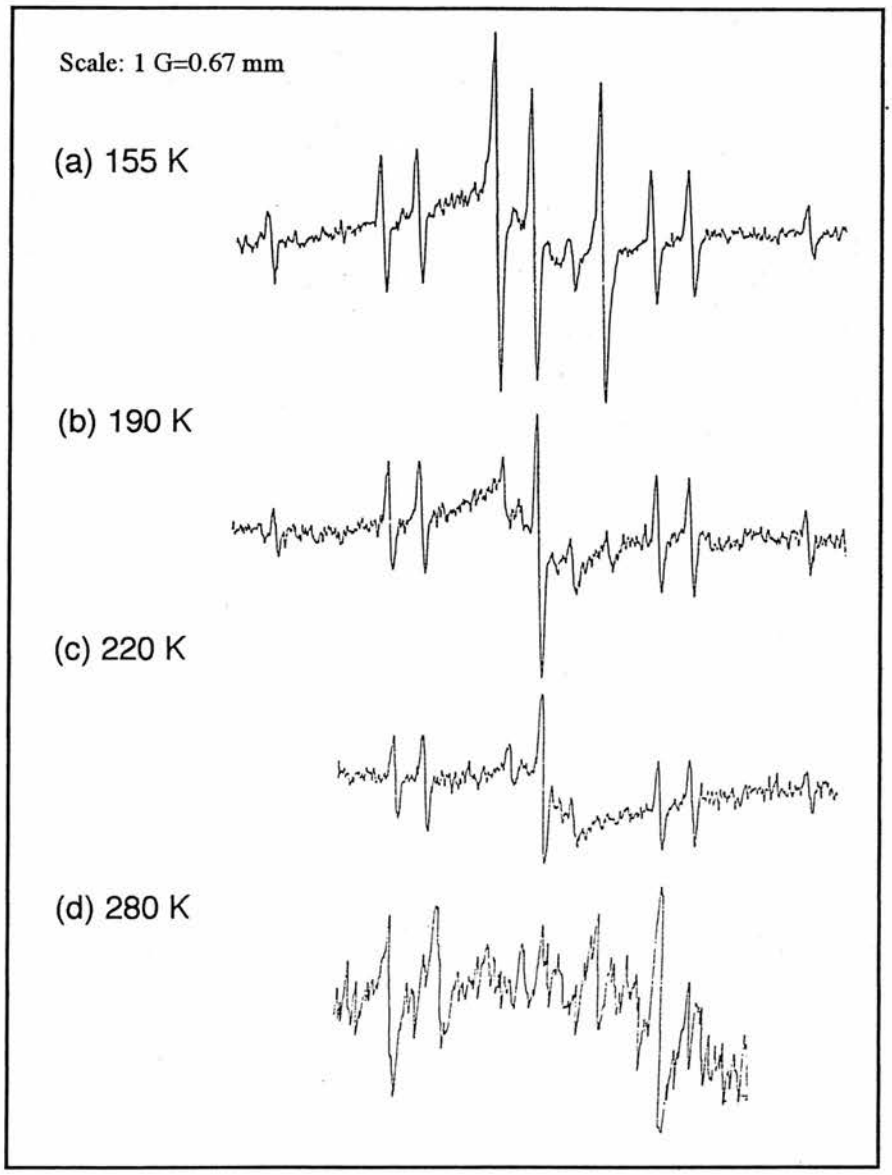
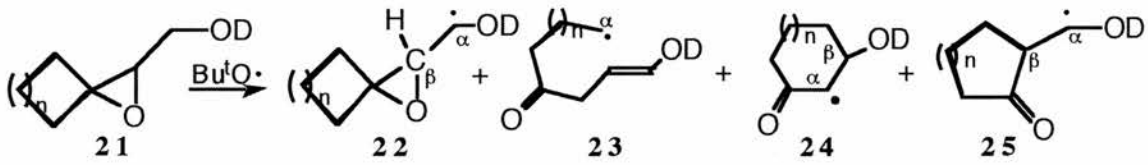


Figure 2: 9.1 GHz EPR spectra of radicals obtained from photolysis of spiroepoxy alcohol **16a** in cyclopropane and di-*tert*-butyl peroxide at the stated temperatures (a) 155 K (b) 190 K (c) 220 K (d) 280 K.

The fact that the anticipated build-up of the cyclised cyclohexyl and cyclopentylhydroxycarbinyl radicals **19a** and **20a** respectively did not occur from 260 K up to 340 K, suggests that the hydroxyl group may be hindering the precursor radical **18a** from undergoing ring-closure.

In order to investigate the effect of the hydroxyl proton on the EPR spectra of cycloalkylepoxy alcohols with di-*tert*-butyl peroxide, the proton in the hydroxyl group was replaced with a deuterium atom. When the deuterated spiroalcohol **21a** was photolysed, no change in the spectral parameters was observed compared with the

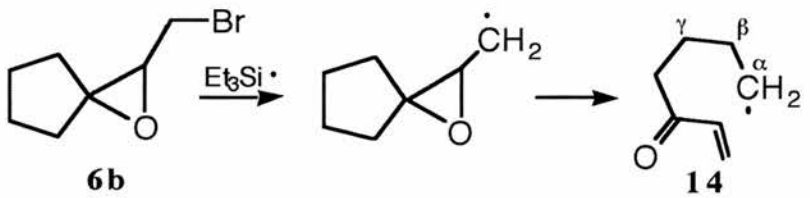
previous data from the corresponding non-deuterated spiroalcohol. Thus, in this case, the hydroxyl hydrogen in the radical does not couple with the unpaired electron.



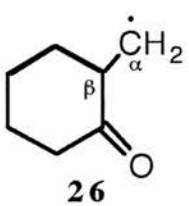
a (n= 1); b (n=2); c (n=4)

Oxaspiro[2.4]heptane derivatives

An attempt to identify the primary 5-oxoheptenyl radical **14**, from the photolysis of cyclopentylepoxy bromide **6b**, with triethylsilane and di-*t*-butyl peroxide, was unsuccessful because the EPR spectra obtained in the temperature range 175-210 K were weak and the spectral peaks were far too broad for an unambiguous interpretation.



With the cyclopentylepoxy bromide **6b** as the starting material and hexamethylditin as the reducing agent, the observed EPR spectrum consisted of a doublet of triplets corresponding to the primary *exo*-cyclised cyclohexanonylcarbinyl radical **26**.



Attempts to identify the formation of the open-chain radical 5-oxo-hept-6-enyl **14** at lower temperatures were unsuccesful with this second reagent. **Table 7** gives the data for the cyclised radical **26**. Note the decrease in hfs relating to $1H_{\beta}$ with increase in temperature resulting from the greater rotation about the C_{α}-C_{β} bond at higher temperatures.

Radical	T/K	hfs/G	
		($2H_\alpha$)	($1H_\beta$)
26	200	22.6	30.9
	210	22.6	30.4
	225	22.6	29.8
	240	22.6	29.6
	260	22.6	29.4
	275	22.6	29.1
	290	22.6	29.0

Table 7: EPR parameters for radical **26** at various temperatures.

The observation of the *exo*-cyclised radical **26** was in agreement with the product studies which showed a preponderance of *exo*-products at all accessible temperatures.

Photolysis of cyclopentylepoxy alcohol **16b** with di-*t*-butyl peroxide gave an EPR spectrum that indicated the presence of three radicals: the primary uncyclised species **18b** which gave a triplet of triplets from coupling of the unpaired electron with the two hydrogen nuclei on each of the α and β carbons. The second radical was an *exo*-cyclised intermediate **20b** resulting in the triplet of doublets from α–hydrogen splitting (doublet) and from the β hydrogen splitting (almost equivalent β hydrogens split the doublet into a triplet). The third radical in this mixture gave a doublet with hfs (18.8 G) characteristic of a single hydrogen attached to the carbon atom bearing the unpaired electron. However, this intermediate could not be identified. Interestingly, the cyclisation proceeds via the route that gives the more stable six-membered ring ketone **20b** rather the stereoelectronically favoured seven-membered ring ketone **19b**. In the latter case the electron is resonance stabilised. Except for the primary radical 5-oxo-hept-6-en-7-ol **18b**, the other two radicals continued to persist at 260 K.

The deuteriospiroalcohol **21b** gave an EPR signal which consisted of a triplet indicating the presence of the uncyclised radical **23b** only in the reaction mixture. Lack of *exo*-cyclised radical in the reaction mixture implies some interaction of the deuterium in this process.

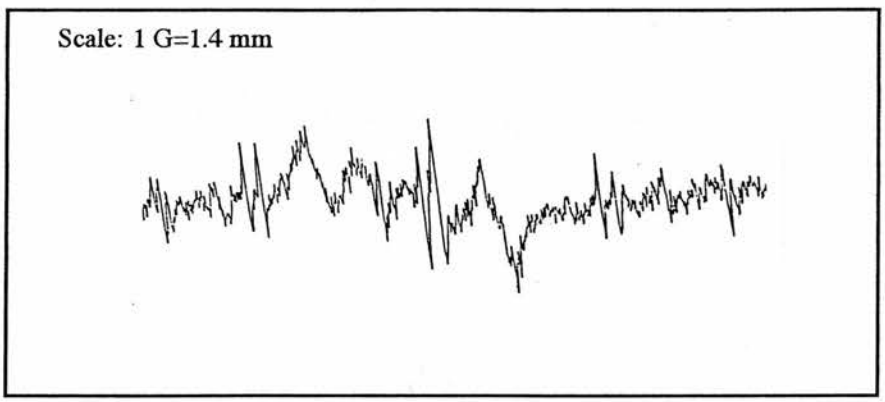
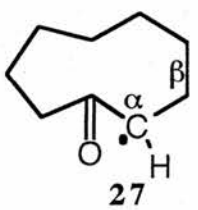


Figure 3: 9.1 GHz EPR spectra of 5-oxo-heptenol **18b** and cyclohexanonylmethyl **20b** radicals from photolysis of spiroalcohol **16b** in at 240 K in Bu^{t}OOBu^t.

Oxaspiro[2.6]nonane derivatives

EPR analysis of the reaction mixtures from cycloheptylepoxy bromide **6c** and hexamethylditin revealed that below 250 K, the uncyclised primary radical 7-oxo-non-8-enyl radical **15** existed whereas above this temperature, the dominant species was the *endo*-cyclised secondary radical cyclononanyl radical **27**. Analytical information for these two radicals is given in **Table 8** and **Figure 4** gives the EPR spectra of all the important radicals observed from photolysis of cycloalkylepoxy bromides **6a-c** with hexamethyldistannane at various temperatures.



27

Radical	T/K	hfs/G	
		($2H_\alpha$)	($2H_\beta$)
15	200	22.6	29.0
	210	22.6	28.9
	235	22.6	28.6
	250	22.6	28.5
27	290	19.0($1H_\alpha$)	21.0

Table 8: EPR spectral data for radicals **15** and **27** obtained from photolysis of cycloheptylepoxy bromide with hexamethyldistannane.

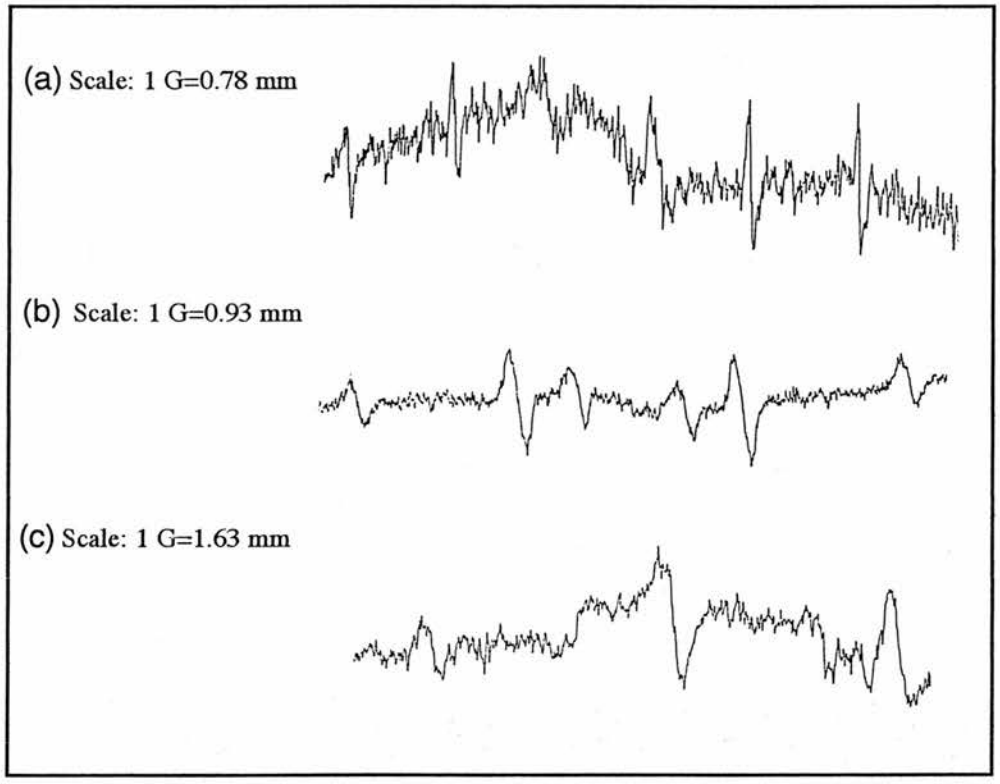


Figure 4: 9.1GHz EPR spectra of the radicals derived from cycloalkylepoxy bromides **6a-c** with hexamethyldistannane at various temperatures.

(a) *Endo*-cyclised radical cyclohexanonyl **13** generated from spirobromide **6a** at 210 K (Note the 4 sharp outer lines and the two inner broadened lines owing to exchange broadening).

(b) *Exo*-cyclised radical **26** formed from spirobromide **6b** at 210 K

(c) *Endo*-cyclised radical cyclononanyl **27** at 290 K from spirobromide **6c**

In an analogous series of photolysis with cycloheptylepoxy alcohol **16c**, an EPR signal, at 240 K, consisted of a 5 line multiplet structure containing additional doublet hfs pattern (**Figure 5**).

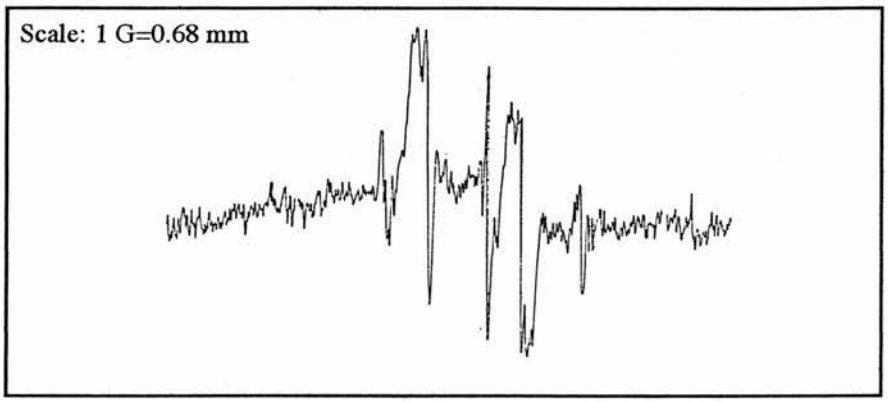
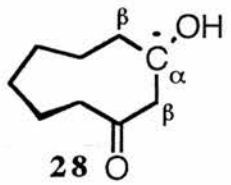


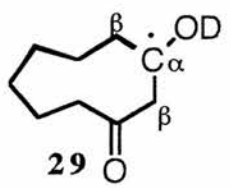
Figure 5: 9.1 GHz EPR spectrum of a radical derived from the photolysis of cycloheptylepoxy alcohol **16c** with di-*tert*-butyl peroxide at 240 K.

This could possibly have originated from the *exo*-cyclised radical **20c** or from the *endo*-intermediate **19c** but more likely from the type of radical **28** shown below. In any event, this radical could not be characterised unequivocally.



28

The deuterated spiroalcohol **21c** gave a simplified weak spectrum containing 5 lines with no secondary structure. The most marked effect on the EPR spectrum was produced by this alcohol. The observed radical produced no doublet which was observed when undeuterated alcohol **16c** was photolysed. Clearly, in this particular case, the hydroxyl hydrogen must couple with the unpaired electron and therefore replacing it with deuterium removes the small doublet hfs. This multiplet probably corresponds to the cyclised radical **29** shown below.



29

The fact that the cyclised radicals are observed from the photolysis of cyclobutylepoxy alcohol **16a** and cycloheptylepoxy alcohol **16c** in the temperature range 240-260 K is in direct contrast to the lack of such radicals, even at higher temperature, in the case of cyclopentylepoxy alcohol **16b**. A likely explanation is that the cyclised radical in the latter case is present at such low concentration that it was not detectable spectroscopically. The analysis of the observed EPR spectra from the photolysis of the three cycloalkylepoxy alcohols is indicated in **Table 9**.

Radical	T/K	$a(2H_\alpha)$	$a(2H_\beta)$	a(other)
18a	155	22.8	30.0	0.5 ($2H_\gamma$)
17a†	150	20.0 ($1H_\alpha$)		
18b	240	22.4	28.4	0.7 ($2H_\gamma$)
20b	240	17.4 ($1H_\alpha$)	17.4 ($1H_\beta$)	1.5 (OH)
28†	240		19.0 (4H)	1.8 (OH)

Table 9: EPR parameters[a,b] for the observed radicals from spiroalcohols **16a-c** with di-*t*-butyl proxide at 150 and 240 K. [a]Radical **18a** produced a spectrum with better resolution at 155 K. [b]hfs in G. †Tentative identification.

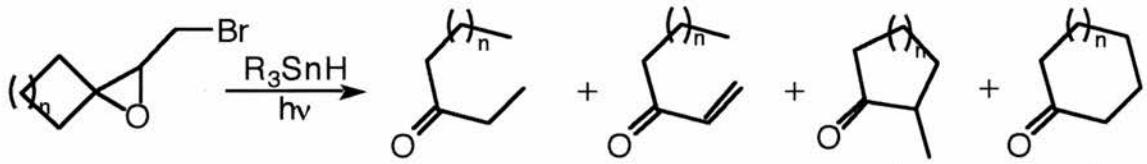
Table 10 lists all the spectral information relating to the photolysis of deuteriospiroalcohols **21a-c**.

Radical	T/K	$a(2H_\alpha)$	$a(2H_\beta)$	a(other)
22a	155	20.0 ($1H_\alpha$)		
23a	155	22.8	30.0	
23b	235	19.5		
29	270		19.0(4H)	<0.3(OD)

Table 10: EPR parameters for the radicals from the photolysis of deuterated alcohols **21a-c** with di-*t*-butyl peroxide at various temperatures.

4.9 Kinetics of the Cyclisation of the 4-Oxo-hex-5-enyl Radical

Product analysis, using a combination of GC-MS and NMR, had shown that the photolysis of cycloalkylepoxy bromides **1a-c**, produced mixtures of cycloalkanones



6 **7** **10** **8** **9**

a (n=1); b (n=2); c (n=4)

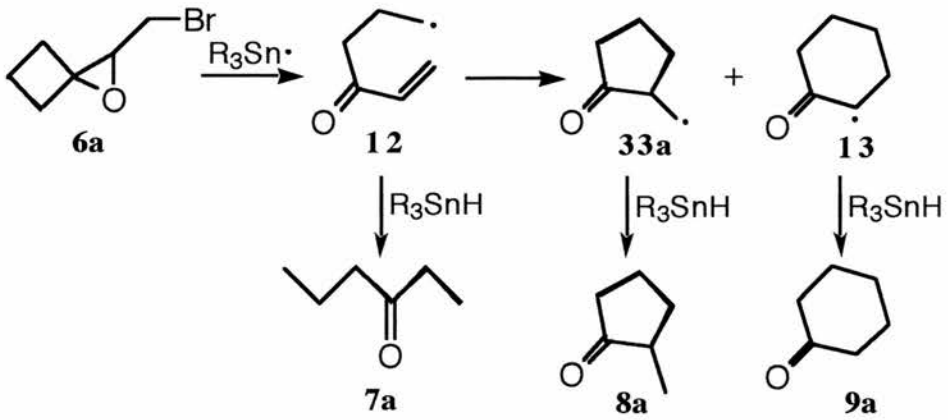
of the type **8** and **9**, open-chain ketone **7** and enone **10**. EPR work on spirobromides **6a-c** had identified the formation of radical **31** as the initial step in the overall process.

30 a (n=1); b (n=2); c (n=4) **31**

The presence of oxo-alkenyl radicals **31** as the precursors for *endo*- and *exo*- cyclised intermediates **32** and **33** respectively has also been spectroscopically demonstrated.

32 a (n=1); b (n=2);c (n=4) **33**

Kinetic studies, using cyclobutylepoxy bromide **6a** as a model compound, were undertaken in order to ascertain the rate parameters and the rate-determining step in the sequence of reactions involving the rearrangement of the carbon skeleton of the initially generated cyclobutylepoxycarbinyl radical **30a**. Photochemical reductions, at a series of temperatures, in hydrocarbon solutions, with a fourfold excess of tributyltin hydride were performed. The relative proportions of the products were measured with GC.



Scheme 17: Rearrangement of cyclobutylepoxy bromide **6a**.

Assuming that the formation of hexan-3-one **7a** is quantitative and that the rate of hydrogen abstraction (k_H) from the organotin reagent is the same for the formation of hexan-3-one **7a**, cyclohexanone **9a** and 2-methylcyclopentanone **8a** and equal to the literature values for the rate constants of secondary and primary radicals[42], the rate constants for the *endo*- (k_C^6) and *exo*- (k_C^5) cyclisation at 298 K were derived from the Arrhenius plot (**Figure 6**) of the data given in **Tables 11** and **12**.

T/K	278	298	323	393[e]	458[f]
$10^3/T$	3.597	3.356	3.096	2.545	2.183
[a]$\log k_H$	6.119	-	6.554	7.032	7.346
$10^{-6}k_H/s^{-1}$	1.32	-	3.58	10.80	22.20
[b][CH]/[HH]	0.089	-	0.099	0.110	0.203
[c]$10^{-6}k_C^6/s^{-1}$	0.08	0.13[d]	0.24	0.81	3.08
$\log k_C^6$	4.90	5.10[d]	5.38	5.91	6.49

Table 11: Kinetic data for the formation of cyclohexanone **9a** from cyclobutylepoxy bromide **6a** with tributyltin hydride in benzene solution.

T/K	278	298	323	393[e]	458[f]
$10^3/T$	3.597	3.356	3.096	2.545	2.183
[a]$\log k_H$	6.169	-	6.573	7.018	7.309
$10^{-6}k_H/s^{-1}$	1.48	-	3.74	10.39	20.40
[b][MH]/[HH]	0.243	-	0.123	0.055	0.049
[c]$10^{-5}k_C^5/s^{-1}$	2.46	2.63[d]	3.16	3.92	6.84
$\log k_C^5$	5.39	5.42[d]	5.49	5.59	5.84

Table 12: Kinetic data for the formation 2-methylcyclopentanone **8a** from cyclobutylepoxy bromide **6a** with tributyltin hydride in benzene solution. Initial $[Bu_3SnH]=0.685$ mol dm^{-3}; initial [**6a**]=0.176 mol dm^{-3}. [a]Calculated using an equation from reference 42. [b][MH]=2-methylcyclopentanone **8a** or [CH]=cyclohexanone **9a**, [HH]=hexan-3-one **7a** from GC analysis using heptane as an internal standard.[c]Calculated from the relationship $k_C^{5or6}=k_H[Bu_3SnH][MH]$ or [CH]/[HH]. [d]Obtained from the Arrhenius plot of $\log k_C^5$ or $\log k_C^6$ vs $10^3/T$. [e]In *tert*-butylbenzene as a solvent. [f]In hexadecane as a solvent.

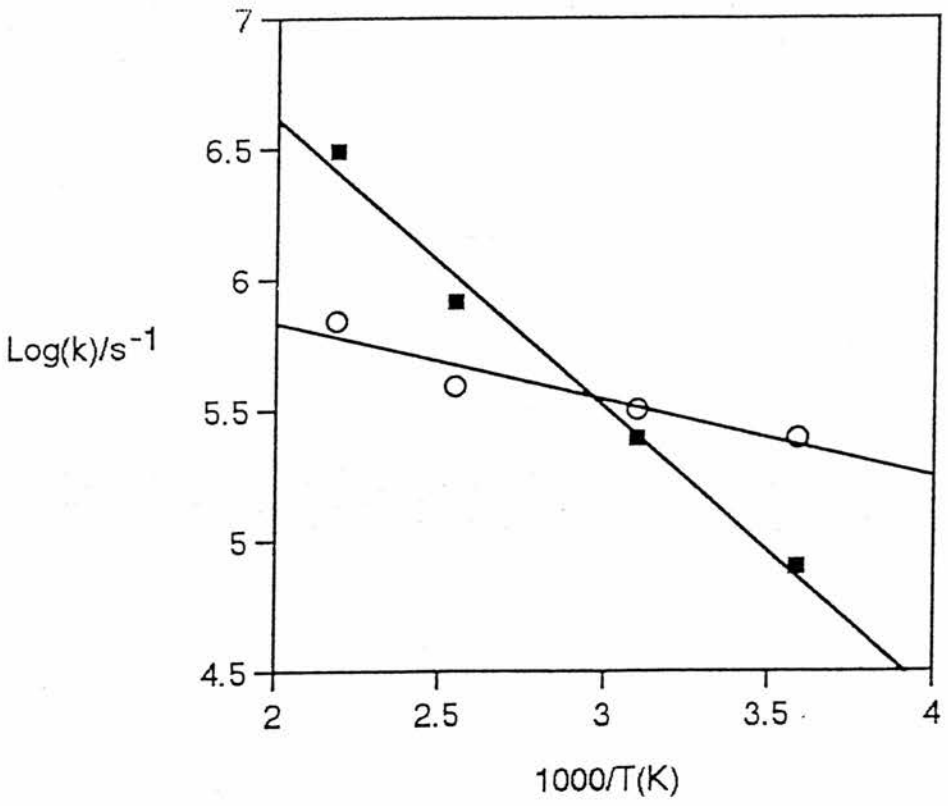


Figure 6: The Arrhenius plot for the cyclisation of 4-oxohex-5-enyl radical **12**.

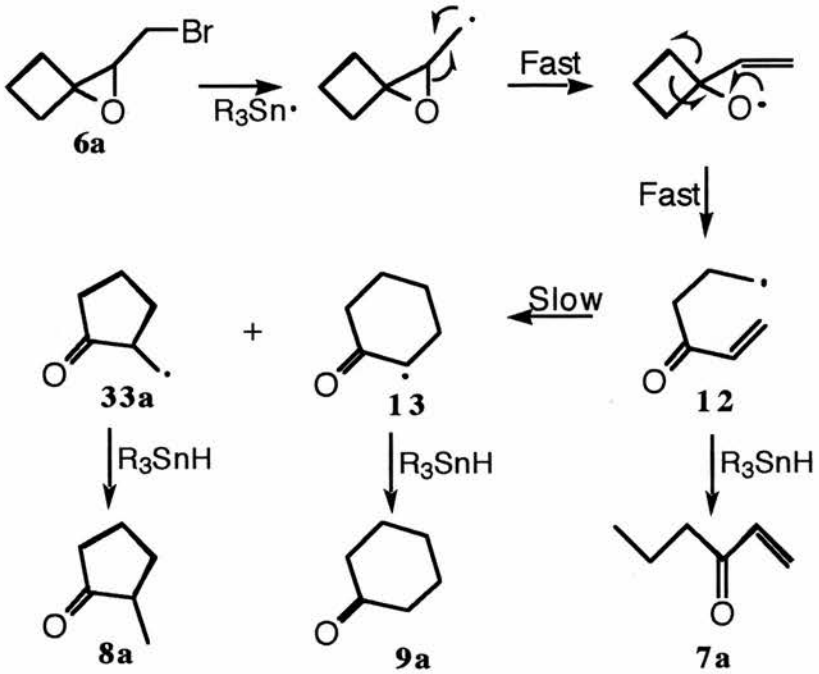
The Arrhenius parameters for the two modes of cyclisation process were also obtained from the plot of $\log k_c^5$ or $\log k_c^6$ against 1/T and these are given in **Table 13**.

Radical	Ea/kJ mol^{-1}	log(A/s^{-1})
13	21.1	8.8
33a	5.64	6.4
Hex-5-enyl[a]	28.6	10.4

Table 13: Arrhenius parameters for 4-oxo-hex-5-enyl **12** cyclisations.
[a]References 42, 44.

The rate constants at 298 K for *endo*- ($k_c^6=1.3\times10^5 s^{-1}$) and for *exo*-($k_c^5=2.6\times10^5 s^{-1}$) cyclisations show that these are relatively slow compared with the known rates of the β–scission steps during the rearrangement of cyclobutylepoxy bromide. Thus, the rate-controlling step in the overall process is the cyclisation of 4-oxo-hex-5-enyl radical. The observed rate constants in this study give values that are comparable to the known rate constant for the hexenyl cyclisation ($2.5 \times 10^5 s^{-1}$ at 298 K)[42,44]. These parameters are also indicative of the fact that the overall rearrangement is fast and this prevents the intermediates from being diverted into alternative reaction channels. It is noteworthy that the pre-exponential factor for the *exo*-cyclisation is lower than the expected value. The correlation coefficient for the data concerning this mode of ring-closure was 0.948 which was significantly poorer than the corresponding figure (0.993) for the *endo*-cyclisation. Thus, the greater scatter associated with the former data helps explain this anomaly.

Although the *exo*-cyclised intermediate was too weak for detection by EPR spectroscopy, nevertheless, the spectroscopic and kinetic data support the mechanism for the rearrangement of cycloalkylepoxy bromides to be a cascade consisting of two β–scissions followed by a cyclisation. The overall effect of this is to enlarge the initial cycloalkyl ring by either one or two carbon atoms (**Scheme 18**).



Scheme 18: Mechanism for the three stage cascade rearrangement of cylobutylepoxy bromide **6a**.

CONCLUSIONS

- This mechanistic study of cycloalkylepoxy bromides confirms that the cleavage of C-O bond in the spiroepoxycarbinyl radicals is fast and that the β–scission of the intermediate alkoxyl radicals formed is also rapid

- It is feasible to incorporate the β–scission of the spiroepoxycarbinyl radicals into a three-stage cascade

- The fragmentation of the vinyl cycloalkoxyl radical occurs selectively so that the more stabilised oxoalkenyl radical is formed

- The fact that the cycloalkanones and enones have been identified as products from the reduction of spiroepoxides containing less strained five- and seven-membered cycloalkyl rings, indicates that excessive ring-strain is not a requirement for β–scissions which yield ketones

- This work has established that the *endo*- mode of cyclisation is favoured from the oxoalkenyl radicals derived from the spiroepoxides containing larger cycloalkyl rings. This method of ring-closure was also observed, at higher temperatures, with the smaller cyclobutylepoxy bromide

- The cascade entails changes in hybridisation at five of the seven original atoms in cyclobutylepoxy bromide and yet the molecule traverses this complex reaction

coordinate with apparent ease

- Cycloalkylepoxy bromides of the type used in this investigation can be easily made by several methods, and so, after suitable optimisation, the cascade constitutes an alternative two carbon ring-enlargement which could be incorporated into a synthetic strategy

EXPERIMENTAL

4.7 Photo-induced Reaction of Spiroepoxy Bromides with Organotin Reagents

The two parameters investigated for the reaction of organotin reagents with spiroepoxy bromides **6a-c** were the temperature of the reaction mixture and the concentration of the reagent.

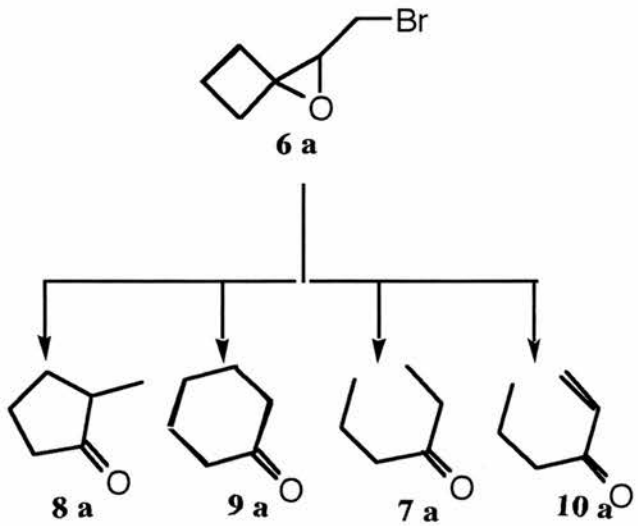
Generally, the reaction vessel consisted of an NMR tube containing the starting material dissolved in a suitable solvent. Photolysis was performed with a 250 W medium pressure Hg arc lamp at the stated temperatures. The reagent was added in excess from the outset or it was introduced into the reaction mixture, in aliquots, as the reaction proceeded. Products were analysed by either GC, GC-MS and/or by NMR spectroscopy. Identity of the compounds formed was established by comparison of the retention times with those obtained from authentic samples separated under identical conditions and by obtaining their mass spectra. For calculation of relative yields from GC data the detector response was calibrated with known amounts of a close analogue, heptane, which was added to the reaction mixture.

4.7.1 Temperature sensitivity of Bu_3SnH-mediated reduction of spiroepoxy bromides

Reduction of 2-bromomethyl-1-oxa-spiro[2.3]hexane ***6a*** *(cyclobutylepoxy bromide)*

Tributyltin hydride (128.0 mg, 0.44 mmol) was weighed into an NMR tube. The reagent was dissolved in benzene (500.0 μl). A single portion of the spirobromide **6a** (20.0 mg, 0.11 mmol) was added to the diluted organotin reagent via a microsyringe. After introducing heptane (10.0 μl), the contents of the tube were thoroughly mixed and a sample was immediately withdrawn for a GC-MS analysis. The rest was photolysed in a

continuously stirred water-bath maintained at 5°C for 4h. The experiment was repeated at 50°C, 120°C and 185°C. In view of the high volatility of benzene, at higher temperatures, *tert*-butylbenzene and hexadecane were used as solvents. The reaction times were appropriately adjusted.

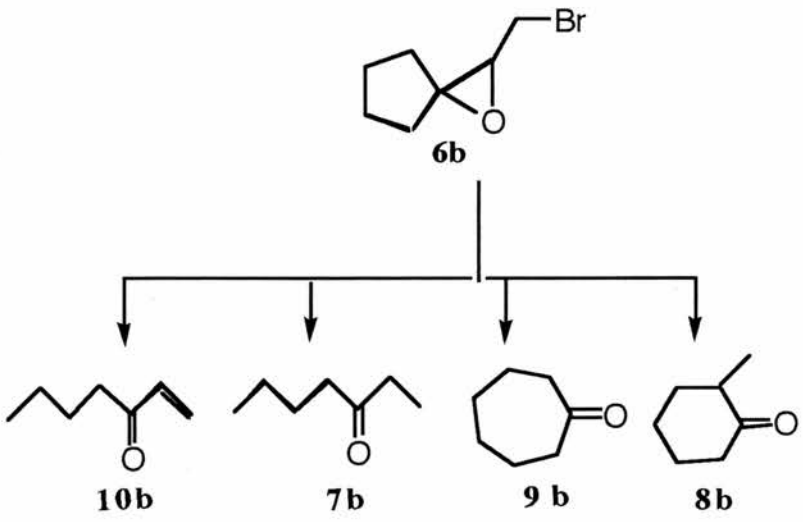


GC-MS peak no. 138, retention time 2.52 min. heptane; peak no. 172, retention time 3.15 min, hexan-3-one **7a**, m/z(relative intensity %) 100(M^+, 11), 71(30), 57(59), 43(100), 42(18), 41(45), 39(31), 29(88), 28(25), 27(91), 26(19); GC-MS peak no. 215, retention time 3.92 min., 2-methylcyclopentanone **8a**, m/z z(relative intensity %) 98(M^+, 13), 78(53), 77(19), 57(29), 56(20), 55(74), 52(20), 51(28), 50(31), 45(25), 43(63), 42(87), 41(67), 39(74), 38(22), 29(47), 28(65), 27(88), 26(29); peak no. 275, retention time 5.03 min., cyclohexanone **9a**, m/z z(relative intensity %) 98(M^+, 14), 78(24), 69(15), 55(71), 43(23), 42(66), 41(52), 39(52), 29(19), 28(48), 27(45), 26(17); GC and GC-MS for the reaction mixtures at 50°C, 120°C and 185°C gave four peaks corresponding to the same compounds.

*Reduction of 2-bromomethyl-1-oxa-spiro[2.4]heptane **6b** (cyclopentylepoxy bromide)*

Cyclopentylepoxy bromide **6b** (50.0 mg, 0.26 mmol) was placed into an NMR tube. After dilution with benzene (500.0 μl), an aliquot of tributyltin hydride (10.51 mg, 36.13 μmol) was introduced into the tube. A sample of this reaction mixture was immediately withdrawn for GC-MS analysis and the remainder was photolysed for 8h at

30°C with the addition of the above mentioned amount of the organotin reagent every 30 mins. until a total of 8 such protions of the reducing agent had been added to the reaction mixture. This experiment was repeated at 80°C, 150°C and 180°C. *tert*-Butylbenzene was used as a solvent at higher temperatures and the duration of the reactions was appropriately adjusted. Products at 30°C were identified by GC-MS which indicated small presence of the unreacted starting material.

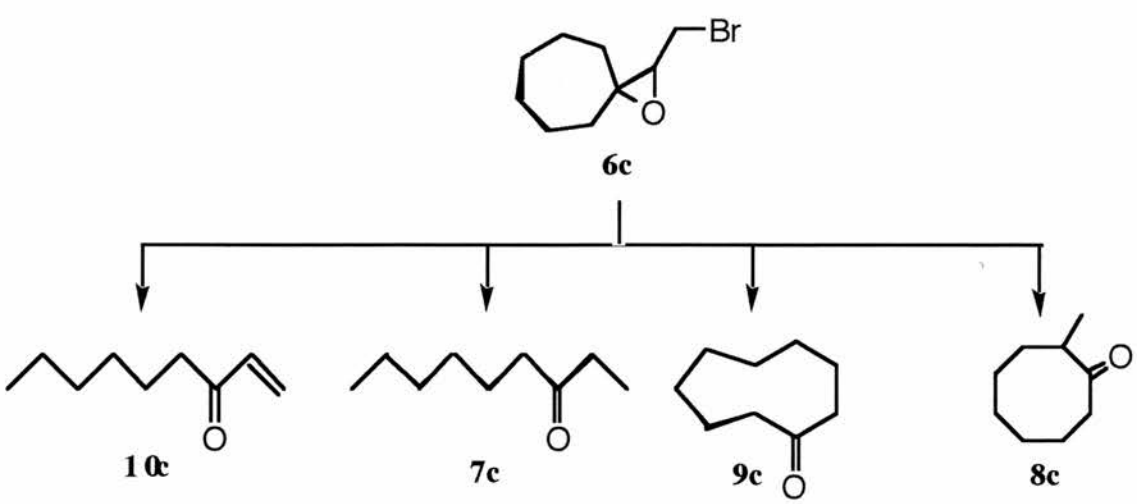


GC-MS peak no. 246, retention time 4.51 min., heptan-3-one **7b**, (3.5 mg, 7%), m/z(relative intensity %) 114(M^+, 6), 85(17), 72(15), 67(24), 57(100), 43(21), 42(12), 41(56), 39(32), 29(79), 28(28), 27(65); peak no. 310, retention time 5.62 min., hept-1-en-3-one **10b**, (5.8 mg, 12%), m/z(relative intensity %) 112(M^+, 14), 71(36), 69(50), 68(21), 43(100), 41(86), 39(39), 27(25); peak no. 340, retention time 6.22 min., 2-methylcyclohexanone **8b**, (17.1 mg, 34%), m/z(relative intensity %) 112(M^+, 18), 84(11), 69(24), 68(48), 56(37), 55(59), 42(40), 41(100), 39(55), 29(20), 28(29), 27(69); peak no. 417, retention time 7.62 min. cycloheptanone **9b**, (17.1 mg, 34%), m/z(relative intensity %) 112(17), 84(15), 68(54), 56(53), 42(54), 41(100), 39(54), 27(68); peak no. 488, retention time 8.95 min., unidentified, 81(34), 67(100), 53(190, 39(45), 27(42); GC-MS for the reaction mixture at 80°C gave all the peaks observed earlier including the one for the starting material. The product proportions were: heptan-3-one **7b** (trace amount), hept-1-en-3-one **10b** (2.0 mg, 5%), 2-methycyclohexanone **8b** (20.0 mg, 50%) and cycloheptanone **9b** (10.0 mg, 25%). At 150°C, no starting material was detectable and the other products were the same as those obtained at 80°C in addition

to the isomeric biphenyls derived from the solvent. The product proportions were: heptan-3-one **7b** (trace amount), hept-1-en-3-one **10b** (1.3 mg, 3%), 2-methycyclohexanone **8b** (5.3 mg, 13%) and cycloheptanone **9b** (25.3 mg, 63%). At 180°C, the only compounds obtained were: the open-chain enone **10b** (1.9 mg, 5%), the α-methylated cycloalkanone **8b** (19.8 mg, 47%) and the cycloalkanone **9b** (11.9 mg, 28%).

*Reduction of 2-bromomethyl-1-oxa-spiro[2.6]nonane **6c** (cycloheptylepoxy bromide)*

Cycloheptylepoxy bromide **6c** (44.09 mg, 0.21 mmol) diluted with benzene (500.0 μl) was placed in an NMR tube. Tributyltin hydride (66.06 mg, 0.23 mmol) was dissolved in benzene (200.0 μl) and divided up into eight equal portions. Following the addition of the first aliquot of the reducing agent, a sample of the reaction mixture was analysed by GC-MS. After commencing the photolysis at 75°C, further aliquots of the reagent were added to the photolysing solution every 30 min. The reaction was terminated after 4h. GC-MS analysis showed that the starting material was only partially converted to the expected products. The experiment was repeated at 150°C using *tert*-butylbenzene as a solvent.



GC-MS peak no. 532, retention time 9.80 min., nonan-3-one **7c**, m/z(relative intensity %) 140(M^+, 1), 113(16), 85(19), 72(37), 57(63), 55(19), 43(100), 29(58), 28(20), 27(43); peak no. 585, retention time 10.80 min., 2-methylcyclooctanone **8c**, m/z(relative

intensity %) 140(M^+, 3), 111(96), 97(76), 83(35), 67(33), 55(80), 41(100), 27(78); peak no. 559, retention time 10.90 min., nonan-1-en-3-one **10c**, m/z(relative intensity %) 111(17), 97(15), 83(57), 70(38), 55(75), 41(62), 27(62); peak no. 744, retention time 12.10 min., cyclononanone **9c**, m/z(relative intensity %) 140(M^+, 4), 111(11), 98(34), 83(25), 69(19), 55(72), 41(100), 39(47), 27(57). The chromatogram also showed several unidentified components. GC-MS analysis performed on the solution obtained from the reaction at 150°C gave three peaks which indicated that all of the starting material had been consumed. However, none of the expected products appeared in the reaction mixture and two peaks corresponded to the impurities present in the solvent. GC-MS peak no. 176, retention time 3.26 min., cycloheptane, m/z(relative intensity %) 98((M^+, 24), 83(19), 70(39), 55(60), 41(100), 39(54), 29(30), 27(44); peak no. 700, retention time, 12.88 min., 1,3-di-*tert*-butylbenzene, m/z(relative intensity %) 190(M^+, 5), 175(36), 9(12), 65(12), 57(100), 41(49), 39(14), 29(30), 27(44); GC-MS peak no. 737, retention time 13.54 min., 1,4-di-*tert*-butylbenzene, m/z(relative intensity %) 190(M^+, 11), 175(93), 160(13), 41(65), 39(22), 29(27).

4.7.2 Effect of Bu_3SnH concentration on the reduction of spiroepoxy bromides

*Reduction of 2-bromomethyl-1-oxa-spiro[2.3]hexane **6a** (cyclobutylepoxy bromide)*

Cyclobutylepoxy bromide **6a** (29.0 mg, 0.16 mmol) was weighed into an NMR tube containing *tert*-butylbenzene (500.0 μl). The tube was equilibrated in an oil-bath at 120°C and quickly a portion of tributyltin hydride (17.75 mg, 0.06 mmol) was added to it. Simultaneously, the photolysis of the reaction mixture was commenced. Three further portions of the reagent were added to the reaction mixture at 15 min. intervals. Upon cooling the contents of the tube, a sample of it, when analysed by GC-MS, gave no peak corresponding to the starting material. GC-MS peak no.174, retention time 3.18 min., hex-1-en-3-one **10a** (28 rel. %); peak no. 209, retention time 3.85 min., 2-methylcyclopentanone **8a** (27 rel. %); peak no. 266, retention time 4.88 min., cyclohexanone **9a** (45 rel. %).

Reduction of 2-bromomethyl-1-oxa-spiro[2.4]heptane ***6b*** *(cyclopentylepoxy bromide)*

The spirobromide **6b** (0.57 g, 2.98 mmol) was placed in an NMR tube containing hexadecane (6.70 cm^3). Tributyltin hydride (0.14 g, 0.47 mmol) was introduced into the tube and, after mixing its contents, a sample of it was subjected to GC-MS analysis. The contents of the tube were photolysed at 180°C and the above sized portions of the reagent were syringed into the reacting solution every five mins. After eight such additions (total reaction time 40 min.) the reaction was terminated and product identification was undertaken by GC-MS which showed that all the starting material had been consumed. GC-MS peak no. 301, retention time 5.51 min., hept-1-en-3-one **10b**; peak no. 316, retention time 5.85 min., 2-methylcyclohexanone **8b**; peak no. 403, retention time 7.44 min., cycloheptanone **9b**.

Reduction of 2-bromomethyl-1-oxa-spiro[2.6]nonane ***6c*** *(cycloheptylepoxy bromide)*

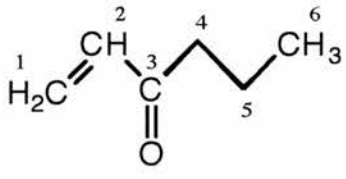
The spirobromide **6c** (40.0 mg, 0.18 mmol), benzene (500.0 μl) and tributyltin hydride (58.20 mg, 0.20 mmol) were all subjected UV irradiation at 75°C for 1h. The progress of the reaction was monitored by GC-MS which revealed that all of the starting material had been consumed. GC-MS peak no. 532, retention time 9.84 min., nonan-3-one **7c**; peak no. 544, retention time 10.06 min., unidentified; peak no. 584, retention time 10.73 min., 2-methylcyclooctanone **8c**; peak no. 590, retention time 10.88 min., nonan-1-en-3-one **10c**; peak no. 743, retention time 13.73 min., unidentified, peak no. 814, retention time 15.06 min., unidentified.

4.7.3 Temperature dependence of Ph_3SnH-initiated reduction of spiroepoxy bromides

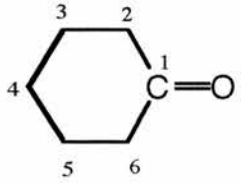
Reduction of 2-bromomethyl-1-oxa-spiro[2.3]hexane ***6a*** *(cyclobutylepoxy bromide)*

A mixture of cyclobutylepoxy bromide (31.0 mg, 0.18 mmol), perdeuteriobenzene (600.0 μl) and triphenyltin hydride (9.26 mg, 26.38 μmol) were

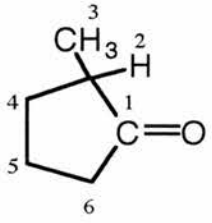
subjected ^{1}H NMR spectroscopy. The tube was then heated to 80°C and aliquots (9.26 mg) of the organotin reagent were added every 30 min. to the reacting solution for 4h. The contents of the tube were cooled and dichloromethane (9.26 mg, 5.0 μl) was added as a standard. A ^{1}H NMR spectrum of the resulting solution showed the composition of the reaction mixture to be hex-1-en-3-one **10a** (2.71 mg, 17%)‡; δ_H(200MHz, C_6D_6) 0.78(3H, t, CH_3-6), 1.53(2H, m, CH_2-5), 2.10(2H, t, CH_2-4), 5.21(1H, dd, CH-2), 5.84(2H, m, CH_2-1);



10a



9a



8a

cyclohexanone **9a** (10.07 mg, 59%)‡; δ_H(200MHz, C_6D_6) 1.14(2H, m, CH_2-4), 1.35(4H, q, CH_2-3,5), 2.0(4H, t, CH_2-2,6); 2-methylcyclopentanone **8a** (3.38 mg, 22%)‡; δ_H(200MHz, C_6D_6) 0.97(3H, d, CH_3-3), 1.06-1.52(4H, m, CH_2-4,5), 1.68(2H, m, CH_2-6), 1.92(1H, m, CH-2).

‡Expressed as mol % of the precursor bromide as determined by NMR spectroscopy.

Reduction of 2-bromomethyl-1-oxa-spiro[2.4]heptane ***6b*** *(cyclopentylepoxy bromide)*

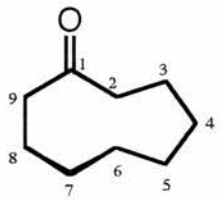
Using an NMR tube as a reaction vessel, cyclopentylepoxy bromide **6b** (25.0 mg, 0.20 mmol) was dissolved in perdeuteriobenzene (650.0 μl). Following the initial addition of triphenyltin hydride (17.17 mg, 0.05 mmol) and withdrawal of a small sample of this for GC-MS and NMR analysis, it was photolysed for 4h at 75°C with the addition of the same amount of the reagent on a hourly basis. The contents of the tube were filtered at the end of the reaction and the solution obtained was examined by NMR spectroscopy and GC-MS. Signals from the ^{1}H NMR spectrum proved very difficult to analyse owing to the severe overlapping of the peaks. GC-MS gave four peaks none of

which corresponded with the starting spirobromide. GC-MS peak no. 310, retention time 5.88 min., hept-1-en-3-one **10b**; peak no. 330, retention time 6.27 min., 2-methylcyclohexanone **8b**; peak no. 378, retention time 7.03 min., unidentified; peak no. 440, retention time 7.82 min., cycloheptanone **9b**.

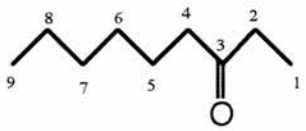
*Reduction of 2-bromomethyl-1-oxa-spiro[2.6]nonane **6c** (cycloheptylepoxy bromide)*

Perdeuteriobenzene (500.0 μl) was syringed into an NMR tube containing cycloheptylepoxy bromide **6c** (55.10 mg, 0.25 mmol) and triphenyltin hydride (88.31 mg, 0.25 mmol). A sample of the reaction mixture was withdrawn for NMR analysis. The contents of the tube were photolysed at 20°C for 1h and re-examined by NMR spectroscopy and GC-MS. ^{1}H NMR spectrum was used to determine the extent of the reaction. Total yield of the products was estimated to be 31% from the integral heights. The proportions of the individual components could be deduced in relative terms from the GC-MS. GC-MS peak no. 178, retention time 3.26 min., cycloheptane, m/z(relative intensity %) 98(M^+, 22), 83(20), 78(38), 41(100), 27(57); peak no. 525, retention time 9.69 min., nonan-3-one **7c**; peak no. 563, retention time 10.36 min., non-1-en-3-one **10c**; peak no. 745, retention time 13.69 min., unidentified; peak no. 811, retention time 14.95 min. unidentified.

The above reaction was scaled up using cycloheptylepoxy bromide (0.43 g, 1.96 mmol), benzene (100 cm^3) and the organotin reagent (1.38 g. 3.92 mmol). This mixture was photolysed at 75°C. The progress of the reaction was monitored with TLC and the spot corresponding to the starting material disappeared after 10h of photolysis. The solvent was completely evaporated under reduced pressure following the removal of solid residues by filtration. The residual solution was separated by column chromatography using silica gel (mesh size 40-63 μm). The column was eluted with a solvent mixture (petroleum ether 40-60/EtOAc) of increasing polarity (5%-20% EtOAc) and this yielded two colourless liquids.



9c



7c

Cyclononanone **9c** (0.15 g, 36%); δ_H(300MHz, $CDCl_3$) 1.36(4H, m, CH_2-5,6), 1.56(4H, m, CH_2-4,7), 1.85(4H, m, CH_2-3,8), 2.43(4H, m, CH_2-2,9); nonan-3-one **7c** (0.04 g, 10%); δ_H(300MHz, $CDCl_3$) 0.86(3H, t, CH_3-9), 1.04(3H, t, CH_3-1), 1.27(6H, m, CH_2-6,7,8), 1.56(2H, m, CH_2-5), 2.36-2.46(4H, m, CH_2-2,4).

4.7.4 Relation between reduction of spiroepoxy bromides and Ph_3SnH concentration

A mixture of perdeuteriobenzene (600.0 μl), cycloheptylepoxy bromide (40.0 mg, 0.18 mmol), contained in a NMR tube was photolysed with UV light at 75°C with addition of triphenyltin hydride (8.86 mg, 0.03 mmol) aliquots every 30 min. GC-MS analysis performed on the reaction mixture at the end showed no starting material but several other peaks corresponding to the products were identified. GC-MS peak no. 180, retention time 3.30 min., cycloheptane; peak no. 530, retention time 9.80 min., nonan-3-one **7c**; peak no. 565, retention time 10.40 min., non-1-en-3-one **10c**; peak no. 587, retention time 10.90 min., 2-methylcyclooctanone **8c**; peak no. 663, retention time 12.20 min., cyclononanone **9c**.

4.8 EPR Characterisation of Transient Radicals Generated from Photolysis of Spiroepoxides

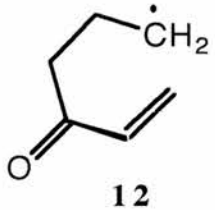
EPR spectra were obtained with a Bruker ER 200D spectrometer operating at 9.1 GHz with 100 kHz modulation. Samples were prepared in spectrosil quartz tubes in cyclopropane solution on a vacuum line or in *tert*-butylbenzene and degassed by bubbling nitrogen for 20 min. Photolysis was performed in the resonant cavity of the spectrometer

with a 500W super pressure mercury arc lamp. Typically, the sample size consisted of the substrate (10-20 mg), cyclopropane or *tert*-butylbenzene (500 μl), di-*tert*-butyl peroxide (30 μl), triethylsilane (30 μl) or hexamethyldistannane (30 μl).

4.8.1 Detection of radicals from photolysis with triethylsilane

Oxaspiro[2.3]hexyl derivatives (cyclobutylepoxy derivatives)

Cyclobutylepoxy bromide **6a** (10.0 mg, 56.80 μmol) was added to an EPR tube containing di-*tert*-butyl peroxide (30 μl) and triethylsilane (30 μl). Cyclopropane gas was condensed (500 μl) into the reaction mixture while the tube was attached to the vacuum line and surrounded by liquid nitrogen. After deaeration by a series of freeze-pump-thaw cycles, the tube was flame sealed and placed in the microwave cavity of an EPR spectrometer. Photolysis was carried out in the temperature range 150-200K. At low temperatures, 150-160K, a strong 9 line signal, originating from the primary radical **12**, was obsrved. A minor radical was also present but it was too weak and/or extensively overlapped for analysis. Information from the spectra, at each temperature studied, is summarised in **Table 5** (Results and Discussion section 4.8.1).

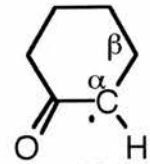


12

Photolysis with Hexamethyldistannane

Cyclobutylepoxy bromide **6a** (10.0 mg, 58.80 μmol), hexamethyldistannane (30 μl) dissolved in *tert*-butylbenzene (500 μl), contained in an EPR tube, were degassed for 20 min. The mixture was photolysed in the cavity of an EPR spectrometer and spectra were recorded within the temperature range 200-235 K. At 200 K, the main signals were owing to the primary radical **12** although another radical, with overlapped peaks, was also visible. The secondary cyclised radical **13** gave four sharp outer lines and broad

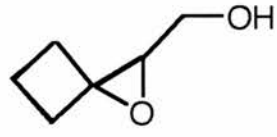
central lines at 210 K and these spectral features continued to persist at a higher temperature of 235 K.



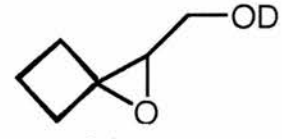
13

EPR parameters of the two radicals observed within the temperature range studied are presented in **Table 6** (Results and Discussion section 4.8.1).

Cyclobutylepoxy alcohol **16a** (*ca* 20 mg) was added to an EPR tube containing di-*tert*-butyl peroxide (50 μl) and cyclopropane (*ca* 500 μl) was intoduced into the mixture in the same manner as described earlier. Following its degassing, the tube was sealed and its contents were photolysed in an EPR spectrometer cavity and spectra were recorded within the temperature range 145-260 K. A comparable experiment was also carried out, after deaeration with nitrogen gas, in the absence of the solvent, in order to gain access to intermediates at a higher temperature range 280-340 K. A mixture of two EPR-active species was observed. A primary radical **18a** which gave a 9 line trace and a radical which gave a doublet possibly **17a**.



16a



21a

Both radicals reached their maximum intensities at 150 K. The primary radical decayed away with increase in temperature until it was no longer visible at approx. 300 K whereas the intensity of the secondary radical reached zero concentration at approx. 200 K. The data derived from the analysis of the spectra from these radicals is contained in **Table 9** (Results and Discussion section 4.8.1)

Alcohol **16a** (*ca* 15 mg) was dissolved in di-*tert*-butyl peroxide (25 μl) and treated with 1-2 drops of D_2O. The mixture, on photolysis at 150 K, gave an EPR spectrum consisting of 9 lines and a doublet, observed previously, from radicals **18a** and **17a** respectively. Increase in temperature to 230K, resulted in fall in the concentration of

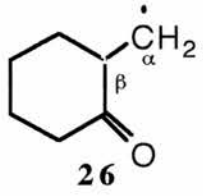
the secondary radical to virtually zero whereas the intensity of the primary radical remained essentially unaltered.

Oxaspiro[2.4]heptyl derivatives (cyclopentylepoxy derivatives)

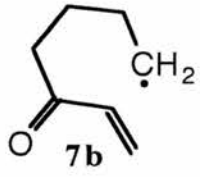
Cyclopentylepoxy bromide **6b**, di-*tert*-butyl peroxide (20 μl), triethylsilane (30 μl) and cyclopropane (500 μl), contained in an EPR tube, were photolysed in the same way as described earlier and in the temperature range 175-210K. The EPR spectra obtained at the temperatures investigated were broad and far too weak for an unambiguous interpretation.

Photolysis with Hexamethyldistannane

When cyclopentylepoxy bromide **6b** (25.0 mg, 0.13 mmol) was treated with distannane, an EPR spectrum consisting of a doublet of triplets was observed. This corresponded to the primary cyclised radical **26**. A minor signal, unidentified, was also

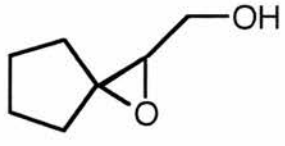


26



7b

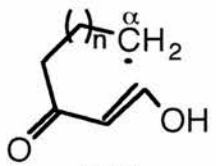
present. The decrease in hfs relating to $1H_\beta$ with increasing temperature is given in **Table 7** (Results and Discussion section 4.8.1). A similar experiment, conducted at lower temperatures (160-185 K), in cyclopropane as a solvent, gave a spectrum from radical **26** instead of the expected triplet of triplets from the uncyclised species **7b** under these conditions.



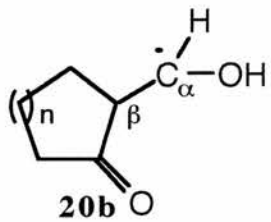
16b

On repeating the above experiment, in turn, with cyclopentylepoxy alcohol **16b** (25.0 mg, 0.19 mmol) dissolved in di-*tert*-butyl peroxide (500 μl), in the temperature

range 230-260 K, gave a spectrum that showed the presence of a primary uncyclised radical **18b**, a cyclised product **20b** and an unidentified intermediate.

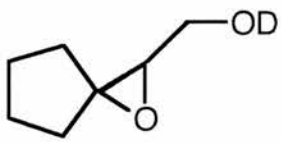


18b



20b

All radicals persisted throughout the temperature range examined except for the primary radical which was not observable at 260 K.



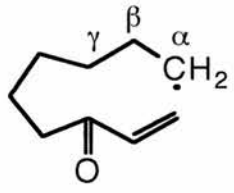
21b

Deuterated spiroalcohol **21b** (*ca* 25 mg), on photolysis at 235 K, gave a weak EPR signal which consisted of a triplet $a(2H_\alpha)$=22.0 G. This originated from the primary radical **23b** which was too weak for observation of the whole spectrum.

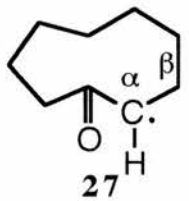
Oxaspiro[2.6]nonanyl derivatives (cyclononanylepoxy derivatives)

Photolysis with Hexamethyldistannane

Cycloheptylepoxy bromide **6c** (15.0 mg, 0.07 mmol) on photolysis in the presence of the distanane reagent and *tert*-butylbenzene in the temperature range 200-300 K produced evidence for two radicals. Below 250 K, a spectrum with 5 main lines was observable. The spectral parameters of this were consistent with the existence of radical **15** in the reaction mixture. Above this temperature, a second species **27** appeared and became dominant at 290 K. Its spectrum consisted of 4 lines with intensity ratios of 1:3:3:1. **Table 8** (Results and Discussion section 4.8.1) gives the spectral parameters of the radicals.

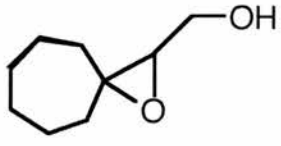


15

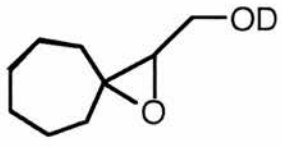


27

In an analogous reaction with cycloheptylepoxy alcohol **16c** (31.10 mg, 0.19 mmol), an EPR signal at 240 K consisted of a doublet with a 5 line multiplet structure, containing an additional doublet hfs. This spectrum was tentatively identified with radical **28** generated by H-abstraction from the intermediate hydroxy-cyclononanone. The analysis of the observed signals can be found in **Table 9** (Results and Discussion section 4.8.1)

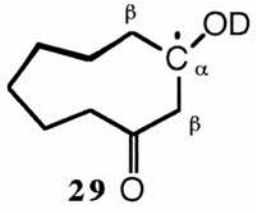


16c

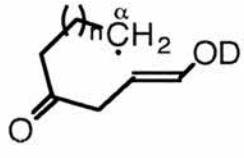


21c

Deuterio alcohol **21c**, when photolysed in the temperature range 270-315 K, produced an EPR spectrum which consisted of a weak 5 line multiplet that corresponded with the cyclised radical **29**, the spectral information is given in **Table 10**.



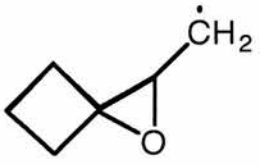
29



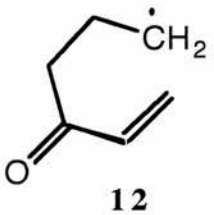
23c

4.9 Kinetic Study of the Cyclisation of 4-Oxo-hex-5-enyl Radical

Photolysis of the cyclobutylepoxy bromide **6a** incorporating two β–scissions of the precursor cyclobutylepoxycarbinyl radical **11**, leads to the production of 4-oxo-hex-5-enyl radical **12**. This can cyclise in both *exo-* and *endo-* fashion to form, after hydrogen transfer, cycloalkanones.



11



12

A kinetic study was undertaken in order to assess the rate constant of the rate-determining step in the sequence of these reactions. Proportions of the products, at a series of temperatures from reactions with fourfold excess of tributyltin hydride,were measured using GC. A standard, heptane, was added to each reaction mixture at the termination of photolysis. The derived rate constants for *endo*- (k_c^6) and *exo*- (k_c^5) at 298 K were 1.3×10^5 s^{-1} and 2.6×10^5 s^{-1} respectively. The related data from, GC analysis, is given in **Tables 11** and **12** (Results and Discussion section 4.9).

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Appendix

Abbreviations and Symbols

NO	Nitric Oxide
NOS	Nitric Oxide Synthase
FAD	Flavin Adenine Dinucleotide
FMN	Flavin Adenine Mononucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
GC	Guanylate Cyclase
cGMP	Cyclic Guanosine Monophosphate
PK	Protein Kinase
ROI	Reactive Oxygen Intermediate
DNA	Deoxyribonucleic Acid
G, C, A, T	Guanine, Cytosine, Adenine, Thymine
CAM	Cytotoxic Activated Macrophage
NONOates	Ionic Salts
bp	Boiling Point
EPR	Electron Paramagnetic Resonance
NMR	Nuclear Magnetic Resonance
GC-MS	Gas Chromatography-Mass Spectrometry
hfs	Hyperfine Splitting Constant
mol	Mole
m/z	Mass to Charge Ratio
M^+	Molecular Ion
s, d, t, q	Singlet, Doublet, Triplet Quartet/Quintet
TLC	Thin Layer Chromatography