A THESIS

entitled

CHIRAL EPOXIDES AND THEIR REACTION WITH MODELS FOR BIOLOGICAL NUCLEOPHILES.

by

Martin Keith Ellis

B.Sc. Chemistry with Biological Chemistry,

University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL,

West Midlands.

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ABBREVIATIONS

A	
aqu.	aqueous
B	
b.p.	boiling point
C	
^{13}C	carbon-13
c.i.	chemical ionisation
cm	centimeter
ca	circa
^{o}C	degree celsius
D	
DNA	deoxyribonucleic acid
D	deuterium
Dimsyl	dimethyl sulphinyl carbanion
DMSO	dimethylsulphoxide
E	
e.i.	electron impact
ee	enantiomeric excess
equiv.	equivalent(s)
$Eu(fod)_3$	tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium III
$Eu(hfbc)_3$	tris(heptafluoropropylhydroxymethylene-d-camphorato)-europium III
$Eu(DMP)_3$	tris(2,2,6,6-tetramethylheptan-3,5-dianato)europium III
e.g.	for example

F

F.A.B.	Fast atom bombardment
Fig.	figure
FT	Fourier transform

G

g.c.	gas chromatography
g.c./m.s.	gas chromatography/mass spectroscopy
GSH	glutathione
g.l.c.	gas liquid chromatography
g	gram(s)

H

^{2}H	deuterium
h.p.l.c.	high performance liquid chromatography
h	hours
HMPA	hexamethylphosphoramide
^{1}H	proton

I

i.r.	infrared
i.d.	internal diameter
I.U.P.A.C.	International Union of Pure and Applied Chemistry

K

k'	pseudo-first-order rate constant

M

m.s.	mass spectroscopy
m/z	mass/charge
MHz	megahertz
m.p.	melting point
Me	methyl

M - cont.

m-	meta-
mg	milligram(s)
mm	millimeter(s)
mmol.	millimole(s)
min	minute
M	molar
mol.	mole(s)

N

n.m.r.	nuclear magnetic resonance
n.O.e	nuclear Overhauser effect

O

o-	ortho-

P

p-	para-
p.p.m.	parts per million
Ph	phenyl
PATC	phenylaminothiocarbonyl
p.l.c.	preparative layer chromatography

R

rac.	racemic
Rf	retardation factor
RT	room temperature

T

THF	tetrahydrofuran
TMS	tetramethyl silane
t.l.c.	thin-layer chromatography
TSS	3-(trimethylsilyl)-tetradeuteropropionic acid sodium salt

U

u.v.	ultraviolet

V

v/v	volume per volume

W

w/v	weight per volume

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DECLARATION

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ABSTRACT

Current questions concerning the safety of industrial chemicals requires that many substances be re-evaluated to quantify the significance of their environmental and occupational hazards. A large number of epoxides have been found mutagenic and carcinogenic. However, for many of these epoxides the stereochemical requirements are still undefined.

After a general introduction into the toxicology of epoxides (Chapter 1), Chapter 2 reviews the general methods developed, and adopted in the syntheses of chiral epoxides and their precursors. In Chapter 3, the materials, methods and instruments used in this project are outlined. A new synthetic route to optically active epoxides is described in Chapter 4. The acid catalysed ketalisation of D-camphorquinone with racemic 1,2-diols is shown to afford a kinetic resolution from which one diastereoisomer is formed predominantly. This dioxolane was hydrolysed to yield an optically active diol or converted into an epoxide. A general route to chiral 1,2-diols is described. The reaction of the enantiomers of methyloxirane with biological nucleophiles (protected N-acetyl esters of amino-acids containing reactive nitrogens [histidine imidazole nitrogens] or sulphur [cysteine] and the amino ester of valine) is described in Chapter 5. This chapter determines the structure and stereochemistry of the products, along with enantioselectivities and kinetic data. This data was used to ascertain the relative rates of nucleophilic attack upon the enantiomeric pairs. For ambifunctional nucleophiles identification of the preferred site of alkylation and the regioselectivity of nucleophilic attack was deduced. A method for deducing the enantioselectivity of nucleophilic groups in peptides using specifically deuterated epoxide racemates is described (Chapter 6). Product mixtures are determined by m.s.. The use of this method to deduce enantioselectivities in peptides and proteins is outlined.

PUBLICATIONS

Part of the work described in this thesis has been published, and a further part is being prepared for publication as follows:

1. M.K. Ellis, B.T. Golding and W.P. Watson, "Intrinsic Reactivities in the Alkylations of Protected Amino-acids by (R)- and (S)-Methyloxirane", J.Chem.Soc.Perkin Trans. II, 1984, 1737.

2. M.K. Ellis, B.T. Golding and W.P. Watson, "Kinetic Resolution of 1,2-Diols with D-Camphorquinone; Preparation of (R)-(Chloromethyl)-oxirane", J.Chem.Soc.Chem.Commun., 1984, 1600.

3. M.K. Ellis and B.T. Golding, "Optically Active Epoxides from Vicinal Diols via Vicinal Acetoxybromides: The Enantiomeric Methyloxiranes", Org.Synth., in press.

4. M.K. Ellis, B.T. Golding and W.P. Watson, "Camphor Derivatives, their Preparation and their Uses", patent application pending.

5. M.K. Ellis, B.T. Golding and W.P. Watson, "The Use of Deuterated Epoxide Racemates to Assess Enantioselectivities in Proteins", Biomed. Mass Spec., in preparation.

TO MY PARENTS

CHAPTER 1

THE TOXICOLOGY OF EPOXIDES

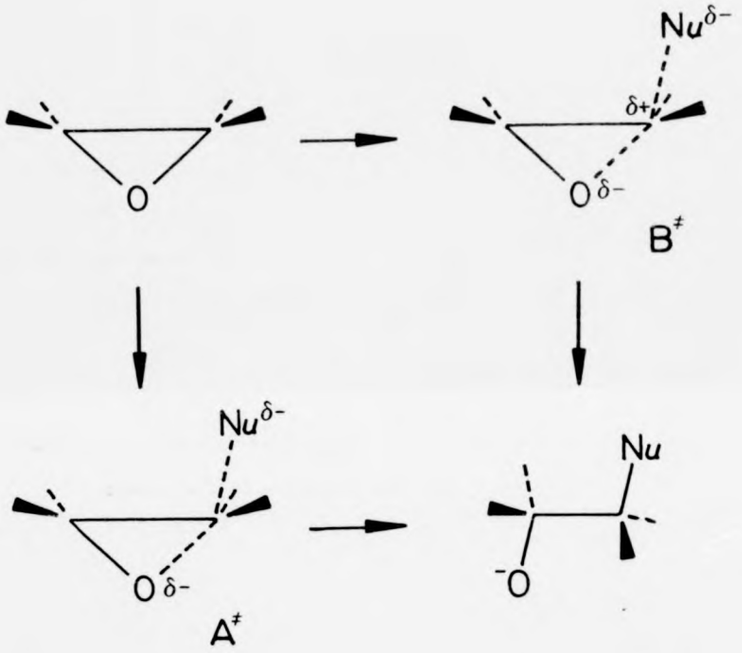
1.A General Reactivity of epoxides

1.B Uses and exposure to industrial and environmental epoxides

1.C Chemical carcinogenesis

1.D Metabolic detoxification of epoxides and related compounds

1.E Haemoglobin as a dose monitor for chemical carcinogens



Scheme 1.A.1

THE TOXICOLOGY OF EPOXIDES

1.A GENERAL REACTIVITY OF EPOXIDES

Epoxide (oxirane), is used to describe a class of cyclic ethers containing a three-membered ring incorporating two carbon atoms and one oxygen atom. Their reactivity is related to the release in strain energy achieved on cleavage of the ring. The direction of cleavage and the stereochemistry of the product is governed by the structure of the epoxide, the structure of the nucleophile and the reaction conditions. The attack of a nucleophilic reagent upon an epoxide follows an S_N2 type mechanism, the theoretical implications of which have been studied by Parker and Isaacs[1]. Their theoretical model states that an epoxide is able to react by either, or both, of two limiting processes which are based on an S_N2 or borderline S_N2 reaction, respectively. This is shown in Scheme 1.A.1. The transition state $B^{\ddagger}$ implies less bond formation than does the transition state $A^{\ddagger}$ and therefore more nearly resembles a conventional S_N2 transition state, being stabilised by inductive and mesomeric effects of substituents. In reactions of this type a fully developed carbonium ion is not formed. The transition state $A^{\ddagger}$ resembles a classical S_N2 transition state. Increasing alkyl substitution decreases the stability of this species and non-polar solvents decrease the activation energy for the pathway via $A^{\ddagger}$ in accordance with their ability to disperse charge. Where $A^{\ddagger}$ does differ from a classical S_N2 transition state is in the departure of the epoxide oxygen. The classical S_N2 reaction geometry requires a linear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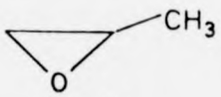

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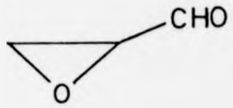

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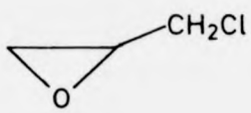

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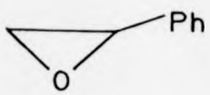

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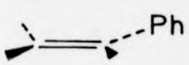

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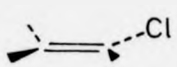

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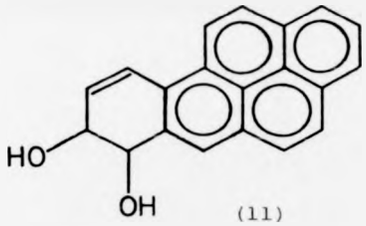

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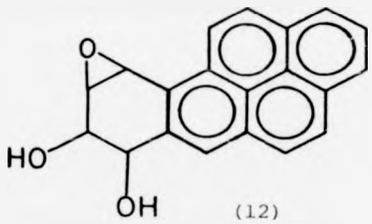

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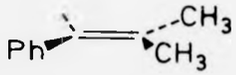

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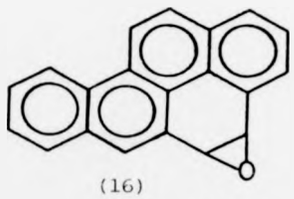
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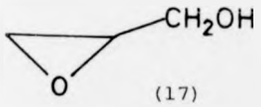

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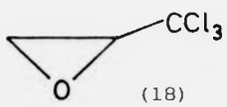

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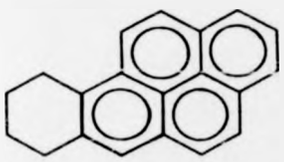
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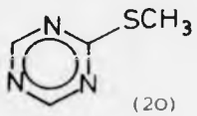

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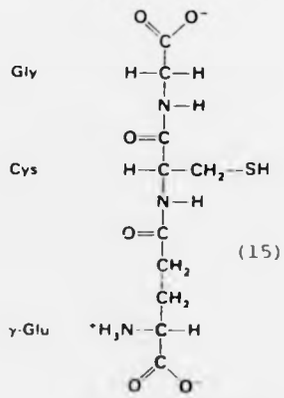

(15)

Reduced glutathione
(γ-Glutamylcysteinylglycine)

disposition of the incoming nucleophile, the carbon atom undergoing substitution and the departing oxygen atom. This criterion cannot be fulfilled in the cleavage of epoxides. With mono-substituted epoxides it can be envisaged that nucleophilic attack can occur at either the methylene or methyne position. Where the substituent is electron-withdrawing and/or when the reaction is conducted in basic solution the preferred mode of attack is at the methylene position. Acid catalysis however, facilitates ring cleavage by formation of an oxonium species and resembles a 'borderline S_N1' reaction mechanism. Protonation of the oxygen facilitates nucleophilic substitution at the methyne position where a more stable, highly substituted carbonium ion can be formed. Reactions at the methyne carbon are also facilitated when electron-donating substituents are present on the ring.

1.B USES AND EXPOSURE TO INDUSTRIAL AND ENVIRONMENTAL EPOXIDES

Oxirane (ethylene oxide, 1), the simplest epoxide, was synthesised for the first time in 1859 by Wurtz, who reacted aqueous potassium hydroxide with ethylene chlorohydrin[2]. A year later, Oser synthesised methyloxirane (epoxypropane, propylene oxide, 2) again utilising the chlorohydrin method[3].

Epoxides are extremely valuable commercially because of the many reactions they undergo. In 1983 approximately 7 million tonnes of oxirane (1) and 5 million tonnes of methyloxirane (2) were used for a variety of purposes in the chemical and related industries. Their major applications are in the production of polyurethane foams,

glycols, lubricants, surfactants, oil demulsifiers and sterilising fumigants for medical plastics. They are present in foodstuffs and used in the production of modified starches which are subsequently incorporated into a wide range of processed foods. Other epoxides are used in the textile industries [diepoxybutane (3), glycidaldehyde (4)] and in the production of paints [epichlorohydrin (5)] and epoxy resins [epichlorohydrin, styrene oxide (6)].

Because of the vast quantities handled, the highest risk of exposure will be to those people who work where these chemicals are produced and used[4]. Human exposure is believed to be most extensive in the sterilisation of health care products, where in the U.S.A. an estimated 75,000 workers are exposed. Workers also come into contact with epoxide stabilisers in chemicals that they handle in large quantities, e.g. trichloroethylene, used for dry cleaning and degreasing machinery. There is little information on the number of people occupationally exposed to epoxides, but production figures suggest that many employees may be affected in many industrial situations and at concentrations significant for chronic, if not acute, exposure. Non-occupational exposure to epoxides is less significant, though atmospheric pollutants and those found in cigarette smoke do constitute potential hazards. Epoxidised fatty acids, e.g. 9,10-epoxy stearate, occur naturally in certain foods and may also be formed during long term storage of e.g. sunflower seeds. These epoxides tend to be highly substituted and have low reactivity as a result of their steric bulk. The toxicity of these compounds is unknown.

1.C CHEMICAL CARCINOGENESIS

Chemical carcinogenesis, the induction of cancer with chemicals, including those found in tobacco smoke, the diet and the workplace, is a biological process that is both initiated and promoted by chemicals. It is now generally accepted that the early precancerous changes can be induced by repeated or long exposure to a chemical which creates a "promoting environment" or by exposure to a single dose of chemical carcinogen. Cancer of any cell type in humans and in experimental animals shows extensive diversity. Exposure of experimental animals to diepoxybutane (3)[5], epichlorohydrin (5)[6] and glycidaldehyde (4)[7] induced skin cancers. Epoxy resins have been shown to induce sarcomas in rats and skin cancers in mice[8]. Ethylene oxide (1) and propylene oxide (2) induce squamous cell carcinomas of the forestomach in rats[9]. Whatever the mechanism, it is the interaction of the active carcinogen with D.N.A. which is important in the initiation[10] and there is evidence to suggest that the induction is site specific. Van Duuren suggested that carcinogenic epoxides, such as diepoxybutane (3), react at the N-7 position of guanine in D.N.A., but so do apparently non-carcinogenic agents[11]. This position is the most reactive nucleophilic site in nucleic acids. It is also known that not all carcinogenic agents react at the N-7 position of guanine and it is thought that reactions at this nitrogen have a negligible effect on the functioning of D.N.A., whereas nucleophilic substitution at oxygen, e.g. O-6 alkylguanine, and possibly the formation of other alkylated bases[12], including N-3

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alkylcytosine[13] and 5-alkylcytosine[14], may lead to the initiation of a premutagenic state in some systems[11]. The toxicological effects of epoxides[15], ethylene oxide (1)[16] and epichlorohydrin (5)[17] have been reviewed.

1.D METABOLIC DETOXIFICATION OF EPOXIDES AND RELATED COMPOUNDS

Chemicals which are foreign to an organism are usually metabolised to make them more readily excretable. This process may involve oxidation, reduction, or hydrolysis, usually in combination with conjugation. Several ubiquitous enzymes *in vivo* participate in the formation or biotransformation of epoxides to more hydrophilic compounds. There are also a number of non-enzymic processes involved (see below). The enzyme-mediated detoxifications are most prevalent in the liver, but are found throughout different tissue types. Their reactivity differs according to the species, strain, sex and developmental age of the animal.

It is now well established that during the metabolic detoxification in mammalian tissues, many aromatic and unsaturated compounds are metabolised *via* epoxides. These endogenous epoxides induce the carcinogenic and mutagenic processes. As early as 1950, Boyland[18] suggested that metabolic phenols and dihydrodiols formed from polyaromatic hydrocarbons, which are found in car exhaust, smog, charcoal-cooked fish and tobacco smoke, might be secondary products of metabolically formed epoxides.

Metabolic activation of a wide range of pollutants (alkenes[19] and substituted alkenes, e.g. styrene (7)[20], vinylchloride (8)[21],

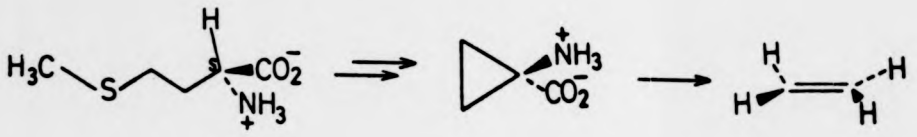


Fig. 1.D.1 Ethylene biosynthesis

benzene (9) and substituted benzenes[22] and polyaromatic hydrocarbons[23,24]) by the mixed functional oxidase (MFO) system converts these substances *in vivo* into epoxides. These metabolically produced epoxides are thought to be more toxic, at least with respect to human carcinogenicity, than their environmental counterparts. When ethylene (10) is administered to rats ethylene oxide (1) is formed and can be detected by g.c. analysis of exhaled air[25]. Low levels of epoxide were also exhaled by non-exposed rats[25] and Ehrenberg has detected low levels of 2-hydroxyethylated amino-acids in haemoglobin obtained from these animals. These phenomena may be explained by endogenous ethylene production and oxidation by the MFO system to the active metabolite, ethylene oxide. Ethylene is a known plant hormone and it is possible that low concentrations of the compound are ingested from eating ripening fruit. Methionine has been shown to be the precursor of ethylene biosynthesis[26] in plants (Fig. 1.D.1) and a similar mechanism could be postulated for its biosynthesis in animals. When human exhaled air was analysed no ethylene oxide was detected but a number of unsaturated alkanes (butenes, pentene and hexene) were present at varying concentrations[27].

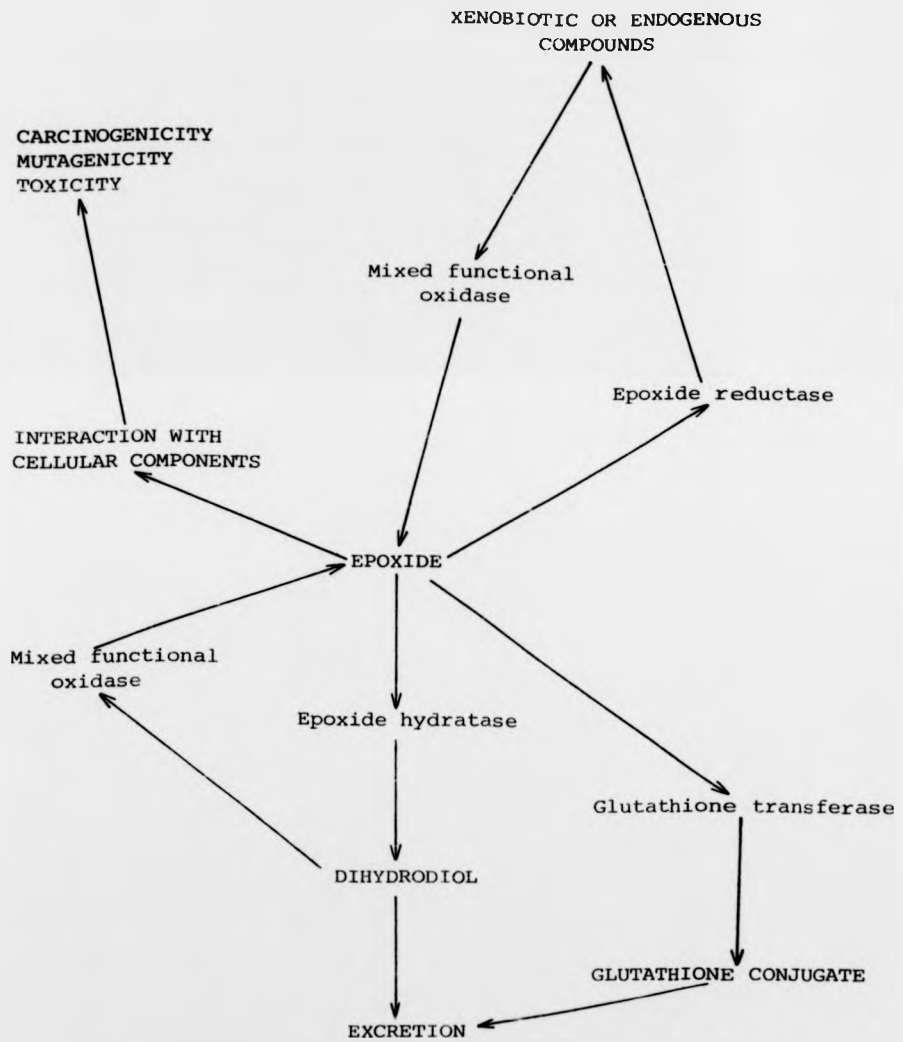
Cytochrome P_{450} is the terminal oxidase of the MFO system and has been shown to metabolise unsaturated chemicals in the presence of the flavoprotein NADPH-Cytochrome P_{450} reductase. Cytochrome P_{450} is a membrane bound protein and has been isolated from many tissue types (e.g. liver, kidney, breast, prostrate, lung, pancreas and brain) and multiple forms exist, each of which may possess different substrate specificity. Other *in vivo* enzymic oxidations of unsaturated hydrocarbons have been suggested. Xanthine oxidase and

ferredoxin reductase have been shown to oxidise styrene (7) to styrene oxide (6) through the production of superoxide ion (O_2^-) and hydrogen peroxide[28], and Prostaglandin H synthetase, which converts arachidonic acid to the endoperoxide PGH_2 has been shown to metabolise benzo[a]pyrene-7,8-dihydrodiol (11) to its corresponding diol epoxide (12)[29]. These oxidations may be potentially more dangerous than the oxidative process involving Cytochrome P_{450}, because epoxide-metabolising enzymes are absent from the site of action of these systems.

The epoxide hydrolases and the glutathione-S-transferases constitute the major metabolic routes to the enzyme mediated detoxification of epoxides. The epoxide hydrolase enzymes are located in both the endoplasmic reticulum and in the cytosol and are found throughout many tissue types. They function to convert epoxide moieties into vicinal dihydrodiols which are more hydrophilic and less toxic than the parent epoxide. Oesch has studied the reactivity of microsomal fractions containing epoxide hydratases towards a number of substituted epoxides[30,31]. It was observed that monosubstituted epoxides with a lipophilic substituent larger than an ethyl group (isopropyl, t-butyl, phenyl) readily interact as substrates or inhibitors, whereas those epoxides with smaller substituents (methyl, ethyl, vinyl) or where the epoxide is sterically hindered [trans-stilbene oxide (13) and dimethylphenyloxirane (14)] show little or no reactivity. This work was performed prior to the discovery of cytosolic epoxide hydrolases and these enzymes may accept epoxides such as oxirane and methyloxirane as substrates. The hydration of mono- and 1,1-disubstituted

oxiranes by enzymes in rat liver microsomes has been shown to be highly regiospecific, involving nucleophilic attack of water on the methylene carbon of the oxirane[32]. Dihydrodiols have been isolated from the urine of rats exposed to styrene (7) and styrene oxide (6)[33]. It has also been postulated that some dihydrodiols derived from polyaromatic hydrocarbons may retain sufficient lipophilicity to serve again as substrates. The dihydrodiols are metabolised to dihydrodiol epoxides [35] which are now believed to be the ultimate carcinogenic metabolite.

The glutathione-S-transferases are a group of cystolic enzymes that show a broad and overlapping substrate specificity[34]. They catalyse the conjugation of epoxides, and compounds possessing labile substituents (e.g. halogen[36]), to glutathione (15). The glutathione conjugate is then degraded enzymically to the cysteine conjugate and excreted, or the cysteine conjugate is N-acetylated to the mercapturic acid and excreted[34]. It is the glutathione S-epoxide transferase that catalyses the conjugation of epoxides. With unsymmetrical epoxides, e.g. styrene oxide (6)[37,38,39], the reaction is not regioselective and gives two positional isomers from attack at the methylene and methyne positions. Highly selective conjugation of glutathione has been observed with dihydronapthalene oxide[40], napthalene oxide[41] and cholesterol-4,5-oxide[42], and regioselective conjugations are observed with benzo-[a]-pyrene-4,5-oxide (16)[43].



Scheme 1.D.1 The Metabolic Detoxification of Epoxides

Epoxides have also been implicated as obligate intermediates in the metabolism of halohydrins to explain the formation of certain mercapturic acid products isolated from rat urine. An alternative mechanism may involve the intermediacy of an episulphonium species[44].

Non-enzymic detoxification by direct hydrolysis of the epoxide gives the vivinal trans-diol which is subsequently excreted[45]. With highly substituted and sterically hindered epoxides this may be the preferred mode of metabolism. It has also been suggested that cleavage of the epoxide, by the weakly nucleophilic chloride ion, may occur in the stomach to give a chlorohydrin[15]. The chlorohydrin may cyclise to the epoxide[46] or be conjugated with glutathione prior to excretion. Ethylene chlorohydrin has been found to be non-mutagenic in mice, but the carcinogenic and mutagenic properties of other chlorohydrins are as yet undefined. The metabolic detoxification of epoxides is illustrated in Scheme 1.D.1.

As a final defence against D.N.A. alkylation the cell has evolved some advanced, enzyme mediated repair systems. These systems function to alleviate or remove damaged D.N.A.. The D.N.A. repair mechanisms have been extensively reviewed[10,47,48], though in individuals where the repair mechanism is impaired, i.e. in those suffering from several recessive inherited disorders, such as xeroderma pigmentosum, ataxia telangiectasia and Fanconi's anaemia there is greater risk of cancer.

1.E HAEMOGLOBIN AS A DOSE MONITOR FOR CHEMICAL CARCINOGENS[49]

When epoxides are administered to laboratory animals, analyses of the various organs indicate a rapid equilibrium of the chemical throughout the animal . The alkylations of nucleophilic sites may therefore be random throughout all tissue components. The probability of specific alkylations depend upon the relative reactivity and steric environment of the nucleophilic centres and the frequency of alkylation depends primarily upon the concentration of the administered dose. Not only do alkylating agents react with the nucleic acid bases, but nucleophilic groups in proteins are also alkylated[49]. The inactivation of alcohol dehydrogenase on administration of styrene oxide is attributed to the alkylation of cysteine residues at the active site[50,51].

The main nucleophilic centres in biological macromolecules are the thiol (cysteine) and thioether (methionine) sulphurs, amino group (N-terminal amino-acid, histidine and lysine) nitrogens and the carboxylate (aspartate and glutamate) oxygens. Nucleophilic reactivity of amines is confined to the unprotonated (base) form and the reactivity of thiols and hydroxyl groups is greatly increased when deprotonation to the thiolate and alkoxide species, respectively occurs. Under physiological conditions (pH 7.2) the reactivity of the various nucleophilic groups will depend upon their pKa's (acid dissociation constants). This can be exemplified by examination of amine reactivities. For amino groups with pKa values higher than the pH of the system their reactivity is proportional to the concentration of free base. At low pH this

concentration will be small and the reactivity is slow, but will increase, as will the reactivity, as the pH increases. The more reactive amines and thiols will be those with pKa values close to the pH of the system. Amongst the nucleophilic groups in proteins the ring nitrogens of histidine (pKa 6-7), the α-amino groups of N-terminal amino-acids (pKa ~7-8) and the thiol of cysteine (pKa ~ 8) fulfil this requirement. The ϵ-NH_2 of lysine (pKa ~ 10) is a strong nucleophile but at pH 7.2 it is essentially present in its protonated form and requires higher pH values for reactivity.

When determining the toxicity of potentially harmful compounds in the human environment it would be advantageous to determine their effects on man himself. This would eliminate metabolic variations in using experimental animals and would allow for variations within the human population itself. The choice of monitoring system faces considerable constraints and analysis has to be performed on samples that can be drawn without inconvenience or serious risk to the person. Such samples are severely restricted to semen, blood and urine.

Primarily it is the alkylation frequency in D.N.A. that is required but direct analysis is difficult because of the low concentration of D.N.A. in the samples mentioned. Approximately 1 mg of sperm D.N.A. may be collected per ejaculation and 10-20 cm^3 of blood is required to yield approximately the same amount from the nucleated cells. A further drawback to the direct monitoring of D.N.A. is that the analytical methods for detecting alkylations are still too insensitive to most needs. However, a fluorometric method has been developed to measure guanine alkylation by simple epoxides: methyloxirane (2), (hydroxymethyl)oxirane (17),

(chloromethyl)oxirane (5), (trichloromethyl)oxirane (18) and phenyloxirane (6). This technique has its limitations since only certain fluorescent types of adducts can be monitored[52]. If verification of D.N.A. alkylation in human subjects is required larger amounts of D.N.A. than available in blood and semen samples is wanted. A more convenient method would be to monitor the alkylations in proteins. A 20 cm^3 blood sample contains approximately 2-3 g of haemoglobin. Ehrenberg has shown that the overall frequency of alkylation in tissue proteins is 1.5-5 times higher than in D.N.A. itself[53].

The advantage of using haemoglobin as a dose monitor for mutagens and carcinogens is primarily the convenience and ease by which it can be drawn from the exposed subject. Since the life-span of haemoglobin is not affected by alkylation, the extent of alkylation can be used to assess exposure levels over a long period, comparable to the life-time of the erythrocyte (ca. 4 months in man). In mice it has been shown that the concentration of alkylated derivatives decreases linearly with time, reaching zero after ca. 40 days (the life-time of the erythrocyte in mice). This observation provides a basis for the use of haemoglobin as a monitor of integral exposures to alkylating agents from both a professional and environmental source. Methods are also available for determining the presence of alkylated amino-acids in haemoglobin. Utilising g.c./m.s. with single ion monitoring, amino-acid adducts have been detected[54,55]. Using this method an N-3'-(2-hydroxypropyl)-histidine adduct was identified in haemoglobin hydrolysate from rats[54] exposed orally to methyloxirane (2). This adduct has also been detected fluorometrically in haemoglobin hydrolysate from mice[56]

and gas chromatographically in blood samples extracted from workers exposed to methyloxirane[57].

Dose-response curves for the alkylation of haemoglobin by a number of alkylating agents show a linear relationship at low doses[57] and the frequency of chemically induced cancers is known to increase in an almost linear manner on increasing the concentration of the administered carcinogen. These results make haemoglobin a very attractive molecule for monitoring D.N.A. alkylation indirectly[58]. Deviations from this norm for haemoglobin alkylation have been observed on administration of high doses. This probably results from the saturation of the detoxification enzymes and results in an unusually high alkylation frequency. These doses are well in excess of any normal exposure level encountered by man. Studies into the alkylation of haemoglobin with ethylene oxide (1)[59], vinyl chloride (8)[21] and benzo[a]-pyrene (19) show the alkylated haemoglobin to be stable throughout the life-span of the erythrocyte. With chemically labile alkylating agents e.g. methyl mercaptotriazine (20), deviations from the norm are observed. This eliviates the use of haemoglobin to rank carcinogens according to their potency because of differences in the stabilities of the alkylating agents and differences in the ability of the alkylating agents to reach the circulating erythrocytes. To date, no carcinogenic or mutagenic compounds have failed to produce covalent reaction products with haemoglobin.

Studies to determine whether the configuration of the administered epoxide has any toxicological significance have not been reported. The reactions of natural double stranded D.N.A.

with certain chiral epoxides, e.g. benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide, show high stereospecificity and enantioselectivity[60]. In principle it is possible that one isomer of an enantiomeric pair could be more mutagenic or carcinogenic than its optical antipode. An aim of this project was to determine any selectivity towards the enantiomers of methyloxirane by the nucleophilic sites in haemoglobin. The results obtained from the alkylation of the protected amino-acids, N-acetyl-L-cysteine methyl ester, N^{α}-benzoyl-L-histidine methyl ester and L-valine methyl ester with (R)- and (S)-methyloxirane are discussed extensively in Chapter 5. Utilising n.m.r. spectroscopy and h.p.l.c. the enantioselectivities of these reactions were deduced. Chapter 6 describes a mass spectroscopic method for establishing enantioselectivities of polypeptides towards (R)- and (S)-methyloxirane. With simple modification to this procedure it could be developed to assess selectivities of proteins *in vivo*.

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CHAPTER 2

THE SYNTHESIS OF OPTICALLY ACTIVE EPOXIDES

THE SYNTHESIS OF OPTICALLY ACTIVE EPOXIDES

2.A GENERAL

The epoxide functional group is one of the most useful in organic synthesis. It is used extensively as a versatile component in the construction of natural products. The utmost reason for the synthetic importance of the epoxide moiety is the existence of stereo- and regioselective methods both for its synthesis and control of its subsequent reactions[1,2]. An epoxide reacts with nucleophiles as an a^2-synthon[3], generating an oxygen functionality at the β-position. Their 1,2-bifuntionality makes epoxides the complement of enol derivatives, which are themselves d^2-synthons. The alcohol resulting from cleavage of the epoxide can also be oxidised to carbonyl derivatives. Thus, depending upon their substitution, epoxides correspond to aldehydes, ketones, acid or ester d^2-synthons. The epoxide is able to function as an Umpolung reagent of carbonyl d^2-reactivity.

This chapter will describe literature methods available for the synthesis of optically active epoxides by asymmetric epoxidations of alkenes, allylic and homoallylic alcohols and carbonyl additions. The use of the 'chiral pool' as a source of epoxide precursors and as chiral resolving agents is outlined, along with asymmetric reductions of prochiral ketones. Finally, the use of enzymes to synthesise epoxides and epoxide precursors with high optical activity is discussed.

2.B ASYMMETRIC EPOXIDATION OF ALKENES

One of the most versatile and useful methods for the synthesis of epoxides is the reaction of peroxy acids with alkenes. These reagents may be added directly to the reaction mixture at reduced temperature (m-chloroperoxybenzoic acid, MCPBA) or generated in situ from the parent acid and hydrogen peroxide (peroxyformic acid, peroxycamphoric acid). The reactivity of the peroxy acid parallels the acidity of the parent acid [Fig. 2.B.1]. Peroxytrifluoroacetic

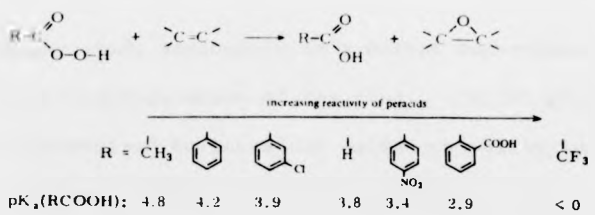
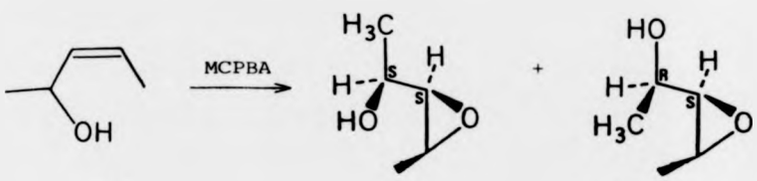


Fig. 2.B.1

acid (trifluoroacetic acid, pKa ~0), one of the most electrophilic peroxy acids, is used to epoxidise electron deficient olefins, whereas for most conventional syntheses m-chloroperoxybenzoic acid (m-chlorobenzoic acid pKa 3.9) is utilised. In general, achiral peroxy acids show no enantiofacial selectivity towards acyclic alkenes, though MCPBA reacts with some (Z)-allylic alcohols (e.g. (Z)-4-hydroxypent-2-ene, 21), to give preferentially the threo-epoxyalcohol (erythro/threo ratio 5:95) in high diastereoisomeric excess[4]. Many cyclic alkenes are stereoselectively epoxidised with

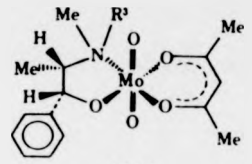


(21) Threo- Erythro-

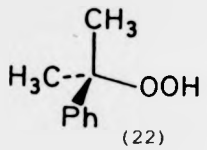
achiral peroxy acids presumably as a direct consequence of the stereochemical configuration of the ring[5]. Chiral peroxy acids have been synthesised to introduce maiden chirality into acyclic alkenes. The selectivity observed with these reagents is rather low. Peroxycamphoric acid gave (S)-styrene oxide (ee 7.8%) from styrene and (1S,2S)-β-methylstyrene oxide (ee 9.2%) from (E)-β-methylstyrene[6]. The maximum enantiomeric excesses observed in epoxidations with chiral peroxypropionic and peroxybutyric acids was 7·5%[7]. More elaborate chiral epoxidising agents have been synthesised incorporating 1,2,4-triazole, carbodiimide and camphor derived pyrazole moieties[8]. The active intermediates in these epoxidations resemble peroxy acids, but their actual structure is uncertain. Epoxidation of (E)-β-methylstyrene with these reagents gave enantiomeric excesses ranging between 2-8%[8]. Metalloporphyrin catalysed reactions of terminal olefins using sodium hypochlorite as oxygen donor gave good yields of the terminal

epoxide[9] and stereospecific epoxidations catalysed by ruthenium bipyridyl complexes have been studied[10].

Enantiofacial selectivity in the epoxidation of straight-chain achiral allylic and homoallylic alcohols has been achieved utilising t-butylhydrogen peroxide (TBHP) in conjugation with chiral transition metal catalysts. The selectivity and rate of reactivity in these reactions is observed to decrease when the substrate possesses bulky substituents at the carbinol centre and an (E)-configuration at the double bond (diastereoselectivity < 80%). The reactivity is also observed to fall with increased substitution at the double bond. The selective epoxidation of secondary (E)-allylic alcohols with TBHP and $VO(acac)_2$ as an achiral catalyst gave preferentially the erythro-product[11], whereas utilisation of $Mo(CO)_6$ as catalyst gave preferentially the threo-isomer[11]. The selectivity was improved when chiral molybdenum catalysts were employed. With chiral catalysts diastereoisomeric chelates are presumed to be formed between the allylic alcohol, the catalyst, and the peroxide. The exact nature of the chelates are uncertain, but association between the substrate and the catalyst occurs to enable selective delivery of the peroxide oxygen to one enantioface of the alkene. Using chiral molybdenum catalysts derived from $Mo(O)_2(acac)_2$ and optically active diols (diesters of tartaric acid and derivatised furanose sugars) enantiomeric excesses of up to 14% were obtained with TBHP[12]. The highest optical yield was achieved on epoxidation of squalene to (3S)-2, 3-oxidosqualene. Selectivities obtained with molybdenum catalysts derived from tartaric acid were superior to those with catalysts derived from furanose sugars. Epoxidations

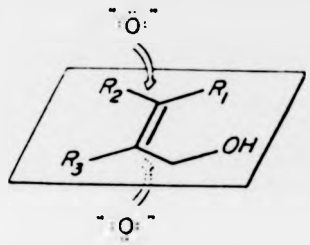


(23)



(22)

D-(-)-diethyl tartrate (unnatural)



L-(+)-diethyl tartrate (natural)

Fig. 2.B.2

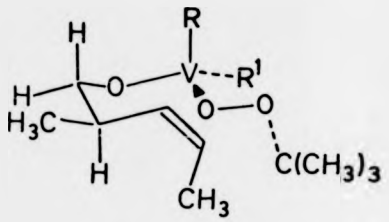


Fig. 2.B.4

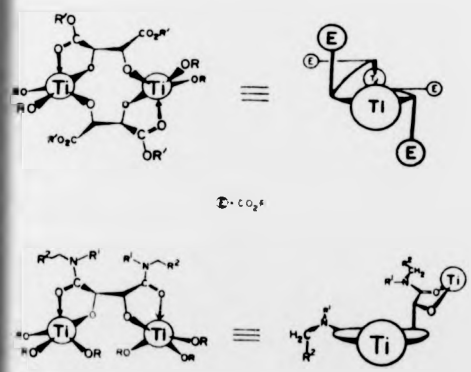
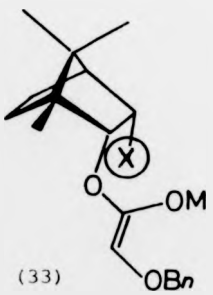


Fig. 2.B.3



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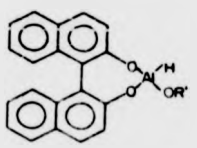

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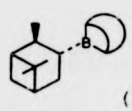

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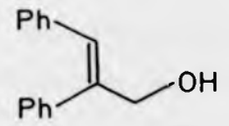
of allylic alcohols with cumene hydroperoxide (22) using (acetylacetonate)[(-)-N-alkylephedrinate]dioxomolybdenum catalysts (23) gave improved selectivity up to 33%[13]. This selectivity has been greatly improved using chiral titanium catalysts derived from titanium isopropoxide [$Ti(O\text{-}i\text{-}Pr)_4$] and (R,R)-diethyl tartrate[14]. This reagent has been found to induce a uniform, high enantiofacial selectivity throughout a range of allylic alcohol substrates when used in conjunction with TBHP. The asymmetric induction is dependant upon the enantiomer of the diethyl tartrate;the oxirane derived from epoxidation of an allylic alcohol is enantiomeric to that oxirane obtained from the enantiomeric tartrate ester[Fig.2.B.2]. Enantiomeric excesses >95% were obtained for a number of substrates[14]. The selectivity was observed to rise on increasing the bulk of the tartrate ester moiety and improved selectivities (ee ~ 100%) were obtained with diisopropyl tartrate. Reactions of racemic allylic alcohols (chirality at the carbinol centre) with the (S,S)-tartrate-derived reagent/TBHP yielded the (R,R)-epoxyalcohol preferentially (attack upon the si-face of the double bond), whereas the (R,R)-tartrate-derived reagent/TBHP selectively epoxidises the (S)-isomer (attack upon the re-face of the double bond) to give the enantiomeric (S,S)-epoxyalcohol [Fig. 2.B.2]. Centres of chirality on the other side of the double bond have little effect upon the selectivity. Substantial rate differences in enantiomer epoxidations with these reagents have been observed and in those epoxidations where k_R/k_S or k_S/k_R >100 the unreacted chiral alcohol is obtained in high enantiomeric purity[15]. From the reaction of (E)-dec-2-en-4-ol (24) with the (R,R)-diisopropyl tartrate-derived reagent the (R)-allylic alcohol



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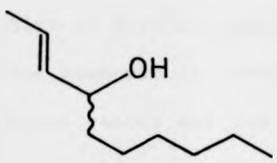

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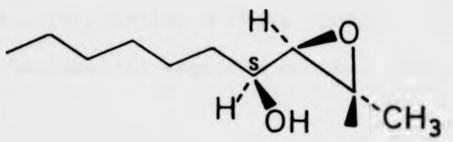


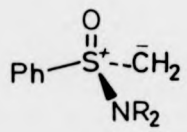
Erythro-(25)

was obtained (ee > 96%) along with the (S)-epoxyalcohol (25, erythro/threo selectivity 99:1). With titanium isopropoxide/TBHP alone the erythro/threo selectivity dropped to 52:48, respectively. Epoxidations using (R,R)-tartaric acid diamides/$Ti(O\text{-}i\text{-}Pr)_4$ (ratio 1:2) gave the (2S,3S)-epoxyalcohol (ee ~96%) on reaction with E-α-phenylcinnamyl alcohol (26). However, when the (R,R)-diamidotartrate/$Ti(O\text{-}i\text{-}Pr)_4$ ratio was reduced to 1:2 the (2R,3R)-epoxyalcohol was preferentially formed (ee 82%)[16]. This remarkable inverse selectivity is attributed to the specific geometries of the chiral catalysts and enables the synthesis of both enantiomeric epoxyalcohols using the relatively inexpensive (R,R)-tartaric acid. The 'standard' (1:1) titanium-tartrate and (2:1) titanium-tartrate asymmetric epoxidation catalysts are shown [Fig. 2.B.3]. The (1:1)-complex is dimeric and forms a ten-membered ring structure, whereas the (2:1)-complex is composed of two 5-membered chelate rings. The inverse selectivity observed with these catalysts is attributed to the mirror image relationships at their 'active sites'.

High stereoselectivity in the epoxidation of acyclic homoallylic alcohols have been obtained using TBHP in the presence of vanadium catalysts[17]. Substitution between the carbinol centre and the proximal olefin Sp^2 centre is observed to govern the stereochemical course of the epoxidation. To predict the stereoselectivity a transition state incorporating a chair conformation between the alcohol and the tetrahedral vanadium complex has been postulated [Fig. 2.B.4][18].

2.C ASYMMETRIC EPOXIDATIONS VIA CARBONYL ADDITION

Since carbonyl compounds are readily available they are attractive intermediates for the synthesis of oxiranes. Sulphur ylides, such as dimethylsulphonium methylide $[(CH_3)_2\overset{+}{S}-\bar{C}H_2]$ react with both saturated and α,β-unsaturated ketones via methylene transfer to generate the epoxide. Analogous reactions with saturated ketones are observed with dimethyloxosulphonium methylide $[(CH_3)_2\overset{+}{S}O-\bar{C}H_2]$, but α,β-unsaturated ketones react preferentially to give the cyclopropane derivative[19]. Intramolecular sulphur ylide addition to ketones have also been studied[20]. Chiral sulphur ylides have been synthesised from optically active (dialkylamino)-alkylaryl-oxosulphonium fluoroborates derived from resolved sulphoximines, and the asymmetric induction in the reactions of chiral (dialkylamino)aryloxosulphonium methylides (27) with aldehydes and ketones was found highly dependent upon the substrate and amino alkyl moieties[21]. Enantiomeric excesses between 7 and 43% were obtained with these reagents. Optically active ylides derived from trialkyl and diarylmethylsulphonium salts have been prepared. These



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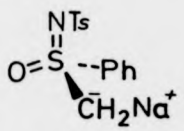

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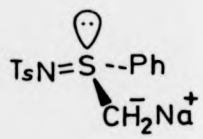


(29)

derivatives were found to racemise too quickly to be capable of significant asymmetric induction, whereas oxosulphonium ylides are configurationally stable[22]. Parellel results were obtained in reactions involving chiral N-tosylsulphoximines (28) and N-tosylsulphilimines (29)[23,24]. Selenium ylides also convert non-enolisable aldehydes and ketones to oxiranes but chiral derivatives have not been reported[25].

Epoxidations of α,β-unsaturated ketones using quaternary ammonium salts derived from alkaloids under phase-transfer conditions with hydrogen peroxide or TBHP gave enantiomeric excesses up to 25%[26].

For further information on the development of new epoxide reagents and advances in the preparation and synthetic applications of oxiranes see recent reviews by Rabek[27] and Rao[28].

2.D THE SYNTHESIS OF CHIRAL EPOXIDE PRECURSORS: UTILIZATION OF THE 'CHIRAL POOL'

It has been recognised for many years that organisms in nature biosynthesise an extensive array of natural products in high enantiomerically pure form and for many of these products both (R)- and (S)-enantiomers are readily available. These natural products represent a 'chiral pool' from which many chiral building blocks can be derived and incorporated into synthesis. Many of these compounds - ascorbic acid[29], amino-acids[30,31], sugars[32,33] and α-hydroxy acids[34] (malic acid[34], tartaric acid[34], lactic acid[35] and mandelic acid), have been utilised as precursors in the synthesis of optically active epoxides. This section outlines the uses of naturally occurring chiral compounds in epoxide syntheses and as chiral resolving agents for epoxide precursors.

Many epoxides have been derived by conversion of natural products into vicinal diols or derivatives thereof. The most widely adopted method for the formation of a terminal epoxide from a 1,2-diol involves regiospecific activation of the primary hydroxyl function by conversion into a good leaving group (e.g. Br, I or OTs) and intramolecular S_N2 cyclisation via the base induced formation of a β-alkoxide. Epoxides of high enantiomeric purity (ee >95%) have been attained by base cyclisation via quaternary ammonium salts derived from the reaction of tertiary β-ethanolamines with dichlorocarbene under phase transfer conditions[36]. Conversion of the 1,2-diol into its 2-acetoxy-1-bromide by treatment with hydrogen bromide/acetic acid prior to base cyclisation also gave epoxides in high enantiomeric purity[35,37]. Where the progenitor

diol readily forms carbenium ion intermediates under acid conditions (e.g. 1,2-diphenylethane-1,2-diol) hydrogen bromide/acetic acid has been substituted by 2-acetoxybenzyl bromide in the synthesis of the acetoxybromide[38].

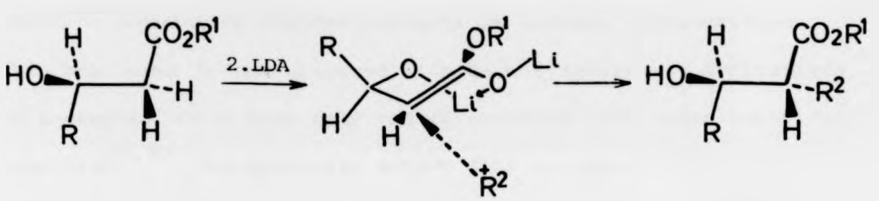
Natural products have also been employed to afford a chemical resolution of one enantiomer of a racemic epoxide precursor. This resolution, involving diastereoisomeric complex formation, affords only a 50% theoretical recovery if just one enantiomer is required. This yield may be improved by epimerisation of the unwanted enantiomer and again resolving the required isomer. The epimerisation and resolution enables a theoretical yield of 100% to be achieved.

Racemic cyanoalcohols have been resolved by conversion into diastereoisomeric carbamate derivatives by reaction with (R)-(-)-1-(1-naphthyl)-ethyl isocyanate and chromatography[39]. This method has been applied to the separation of racemic α-hydroxy sulphides which were subsequently converted into the epoxide by treatment with trimethyloxonium tetrafluoroborate and cyclisation with base[40]. Racemic diols have also been separated by ionic interaction with amino salts[41] and vicinol 1,2-diols have been separated both chromatographically and by recrystallisation via derivatisation with camphorquinone (Chapter 4). Where one enantiomeric alcohol or diol is available in optically active form its antipode may be obtained by inversion of chirality at the asymmetric centre[42,43].

Enantiomers of epoxides have been resolved chromatographically with chiral stationary phases, although the method is rather limited and such techniques are essentially restricted to determinations of enantiomeric purity. The enantiomers of epoxides have been separated

by g.c. using chiral manganese (II)[30], nickel (II)[44], and Lanthanide complexes[45].

Chiral diol precursors may also be derived from lithium enolates of β-hydroxy esters [Scheme 2.D.1]. Treatment of the



(30) (31) (32)

Scheme 2.D.1

diester of malic acid (30, R = CO_2R') with lithium diisopropylamide (two mol. equiv.) at reduced temperature (-20 to -50°C) generates the dianion (31, R = CO_2R') which is quenched diastereoselectively with alkyl halides to yield the alkylated hydroxy ester (32, R = CO_2R'). Reduction of (32) with $LiAlH_4$ yields the optically active triol which may be subsequently converted into the epoxide[34]. Quenching of the dianion derived from dimethyl or diethyl malate (31, R = CO_2R', R' = CH_3 or CH_2CH_3) with methyl iodide gave preferentially the <u>erythro</u>-product (<u>erythro</u>/<u>threo</u> selectivity > 10:1). This selectivity was greatly increased when the dianion of diethyl malate was quenched with allylbromide (erythro/threo > 20:1)[46,47]. β-Hydroxy esters of type (30, R = H, CH_3) are converted into epoxides of high diastereoisomeric purity by quenching the dianion

(31, R = H, CH_3) with I_2[48]. The ensuing chiral iodohydrin is base cyclised to the epoxide. High diastereoselectivity in the alkylation of propionate derivatives of L-camphor has been achieved via their lithium enolates[49]. Metalation of these camphor derivatives in THF (-80°C) was reported to give highly selective (Z)-enolates, whereas reactions conducted in THF/HMPA (4:1) gave (E)-enolates which on alkylation yielded products of inverse configuration. This behaviour is not observed with O-benzylglycolate derivatives of L-camphor which give only the (E)-enolate (33) under identical conditions[49,50]. The optically active diol is obtained from the chiral auxilary in these reactions by reduction with $LiAlH_4$ and removal of the benzyl moiety by catalytic hydrogenation.

2.E ASYMMETRIC REDUCTION OF PROCHIRAL KETONES

This section describes methods that have been utilised to introduce chirality into prochiral molecules which can subsequently be converted into optically active epoxides.

2.E.1 Metal Catalysed Hydroxysilylations

Asymmetric reductions of β-keto esters via hydroxysilylations catalysed by chiral rhodium-phosphine complexes have been shown to give optically active β-hydroxy ester (ee 72-84%)[51]. The optical yields and direction of asymmetric induction were found dependent upon the nature of the hydroxysilane, the chiral phosphine ligand and the size of the ester moiety. Titanium tetrachloride-catalysed reactions of allyltrimethylsilane with α-keto esters afforded (S)-δ,γ-unsaturated-α-hydroxyvalerates (ee 16-55%) and high

diastereoselectivities have been achieved in the reduction of asymmetric ketones with phenyldimethylsilane in the presence of a catalytic quantity of tetrabutylammonium fluoride in HMPA. Ratios of threo:erythro isomers ranged between 84:16 and › 99:1 for a number of substrates[52].

2.E.2 Metal Hydride Reductions

The asymmetric reduction of chiral α-keto esters by catalytic hydrogenation and metal hydride reductions have been extensively reviewed. The principle behind these reductions involves coordination of a chiral bidentate or polydentate ligand to a metal (Al, Rh, Li, K) that formally transfers hydride to the electrophile. Incorporation of chiral ferrocenyl diphosphines into rhodium complexes gave effective asymmetric hydrogenation (pyruvic acid was catalytically hydrogenolysed to (R)-lactic acid, ee 55-58%)[53] and asymmetric hydride reductions of prochiral ketones have been achieved with aluminium hydrides incorporating 2,2'-disubstituted-1,1'-binaphthyl as the auxiliary ligand (34)[54]. Substantial enantio- and diastereoselectivities have also been attained in reductions with Grignard reagents[55] and modified lithium tetrabutylaluminates[56].

2.E.3 Hydroborations

The asymmetric reductions of prochiral ketones with t-butyloxy isopinocampheylborane derived from (+)-α-pinene gave exclusively optically active (R)-alcohols[57] (largest ee 23% for

acetophenone), whereas monoisopinocampheylborane (Ipc BH_2) from (+)-α-pinene gave preferentially the (S)-alcohol (highest ee 46% for 3-methylbutan-2-one). The largest enantiomeric excesses have been achieved in the reduction of α-keto esters with α-(3-pinanyl)-9-borobicyclo[3.3.1]nonane (35)[58]. This reagent gave enantiomeric excesses approaching 100% for t-butylpyruvate, t-butyl-2-oxobutyrate, t-butyl-4-methyl-2-oxopentanoate and t-butylbenzoylformate and the selectivities for other α-keto esters ranged from 82-96%. Decreasing the bulk of the ester moiety and increasing the reaction temperature ($> 0^{o}C$) reduced the optical induction attained. Asymmetric syntheses via chiral organoboranes have been reviewed[59].

2.F ENZYMIC TRANSFORMATIONS: SYNTHESIS OF CHIRAL EPOXIDES AND PRECURSORS

There are many examples where microbial enzymic conversions have been used to introduce maiden chirality into synthetically useful reagents and such transformations have been extensively reviewed.

The reactive centre of an enzyme is embedded within a protein 'coat', which constructs a highly chiral environment about its active site. The reactions catalysed by enzymes are often highly regio- and stereospecific and since some enzymes show a varying degree of substrate specificity these qualities make enzymes extremely useful chiral catalysts for organic transformations. Enzymes can thus be used to convert prochiral substrates into

enantiomerically pure products or adopted to infer an enzymic resolution in which one enantiomer of a racemate is transformed of degraded preferentially leaving its antipode optically pure.

Esters of α- and β-hydroxy acids have been synthesised in high optical purity by reductions of α- and β-keto esters with actively fermenting yeast, Saccharomyces cerevisiae. Methyl-2-oxo-2-phenylacetate was converted into (R)-methyl-2-hydroxy-2-phenylacetate (ee $\simeq$ 100%) and 2-oxo-2-thienylacetate to (R)-2-hydroxy-2-thienylacetate (ee $\simeq$ 100%), whereas the reduction of ethyl pyruvate gave only a 92% enantiomeric excess of (+)-ethyl lactate[60]. It was later found that optically active (+)-ethyl lactate was epimerised by fermenting yeasts which made reductions of simple α-keto esters by such a method impractical[60]. Simple α-hydroxy ketones have also been reduced with actively fermenting yeasts to yield optically active terminal 1,2-diols which can be converted into epoxides[61,62].

Enantioselective transesterifications have been achieved using hogs' liver carboxyesterase supported on Sepharose or Chromosorb. Methyl propionic acid was converted into a mixture of (R)-methyl propionic acid and (S)-methyl propionic ester in high enantiomeric excess[63]. Carboxyesterases have been employed as a method to resolve racemic alcohols. Hogs' liver carboxyesterase shows a high alcohol specificity in its transformations and this physical property is detrimental to its use in alcohol resolutions. This problem was circumvented by using bound lipase enzymes from the yeast Candida cylindracea which naturally catalyses the degradation of tributyrin (glyceryl tributyrate) to glycerol and butyrate esters. Degradation

of tributyrin in the presence of 1-chloropropan-2-ol or 2,3-dichloropropan-1-ol yielded the (R)-butyrate ester and (S)-alcohol. The two pairs of enantiomeric alcohols were separated and converted into (R)- and (S)-methyloxirane and (R)- and (S)-(chloromethyl)-oxirane in high enantiomeric excess[63].

Microbial oxidations of olefins to epoxides have also been studied[64]. The fungus Penicillium spinulosum was found to convert cis-propylenephosphonic acid into (-)-cis-epoxypropylphosphonic acid, a broad spectrum orally active antibiotic[64].

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CHAPTER 3

MATERIALS, METHODS AND INSTRUMENTAL

3.A Materials

3.B Methods

3.C Instrumental

3.D References

MATERIALS, METHODS AND INSTRUMENTAL

3.A MATERIALS

i) Solvents

Solvents used in h.p.l.c. analysis were h.p.l.c. grade and were purchased from Rathburn Chemicals Ltd., Walkerburn, Scotland. Hygroscopic deuterated solvents used for n.m.r. purposes were stored in sealed bottles in a dry box. Other solvents were of AnalaR or laboratory grade. Where solvent purification was necessary, laboratory grade solvents were purified by standard procedures[1,2]. Anhydrous solvents were purified and dried according to standard procedures and distilled immediately prior to use or stored under nitrogen in tightly stoppered bottles with parafilm seals[1,2]. Chloroform was obtained ethanol-free by passage through a column of basic alumina.

ii) Chemicals

All chemicals were of the highest purity commercially available and where degradation had occurred were purified prior to use[2].

3.B METHODS

i) Solutions of sodium and potassium alkoxides were prepared by dissolving a freshly cut, clean piece of sodium or potassium in the appropriate anhydrous solvent under nitrogen. The metal was cleaned by washing in 40-60°C petrol to remove oil, followed by activation in a small volume of the appropriate anhydrous alcohol, e.g. sodium methoxide, activated in methanol. Calculations of molarities were performed by titrating against standard hydrochloric acid solutions using phenolphthalein indicator. Sodium dimsyl was prepared by dissolving a known amount of sodium in anhydrous DMSO to generate a ~0.5 M solution.

ii) All glassware used in moisture-sensitive reactions was dried at 110°C overnight, flamed and then allowed to cool at R.T. under a blanket of dry nitrogen.

iii) 3A and 4A molecular sieves were activated by flaming for 15 min and cooling to R.T. in a dry box. Activated sieves were stored at 110°C in a dry oven.

iv) Solutions of organic chemicals were dried using $MgSO_4$, that had been stored at 110°C for at least 48h. "Solvents were removed at reduced pressure (rotary evaporation)" . . . refers to the removal of bulk solvent using a Buchi rotary evaporator at 15-22 mm Hg (water-pump pressure). Otherwise, conditions of solvent removal are stated.

v) P.l.c. was preformed on home-made plates of Kieselgel 60 F_{254} (Merck Cat. No. 5554) which were activated at 110°C overnight before use. T.l.c. was performed on aluminium backed Kieselgel 60 F_{254} (Merck Cat. No. 5554). The preparative plates were 1.0 mm, whilst the analytical plates were 0.2 mm in thickness.

3.C INSTRUMENTAL

i) Combustion analysis

C.H.N. combustion analyses were performed by C.H.N. laboratories, Leicester or by the services at Shell Research Ltd., Sittingbourne, Kent or The University of Newcastle upon Tyne.

ii) High performance liquid chromatography

H.p.l.c. analyses were performed on either a Waters Associates (model 204) instrument fitted with a 6000A solvent delivery system and a variable wavelength U.V. detector or a Gilson instrument fitted with Rheodyne (model 7125) septumless injector, two Gilson 303 liquid delivery modules (25 cm^3 pump heads) and Gilson Holochrome variable wavelength detector.

iii) Infrared spectra

Infrared (i.r., ν max) spectra were recorded on a Perkin-Elmer (model 580B) grating spectrophotometer. Samples were either mulls (medicinal white oil), films or solutions and were run using NaCl plates. Peaks are assigned by their wavenumber (cm^{-1}) followed by s, strong; m, medium; w, weak or br, broad.

iv) Mass spectra

Mass spectra (c.i. and e.i.) were recorded on a Kratos MS80 spectrometer. F.a.b. mass spectra were recorded on a V.G. 7070 mass spectrometer. Peaks are quoted as m/z followed by their percentage abundance relative to the base peak. The molecular ion is designated M^+.

v) Melting points

Melting points were determined using a Kofler block or an electrothermal apparatus and are uncorrected.

vi) Nuclear magnetic resonance spectra

^1H n.m.r.spectra were recorded on one of the following instruments: a Bruker (model WH-360) 360 MHz, a Bruker (model WH-300) 300 MHz, a Perkin-Elmer (model R34) 220 MHz, a Varian (model EM-360) 90 MHz and a Hitachi Perkin-Elmer (model R-24B) 60 MHz spectrometer.

Peaks are assigned by their chemical shift (δ) in parts per million, followed in brackets by their relative integral values (e.g. 3H) in hydrogens, their multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet), and the spin-spin coupling constants (J) in Hertz, where appropriate. Tetramethylsilane was used as the internal standard in organic solvents and 3-(trimethylsilyl)-tetradeuteropropionic acid sodium salt in D_2O (zero δ).

^{13}C n.m.r. spectra were recorded on one of the following instruments: a Bruker (model WH-400) 100.62 MHz, Bruker (model WH-360) 90.52 MHz, and a Bruker (model WH-90) 22.63 MHz spectrometers. All spectra were run with broad band ^{1}H decoupling, and consist of singlets unless otherwise indicated.

vii) Optical rotations

Optical rotations were recorded with a Bendix NPL automatic polarimeter (model 143D). The instrument was calibrated against a standard sucrose solution prior to each measurement. Values are expressed as specific rotations ($[\alpha]_D^t$).

viii) Ultraviolet spectra

Ultraviolet spectra (λ max) were recorded with a Pye-Unicam (model SP-1800) ultraviolet spectrophotometer.

3.D REFERENCES

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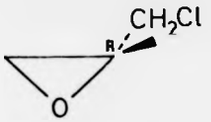
CHAPTER 4

THE KINETIC RESOLUTION OF 1,2-DIOLS WITH CHIRAL KETONES: A NEW ROUTE TO OPTICALLY ACTIVE 1,2-DIOLS AND EPOXIDES

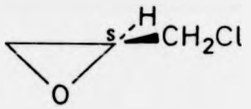
THE KINETIC RESOLUTION OF 1,2-DIOLS WITH CHIRAL KETONES: A NEW SYNTHESIS OF OPTICALLY ACTIVE 1,2-DIOLS AND EPOXIDES

4.A INTRODUCTION

For studies into the toxicology of epoxides we required samples of (R)- and (S)-(chloromethyl)oxirane [epichlorohydrins (5a) and (5b) respectively]. These epoxides are also valuable



(5a)

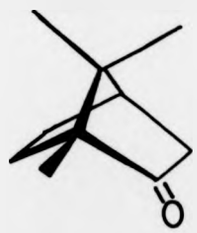


(5b)

precursors in the syntheses of many other optically active compounds. Although routes to these materials from D-mannitol have been described (J.J. Baldwin[1]) the syntheses are rather lengthy and two groups (Kawakami[2] et al and Russell[3] et al) have reported difficulties in reproducing the optical purity claimed. We therefore sought an alternative route to these compounds.

A convenient route to optically active oxiranes developed by Golding and his coworkers[4] involves the use of chiral 1,2-diols as precursors. The precursor diol in the synthesis of (chloromethyl)-oxirane is 3-chloropropane-1,2 diol which is relatively cheap and readily available in racemic form. We have sought to prepare either oxirane (5a) or (5b) by kinetic resolution[5] of this diol via a

suitable intermediate that can be converted directly into the oxirane. Our strategy was to invoke this resolution by the reaction of the racemic diol with one enantiomer of a chiral ketone. Although there are many chiral ketones available our immediate studies have involved the use of D-camphor[1,7,7-trimethylbicyclo[2.2.1]-heptan-2-one,D-bornane-2-one (36)] and D-camphorquinone [1,7,7-trimethylbicyclo [2.2.1]heptan-2,3-dione,D-bornane-2,3-dione (37)], synthesised by oxidation of camphor (36) with selenium dioxide[6].



(36)

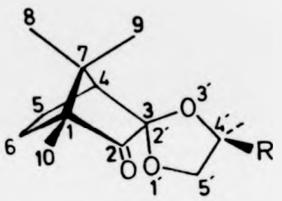
(37)

This chapter will describe in detail the reactions of a number of chiral and racemic 1,2-diols with (36) and (37), a new synthetic route to optically active (chloromethyl)oxirane and the synthesis of a new chiral diol precursor. Finally, there will be discussed some future work to enhance further the usefulness of these reactions in organic synthesis.

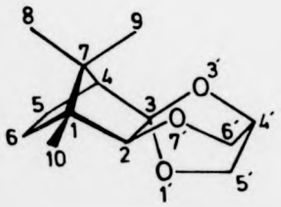
4.B NOMENCLATURE

The numbering system used to indicate the structural relationships of the functionalised bornanes is in accordance with the I.U.P.A.C. system for naming organic compounds. The numbering of the carbon atoms of camphor (36) and the di- and trioxolanes is illustrated (Fig. 4.B.1).

(36)



Dioxolane



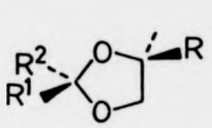
Trioxolane

Fig. 4.B.1

4.C THE REACTION OF 1,2-DIOLS WITH CHIRAL KETONES

The acid catalysed reaction of a 1,2-diol with an aldehyde or ketone generates a five-membered dioxolane ring system. This reaction is reversible but under conditions where water is constantly removed from the reaction mixture (Dean-Stark apparatus) the equilibrium is driven towards the dioxolane.

The reaction of a racemic 1,2-diol ($RCHOHCH_2OH$) with one enantiomer of a chiral ketone (R^1COR^2) will generate four diastereoisomeric dioxolanes (38-41), two from each enantiomer of the diol.



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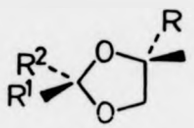

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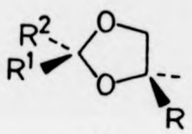

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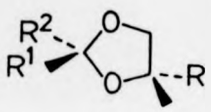


(41)

The proportions of each diastereoisomer formed will be governed by the extent of interaction between R and R^1 and R and R^2 under conditions of both thermodynamic and kinetic control. Ideally one diastereoisomer must predominate to permit its efficient separation, preferably by direct crystallisation from the mixture.

In previous work, Corey was able to separate the two diastereoisomers formed in the reaction of (+)-camphor with D-(-)-butane-2,3-diol by g.l.c.[7]. This enabled the separation of the two enantiomeric forms of camphor. Corey also used (rac.)-3-bromopropane-1,2-diol as a carbonyl protecting group[8]. This group could be easily removed under mild, neutral conditions (activated zinc in refluxing methanol), though there was no reported attempt to separate the diastereoisomeric products formed. Ketalisations have also been explored as a method of assessing the enantiomeric purity of chiral ketones. Wynberg reacted a number of chiral ketones

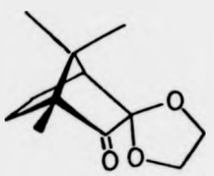
with (R,R)-(-)butane-2,3-dione[9]. By analysis of the ^{13}C n.m.r. spectra obtained for the diastereoisomeric mixtures he was able to deduce the enantiomeric purity of the starting ketones. Mayers later reversed this technique, using 2-substituted cyclohexanones to assess the enantiomeric purity of 1,2-diols by ^{13}C n.m.r. spectroscopy and h.p.l.c. analysis of the diastereoisomeric products[10]. No enantioselectivity was observed in the reaction of (S)-(+)-2-propylcyclohexanone with (rac.)-propane-1,2-diol, (rac.)-n-hexane-1,2-diol, (rac.)-n-octane-1,2-diol or (rac.)-4-methoxybutane-1,2-diol.

We have found no previous reference to work where enantiomers of diastereoisomeric ketones have been used to induce a kinetic resolution towards one of a pair of diol enantiomers.

4.D THE REACTIONS OF 1,2-DIOLS WITH CAMPHOR AND CAMPHORQUINONE

4.D.1 Ethane-1,2-diol

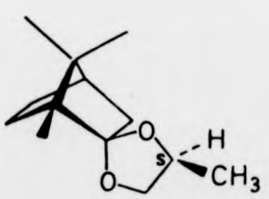
A preliminary study into the acid-catalysed reaction of D -camphorquinone (37) and ethylene glycol (ethane-1,2-diol, CH_2OHCH_2OH) showed the formation of only one dioxolane derivative (m.p. 80-82°C, recrystallisation from 60-80°C petrol). This dioxolane(42) has previously been synthesised as an intermediate in the formation of α-ketols possessing the bornane skeleton and in the transformation of camphor (36) into epi-camphor[11].



(42)

4.D.2 Propane-1,2-diol

Reactions of either (R)- or (S)-propane-1,2-diol[4,12] with D-camphor (36) in refluxing benzene or toluene containing a catalytic quantity of p-toluenesulphonic acid gave ca. 1:1 mixtures of the diastereoisomeric dioxolanes (43) and (44) and, (45) and (46), respectively. However, the acid catalysed reaction of



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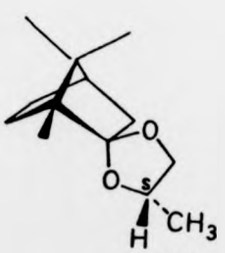

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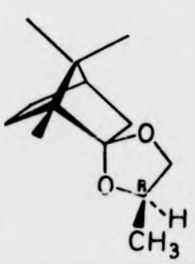

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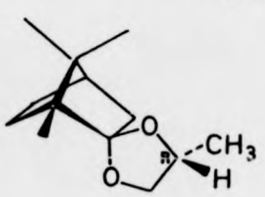

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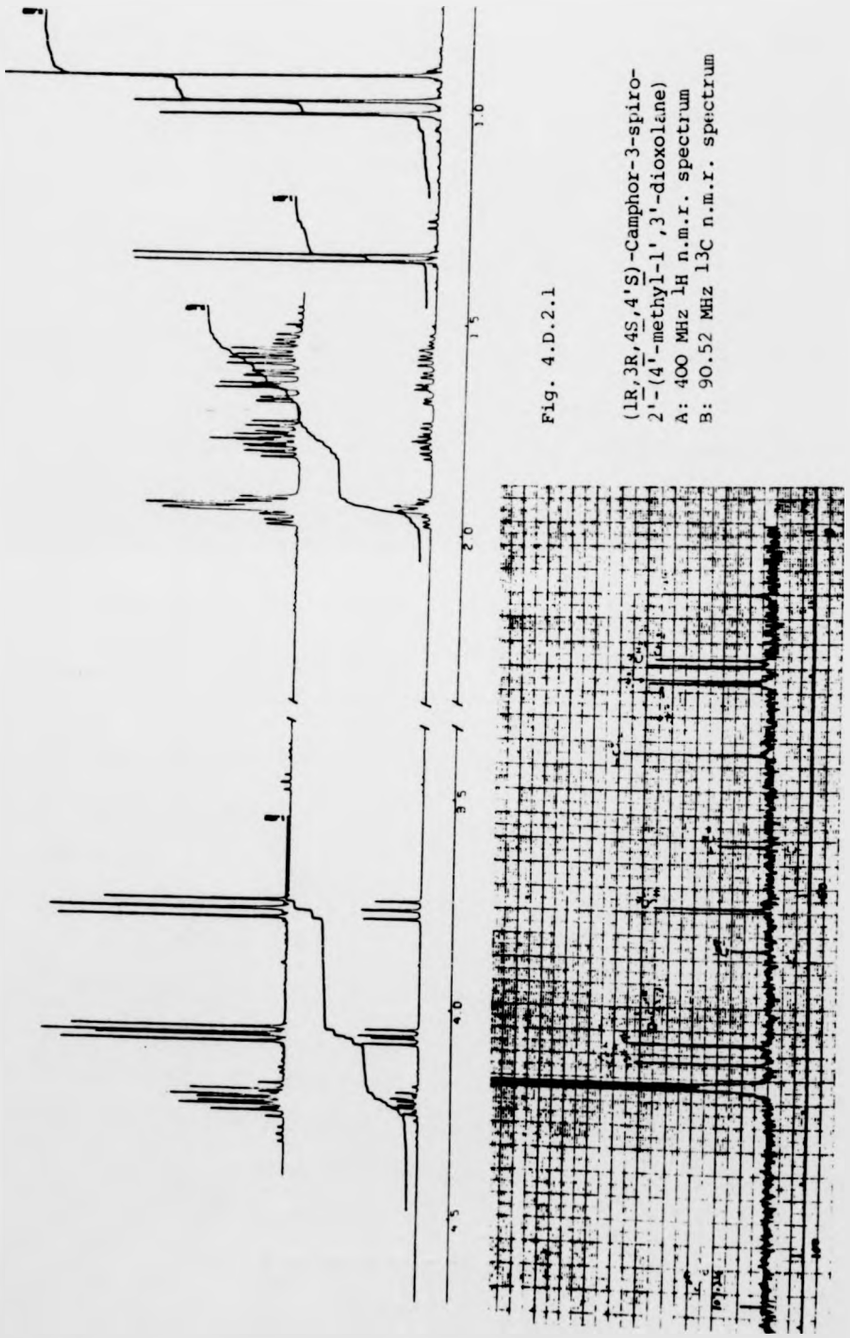
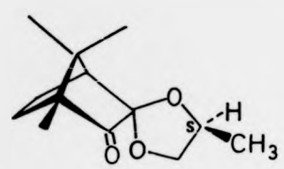


Fig. 4.D.2.1

(1R,3R,4S,4'S)-Camphor-3-spiro-2'-(4'-methyl-1',3'-dioxolane)
A: 400 MHz ^{1}H n.m.r. spectrum
B: 90.52 MHz ^{13}C n.m.r. spectrum

(S)-propane-1,2-diol with D-camphorquinone[6] (37) in refluxing benzene for 20 h gave a 4:1 mixture of diastereoisomeric dioxolanes (47) and (48).



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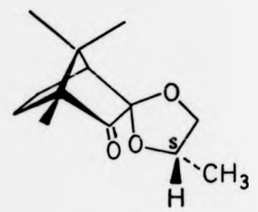


(48)

Upon work-up of this mixture a slightly yellow oil was obtained, the yellow colour being indicative of unreacted camphorquinone (37). Our attempts to remove the quinone involved dissolution of the mixture in ether and extraction of the quinone as its sulphite by repeated shaking with 40% sodium bisulphite solution[13]. This method was only partially successful and a much improved purification was obtained simply by gravity column chromatography. On completion of the work-up the predominant dioxolane was easily crystallised from petrol: m.p. 35-37°C [Fig. 4.D.2.1]. When this dioxolane was heated in toluene containing a catalytic amount of p-toluenesulphonic acid (105°C/96 h) it yielded (47) and (48) in a ratio 2:3 [Fig. 4.D.2.2]. This suggests that the dioxolanes produced in refluxing benzene (84°C) are products of kinetic control, whereas the product ratios in toluene (105°C) are a result of thermodynamic control.

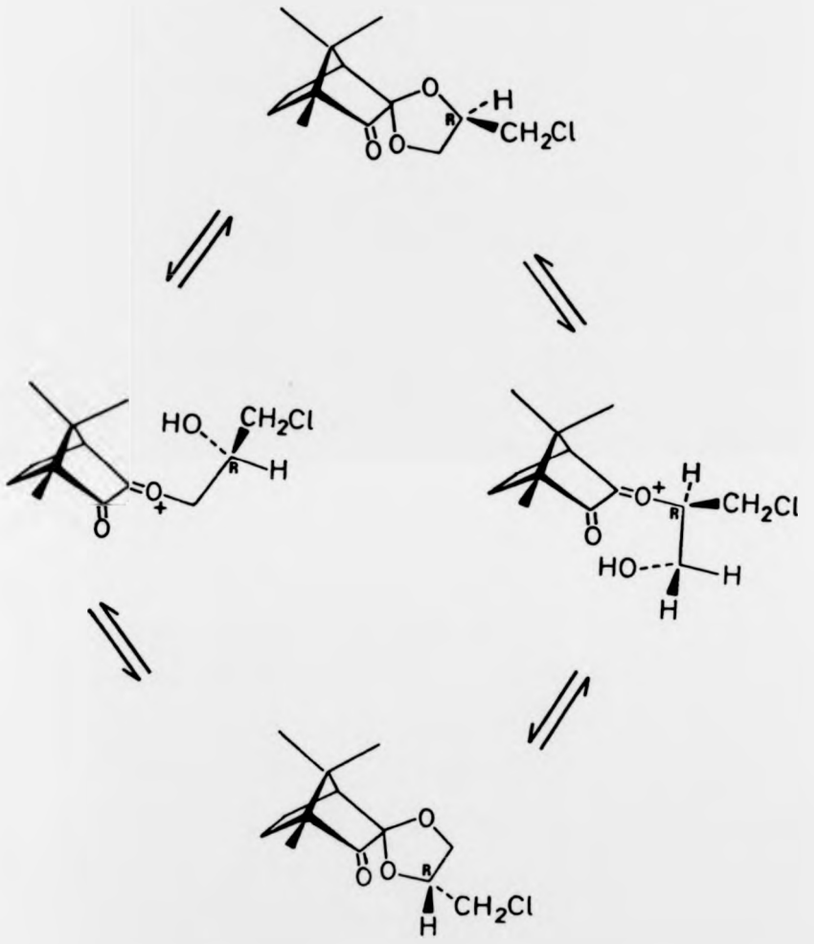
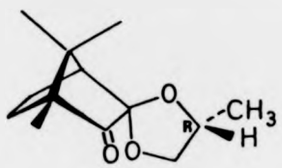
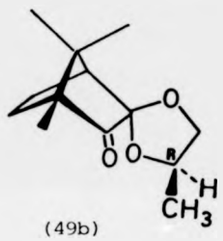


Fig. 4.D.2.2 The acid catalysed rearrangement of (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane)

The acid catalysed reaction of (R)-propane-1,2-diol with D-camphorquinone (37) in refluxing benzene gave a 4:3 mixture of dioxolanes (49a) and (49b). No crystalline product could be obtained from this mixture.



(49a)



(49b)

The reaction of (rac.)-propane-1,2-diol with camphorquinone gave a complex ^{1}H n.m.r. spectrum from which the reaction composition could not be determined. Purification of this mixture by column chromatography and repeated attempts to recrystallise the fractions obtained gave a crystalline product. This product had identical chemical and physical properties to those shown by dioxolane (47 or 48). Unfortunately, this result could not be repeated subsequently.

4.D.3 3-Chloropropane-1,2-diol

We found that an excess of (rac.)-3-chloropropane-1,2-diol (10 mol. equiv.) reacts with D-camphorquinone (37) in refluxing benzene containing a catalytic quantity of p-toluenesulphonic acid to give a kinetically controlled mixture of the diastereoisomers (50-53) in

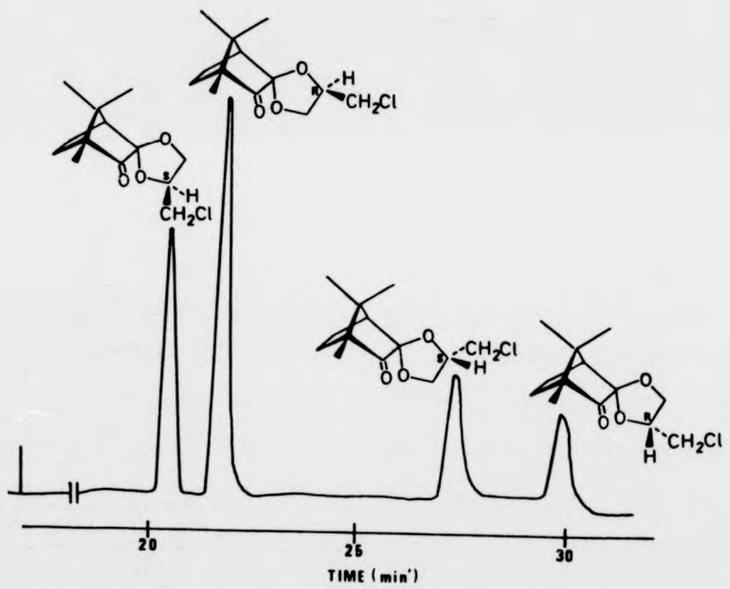
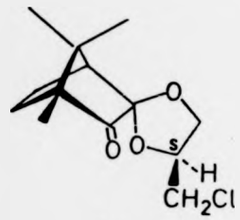
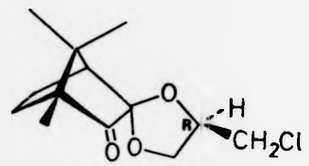


Fig. 4.D.3.1 H.p.l.c. separation of dioxolanes (50 - 53)

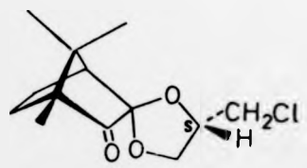
yields of 27, 45, 17 and 12%, respectively, determined by h.p.l.c. [Fig. 4.D.3.1].



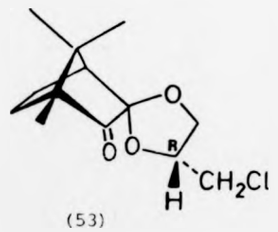
(50)



(51)



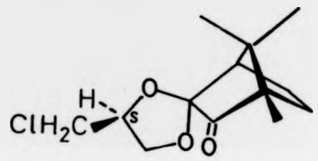
(52)



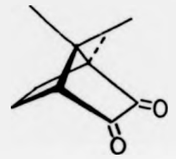
(53)

On standing, the dioxolane mixture became crystalline and from this the major diastereoisomer could be separated by repeated fractional crystallisation from petrol. An analogous reaction conducted in toluene gave product ratios of ca 28, 43, 14 and 14% after 8 h; 27, 43, 15 and 15% after 12 h; and 16, 32, 24 and 27% after 17.5 h for (50-53), respectively. All four diastereoisomers were baseline separated in one pass through a preparative h.p.l.c. column. The reaction of L-camphorquinone (54) with an excess of (rac.)-3-chloropropane-1,2-diol (10 mol. equiv.) gave upon work-up

a crystalline product (55) whose chemical and physical properties were identical to those shown by dioxolane (51). The dioxolanes (51) and (55) must therefore be one pair of enantiomers, each possessing a different chirality at the carbon-atom bearing chloromethyl.



(55)



(54)

When dioxolane (51) was heated in toluene (103°C/96 h) containing a catalytic amount of p-toluenesulphonic acid it equilibrated with dioxolane (53), [ratio 1:1 at equilibrium as determined by ^{1}H n.m.r. spectroscopy and h.p.l.c.]. Thus, dioxolanes (51) and (53) have the same chirality at the carbon atom bearing the chloromethyl group.

Dioxolane (51) is a source of chiral 3-chloropropane-1,2-diol but was not readily hydrolysed with 2 M sulphuric acid. A much milder hydrolysis was obtained by reduction of (51) with sodium borohydride to the alcohols (56) and (57), [ratio 3:1 by ^{1}H n.m.r. spectroscopy], which on treatment with 2 M HCl in methanol (3h/ reflux) and work-up gave 3-chloropropane-1,2-diol, 55% based on dioxolane (51). Direct hydrolysis of dioxolane (51) with 2 M HCl in

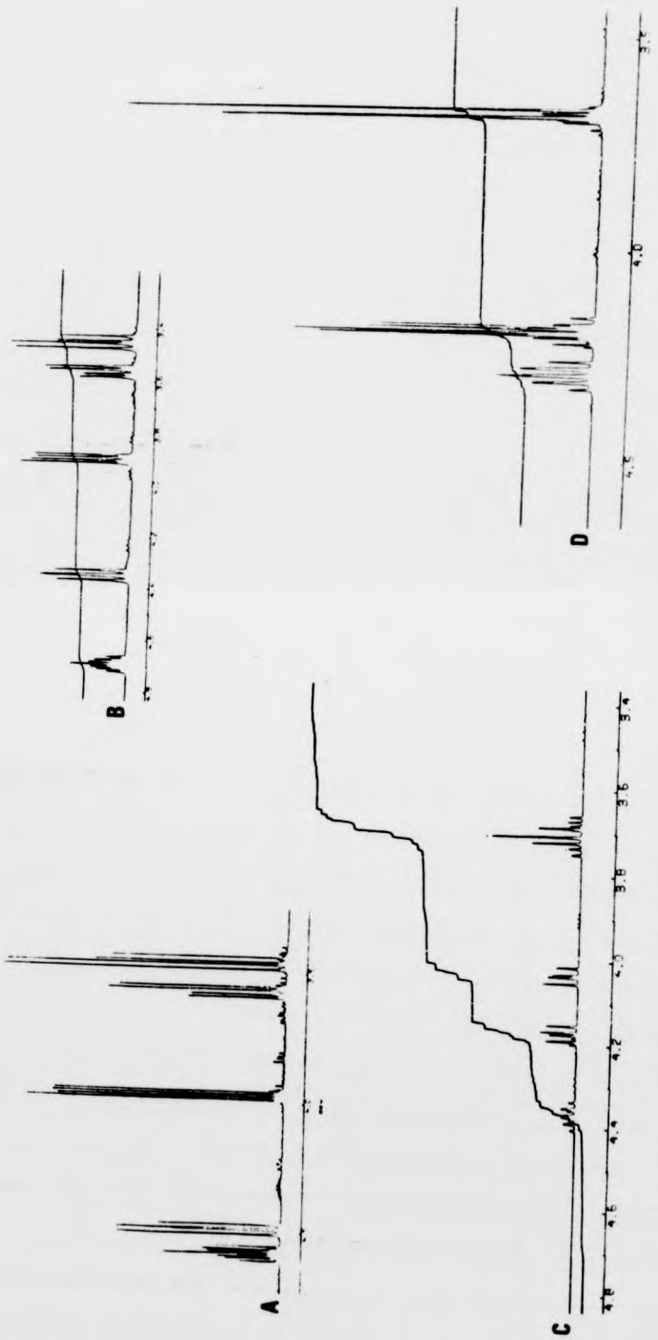
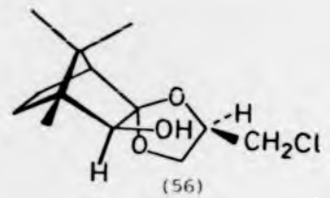


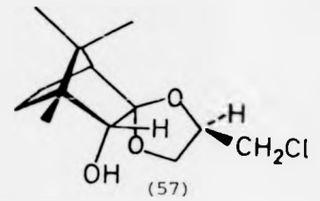
Fig. 4.D.3.2 400 MHz ^{1}H n.m.r. of the dioxolane moiety of:
A (1R,3S,4S,5'R)-Camphor-3-spiro-2'-(5'-chloromethyl-1',3'-dioxolane),
B (1R,3R,4S,4'S)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane),
C (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane),
D (1R,3S,4S,5'S)-Camphor-3-spiro-2'-(5'-chloromethyl-1',3'-dioxolane).

methanol showed little reaction after 6h at 80°C. The lack of reactivity of dioxolane (51) to acid hydrolysis probably results from the stabilisation of the intermediate carbocation (58) by the adjacent carbonyl at C-2.

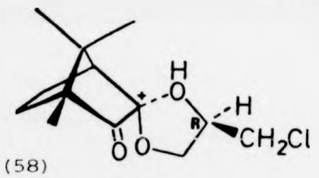
The diol obtained from the alcohols (56) and (57) was found



(56)



(57)



(58)

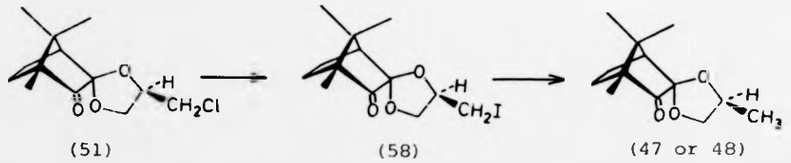
to be chemically and optically pure by ^{1}H n.m.r. spectroscopy (only one enantiomer was detected after addition of the chiral shift reagent Eu(hfbc)$_3$) and gave $[\alpha]_D^{19}$ - 7.4°, lit.[14] $[\alpha]_D^{20}$ + 7.3° for the (S)-isomer . This proves the carbon bearing chloromethyl in (51) and (53) possesses an (R)- configuration, whereas the enantiomeric dioxolane (55) must possess an (S)- configuration at this centre.

The structural assignments made for dioxolanes (51 - 53) are additionally based on careful analysis of their 400 MHz ^{1}H n.m.r. spectra, the chemical shifts of the dioxolane protons being exceptionally informative [Fig. 4.D.3.2].

An interesting question now arose. Were the stereochemistries of the two crystalline dioxolanes obtained from the reaction of

(S)-propane-1,2-diol and (rac.)-3-chloropropane-1,2-diol with D-camphorquinone (37) identical?

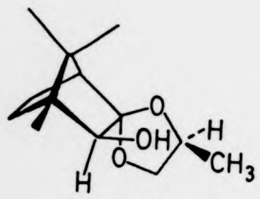
A nOe difference spectrum irradiating at the methyl doublet (1.34 ppm) of dioxolane (47 or 48) gave a positive effect on the dioxolane protons at δ 3.77, 4.07 and 4.22 ppm, though the result at high field (observation of the methyl signals at δ 0.90, 0.97 and 1.00 ppm) was inconclusive. The answer to this question was therefore sought by conversion of dioxolane (51) into its propane-1,2-diol analogue (47 or 48) [Scheme 4.D.3.1].



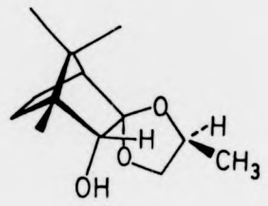
(51) (58) (47 or 48)

Scheme 4.D.3.1

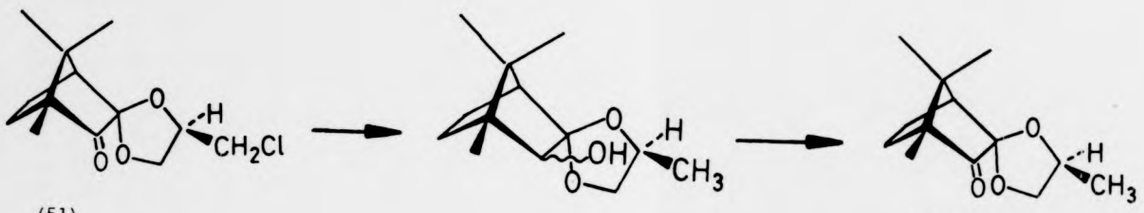
Our initial strategy was to convert the chloromethyl group of dioxolane (51) into its iodide (58) using the well known Finkelstein substitution reaction. The iodide (58) could then be reduced with tri-n-butyltin hydride[15] to a methyl group. The Finkelstein substitution[16] was only partially successful. The reaction of dioxolane (51) with sodium iodide or potassium iodide in refluxing acetone (under N_2) gave no required product and only partial success was obtained with potassium iodide in HMPA (65°C/3 weeks). The ^1H n.m.r. spectrum of the reaction mixture indicated the presence of both chloro- and iodo-dioxolanes (51) and (58) in a ratio of



(59)



(60)



(51) (47)

Scheme 4.D.3.2

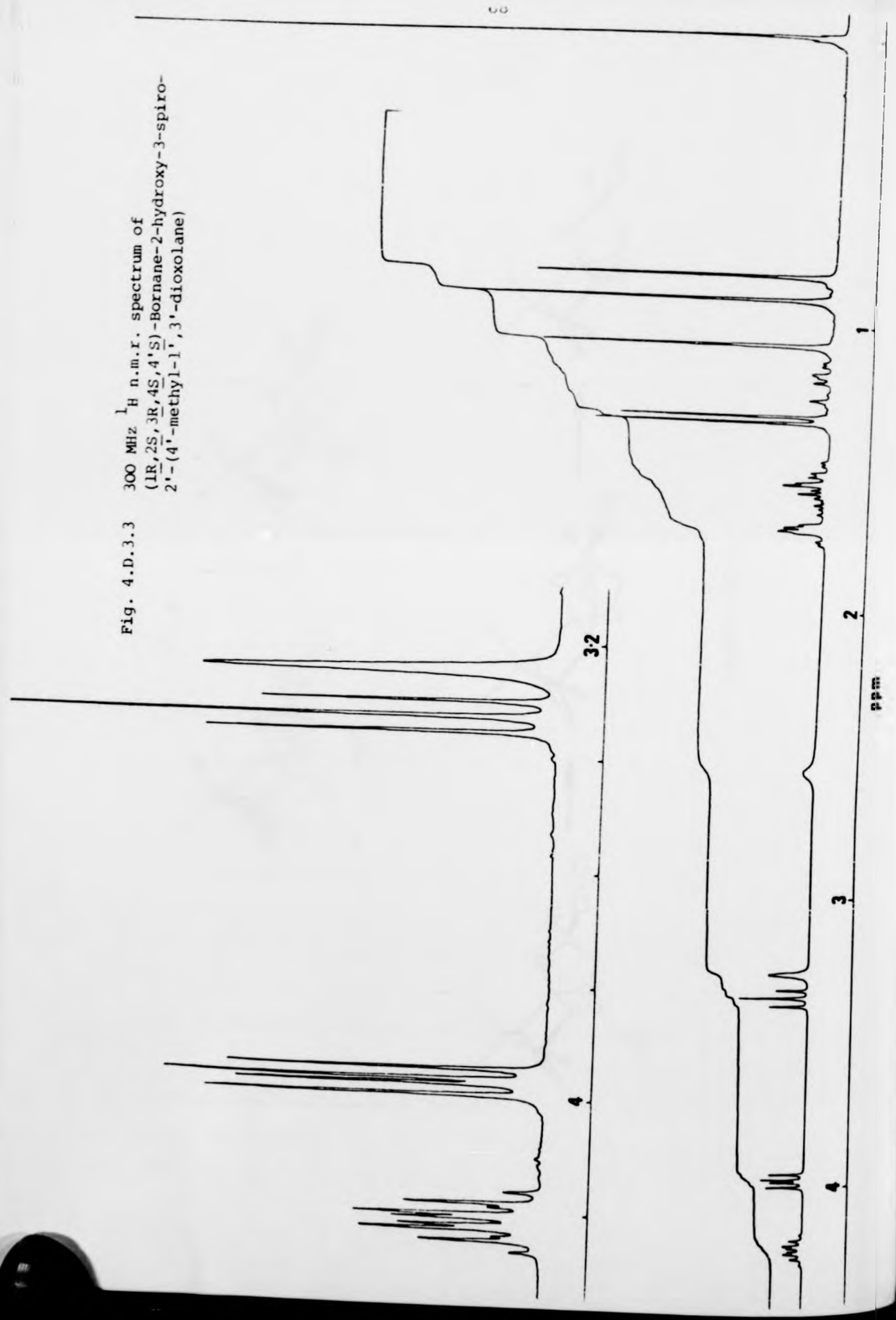
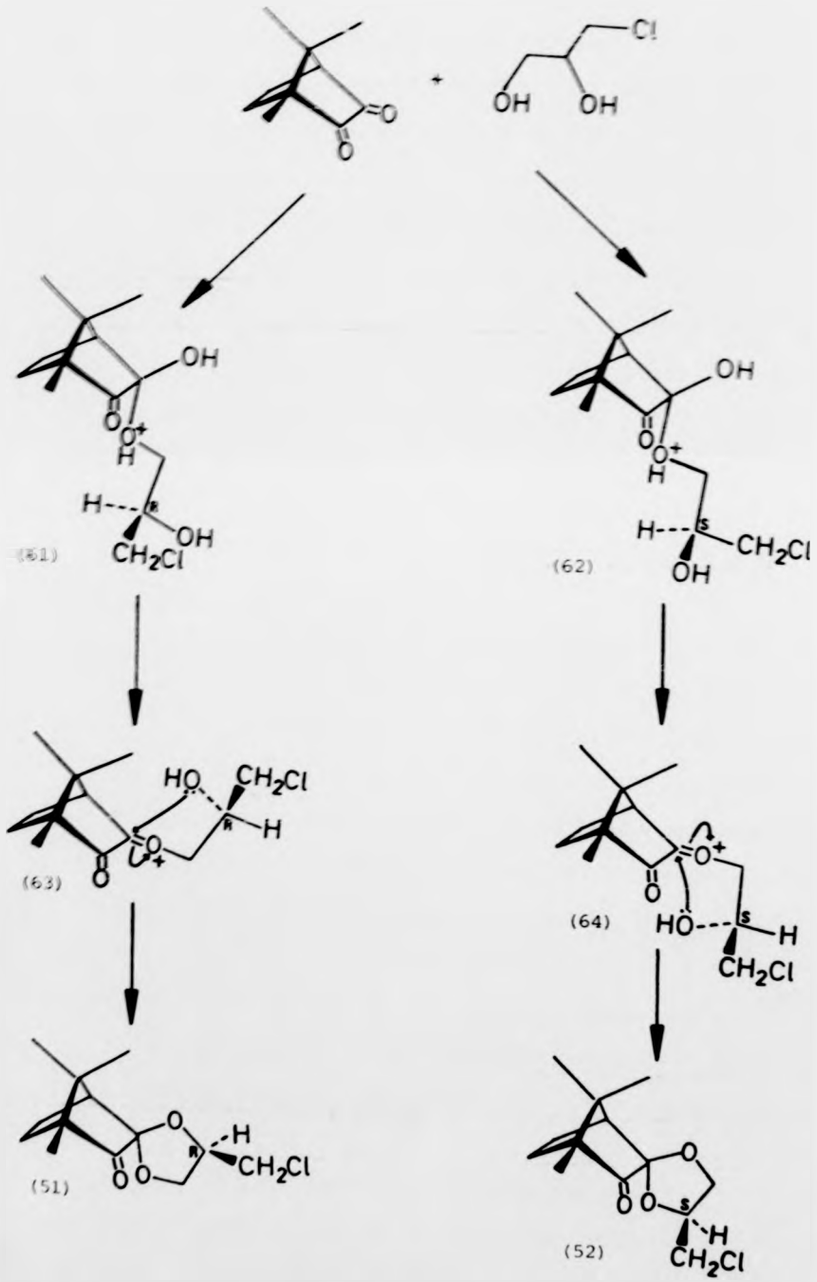


Fig. 4.D.3.3 300 MHz ^{1}H n.m.r. spectrum of (1R,2S,3R,4S,4'S)-Bornane-2-hydroxy-3-spiro-2'-(4'-methyl-1',3'-dioxolane)

ca 1:2, respectively. The dioxolane (58) was identified by the appearance of new resonances at δ 3.36 (d, CH_2I), 3.95 (dd, one of dioxolane CH_2), 4.22 (dd, one of dioxolane CH_2) and 4.46 ppm (m, $CHCH_2I$). Dioxolanes (51) and (58) gave a single spot by t.l.c. and neither could be separated by fractional crystallisation.

Our second choice of reactions (51→47 or 48, Scheme 4.D.3.2) involved reduction of the chloromethyl moiety with L-selectride (lithium tri-sec-butylborohydride). This reagent would reduce the C-2 ketone to a mixture of exo- and endo-alcohols (59) and (60) but these could readily be converted back to the ketone by oxidation. Reduction of dioxolane (51) with L-selectride (20h/45°C) gave upon work-up and column chromatography dioxolane (59, >90% exo, $[\alpha]_D^{22}$ + 26°), the 300 MHz ^{1}H n.m.r. spectrum showed no visible contamination from dioxolane (60) [Fig. 4.D.3.3]. Oxidation of (59) with pyridinium dichromate in DMF[17] (20h/RT) gave on t.l.c. a single spot, Rf 0.57 (visualised by I_2 staining), 67.2% from dioxolane (51). This product had identical chemical and physical properties to those possessed by dioxolane (47), [Fig. 4.D.2.1.]. The mechanism of formation of dioxolane (51) presumably involves initial endo-attack of the primary alcohol of (R)-chloropropane-1,2-diol on the C-3 carbonyl-carbon of camphorquinone to generate the hemiketal (61). The reaction with (S)-chloropropane-1,2-diol is presumably initiated by attack to give hemiketal (62). The hemiketals then lose water to give the oxonium species (63) and (64), respectively. It then has to be postulated that dioxolane (51) is formed by exocyclic attack of the secondary hydroxyl group on C-3 followed by loss of proton to give the neutral species and



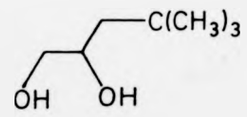
Scheme 4.D.3.3

dioxolane (52) is formed in an analogous fashion involving cyclisation by endocyclic attack of the secondary hydroxyl group [Scheme 4.D.3.3.]. The actual mechanism is uncertain.

4.D.4 2,2-Dimethylbutane-3,4-diol

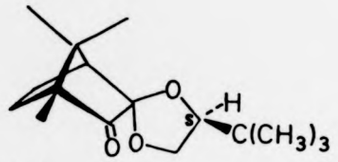
Increasing the bulk of the R group in $RCHOHCH_2OH$ should increase the steric interaction between R and R^1 and R and R^2 in dioxolanes (38-41). This increase in interaction should effect the ratio of diastereoisomers produced on dioxolane formation. If the proportion of one diastereoisomer can be increased (ideally >95% one diastereoisomeric product) the viability of these reactions in synthesis increases substantially.

The acid-catalysed reaction of (rac.)-2,2-dimethylbutane-3,4-diol[18] (65) with D-camphorquinone gave upon work-up a mixture



(65)

of dioxolanes (66-69). Attempts to separate these products by column chromatography or fractional crystallisation were unsuccessful but all four diastereoisomers were baseline separated by h.p.l.c.



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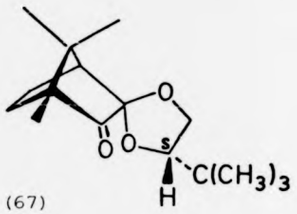

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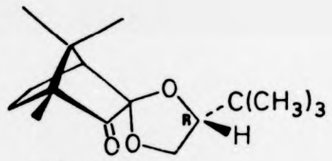

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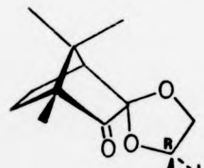

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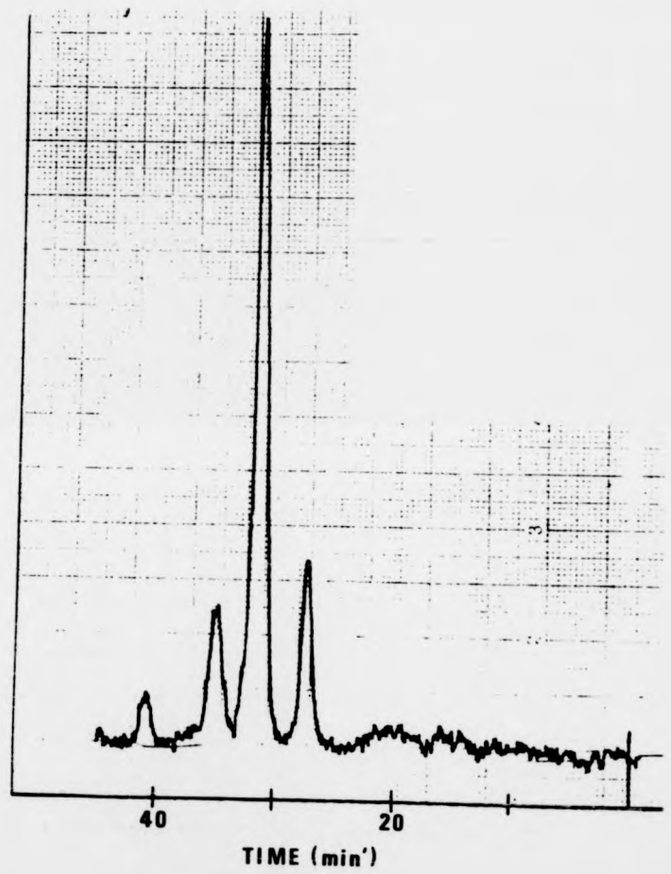
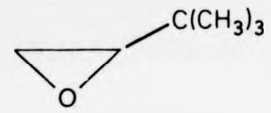


Fig. 4.D.4.1 H.p.l.c. separation of dioxolanes (66-69)

Analysis of the product mixture by h.p.l.c. indicated the major dioxolane to be formed in 73% yield, [Fig. 4.D.4.1.] a pure sample of which was separated on a Techoprep 5/20 preparative column.

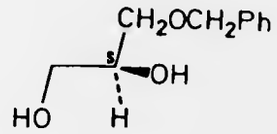
This compound is of interest because t-butyloxirane (70) is required to assess the enantioselectivity of biological nucleophiles towards epoxides, and should be readily available from this dioxolane [See section 4.F.].



(70)

We also examined the selectivity of D-camphorquinone (37) towards (S)-3-benzyloxypropane-1,2-diol[19] (71) and (S)-4-benzyloxybutane-1,2-diol (72) of interest to a colleague on the synthesis of optically active lipoic acid.

When D-camphorquinone (37) was reacted with (S)-3-benzyloxypropane-1,2-diol (71) [1 mol. equiv.] in benzene over 36h a mixture of the dioxolanes (73) and (74) were formed. After purification (Kugelröhr distillation) the ^{1}H n.m.r. spectrum gave a product ratio of ca 3:1. In an analogous reaction D-camphorquinone was reacted with (S)-4-benzyloxybutane-1,2-diol[20] in benzene over 50h. The resulting dioxolanes (75) and (76) were purified by column chromatography and a product ratio of ca 4:1 was estimated by ^{1}H n.m.r. spectroscopy. Neither dioxolane mixture (73 and 74) or (75 and 76) afforded a crystalline product on attempted fractional crystallisation.



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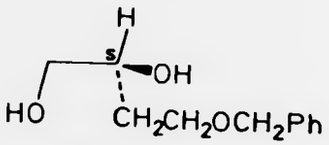

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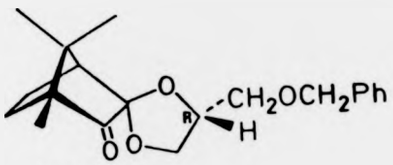

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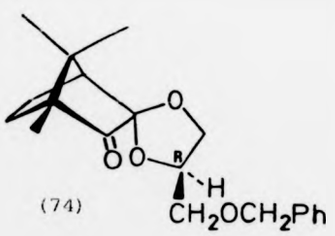
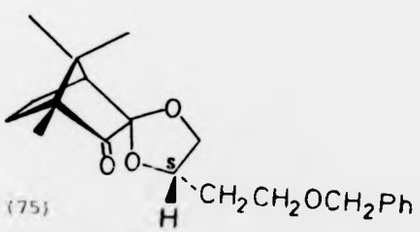


(74)



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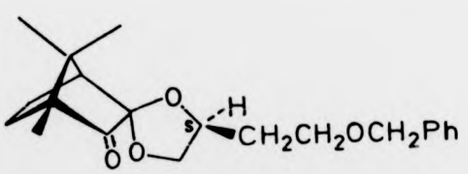


(76)

Acid-catalysed rearrangement of the mixtures (73 and 74) and (75 and 76) in toluene at 105°C gave product ratios of ca 1:1 for both sets of diastereoisomeric dioxolanes.

4.E ANALYSIS OF PRODUCT MIXTURES

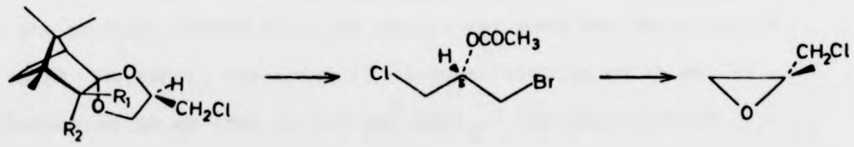
The diastereoisomeric ratios in the reactions of camphor (36) and camphorquinone (37) with (R)-and (S)-propane-1,2-diol, (S)-3-benzyloxypropane-1,3-diol and (S)-4-benzyloxybutane-1,2-diol were all measured by 220 MHz ^{1}H n.m.r. spectroscopy. All mixtures were analysed as solutions in $CDCl_3$ (using TMS as the internal standard) at concentrations of ca 40 mg/cm^3, except for dioxolanes (75) and (76) which were analysed in $[^{2}H_8]$-toluene. Dioxolanes (43) and (44) were analysed by integration of the methyl singlets at δ 0.99 and 1.01 ppm and (45) and (46) by integration of the diastereotopic methylene protons at δ 3.33 and 3.90 (one diastereotopic pair) and δ 3.51 and 4.02 ppm (the other diastereotopic pair). Dioxolanes (47-50) were analysed by integration of their methyl doublets at δ 1.18 and 1.25 (for 49a and 49b respectively) and 1.28 and 1.34 ppm (for 48 and 47). Dioxolanes (73) and (74) were analysed from their methyl signals at δ 1.00 (major product) and 1.02 ppm (minor product).

Product mixtures obtained from the reaction of camphorquinone (37) with (rac.)-3-chloropropane-1,2-diol and (rac.)-2,2-dimethylbutane-3,4-diol were determined by h.p.l.c. using a Gilson instrument (equipped with variable wavelength UV detector) and a μ-Porasil (normal phase) column (250 mm x 0.635 i.d.), eluting with 1% ethylacetate-hexane (v/v) at a flow rate of 0.5 cm^3/min. [Fig. 4.D.3.1. and 4.D.4.1.].

4.F SYNTHESIS OF (R)-(CHLOROMETHYL)OXIRANE [(R)-EPICHLOROHYDRIN] [21,22]

Previous work by Golding et al has shown that the reaction of a 1,2-diol with 45% HBr in acetic acid generates a vicinal acetoxybromide which on treatment with base cyclises to give an oxirane. Our strategy was to treat the dioxolane (51) in an analogous manner and base cyclise the intermediate vicinal acetoxybromide to (chloromethyl)oxirane (5a).

Treatment of dioxolane (51, section 4.D.3) with 45% HBr in acetic acid (60°C/5h) gave a mixture of products containing camphorquinone (37) and (S)-2-acetoxy-1-bromo-3-chloropropane [77, scheme 4.F.1., the chirality of (77) being inferred from the chirality of dioxolane (51) and the product oxirane]. It was later found that a much faster and milder reaction (25°C/2.5h) could be performed by reduction of the C-3 ketone of dioxolane (51) prior to HBA treatment (see section 4.H). Attempts to purify the acetoxybromide (77) by either fractional distillation (90-92°C/13 mm) or flash chromatography down a short column of silica gel was only mildly successful and it was therefore decided to synthsise the epoxide directly from the reaction mixture.



(51) $R_1 = R_2 = O$
(52) $R_1 = H$ $R_2 = OH$
(56) $R_1 = OH$ $R_2 = H$

(77) (5a)

Scheme 4.F.1

One possible drawback to our synthesis of (R)-(chloromethyl)-oxirane (5a) would arise if on treatment of (77) with base a mixture of (R)-(chloromethyl)oxirane (from S_N2 displacement of bromine) and (S)-(bromomethyl)oxirane (from S_N2 displacement of chlorine) were formed. This is unlikely as the CH_2Br bond (284 KJ/mol.) is some 54 KJ/mol. weaker than a CH_2Cl bond (338 KJ/mol.) but a preliminary reaction with (rac.-77) was performed to assess this possibility. Treatment of (rac.-77) with 1.2 M sodium ethan-1,2-diolate in ethane-1,2-diol (1 mol. equiv.) gave upon work up a product oxirane whose ^1H n.m.r. spectrum was consistent with the formation of (rac.)-(chloromethyl)oxirane, no resonances indicating any presence of (bromomethyl)oxirane being observed (δ 3.33 ppm, CH_2Br). This result confirmed that our method for the synthesis of (R)-(chloromethyl)oxirane was valid.

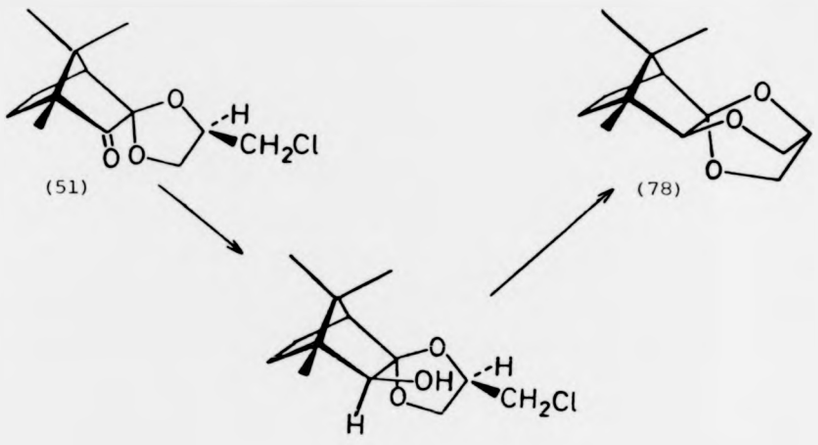
The product mixture from the reaction of dioxolane (51) with HBA was now treated with 1.2 M sodium ethan-1,2-diolate in ethane-1,2-diol (1 mol. equiv. $NaOCH_2CH_2OH$) and gave (R)-(chloromethyl)oxirane (5a) that was distilled directly from the reaction mixture, $[\alpha]_D^{24}$ - 33°, comparable to the highest literature values[1] [-34.3° for (R)-isomer, + 33° for (S)-isomer]. ^1H n.m.r. analysis of the product showed only one enantiomer when the chiral shift reagent $Eu(hfbc)_3$ was added (3 mg quantities up to 15 mg) to a solution of 10 mg (5a) in 0.5 cm^3 $CDCl_3$. The use of shift reagents $Eu(fod)_3$ and $Eu(DPM)_3$ resolved the diastereotopic methylene protons of the chloromethyl group of (rac.)-(chloromethyl)oxirane (J = 4.8 and 11.7 Hz) and these coupling constants were consistent

with those observed for the chloromethyl protons on addition of Eu(hfbc) to (5a).

The method described is convenient for the preparation of optically active oxiranes from readily available racemic diols, when alternative methods (e.g. Sharpless epoxidation[23], derivation from the 'chiral pool'[24]) are not directly applicable. Treatment of dioxolanes (47) and (67) in a similar manner should give optically active methyl- and t-butyloxirane and further derivatisation of the chloromethyl group of dioxolane (51) should permit the synthesis of many other optically active diols and epoxides (see preceeding section).

4.G SYNTHESIS OF (1R,2S,3R,4S,4'S)-BORNANE-3-SPIRO-2'-(1',3',7'-TRIOXOLANE): A NEW CHIRAL 1,2-DIOL PRECURSOR

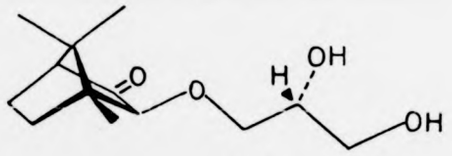
If our analysis of the 400 MHz ^{1}H n.m.r. spectra of dioxolanes (50 - 53) was correct it should be possible to synthesise the trioxolane (78) by reduction of (51) to its C-2 exocyclic alcohol (56) and base cyclise this alcohol (6-exo-tet process[25]) to (78, Scheme 4.G.1.). Not only would the trioxolane (78) prove conclusively the stereochemistry of the dioxolanes (51, 53 47 and 55) but on hydrolysis of the dioxolane ring it would release a chiral 1,2-diol (79). This chiral diol could be derivatised and released from the epi-camphor moiety by either an oxidative[26] or



(51) (78)

(56)

Scheme 4.G.1



(79)

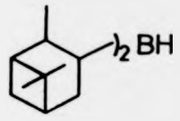
reductive process. If this were successful the reaction of camphorquinone with (rac.)-3-halogenopropane-1,2-diols (halogen = Cl, Br, I) would give a general method for the synthesis of a vast number of chiral 1,2-diol products which are difficult to obtain by existing methods.

4.H THE STEREOSELECTIVE REDUCTION OF (1R,3R,4S,4'R)-CAMPHOR-3-SPIRO-2'-(4'CHLOROMETHYL -1',3'-DIOXOLANE)

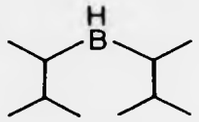
Previously, dioxolane (51) had been reduced with sodium borohydride in ethanol (section 4.F and 4.D.3). ^{1}H n.m.r. spectroscopic analysis of the product mixture indicated the alcohols (56) and (57) to be formed in a ca 3:1 ratio, by integration of the methyl singlets at δ 1.09 (exo-product) and 1.03 ppm (endo-product), respectively.

The selectivity attained by reduction of the C-2 ketone of dioxolane (51) with sodium borohydride is modest (75% exo) and we therefore required a reagent that would increase the percentage of the exo-alcohol (56) in the product mixture. Work by H.C. Brown and coworkers showed that a high selectivity towards the less stable, exo-alcohol of camphor could be obtained by reductions with dialkylboranes[27,28]. It was shown that the reduction of camphor at 0^{o}C with diborane in THF gave only 52% of the exo-alcohol, but the stereoselectivity could be improved by using diisoamylborane (65% exo-product) or diisopinocampheylborane (100% exo-product). These highly hindered boranes were therefore used to reduce dioxolane (51).

Attempts to reduce the C-2 ketone of dioxolane (51) with diisopinocampheylborane (80, 24h/RT) or diisoamylborane (81, 96h/RT) in diglyme were unsuccessful and upon work-up only starting material was obtained. The reduction of dioxolane (51) with diborane (82) was examined.



(80)



(81)

B_2H_4

(82)

Following the reduction by t.l.c. showed no reaction at -20°C (12h) and only little reaction at 0°C (48h) and RT (24h). The complete reduction of dioxolane (51) with diborane required heating at 40°C for 96h. Analysis of the product (^{1}H n.m.r. spectroscopy) showed it to be a mixture of exo- and endo-alcohols (56) and (57), formed in a ratio of 14:3, respectively (82.3% exo).

The severe conditions required for the reduction of dioxolane (51) with diborane provided the answer as to why our initial attempted reductions with diisopinocampheylborane and diisoamylborane were unsuccessful. The C-2 ketone of dioxolane (51) must be very sterically hindered and this hindrance prevents its reduction with the bulky alkylboranes.

We now turned our attention to the lithium trialkoxyaluminium hydrides[28,29]. These reducing agents are less powerful than the parent lithium aluminium hydride and therefore enable a much milder reduction of the ketone[30]. These reagents are synthesised by addition of the appropriate alcohol (3 mol. equiv.) to a suspension of lithium aluminium hydride in ether or THF at reduced temperature. H.C. Brown and his coworkers[30] have shown that the reduction of camphor with these reagents is highly stereoselective. Lithium

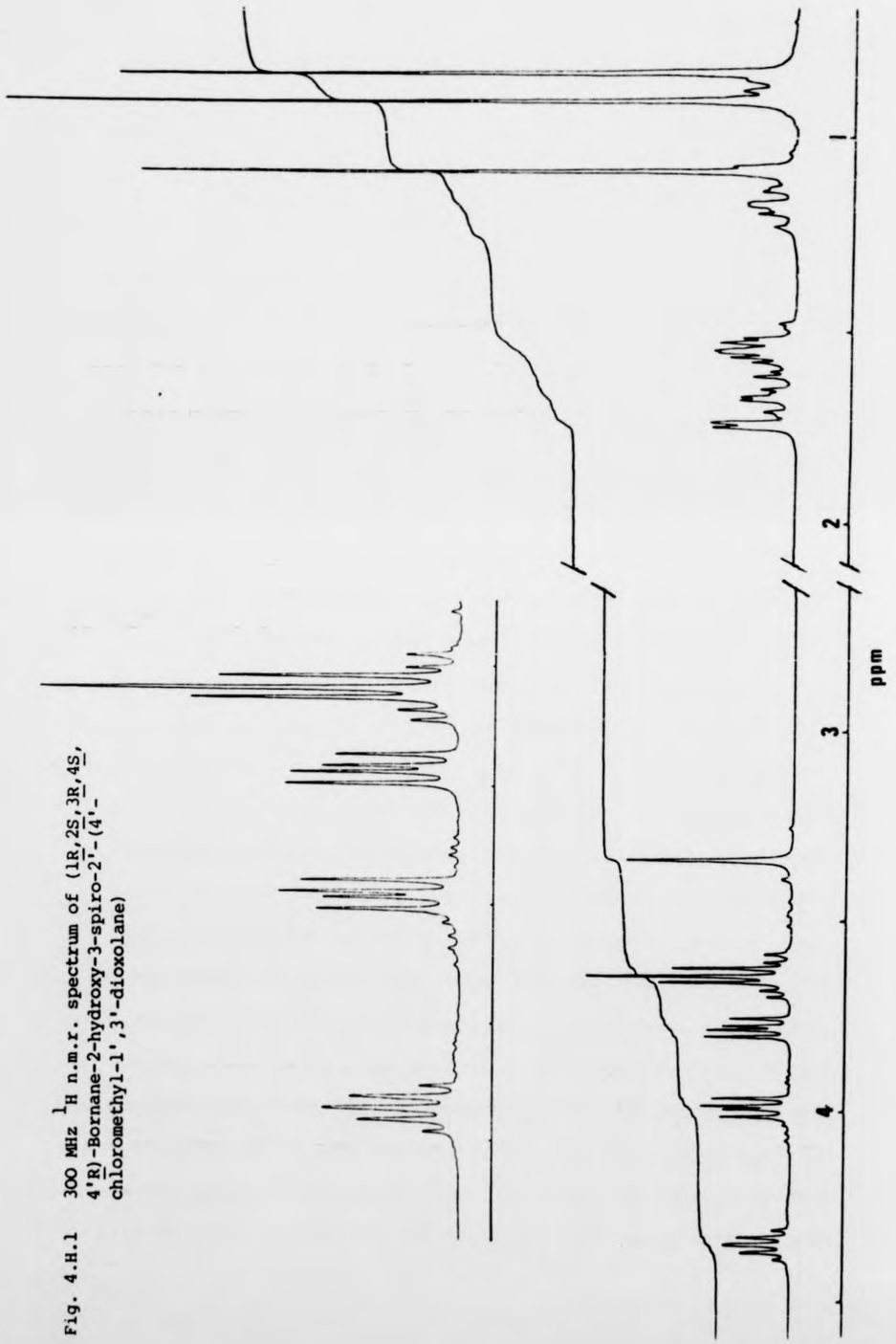
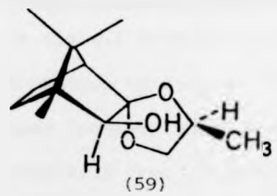


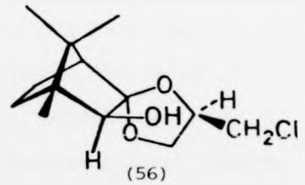
Fig. 4.H.1 300 MHz ^{1}H n.m.r. spectrum of (1R,2S,3R,4S, 4'R)-Bornane-2-hydroxy-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane)

tri-t-butoxyaluminium hydride[31] (Li^+ $Al(O\text{-}t\text{-}Bu)_3H^-$) gave 93% of the least stable exo-alcohol, whereas the lithium trimethoxyaluminium hydride[32,33] (Li^+ $Al(OMe)_3H^-$) gave 99% of the exo-product.

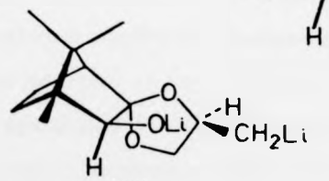
The reaction of dioxolane (51) with lithium trimethoxyaluminium hydride in THF was found to have gone to completion after 20h at RT (single spot by t.l.c.). Analysis of the oil obtained (^{1}H n.m.r. spectroscopy) indicated the presence of two product alcohols identified as (59) and (56), ratio 5:2, respectively.



(59)



(56)



(83)

Both these derivatives possess an exocyclic alcohol group at the C-2 position (> 95% exo) but the presence of dioxolane (59) indicated that lithium trimethoxyaluminium hydride is much too powerful a reducing agent for this transformation. The exocyclic alcohol (56) was synthesised by reduction of dioxolane (51) with lithium tri-t-butoxyaluminium hydride in THF. T.l.c. analysis showed a single spot after 20h (RT) and on work-up a colourless oil was obtained (96.3%). ^{1}H n.m.r. analysis of the oil [Fig.4.H.1] showed it to be dioxolane (56, > 95% exo) with no visible contamination by the endo-alcohol. The dioxolane (56) was now cyclised to give the trioxolane (78).

4.J THE CYCLISATION OF (1R,3R,4S,4'R)-CAMPHOR-3-SPIRO-2'-(4'-CHLOROMETHYL-1',3'-DIOXOLANE)

The reaction of dioxolane (56) with n-butyl lithium (1 mol. equiv.) in hexane (72h/RT, under nitrogen) gave a white precipitation but analysis of the reaction mixture by t.l.c. showed only a single spot, identified as unreacted starting material. In an analogous reaction conducted in THF (55-60°C, 72h, 1 or 10 mol. equiv. n-butyl lithium) a major product was obtained which on analysis (e.i.m.s.) gave a molecular ion m/z 226 mmu, attributed to the formation of dioxolane (59). This transformation (56 ⟶ 59) must involve the formation of a lithio-alkyl intermediate (83) resulting from a metal-halogen interchange between n-butyl lithium and dioxolane (56)[34]. On addition of water during work-up the lithio-alkyl species is hydrolysed to yield dioxolane (59). Although this reaction was unsuccessful at forming trioxolane (78) it may prove useful in the synthesis of chiral diols. If rather than the addition of water the lithio-alkyl species is quenched with an excess of an alkylbromide or iodide the dioxolane C-4' alkyl group could be modified. Hydrolysis would then release the chiral 1,2-diol. These reactions have yet to be developed.

The target molecule (78) was synthesised by treatment of dioxolane (56) with sodium hydride (10 mol. equiv.) in THF. T.l.c. analysis showed a single spot after 24h at RT and upon work-up trioxolane (78) was isolated as a colourless oil (75.6% from 51). An interesting feature of the ^{1}H n.m.r. spectrum of (78) is the

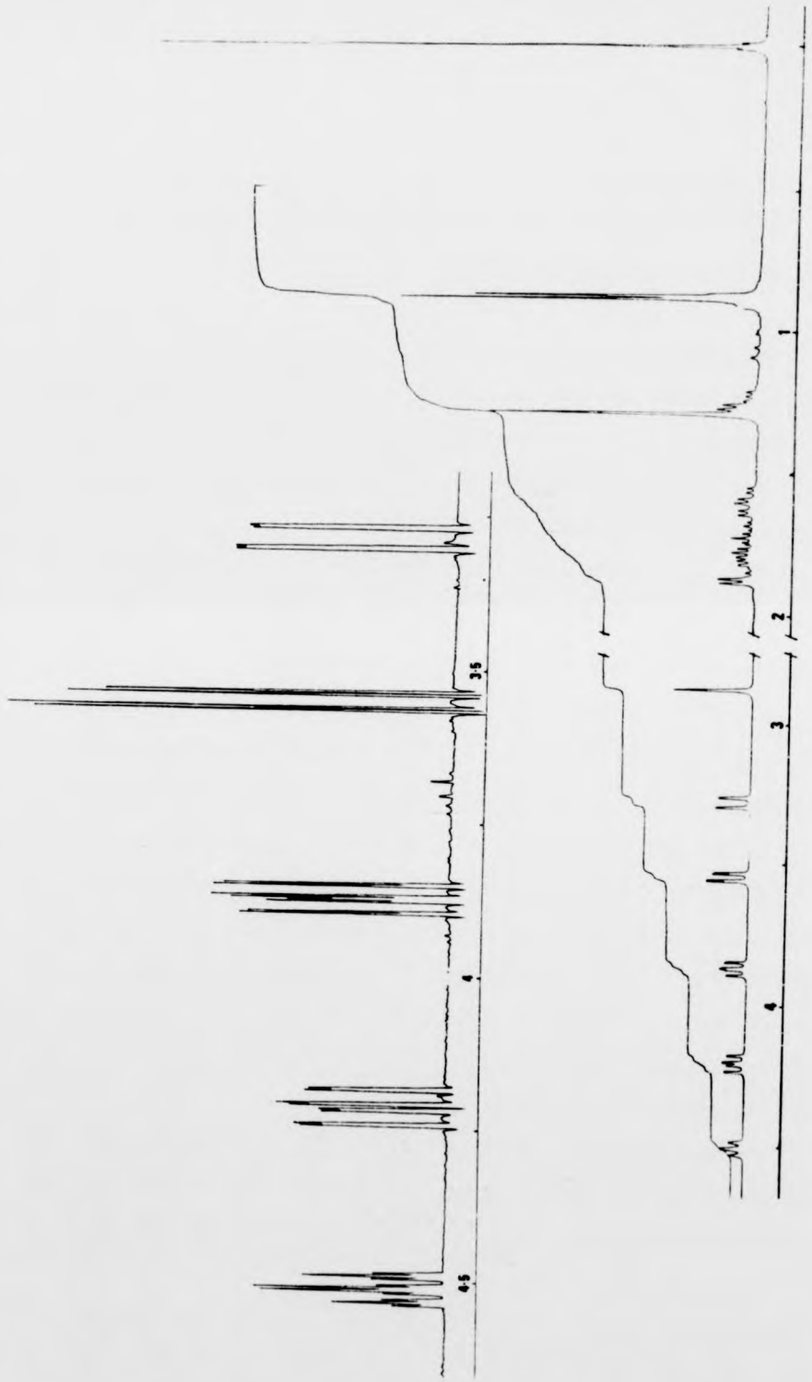


Fig. 4.J.1 300 MHz ^{1}H n.m.r. spectrum of (1R,2S,3R,4S,4'S)-Bornane-3-spiro-2'-(1',3',7'-trioxolane)

presence of an additional 1 Hz coupling shown by resonances at δ 3.86 and 4.2 ppm [Fig. 4.J.1.]. This coupling results from a long range interaction between the exocyclic CH_2 protons at C-5' and C-6' [Fig. 4.J.2.] and aids in their identification. No attempt was made to distinguish between these protons.

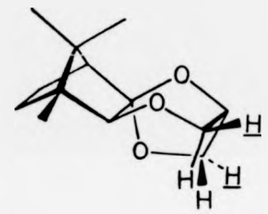
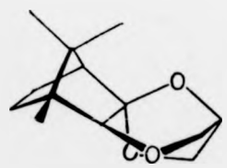
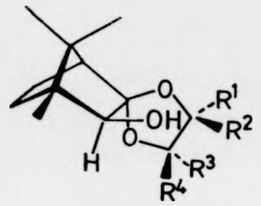


Fig. 4.J.2

Examination of molecular models of the dioxolane mixture (50-53) suggested that the C-2 exo-alcohol would cyclise with the (R)-4'-chloromethyl-1',3'-dioxolane (proved by synthesis) but cyclisation with the (S)-5'-chloromethyl-1',3'-dioxolane (87) to give trioxolane (84) would be less favourable because of the greater distance between the hydroxy and chloromethyl functional groups.



(84)



(85) $R^1 = CH_2Cl$, $R^2 = R^3 = R^4 = H$

(56) $R^2 = CH_2Cl$, $R^1 = R^3 = R^4 = H$

(86) $R^3 = CH_2Cl$, $R^1 = R^2 = R^4 = H$

(87) $R^4 = CH_2Cl$, $R^1 = R^2 = R^3 = H$

The mixture of dioxolanes (50-53) was reduced with lithium tri-t-butoxyaluminium hydride in THF to the corresponding exo-alcohols (56 and 85-87) and without further purification this mixture was treated with sodium hydride in THF. The reaction was followed by h.p.l.c. and showed a steady decrease in the concentration of dioxolane (56), whereas the peak corresponding to dioxolane (87) remained unchanged. As the reaction progressed a new peak corresponding to the formation of trioxolane (78) was observed. Upon work-up, t.l.c. analysis indicated the presence of unreacted alcohols and trioxolane, but these compounds could not be efficiently separated. Attempts were made to purify the trioxolane by oxidising the alcohols to ketones. This would allow separation by chromatography.

Oxidation of the reaction mixture with pyridinium dichromate in DMF or CH_2Cl_2 gave upon work-up a yellow oil, which, after column chromatography, gave trioxolane, free of dioxolanes (85,86 and 87). Unfortunately, the trioxolane co-chromatographed with a yellow impurity, which was identified as camphorquinone by ^1H n.m.r. spectroscopy. The camphorquinone was found to be a product of trioxolane oxidation. A more selective oxidant was therefore required because the formation of camphorquinone severely reduces the yield of trioxolane. Alternative oxidants have yet to be explored, but Moffat[35] or Swern[36] oxidations may solve this problem.

Kugelröhr distillation of the trioxolane/camphorquinone mixture gave a colourless liquid. The ^1H n.m.r. spectrum of this product was identical to that obtained for trioxolane (78). Hydrolysis of (78) with 2M HCl in methanol gave a product whose i.r. spectrum

showed the presence of hydroxyl groups and a carbonyl group suggesting cleavage of the dioxolane moiety to release the diol (79). These preliminary reactions into the synthesis of trioxolane (78) from the diastereoisomeric dioxolane mixture (50 - 53) and its conversion into the diol (79) are promising. Further work is being directed into the use of the diol (51) as a potential drug precursor. When dioxolane (51) was administered to rice leaf fungi it was found to be a potent antifungal agent. Its mode of action probably results from the reduction of the C-2 ketone followed by cleavage at the dioxolane moiety to liberate 3-chloropropane-1,2-diol. It is most probable that this is the active constituent as chloropropanediol is a known enzyme inhibitor. Trioxolane (78) has been submitted for testing and we await the results.

4.K EXPERIMENTAL

4.K.1 Preparation of D-Camphorquinone (37)

To a stirred solution of D-camphor (250 g, 1.64 mol) in acetic anhydride (250 cm^3) was added selenium dioxide (300 g, 2.97 mol) and the resulting suspension boiled under reflux for 24h. A colour change from red-purple to greenish grey was observed. After cooling, the suspension was filtered and the filter-cake washed with glacial acetic acid (ca 200cm^3). Camphorquinone was precipitated by careful neutralisation of the orange filtrate with 12 M potassium hydroxide solution (ca 500 cm^3). The camphorquinone was filtered off, washed with water, dried (vacuum) and recrystallised

to afford yellow needles (from 60-80°C petroleum ether) [228.7 g, 84%], m.p 195-196°C [Lit. 198°C]; δ_H ($CDCl_3$) 0.94 (s, 3H, CH_3), 1.07 (s,3H, CH_3), 1.11 (s, 3H, CH_3), 1.65 (m, 2H, $^5CH_2$ exo and $^6CH_2$ exo protons), 1.95 (m,1H, $^5CH_2$ endo or $^6CH_2$ endo proton), 2.19 (m, 1H, $^5CH_2$ endo or $^6CH_2$ endo proton) and 2.65 ppm (d, 1H CH); δc ($CDCl_3$) 8.59 (CH_3), 17.27 (CH_3), 20.91 (CH_3), 22.13 (CH_2),29.84 (CH_2), 42.44 (C-7), 57.89 (CH), 58.54 (C-1), 202.67 (CO) and 204.7 ppm (CO); νmax (Mull, Nujol) 2,925s, 2,855s, 1,760s, 1,395s, 1,324m, 1,200m, 1,107m, 1,052m, 737m and 696m cm^{-1}; m/z (e.i.) 166 (M^+), 138 ($M-CO^+$), 110 ($M-2CO^+$) and 95 ($C_7H_{11}^+$); $[\alpha]_D^{20}$ + 106.2° (c 1 in toluene) .

4.K.2 Preparation of L-Camphorquinone (54)

This was prepared from L-camphor (5 g, 3.28 x 10^{-2} mol) and selenium dioxide (6 g, 6 x 10^{-2} mol) in the manner described for quinone (37) to give (54) as yellow needlelike crystals (3.93 g, 72%), m.p. 194-196°C [lit. 198°C]; $[\alpha]_D^{20}$- 108.4° (c 1 in toluene) {lit. $[\alpha]_D^{20}$- 109.8° (c 1 in toluene)}; all other physical properties were identical to those shown by D-camphorquinone (37).

4.K.3 Preparation of (1R,4S)-Camphor-3-spiro-2'-(1'3'-dioxolane) (42)

A stirred solution of D-camphorquinone (1 g,6.02 x 10^{-3} mol), ethane-1,2-diol (0.55 g, 8.8 x 10^{-3} mol) and p-toluenesulphonic

acid (0.1 g, 5.2×10^{-4} mol) was boiled under reflux in benzene (25 cm^3) through a thimble of oven-dried magnesium sulphate for 24h, under an atmosphere of nitrogen. After cooling the resulting solution, anhydrous sodium carbonate (0.5 g) was added to neutralise the acid, the solid was filtered and solvent was removed at reduced pressure (rotary evaporator) to give a yellow oil. The oil was dissolved in diethyl ether (10 cm^3) and unreacted quinone was removed by repeated shaking with 40% sodium bisulphite solution until the ether layer became very pale yellow. The ether layer was dried (sodium sulphate) and evaporated to give a white solid. Recrystallisation from 60-80°C petrol gave colourless needle-like crystals (0.66g, 52%), m.p. 80-82°C [lit.[10] 81-83°C]; δ_H ($CDCl_3$) 0.89 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.45-2.08 (m, 5H, ^4CH, $^5CH_2$ and $^6CH_2$), 3.97 (m, 2H, exo protons $-OCH_2CH_2O-$) and 4.21 ppm (m, 2H, endo protons $-OCH_2CH_2O-$); νmax. (Mull, HCB) 2,960s, 2,899s, 1,754s, 1,468m, 1,452m, 1,393m, 1,374m, and 1,319s cm^{-1}; m/z (e.i.) 182 ($M-CO^+$), 99 and 55; (+ive c.i., reagent NH_3), 228 ($M+NH_4^+$), 211 ($M+H^+$), 99 and 55.

4.K.4 Preparation of (1R,2R,4S,4'S)-Bornane-2-spiro-2'-(4'-methyl-1',3'-dioxolane) (43) and its diastereoisomer (44)

A stirred solution of D-camphor (1 g, 6.57×10^{-3} mol), (S)-(+)-propane-1,2-diol (0.5 g, 6.5×10^{-3} mol) and p-toluenesulphonic acid (0.1 g, 5.2×10^{-4} mol) was boiled under reflux in benzene (ca 25 cm^3) through a thimble of oven-dried magnesium sulphate for 18h, under an atmosphere of nitrogen. After cooling,

anhydrous sodium carbonate was added, the solid was filtered off and solvent was removed at reduced pressure (rotary evaporation) to leave a colourless oil. Purification by column chromatography [Merck Kieselgel 60, Art 7734 (70-230 mesh)], eluting with 40-60°C petrol-ethylacetate, 3:1 (v/v) gave a mixture of diastereoisomers (43) and (44). No crystalline product could be obtained on attempted fractional crystallisation. The mixture was analysed by 220 MHz ^{1}H n.m.r. spectroscopy (see sections 4.D.2 and 4.E) with no further purification.

4.K.5 Preparation of (1R,2R,4S,4'R)-Bornane-2-spiro-2'-(4'-methyl-1',3'-dioxolane) (45) and its diastereoisomer (46)

This was prepared from D-camphor (1 g, 6.57 x 10^{-3} mol), and (R)-(-)-propane-1,2-diol (0.5 g, 6.5 x 10^{-3} mol) in the manner described for dioxolanes (43) and (44). After work up no crystalline product could be obtained and the mixture was analysed by 220 MHz ^{1}H n.m.r. spectroscopy (see section 4.D.2 and 4.E) with no further purification.

4.K.6 Preparation of (1R,3R,4S,4'S)-Camphor-3-spiro-2'-(4'-methyl-1',3'-dioxolane) (47)

A stirred solution of D-camphorquinone (1 g, 6.02 x 10^{-3} mol), (S)-(+)-propane-1,2-diol (0.55 g, 6.02 x 10^{-3} mol) and p-toluenesulphonic acid (0.1 g, 5.2 x 10^{-4} mol) was boiled under reflux in benzene (ca 25 cm^3) for 24h under nitrogen. Water was removed by azeotropic distillation through a thimble of oven-dried magnesium sulphate. After cooling, anhydrous sodium carbonate (0.5 g) was added, the solid was filtered off and solvent was removed at reduced pressure (rotary evaporation) to leave a yellow oil. Purification by gravity column chromatography [Merck Kieselgel 60, Art 7734 (70-230 mesh)], eluting with 40-60°C petrol-ethylacetate, 7:1 (v/v) gave a colourless oil which on dissolution in a minimum volume of 40-60°C petrol and cooling (-18°C) yielded (47) as a colourless crystalline solid (0.35 g, 26%), m.p. 35-37°C, Rf 0.53 (silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v); δ_H ($CDCl_3$) 0.90 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.0 (s, 3H, CH_3), 1.34 (d, 3H, dioxolane CH_3), 1.55 (m, 1H, $^5CH_2$ exo), 1.64 (m, 1H, $^6CH_2$ exo), 1.77 (m, 1H, $^6CH_2$ endo), 1.94 (d, 1H, CH), 1.95 (m, 1H, $^5CH_2$ endo), 3.77 (dd, 1H, one of dioxolane CH_2, J = 8.1 Hz), 4.07 (dd, 1H, one of dioxolane CH_2, J = 8.1 and 6.0 Hz) and 4.22 ppm (m, 1H, $CHCH_3$): δ_C ($CDCl_3$) 9.13 (CH_3), 18.14 (CH_3), 18.92 (dioxolane CH_3), 2.39 (CH_3), 21.35 (CH_2), 30.37 (CH_2), 43.33 (C-7), 52.09 (^4CH), 57.92 (C-1), 71.08 ($CHCH_3$), 73.64 (dioxolane CH_2) and 107.24 ppm (C-3) carbonyl resonance not recorded [Fig. 4.D.2.1] ; ν_{max} (film) 2,960 s, 2,930 s, 1,760s, 1,458m,

1,397m, 1,375m, 1,318m, 1,160m, 1,130m, 1,040s and 1,030s cm^{-1}; m/z (e.i.) 224 (M^+) and 196 ($M-CO^+$); $[\alpha]_D^{20}$ + 93.3° (c 1.5 in $CHCl_3$). (Found: C, 69.58; H, 9.04. $C_{13}H_{20}O_3$ requires C, 69.61; H, 8.98).

4.K.7 Preparation of (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-methyl-1',3'-dioxolane) (49a) and its diastereoisomer (49b)

This was prepared from D-camphorquinone (1 g, 6.02×10^{-3} mol) and (R)-(-)-propane-1,2-diol (0.55 g, 6.02×10^{-3} mol) in the manner described for dioxolane (47). After work up no crystalline product could be obtained and the mixture was analysed by 220 MHz ^1H n.m.r. spectroscopy (see section 4.D.2 and 4.E for results) with no further purification.

4.K.8 Preparation of (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane) (51)

A stirred solution of D-camphorquinone (100 g, 0.6 mol), (rac.)-3-chloropropane-1,2-diol (314 g, 238 cm^3, 2.86 mol) and p-toluenesulphonic acid (10 g, 0.052 mol) in benzene (1,750 cm^{-3}) was boiled under reflux for 18h in a flask fitted to a Dean-Stark apparatus. After cooling, anhydrous sodium sulphate (15 g) was added, the solid was filtered off and solvent was removed at reduced pressure (rotary evaporation) to leave a yellow liquid. This liquid was dissolved in water (1,000 cm^3) and the product was

extracted with petrol (b.p. 40-60°C, 3 x 500 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated to yield a yellow oil. Purification by gravity column chromatography [Merck Kieselgel 60, Art 7734 (70-230 mesh)] , eluting with 40-60°C petrol-ethylacetate, 7:1 (v/v) gave a colourless mixture of dioxolanes (50 - 53) from which (51) crystallised. Repeated recrystallisation [from petrol (b.p. 40-60°C)] gave dioxolane (51) as a colourless crystalline solid (14.6 g, 9.4%), m.p. 75.5-76.5°C (starts to sublime at 61°C); Rf 0.73 (silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v); δ_H (400 MHz, $CDCl_3$) 0.91 (s, 3H, CH_3), 0.98 (s, 6H, 2 x CH_3), 1.56 (m, 1H, $^5CH_2$ exo), 1.66 (m, 1H, $^6CH_2$ exo), 1.80 (m, 1H, $^6CH_2$ endo), 1.94 (m, 1H, $^5CH_2$ endo), 1.99 (d, 1H, ^4CH), 3.71 (m, 2H, CH_2Cl), 4.03 (dd, 1H, one of dioxolane CH_2, J = 6.67 and 8.4 Hz), 4.17 (dd, 1H, one of dioxolane CH_2, J = 6.12 and 8.4 Hz) and 4.37 ppm (m, 1H, $CHCH_2Cl$); δ_C ($CDCl_3$) 9.23 (CH_3), 18.97 (CH_3), 21.18 (CH_2), 21.38 (CH_3), 30.81 (CH_2), 43.48 (C-7), 44.13 (CH_2Cl), 52.06 (^4CH), 58.17 (C-1), 68.83 (CH_2 dioxolane), 77.21 ($CHCH_2Cl$), 108.41 (C-3), and 216.31 ppm (CO); ν max (film) 2,960s, 2,920s, 2,870s, 1,754s, 1,464s, 1,398m, 1,374m, 1,320m, 1,222m, 1,130m, 1,030m, 736m and 707w cm^{-1}; m/z (c.i. reagent NH_3) 278 and 276 ($M + NH_4^+$, 45.8%), 261 and 259 ($M + H^+$, 56.8%), 232 and 230 ($M-CO^+$ 5.2%) and 149 and 147 ($C_5H_5ClO_3^+$, 100%), ratios 1:3, respectively; $[\alpha]_D^{22}$ + 90° (c 2 in CCl_4). (Found: C, 60.36; H, 7.45; Cl, 13.61%. $C_{13}H_{19}O_3Cl$ requires C, 60.34; H, 7.40; Cl, 13.70%).

extracted with petrol (b.p. 40-60°C, 3 x 500 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated to yield a yellow oil. Purification by gravity column chromatography [Merck Kieselgel 60, Art 7734 (70-230 mesh)] , eluting with 40-60°C petrol-ethylacetate, 7:1 (v/v) gave a colourless mixture of dioxolanes (50 - 53) from which (51) crystallised. Repeated recrystallisation [from petrol (b.p. 40-60°C)] gave dioxolane (51) as a colourless crystalline solid (14.6 g, 9.4%), m.p. 75.5-76.5°C (starts to sublime at 61°C); Rf 0.73 (silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v); δ_H (400 MHz, $CDCl_3$) 0.91 (s, 3H, CH_3), 0.98 (s, 6H, 2 x CH_3), 1.56 (m, 1H, $^5CH_2$ exo), 1.66 (m, 1H, $^6CH_2$ exo), 1.80 (m, 1H, $^6CH_2$ endo), 1.94 (m, 1H, $^5CH_2$ endo), 1.99 (d, 1H, ^4CH), 3.71 (m, 2H, CH_2Cl), 4.03 (dd, 1H, one of dioxolane CH_2, J = 6.67 and 8.4 Hz), 4.17 (dd, 1H, one of dioxolane CH_2, J = 6.12 and 8.4 Hz) and 4.37 ppm (m, 1H, $C\underline{H}CH_2Cl$); δ_C ($CDCl_3$) 9.23 (CH_3), 18.97 (CH_3), 21.18 (CH_2), 21.38 (CH_3), 30.81 (CH_2), 43.48 (C-7), 44.13 (CH_2Cl), 52.06 (^4CH), 58.17 (C-1), 68.83 (CH_2 dioxolane), 77.21 ($\underline{C}HCH_2Cl$), 108.41 (C-3), and 216.31 ppm (CO); ν max (film) 2,960s, 2,920s, 2,870s, 1,754s, 1,464s, 1,398m, 1,374m, 1,320m, 1,222m, 1,130m, 1,030m, 736m and 707w cm^{-1}; m/z (c.i. reagent NH_3) 278 and 276 ($M + NH_4^+$, 45.8%), 261 and 259 ($M + H^+$, 56.8%), 232 and 230 ($M-CO^+$ 5.2%) and 149 and 147 ($C_5H_5ClO_3^+$, 100%), ratios 1:3, respectively; $[\alpha]_D^{22}$ + 90° (c 2 in CCl_4). (Found: C, 60.36; H, 7.45; Cl, 13.61%. $C_{13}H_{19}O_3Cl$ requires C, 60.34; H, 7.40; Cl, 13.70%).

4.K.9 H.p.l.c. separation of Chloromethyldioxolanes (50 - 53)

All four diastereoisomers were completely separated in one pass through a preparative h.p.l.c. column [Whatman Partisil M20:10/50 (50 cm x 22 mm i.d.)] , elution with 2% (v/v) ethylacetate in hexane at a flow rate of 8.5 cm^3/min, UV detection 325 nm.

(1R,3S,4S,5'S)-Camphor-3-spiro-2'-(5'-chloromethyl-1',3'-dioxolane) (50)

Colourless crystalline solid, m.p. 72-73°C; δ_H (400 MHz, $CDCl_3$) 0.91 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.53 (m, 1H, $^5CH_2$ exo), 1.66 (m, 1H, $^6CH_2$ exo), 1.79 (m, 1H, $^5CH_2$ endo), 1.95 (d, 1H, ^4CH), 1.96 (m, 1H, $^5CH_2$ endo) 3.68 (d, 2H, CH_2Cl), 4.185 (m, 2H, dioxolane CH_2) and 4.29 ppm (m, 1H, $CHCH_2Cl$); ν max (film) 2,960s, 2,920s, 2,870s, 1,754s, 1,464s, 1,398m, 1,374m, 1,320m, 1,222m, 1,130m, 736m and 707w cm^{-1}; m/z (c.i., reagent NH_3) 278 and 276 ($M+NH_4^+$, 26.4%), 261 and 259 ($M+H^+$, 4.8%), 232 and 230 ($M-CO^+$, 0.8%) and 149 and 147 ($C_5H_5ClO_3^+$, 8.8%) ratio 1:3, respectively, 18 = 100%.

(1R,3R,4S,4'S)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane) (52)

Colourless crystalline solid, m.p. 52-53°C; δ_H (400 MHz, $CDCl_3$) 0.90 (s, 1H, CH_3), 0.97 (s, 1H, CH_3), 0.99 (s, 1H, CH_3), 1.56 (m, 1H, $^5CH_2$ exo), 1.65 (m, 1H, $^6CH_2$ exo), 1.81 (m, 1H, $^5CH_2$ endo), 1.97 (m, 1H, $^6CH_2$ endo), 1.98 (d, 1H, ^4CH), 3.46 (dd, 1H, one of CH_2Cl, J = 7.56 and 10.85 Hz), 3.57 (dd, 1H, one of CH_2Cl,

4.K.9 H.p.l.c. separation of Chloromethyldioxolanes (50 - 53)

All four diastereoisomers were completely separated in one pass through a preparative h.p.l.c. column [Whatman Partisil M20:10/50 (50 cm x 22 mm i.d.)] , elution with 2% (v/v) ethylacetate in hexane at a flow rate of 8.5 cm^3/min, UV detection 325 nm.

(1R,3S,4S,5'S)-Camphor-3-spiro-2'-(5'-chloromethyl-1',3'-dioxolane) (50)

Colourless crystalline solid, m.p. 72-73°C; δ_H (400 MHz, $CDCl_3$) 0.91 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.53 (m, 1H, $^5CH_2$ exo), 1.66 (m, 1H, $^6CH_2$ exo), 1.79 (m, 1H, $^5CH_2$ endo), 1.95 (d, 1H, ^4CH), 1.96 (m, 1H, $^5CH_2$ endo) 3.68 (d, 2H, CH_2Cl), 4.185 (m, 2H, dioxolane CH_2) and 4.29 ppm (m, 1H, $CHCH_2Cl$); νmax (film) 2,960s, 2,920s, 2,870s, 1,754s, 1,464s, 1,398m, 1,374m, 1,320m, 1,222m, 1,130m, 736m and 707w cm^{-1}; m/z (c.i., reagent NH_3) 278 and 276 ($M+NH_4^+$, 26.4%), 261 and 259 ($M+H^+$, 4.8%), 232 and 230 ($M-CO^+$, 0.8%) and 149 and 147 ($C_5H_5ClO_3^+$, 8.8%) ratio 1:3, respectively, 18 = 100%.

(1R,3R,4S,4'S)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane) (52)

Colourless crystalline solid, m.p. 52-53°C; δ_H (400 MHz, $CDCl_3$) 0.90 (s, 1H, CH_3), 0.97 (s, 1H, CH_3), 0.99 (s, 1H, CH_3), 1.56 (m, 1H, $^5CH_2$ exo), 1.65 (m, 1H, $^6CH_2$ exo), 1.81 (m, 1H, $^5CH_2$ endo), 1.97 (m, 1H, $^6CH_2$ endo), 1.98 (d, 1H, ^4CH), 3.46 (dd, 1H, one of CH_2Cl, J = 7.56 and 10.85 Hz), 3.57 (dd, 1H, one of CH_2Cl,

J = 4.48 and 10.85 Hz), 3.90 (dd, 1H, one of dioxolane CH_2, J = 3.92 and 8.2 Hz), 4.35 (dd, 1H, one of dioxolane CH_2, J = 6.96 and 8.2 Hz) and 4.70 ppm (m, 1H, $C\underline{H}CH_2Cl$); δ_c ($CDCl_3$) 9.0 (CH_3), 18.8 (CH_2), 21.2 (CH_3), 21.3 (CH_3), 30.7 (CH_2), 43.5 (C-7), 44.2 (CH_2Cl), 51.8 (^4CH), 58.1 (C-1), 67.25 (dioxolane CH_2), 108 (C-3) and 216 ppm (CO), dioxolane CH hidden under the $CDCl_3$ triplet; ν max (film) 2,992s, 2,960s, 2.923s, 2,878s, 1,754s, 1,482s, 1,456s, 1,443s, 1,397m, 1,372m, 1,320m, 1,222m, 1,138m, and 707w, cm^{-1}; m/z (c.i., reagent NH_3) 278 and 276 ($M+NH_4^+$, 63.2%), 261 and 259 ($M+H^+$, 30.8%), 232 and 230 ($M-CO^+$, 15.4%) and 149 and 147 ($C_5H_5ClO_3^+$, 76%) ratio 1:3, respectively, 18 = 100%; $[\alpha]_D^{28}$ +48° (c 1.6 in $CHCl_3$).

(1R,3S,4S,5'R)-Camphor-3-spiro-2'-(5'-chloromethyl-1'3'-dioxolane) (53)

Colourless crystalline solid, m.p. 53-54°C; δ_H (400 MHz, $CDCl_3$) 0.91 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.107 (s, 3H, CH_3), 1.56 (m, 1H, $^5CH_2$ exo), 1.66 (m, 1H, $^6CH_2$ exo), 1.81 (m, 1H, $^6CH_2$ endo), 1.98 (d, 1H, ^4CH), 1.99 (m, 1H, $^5CH_2$ endo), 3.46 (dd, 1H, one of CH_2Cl, J = 7.2 and 10.8 Hz), 3.57 (dd, 1H, one of CH_2Cl, J = 4.56 and 10.8 Hz), 3.97 (dd, 1H, one of dioxolane CH_2, J = 4.0 and 8.2 Hz), 4.49 (dd, 1H, one of dioxolane CH_2, J = 6.6 and 8.2 Hz) and 4.58 ppm (m, 1H, $C\underline{H}CH_2Cl$); δ_c ($CDCl_3$) 8.97 (CH_3), 18.84 (CH_2), 21.24 (CH_3), 21.40 (CH_3), 30.81 (CH_2), 43.52 (C-7), 44.25 (CH_2Cl), 51.91 (^4CH), 58.2 (C-1), 69.22 (dioxolane, CH_2), 74.67 ($\underline{C}HCH_2Cl$), 108.1 (C-3) and 216 ppm (CO); ν max (film) 2,992s, 2,960s, 2,924s,

2,878s, 1,755s, 1,482s, 1,456s, 1,443s, 1,398m, 1,374m, 1,321m, 1,222m, 1,138m and 707m cm^{-1}; m/z (c.i., reagent NH_3) 278 and 276 ($M+NH_4^+$, 35%), 261 and 259 ($M+H^+$, 12%), 232 and 230 ($M-CO^+$, 5.8%) and 149 and 147 ($C_5H_5ClO_3^+$, 58.8%) ratio 1:3, respectively, 18 = 100%; $[\alpha]_D^{29}$ + 69° (c 1.09 in $CHCl_3$).

4.K.10 Preparation of (1S,3S,4R,4'S)-Camphor-3-spiro-2-(4'-chloromethyl-1',3'-dioxolane) (55)

This was prepared from L-camphorquinone (1.926 g, 1.1 x 10^{-2} mol) and (rac.)-3-chloropropane-1,2-diol (12.76 g, 0.116 mol) in the manner described for dioxolane (51) except that water was removed by azeotropic distillation through a thimble of oven-dried magnesium sulphate. Recrystallisation from petrol gave a colourless crystalline solid (0.32 g, 11.4%) m.p. 74-76°C; $[\alpha]_D^{20}$ - 71° (c 1.5 in $CHCl_3$) and spectral properties identical to dioxolane (51).

4.K.11 Preparation of (1R,3R,4S,4'S)-Camphor-3-spiro-2'-(4'-benzyloxymethyl-1',3'-dioxolane) (73) and its diastereoisomer (74)

This was prepared from D-camphorquinone (1 g, 6.02 x 10^{-3} mol) and (S)-3-benzyloxypropane-1,2,diol (1.09 g, 6.02 x 10^{-3} mol) in the manner described for dioxolane (47) [reflux 36h, benzene]. After work up the yellow oil was Kugelrohr distilled (120-130°/0.1mm) to give a colourless oil (1.44 g, 72.4%) which did not yield a

crystalline product on attempted fractional crystallisation. No further purification was attempted. δ_H ($CDCl_3$, major product) 0.90 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.00 (s, 3H, CH_3), 1.64 (m, 2H, $^5CH_2$ and $^6CH_2$ exo protons), 1.80 (m, 1H, $^6CH_2$ endo proton), 1.97 (m, 1H, $^5CH_2$ endo proton), 1.98 (d, 1H, ^4CH), 3.63 (dd, 1H, one of CH_2OCH_2Ph, J = 9.5 and 5.5 Hz), 3.76 (dd, 1H, one of CH_2OCH_2Ph, J = 9.5 and 5.5 Hz), 3.94 (dd, 1H, one of dioxolane CH_2, J = 7.9 Hz), 4.10 (dd, 1H, one of dioxolane CH_2, J = 7.9 and 6.7 Hz), 4.36 (m, 1H, dioxolane CH), 4.56 (s, 2H, CH_2Ph) and ArH resonances; ν_{max} 1,755s cm^{-1} (C=O); m/z (e.i.) 330 (M^+), 316 ($M-CH_2^+$), 302 ($M-CO^+$), 233, 219 and 91 ($C_7H_7^+$), 330.1839 (M^+, $C_{20}H_{26}O_4$ requires 330.1831).

4.K.12 Preparation of (1R,3R,4S,4'S)-Camphor-3-spiro-2'-(4'-benzyloxyethyl-1',3'-dioxolane) (75) and its diastereoisomer (76)

This was prepared from D-camphorquinone (0.85 g, 5.1 x 10^{-3} mol) and (S)-4-benzyloxybutane-1,2-diol (1 g, 5.1 x 10^{-3} mol) in a manner described for dioxolane (47) [reflux 5h benzene]. After work-up the resulting yellow oil was purified by column chromatography [Merck Keiselgel 60, Art 7734 (70-230 mesh)] , eluting with 40-60°C petrol-ethylacetate 7:1 (v/v) to afford a colourless oil (1.46 g, 83.7%), Rf 0.41 (silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v); δ_H ($CDCl_3$, major product) 0.90 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.5-2.2 (m, 5H, ^4CH, $^5CH_2$-$^6CH_2$), 3.58 (m, 4H, CH_2CH_2OPh), 3.87 (dd, 1H, one of dioxolane

CH_2, J = 7.9 Hz), 4.09 (dd, 1H, one of dioxolane CH_2, J = 7.9 and 6.7 Hz), 4.28 (m, 1H, dioxolane CH), 4.49 (s, 2H, OCH_2Ph) and ArH resonances; νmax 1,754s cm^{-1} (C=O); m/z (e.i.) 344 (M^+), 330 ($M-CH_2^+$), 316 ($M-CO^+$), 247, 233, 219 and 91 ($C_7H_7^+$), 344.1989 (M^+, $C_{21}H_{28}O_4$ requires 344.1987).

4.K.13 Preparation of (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-t-butyl-1',3'-dioxolane) and its diastereoisomers (66 - 69)

A stirred solution of D-camphorquinone (0.5 g, 3.01 x 10^{-3} mol), (rac.)-2,2-dimethylbutane-3,4-diol (3.0 g, 2.3 x 10^{-2} mol) and p-toluenesulphonic acid (0.05 g, 2.6 x 10^{-4} mol) was boiled under reflux in benzene (ca 30 cm^3) through a thimble of oven-dried magnesium sulphate for 18h under an atmosphere of nitrogen. After cooling, anhydrous sodium carbonate was added, the solid was filtered off and solvent was removed at reduced pressure (rotary evaporation) to give a yellow oil. The oil was dissolved in 40-60°C petrol (20 cm^3), washed with water (3 x 20 cm^3), dried ($MgSO_4$), filtered and evaporated at reduced pressure (rotary evaporation). Purification by gravity column chromatography [Merck Kieselgel 60, Art 7734 (70 - 230 mesh)] eluting with 40-60°C petrol-ethylacetate, 7:1 (v/v) gave a colourless oil (0.686 g, 85.7%) containing four diastereoisomeric dioxolanes (see text) which did not yield a crystalline derivative on attempted fractional crystallisation. An analytical sample of the major dioxolane was purified by h.p.l.c. (injection amount, 50 µl of a 60 mg/100 µl sample in

hexane) using a Gilson instrument (fitted with a variable wavelength UV detector) and a normal phase preparative column [Whatman Partisil M20: 10/50 (50 cm x 22 mm i.d.)], eluting with 1% ethyl-acetate in hexane (v/v), flow rate 7.5 cm^3/min and monitoring at 325 nm. The product had the following physical properties; b.p. (Kugelröhr distillation) 75-79°C/0.2 mm; Rf 0.65 (silica gel, 40-60°C petrol-ethylacetate 7:1 v/v); δ_H (300 MHz, $CDCl_3$) 0.91 (s, 3H, CH_3), 0.93 (s, 9H, t-But), 0.97 (s, 1H, CH_3), 1.00 (s, 3H, CH_3), 1.52-1.98 (m, 4H, $^5CH_2{}^6CH_2$), 1.94 (d, 1H, ^4CH), 3.73 (dd, one of dioxolane CH_2, J = 5.7 and 9.4 Hz) and 3.93 ppm (m, 2H, $CHC(CH_3)_3$ and one of dioxolane CH_2); νmax (film) 2,963s, 2,910s, 2,875s, 1,764s, 1,482m, 1,398m, 1,369m, 1,320m, 1,225m, 1,127m, 1,026s and 947m cm^{-1}; m/z 266 (M^+), 251 ($M-CH_3$), 238 ($M-CO^+$), 223 ($M-CO-CH_3^+$) and 155; $[\alpha]_D^{22}$ + 30.4° (c 1.56 in CCl_4); m/z (e.i.) 266.1885 (M^+, $C_{16}H_{26}O_3$ requires 266.1882).

4.K.14 Preparation of (1R,2S,3R,4S,4'S)-Bornane-2-hydroxy-3-spiro-2'-(4'-methyl-1',3'-dioxolane) (59)

To a stirred solution of dioxolane (51, 0.2 g, 7.7 x 10^{-4} mol) in dry THF (5 cm^3) under nitrogen was added lithium tri-sec-butylborohydride (L-selectride, 3.08 cm^3, 3.08 x 10^{-3} mol of a 1 M solution in THF) and the reaction heated to 40°C. T.l.c. analysis (silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v) showed the reaction to have gone to completion after 14h. Water (10 cm^3) was carefully added and the product was extracted into 40-60°C petrol (3 x 10 cm^3). The petrol fractions were combined, washed

with water (2 x 10 cm^3), dried ($MgSO_4$) and evaporated at reduced pressure (rotary evaporation) to give a crude oily product. The product was purified by gravity column chromatography [Merck Kieselgel 60, Art 9385 (230-400 mesh)], eluting with 40-60°C petrol-ethylacetate, 7:1 (v/v) to give alcohol (59) as a colourless oil (140 mg, 83%), b.p. 36-38°C/0.1 mm; Rf 0.41; δ_H (300 MHz, $CDCl_3$) 0.83 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 1.07 (s, 3H, CH_3) 1.18 (m, 1H, $^6CH_2$ endo), 1.32 (d, 2H, dioxolane CH_3), 1.45-1.80 (m, 4H, $^5CH_2$, $^6CH_2$ exo and ^4CH), 2.54 (bs, 1H, CHOH), 3.24 (s, 1H, CHOH), 3.33 (dd, 1H, one of dioxolane CH_2, J = 8.17 Hz), 3.97 (dd, 1H, one of dioxolane CH_2, J = 5.5 and 8.17 Hz) and 4.24 ppm (m, 1H, $CHCH_3$); ν max(film) 3,530br, 2,930s, 2,870s, 1,478m, 1,454m, 1,393m, 1,371m, 1,309m, 1,226m, 1,094s and 994m cm^{-1}; m/z (e.i.) 226 (M^+), 211 ($M-CH_3^+$), 155 and 141; $[\alpha]_D^{22}$ + 26.4° (c 1.45 in CCl_4); m/z (e.i.) 226.1574 (M^+, $C_{13}H_{22}O_3$ requires 226.1569).

4.K.15 Oxidation of (1R,2S,3R,4S,4'S)-Bornane-2-hydroxy-3-spiro-2'-(4'-methyl-1',3'-dioxolane) (59)

To a solution of dioxolane (59, 20 mg, 8.85 x 10^{-5} mol) in DMF (0.5 cm^3) was added pyridinium dichromate (0.33 g, 8.85 x 10^{-4} mol) and the reaction stirred under nitrogen at RT. T.l.c. analysis (silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v) showed the reaction to be complete after 20h. The reaction mixture was filtered through a short column of magnesium sulphate (ca 1 g) and the column was washed with 40-60°C petrol (10 cm^3). The petrol was washed with water (4 x 5 cm^3), dried ($MgSO_4$) and evaporated at reduced pressure

to leave a colourless oil (16 mg, 81%) which crystallised on standing, m.p. 35-36°C (recrystallisation from 40-60°C petrol); Rf 0.57 and all other physical properties identical to those shown by dioxolane (47 , Expt. 4.K.6).

4.K.16 Attempted Reduction of (1R,3R,4S,4'R)-Camphor-3-spiro--2'-(4'-chloromethyl-1',3'-dioxolane) (51) with Diisopinocampheylborane and Diisoamylborane

1. Diisopinocampheylborane

To a stirred suspension of α-pinene (0.423 g, 0.484 cm^3, 3.1×10^{-3} mol) and sodium borohydride (4.37×10^{-2} g, 1.1×10^{-3} g) in diglyme (0.5 cm^3) under nitrogen at 0°C was added boron trifluoride: diethyletherate (0.208 g, 0.184 cm^3, 1.47×10^{-3} mol) in diglyme (0.25 cm^3) dropwise over ca 15 min. The reagent was maintained at 0°C for a futher 3h prior to use. Dioxolane (51, 0.2 g, 7.75×10^{-5} mol) in diglyme (0.5 cm^3) was added and the reaction further stirred for 24h at RT. Water (0.5 cm^3) was slowly added and the organoborane formed was hydrolysed at 30-50°C by adding 3 M sodium hydroxide (0.5 cm^3) followed by dropwise addition of 30% hydrogen peroxide (0.32 cm^3). The product was extracted into 40-60°C petrol, dried ($MgSO_4$) and solvent removed at reduced pressure (rotary evaporation) to leave a colourless oil. ^1H n.m.r. spectroscopic analysis showed the oil to contain a mixture of unreacted dioxolane (51) and hydroxypinene.

2. Diisoamylborane

To a stirred suspension of 2-methylbutene (0.416g, 0.63 cm^3, 5.88 x 10^{-3} mol) and sodium borohydride (8.74 x 10^{-2} g, 2.2 x 10^{-3} mol) in diglyme (2 cm^3) under nitrogen at -15°C was added boron-trifluoride:diethyletherate (0.416 g, 0.368 cm^3, 2.94 x 10^{-3} mol) in diglyme (2 cm^3) over ca 15 min. A colourless precipitate of diisoamylborane formed and the reagent was further stirred for 3h at 0°C prior to use. The reagent was cooled to -10°C and dioxolane (51, 0.2 g, 7.7 x 10^{-4} mol) in diglyme (0.5 cm^3) was added over 15 min. After 96h at RT water (1 cm^3) was slowly added and the organoboranes formed were hydrolysed at 30-50°C by adding 3 M sodium hydroxide (1 cm^3) followed by dropwise addition of 30% hydrogen peroxide (0.64 cm^3). The product was extracted into 40-60°C petrol, was dried ($MgSO_4$) and evaporated (rotary evaporation, bath temperature 55°C) to leave a colourless oil which spontaneously crystallised on cooling to RT. ^1H n.m.r. analysis of the product showed it to be starting material.

4.K.17 Reduction of (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane (51) with Diborane

To a stirred solution of dioxolane (51, 0.2 g, 7.74 x 10^{-4} mol) in THF (2 cm^3) under nitrogen at -10°C was added diborane (3.7 cm^3, 3.7 x 10^{-3} mol of a 1 M solution in THF) and the reaction warmed to 40°C. T.l.c. analysis (silica gel, 40-60°C petrol-ethyl-acetate, 7:1 v/v) indicated the reaction to be complete after 96h. Water (0.5 cm^3) was slowly added and the organoboranes were

hydrolysed at 30-50°C by addition of 3 M sodium hydroxide (0.5 cm^3) followed by dropwise addition of 30% hydrogen peroxide (0.4 cm^3). The product was extracted into 40-60°C petrol (20 cm^3) and the petrol layer was washed with water (2 x 5 cm^3), dried ($MgSO_4$) and evaporated at reduced pressure (rotary evaporation) to leave a colourless oil (0.18 g, 87.1%), Rf 0.45. ^1H n.m.r. analysis showed the product to be a mixture of C-2 exo- and endo-alcohols in a ratio 14:3 (82% exo), respectively; δ_H ($CDCl_3$, exo-alcohol) 0.83 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.18 (m, 1H, $^6CH_2$ endo), 1.48-1.75 (m, 4H, $^5CH_2$, $^6CH_2$ exo and ^4CH), 2.38 (bs, 1H, CHOH), 3.33 (s, 1H, CHOH), 3.63 (m, 2H, CH_2Cl), 3.77 (dd, 1H, one of dioxolane CH_2, J = 5.7 and 8.73 Hz), 3.98 (dd, 1H, one of dioxolane CH_2, J = 5.9 and 8.73 Hz) and 4.34 ppm (m, 1H, $CHCH_2Cl$), the endo alcohol was identified by resonances at δ_H 0.87 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 3.48 (dd, 1H, one of CH_2Cl, J = 8.1 and 10.8 Hz), 3.86 (dd, 1H, one of dioxolane CH_2, J = 5.1 and 8.3 Hz) and 4.31 ppm (m, 1H, $CHCH_2Cl$); m/z (e.i.) 262 and 260 (M^+, ratio 1:3 respectively).

4.K.18 Reduction of (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane) (51) with sodium borohydride

To a stirred solution of dioxolane (51, 0.5 g, 1.9×10^{-3} mol) in ethanol (10 cm^3) was added sodium borohydride (8.66×10^{-2} g, 2.34×10^{-3} mol) over ca 10 min and the reaction mixture heated to 40°C. Monitoring by t.l.c.(silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v) showed the reaction to be complete after 4h. Water (2cm^3)

was added and volatiles were removed at reduced pressure. The resulting oil was dissolved in diethyl ether (10 cm^3), washed with water (3 x 5 cm^3), dried (Na_2SO_4) and evaporated to give a colourless oil (4.42 g, 89.4%), Rf 0.43. ^1H n.m.r. analysis showed the product to be a mixture of C-2 exo- and endo-alcohols in a ratio 3:1 (75% exo), respectively. See preparation 4.K.17 for spectral analysis.

4.K.19 Reduction of (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane) (51) with lithiumtri-t-butoxyaluminium hydride

To a stirred suspension of lithium aluminium hydride (0.368 g, 9.68 x 10^{-3} mol) in THF (5 cm^3) under N_2 at -10°C was added dropwise a solution of t-butanol (2.15 g, 2.90 x 10^{-2} mol) in THF (0.5 cm^3) over ca 30 min. The reaction was allowed to warm to RT and after 2h a solution of dioxolane (51, 0.5 g, 1.93 x 10^{-3} mol) in THF (2 cm^3) was added over 2 min. Monitoring by t.l.c. (silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v) indicated the reaction to have gone to completion after 20h. Water (3 cm^3) was slowly added and the white precipitate (LiOH) was filtered off (celite) and washed with 40-60°C petrol (50 cm^3). The supernatant was evaporated at reduced pressure (rotary evaporation) to give a crude oily product. This oil was redissolved in 40-60°C petrol (15 cm^3), was washed with water (2 x 3 cm^3), dried ($MgSO_4$), filtered and evaporated at reduced pressure to give a colourless oil (0.47 g, 93%); Rf 0.45; δ_H (300 MHz, $CDCl_3$) 0.83 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.18 (m, 1H, $^6CH_2$ endo), 1.48-1.75 (m, 3H,

$^{5}CH_2$, $^{6}CH_2$ exo), 1.74 (d, 1H, ^{4}CH), 2.54 (bs, 1H, CHOH), 3.33 (s, 1H, CHOH), 3.63 (m, 2H, CH_2Cl), 3.77 (dd, 1H, one of dioxolane CH_2, J = 5.7 and 8.73 Hz), 3.98 (dd, 1H, one of dioxolane CH_2, J = 5.9 and 8.73 Hz) and 4.34 ppm (m, 1H, $CHCH_2Cl$); ν max (film) 3,540br, 2,980s, 2,892s, 1,479m, 1,454m, 1,393m, 1,372m, 1,308m, 1,094s, 993s, 742w cm^{-1}; m/z (e.i.) 260 and 262 (M^+, ratio 3:1 respectively), 245 and 247 ($M-CH_3^+$, ratio 3:1, respectively), 189 and 191 (ratio 3:1, respectively) and 175 and 177 (ratio 3:1, respectively); $[\alpha]_D^{18}$ + 36° (c 2.13 in CCl_4); m/z (e.i.) 260.1189 (M^+, $C_{13}H_{21}O_3Cl$ requires 260.1179).

4.K.20 Reduction of (1R,3R,4S,4'R)-Camphor-3-spiro-2'-(4'-chloromethyl-1',3'-dioxolane) (51) with Lithium trimethoxylaluminium hydride

To a stirred suspension of lithium aluminium hydride (7.35×10^{-2} g, 1.93×10^{-3} mol) in THF (0.5 cm^3) under nitrogen at -10°C was added methanol (0.186 g, 237μl, 5.8×10^{-3} mol) over ca 30 min. The reaction was allowed to warm to 0°C and after 2h a solution of dioxolane (51, 0.1 g, 3.87×10^{-4} mol) in THF (0.5 cm^3) was added dropwise. The reaction was allowed to warm to RT and followed by t.l.c.(silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v). After 20h water (0.2 cm^3) was slowly added and the white precipitate was filtered off (celite) and washed with 40-60°C petrol (15 cm^3). The supernatant was washed with water (3 x 5 cm^3) and the petrol layer was dried ($MgSO_4$), filtered and evaporated at reduced pressure (rotary evaporation) to give a colourless oil

(74.2 mg), Rf 0.43, ^{1}H n.m.r. analysis showed the product to be a mixture of dioxolane (59, see preparation 4.K.14) and dioxolane (56, preparation 4.K.19) in the ratio 5:2, respectively.

4.K.21 Preparation of (1R,2S,3R,4S,4'S)-Bornane-3-spiro-2'-(1',3',7'-trioxolane) (78)

To a stirred solution of sodium hydride (0.11 g, 4.66 x 10^{-3} mol) in THF (4 cm^3) under nitrogen was added a solution of dioxolane (59, 59.7 mg, 2.29 x 10^{-4} mol) in THF (1 cm^3) and the reaction heated to 55-60°C. Aliquots were removed at intervals and analysis by t.l.c. (silica gel, 40-60°C petrol-ethylacetate, 7:1 v/v) showed the reaction to be complete after 24h. Water (15 cm^3) was slowly added and the product was extracted into 40-60°C petrol (3 x 10 cm^3). The petrol layers were combined, washed with water (2 x 5 cm^3), dried ($MgSO_4$), filtered and evaporated at reduced pressure (rotary evaporation) to give trioxolane (78) as a colourless oil (41.8 mg, 81.3%), b.p. 50-55°C/0.2 mm (Kugelröhr distillation); Rf 0.31; δ_H (300 MHz, $CDCl_3$) 0.87 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 1.24 (m, 1H, $^{6}CH_2$ endo), 1.28 (s, 3H, CH_3), 1.54-1.83 (m, 3H, $^{5}CH_2$ and $^{6}CH_2$ exo), 1.87 (d, 1H, ^{4}CH), 2.87 (s, 1H, ^{2}CH endo), 3.28 (dd, 1H, one of trioxolane CH_2 endo protons, J = 1.26 and 10.22 Hz), 3.54 (dd, 1H, one of trioxolane endo protons, J = 1.54 and 7.83 Hz), 3.86 (ddd, 1H, one of trioxolane exo protons, J = 1.00, 5.97 and 7.83 Hz), 4.20 (ddd, 1H, one of trioxolane exo protons, J = 1.00, 7.23 and 10.2 Hz) and 4.50 ppm (ddt, 1H, $(-OCH_2)_2CHO-$); νmax (film) 2,978s, 2,896s, 1,479m, 1,459m, 1,394m, 1,374m, 1,338m,

1,228w, 1,204m, 1,140s, 1,124s, 1,099m, 1,086m, 923m and 857m cm^{-1}; m/z (e.i.) 224 (M^+), 209 ($M-CH_3^+$), 193 ($M-CO^+$), 181 ($M-CH_3-CO^+$), 141 and 95; $[\alpha]_D^{22}$ - 24° (c 1.99 in CCl_4); m/z (e.i.) 224.1414 (M^+, $C_{13}H_{20}O_3$ requires 224.1412). (Found: C, 69.66; H, 8.84. $C_{13}H_{20}O_3$ requires C, 69.61; H, 8.98%).

4.K.22 Preparation of (Rac.)-2-Acetoxy-1-bromo-3-chloropropane

To 3-chloropropane-1,2-diol (11.0 g, 0.1 mol) at 0°C was added, with stirring, 45% hydrogen bromide in acetic acid (106.5 g, 76.6 cm^3, 0.45 mol) over 5 min. After stirring at RT for 1.5 h the solution was quickly added to ice-cooled water (150 cm^3) and was immediately neutralised with solid sodium carbonate. The neutral solution was extracted with diethyl ether (3 x 150 cm^3), the extracts were combined, dried ($MgSO_4$) and evaporated at reduced pressure (rotary evaporation) to give a yellowish liquid (20.81 g, 97.4%). Fractional distillation gave the desired product as a colourless liquid (19.66 g, 92%), b.p. 90-92°C/13 mm; δ_H ($CDCl_3$) 2.12 (s, 3H, $OCOCH_3$), 3.60 (d, 2H, CH_2Br), 3.76 (d, 2H, CH_2Cl) and 5.15 ppm (m, 1H, $CHOCOCH_3$); ν max (film) 3,020w, 2,965w, 1,748s, 1,430m, 1,372s, 1,230s, and 1,030s cm^{-1}. (Found C, 28.0; H, 3.9. $C_5H_8BrClO_2$ requires C, 27.9; H, 3.8%).

4.K.23 Preparation of (R)-(-)-3-Chloropropane-1,2-diol

Dioxolane (51, 0.5 g, 1.9×10^{-3} mol) was reduced with sodium borohydride (1.4×10^{-1} g, 3.8×10^{-3} mol) in the manner described in Preparation 4.K.18. To the product diols was added 2 M HCl (3 cm^3) followed by methanol until the reaction mixture became homogeneous. After refluxing for 3h volatiles were evaporated and water (10 cm^3) was added. The water was washed with 40-60°C petrol (3 x 10 cm^3) and evaporated to leave a pale yellow oil which was taken up into dichloromethane, dried ($MgSO_4$), filtered and evaporated at reduced pressure (rotary evaporation) to give a crude product. Kugelröhr distillation gave (R)-(-)-3-chloropropane-1,2-diol [115 mg, 55% from dioxolane (51)] as a colourless liquid, b.p. 74-76°C/15 mm; δ_H ($CDCl_3$) 3.0 (bs, 2H, 2 x OH), 3.62 (m, 2H, CH_2Cl), 3.73 (m, 2H, CH_2OH), and 3.94 ppm (m, 1H, CHOH); $[\alpha]_D^{19}$ - 7.4° (c 1.0 in H_2O), { lit.[14] $[\alpha]_D^{20}$ + 7.3° (c 1.0 in H_2O) for (S)-isomer }.

4.K.24 Preparation of (R)-(Chloromethyl)oxirane

To dioxolane (51, 3 g, 1.16×10^{-2} g) was added with stirring, 45% hydrogen bromide in acetic acid (w/v) [13.02 g, 9.5 cm^3, 5.5×10^{-2} mol] and the mixture was heated to 65°C for 5h. After cooling, the solution was quickly added to ice-cooled water (100 cm^3) and was immediately neutralised with solid sodium carbonate. The neutral solution was extracted with diethyl ether (3 x 30 cm^3), the extracts were combined, dried ($MgSO_4$) and evaporated at reduced pressure

(rotary evaporation) to leave a yellow oil. ^{1}H n.m.r. analysis showed the presence of 2-acetoxy-1-bromo-3-chloropropane: δ_H ($CDCl_3$) 2.13 (s, 3H, OCH_3), 3.60 (d, 2H, CH_2Br), 3.77 (d, 2H, CH_2Cl) and 5.15 ppm (m, 1H, $CHOCOCH_3$).

The yellow oil (containing ca 2.37 g, 1.1 x 10^{-2} mol) was vigourously stirred in dry ethane-1,2-diol (5 cm^3) to give a homogeneous suspension and 1.2 M sodium ethane-1,2-diolate in ethane-1,2-diol (9.17 cm^3, 1.1 x 10^{-2} mol) was added dropwise over 15 min. The reaction was stirred for 15 min after which the pressure was reduced to 0.2 mm and the product collected in an efficiently cooled receiver (-196°C). After 30 min a further aliquot of 1.2 M sodium ethane-1,2-diolate in ethane-1,2-diol (2.29 cm^3, 2.9 x 10^{-3} mol) was added and the reaction was allowed to proceed a further 30 min. Pressure was equalised and the receiver was allowed to warm to RT. Kugelrohr distillation of the crude product gave (R)-(chloromethyl)oxirane as a colourless liquid [0.594 g, 58% from dioxolane (51)]; b.p. 115-116°C; δ_H ($CDCl_3$) 2.7 (dd, 1H, one of oxirane CH_2), 2.90 (dd, 1H, one of oxirane CH_2), 3.25 (m, 1H, CH) and 3.59 ppm (d, 2H, CH_2Cl); $[\alpha]_D^{24}$ - 33° (c 1.5 in methanol) {lit.[1] $[\alpha]_D^{20}$ + 33° (c 1.13 in methanol) for (S)-isomer}.

4.K.25 Acid-Catalysed Rearrangements

To the dioxolane (20 mg) in [$^2H_8$]-toluene (0.5 cm^3) was added p-toluenesulphonic acid (0.1 mol. equiv.) and the reaction mixture incubated at 105°C. The reaction was followed to equilibrium by ^{1}H n.m.r. spectroscopy.

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CHAPTER 5

THE INTRINSIC REACTIVITY OF BIOLOGICAL NUCLEOPHILES TOWARDS METHYLOXIRANE

THE INTRINSIC REACTIVITY OF BIOLOGICAL NUCLEOPHILES TOWARDS METHYLOXIRANE

5.A INTRODUCTION

Although there is no epidemiological evidence correlating human exposure to methyloxirane with cancer in man, reports have indicated that it is carcinogenic in mice and rats[1,2]. Recent studies have also shown that it is capable of modifying DNA *in vitro*[3,4] and that it is mutagenic in yeasts (*Schizosaccharomyces pombe*)[5], bacteria[6,7] (*Salmonella typhimurium*, *Klebsiella pneumoniae*, *Eschenchia coli*) and mammalian cells *in vitro*[8]. Ehrenberg *et al*.[9,10] have demonstrated that levels of human exposure to alkylating agents can be monitored by quantitating the reaction products of these reagents with haemoglobin.

Following the exposure of a protein to methyloxirane there are four reaction parameters that could be investigated:

(i) the relative extents of alkylation at different nucleophilic sites,

(ii) the relative extent of mono- and di-alkylation at a particular site,

(iii) the enantioselectivity of a particular site for (*R*)- or (*S*)-methyloxirane [(2a) and (2b), respectively],

(iv) the regioselectivity of a particular site in its attack on methyloxirane; in principle, (*R*)- or (*S*)-methyloxirane could exhibit different regioselectivities.

In connection with our studies into the reactions of electrophilic reagents with cellular macromolecules, we have investigated the above parameters using the protected amino-acids

(R)-N-acetylcysteine methyl ester (88), (S)-valine methyl ester (89) and (S)-N-benzoylhistidine methyl ester (90), corresponding to the three likely sites of covalent modification in haemoglobin: cysteine SH, terminal valine NH_2 and histidine N (of imidazole). Protected amino-acids were used so that reactions with methyloxirane could be conducted in homogeneous methanolic solutions and problems that might arise from reactions occurring at more than one site were avoided. The reactivities observed in methanol can probably be extrapolated to aqueous media. It is known that the reactions between epoxides and amine nucleophiles occur very much faster in protic compared with those in aprotic media, because of proton transfer to oxirane oxygen in the transition state for ring-opening[11].

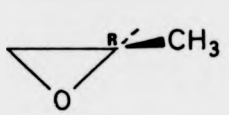
A comparative study is described of the reactions between the protected amino-acids and the enantiomeric methyloxiranes. It is of interest to determine the intrinsic reactivities of these functional groups in model compounds, although relative reactivities may change in the protein. Data about the relative rates of reactions of (R)- and (S)-methyloxirane with the functional groups of the model compounds and the amino-acid adducts obtained will provide useful references for comparison with *in vivo* systems. The relevance of the present study to the toxicology of methyloxiranes will be discussed. Finally, we shall attempt to use the spectroscopic data gathered to infer enantioselectivity from the alkylation with racemates.

5.B REACTIONS BETWEEN (R)-N-ACETYLCYSTEINE METHYL ESTER AND THE ENANTIOMERS OF METHYLOXIRANE

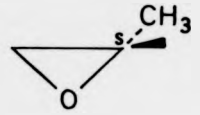
It has long been known that cysteine in aqueous solution reacts with simple epoxides, e.g. oxirane[12,13], methyloxirane[14] and ethyloxirane[15], to give an S-2-hydroxyalkyl cysteine derivative. The kinetics of the reactions of oxirane with a variety of thiols, including cysteine, have been studied over a range of pH. Values for the rate constants were found to increase with increasing pH and it was inferred that it is the thiolate ion (RS^-) that reacts with the epoxide[16]. At a given pH (e.g.'physiological pH' of 7.4) the concentration of thiolate, and hence its rate of reaction with epoxide, will depend on the pKa of the thiol (RSH). The pKa values vary widely according to substituents near the thiol and their state of ionisation[17]. Thiol groups in proteins also exhibit a range of pKa values and can be alkylated by epoxides[17].

Difficulties were experienced in obtaining pure S-2-hydroxyalkyl cysteines from reactions between epoxides and cysteine[12b], because these products were very soluble in water and alcohol. We have circumvented this problem by the use of (R)-N-acetylcysteine methyl ester (88) which in reactions with the enantiomeric methyloxiranes gave S-2-hydroxypropyl adducts having favourable physical properties (see below). Furthermore, only the ionisation state of the thiol group of ester (88) requires consideration in these reactions and there are no complications from competing alkylations at other functional groups within the molecule, e.g. some N-alkylation was observed with cysteine[12b].

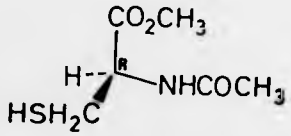
Preliminary reactions of (rac.)-methyloxirane with (R)-N-acetylcysteine methyl ester (88) in propan-2-ol were complicated by the incomplete transesterification of (88) into its iso-propylester. This problem was circumvented by using methanol as solvent. Both (R)- and (S)-methyloxirane [(2a) and (2b), respectively] reacted with N-acetylcysteine methyl ester in



(2a)



(2b)



(88)

methanol containing triethylamine. For a 0.125 M solution of (88) with 1 mol. equiv. triethylamine and 5 mol. equiv. methyloxirane, the reaction was complete after 4h/0°C (assessment by ^{1}H n.m.r. spectroscopy and h.p.l.c.). The principal product from (R)-methyloxirane (2a), characterised by its spectroscopic properties, was (R,αR)-N-acetyl-S-(2-hydroxypropyl)cysteine methyl ester (91). The 300 MHz ^{1}H n.m.r. spectrum of adduct (91) showed resonances at δ 1.23 (d, CH_3), 3.85 (m, CHOH) 2.96 (dd, one of diastereotopic CH_2CHOH)

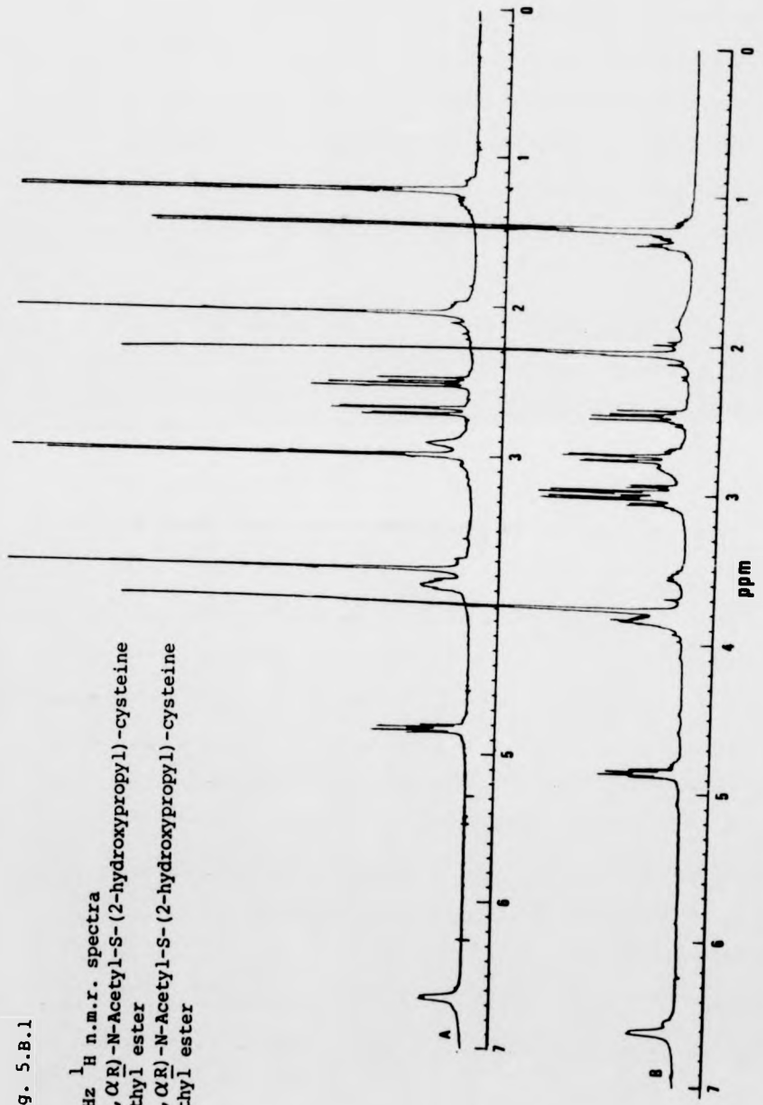
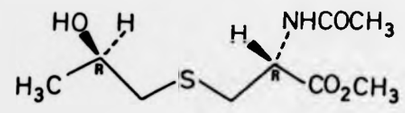


Fig. 5.B.1

360 MHz ^{1}H n.m.r. spectra
A (S,αR)-N-Acetyl-S-(2-hydroxypropyl)-cysteine methyl ester
B (R,αR)-N-Acetyl-S-(2-hydroxypropyl)-cysteine methyl ester



(91)

and 3.04 (dd, one of diasterotopic CH_2CHOH) proving that the nucleophilic attack of the thiolate anion on methyloxirane is highly regioselective for the methylene carbon[Fig. 5.B.1.] . The spectrum showed no evidence for the presence of an isomeric impurity derived by thiolate attack on the methine carbon of methyloxirane. This conclusion was supported by h.p.l.c. analysis which showed a single peak for (91). This is the expected behaviour of an alkyl-substituted epoxide[18], in contrast to that of phenyl-oxirane, for which products from attack by glutathione at both carbon atoms of the oxirane ring have been observed[19,20] ($GSCH_2CHOHPh/GSCHPhCH_2OH$ ~ 2:3 where GSH = glutathione). Similarly, the (S,αR)-diastereoisomer (92), from the reaction with (S)-methyloxirane was obtained. This diastereoisomer, having distinctive spectral properties from those of the (R,αR)-isomer gave resonances, by ^{1}H n.m.r. spectroscopy at δ1.21 (d, CH_3) 3.0 (d, CH_2CHOH) and

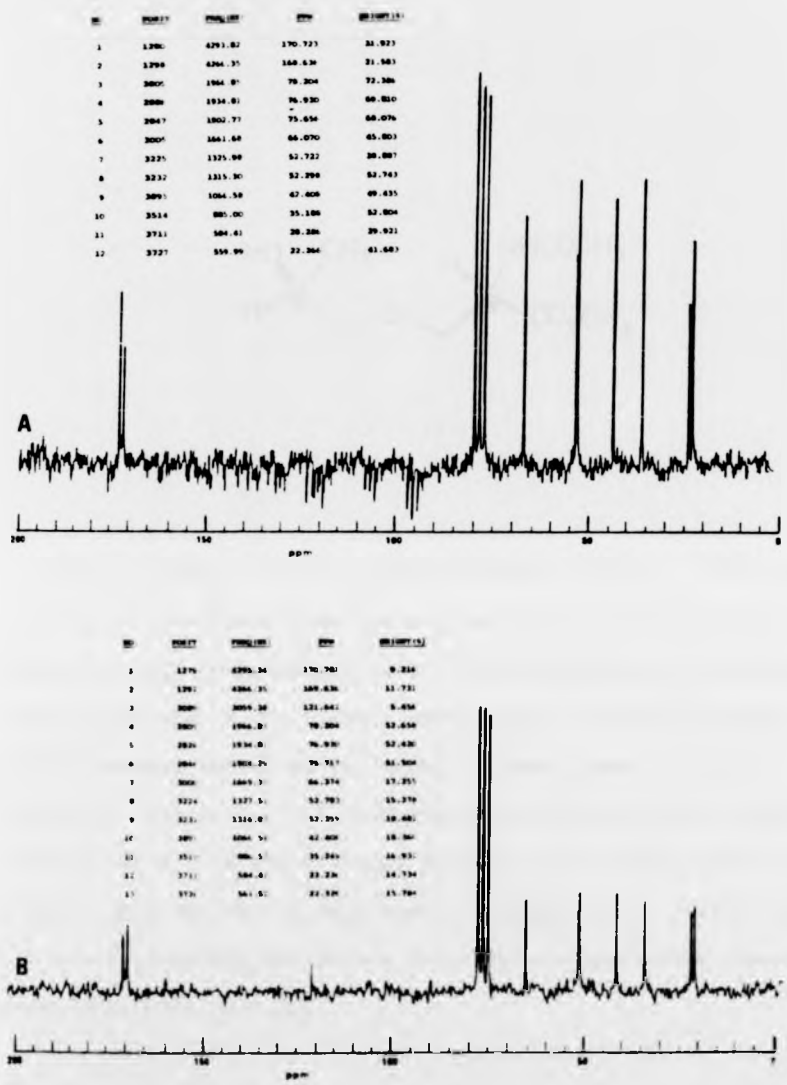
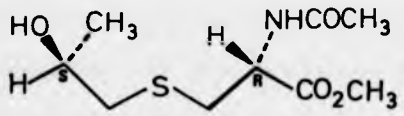


Fig. 5.B.2 90-52 MHz ^{13}C n.m.r. spectra

A (S,αR)-N-Acetyl-S-(2-hydroxypropyl)-cysteine methyl ester

B (R,αR)-N-Acetyl-S-(2-hydroxypropyl)-cysteine methyl ester



(92)

3.87 ppm (m, CHOH) for its 2-hydroxypropyl moiety. The ^{1}H n.m.r. spectra of the diastereoisomers are particularly diagnostic for the diastereotopic methylene protons. The hydroxypropyl methylene protons for the (S,αR)-isomer have nearly identical chemical shifts whereas those for the (R,αR)-isomer appear as double doublets. Similarly, the thiomethylene protons appear as double doublets at δ 2.50 and 2.70 ppm for the (S,αS)-isomer and at δ 2.46 and 2.76 ppm for the (R,αS)-isomer, respectively. Differences in ^{13}C n.m.r. chemical shifts for structurally equivalent carbons were small [Fig. 5.B.2.].

5.C DETERMINATION OF THE RELATIVE RATES OF REACTION OF (R)- AND (S)-METHYLOXIRANE WITH (R)-N-ACETYLCYSTEINE METHYL ESTER

The relative rates of the reactions of the enantiomeric methyloxiranes with ester (88) were determined by h.p.l.c.[Fig. 5.C.1.]. The diastereoisomers (91) and (92) formed by reaction

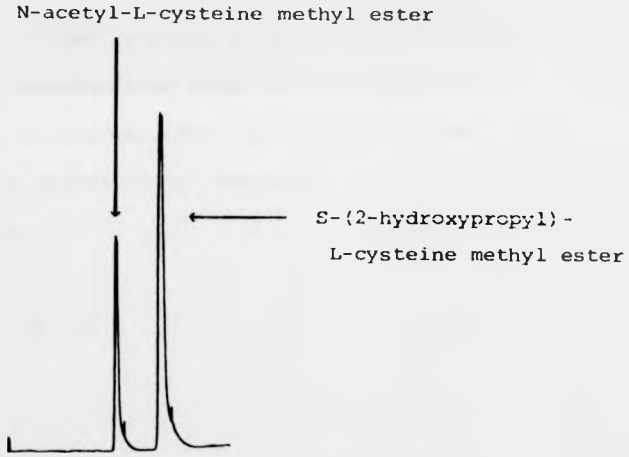


Fig. 5.C.1 H.p.l.c. trace of the reaction between N-acetyl-L-cysteine methyl ester and (R)- or (S)-methyloxirane

of ester (88) with racemic methyloxirane were unresolved with the system used.

The reaction of methyloxirane with the thiol (or thiolate anion) of ester (88) obeys a second order rate process, being overall first order in both epoxide and ester (V.1).

$$-d[RSH]/dt = k[RSH].[oxirane] \qquad V.1$$

In reactions where a large excess of one reactant is present the change in concentration of this reactant is negligible, and for simplicity may be omitted from the rate equation. With a large excess of methyloxirane (> 10 mol. equiv.) the rate equation (V.1) simiplifies to:

$$-d[RSH]/dt = k'[RSH] \qquad V.2$$

where $k' = k[\text{oxirane}]$. This is known as a pseudo-first order rate process and k' is the pseudo-first order rate constant.

If at time zero we express $[RSH]$ as 'a' and at time 't' by (a-x), where x is the amount of 'a' reacted at time 't', then equation (V.2) becomes:

$$-dx/dt = k'(a - x) \qquad V.3$$

rearranging

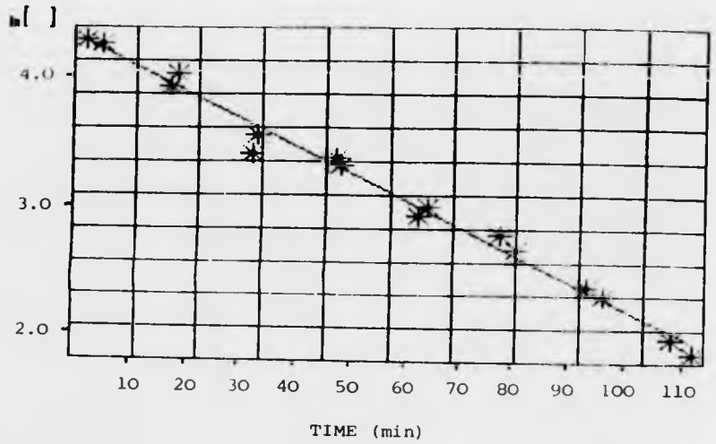
$$-dx/(a - x) = k'dt \qquad V.4$$

integrating

$$-\ln(a - x) + \ln a = k't \qquad V.5$$

and consolidating

$$-\ln[A] = k't \qquad V.6$$



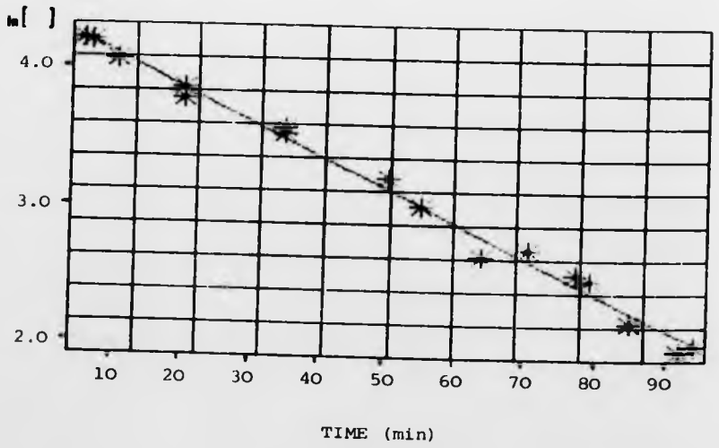
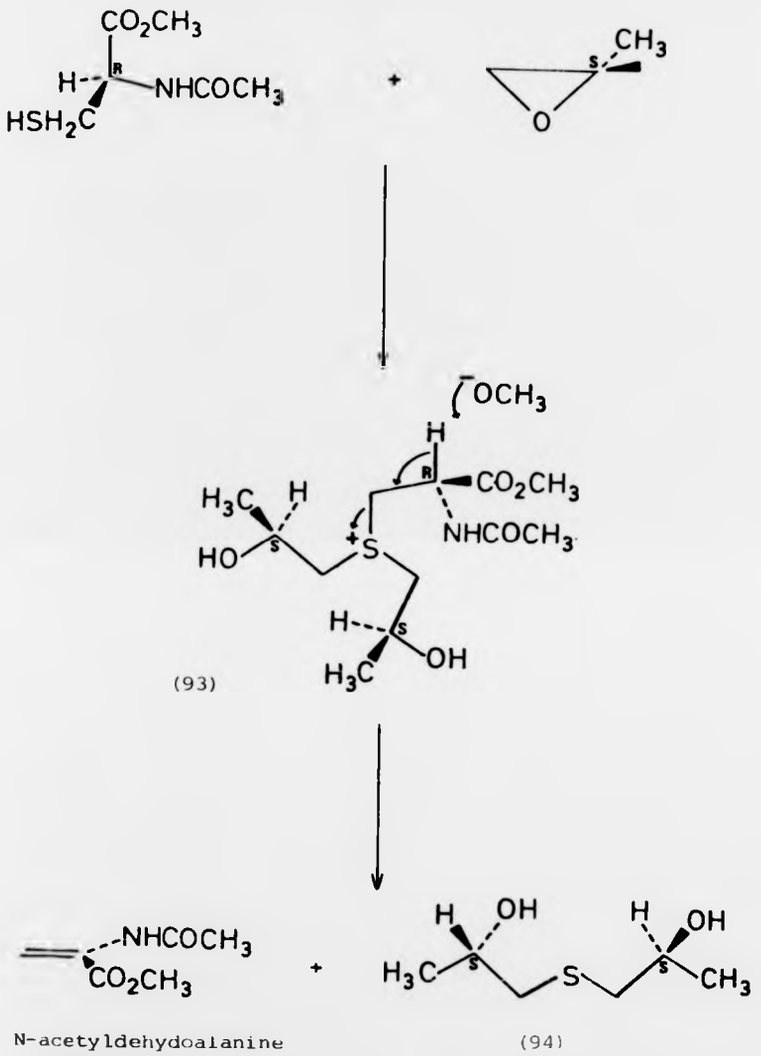


Fig. 5.C.2 The pseudo-first-order rate plots for the reaction of N-acetyl-L-cysteine methyl ester with:
A (R)-methyloxirane
B (S)-methyloxirane

Therefore a plot of In [A] against time gives a straight line from which the slope enables the calculation of k'^{21}.

With a 10 fold excess of (R)- or (S)-methyloxirane, good first-order plots for the disappearance of ester (88) were obtained, from which $k_R = 3.52 \pm 0.10 \times 10^{-4}\ s^{-1}$, $k_S = 4.12 \pm 0.10 \times 10^{-4}\ s^{-1}$ and $k_R/k_S = 0.85 \pm 0.05$ for triethylamine-catalysed reactions in methanol [Fig. 5.C.2.]. This result was confirmed by performing a competitive reaction between ester (88) and an excess of (rac.)-methyloxirane in methanol containing triethylamine. Analysis by 400 MHz ^1H n.m.r. spectroscopy indicated approximately equal amounts of the diastereoisomers (91) and (92) by integration of the diastereotopic thiomethylene protons. The effect of varying the concentration of triethylamine was determined for reactions of ester (88) and an excess of each methyloxirane. It was found that without triethylamine the extent of reaction during 4h/0°C was negligible whereas reactions containing 10 mol. equiv. triethylamine were complete within ca 90 min under identical conditions. Both results were judged by ^1H n.m.r. spectroscopy and h.p.l.c..

Evidence for the formation of a sulphonium species (93) was obtained from reactions lacking triethylamine that were allowed to proceed for extended periods (2 days/45°C). Thus monitoring such a reaction in [$^2H_4$]-methanol by ^1H n.m.r. spectroscopy showed new resonances at δ 5.89 and 6.59 ppm which were assigned to the formation of N-acetyldehydroalanine methyl ester. A mechanism of formation of this substance via an intermediate sulphonium species (93) is shown in Scheme 5.C.1. This was supported by ^1H n.m.r. spectroscopic

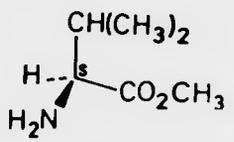


Scheme 5.C.1 Formation of N-acetyldehydroalanine methyl ester

evidence for the formation of (S,S)-bis(2-hydroxypropyl)sulphide (94) from a reaction between ester (88) and (S)-methyloxirane. During the formation of the sulphonium species base is generated and is observed to induce an ester exchange (observed by the disappearance of the OMe resonance at δ 3.85 ppm between 30 and 42h) and initiate the β-elimination shown in Scheme 5.C.1. An interesting consequence of the formation of a sulphonium species, followed by its β-elimination leading to dehydroalanine is that this process would cause 'sulphur stripping' from the cysteine group(s) of a protein. This has been proposed as a possible consequence of reacting certain bifunctional alkylating agents e.g. (chloromethyl)oxirane with cysteine containing proteins[22].

Conversion of ester (88) into its thiolate anion by addition of a stoichiometric quantity of triethylamine increases the rate of reaction with methyloxirane by at least 100-fold. It was found that, for ester (88) $\left[\right.$ thiolate $\left.\right]$ the products of mono-alkylation with methyloxirane, are further alkylated much slower than their rate of formation. This is because further alkylation takes place on the dialkyl sulphide, e.g. (91) and (92), whereas the initial alkylation involves the thiolate of (88).

5.D REACTIONS BETWEEN (S)-VALINE METHYL ESTER AND THE ENANTIOMERS OF METHYLOXIRANE



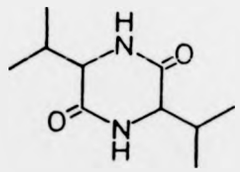
(89)

The reaction between an alkyl-substituted epoxide and a primary amine is well-known to occur with high regioselectivity at the methylene carbon of the epoxide and to give a mono-(2-hydroxyalkyl)amine in high yield[23]. This reaction is favoured by protic solvents compared to aprotic solvents and the much greater rates of reaction are attributed to the ability of the protic solvent to hydrogen bond to the epoxide oxygen, thereby electrophilically assisting ring fission [Fig. 5.D.1.].

(S)-valine methyl ester (89) was liberated from its hydrochloride salt by treatment with 1 mol. equiv. base. Preliminary reactions were performed using triethylamine, *in situ*, but this made monitoring the reaction with epoxide difficult (t.l.c., ^{1}H n.m.r. spectroscopy) due to the presence in solution of triethylamine hydrochloride unreacted triethylamine and (S)-valine methyl ester hydrochloride. This problem was overcome by

generating the amino ester with 1 mol. equiv. sodium methoxide in methanol prior to the reaction with epoxide.

A possible complication with amino-acid esters arises from their dimerisation to give diketopiperazines, e.g. (95). The extent to which diketopiperazine formation occurs was assessed by dissolving (S)-valine methyl ester (25 mg, 1.9×10^{-4} mol) in $[^2H_4]$-methanol (0.5 cm^3) and heating the solution to 45°C. Monitoring the reaction by ^1H n.m.r. spectroscopy showed only ester exchange to have occurred (loss at OMe resonance at δ 3.67 ppm) over 72h and a similar reaction in $[^2H_2]$-water gave a parallel result, yielding only the free amino-acid.



(95)

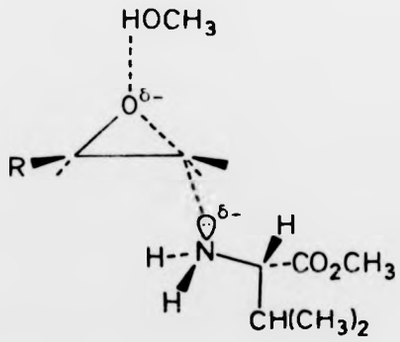
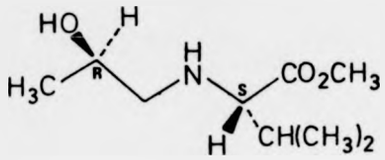
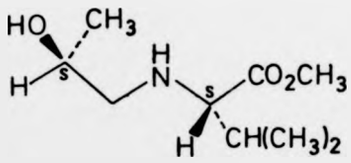


Fig. 5.D.1

The reaction between (S)-valine methyl ester and (R)- and (S)-methyloxirane, respectively, proceeded smoothly in methanol at 45°C to give products of mono-alkylation: (R,α S)-N-(2-hydroxypropyl)valine methyl ester (96) from (R)- and (S,α S)-N-(2-hydroxypropyl)valine methyl ester (97) from (S)-methyloxirane,

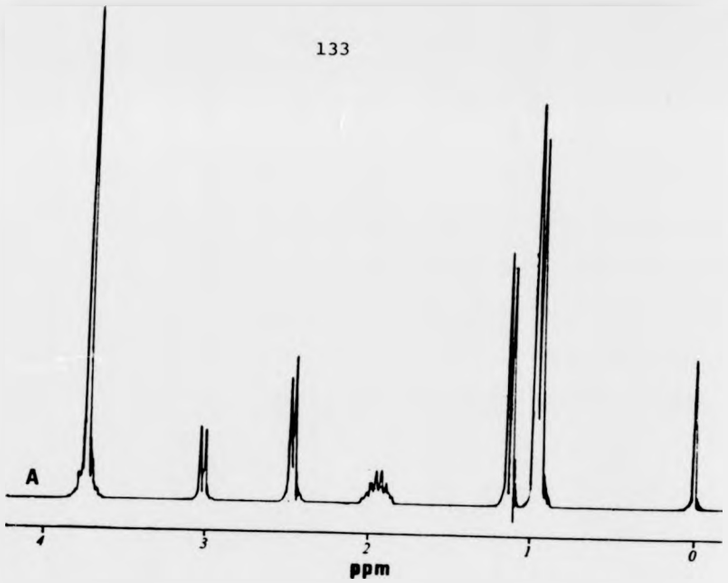


(96)



(97)

respectively. Monitoring these reactions (in $[^2H_4]$ methanol) by ^1H n.m.r. spectroscopy showed that further alkylation of both (96) and (97) was much slower than their rates of formation. This is probably due to steric reasons as it is known that the rate of alkylation of amines is highly susceptible to the size of the alkyl groups present in the amine[24]. The diastereoisomers (96) and (97) were characterised by their n.m.r. spectroscopic properties



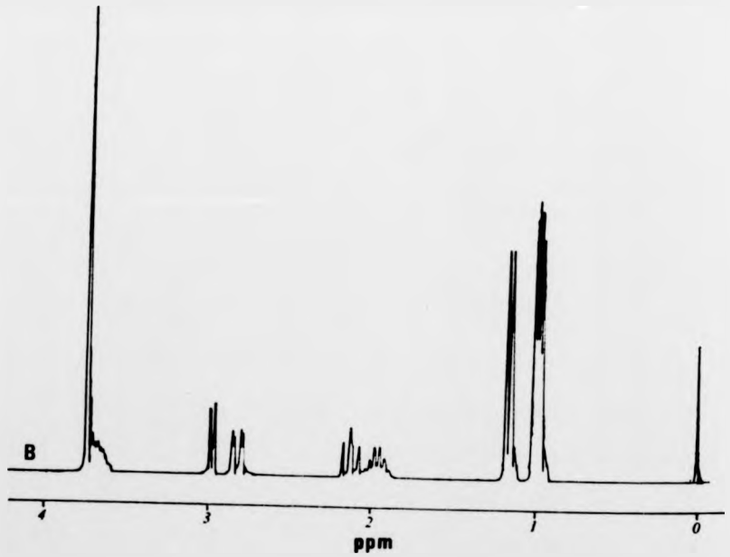
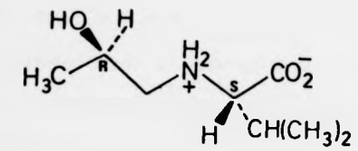
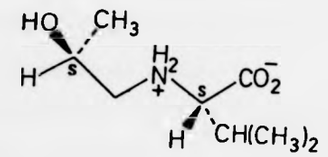


Fig. 5.D.2 220 MHz ^{1}H n.m.r. spectra
A (R,αS)-N-(2-hydroxypropyl)-L-valine methyl ester
B (S,αS)-N-(2-hydroxypropyl)-L-valine methyl ester



(98)

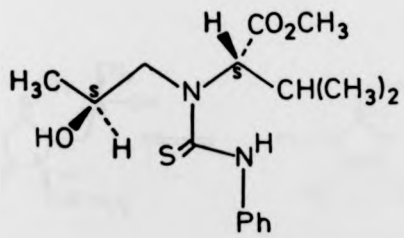


(99)

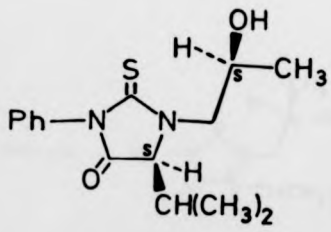
(^{1}H and ^{13}C) and by electron impact mass spectrometry. Their ^{1}H n.m.r. spectra have distinctive properties and were particularly diagnostic for the diastereotopic methylene protons [Fig. 5.D.2.]. For the (S,αS)-isomer these protons appear as double doublets (δ 2.11 and 2.83 ppm), whereas for the (R,αS)-isomer these protons were indistinguishable and appeared as a doublet (δ2.46 ppm). Hydrolysis of (96) and (97) in distilled water (4 days, RT) gave the corresponding N-(2-hydroxypropyl)valines (98) and (99) which

were obtained as pure crystalline materials and were fully characterised. The ^1H n.m.r. spectra of esters (96) and (97), respectively, proved that attack on methyloxirane by (S)-valine methyl ester occurs with high regioselectivity at the oxirane CH_2. This was also supported by h.p.l.c. analysis of the esters (96) and (97) which gave only a single peak. No evidence was obtained for the formation of isomers of (96) and (97) containing a N-(2-hydroxy-1-methylene) group.

A valuable adjunct to the characterisation of esters (96) and (97) was discovered when it was attempted to convert them into N-phenylaminothiocarbonyl (P.A.T.C.)derivatives. Treatment of ester (97) with isothiocyanatobenzene[24] in ethanol gave on cooling a crystalline derivative, whose spectral properties were consistent with the formation of thiohydantoin (101). The thiohydantoin presumably arises by intramolecular cyclisation of the initially formed P.A.T.C. derivative (100) by a type of reaction analogous to Edman degradation. An experiment monitored by ^1H n.m.r.

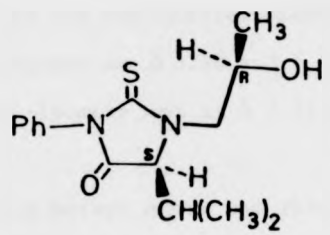


(100)



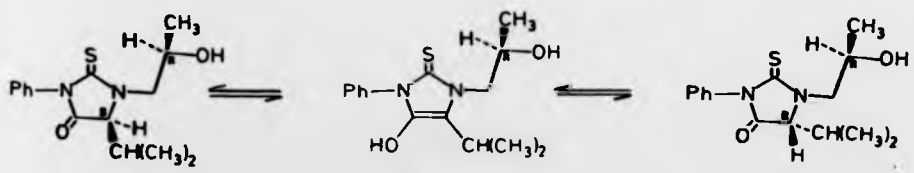
(101)

spectroscopy in which ester (96) was reacted with isothiocyanatobenzene (3 mol. equiv.) in $CDCl_3$ at RT gave results consistent with the formation of thiohydantoin (102). No resonances for the intermediacy of a P.A.T.C. derivative were observed.



(102)

Thiohydantoin (102) was an oil and on storage (ethanol solution, 6 months, 4°C) or when heated to 80°C was observed to epimerise at C-5 to the diastereoisomeric product (103). This epimerisation is illustrated in Scheme 5.D.1.

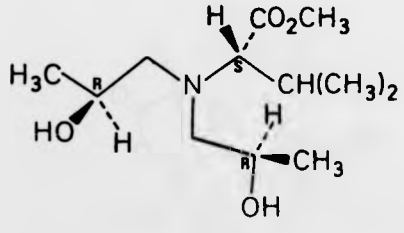


(102) (103)

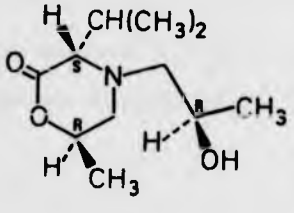
Fig. 5.D.1

Thiohydantoins (101) and (103) are enantiomers and therefore cannot be discriminated by n.m.r. spectroscopy (^{1}H and ^{13}C) whereas thiohydantoin (102) is diastereoisomeric to both (101) and (103). The diastereotopic methylene protons of (101) and (103) are particularly diagnostic for the two diastereoisomers. These protons appear as double doublets at δ 3.38 and 4.57 ppm for the (R,αR)- and (S,αS)-isomers and at δ 3.32 and 4.26 ppm for the (R,αS)-isomer.

When (S)-valine methyl ester was allowed to react with (R)-methyloxirane (10 mol. equiv.) in methanol at 45°C for 3 weeks, a mixture of two products of dialkylation were obtained. The spectral properties of the mixture showed these substances to be (R,R,αS)-N,N-di(2-hydroxypropyl)-valine methyl ester (104) [ca. 1 part] and (R,R,αS)-N,N-di(2-hydroxypropyl)-valine δ-lactone (105) [ca. 3 parts].

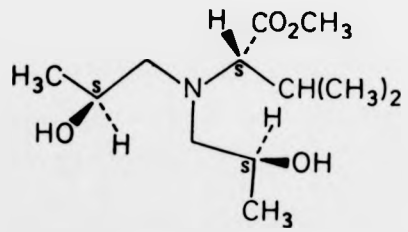


(104)

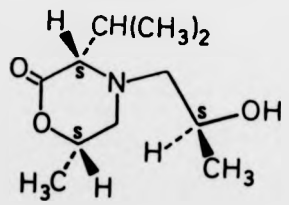


(105)

These compounds were separated by preparative layer chromatography. The morpholone (105) was obtained in pure form, whereas the ester (104) could not be obtained completely pure because of its ready conversion into the morpholone. In an analogous reaction (S)-methyloxirane was observed to react with (S)-valine methyl ester to give (S,S,αS)-N,N-di-(2-hydroxypropyl)-valine methyl ester (107) and morpholone (108). For esters (96) and (97) the rate of dialkylation with methyloxirane is much slower than their rate of formation.

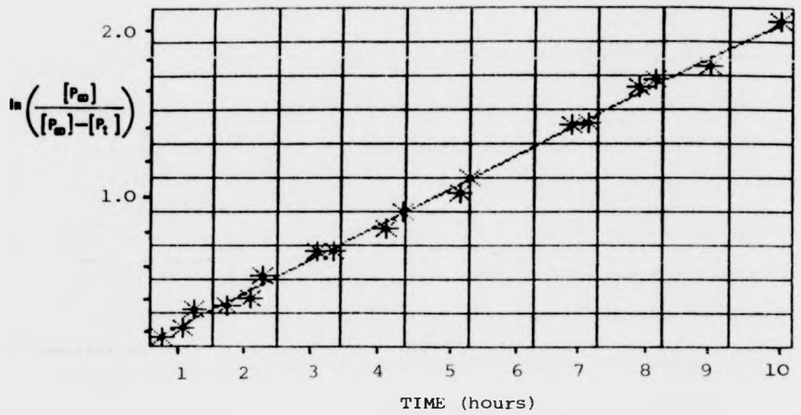


(107)



(108)

N-(2-hydroxyethyl)-amino acid esters have been prepared by reacting amino-acid esters with oxirane[25]. These compounds were shown to react further with oxirane in methanol to give N-(2-hydroxyethyl)-morpholo-2-ones[25].



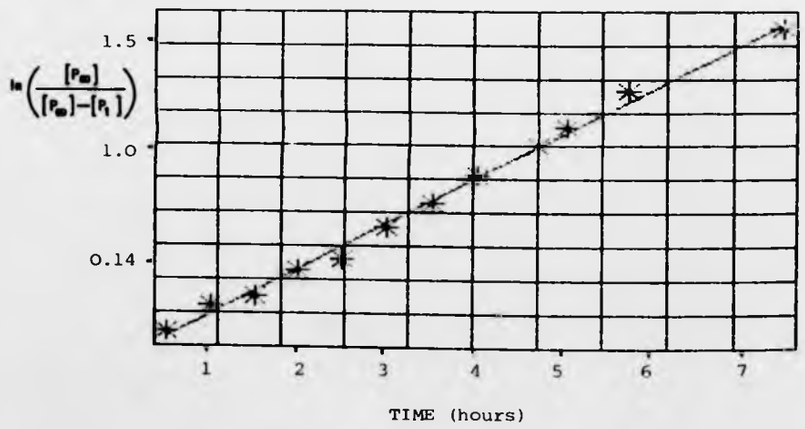


Fig. 5.E.1 The pseudo-first order rate plots for the reaction of L-valine methyl ester with:
A (R)-methyloxirane
B (S)-methyloxirane

5.E DETERMINATION OF THE RELATIVE RATES OF REACTION OF (R)- AND (S)-METHYLOXIRANE WITH (S)-VALINE METHYL ESTER

The reaction of methyloxirane with the primary amine of ester (89) follows a second order rate process being first order in both oxirane and amine. In a reaction where a large excess of one reactant is used the reaction simplifies and can be judged to follow a first order rate process (see Section 5.C for analysis)[21].

The relative rates of reaction of (S)-valine methyl ester with (R)- or (S)-methyloxirane were determined by monitoring the reaction between an excess of each enantiomer of the epoxide with ester (89) in $[^2H_4]$-methanol at 45°C ± 0.1°C by 220 MHz ^1H n.m.r. spectroscopy. The formation of the (R,αS)-diastereoisomer (96) was followed by the appearance of the methyl doublet at δ 1.14 ppm. Similarly, for the (S,αS)-isomer (97) the appearance of the methyl doublet at δ 1.14 ppm was used. Good first order kinetic plots were obtained by plotting In[conc'] against time, from which k_R = 6.40 ± 0.10 x 10^{-5} s^{-1} and k_S = 6.68 ± 0.12 x 10^{-5} s^{-1} were obtained [Fig. 5.E.1.]. Hence k_R/k_S = 0.96 ± 0.03.

Although these reactions with methyloxirane occur at a nucleophilic centre adjacent to a chiral centre, this is insufficient to give rise to a significant degree of enantioselectivity. This conclusion was confirmed by carrying out a reaction between (S)-valine methyl ester and an excess of (rac.)-methyloxirane in $[^2H_4]$-methanol. Analysis of the product mixture by 400 MHz ^1H n.m.r. spectroscopy showed the formation at approximately equal amounts of esters (96) and (97).

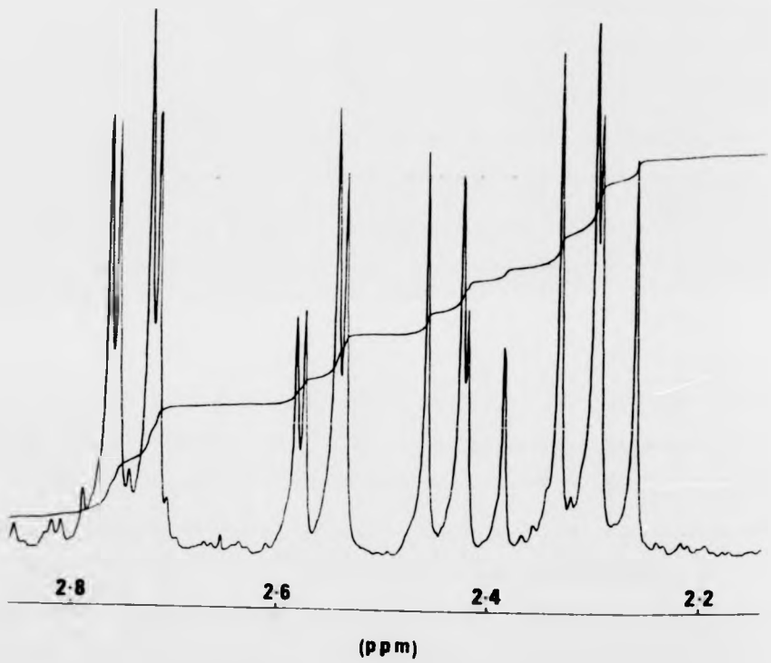
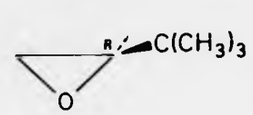


Fig. 5.E.2 300 MHz ^{1}H n.m.r. spectrum of the diastereoisomeric methylene protons of (S,αS)- and (R,αS)-N-(2-hydroxy-3,3-dimethylbutyl)-valine methyl ester

To test whether an enantioselectivity could be observed for N-alkylation a reaction was performed between (rac.)-t-butyloxirane (109 and 110) and (S)-valine methyl ester. Our strategy was to use the existing data already gathered to assess any selectivity by ^{1}H n.m.r. spectroscopy. (S)-valine methyl ester

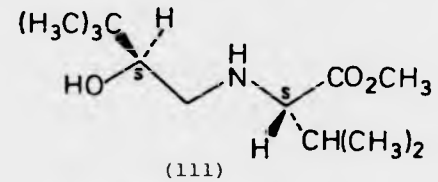


(109)

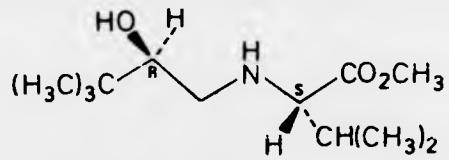
(110)

was reacted with t-butyloxirane (10 mol. equiv.) in $[^{2}H_4]$-methanol (0.5 cm^3) at 45°C and the reaction followed by 300 MHz ^{1}H n.m.r. spectroscopy. After 48h the reaction was assessed to have gone to ca. 20% completion. Volatiles were removed and ^{1}H n.m.r. spectroscopic analysis of the resulting oil gave clearly resolved double doublets for the diastereotopic methylene protons of the products (111) and (112) at δ 2.29, 2.42, 2.55 and 2.74 ppm [Fig. 5.E.2]. By comparison of coupling constants the resonance at δ 2.29 ppm (J = 10.06 and 11.55 Hz) was paired with that at δ 2.74 ppm (J = 2.35 and 11.55 Hz) and the resonance at δ 2.42 ppm (J = 10.00 and 11.73 Hz) with that at δ 2.55 ppm (J = 2.50 and 11.73 Hz). If a direct comparison of these resonances can be made with those for (S,αS)-N-(2-hydroxypropyl)valine methyl ester (δ 2.11 and 2.83 ppm, diastereotopic CH_2) and (R,αS)-N-(2-hydroxypropyl)valine methyl

ester (δ 2.46 ppm, diastereotopic CH_2) then the resonances at δ 2.29 and 2.74 ppm would belong to (S,α S)-N-(2-hydroxy-3,3-dimethylbutyl)valine methyl ester (111) and those at δ 2.42 and 2.55 ppm to (R,αS)-N-(2-hydroxy-3,3-dimethylbutyl)valine methyl ester (112). Integration indicated esters (111) and (112) to be



(111)



(112)

formed in a ratio of-3:2, respectively. A possible transition state for the reaction of (R)-t-butyloxirane and (S)-valine methyl ester

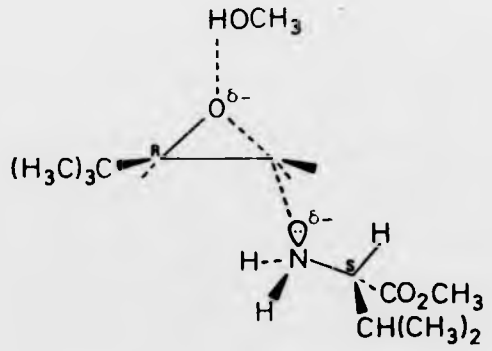


Fig. 5.E.3

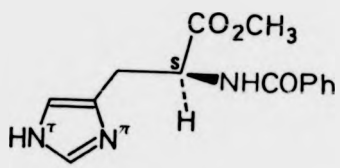
is shown in Fig. 5.E.3. This illustrates that there is an enhanced steric interaction between the iso-propyl group of ester (89) and the t-butyl group of the (R)-epoxide compared to the identical transition state for the reaction with the (S)-epoxide. This is in agreement with our observed result. No products from attack at the epoxide methine carbon were observed.

This study shows that by use of our model compounds, extrapolated information from one set of results can be used to predict the outcome of similar reactions. From the spectral data obtained for esters (96) and (97) it should be possible to analyse, correctly, the enantioselectivity of nucleophilic attack by valine methyl ester (and related compounds) on epoxide racemates.

5.F REACTIONS BETWEEN (S)-N$^{\alpha}$-BENZOYLHISTIDINE METHYL ESTER AND THE ENANTIOMERS OF METHYLOXIRANE

Campbell[26] has recently synthesised (S)-N(τ)-(2-hydroxypropyl)histidine as a mixture of diastereoisomers by alkylation of the silver salt of (S)-N(α)-benzyloxycarbonylhistidine methyl ester with bromoacetone to give (S)-N(α)-benzyloxycarbonyl-(2-oxopropyl)-histidine methyl ester which was reduced and hydrolysed. He required N(τ)-(2-hydroxypropyl)histidines as standards for analysis by g.l.c. of the products of alkylation of haemoglobin by methyloxirane. It was assumed that N(τ)-alkylation of haemoglobin by nucleophilic attack of the histidine N(τ)-imidazole nitrogen would greatly predominate (but see results below).

We have studied the alkylation of (S)-N(α)-benzoylhistidine methyl ester (90) by (R)- and (S)-methyloxirane and have isolated discrete diastereoisomeric products.



(90)

Dissolution of ester (90) in [$^2H_4$]methanol and observation by ^1H n.m.r. spectroscopy showed that over 96h at 45°C, both trans-esterification and exchange of the histidine imidazole C-2 proton had occurred. This result was confirmed by analysis of the reaction by e.i.m.s. which gave m/z values of 274 (imidazole C-2 exchange), 276 (ester exchange) and 277 (imidazole C-2 and ester exchange). Reactions conducted in propan-2-ol were also complicated by the conversion of methyl ester into prop-2-yl ester. From the reaction of (R)-methyloxirane (10 mol. equiv.) with ester (90) in methanol at 45°C it was possible, by 220 MHz ^1H n.m.r. spectroscopy, to observe the region between δ 6.7 - 7.5 ppm and showed the formation of at least three products (new signals for histidine C-5 protons at δ 6.7, 6.93 and 7,34 ppm) [Fig. 5.F.1.]. Analysis of the reaction

mixture by t.l.c. (silica gel F_{254}, elution with chloroform:methanol, 4:1) showed three new spots at Rf 0.04, 0.52 and 0.66. From a reaction allowed to proceed for 10h at 45°C, the products having Rf 0.66 and 0.52 were separated chromatographically and their structures were assigned as the monoalkylated histidines (113) and (114), respectively[27]. It was assumed that (113) is the product of alkylation at the less hindered N(τ) of ester (90), because it predominated over (114) derived from alkylation at N(π) [ratio of

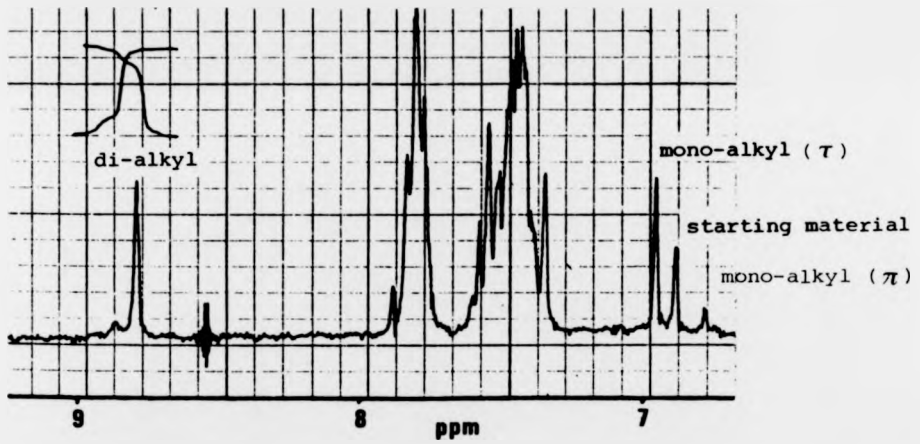
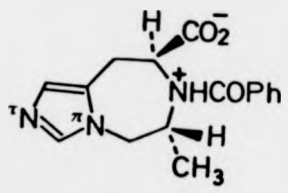
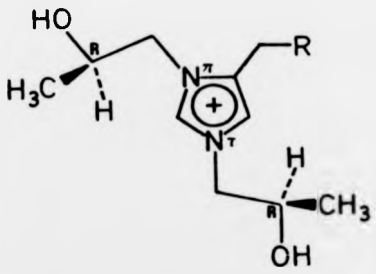


Fig. 5.F.1

(113):(114)= ca. 2:1]. Both (113) and (114) showed characteristic differences in their ^1H n.m.r. spectra. For the N(τ)-isomer the position of the C-5 proton peak (δ 6.93 ppm) exhibits a downfield shift of 0.2 ppm relative to the resonance for the N(π)-isomer (δ 6.7 ppm). Monoalkylated derivatives (113) and (114) were clearly distinguishable by differences in the e.i. mass spectra. Both derivatives gave m/z 331 [M^+] and identical fragment ions, but, (114) showed an additional peak at m/z 299. This peak is attributed to the formation of the bicyclic fragment (115) which can only form



(115)



(118) R=H

(119) R= CH=NH

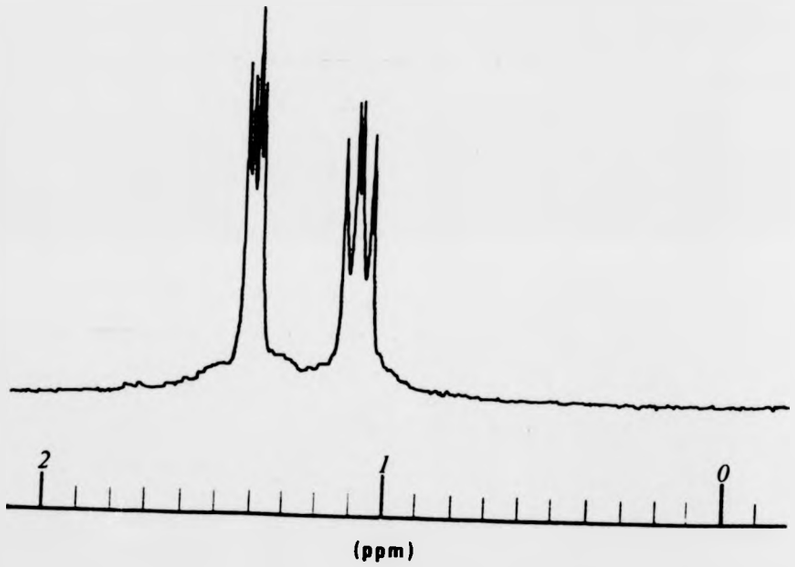
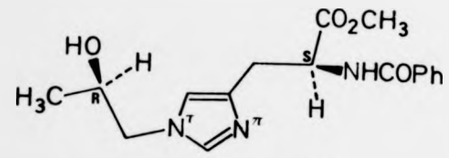
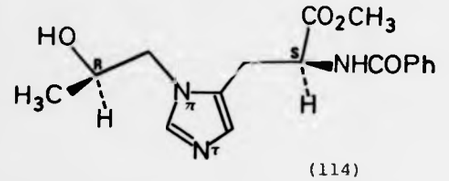


Fig. 5.F.2 360 MHz ^{1}H n.m.r. of the diastereoisomeric histidinylimidazolium carboxylate methyl resonances.



(113)



(114)

from (114)[28]. Examination of molecular models and considerations of the steric factors involved indicate that alkylation at the N(π) position should proceed slower than at the less hindered N(τ) site. This is in agreement with our observations and there is literature precedence giving credence to this assumption[29].

From the reaction of ester (90) with (R)-methyloxirane in methanol which was allowed to proceed for ca. 42h at 45°C it was observed by t.l.c. that the spots at Rf 0.66 and 0.52 had diminished in intensity whilst the spot at Rf 0.04 had increased in intensity. Evaporation of the reaction mixture and recrystallisation of the resulting oil gave a sharp melting point substance whose ^{1}H n.m.r. spectrum indicated it to be a mixture of the diastereoisomeric histidine imidazolium carboxylates (116) and (117) [Fig. 5.F.2.]. The rate of further reaction of the monoalkylated derivatives (113) and (114) with methyloxirane to give imidazolium carboxylates [(116) + (117)] is comparable with the rate of formation of these adducts from ester (90). [See Section 5.G.].

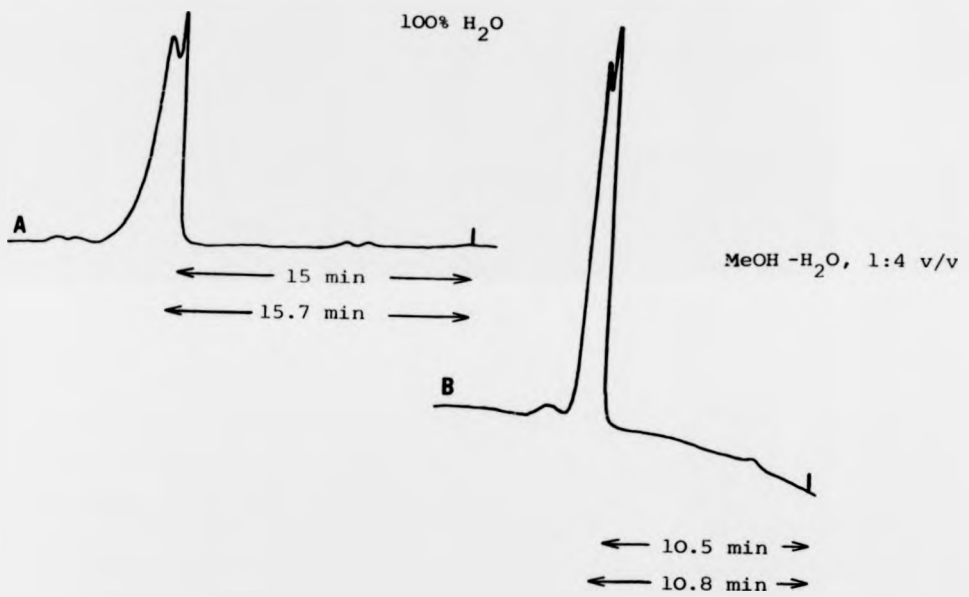
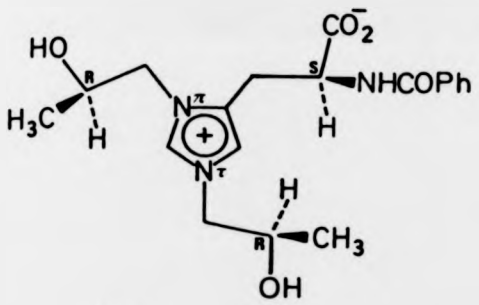
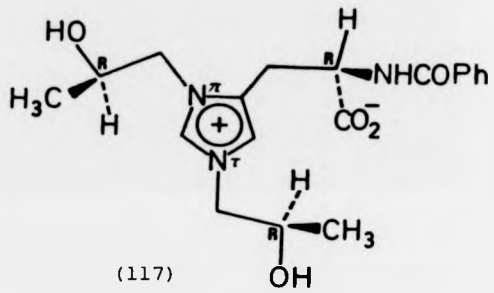


Fig. 5.F.3 Partial separation of the diastereoisomeric mixture of histidinylimidazolium carboxylates by h.p.l.c., stationary phase N-3,5-Dinitrobenzoyl-D-phenylalanine bonded to a 5μ -aminopropylsilica packing

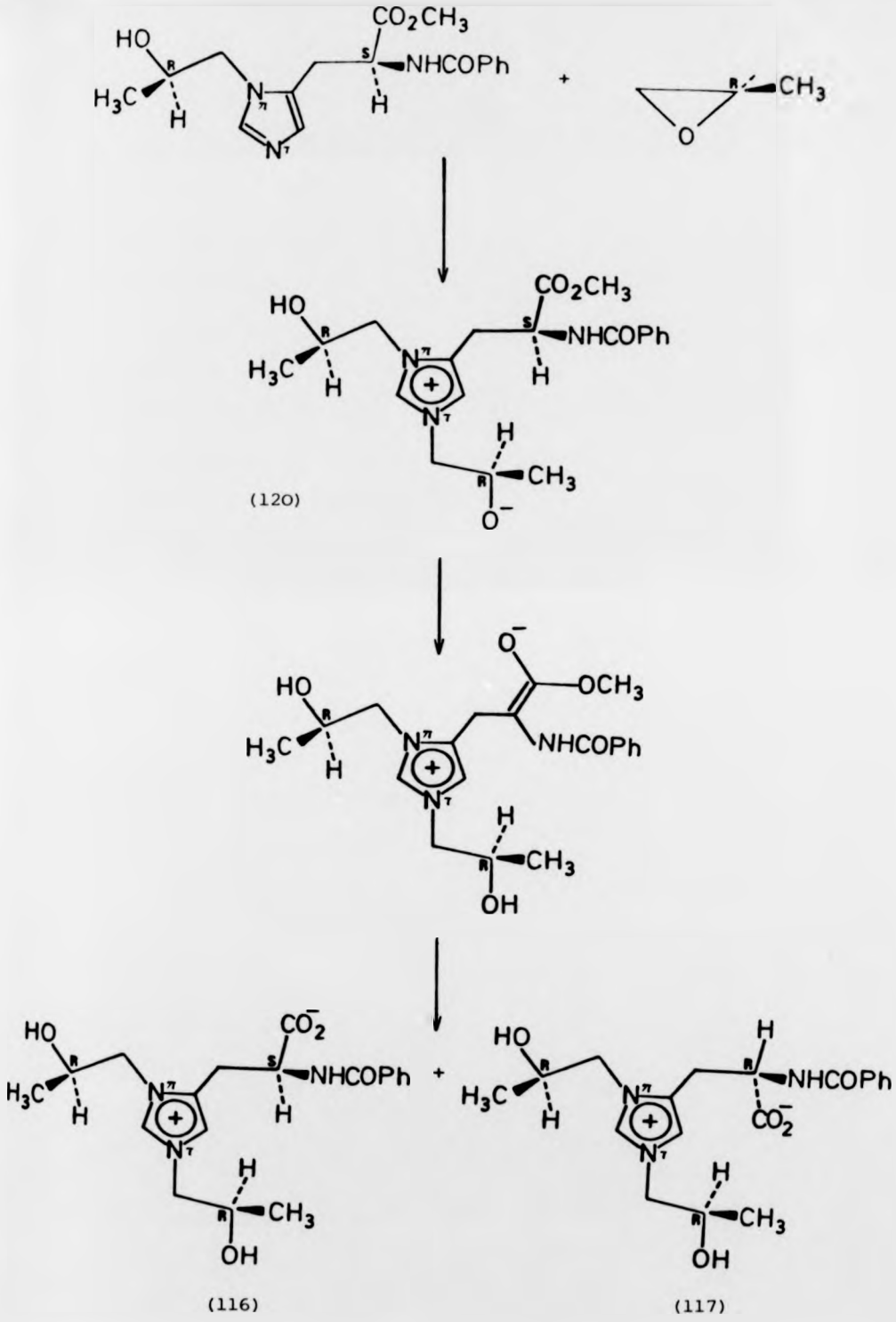


(116)



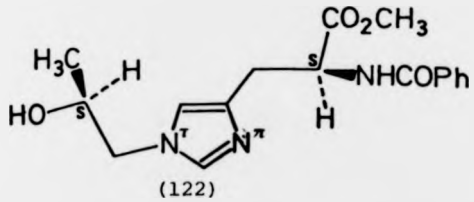
(117)

Attempts to separate the two carboxylates (116) and (117) by h.p.l.c. using a reverse phase, normal phase or cation exchange column was unsuccessful. However, partial resolution of the carboxylates was obtained using a chiral h.p.l.c. column containing (R)-N(α)-3,5-dinitrobenzoylphenylglycine bonded to a 5μ-amino-propyl silica stationary phase [Fig. 5.F.3]. The fast atom bombardment spectrum of the mixture, in the positive ion mode, confirmed the molecular weight as 375 and fragment ions at m/z 199 and 226 can be assigned to the ions (118) and (119), respectively. The mechanism of formation

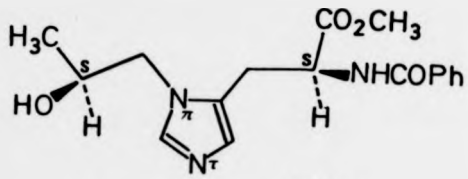


Scheme 5.F.1

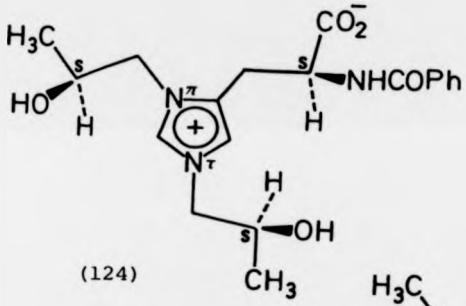
of (116) and (117) is illustrated in Scheme 5.F.1. Formation of the dialkylated intermediate (120) by alkylation of esters (113) and (114) will produce an alkoxide . This alkoxide could then abstract the α-proton intramolecularly, or more favourably, generate methoxide [by solvent quenching of (120)] which would abstract the α-proton by an intermolecular process causing epimerisation of the α-CH. The carboxylate group of (116) and (117) could arise either by methoxide-induced alkyl-oxygen fission of the methyl ester of (120) or by the adventitious presence of sufficient hydroxide in the reaction mixture. The analogous dialkylation of a histidine residue of a protein by methyloxirane would generate hydroxide, which would probably be neutralised by deprotonation of an acidic group (e.g. aspartate or glutamate) of the protein. A comparable set of results was obtained from studies of the reaction between ester (90) and (S)-methyloxirane. The monoalkylated histidines (122) and (123), corresponding to (113) and (114) were obtained, as well as a crystalline mixture of dialkylated histidine imidazolium carboxylates (124) and (125) This mixture contains the enantiomers of the components of the mixture obtained from reacting ester (90) with (R)-methyloxirane. This was confirmed by analysis of their respective optical rotations. The diastereoisomeric mixture (116) and (117) gave $[\alpha]_D^{15}$ - 27.7° whilst the mixture obtained from the alkylation



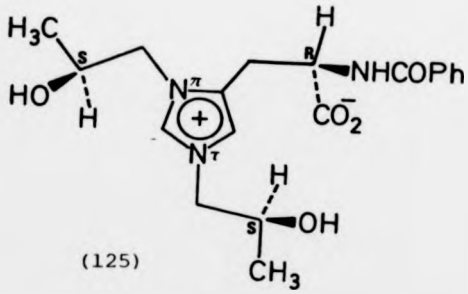
(122)



(123)



(124)



(125)

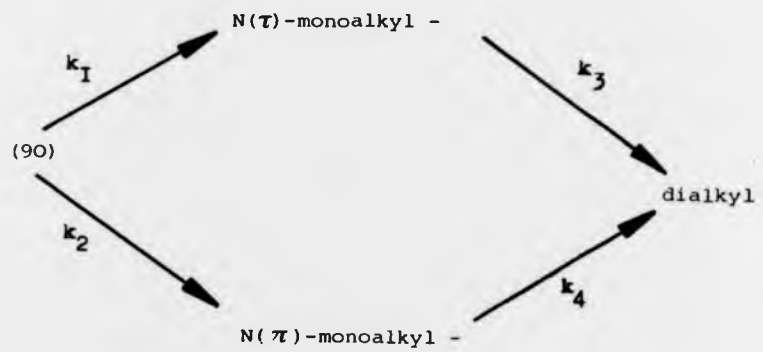
of ester (90) with (S)-methyloxirane, (124) and (125) showed an almost equal but opposite rotation $[\alpha]_D^{17}$ + 30.8°. The stereochemical designations of compounds (116), (117), (124) and (125) are (R,R,αS), (R,R, αR), (S,S, αS) and (S,S, αR), respectively. Thus, the mixture [(116) + (117)] contains the enantiomers of the mixture [(124) + (125)]. Following a reaction between ester (90) and (R)-methyloxirane in phosphate-citrate buffer, pH 7.2, (13.05 cm^3 0.1 M citric acid [21 g/l] and 86.95 cm^3 0.2 M disodium phosphate, Na_2HPO_4-$2H_2O$ [36.5 g/l]) by ^1H n.m.r.

spectroscopy showed that epoxide hydrolysis by the buffer is a stongly competing factor. An analogous reaction containing only epoxide showed ca. 60% hydrolysis after 18h at 45°C.

Jones and Hysert[29] studied the reactions between ester (90) and alkyl halides (e.g. allyl bromide, propagryl bromide) and obtained products analogous to those reported here. They did not report optical rotations for their histidine imidazolium carboxylates and so their assignment of L-configuration to these products must be regarded as suspect.

5.G DETERMINATION OF THE RELATIVE RATES OF REACTION OF (R)- AND (S)-METHYLOXIRANE WITH (S)-N$^{\alpha}$-BENZOYLHISTIDINE METHYL ESTER

The kinetics of formation of compounds (122) - (125), (113) and (114), (116) and (117) were determined by monitoring reactions between ester (90) and an excess of (R)- or (S)-methyloxirane in methanol at 45°C ± 0.1°C by ^{1}H n.m.r. spectroscopy. A plot of concentration against time obtained from the integration of the C-5 proton singlets between δ 6.7 - 7.5 ppm and the imidazolium carboxylate C-3 proton singlet at δ 8.77 ppm is shown in Fig. 5.F.1. Analysis of the results according to Scheme 5.G.1 shows the reaction to consist of two consecutive, parallel pseudo-first order processes[30].



Scheme 5.G.1

which gave for (R)-methyloxirane:

$$k_1 = 1.46 \pm 0.04 \times 10^{-5}\ s^{-1}$$

$$k_2 = 7.17 \pm 0.46 \times 10^{-6}\ s^{-1}$$

$$k_3 = 1.05 \pm 0.06 \times 10^{-5}\ s^{-1}$$

$$k_4 = 4.95 \pm 0.54 \times 10^{-5}\ s^{-1}$$

and for (S)-methyloxirane:

$$k_1 = 1.41 \pm 0.04 \times 10^{-5}\ s^{-1}$$

$$k_2 = 6.93 \pm 0.46 \times 10^{-6}\ s^{-1}$$

$$k_3 = 1.00 \pm 0.07 \times 10^{-5}\ s^{-1}$$

$$k_4 = 4.77 \pm 0.56 \times 10^{-5}\ s^{-1}$$

by computer simulation of Fig. 5.G.1 [Fig.5.G.2]. We thank Dr. J. Sachinidis for his assistance with this analysis.

Comparison of these results show that the alkylation of each imidazole nitrogen shows no detectable enantioselectivity towards (R)- or (S)-methyloxirane, as expected for reactions relatively distant from the chiral centre of histidine and that the rate of alkylation at N(τ) is approximately twice that at N(π).

5.H SOME BIOLOGICAL IMPLICATIONS OF THE REACTIONS OF ESTERS (88), (89) AND (90) WITH (R)- AND (S)-METHYLOXIRANE

For the amino-acid derivatives (88), (89) and (90) the relative rates of their reaction with methyloxirane in methanol at 45°C are (89) ~ (90) > (88). Conversion of ester (88) into its thiolate anion by addition of triethylamine (1 mol. equiv.) increases the rate of reaction with methyloxirane by at least 100-fold.

Industrially used chiral epoxides are racemates. When these materials react with a nucleophilic group of a protein on nucleic acid, or undergo an enzyme-catalysed reaction, it is expected that the rates of reaction of the epoxide enantiomers will differ. For a protein or nucleic acid, two diastereoisomeric products of regiospecific mono-alkylation are possible. Epoxides can also be produced metabolically by a cytochrome P_{450} mediated oxidation of unsaturated alkanes. Such an enzyme mediated oxidation is likely to generate one enantiomer of chiral epoxide preferentially[31], which will lead to predominantly one diastereoisomer of mono-

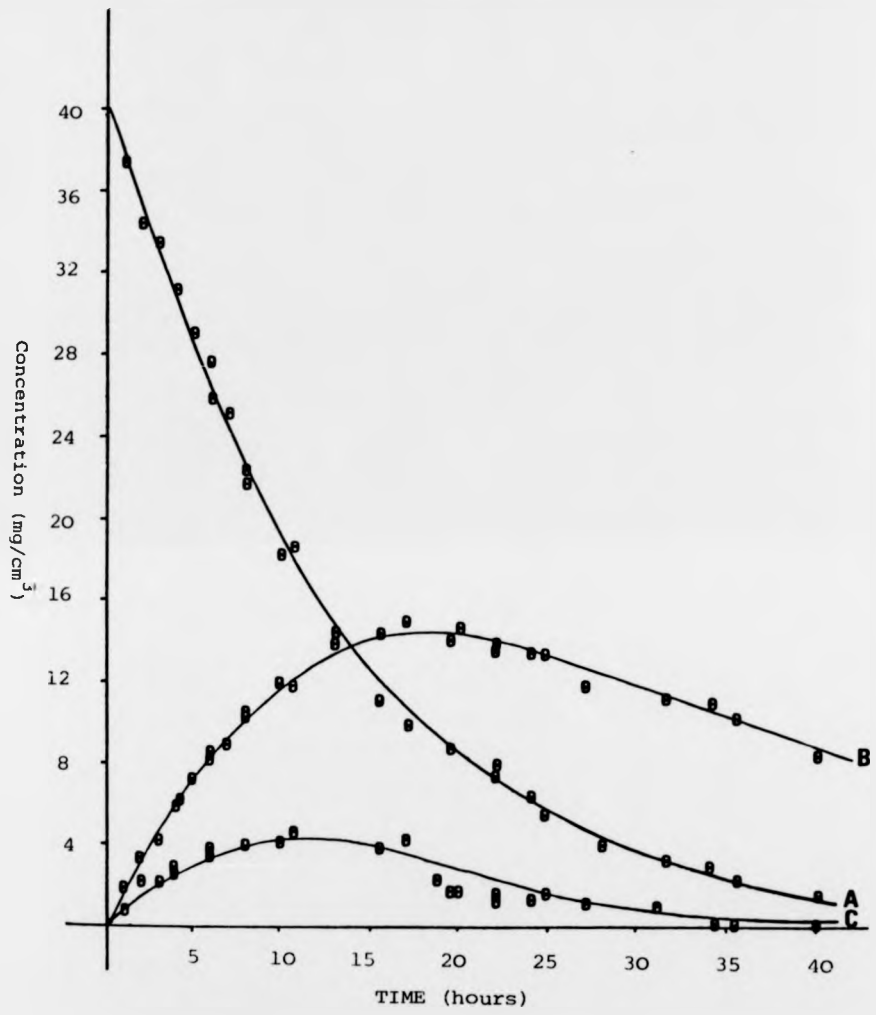


Fig. 5.G.1 Plot of the reaction between N-benzoyl-L-histidine methyl ester with methyloxirane:

A) rate of loss of N-benzoyl-L-histidine methyl ester,

B) rate of formation of (S, αS)-N -benzoyl-N^{τ}-(2-hydroxypropyl)-histidine methyl ester,

C) rate of formation of (R, αS)-N -benzoyl-N^{τ}-(2-hydroxypropyl)-histidine methyl ester

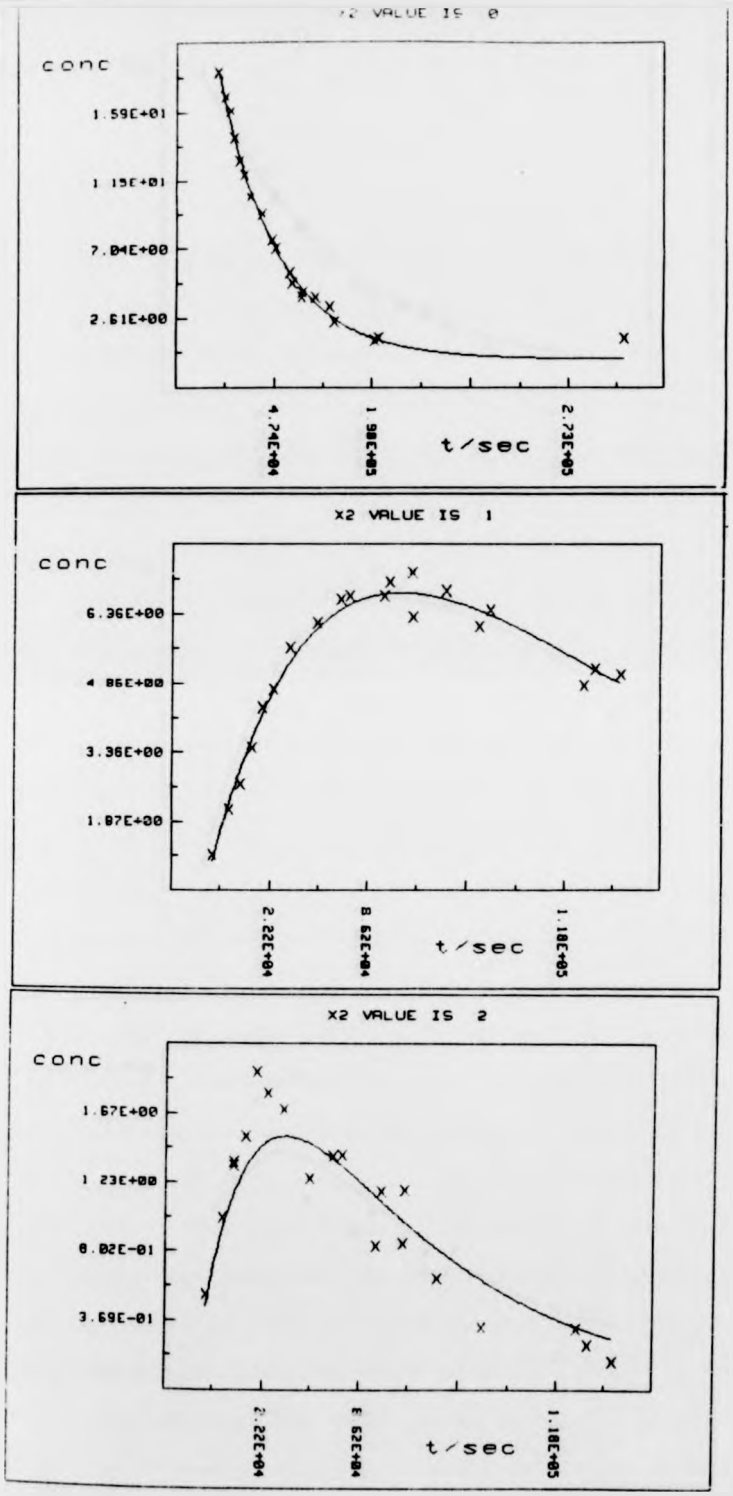


Fig. 5.G.2a Computer simulation of Fig. 5.G.1. The reaction of N-benzoyl-L-histidine methyl ester with (R)-methyloxirane

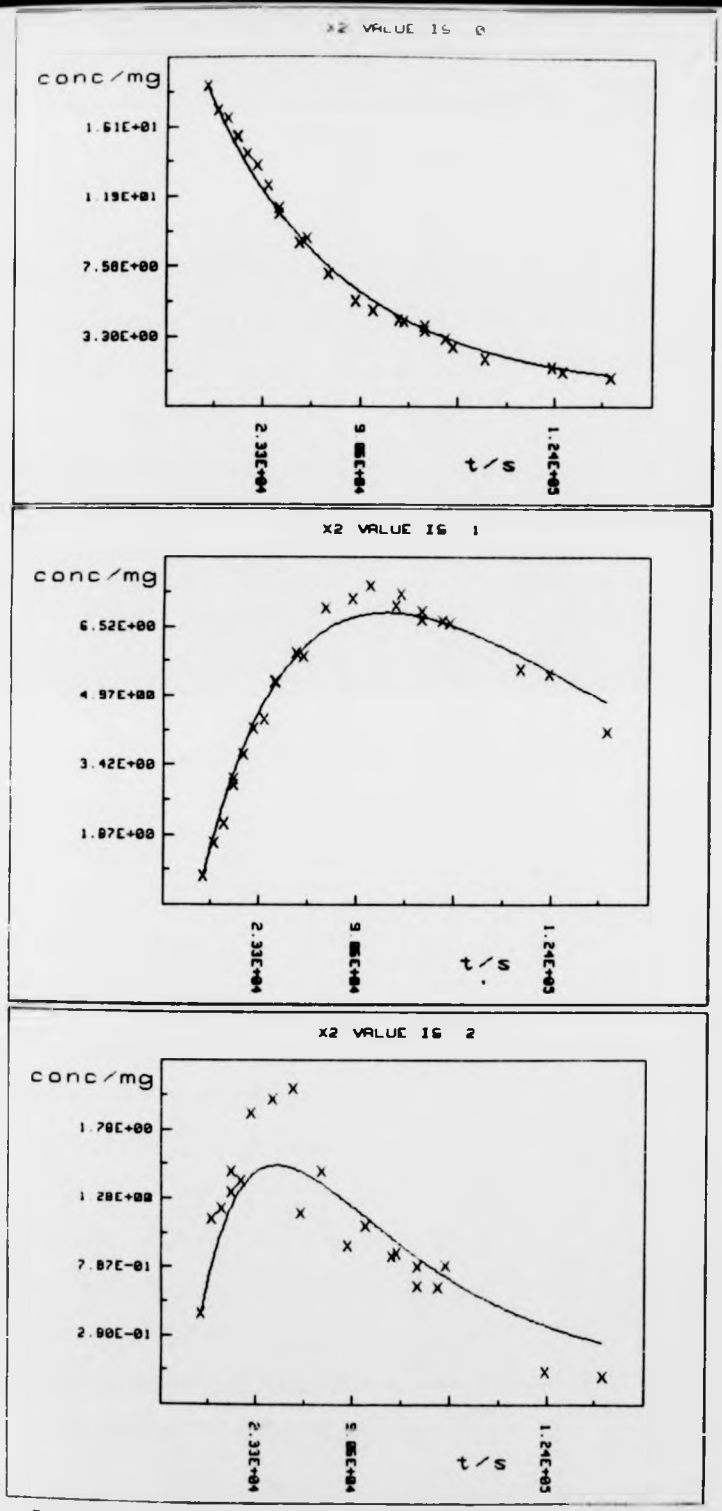


Fig. 5.G./2b Computer simulation of Fig. 5.G.1. The reaction of N-benzoyl-L-histidine methyl ester with (R)-methyloxirane

alkylated protein or nucleic acid. This predominance will occur irrespective of any enantioselectivity of these substrates towards the enantiomers of the epoxide. It is possible that a stereochemical distinction might be made between alkylation of a biological nucleophile by an epoxide, according to whether one (metabolically generated epoxide) or two (environmentally generated epoxide) diastereoisomeric product(s) of monoalkylation is (are) formed.

It was not expected that amino-acid derivatives (88), (89) and (90) would show appreciable enantioselectivity towards the enantiomers of methyloxirane. The kinetic data obtained for reactions of esters (88), (89) and (90) with (R)- and (S)-methyloxirane show that for each case the degree of enantioselectivity $[k_R/k_S]$ is for (88): 0.85 $\pm$ 0.05, for (89): 0.96 $\pm$ 0.03 and for (90) (116 and 117) or (124 and 125): 1.04 $\pm$ 0.05. This is not surprising for the reactions of esters (88) and (90), because the nucleophilic attack on the oxirane ring occurs distant from the α-chiral centre. For the reaction of ester (89) with t-butyloxirane an enantioselectivity $[k_R/k_S]$ was estimated by ^1H n.m.r. spectroscopy to be-0.66. For ester (89) the nucleophilic amino group is attached to the chiral centre and a greater interaction between the valine iso-propyl group and a bulky alkyl substituent of an epoxide is expected. It has been found that when the powerful nucleophile cob(I)alamin reacts with an excess of racemic methyloxirane a diastereoisomeric mixture of 2-hydroxypropylcobalamins is formed. Analysis by 400 MHz ^1H n.m.r. spectroscopy has shown the (R)-isomer of methyloxirane to react ca. 3-fold faster than the (S)-isomer[32]. This selectivity probably results from the interactions of the cob(I)alamin 'sentinel'

methyl- and ethylcarboxylate side chains of the chiral, reacting β-face with methyloxirane at the transition state for the reaction.

In a physiological milieu the intrinsic greater reactivity of a particular functional group of a protein for (R)- or (S)-methyloxirane can only be expressed as the predominant formation of one diastereoisomeric adduct if the ratio of racemic methyloxirane to protein is > 2. The importance of the above stereochemical considerations have not been discussed in previous studies of reactions between epoxides and proteins[33] or nucleic acids[34].

The regioselectivity for nucleophilic attack on an unsymmetrically substituted epoxide is known to depend on the nature of the nucleophile, solvent and whether or not the oxirane oxygen is protonated[18]. Epoxides possessing electron donating substituents on the ring show a greater extent of reactivity at the methine carbon due to stabilisation of positive charge α- to the substituent, e.g. phenyloxirane. This behaviour is reversed when electron withdrawing substituents are present. For the reactions described, regioselective attack on the methylene carbon of methyloxirane is expected and was observed. No products from attack at the methine carbon were detected and it is estimated that $k_{CH_2}/k_{CH} > 20$ for all reactions described.

In the reaction of a protein and methyloxirane the regioselectivity could differ from that observed with esters (88), (89) and (90). There could be an increased extent of attack at oxirane methine either because of protonation of the oxirane oxygen concomitant with nucleophilic attack, or because the oxirane binds to the protein in such a way that sterically attack at CH is favoured, e.g. intercalation of benzo [a] pyrene-7,8-dihydrodiol-9,10-epoxide between the α- and β-chains of DNA prior to alkylation.

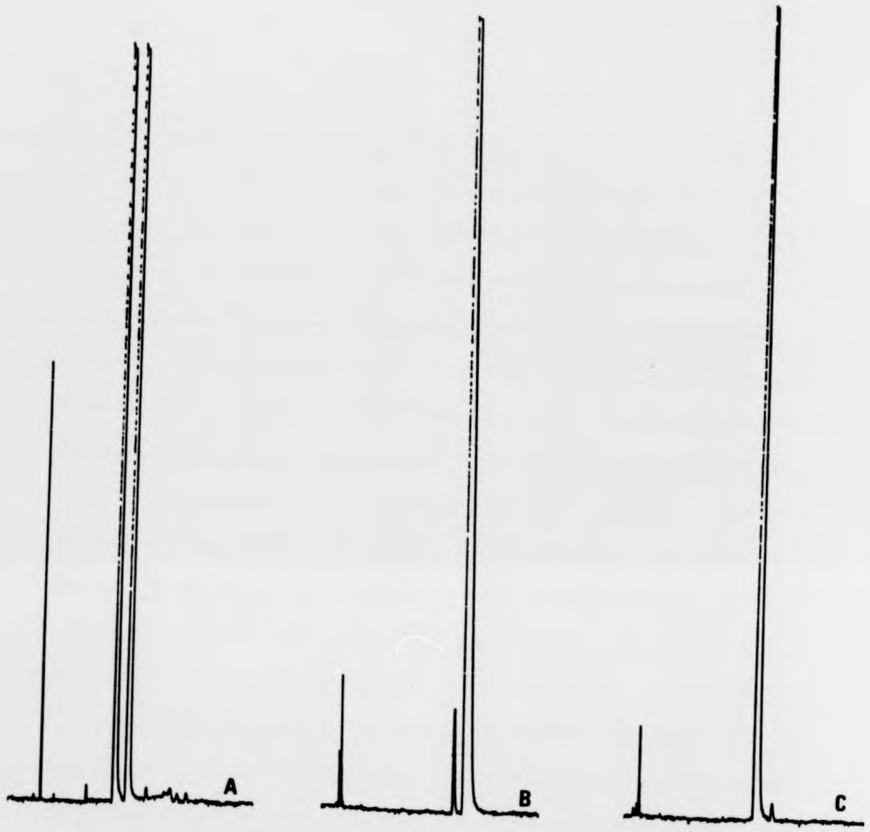


Fig. 5.J.1.1 A (rac.)-methyloxirane
B (S)-methyloxirane
C (R)-methyloxirane

5.J EXPERIMENTAL

5.J.1 Preparation of (R)- and (S)-Methyloxirane

The optically active methyloxiranes were prepared as described[35]. We thank Dr. B. Koppenhoefer for analysing the enantiomer composition of these samples by g.c. (chiral column) and the results are shown in Fig. 5.J.1.1. The (S)-methyloxirane (2b), prepared from L-ethyl lactate was shown to contain ca. 3% (R)-isomer. This is rather high (1-2% norm) and may result from aging of the ethyl lactate. The (R)-methyloxirane (2a), prepared from the yeast reduction of hydroxyacetone contained ca. 1% (S)-isomer.

5.J.2 Preparation of N-Acetyl-L-cysteine methyl ester

To a solution of N-Acetyl-L-cysteine (2 g, 1.2×10^{-2} mol) in dry methanol (10 cm^3) at 0^oC is added acetylchloride (0.31 cm^3 of a 1.01 g/cm^3 solution) to generate an 0.2 M HCl solution[36]. The reaction was allowed to warm to RT and monitored by t.l.c. (silica gel, ether) and in the later stages by 220 MHz ^1H n.m.r. spectroscopy. After 3 days volatiles were evaporated and the oil crystallised (diethyl ether:petrol ether, 1:1) to give a colourless crystalline product (1.12 g, 52.8%), m.p. 76-78°C and after sublimation [50-55°C (bath temperature)/ 0.01 mm], m.p. 80-81°C (lit.[37] m.p. 79-80°C); Rf 0.27; δ_H (D_2O) 2.06 (s, 3H, CH_3CO), 2.98 (d, 2H, CH_2), 3.79 (s, 3H, CH_3O) and 4.68 ppm (t, 1H, CH); δ_C ($CDCl_3$) 23.17 ($CO\underline{C}H_3$), 26.92 (SCH_2), 52.84 ($CO_2\underline{C}H_3$), 53.67 (CH), 170.0 ($\underline{C}OCH_3$) and 170.81 ppm ($\underline{C}O_2CH_3$); ν max (nujol) 3,300m,

3,080w, 2,850s, 1,735s,1,643m, 1,553m and 1,445m cm^{-1}; m/z (c.i.) 178 ($M + H^+$), 118 ($M - CO_2CH_3^+$), 88 ($C_3H_6NO_2^+$), and 76 ($C_2H_6NS^+$).

5.J.3 Preparation of N(α)-Benzoyl-L-histidine methyl ester

N(α)-Benzoyl-L-histidine methyl ester was prepared as described[38]: m.p. 156-157°C, (5.76 g, 55%); Rf 0.59 (silica gel F_{254}) elution with CH_2Cl_2-MeOH, 4:1 v/v); δ_H ([$^2H_4$]-methanol) 3.17 (m, 2H, CH_2), 3.62 (s, 3H, CO_2CH_3), 4.86 (q, 1H, CHND), 6.90 (s, 1H, ^5CH), 7.61 (s, 1H, ^2CH) and Ar resonances; ν max (h.c.b.) 3,170m, 3,010w, 1,742m, 1,730m, 1,637s and 1,563s cm^{-1}; m/z (e.i.) 273 (M^+), 214 ($M-CO_2CH_3^+$), 168 ($M-C_7H_5O^+$), 152, 105 ($C_7H_5O^+$) and 77 ($C_6H_5^+$); $[\alpha]_D^{15}$ - 32.4° (c 2.5 in MeOH).

5.J.4 Preparation of t-Butyloxirane

To a stirred solution of 3,3-dimethylbut-1-ene (6.53 g, 10 cm^3, 77.7 mmol) in o-dichlorobenzene (90 cm^3) at 0°C was added m-chloroperoxybenzoic acid (20 g, 0.12 mol). The mixture was stirred at 0°C for 3 days after which solids were filtered off and the supernatant washed with saturated sodium metabisulphite solution (2 x 30 cm^3), saturated sodium bicarbonate solution (20 cm^3) and brine (2 x 20 cm^3). The organic layer was dried ($MgSO_4$), was filtered and the product distilled out from the solvent (oil bath temperature ca. 190°C) to give a colourless liquid. Redistillation from CaH_2 gave pure oxirane (4.26 g, 54.8%), b.p. 96-99°C; δ_H (300 MHz, [$^2H_4$]-methanol) 0.93 (s, 9H, $C(CH_3)_3$), 2.61 (m, 2H, CH and one of CH_2), and 2.74 ppm (dd, 1H, one of CH_2): (60 MHz, $CDCl_3$)

0.89 (s, 9H, $C(CH_3)_3$) and 2.57 ppm (m, 3H, CH and CH_2); ν max (solution, CCl_4) 3,038w, 2,965s, 2,910m, 2,872m, 1,481s, 1,367s, 1,250w, 1,210w, 919s and 846m cm^{-1}.

5.J.5 Preparation of (S,αR)-N-Acetyl-S-(2-hydroxypropyl)cysteine methyl ester

To a stirred solution of N-acetyl-L-cysteine methyl ester (0.1 g, 5.6×10^{-4} mol) in dry methanol (5 cm^3) under nitrogen was added triethylamine (0.056 g, 5.6×10^{-4} mol). A pH change from 6.49 to 10.25 was observed. After 30 min the solution was cooled to 0°C and (-)-(S)-methyloxirane (0.18 g, 3.10×10^{-3} mol) was added. T.l.c. analysis (silica gel, CH_2Cl_2/CH_3OH, 4:1) showed the reaction to be complete within ca. 4h. The volatile components of the reaction were evaporated under reduced pressure, giving a colourless oil (0.119 g, 98%). This was pure (S,αR)-N-acetyl-S-(2-hydroxypropyl)-cysteine methyl ester (92) according to its ^1H and ^{13}C n.m.r. spectra. An analytical sample of the ester (92) was obtained by Kugelröhr distillation : colourless oil, b.p. 90°C/0.01 mm; δ_H ($CDCl_3$) 1.21 (d, 3H, MeCH), 2.04 (s, 3H, MeCO), 2.50 (dd, 1H, J 13.8 and 7.2 Hz, H of cys CH_2), 2.70 (dd, 1H, J 13.8 and 4.2 Hz, H of cys CH_2), 3.0 (d, 2H, CH_2CHOH), 3.76 (s, 3H, MeO), 3.87 (m, 1H, CHOH), and 4.83 ppm (m, 1H, CHNH), δ_C ($CDCl_3$) 22.26 (MeCH), 23.23 (MeCO), 35.18 and 42.40 (2 x CH_2), 52.29 (MeO), 52.72 (CHNH), 66.07 (CHOH), 169.63 and 170.7 (2 x CO); ν max (film) 3,300m, 2,940w, 1,745s, 1,660s, 1,550s and 1,440m cm^{-1}; M^+, 217.0776 ($C_9H_{15}NO_3S$ requires 217.0772); $[\alpha]_D^{27}$ - 20.1° (c 2.98 in MeOH).

5.J.6 Preparation of (R,OR)-N-Acetyl-S-(2-hydroxypropyl)cysteine Methyl Ester (91)

This was prepared from N-acetyl-L-cysteine methyl ester and (R)-methyloxirane in the manner described for ester (92) to give essentially pure ester (91) as a colourless oil (98%). An analytical sample was obtained by Kugelröhr distillation: colourless oil, b.p. 90°C/0.01 mm; δ_H ($CDCl_3$) 1.23 (d, 3H, MeCH), 2.05 (s, 3H, MeCO), 2.46 (dd, 1H, J 13.8 and 8.8 Hz, H of cys CH_2S), 2.76 (dd, 1H, J 13.8 and 3.2 Hz, H of cys CH_2S), 2.96 (dd, 1H, J 14.0 and 5.8 Hz, H of CH_2CHOH), 3.04 (dd, 1H, J 14.0 and 4.6 Hz, H of CH_2CHOH), 3.77 (s, 3H, MeO), 3.85 (m, 1H, CHOH) and 4.86 ppm (m, 1H, CHNH); δ_C ($CDCl_3$) 22.32 (MeCH), 23.23 (MeCO), 35.24 and 42.40 (2 x CH_2), 52.35 (MeO), 52.78 (CHNH), 66.37 (CHOH), 169.63 and 170.78 (2 x CO); νmax (film) 3,300m, 2,940w, 1,745s, 1,660s, 1,550s and 1,440m cm^{-1}; M^+, 217.0784 ($C_9H_{15}NO_3S$ requires 217.0772); $[\alpha]_D^{25}$ -21.8° (c 2.94 in MeOH).

5.J.7 Prolonged Reaction of (S)-N-Acetylcysteine Methyl Ester with (S)-Methyloxirane: Evidence for the Formation of N-Acetyldehydroalanine methyl ester and (S,S)-di-(2-hydroxypropyl)-sulphide

N-Acetyl-L-cysteine methyl ester was reacted with a 5-fold excess of (S)-methyloxirane in [$^2H_4$]methanol at 45°C monitoring by ^1H n.m.r. spectroscopy. The solvent was evaporated and the resulting oil was redissolved in $CDCl_3$. Singlets at δ 2.13 ($COCH_3$), 5.89 and 6.59 ($H_2C{=}C$) were attributed to N-acetyldehydroalanine methyl ester reactions carried out in dry methanol also showed the methyl ester resonance at δ 3.85 ppm. Resonances at δ 1.25 (d, 6H, 2 x CH_3),

2.51 (dd, 4H, 2 x CH_2), 2.77 (dd, 4H, 2 x CH_2) and 3.89 ppm (m, 2H, 2 x CHOH) were assigned to (S,S)-di-(2-hydroxypropyl)sulphide.

5.J.8 Preparation of (S,OS)-N-(2-Hydroxypropyl)-valine methyl ester (97)

L-valine methyl ester [prepared by neutralising its hydrochloride, 0.638 g, 3.82 x 10^{-3} mol with sodium methoxide] in dry methanol (5 cm^3) under nitrogen was treated with (S)-methyloxirane (0.22 g, 257 μl, 3.80 x 10^{-3} mol). The reaction was incubated at 45°C for 108h, when t.l.c. analysis showed it to be complete. Evaporation gave ca. 95% pure ester (97) as a pale yellow oil (0.461 g, 68%), δ_H ($CDCl_3$) 0.94 (d, 3H, Me), 0.96 (d, 3H, MeCH), 1.14 (d, 3H, MeCH), 1.94 (m, 1H, Me_2CH), 2.11 (dd, 1H, J 11.65 and 9.88 Hz, H of CH_2), 2.83 (dd, 1H, J 11.65 and 2.77 Hz, H of CH_2), 2.98 (d, 1H, CHNH), 3.67 (m, 1H, CHOH) and 3.72 ppm (s, 3H, MeO). This compound was characterised by conversion into the thiohydantoin (101) and by hydrolysis to (S,OS)-N-(2-hydroxypropyl)-L-valine (99) [see below].

5.J.9 Preparation of (R,OS)-N-(2-Hydroxypropyl)-valine methyl ester (96)

This was prepared from L-valine methyl ester and (R)-methyloxirane in the manner described for ester (97) to give ca. 95% pure ester (96) as a pale yellow oil (64%): δ_H 0.97 (d, 6H, Me_2), 1.14 (d, 3H, MeCH), 1.95 (m, 1H, Me_2CH), 2.46 (d, 2H, CH_2, J 6.0 Hz), 3.04 (d, 1H, CHNH), 3.74 (s, 3H, MeO) and 3.75 (m, 1H, CHOH). This compound was characterised by hydrolysis to (R,OS)-N-(2-hydroxypropyl)-valine (98) [see below]. It gave an oily hydantoin (102) on treatment with isothiocyanatobenzene in ethanol. δ_H ($CDCl_3$): 0.96 (d, 3H, MeCH), 1.20 (d, 3H, CH_3), 1.23 (d, 3H, CH_3), 2.47 (m, 1H, Me_2CH), 3.25 (dd, 1H,

J 14.6 and 9.8 Hz, H of CH_2), 4.28 (m, 1H, CHOH), 4.31 (dd, 1H, J 14.6 and 2.4 Hz, H of CH_2), 4.48 (d, 1H, CHNH) and ArH resonances; M$^+$, 292.1240 ($C_{15}H_{20}N_2O_2S$ requires 292.1245) [see data for crystalline hydantoin (101) below].

5.J.10 Preparation of 1-[(S)-2-Hydroxypropyl]-5-(S)-isopropyl-3-phenyl-2-thiohydantoin (101)

(S,αS)-N-(2-Hydroxypropyl)-valine methyl ester (97) [0.1 g, 5.29 x 10^{-4} mol] in dry ethanol (1 cm^3) was treated with isothiocyanatobenzene (0.143 g, 1.06 x 10^{-3} mol). The mixture was stirred at room temperature for 1h and then heated at 40°C for 15 min. On cooling, crystallisation of hydantoin (101) occurred. An analytical sample of the title compound was obtained by recrystallisation from ethanol: colourless needles (36%), m.p. 146-148°C, Rf 0.61 (silica gel F_{254}, CH_2Cl_2/MeOH, 10:1) $[\alpha]_D^{27}$ -1.4° (c 2.0 in MeOH), δ_H ($CDCl_3$) 0.96 (d, 3H, MeCH), 1.26 (d, 3H, CH_3), 1.29 (d, 3H, CH_3), 2.25 (s, 1H, OH), 2.48 (m, 1H, Me_2CH), 3.38 (dd, 1H, J 14.4 and 3.0 Hz, H of CH_2), 4.19 (m, 1H, CHOH), 4.26 (d, 1H, CHN), 4.57 (dd, 1H, J 14.4 and 7.8 Hz, H of CH_2) and ArH resonances; νmax (Nujol) 3,495s, 3,440m, 3,050m, 1,725s, 1,600w, 1,425m, 1,295s, 878w, 750s and 694m cm^{-1}; M$^+$ 292 (Found: C, 61.45; H, 6.9; N, 9.5; S, 11.0; O, 11.3. $C_{12}H_{15}N_2OS$ requires C, 61.6; H, 6.9; N, 9.6; S, 10.95; O, 10.95%). The 1-[(R)-2-hydroxypropyl]-5-(S)-isopropyl-3-phenyl-2-thiohydantoin (102) prepared from (R,αS)-N-(2-hydroxypropyl)-valine methyl ester was a colourless oil, δ_H($CDCl_3$) 0.95 (d, 3H, CH_3), 1.22 (2 x d, 6H, $CH(CH_3)_2$), 2.46 (m, 1H, $CH(CH_3)_2$), 3.25 (dd, 1H, H of CH_2), 4.3 (m, 2H, CHOH and H of CH_2) and 4.48 ppm (d, 1H, CHN) and ArH resonances.

5.J.11 Preparation of (S,αS)-N-(2-Hydroxypropyl)-valine (99)

This was obtained by hydrolysis of ester (97) in water at room temperature for 6 days. Evaporation and recrystallisation of the residue from aq. acetone gave the title compound as colourless rectangular crystals: sublimes at 165-166°C, m.p. 231-232°C (in closed capillary); δ_H (360 MHz, D_2O) 1.01 (d, 3H, CH_3), 1.05 (d, 3H, CH_3), 1.22 (d, 3H, MeCH), 2.24 (m, 1H, Me_2CH), 2.92 (dd, 1H, J 12.8 and 9.9 Hz, H of CH_2), 3.15 (dd, 1H, J 12.8 and 2.7 Hz, H of CH_2), 3.52 (d, 1H, J 5.0 Hz, CHND) and 4.10 ppm (m, 1H, CHOD); δ_C (D_2O) 18.1 and 18.7 (Me_2), 20.5 (MeCH), 54.3 (CH_2), 63.4 (CHND), 68.7 (CHOD) and 173.3 (CO); ν max (Nujol) 3,450s, 3,320s, 2,700-2,200br, 1,610s, 1,560s, 1,390s, 1,334s and 1,030s cm^{-1}; m/z (FAB) 214 $(M + K)^+$, 198 $(M + Na)^+$, 176 $(M + H)^+$; $[\alpha]_D^{27}$ -0.32° (c 1.55 in MeOH). (Found: C, 54.9; H, 9.7; N, 7.95. $C_8H_{17}NO_3$ requires C, 54.85; H, 9.75; N, 8.0%).

5.J.12 Preparation of (R,αS)-N-(2-hydroxypropyl)-valine (98), see page 176.

5.J.13 Preparation of (R,R,αS)-N,N-Di-(2-hydroxypropyl)-valine methyl ester and δ-Lactone [ester (104) and morpholone (105) respectively].

L-Valine methyl ester was reacted with a 10-fold excess of (R)-methyloxirane in methanol for 3 weeks at 45°C. The product mixture was separated by preparative t.l.c.[silica gel, elution with CH_2Cl_2/methanol (10:1)] to give:

(i) (R,R,αS)-N,N-di-(2-hydroxypropyl)-valine methyl ester (104) as a colourless oil (40%): b.p. ca. 100°C/0.1 mm, δ_H ($CDCl_3$) 0.87 and 1.06 (2 x d, 6H, Me_2CH), 1.14 (d, 6H, 2 x MeCHOH), 2.04 (m, 1H, Me_2CH), 2.48 (m, 4H, 2 x $CHOHCH_2$), 2.88 (d, 1H, -CH), 3.71 (s, 3H, OMe) and

3.86 ppm (m, 2H, 2 x CHOH); the c.i. mass spectrum (reagent: ammonia) of the trideuteriomethyl ester of (104) obtained from a reaction run in [$^2H_4$] methanol showed $M+H^+$ at 251 (most abundant peak of mass > 18). This product was contaminated with morpholone (105).

(ii) (R,R,S)-N,N-di-(2-hydroxypropyl)-valine-δ-lactone (105): b.p. ca. 100°/0.1 mm (60%), δ_H ($CDCl_3$) 1.01 and 1.14 or 1.16 (2 x d, 6H, Me_2CH), 1.16 or 1.14 (d, 3H, MeCHOH), 1.33 (d, 3H, MeCHOCO), 2.0 (m, 1H, Me_2CH), 2.50 (m, 3H, $CHOHCH_2$ and one of ring CH_2), 3.14 (m, 2H, one of ring CH_2 and α-CH), 3.88 (m, 1H, CHOH) and 4.60 ppm (m, 1H, MeCHOCO); δ_C ($CDCl_3$), 17.48, 18.79, 20.01, 20.82, 32.39, 56.65, 65.22, 65.87, 70.89, 74.04 and 169.92; νmax (film) 3,442br, 2,965s, 2,935s, 2,878s, 2,820m, 1,725s, 1,460s, 1,369s, 1,275s, 1223s, 1,150s, 1,052s, 990m and 950m cm^{-1}; m/z (e.i.) 215.1530 (M^+, $C_{11}H_{21}NO_3$ requires 215.1521); $[\alpha]_D^{27}$ +60° (c 0.7 in methanol).

5.J.14 Preparation of (S,S,αS)-N,N-Di-(2-hydroxypropyl)-valine methyl ester and δ-Lactone ester (107) and morpholone (108), respectively.

L-valine methyl ester was reacted with (S)-methyloxirane following the procedure given in the previous section, to give (107) and (108).

(107): colourless oil, b.p. ca. 70°C/0.04 mm , δ_H ($CDCl_3$) 0.88 and 1.10 (2 x d, 6H, Me_2CH), 1.13 (d, 6H, 2 x MeCHOH), 2.09 (m, 1H, Me_2CH), 2.45 (dd, 2H, 2 x one of $CHOHCH_2$, J 9.9 and 13.8 Hz), 2.89 (dd, 2H, 2 x one of $CHOHCH_2$, J 3.3 and 13.8 Hz), 3.72 (s, 3H, OMe), 3.84 (m, 2H, 2 x CHOH) and 4.75 ppm (m, 1H, α-CH).

(108) :colourless oil (72%), b.p. ca. 70°/0.04 mm. δ_H ($CDCl_3$) 1.05 and 1.09 (2 x d, 6H, $\underline{Me}_2CH$), 1.18 (d, 3H, $\underline{Me}CHOH$), 1.39 (d, 3H, $\underline{Me}CHOCO$), 2.20 (m, 1H, $Me_2\underline{CH}$), 2.38 (dd, 1H, one of $CHOH\underline{CH}_2$, $\underline{J}$ 10.0 and 12.9 Hz), 2.76 (dd, 1H, one of $CHOH\underline{CH}_2$, $\underline{J}$ 3.2 and 12.9 Hz), 2.83 (m, 2H, ring CH_2), 3.0 (m, 1H, α-CH), 3.82 (m, 1H, $\underline{CH}OH$) and 4.74 ppm (m, 1H, $MeC\underline{H}OCO$); νmax (film) 3,440br, 2,970s, 2,935s, 2,878s, 2,825m, 1,725s, 1,457s, 1,372s, 1,275s, 1,220s, 989m and 945m cm^{-1}; $\underline{m/z}$ (e.i.) 215.1521; $[\alpha]_D^{24}$ -10.77° (c 0.7 in methanol).

5.J.15 Preparation of (S,S,αR/αS)-N(α)-Benzoyl-N(π),N(τ)-di-(2-hydroxypropyl)-histidine imidazolium carboxylate [(124) + (125)]

N-Benzoyl-L-histidine methyl ester (0.5 g, 1.83 x 10^{-3} mol) in dry methanol (5 cm^3) was treated with (S)-methyloxirane (0.53 g, 9.1 x 10^{-3} mol). The mixture was incubated at 45°C for 4 days. Removal of the solvent gave a pale yellow oily residue that was crystallised from acetone. Recrystallisation from methanol/ether gave the imidazolium carboxylates [(124) + (125)] (0.418 g, 61%) as colourless crystals: m.p. 146-147°C; δ_H (360 MHz, D_2O) 1.086 (d, 3H, $\underline{Me}CH$), 1.113 (d, 3H, $\underline{Me}CH$), 1.26 (d, 3H, $\underline{Me}CH$), 1.28 (d, 3H, $\underline{Me}CH$), 3.21 (dd, 2H, H of his CH_2), 3.45 (2.t, 2H, H of his CH_2), 3.94-4.04 (m, 6H, $C\underline{H}(OH)C\underline{H}_2$), 4.70 (m, 2H, CHND), 7.34 (s, 2H, H-5), 8.77 (s, 2H, H-2) and Ar resonances; δ_C (D_2O) 19.55 and 19.9 (Me), 27.1 (his CH_2), 53.8 and 56.3 (CH_2), 54.4 (CHND), 66.3 and 66.5 (CHOD), 121.9 (C-5), 127.9 ($ArC_{\underline{meta}}$), 129.6 ($ArC_{\underline{ortho}}$), 132.7 (C-4), 133.2 ($ArC_{\underline{para}}$), 133.7 (C-2), 137.3 (ArC-1), 170.7 ($Ph\underline{C}O$) and 176.7 (CO_2^-); νmax (Nujol) 3,350m, 3,240m, 3,090m, 2,845s, 1,680m, 1,595s, 1,540m, 1,490m, 775m and 690m cm^{-1}; λmax 227 nm (ϵ = 4,100); $\underline{M}^+$, 375, $[\alpha]_D^{17}$ +30.8° (c 2.4 in MeOH).

5.J.16 Preparation of (R,R,αR/αS)-N(π), N(τ)-Benzoyl-N(α)-di-(2-hydroxypropyl)-histidine imidazolium carboxylate [(116) + (117)]

This mixture was prepared in 66% yield from N-benzoyl-L-histidine methyl ester and (R)-methyloxirane in the manner described for imidazolium carboxylate mixture [(124) + (125)]: m.p. 145.5 - 146.5°C δ_H, δ_C, νmax values identical to those reported for [(124) + (125)], $[\alpha]_D^{15}$ -27.7° (c 2.4 in MeOH), m/z (FAB) 398 $(M + Na)^+$, 376 $(M + H)^+$, 332 $(MH-CO_2)^+$, 330 $(MH-HCO_2H)^+$, 274 $(MH-CO_2-MeCHOHCH_2)^+$, 226 (see text), 199 (see text) and 105 $(PhCO)^+$.

5.J.17 Preparation of (S,αS)-N(α)-Benzoyl-N(τ)-(2-hydroxypropyl)-histidine methyl ester (122) and its N(π)-isomer (123)

These compounds were obtained from reactions between N(α)-benzoyl-L-histidine methyl ester and (S)-methyloxirane in methanol at 45°C, which were monitored by t.l.c. and stopped before an appreciable quantity of the imidazolium carboxylate mixture [(124) + (125)] had formed. They were separated by preparative layer chromatography (silica gel PF_{254}, multiple development with CH_2Cl_2/MeOH, 16:1) to give esters (122) and (123) as colourless oils.

(122): δ_H (400 MHz, D_2O) 1.05 (d, 3H, MeCH), 3.13 (dd, 1H, J 14.6 and 8.6 Hz, H of his CH_2), 3.22 (dd, 1H, J 14.6 and 5.8 Hz, H of his CH_2), 3.78 (s, 3H, MeO), 3.87 (dd, 1H, J 14.4 and 6.6 Hz, H of CH_2), 3.97 (dd, 1H, J 14.4 and 3.8 Hz, H of CH_2), 4.02 (m, 1H, CHOD), 4.87 (dd, 1H, J 8.6 and 5.8 Hz, CHND), 6.99 (s, 1H, H-5), 7.58 (s, 1H, H-2) and ArH resonances; νmax (film) 3,330m, 2,935m, 1,740s, 1,650s, 1,604s, 1,580s, 1,219m, 1,178m; m/z (e.i.) 331 (M^+, 41.6), 272

(24.4), 226 (15.4), 210 (29.6), 139 (42.0), 121 (13.6), 105 (100) and 77 (62.8).

(123): δ_H (400 MHz, D_2O) 1.20 (d, 3H, MeCH), 3.24 (dd, 1H, J 15.6 and 9.8 Hz, H of his CH_2), 3.41 (dd, 1H, J 15.6 and 5.0 Hz, H of his CH_2), 3.79 (s, 3H, OMe), 3.94 (dd, H, H of CH_2), 4.10 (m, 1H, CHOD), 4.11 (dd, 1H, H of CH_2), 4.94 (dd, 1H, J 9.8 and 5.0 Hz, CHND), 6.93 (s, 1H, H-5) and ArH resonances; νmax (film) 3,330s, 3,360m, 2,925m, 1,740s, 1,650s, 1,605s, 1,580s, 1,224m, 719m, and 698 cm^{-1}; m/z (e.i.) 331 (M^+, 1.6), 299 (3.5), 272 (1.4), 210 (20.0), 139 (34.8), 121 (12.8), 105 (100) and 77 (68.0).

5.J.18 Preparation of (S,αS)- and (R,αS)-N-(2-hydroxy-3,3-dimethylbutyl)valine methyl esters

To L-valine methyl ester (10 mg, 7.63 x 10^{-5} mol) in $[^2H_4]$-methanol was added (rac.)-t-butyloxirane (76 mg, 92.7μl, 7.63 x 10^{-4} mol) and the reaction followed by ^1H n.m.r. spectroscopy. After 48h volatiles were evaporated at reduced pressure (rotary evaporation) to give a colourless oil, the composition of which was determined by 300 MHz ^1H n.m.r. spectroscopy (see text).

5.J.19 Kinetic Studies of Reactions between Enantiomeric Methyloxiranes and Esters (88), (89) and (90)

Reactions were studied quantitatively in dry methanol at 0 ± 0.2°C for (88) and 45 ± 0.1°C for (89) and (90). The initial concentrations of esters were 0.125 M (88), 0.30 M (89) and 0.146 M (90). For all reactions the initial concentration of epoxide was 10x that of the ester. Triethylamine (0.125 M) was

present in the reactions of ester (88). For the reactions of esters (89) and (90) ^{1}H n.m.r. spectroscopy was used to monitor the reaction in regions of the spectrum where there was no interference from solvent or epoxide absorptions (see text). For the reactions of ester (88) analysis by h.p.l.c. Waters instrument (fitted with a variable wavelength U.V. detector) and a Partisil 5 C8 column (100 mm x 9 mm i.d.) eluting isocratically with 20% (v/v) methanol in water at a flow-rate of 1 cm^3/min was used. With esters (88), (89) and (90), pseudo-first-order rate constants were obtained by least squares analysis of the data by a desk computer. The reactions of ester (90) with the enantiomeric methyloxiranes were analysed as consecutive, parallel, pseudo-first-order reactions (c.f. Scheme 5.G.1), measuring rates of change of the species (90) and products[39*].

By the procedures described, qualitative information about di-alkylations of esters (89) and (90) was obtained and is discussed in the text.

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* We thank Dr. J. Sachinidis for assistance with this analysis.

5.J.12 Preparation of (R, αS)-N-(2-Hydroxypropyl)-valine (98)

This was obtained by hydrolysis of ester (96) and was recrystallised from aq. acetone : sublimes at 178-179°C, m.p. 237-238°C (in closed capillary); δ_H (360 MHz, D_2O) 1.01 (d, 3H, CH_3), 1.05 (d, 3H, CH_3), 1.22 (d, 3H, MeCH), 2.24 (m, 1H, Me_2CH), 2.92 (dd, 1H, J 12.7 and 10.0 Hz, H of CH_2), 3.15 (dd, 1H, J 12.7 and 2.7 Hz, H of CH_2), 3.52 (d, 1H, J 4.9 Hz, CHND) and 4.10 (m, 1H, CHOD); δ_C (D_2O) 18.09 and 18.71 (Me_2), 20.5 (MeCH), 54.3 (CH_2), 63.4 (CHND), 68.7 (CHOD) and 173.2 (CO); ν max. (Nujol) 3,450s, 3,320s, 2,700 - 2,200br, 1,610s, 1,560s, 1,390s, 1,334s and 1,030s cm^{-1}; m/z (FAB) 198 $(M + Na)^+$, 176 $(M + H)^+$; $[\alpha]_D^{26}$ -9.0° (c 1 in MeOH), (Found: C, 54.8; H, 9.8; N, 7.8. $C_8H_{17}NO_3$ requires C, 54.85; H, 9.75; N, 8.0%).

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CHAPTER 6

THE USE OF DEUTERATED EPOXIDE RACEMATES TO ASSESS ENANTIOSELECTIVITIES IN PEPTIDES AND PROTEINS

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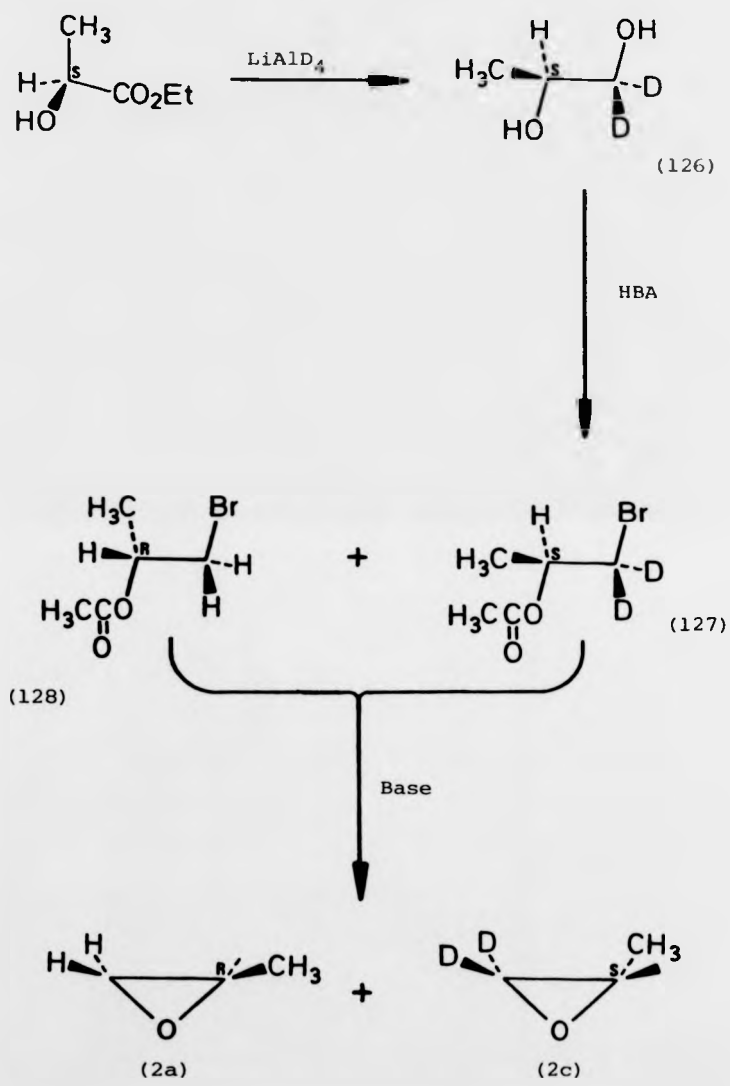
6.A INTRODUCTION

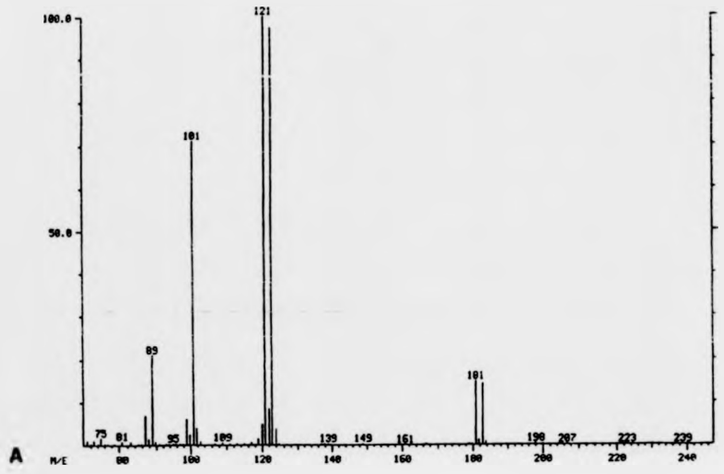
When racemic methyloxirane reacts with a nucleophilic group of a protein (Chapter 1) the enantioselectivity may be enhanced, leading to the preferred formation of one diastereoisomeric hydroxypropylated product. The extent of enantiomeric discrimination will depend upon the microenvironment of the reacting amino-acid residue. For a functional group deeply embedded within a protein its environment will be highly structured (e.g. the chiral cavity of an active site) and would be expected to show greater discrimination than a residue exposed to the bulk solvent. In physiological milieu there are many chiral molecules, each of which may exert an enantioselectivity towards one antipode of a chiral epoxide. Under these conditions, when examining the reactivity at a specific nucleophilic site, the *in vivo* composition of an "epoxide racemate" may contain a preponderance of one enantiomer. It is known that the erythrocyte contains a high concentration of glutathione (15). This tripeptide, whose function lies in metabolic detoxification (Chapter 1, section D), could show an enantioselectivity towards one member of an epoxide racemate. When determining the enantioselectivity of a specific nucleophilic group in a protein, e.g. the N-terminal amino-acid of haemoglobin, it must be recognised that the observed selectivity may, in part, result from the protein being exposed to a "racemate" enhanced in one enantiomer.

In the previous chapter (Chapter 5) the enantioselectivities in the reaction of methyloxirane with the nucleophilic groups of protected cysteine, valine and histidine were evaluated from kinetic data obtained by ^{1}H n.m.r. and h.p.l.c. When determining enantioselectivities *in vivo* a much more sensitive method is required to enable the detection of alkylated products at very low concentrations. This chapter describes a method applicable for detecting enantioselectivity in the reaction of peptides and proteins with chiral epoxides and employs mass spectroscopy as the analytical technique. This method could, in principle, be further developed to detect selectivities towards many chiral alkylating agents. Hydroxypropylated amino-acid derivatives have been detected in haemoglobin hydrolysate obtained from animals exposed to methyloxirane by g.c./m.s. (Chapter 1, ref. 54-57).

To determine enantioselectivity in peptides and proteins a mixture of (*R*)- and (*S*)-methyloxirane was synthesised in which one enantiomer is specifically labelled with deuterium. The enantioselectivity would be confirmed from the ratios of the molecular ions, one being 'n' mass units (where 'n' is the number of deuterons incorporated) greater than the other. This method does not permit the discrimination of alkylated products resulting from non-regioselective opening of the epoxide ring, because both the 2-hydroxypropyl- and 2-hydroxy-1-methylethyl moieties have the same relative molecular masses.


CH3
H
S
CO2Et
HO
LiAlD4
H
OH
H3C
S
D
HO
D
(126)
HBA
H3C
Br
H
R
H
H3CCO
O
H
Br
H3C
S
D
H3CCO
D
O
(127)
(128)
Base
H
H
R
CH3
O
(2a)
D
D
CH3
S
O
(2c)

Scheme 6.B.1



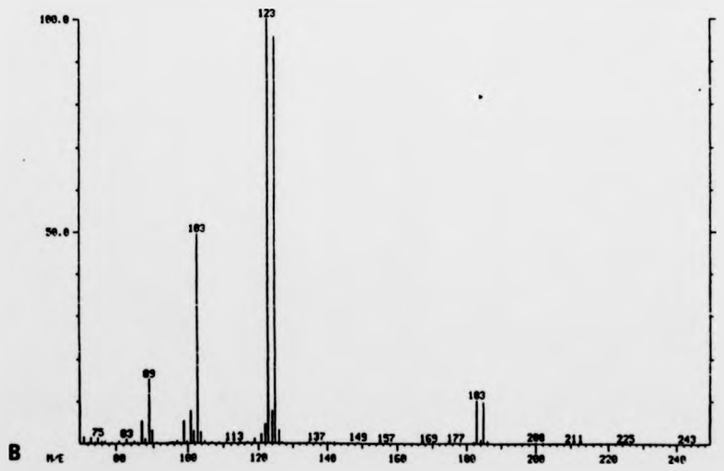


Fig. 6.B.2 The mass spectra (c.i.) of 2-acetoxy-1-bromo-propane.
A. (S)-2-acetoxy-1-bromopropane
B. (S)-2-acetoxy-1-bromo-$[1,1\text{-}^{2}H_{2}]$-propane

6.B SYNTHESIS OF THE EPOXIDE RACEMATE

The synthesis of the 'deuterated racemates' is illustrated in Scheme 6.B.1. (S)-ethyl lactate was reduced with lithium aluminium deuteride in ether to give (S)-$[2,2\text{-}^{2}H_{2}]$-propane-1,2-diol (126). The ^{1}H n.m.r. spectrum of the product showed the absence of resonances at δ 3.35 and 3.56 ppm which correspond with the two diastereotopic methylene protons of the unlabelled diol. The methine resonance (δ3.84, J = 6.3 Hz) was also observed to have collapsed to a quartet [Fig. 6.B.1].

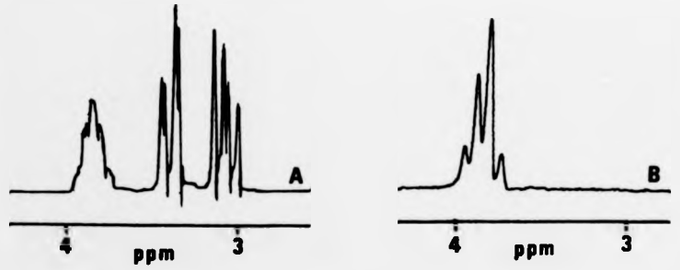


Fig. 6.B.1 A Propane-1,2-diol
B $[2, 2\text{-}^{2}H_{2}]$-propane-1,2-diol

Analysis of the product by m.s. gave a molecular ion (m/z 96, >98% deuterium incorporation) consistent with the desired product. The deuterated diol (126) was treated with 45% (w/v) hydrogen bromide in acetic acid to give (S)-2-acetoxy-1-bromo-$[1,1\text{-}^{2}H_{2}]$-propane (127) that was mixed with (R)-2-acetoxy-1-bromopropane (128, 1 mol. equiv.) and base cyclised (treatment with sodium or potassium pentylate in pentyl alcohol) to the epoxide. The mass spectrum of the acetoxybromide (128)[Fig. 6.B.2] shows mass ions (m/z 183 and

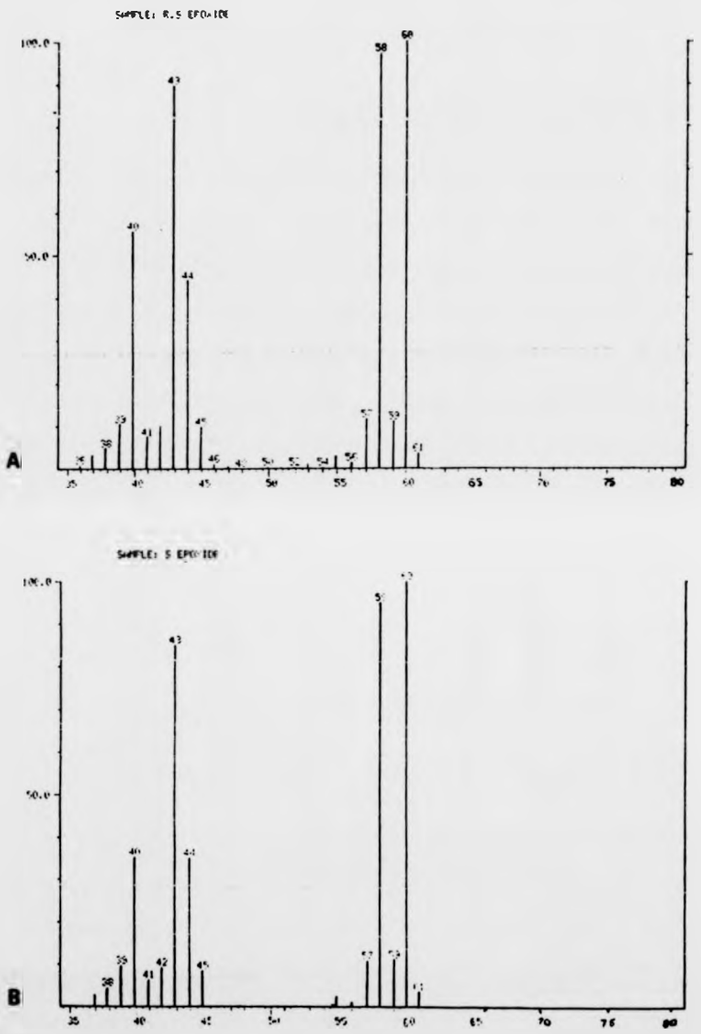
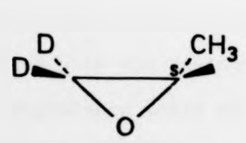


Fig. 6.B.3 The mass spectra (e.i.) of:
A. (R)-methyloxirane/(S)-[2,2-$^{2}H_2$]-methyloxirane
B. (S)-methyloxirane/(S)-[2,2-$^{2}H_2$]-methyloxirane

185) and the ^{1}H n.m.r. spectrum resonances consistent with the formation at the dideuterated product. Analysis of the 'epoxide racemate' by 360 MHz ^{1}H n.m.r. spectroscopy (integration of the methine and methylene resonances) showed the epoxide to be composed of a 1.03:1.00 mixture of (S)-(+)-[2,2-$^{2}H_2$]-methyloxirane (2c) and (R)-(-)-methyloxirane (2a), respectively. This was confirmed by g.c./m.s. analysis which after allowing for isotope contributions from ^{13}C, ^{14}C, ^{17}O and ^{18}O showed the mixture to contain 47.0% protonated, 3.36% monodeuterated and 49.6% dideuterated epoxide [Fig. 6.B.3a].



(2c)

(2a)

6.C DETERMINATION OF THE SECONDARY ISOTOPE EFFECT

In a chemical reaction where an isotope substitution has been made, e.g. $^{1}H \longrightarrow {}^{2}H$, $^{12}C \longrightarrow {}^{13}C$, there is a primary isotope effect when the isotopic change is in the bond being formed or broken in the transition state and a small secondary isotope effect when the isotope-carbon bond remains intact. These effects are large for isotopic substitutions of hydrogen atoms by deuterium or tritium atoms because of the large difference in the relative masses

of these isotopes. For a primary isotope effect where cleavage of a C-H bond is involved, substitution by deuterium gives $k_H/k_D \sim 8$ and by tritium $k_H/k_T \sim 20$ at 300 K. In contrast, carbon isotope substitutions give a value of $k_{12_C}/k_{13_C} \sim 1.02$. Secondary isotope effects are much smaller, $k_H/k_D = 1.00 \pm 0.1$ in the majority of reactions. In general one must be cautious of drawing concise conclusions from what appear as very small isotope effects because the origins of these might be doubtful.

The reaction of an achiral nucleophile upon the methylene carbon of (S)-[2,2-$^2H_2$]-methyloxirane would be expected to show a small rate difference in the cleavage of the oxirane ring when compared with the analogous reaction with (S)-methyloxirane. This difference would be consistent with any secondary isotope effect involvement.

Our initial strategy was to determine the magnitude of the secondary isotope effect by reacting the deuterated epoxide mixture with glycine ethyl ester (129) in methanol.

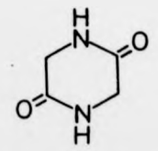
$H_2NCH_2CO_2CH_2CH_3$ (129)

$H_2NCH_2CO_2CH_3$ (130)

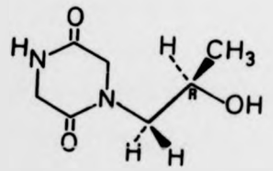
$H_3\overset{+}{N}CH_2CO_2^-$ (134)

Glycine is an ideal model compound for this reaction because of its achiral nature and similarity to valine, the N-terminal amino-acid of the human haemoglobin β-chain. The reaction of glycine

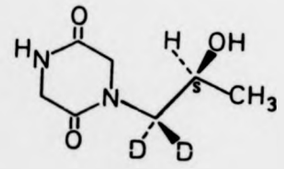
ethyl ester with (rac.)-methyloxirane in methanol was complicated by transesterification to the methyl analogue. A model reaction of glycine ethyl ester in $[^2H_4]$-methanol showed the transesterification to be half complete after ca. 20h at R.T.. In an analogous reaction glycine ethyl ester was hydrolysed in $[^2H_2]$-water, $\tau_{\frac{1}{2}}$ ca. 18h at R.T.. When glycine methyl ester (130) was reacted with racemic methyloxirane (2) (methanol/R.T.) a complex 60 MHz ^1H n.m.r. spectrum was obtained which suggested the possibility of diketopiperazine formation. Mass spectral analysis of the product mixture from the reaction with the deuterated epoxide mixture showed no peaks for the mono- or dialkylated glycine methyl esters but peaks were observed (m/z 115, 173 and 175) which could be attributed to mass ions and suggest the presence of diketopiperazine (131) and the mono-alkylated species (132 and 133). Other peaks at higher mass were also present which possibly arise from larger polymeric products.



(131)



(132)



(133)

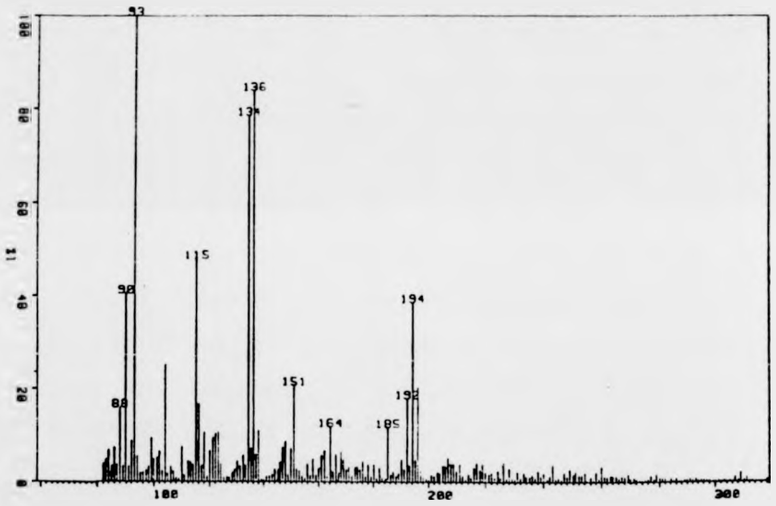
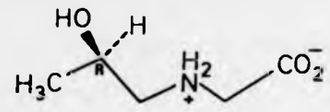
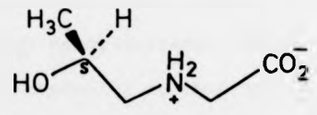


Fig. 6.C.1 The mass spectrum (c.i.) of the product mixture from the reaction of glycine with (R)-methyloxirane/(S)-[2,2-$^2H_2$]-methyloxirane

The reaction of glycine(134) with (rac.)-methyloxirane (10 mol. equiv., RT) in water was slow. Analysis of the mixture after 2 weeks by ^1H n.m.r. spectroscopy showed the major product to be (R)- and (S)-N-(2-hydroxypropyl)glycine(135) and (136), respectively, but the presence of methyl doublets at δ 1.237 and 1.241 ppm were



(135)



(136)

indicative of the presence of diastereoisomeric dialkylated material. Analysis by mass spectroscopy of a reaction between glycine and the deuterated epoxide mixture (2c + 2a) after 12h showed peaks for the presence of mono-alkylated derivative (m/z 134 and 136, $M+H^+$, ratio 1:1) and dialkylated derivatives (m/z 192, 194 and 196, $M+H^+$, ratio 1:2:1, respectively) [Fig. 6.C.1], whereas a reaction run over 7 days showed additional peaks (m/z 250, 252, 254 and 256, $M+H^+$, ratio 1:2:2:1, respectively) which presumably result from further alkylation of the carboxylate.

The presence of dialkylated products at short reaction times makes this method of determining isotope effects unsuitable, because the initially formed enantiomers from monoalkylation (135 and 136) could show enantioselectivity on dialkylation. Since secondary isotope

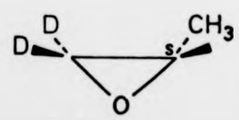
effects are small, what may in fact be observed are enantioselectivity differences that are misconstrued as isotope effects.

A useful adjunct to the use of deuterated epoxide mixtures is that products of mono-alkylation appear as double peaks, those of dialkylation as triple peaks and trialkylation quadruple peaks, which enables quick identification of molecular and fragmentation ions. Where no selectivity is apparent the ratios of these peaks are 1:1, 1:2:1 and 1:2:2:1,respectively.

The method chosen to assess the secondary isotope effect was to react (S)-valine methyl ester (89) with a mixture of (S)-[2,2-$^{2}H_{2}$]-methyloxirane and (S)-methyloxirane. The epoxide mixture was synthesised from an equimolar mixture of (S)-2-acetoxy-1-bromo-[1,1-$^{2}H_{2}$]-propane and (S)-2-acetoxy-1-bromopropane in an analogous manner described for mixture (2a + 2c). Analysis of the mixture by 300 MHz ^{1}H n.m.r. spectroscopy showed the epoxide to be present in a ca. 1:1 ratio. Analysis by g.c./m.s. and allowing for isotope contributions from ^{13}C, ^{14}C, ^{17}O and ^{18}O showed the mixture to contain 47.6% protonated, 3.7% monodeuterated and 48.7% dideuterated epoxide [Fig. 6.B.3b].

A reaction of (S)-valine methyl ester with the deuterated epoxide mixture (2b + 2c) was incubated for 4h at 45°C and the monoalkylated

(2b)



(2c)

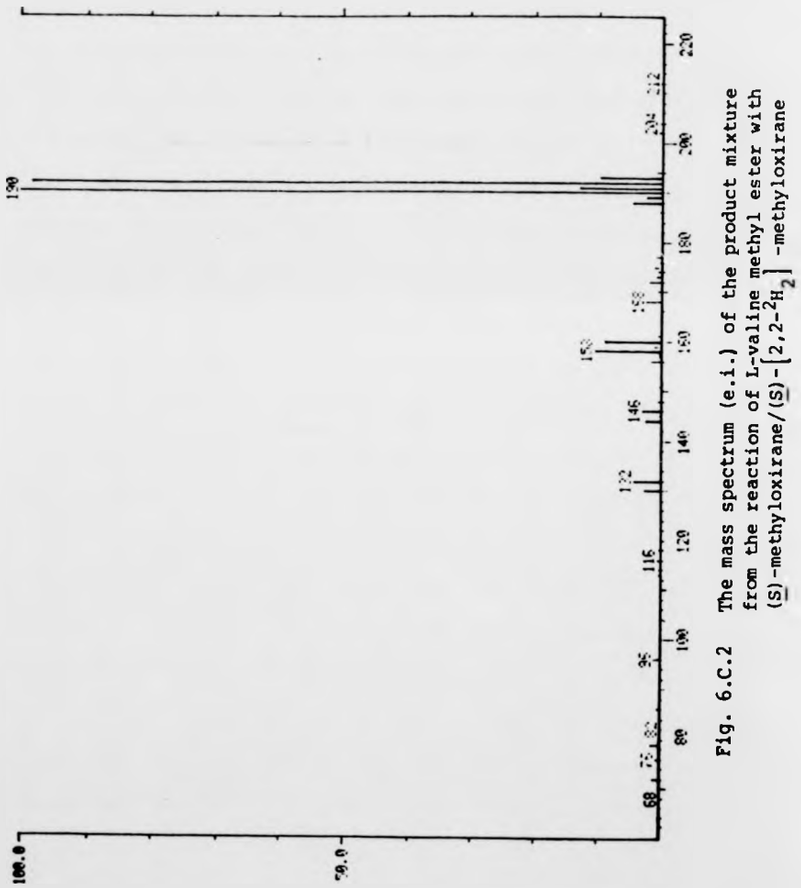
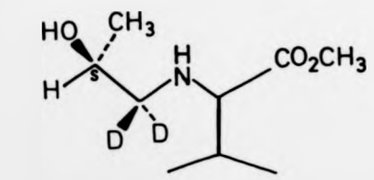
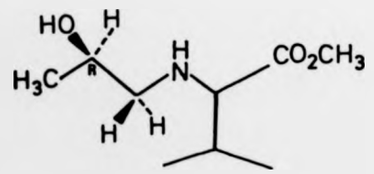


Fig. 6.C.2 The mass spectrum (e.i.) of the product mixture from the reaction of L-valine methyl ester with (S)-methyloxirane/(S)-[2,2-$^2H_2$]-methyloxirane

material purified by h.p.l.c. prior to m.s.. The c.i. mass spectrum obtained showed two major peaks[m/z 190 (100%) and 192 (98.1%)] which correspond to the mass ions ($M+H^+$) of esters (137) and (96) respectively [Fig. 6.C.2]. Calculation of the secondary isotope effect gave k_H/k_D = 1.02 ± 0.01. As was expected, the secondary isotope effect in the reaction of an epoxide with a primary amine is small, showing a 2 ± 1% preference towards the undeuterated isomer. This value is within the error limits of our analysis of the degree of enantioselectivity. The contribution of a secondary isotope can be ignored.



(137)



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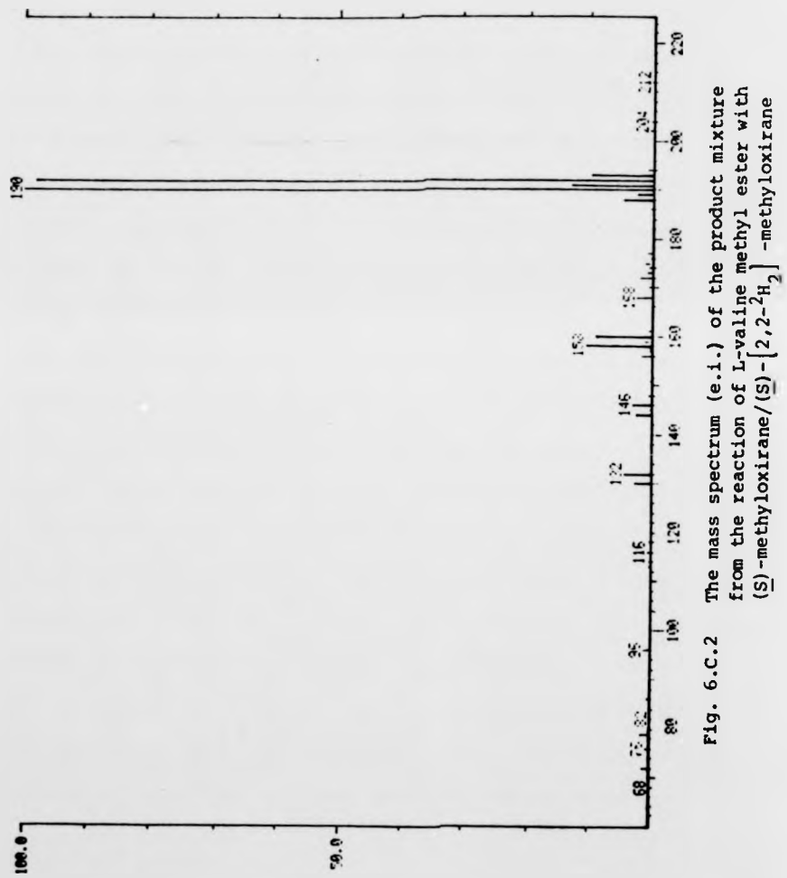
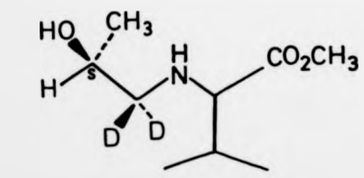
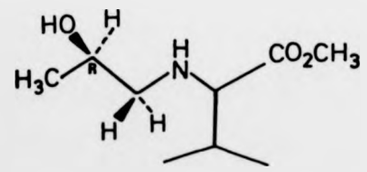


Fig. 6.C.2 The mass spectrum (e.i.) of the product mixture from the reaction of L-valine methyl ester with (S)-methyloxirane/(S)-[2,2-$^2H_2$]-methyloxirane

material purified by h.p.l.c. prior to m.s.. The c.i. mass spectrum obtained showed two major peaks [m/z 190 (100%) and 192 (98.1%)] which correspond to the mass ions ($M+H^+$) of esters (137) and (96) respectively [Fig. 6.C.2]. Calculation of the secondary isotope effect gave k_H/k_D = 1.02 ± 0.01. As was expected, the secondary isotope effect in the reaction of an epoxide with a primary amine is small, showing a 2 ± 1% preference towards the undeuterated isomer. This value is within the error limits of our analysis of the degree of enantioselectivity. The contribution of a secondary isotope can be ignored.



(137)



(96)

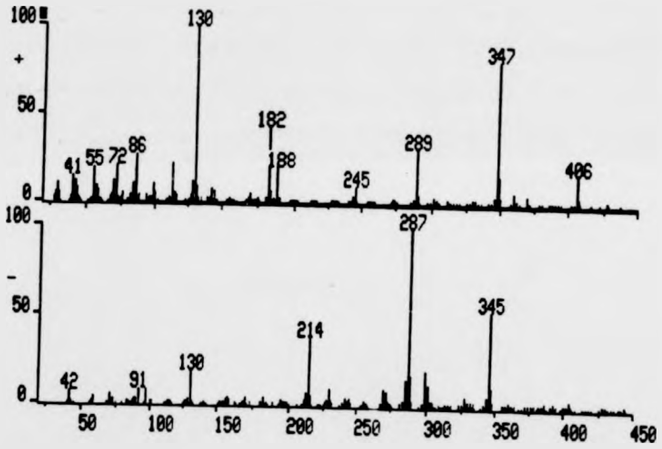
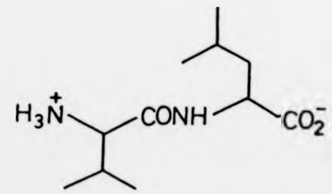


Fig. 6.D.1 The mass spectrum (f.a.b.) of the product mixture from the reaction of L-valyl-L-leucine with (S)-methyloxirane

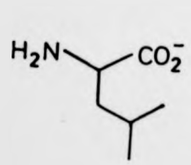
6.D REACTIONS BETWEEN L-VALYL-L-LEUCINE AND THE DEUTERATED EPOXIDE MIXTURE (2a + 2c)

The reaction of L-valyl-L-leucine(138), the N-terminal dipeptide of human haemoglobin (α-chain), with (S)-methyloxirane (10 mol. equiv.) in water showed two new spots when analysed by t.l.c.: Rf 0.52 and 0.46 (SiO_2, CH_2Cl_2-MeOH, 10:1, starting material Rf 0.04). The f.a.b. mass spectrum of the reaction mixture gave ions (negative ion mode) at m/z 287 and 345 corresponding to the $[M-H^-]$ ions for both the mono- and dialkylated dipeptides(139) and (140), respectively [Fig. 6.D.1].

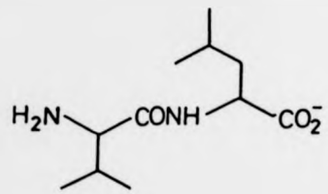


(138)

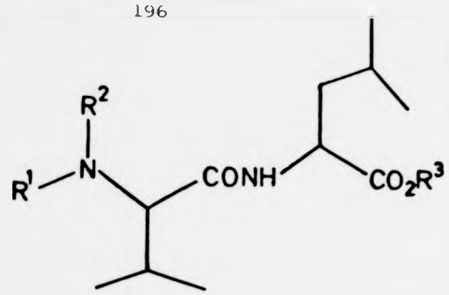
Ions at m/z 130 and 214 were assigned to (141) and (142) which arise from the unreacted dipeptide (138).

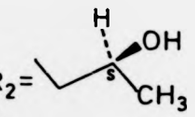


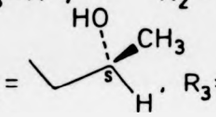
(141)

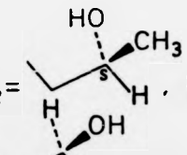


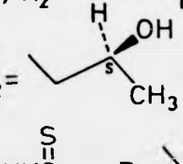
(142)

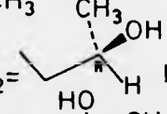


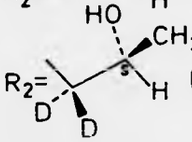
(139) $R_1 = R_3 = H$, $R_2 =$ 

(140) $R_1 = R_2 =$  , $R_3 = H$

(143) $R_1 = H$, $R_2 =$  , $R_3 = CH_3$

(144) $R_1 = R_2 =$  , $R_3 = CH_3$

(155) $R_1 =$, $R_2 =$  , $R_3 = H$

(156) $R_1 =$, $R_2 =$  , $R_3 = H$

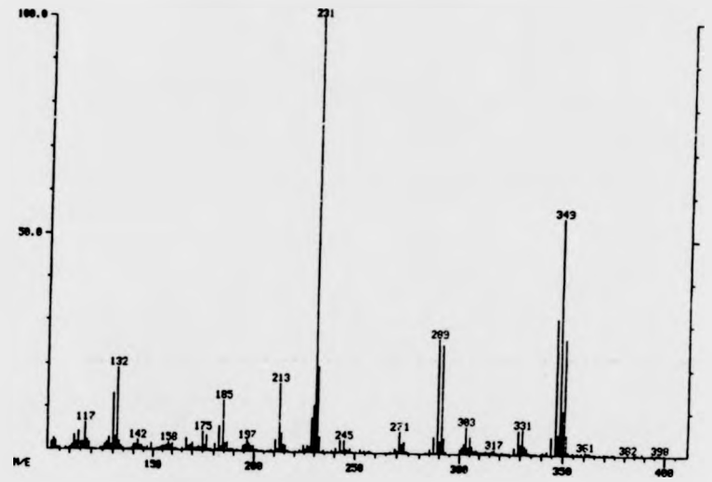


Fig. 6.D.2 The mass spectrum (c.i.) of the product mixture from the reaction of L-valyl-L-leucine with (R)-methyloxirane/(S)-[2,2-$^2H_2$]-methyloxirane

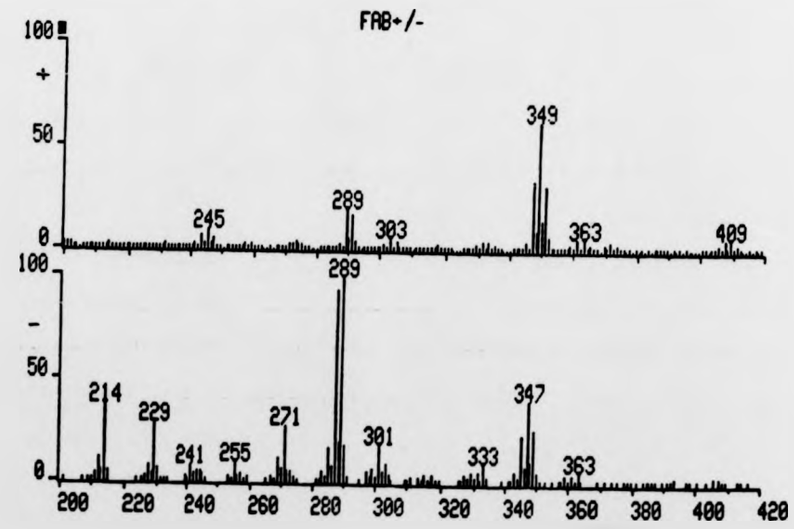
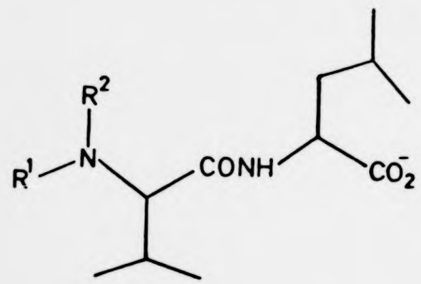


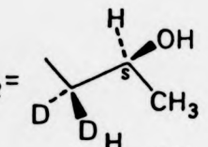
Fig. 6.D.3 The mass spectrum (f.a.b.) of the above reaction mixture

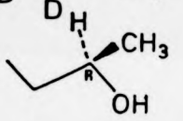
From a reaction allowed to proceed for 19h at 45°C, the products having Rf 0.52 and 0.46 were separated chromatographically and their structures assigned as the mono- and dialkylated dipeptides (139) and (140), respectively. The f.a.b. mass spectrum of the monoalkylated derivative showed peaks [m/z 303 (positive ion mode) and 301 (negative ion mode)] which suggest the formation of the methyl ester (143). Analogous peaks for the methylated dipeptide (144) were also observed [m/z 361, $M+H^+$ and 383, $M+Na^+$ (positive ion mode)]. These peaks are absent from the direct analysis of the product mixture and presumably arise from acid catalysed esterification on the silica plate during p.l.c.

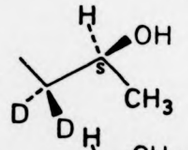
The reaction between L-valyl-L-leucine and the deuterated epoxide mixture (2a + 2c) in water at 45°C was analysed by c.i.m.s.. After two hours the mass spectrum showed peaks [m/z 289 (100%) and 291 (95%)] which indicate the formation of monoalkylated derivatives (145 and 146), and dialkylated derivatives (147-149) [m/z 347 (4.2%), 349 (7.5%) and 351 (4.2%)], respectively. As the reaction progressed the intensities of the dialkylated product peaks were observed to increase with respect to the monoalkylated derivatives [Fig. 6.D.2]. These results are in marked contrast to those obtained from the alkylation of (S)-valine methyl ester (89) with methyloxirane which show predominantly monoalkylation prior to dialkylation. The f.a.b. mass spectra for the reaction mixture is shown in Fig. 6.D.3.

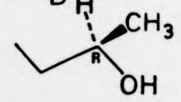
A 360 MHz ^1H n.m.r. spectrum of the diastereoisomeric dialkylated dipeptide derivatives from the reaction of L-valyl-L-leucine with the deuterated epoxide mixture [4 days, 45°C] was obtained. The

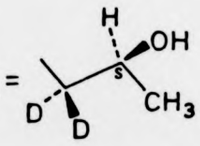
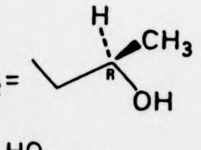


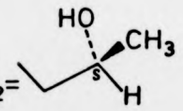
(145) $R_1 = H$ $R_2 =$ 

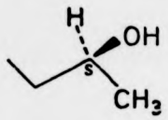
(146) $R_1 = H$ $R_2 =$ 

(147) $R_1 = R_2 =$ 

(148) $R_1 = R_2 =$ 

(149) $R_1 =$  $R_2 =$ 

(153) $R_1 = PhNHC(=S)-$, $R_2 =$ 

(154) $R_1 = R_2 =$ 

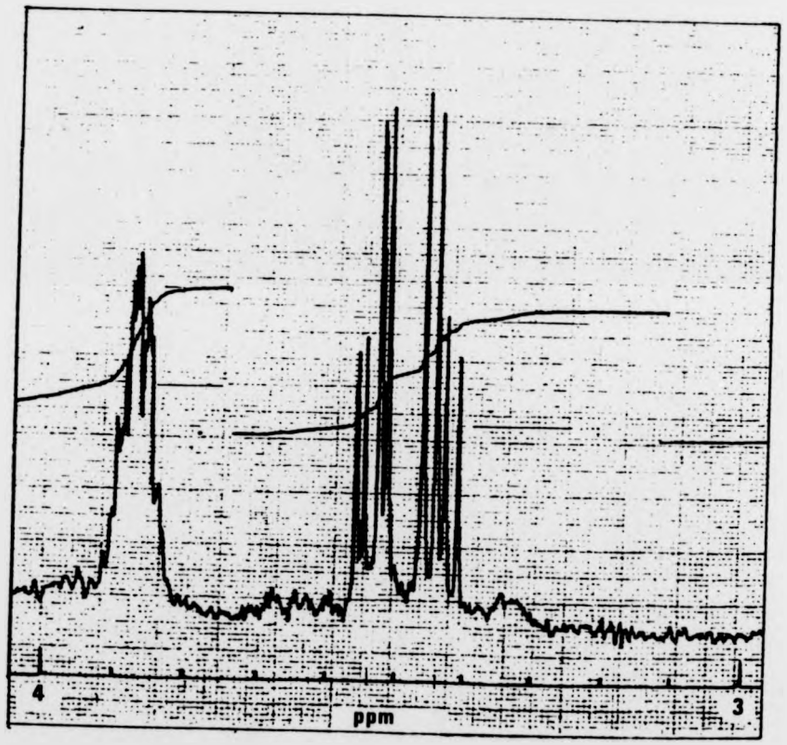
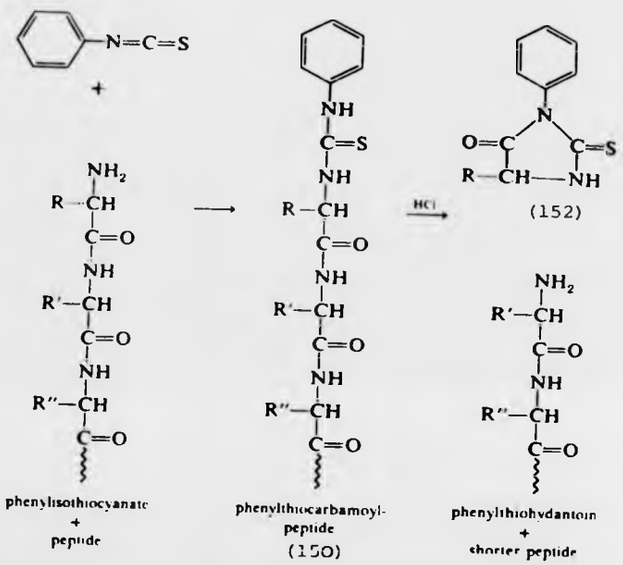


Fig. 6.D.4 The ^{1}H n.m.r. spectrum of the 2-hydroxypropyl-moiety of dialkylated L-valyl-L-leucine: L-valyl-L-leucine + (R)-methyloxirane/ (S)-[2,2-$^{2}H_{2}$]-methyloxirane

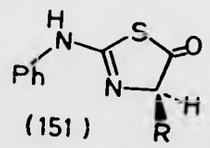
spectrum at low field (δ 0.8-1.4 ppm) was complex due to the diastereoisotopic nature of the observed functional groups, but at higher field clear double doublets (δ 3.54 and 3.87 ppm) were observed for the diastereotopic methylene protons of the (R)-2-hydroxypropyl moiety [Fig. 6.D.4]. By comparison of integral heights, the double doublet was found equivalent to one proton (the deuterated (S)-2-hydroxypropyl would pertain to the second proton but is 'unseen'). This result indicates no enantioselectivity on dialkylation and is in agreement with m.s. results.

6.E ENANTIOSELECTIVITY IN PROTEINS: MODEL STUDIES WITH L-VALYL-L-LEUCINE

To determine an enantioselectivity upon alkylation of the N-terminal amino-acid of a peptide or protein a method is required for analysing the alkylated derivatives. Analysis of the amino-acid composition of peptides by sequential cleavage of the N-terminal amino-acid has been developed by Edman and mass spectroscopic techniques for analysing small peptides up to ten amino-acids in length are available. These methods were adopted to analyse alkylated products using L-valyl-L-leucine as a model compound.



Scheme 6.E.1

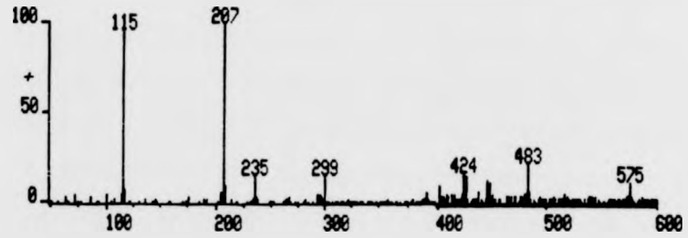


(151)

6.E.1 Edman Degradation

Edman[1] has shown that the N-terminal amino-acid of a peptide can be cleaved by treatment with isothiocyanatobenzene. The initial step in the degradation involves nucleophilic attack of the N-terminal amino group on the isothiocyanatobenzene to generate a phenylamino-thiocarbamyl derivative (150), which under acidic conditions cyclises to the thiazolinone (151). Fairwell attempted to identify this intermediate by mass spectroscopy but found it to rearrange thermally under the analytical conditions to the hydantoin (152)[2]. Treatment of (151) with acid causes it to rearrange to the thermodynamically more stable hydantoin (152) [Scheme 6.E.1].

Isothiocyanatobenzene was added to a sample of unpurified alkylated L-valine-L-leucine (139) in methanol (5h, RT). Analysis of the product mixture by f.a.b. m.s. showed the predominant product to be the N-phenylaminothiocarbamyl (P.A.T.C.) derivative (153) which was contaminated with thiohydantoin (101) and (S,S,)-N,N-di-(2-hydroxy-propyl)-L-valyl-L-leucine (154). The most probably source of fortuitous thiohydantoin contaminants is from the thermal decomposition of the P.A.T.C. derivative (153) under the m.s. conditions. Treatment of the P.A.T.C. mixture with 3 M HCl gave after chromatography the thiohydantoin (101) that was identified from its e.i.m.s.. Thermal decomposition of P.A.T.C. derivatives of small peptides by e.i.m.s. has been observed by Fairwell et al.[3]. He explored this phenomenon to enable sequencing of small quantities of peptides obtained from protein degradation[4], though a more versatile method involves the use of h.p.l.c. as the analytical tool[5,6].



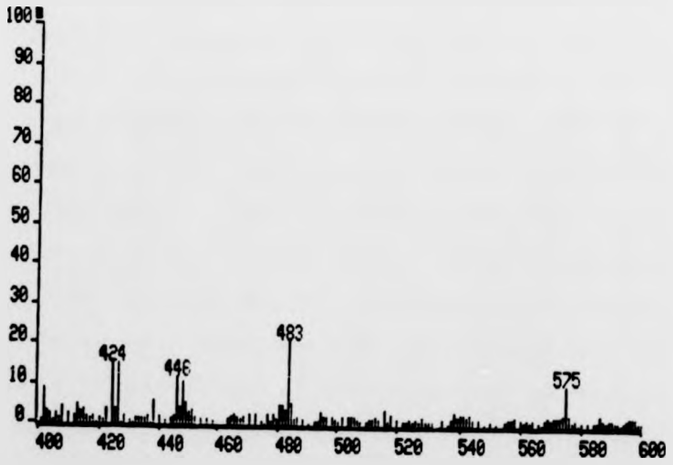


Fig. 6.E.1.1 The mass spectrum (f.a.b.) of the PATC derivatives from the reaction of L-valyl-L-leucine with (R)-methyloxirane/(S)-[2,2-$^2H_2$]-methyloxirane

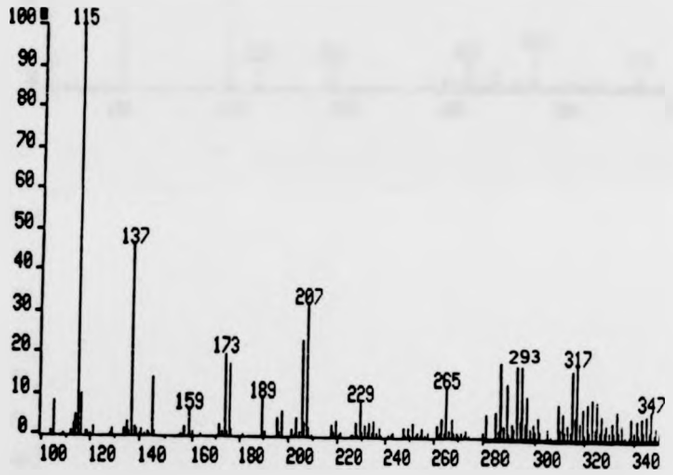
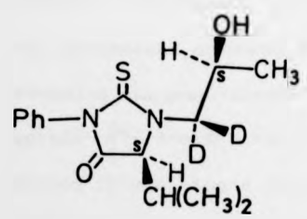
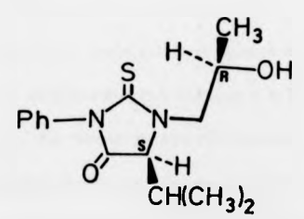


Fig. 6.E.1.2 The mass spectrum (f.a.b.) of the thiohydantoins from the reaction of L-valyl-L-leucine with (R)-methyloxirane/(S)-[2,2-$^2H_2$]-methyloxirane

The chromatographically purified P.A.T.C. derivatives (155) and (156) were obtained from the reaction mixture of L-valyl-L-leucine with the deuterated epoxide mixture (2a + 2c) and analysis by f.a.b. m.s. (positive ion mode) gave mass ions [m/z 424 (16%) and 426 (15.8%), $M+H^+$ for (155) and (156), respectively] and $M+Na^+$ ions [m/z 446 (12.7%) and 448 (11.0%) for (155) and (156), respectively] consistent with the expected products [Fig. 6.E.1.2]. The major products from the reaction mixture (Rf 0.6) were identified as the thiohydantoins (157) and (102) [m/z 293 and 295, $M+H^+$ ratio 1:1 for (157) and (102), respectively] [Fig.6.E.1.2]. Unexpectedly, the



(157)



(102)

^1H n.m.r. spectrum of the thiohydantoins (157) and (102) showed two sets of double doublets corresponding to the diastereoisotopic protons of the (R)-2-hydroxypropyl moiety (δ 3.25 and 4.3 ppm and δ 3.38 and 4.57 ppm). This phenomenon results from the epimerisation at the C-5 position to give two sets of diastereoisomeric products (Chapter 5, section D). Epimerisations of hydantoins have been

investigated by Dakin[7] and Bovarnick[8] and observed by Edman[9] when it was found that thiohydantoins synthesised from chiral amino-acids had small or zero optical rotations. Recently, Davies et al[10] have investigated this epimerisation using a chiral isothiocyanate derived from L-phenylalanine. From the results it was concluded that epimerisation occurs at the thiazolinone stage due to 'aromatization' within the 5-membered ring and that thiohydantoin epimerisation occurs on silica gel. These observations complement our results and we also found thermal racemisation of the thiohydantoin to occur (Chapter 5, section D). The ease by which epimerisation of these systems occurs precludes the use of n.m.r. spectroscopy (^{1}H, ^{13}C) to measure the enantioselectivities because the product ratio observed is dependent upon the stereochemistry about the C-5 carbon. This phenomenon enhances the usefulness of deuterated epoxides in assessing the enantioselectivity of a nucleophilic group of a peptide or protein towards an oxirane, because epimeric products derived from a single epoxide enantiomer have the same masses when analysed by m.s. and are therefore independent of the stereochemistry at C-5.

6.E.2 Chemical Modification for Mass Spectroscopy

The high polarity and zwitterionic character of peptides endows them with low volatility, which makes direct analysis by m.s. difficult, if not impossible. This obstacle has been overcome by simple chemical modification. The most commonly used methods involve derivatization to eliminate the basicity of the amino group(s) and acidity of the carbonyl group(s), or both. This has been

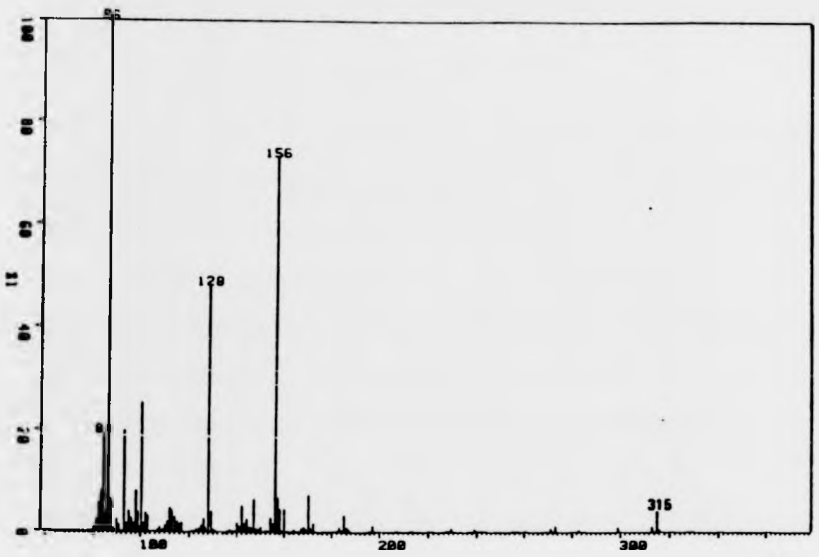


Fig. 6.E.2.1 The mass spectrum (e.i.) of N-acetyl-N,O-permethylated-L-valyl-L-leucine

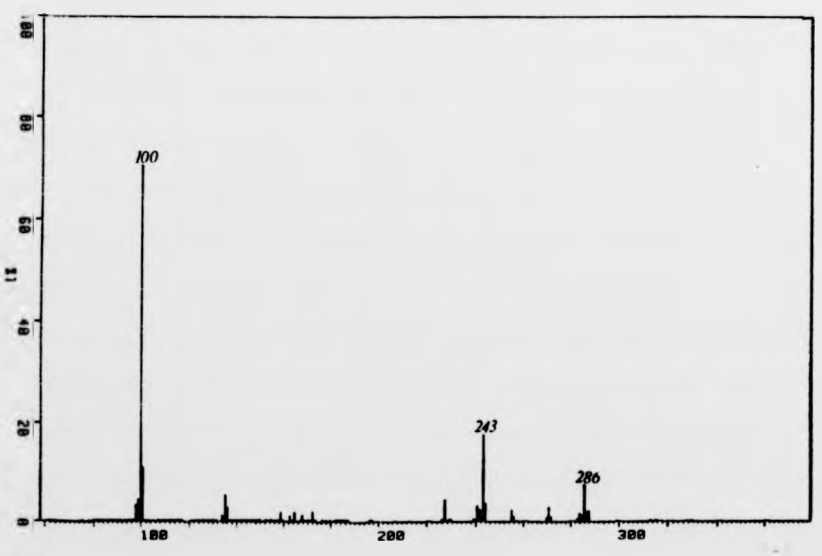
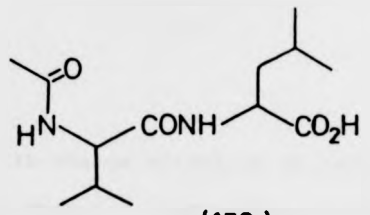


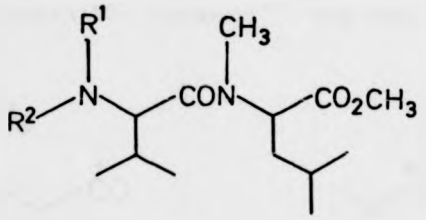
Fig. 6.E.2.2 The mass spectrum (e.i.) of N,O-permethylated L-valyl-L-leucine

achieved by conversion of the amino group to its N-acetyl-derivative and/or esterification of the carboxyl[11]. These modifications are the basis of all peptide derivatizations for m.s. analysis. Two general methods are available to enhance peptide volatility. These are the Biemann and N-acetylation-O,N-permethylation methods. The Biemann method[12,13] involves N-acetylation and esterification followed by reduction with lithium aluminium hydride or lithium aluminium deuteride. The reduction converts the ester into a primary alcohol, the amides into substituted ethylenediamine units and the N-acetylated groups into monoalkylamines. Reducible amino-acid side chain functionalities are also affected[14]. The second method involves simple N-acetylation followed by amide and carboxyl methylation which renders the peptide more volatile than the N-acetylated peptide ester[15]. This was our choice for the derivatization of the L-valyl-L-leucine analogues.

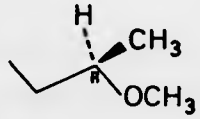
The N-acetylated dipeptide (158) was treated with sodium dimsyl in DMSO (1h, RT) and methylated by addition of methyl iodide (10 mol. equiv.). After 12h, analysis of the product by e.i.m.s. showed the major product to be the N-acetylated-O,N-permethylated derivative. Additional ions were also observed (m/z 170 and 142) suggesting possible C-methylation at the α-C-H positions, an artefact resulting from the presence of excess base and extended reaction times[15,16]. Using shorter permethylation times[17] [15 min, RT] L-valyl-L-leucine was derivatised to the N-acetyl-N,O-permethylated dipeptide (159). Analysis by e.i.m.s. [Fig. 6.E.2.1] gave ions identifiable with the desired product (m/z 315 [$M+H^+$], 156 and 128). The major fragmentation ion (m/z 156) results from the cleavage of the

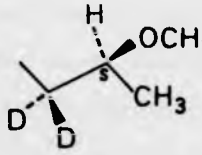


(158)

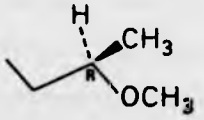


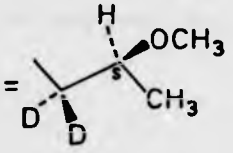
(159) $R_1 = CH_3$ $R_2 = COCH_3$

(145a) $R_1 =$  $R_2 = COCH_3$

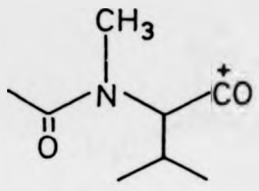
(146a) $R_1 =$  $R_2 = COCH_3$

(162) $R_1 = R_2 = CH_3$

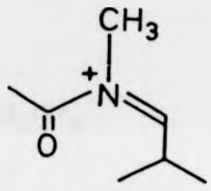
(145b) $R_1 = CH_3$ $R_2 =$ 

(146b) $R_1 = CH_3$ $R_2 =$ 

-CO-N(CH_3)-bond with charge retention on carbon to give the acylium ion (160). This ion readily loses CO to form the mesomerically stabilised acyliminium ion (161, m/z 128). Low intensity ions, m/z 299 and 271 are also observed for the loss of $CH_3I^{\cdot}$ and $CH_3COI^{\cdot}$ from the parent molecule (159). Hakamori[18], and Barber and Lederer[19] have



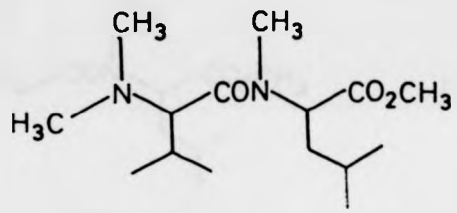
(160)



(161)

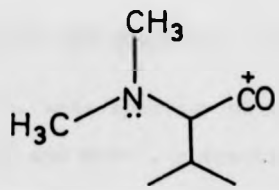
shown that an N-alkylated peptide can be analysed using the N-acetyl-O,N-permethylation method but when the mixture of alkylated dipeptides (145) and (146) were derivatised in a similar manner the e.i. mass spectrum obtained was inconsistent with the desired product (145a and 146a). To simplify the derivatization the N-acetylation was omitted and the dipeptide (138) was permethylated directly. One possible drawback to this technique is that the N-terminal amino group could be converted into its quaternary ammonium salt. This would reduce the volatility of the product, but methylation of a tertiary amine is known to be much slower than its initial formation and under the short reaction times used this should be

negligible. Analysis of the product from the permethylation of L-valyl-L-leucine by e.i.m.s. was consistent with the formation of the desired product (162) [Fig. 6.E.2.2]. A striking difference



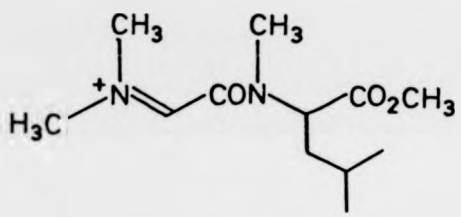
(162)

between the e.i. mass spectra of the N-acetylated-N,O-permethylated and permethylated dipeptides (159) and (162), respectively, is the absence of the acylium ion (163) from the permethylated product spectrum, whereas the acylium ion (160) is a major fragmentation ion of (159). This difference is attributed to the stabilisation

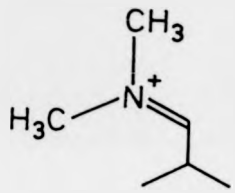


(163)

of the iminium ions (164 and 165) by the nitrogen lone-pair electrons of (162). These electrons are involved in amide resonance in (159) and are thus less readily available to stabilise acyliminium ion (161) formed by loss of CO from the acylium ion (160).



(164)



(165)

Direct permethylation of the alkylated dipeptides (145) and (146) and analysis by e.i.m.s. gave product ons which were inconsistent with the desired product.

6.F PRELIMINARY STUDIES INTO THE REACTION OF L-VALYL-L-LEUCYL-L-SERYL-L-PROLYL-L-ALANYL-L-ASPARTYL-L-LYSINE WITH THE DEUTERATED EPOXIDE MIXTURE (2a + 2c)

6.F.1 Analysis of the heptapeptide* (166)

A f.a.b. mass spectrum of the heptapeptide (166) gave m/z 729 and 741 ($M+H^+$ and $M+Na^+$, respectively) consistent with the mass ion of the proposed structure. Informative fragment ions were

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* We thank Mr M. Wraith, for his kind donation of heptapeptide (166).

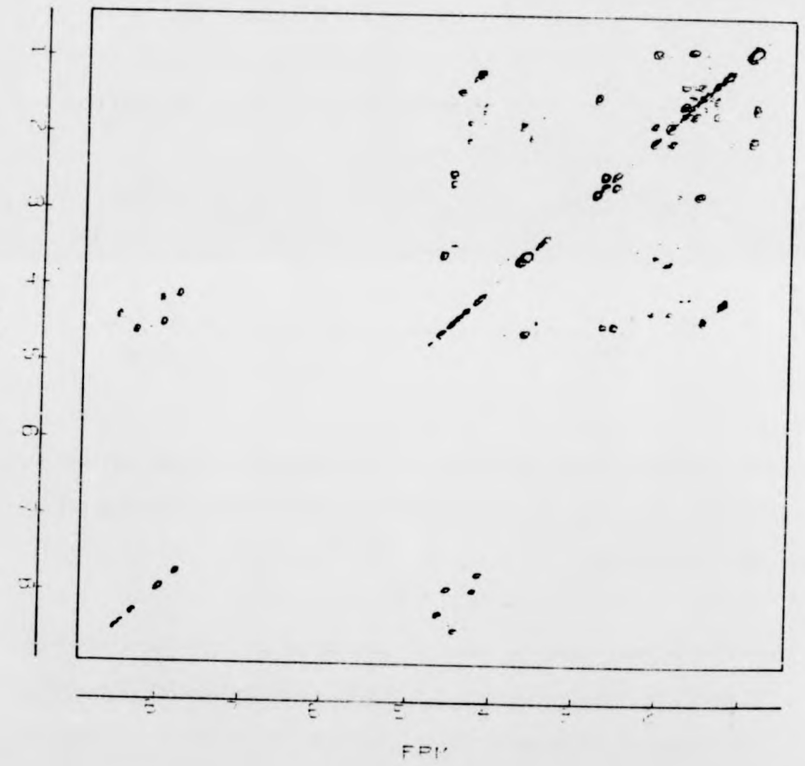


Fig. 6.F.1.1 The two-dimensional cross-relaxation spectrum of heptapeptide (166)

not observed. The stereochemical integrity was determined by ^{1}H n.m.r. spectroscopy by analysis of the two-dimensional cross-relaxation spectrum. This spectrum is shown [Fig. 6.F.1.1]. The normal one-dimensional Fourier Transformed ^{1}H n.m.r. spectrum of (166)is represented by the diagonal bisecting the two halves of the contour map. Two sets of cross peaks appear in symmetrical locations with respect to the diagonal.

In conventional Fourier Transformed (FT) n.m.r. spectroscopy the spectrum is obtained by applying a pulse or radio frequency (RF) power to a sample whose nuclear spin system is at equilibrium and collecting the FID information as the spins relax[21]. This information is converted into a classical spectrum by FT[21]. In two-dimensional FT n.m.r. the spin system is initially perturbed by either a continuous or pulsed radiation. This 'preparation' is followed by a time period (t_1) during which the spins respond to the pertubation by spin-lattice and spin-spin relaxation (spin-spin relaxation between closely associated nuclei is the predominant relaxation mode). Finally, during the detection period (t_2) the FID is collected to monitor the state of the system. The spectrum depends upon the 'preparation', evaluation and detection periods. By varying the time (t_1) and collecting a series of spectra the spin behaviour during t_1 and t_2 can be evaluated. Signals that are a function of the two independant time perameters can be converted into two frequency variables. The double Fourier Transformed spectrum is therefore dependent on two frequencies[22]. Exchange of energy between two components due to spin-spin relaxation is manifested by the cross-peaks between coupled components.

Fig. 6.F.1.2a

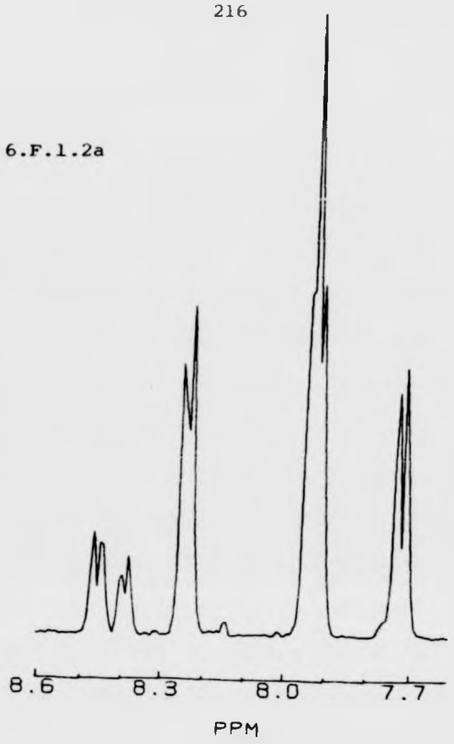


Fig. 6.F.1.2

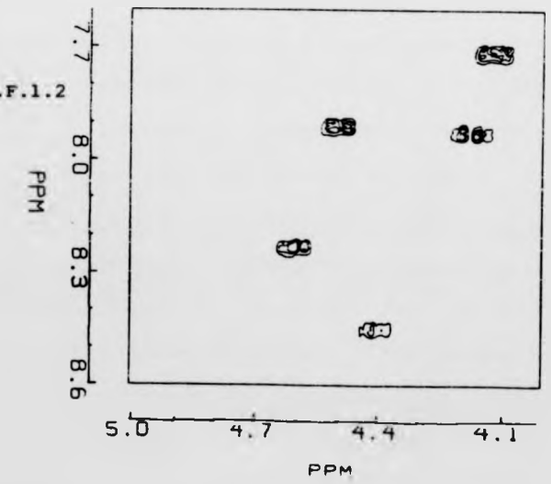


Fig. 6.F.1.2 The two-dimensional cross-relaxation spectrum showing the NH resonances of heptapeptide (166)

Fig. 6.F.1.2a The normal one-dimensional cross-relaxation spectrum of the above resonances

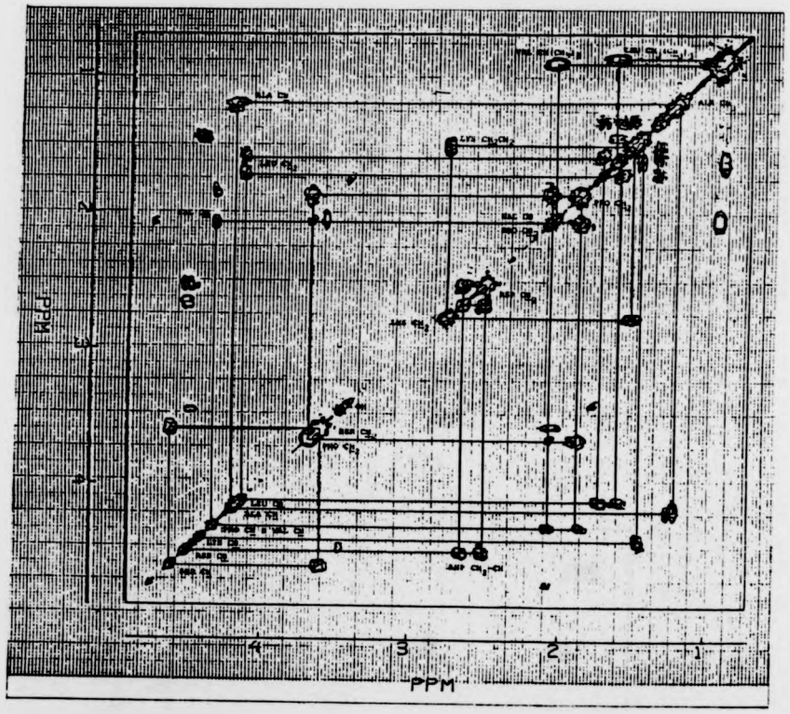


Fig. 6.F.1.3 A tentative assignment of the two-dimensional cross-relaxation spectrum of heptapeptide (166)

Two-dimensional n.m.r. has the advantage over the more conventional one-dimensional method in that spectra that are complicated by extensive and multiple overlap of spin multiplets can be analysed. A further application of two-dimensional n.m.r. is in the analysis of peptide and protein structure in solution. This is achieved by the use of two-dimensional nOe spectroscopy[23]. This is a spin-lattice relaxation phenomenon in which signal intensity changes when a nearby nucleus is irradiated. The nOe is therefore a through space effect, its magnitude is proportional to r^{-6} (where r is the distance between two nuclei) and hence the effect falls rapidly as r increases. The effect is not normally observed when $r > 0.3$ nm. The use of two-dimensional nOe has the advantage of providing a complete set of nOe's between all closely spaced groups in a macromolecule and avoids adverse effects arising from non-selective pre-irradiation of nearby resonances in a complex spectral region.

The two-dimensional cross relaxation spectrum of heptapeptide (166)is shown [Fig. 6.F.1.1] and indicated the sample to be essentially pure. Spurious resonances are observed in both the 400 MHz ^{1}H n.m.r. spectrum and two-dimensional plot indicating the trace contamination from a possible smaller peptide. The five amide NH resonances are clearly visualised by observation of the region δ 7.7-8.6 by δ 4.1-5.0 ppm and their individual assignments are shown [Fig.6.F.1.2]. The one-dimensional n.m.r. spectrum of these resonances is represented by the F2 axis [Fig.6.F.1.2a]. A tentative analysis of the remaining spectrum is shown [Fig.6.F.1.3]. Further analysis requires comparisons against smaller peptide moieties of (166).

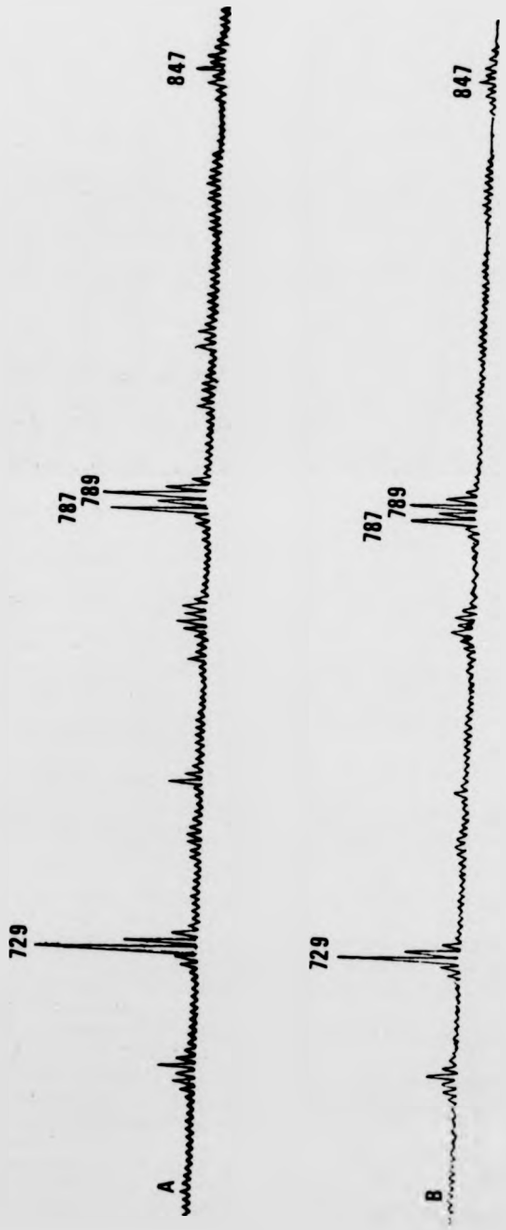


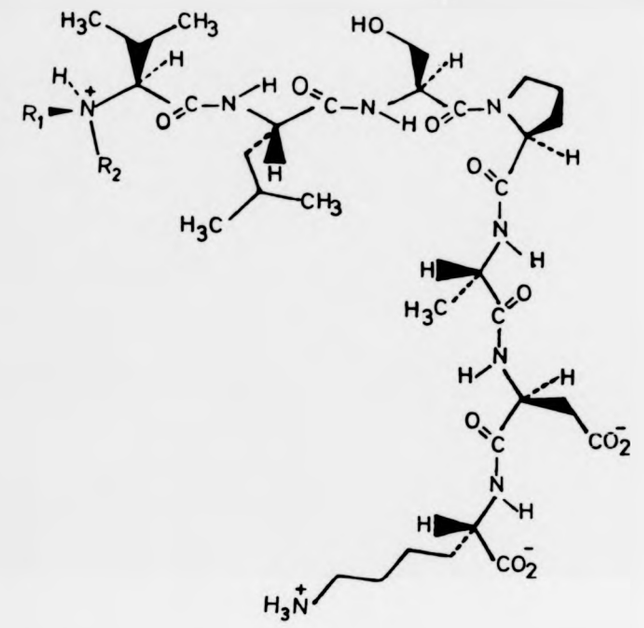
Fig. 6.F.2.1 The mass spectrum (f.a.b.) of the re mixture from the reaction of heptapeptide (166) with (R)-methyloxirane/(S)-[2,2-$^2H_2$]-methyloxirane:
A) after 2 days
B) after 4 days

6.F.2 Reactions of the heptapeptide (166)

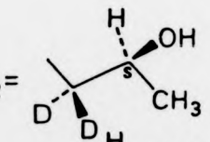
The heptapeptide (166) represents the N-terminal sequence of human haemoglobin α-chain and was chosen because this fragment is cleaved on enzyme digestion of globin with trypsin. Thus, heptapeptide (166) or its alkylated derivative is readily available from extracted human blood.

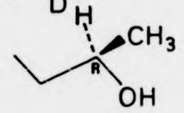
The reaction of heptapeptide (166) with the deuterated epoxide mixture was allowed to proceed in water at RT for 4 days. Aliquots were removed at two and four days and analysed by f.a.b. mass spectroscopy (positive ion mode) [Fig. 6.F.2.1]. From the two day sample mass peaks ($M+H^+$) were observed for the heptapeptide, monoalkylated heptapeptide (167 and 168) and dialkylated heptapeptide (169, 170 and 171) indicating the rate of dialkylation to be comparable to that of monoalkylation. Mass peaks for the monoalkylated derivatives (167 and 168) were observed (m/z 787 and 789) as a 'doublet' of approximately equal intensity and those for the dialkylated derivative (m/z 845, 847 and 849) as a 1:2:1 'triplet' Examination of the four day aliquot showed the relative concentrations of the mono- and dialkylated derivatives to have increased and the product ratios were essentially unchanged.

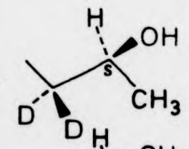
The heptapeptide (166) has two potential alkylation sites, the N-terminal amino group and the ϵ-NH_2 group of the C-terminal lysine. It is therefore conceivable that alkylation of the lysine ϵ-NH_2 could mask any enantioselectivity at the N-terminal amino function when examined by m.s.. Under the reaction conditions the reactivity of the ϵ-NH_2 group of lysine will be slow (Chapter 1, section 1.) and because it is relatively distant from a chiral centre, no

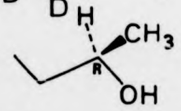


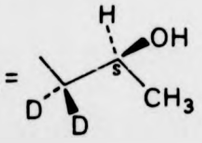
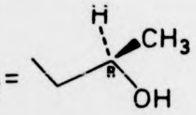
(166) $R_1 = R_2 = H$

(167) $R_1 = H$ $R_2 =$ 

(168) $R_1 = H$ $R_2 =$ 

(169) $R_1 = R_2 =$ 

(170) $R_1 = R_2 =$ 

(171) $R_1 =$  $R_2 =$ 

chiral recognition towards the enantiomers of methyloxirane will be observed. (It has been shown that for reactions of methyloxirane with nucleophilic groups relatively distant from a chiral centre the enantioselectivity is negligible, see Chapter 5.)

Alkylation at a second site (showing enantioselectivity or not) will render uncertain the validity of enantioselectivity observed in the alkylation at the first site. Our initial attempts to differentiate between possible alkylation of lysine and the N-terminal valine involved Edman type degradation and N-acetylation-O,N-permethylation (section 6.E.2) of the alkylated heptatpeptide. These reactions have thus far been unsuccessful but other methods are available (e.g. enzymic digestion and examination of the amino-acids either directly or post-derivatisation) which may prove valuable in this analysis.

The preliminary studies into the use of a deuterated epoxide mixture to determine enantioselectivities of biological macromolecules towards methyloxirane are encouraging. No enantioselectivity towards (R)- or (S)-methyloxirane was observed in the reactions of L-valyl-L-leucine (138) and heptapeptide (166) with this mixture . These results are in agreement with those obtained for the alkylation of L-valine methyl ester with (R)- and (S)-methyloxirane.

To test the usefulness of the deuterated racemates in assessing enantioselectivities a system was required in which a known enantioselectivity towards one antipode of methyloxirane is observed. It has been shown that when cob(I)alamin reacts with (rac.)-methyloxirane there is a preference for the (R)-isomer (R/S rate ratio = 3:1)[24].

The deuterated epoxide mixture was reacted with cob(I)alamin[‡] and we await the f.a.b. m.s. analysis of the product mixture.

The reaction of (2a + 2c) with L-valyl-L-leucine and Edman degradation to cleave the alkylated N-terminal amino-acid is promising, though difficulties have been reported in the cleavage of N-blocked terminal amino-acids of oligopeptides by this method[25]. We have also experienced difficulties with the alkylated heptapeptide. Chemical derivatisation of the dipeptide was successful but the derivatisation of the alkylated species (145 and 146) were not. Future work requires improvements to the purification procedures, possibly involving h.p.l.c. and/or g.c. prior to or post derivatisation before analysis.

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‡ We thank Miss R.M. Dixon for her assistance in performing this reaction.

6.G EXPERIMENTAL

6.G.1 Preparation of (S)-(+)-1,2-Propane-[2,2-$^2H_2$]-diol

L-(-)-Ethyl lactate (14 g, 0.12 mol) was reduced with lithium aluminium deuteride (5.5 g, 1.31 mol) in dry ether in the manner described for (S)-(+)-propane-1,2-diol[26] to give a colourless liquid (5.92 g, 63.2%), b.p. 58 60°C/0.7 mm; δ_H ($CDCl_3$) 1.24 (d, 3H, CH_3) and 3.85 ppm (q, 1H, CHOH); νmax (film) 3,350br, 2,979m, 2,955m, 2,880m, 2,095w, 1,450w, 1,377m, 1,090s and 970s cm^{-1}; m/z (c.i.) 112 ($M+NH_4^+$), 96 (M^+); $[\alpha]_D^{20}$ + 20.8° (c 7.5 in H_2O) [Found: C, 46.0; H, 10.4%. $C_3H_4D_2O$ requires C, 46.1; H, 10.4%]. Percentage incorporation estimated >98% (c.i.m.s.).

6.G.2 Preparation of (S)-(-)-2-Acetoxy-1-bromo-[1,1-$^2H_2$]-propane

(S)-(-)-2-Acetoxy-1-bromo-[1,1-$^2H_2$]-propane was prepared from (S)-(+)-1,2-propane-[2,2-$^2H_2$]-diol (3.9 g, 0.05 mol) in the manner described for (R)-(-)-2-acetoxy-1-bromopropane[26] to give a colourless oil (5.8 g, 60.8%), b.p. 57-59°C/10 mm; δ_H ($CDCl_3$) 1.30 (d, 3H, CH_3), 2.18 (s, 1H, $OCOCH_3$) and 5.03 ppm (q, 1H, CH); νmax (film) 2,990m, 2,940m, 2,170w, 1,740s, 1,448m, 1,372s and 935m cm^{-1}; m/z (c.i.) 185 and 187 (M^+, ratio 1:1) [Found: C, 32.9; H, 5.1%. $C_5H_7BrD_2O_2$ requires C, 32.8; H, 4.95%].

6.G.3 Preparation of the Deuterated Epoxide Mixtures:

(S)-(-)-[2,2-$^2H_2$]methyloxirane/(R)-(+)-methyloxirane and

(S)-(-)-[2,2-$^2H_2$]methyloxirane/(S)-(-)-methyloxirane

These were synthesised from equimolar mixtures of (S)-(-)-2-acetoxy-1-bromo-[1,1-$^2H_2$]-propane/(R)-(+)-2-acetoxy-1-bromopropane and (S)-(-)-2-acetoxy-1-bromo-[1,1-$^2H_2$]-propane/(S)-(-)-2-acetoxy-1-bromopropane in the manner described for (S)-(-)-methyloxirane[26], respectively.

(R)-,(S)-mixture: b.p. 34-35°C, (2.3 g, 78%); δ_H ($CDCl_3$) 1.31 (d, 6H, CH_3), 2.43 (dd, 1H, one of CH_2), 2.76 (dd, 1H, one of CH_2) and 2.99 ppm (m, 2H, CH); $[\alpha]_D^{20}$ 0.00° (c 5.79 in CCl_4).

(S)-,(S)-mixture: b.p. 34-35°C, (2.4 g, 81.3%); δ_H ($CDCl_3$) 1.31 (d, 6H, CH_3), 2.43 (dd, 1H, one of CH_2), 2.76 (dd, 1H, one of CH_2) and 2.99 ppm (m, 2H, CH); $[\alpha]_D^{22}$ -18.8° (c 5.67 in CCl_4).

6.G.4 The Reaction of L-Valine methyl ester with (S)-methyloxirane/(S)-[2,2-$^2H_2$]methyloxirane

Determination of the Secondary Isotope Effect

To L-valine methyl ester (13.3 mg, 1.01 x 10^{-4} mol) in dry methanol (0.5 cm^3) was added an equimolar mixture of (S)-methyloxirane/(S)-[2,2-$^2H_2$]methyloxirane (5.86 x 10^{-2} g, 68.2 μl, 1.01 x 10^{-3} mol) and the reaction incubated at 45°C for 5h. Volatiles were removed at reduced pressure (rotary evaporation) and the resulting oil was dissolved in dry methanol (0.5 cm^3) and the

alkylated derivatives purified by h.p.l.c. using a Gilson instrument (fitted with a variable wavelength UV detector) and a Partisil 5 ODS-3 column (250 mm x 4.6 mm i.d.) eluting isocratically with 30% (v/v) methanol in water at a flow rate of 1 cm^3/min. Monitoring at 195 nm a single product was obtained (retention time 34.75 min) which on analysis by c.i.m.s. (NH_3) gave m/z 190 and 192 [$M+H^+$, for (137)and (97), respectively], k_H/k_D = 1.02 ± 0.01 (see text).

6.G.5 Reaction of L-Valyl-L-leucine with the Deuterated Epoxide Mixture (2a + 2c)

To L-valyl-L-leucine (20 mg, 9.2 x 10^{-5} mol) in water (0.5 cm^3) was added deuterated epoxide mixture (2a + 2c, 63 µl, 91 9.2 x 10^{-4} mol) and the reaction heated to 45°C. Aliquots were removed, solvent was evaporated at reduced pressure (rotary evaporation) and the resulting oils analysed by c.i.m.s. (see text). After 4 days, a crystalline mixture of the dialkylated dipeptide was obtained which gave by ^1H n.m.r. spectroscopy: δ_H (360 MHz, D_2O) 0.84-1.30 (m, $CH(CH_3)_2$ and $CHOHCH_3$), 1.55-1.88 (m, $CH(CH_3)_2$, and $CH_2CH(CH_3)_2$), 3.54 (dd, diastereoisotopic CH_2CHOH, J = 6.9 and 11.4 Hz) 3.87 (dd, diastereoisotopic CH_2CHOH, J = 4.2 and 11.4 Hz) and 3.87 ppm (m, CHOH).

6.G.6 Formation of Thiohydantoins (157) and (102)

To L-valyl-L-leucine (20 mg, 9.2 x 10^{-5} mol) in water (1 cm^3) was added deuterated epoxide mixture (2a + 2c, 54.2 mg, 63 l, 9.2 x 10^{-5} mol) and the reaction heated to 45°C. After 8h the volatiles were evaporated at reduced pressure (rotary evaporation) to give a colourless oil that was dissolved in dry methanol and stirred overnight with isothiocyanatobenzene (50 mg, 42 μl, 3.7 x 10^{-4} mol) at RT. The product was purified by p.l.c. (silica gel PF_{254}, CH_2Cl_2-MeOH, 9:1 v/v, Rf 0.6) and analysed by m.s. and ^1H n.m.r. spectroscopy (see text).

6.G.7 N-Acetylation and O,N-permethylation of L-valyl-L-leucine

L-Valyl-L-leucine (10 mg, 4.63 x 10^{-5} mol) was dissolved in methanol-acetic anhydride (4:1, v/v). After 10h volatiles were evaporated at reduced pressure (0.2 mm/RT). To the N-acetylated dipeptide (2.0 mg, 7.4 x 10^{-6} mol) in DMSO was added 0.33 M sodium dimethyl sulphinyl carbanion in DMSO (134 μl). After 1h the reaction mixture was quenched with methyl iodide (0.315 g, 0.14 cm^3, 2.2 x 10^{-3} mol) and after a further 15 min water (1 cm^3) was added. The product was extracted into CCl_4 and the organic layer was washed with water (2 x 1 cm^3), was dried ($MgSO_4$), was filtered and evaporated at reduced pressure (rotary evaporation) to leave a colourless oil which gave a single spot by t.l.c. (silica gel, CH_2Cl_2 MeOH, 20:1, v/v), Rf 0.28, m/z (e.i.) 314 (M^+). 299 ($M-CH_3^+$), 271 ($M-COCH_3^+$), 255 ($M-CO_2CH_3^+$), 156 (see text) and 128 (see text).

6.G.8 Permethylation of L-valyl-L-leucine

To a suspension of L-valyl-L-leucine (2.0 mg, 8.7 x 10^{-6} mol) in DMSO (0.5 cm^3) under nitrogen was added 0.6 M sodium dimethyl sulphinyl carbanion in DMSO (0.116 cm^3, 6.96 x 10^{-5} mol) and the reaction allowed to stand for 1h. Methyl iodide (0.315 g, 0.138 cm^3, 2.2 x 10^{-3} mol), and after 10 min, water (5 cm^3) were added and the product extracted into CCl_4 (10 cm^3). The CCl_4 layer was washed thrice with water (3 x 5 cm^3), was dried ($MgSO_4$), was filtered and solvent removed at reduced pressure (rotary evaporation) and the product analysed by e.i.m.s.; m/z 286 (M^+), 271 ($M-CH_3^+$), 243 ($M-C_3H_7^+$), 227 ($M-CO_2CH_3^+$), and 100 (see text).

6.G.9 Reaction of L-Valyl-L-leucyl-L-seryl-L-prolyl-L-alanyl-L-aspartyl-L-lysine (166) with the Deuterated epoxide mixture (2a + 2c)

To heptapeptide (166, 0.5 mg, 7 x 10^{-7} mol) in water (0.5 cm^3) was added deuterated epoxide mixture (20 mg, 23.3 μl, 3.4 x 10^{-4} mol) and the reaction allowed to stand at RT. Aliquots were removed at 2 days and 4 days and the product mixture analysed by f.a.b. m.s. (+ive ion mode); m/z (f.a.b.) 729 and 741 ($M+H^+$ and $M+Na^+$ of heptapeptide, respectively), 787 and 789 ($M+H^+$, monoalkylated heptapeptide), 809 and 811 ($M+Na^+$, monoalkylated heptapeptide, ratio 1:1) and 845, 847 and 849 ($M+H^+$, dialkylated heptapeptide, ratio 1:2:1, respectively).

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