SYNTHETIC AND BIOSYNTHETIC STUDIES

ON SULPHUR-CONTAINING HETEROCYCLES

A Thesis presented in part fulfilment of the requirement for the Degree of Doctor of Philosophy

by

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I thank the J.D. Loudon Bequest for a Studentship.

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Part 2 FLUORINATED DERIVATIVES AS BIOSYNTHETIC PRECURSORS OF GLIOTOXIN ANALOGUES

SUMMARY

This thesis records an investigation into synthetic and biological aspects of sulphur chemistry.

A study of thioaldehyde S-oxides (sulphines), a family of labile, dienophilic heterocumulenes, RCH=S=O, was undertaken. The feasibility of generating the reactive (Z)- and (E)-isomers of ethyl thioxoacetate S-oxide, $EtO_2C.CHSO$, by retro-Diels-Alder reactions was successfully demonstrated for the first time. The required precursors were obtained by S-oxidation of the cycloadducts formed from the thioaldehyde, $EtO_2C.CHS$, and anthracene, cyclopentadiene, and the alkaloid thebaine.

The (E)-sulphine was released from its anthracene cycloadduct at 60 °C and from its cyclopentadiene or thebaine cycloadducts at 80 °C. Higher temperatures were necessary to generate the (Z)-sulphine; 80 °C for the anthracene and 111 °C for the cyclopentadiene cycloadduct. At the highest temperature (111°C) signs of Z-E isomerism were observed. However, the (E)-sulphine, formed at 60-80 °C, was trapped by conjugated dienes to give trans-sulphoxides exclusively.

When the (Z)- or (E)- sulphine was trapped with cyclopentadiene, the resulting cycloadducts were exo-S-oxides. Cycloadducts of 2,3-dimethylbuta-1,3-diene were transformed via a Pummerer rearrangement to a thiapyran which, on oxidation with iodine yielded a novel thiopyrylium salt.

The biological studies centred on the biosynthesis of gliotoxin, a member of the sulphur containing dioxopiperazine metabolites produced by the fungus Gliocladium virens. Later steps in the biosynthetic pathway involve ring closure between the sulphur bridged dioxopiperazine and a proposed arene oxide, which arises from the known precursor cyclo-(L-Phe-L-Ser).

A series of analogues, cyclo-(L-Ala-L-Fluoro-Phe), substituted with fluorine at the o, m, and p positions were prepared and fed to the fungus, both unlabelled and labelled with ^{14}C. Their metabolism was followed by ^{19}F n.m.r. spectroscopy and radio chromatography. The o- and p fluorinated analogues were transformed into a range of fluorinated metabolites, but the m-fluorinated analogue was not. A stepwise mechanism for arene oxide formation is proposed to explain these results.

ABBREVIATIONS USED IN THE TEXT

n.m.r.	=	nuclear magnetic resonance.
p.p.m.	=	parts per million.
t.l.c.	=	thin layer chromatography.
u.v.	=	ultraviolet.
i.r.	=	infra red.
mcpba	=	m-chloroperbenzoic acid.
DMF	=	N,N-dimethylformamide.
Phe	=	phenylalanyl.
Ala	=	alanyl.
Gly	=	glycyl.
Ser	=	seryl.
Tyr	=	tyrosyl.
D	=	deuterium.
g.c.	=	gas chromatography.
g.c.-m.s.	=	gas chromatography mass spectrometry.

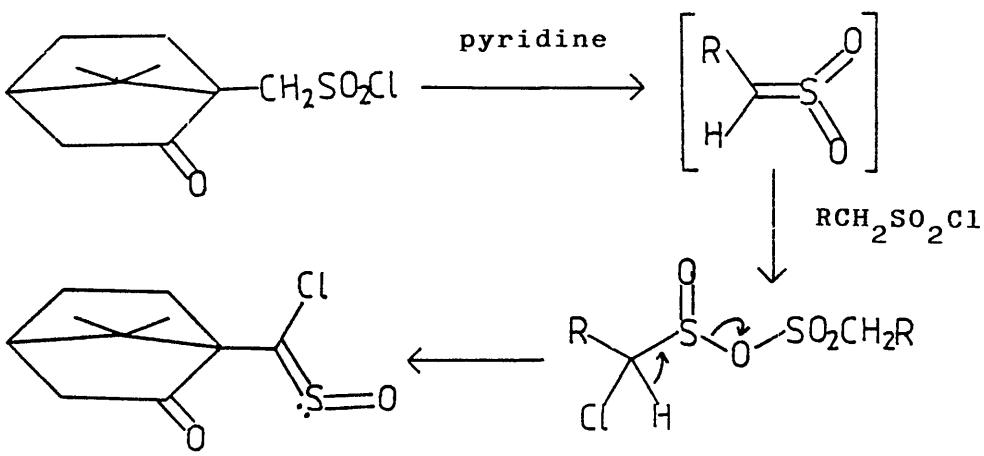
PART 1 GENERATION OF THIOALDEHYDE S-OXIDES (SULPHINES) BY RETRO-DIELS-ALDER REACTIONS

Chapter 1 Introduction

1.1 General

Heterocumulenes with the general formula XYC=S=O are commonly called sulphines. The name for these thiocarbonyl oxides was coined to express the relationship with thiocarbonyl dioxides ($XYC=SO_2$), known as sulphenes. It is unfortunate that this nomenclature does not follow the usual pattern for sulphur compounds, namely sulphenyl (RSX), sulphinyl (RSOX), and sulphonyl (RSO_2X).

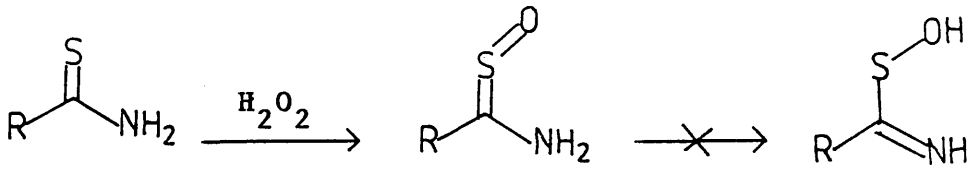
The first stabile sulphine (1) was prepared in 1923 [2] by a curious reaction between camphor-10-sulphonyl chloride and pyridine (Scheme 1). The reaction was reinvestigated [3] 40 years later, leading to the discovery that syn (Z) and anti (E) isomers can exist independently with a significant barrier to interconversion. A single isomer, assigned as Z was produced in this case. Thus sulphines, like SO_2, are bent, due to the sulphur lone pair. The observation [4] that the yield of chlorosulphine (1) was almost doubled by the addition of p-toluenesulphonyl chloride strongly supports the mechanism involving a sulphene intermediate.



Scheme 1

(1)

In the intervening years sulphines seldom appeared in the literature; one significant exception was the synthesis of thioamide S-oxides[5] by oxidation of thioamides. The

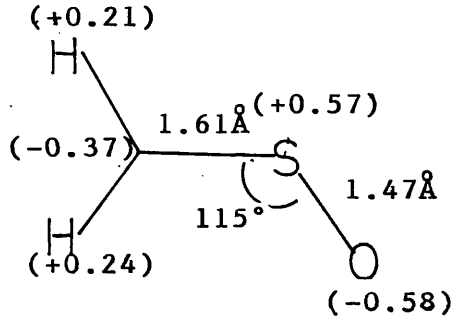


R=Ph,Bn (3) (2)

original workers [5] in 1938 suggested the imino sulphenic acid structure (2); the correct sulphine tautomer (3) was not deduced [6] until the early 1960's. Since then work in the area has proceeded rapidly with the publication of several reviews[7-11]. The most recent comprehensive review in 1982[11] contained 185 references.

Structure and Bonding

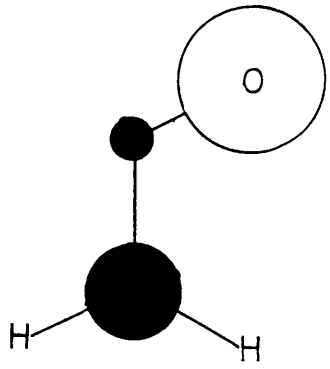
The structure of sulphine (4) itself, the simplest member of the family has been determined by microwave spectroscopy[12]. The C-S bond length is almost identical to



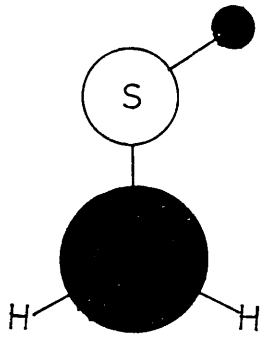
(4)

(Calculated charge distribution shown in parenthesis)

that of thioformaldehyde while the S-O bond length is equal to that in dimethylsulphoxide. The MO's are π in



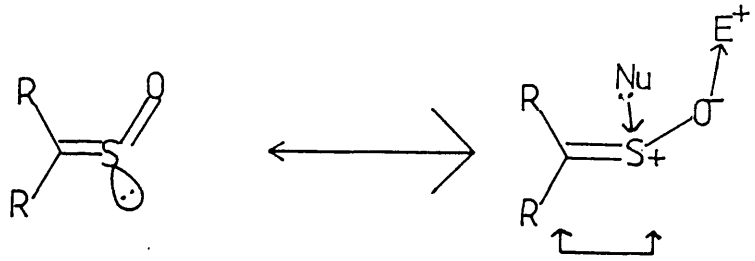
HOMO



LUMO

character[13] with a node close to oxygen in the HOMO and opposite coefficients at carbon and oxygen.

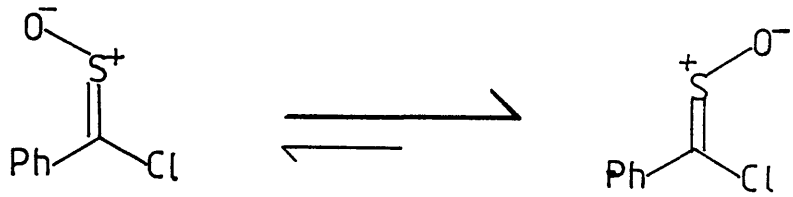
As in thiocarbonyl compounds, the weak overlap between the C-2p and S-3p orbital gives rise to a reactive double bond and sulphines react typically as dienophiles. Reaction with nucleophiles usually occur at sulphur, and with electrophiles at oxygen[10] (Scheme 2). The observed physical and chemical properties of sulphines favour considering them as oxidised thiocarbonyl compounds rather than heterocumulenes. In general the dipolar resonance form of the S-oxide, as commonly used for sulphoxides, better reflects their nature.



Cycloaddition

Scheme 2

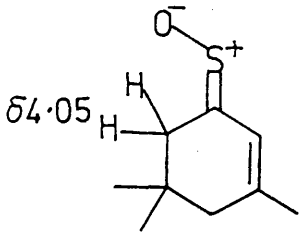
Although it has long been realised that interconversion between E and Z forms could, in principle, take place, this isomerisation has been little studied. The conversion of (E)-chlorophenylsulphine[14] (5) into the thermodynamically more stable (Z)-form (6) had a half-life of 0.75h in refluxing toluene and 8h in refluxing carbon tetrachloride. In general, when stable sulphines are isolated Z - E isomerisation does not occur at ambient temperatures.



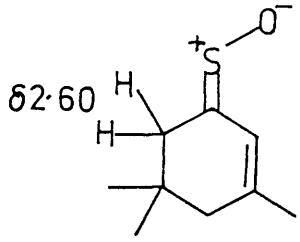
(E) (5)

(Z) (6)

The assignment of E or Z stereochemistry has been based on n.m.r. evidence. Sulphines have a similar anisotropic deshielding cone to nitrones[15]. The effect is due to the SO group; the CS portion produces a negligible effect[16]. In the (E)-isomer (7)[17] the proton syn to the SO group resonated downfield from the anti-proton in the (Z)-isomer (8).

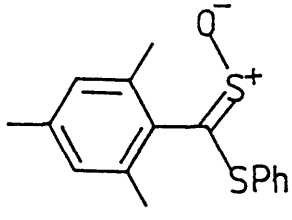


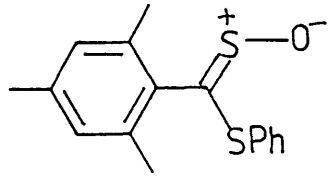
(7) (E)



(8) (Z)

Lanthanide shifts (LIS) and aromatic solvent induced shifts (ASIS) have also been used to determine the configuration. Lanthanide shifts are calculated as, $\Delta\delta$ (LIS)=$\delta[CDCl_3$ + 0.3 equiv. $Eu(DPM)_3]-\delta(CDCl_3)$[20] and aromatic solvent induced shifts (for benzene) as $\Delta\delta(ASIS)=\delta(CDCl_3)-\delta(C_6D_6)$. In the examples (9) and (10)[18] the lanthanide complexes to the oxygen of the CSO portion. A larger downfield shift is observed for the methyl protons closer in space to the oxygen i.e. the syn-position of the E-isomer (9). Aromatic solvents such as benzene associate with the positive end of the SO dipole. The ring current leads to a higher upfield shift of the o-methyl signal in the (Z)-isomer (10).





	(9) (E)	**(10) (Z)**
Δδ (LIS)	1.40	0.96
Δδ (ASIS)	0.07	0.18

Unstable sulphines are usually trapped as Diels-Alder cycloadducts; the geometry of the diastereomeric sulphoxides produced are determined by the same n.m.r. techniques and will be discussed in due course.

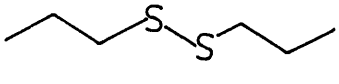
An idea of the general stability of sulphines would be advantageous before discussing their synthesis and reactions in detail. Aromatic sulphines are often shelf stable compounds and are considerably less labile than their aliphatic counterparts. The latter, although isolable, decompose at low temperatures and are usually trapped as adducts with dienes or are allowed to react with nucleophiles.

A hetero substituent at the sulphine carbon atom, e.g. Cl, S, SO, SO_2, P, NHR, enhances stability. Sulphines are usually more stable than their corresponding thiocarbonyl compounds.

1.2 In vivo Synthesis of Sulphines:

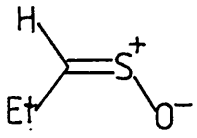
The onion lachrymatory factor

The peculiar ability of the onion (Allium cepa) to bring tears to the eyes of those who would cut it open must surely have been noticed since the dawn of civilisation, when these plants were first cultivated. The suggestion that sulphur compounds might be responsible was first made in 1892[19]. The steam distillation of 5000kg of onions produced a variety of compounds including propionaldehyde and dipropyl disulphide (11)[20]. Although compounds such as (11) were remarkably pungent they were not lachrymatory. It

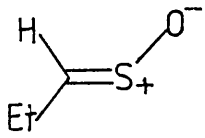


(11)

required extraction of onions at 0 °C with Freon to isolate the onion lachrymatory factor (LF) [21] as a mixture of (Z)-(12) and (E)-propanethial S-oxide (13)[21]. N.m.r.[22] and microwave[23] spectroscopic studies have shown that the Z isomer predominates in the ratio 19:1 (Z:E).



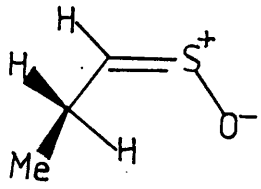
(12) (Z)



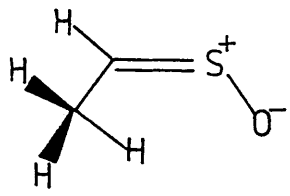
(13) (E)

The precursor was identified as (+)-S-(1-propenyl)-L-cysteine sulphoxide (14) (Scheme 3)[24]. It was proposed[23] that the onion allinase enzyme converts (14) via a pyridoxal phosphate dependent process to the sulphenic acid (15). The latter rapidly rearranges into the lachrymatory factor via a [1.4] sigmatropic rearrangement.

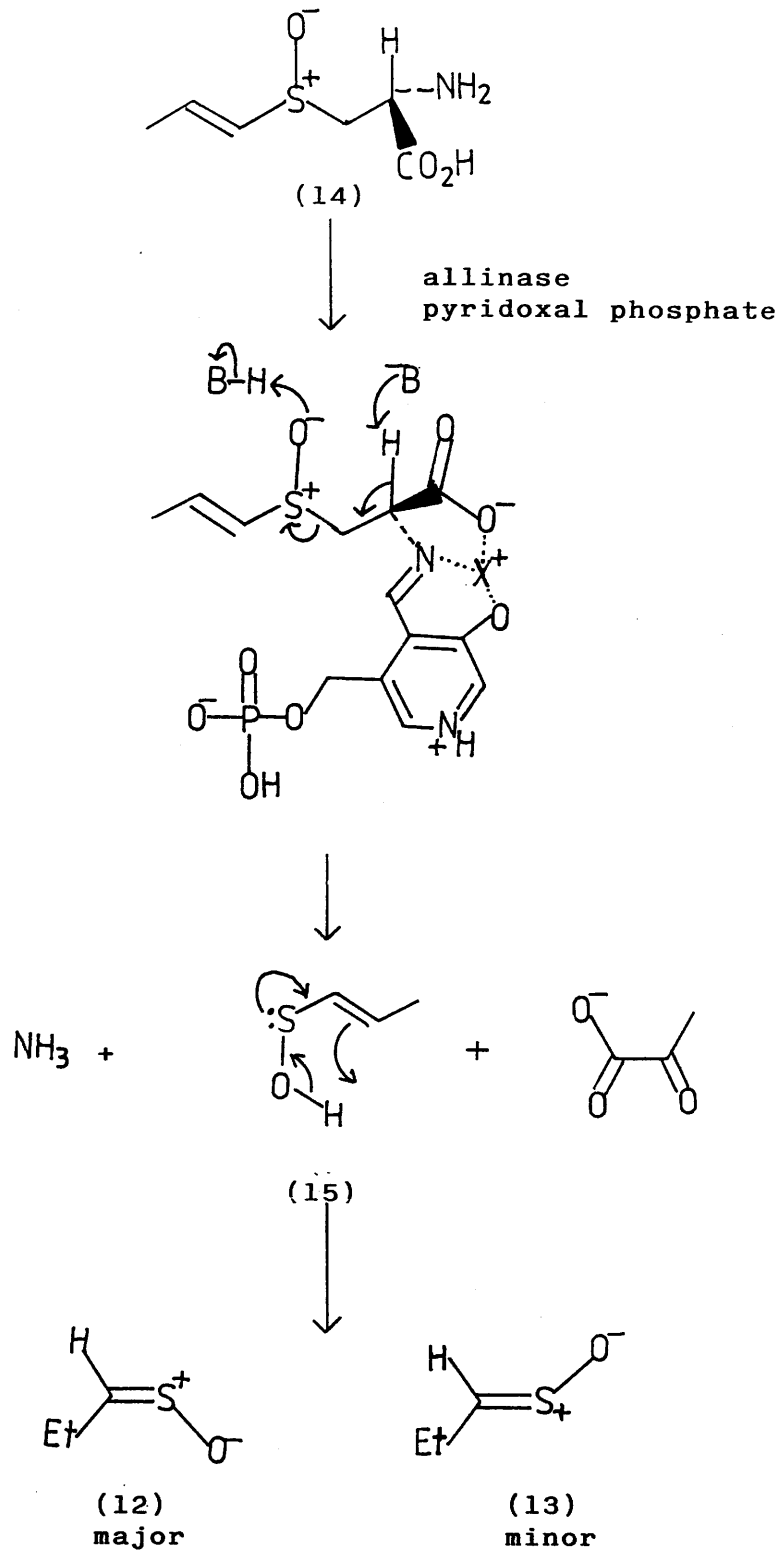
The predominance of the (Z)-isomer (12), presumably the less stable isomer, has been the area of some discussion. Microwave studies[23] on (12) revealed that the preferred conformation is "syn staggered" as shown in (16).



(16)

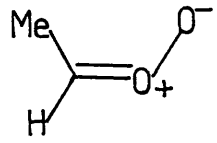


(17)



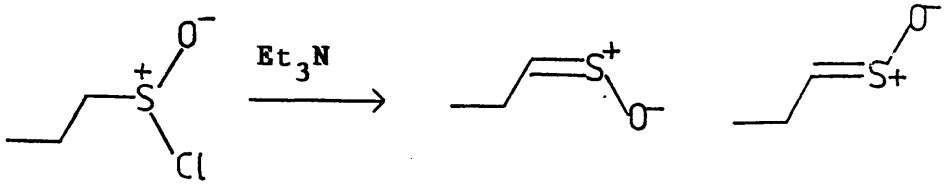
Scheme 3

Calculations (using Hartree-Fock theory) on the related ethanethial S-oxide (17) show that the given conformation (17) is more stable than the corresponding (E)-isomer by 7.1kJmol^{-1} [10]. It has been suggested that the steric repulsion in the (Z)-form is counterbalanced by a σ - stabilization between the oxygen and the hydrogen syn to the SO group.[22] Similar results have been obtained from calculations on the isoelectronic carbonyl oxide (18)[25].



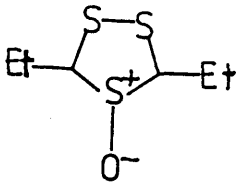
(18)

The lachrymatory factor has also been synthesised in the 'test tube'. Low temperature dehydrochlorination of the sulphinyl chloride (19) led again to the predominance of the (Z)-isomer (12) (98%)[22]. Flash vacuum pyrolysis (FVP) of



(19) (12) (Z) major (13) (E) minor

the compounds (20-22)[26] also led to the detection of the (Z)-sulphine (12).



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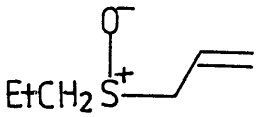
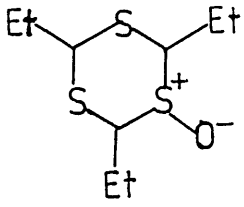


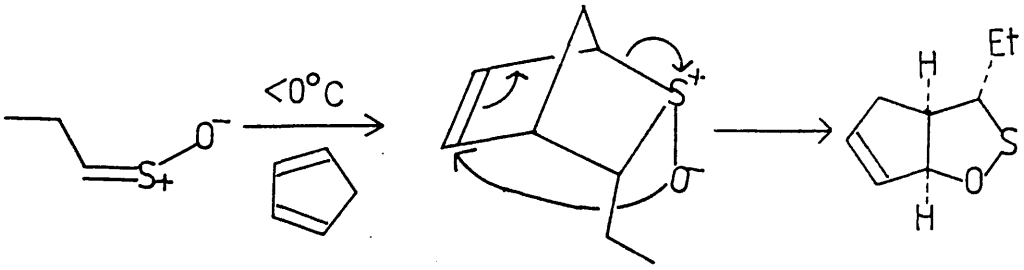
(21)



(22)

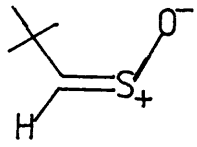
In conclusion the (Z)-preference in the onion lachrymatory factor appeared to arise from kinetic control i.e., the concerted [1,4] sigmatropic hydrogen shift of the sulphenic acid (15). However, the same isomer was synthesised by a variety of unrelated chemical methods and calculations have shown the (Z)-isomer to be the most stable. Thus thermodynamic control cannot be ruled out in the formation of (Z)-propanethial S-oxide (12).

To aid characterisation of the lachrymatory factor (12), cyclopentadiene was added to a solution of this sulphine at low temperature. A single cycloadduct (23) was obtained[27]; this strong endo preference in cycloaddition to cyclopentadiene has been observed with other sulphines. The adduct (23) was not itself stable and underwent a facile [2,3] sigmatropic rearrangement, even at 0 °C, to give the unusual sultene (24)[27].



(23) (24)

The mechanism of the lachrymatory action of the simple sulphines is not yet fully understood. There is strong evidence that it is not just a generalised irritation caused by decomposition products such as sulphuric acid. With increasing steric bulk on the alkyl chain, lachrymatory effects drop off significantly and the ^{t}butyl derivative (25) showed no effect[22].

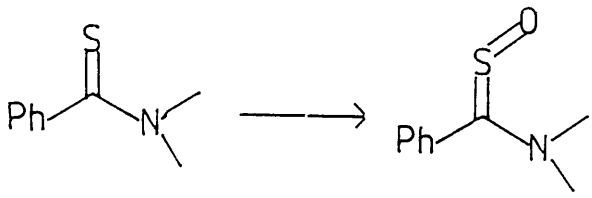


(25)

1.3 The Non-Enzymic Synthesis and Reactivity of Sulphines

1.3.1 Oxidation of Thiones

In general, when a stable thiocarbonyl compound is available, treatment with H_2O_2 or a peracid often leads to a stable sulphine. The thioamide (26) was oxidised with hydrogen peroxide to the sulphine (27)[28].

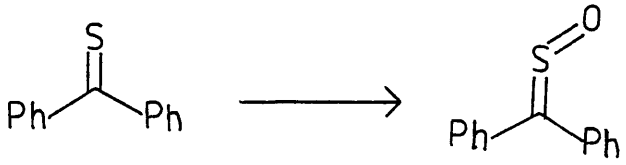


(26) (27)

When an NH group is present, internal hydrogen bonding offers a considerable stabilising effect. This was demonstrated by the increased stability of thioformamide S-oxide (28)[29] over the N,N-dimethyl derivative (29). Stable thioketones such as thiobenzophenone (30)[30] and non-enethiolisable, aliphatic thiones e.g., di-^{t}butylthioketone

(28) (29)

(31)[31] were readily oxidised to sulphines with m-chloroperbenzoic acid in a similar manner to the oxidation of sulphides.



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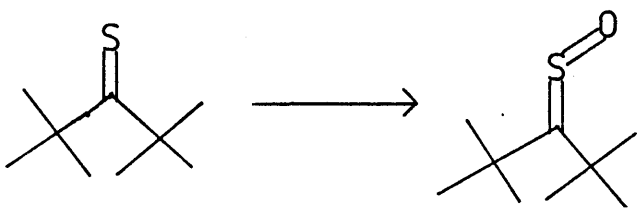
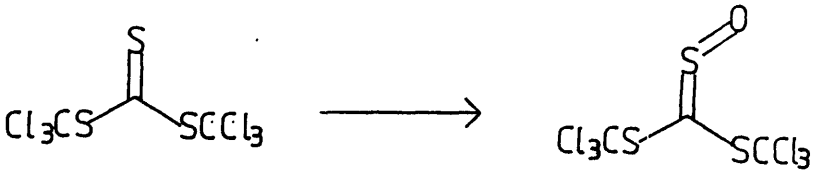


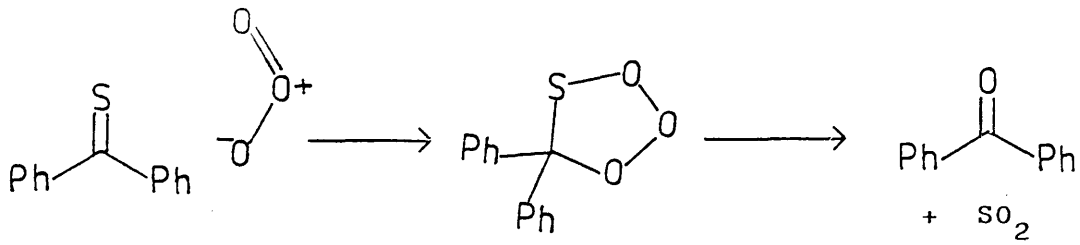
(31)

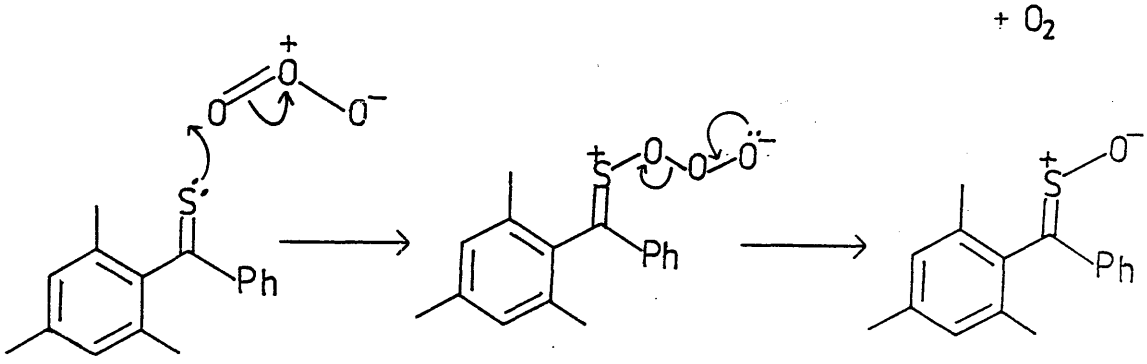
Treatment of thiocarbonates such as the crystalline compound (32) with peracids also give stable sulphines. It is worth noting that the singly bonded sulphur atoms were not oxidised[32]. Ozone has been employed (Scheme 4) as an



(32)

alternative to peracids with hindered substrates. Unhindered thioketones gave rise to the corresponding carbonyl compound while thiobenzophenone yielded a sulphine. The rationale for the different behaviour was as follows: unhindered substrates undergo a cycloaddition reaction to form an 'ozonide' which breaks down producing SO_2 and a ketone *via* a Criegee type mechanism; with hindered thioketones, nucleophilic attack of sulphur on ozone occurs, followed by elimination of oxygen[33].

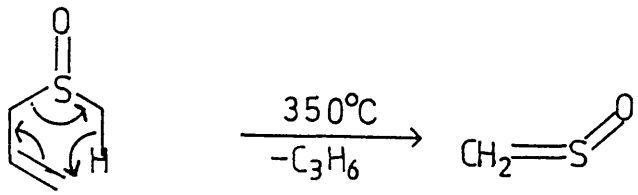




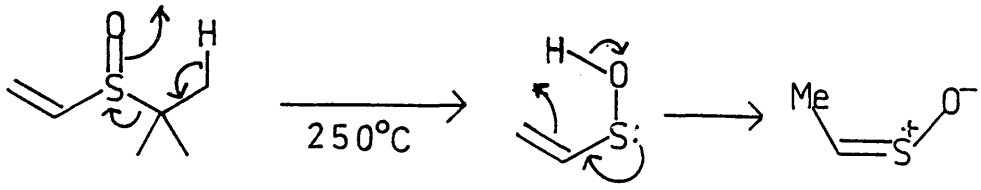
Scheme 4

1.3.2. Thermolysis

The flash vacuum pyrolysis technique (FVP) has been employed on several precursors to prepare sulphines. The thermal (FVP) 'retro-ene' reaction of allyl methyl sulphoxide (33) gave (34)[34]; Cope elimination of isobutene from the vinyl^{t}butyl sulphoxide (35) gave vinyl sulphenic acid (36), which rearranged to (17), in a similar manner to the *in vivo* production of the LF (12)[22].



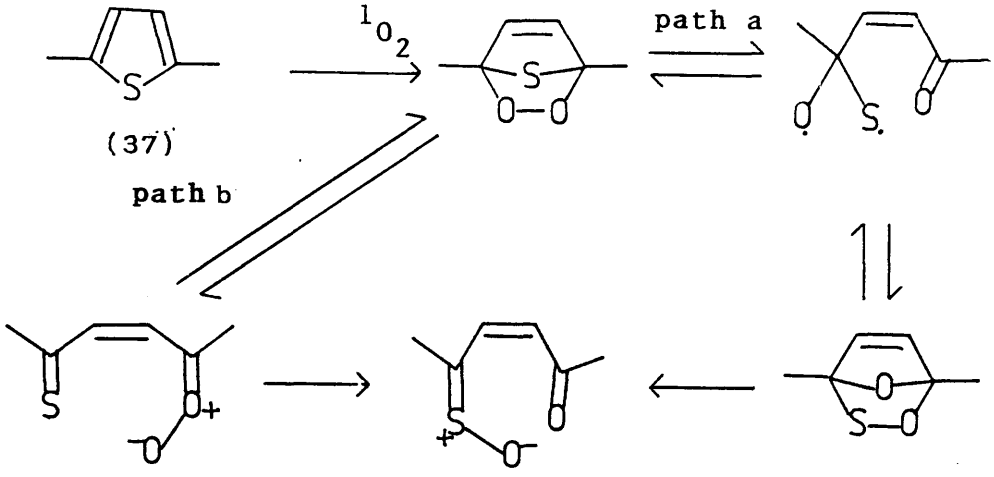
(33) (34)



(35) (36) (17)

1.3.3 Singlet-Oxygen Oxidation of Thiophenes

Treatment of 2,5-dimethylthiophene (37) with singlet-oxygen gave the unsaturated sulphine (38). The proposed mechanism involves initial formation of a thiazonide which may rearrange by radical or ionic pathways to the sulphine (38). Labelling studies have shown that the process is entirely intramolecular. Unfortunately the thiophene route is not general and requires the presence of both methyl groups[35].

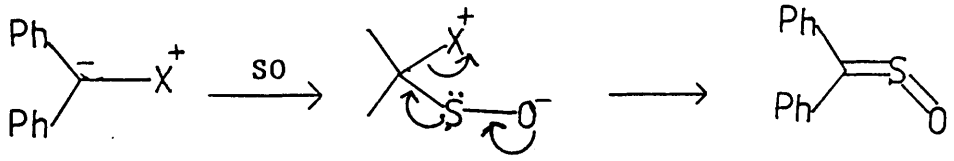


(37)

(38)

1.3.4 Reaction of Sulphur Monoxide with Diazoalkanes and Ylids

Sulphur monoxide can be conveniently generated by the mild thermolysis of trans-2,3-diphenylthiirane S-oxide[36]. Reactions of SO in the presence of various ylids (Scheme 5) produced sulphines in moderate yield. Presumably, electrophilic sulphur monoxide attacked the nucleophilic carbon atom; elimination of the X group led to the sulphine[37]. Most of the approaches detailed so far,



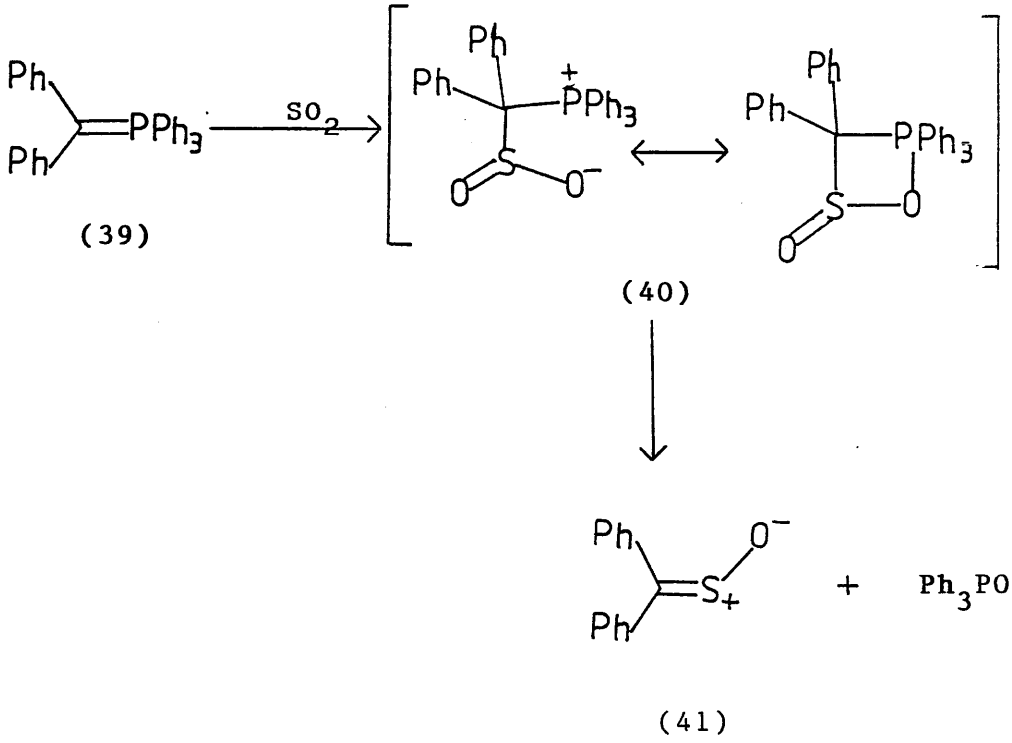
X=N_2, PPh_3, NC_5H_5, SPh_2

Scheme 5

although mechanistically interesting, have not been widely applicable. More recently many of the methods used in carbon carbon double bond formation have been successfully transferred to the formation of carbon sulphur double bonds in sulphines.

1.3.5. Wittig Alkylidenation of Sulphur Dioxide

By analogy with the Wittig reaction the reaction of (diphenylmethylene)triphenylphosphorane (39) with an excess of SO_2 in benzene led to the formation of a sulphobetaine (40). This fragmented to the sulphine (41) and triphenylphosphineoxide [38].

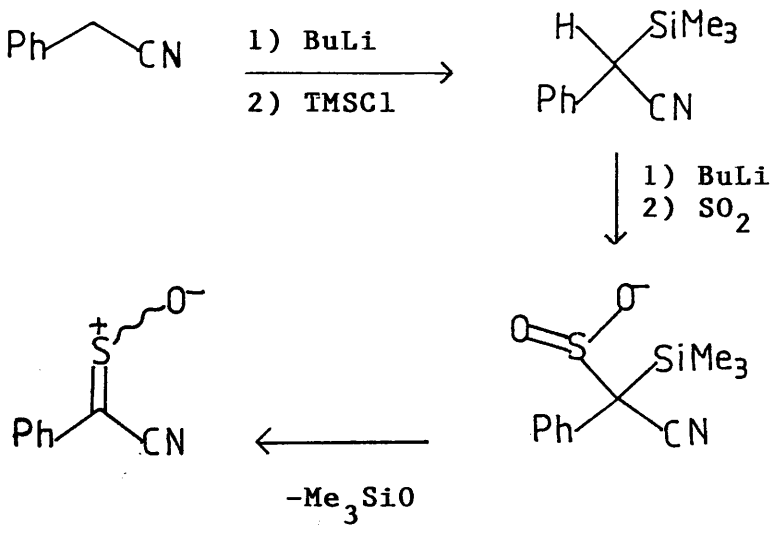


(39) (40) (41)

1.3.6 Alkylidenation of Sulphur Dioxide using α-Silyl Carbanions

The Peterson olefination[39] may be considered as silicon's answer to the Wittig reaction. The same methodology has been applied to form sulphines [40]. A major

attraction of this approach is the variety of routes available in generating α-silyl carbanions. A typical reaction sequence is shown (Scheme 6) and demonstrates that sulphines with electron withdrawing groups can be prepared with moderate success by this route.

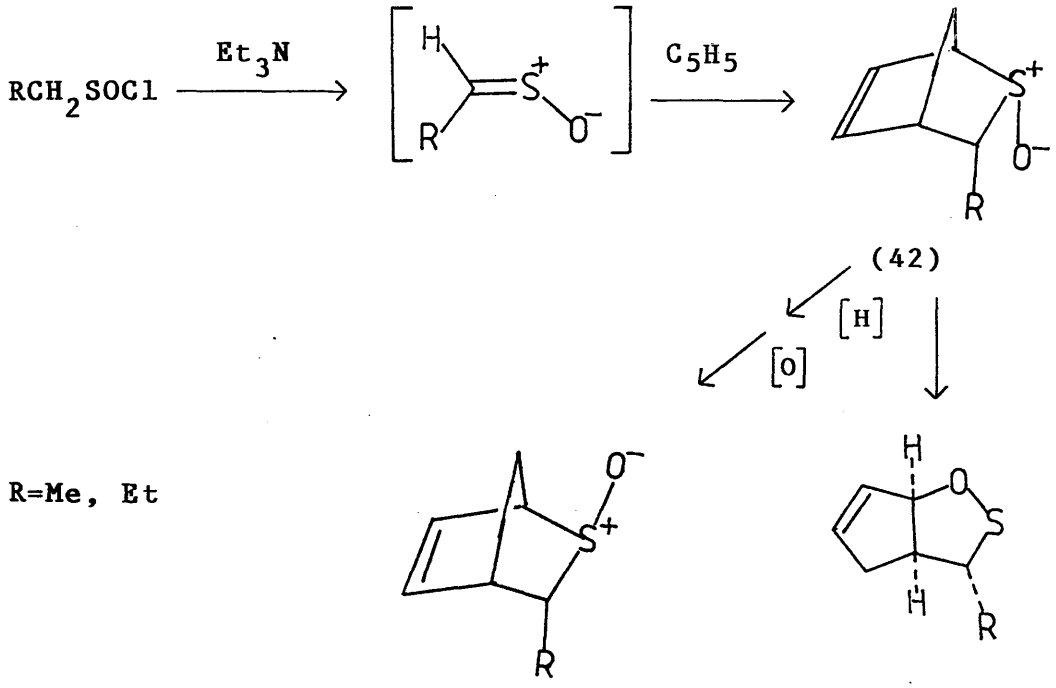


Scheme 6

1.3.7 Dehydrohalogenation of Sulphinyl Chlorides

One of the first methods used to produce thioaldehyde S-oxides was the base induced elimination of hydrogen chloride from sulphinyl chlorides[41]. The method has been successfully employed in preparing the simple alkanethial S-oxides (Scheme 7) including the LF (12); the parent sulphine (CH_2SO), however, cannot be prepared in this manner. A marked preference for the Z-isomer was observed as was seen in the *in vivo* formation of the LF (12). For a series of compounds (RCH=SO) prepared by triethylamine treatment of the corresponding sulphinyl chlorides the observed *syn* preference was: R=^{t}Bu (75%), ^{i}Pr (92%), Et (98%), and Me (97%)[22].

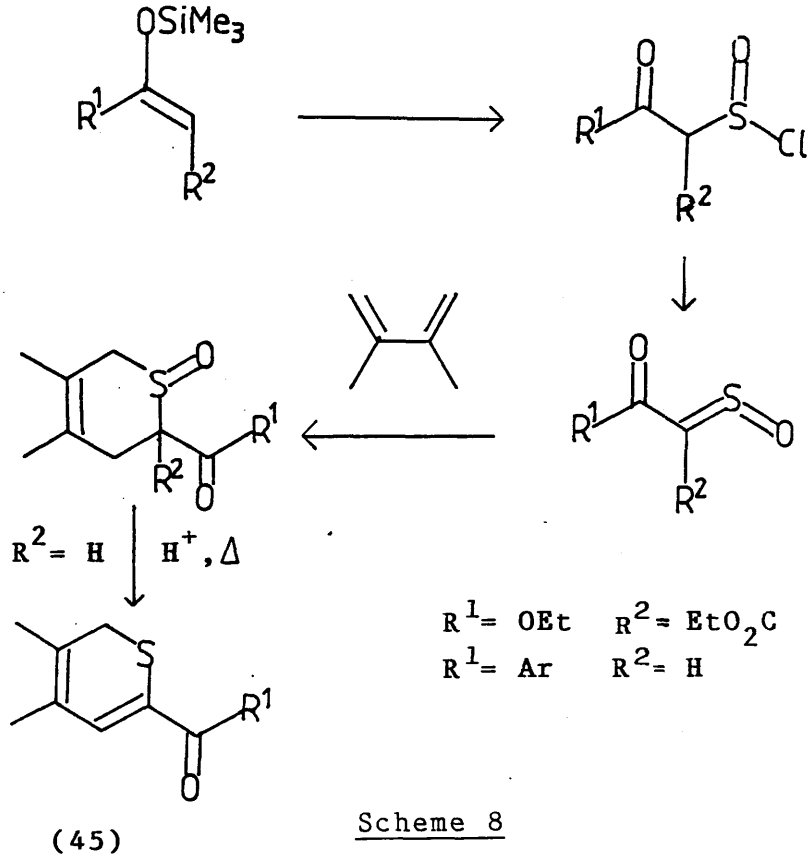
These unstable sulphines added to cyclopentadiene entirely in the endo fashion (Scheme 7) [42]. The resulting sulphoxide (42) rearranged via a [2,3]-sigmatropic shift at room temperature to give a bicyclic sultene (43). When the endo-adduct was reduced and the resulting sulphide then reoxidised with m-chloroperoxybenzoic acid (mcpba) the exo-isomer (44) was formed. This isomer was unchanged after being heated in refluxing toluene for 20h [27,42].



Scheme 7

A route to α-oxo sulphines was achieved in a similar way [43] (Scheme 8). The reaction of α-methylene ketones with thionyl chloride yielded sulphinyl chlorides in variable yield. The presence of the enol form of the methylene ketone was necessary for efficient reaction. Therefore the use of trimethylsilyl enol ethers was employed, providing a more versatile and efficient route. The sulphinyl chlorides, in some cases, spontaneously eliminated hydrogen chloride to give sulphines whilst others required the

presence of base (2,6-lutidine being preferred). The sulphines were not generally isolated but were trapped with 2,3-dimethylbuta-1,3-diene yielding dihydrothiapyran-S-oxides as diastereomeric mixtures. Pure E or Z sulphoxides did not epimerise with base so the isomers presumably arose from E and Z sulphines. Heating the adducts with a catalytic amount of p-toluenesulphonic acid gave thiapyrans (45), presumably via a Pummerer rearrangement.



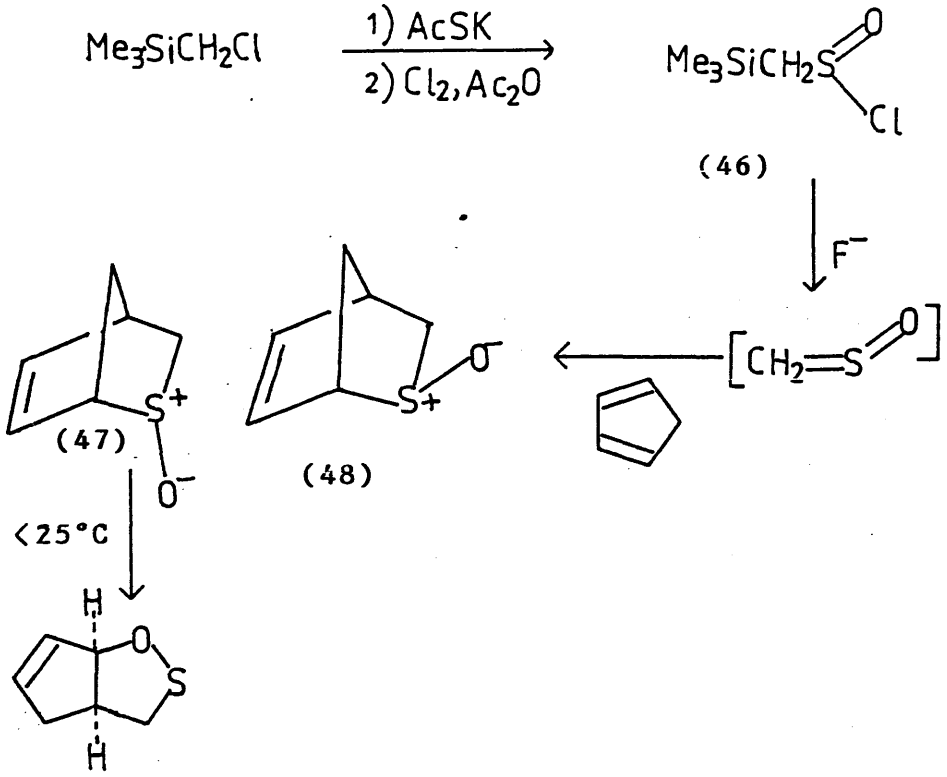
(45)

Scheme 8

1.3.8 Fluorodesilylation of (Trimethylsilyl) Methanesulphinyl Chloride

The previous route has proved useful in preparing alkanethial S-oxides but not the parent sulphine. Only gas phase methods[12] were available to form $CH_2=SO$. Fluorodesilylation of compounds bearing a leaving group in the β-position has proved useful in olefin synthesis; the same strategy has been used to form thiocarbonyl bonds.

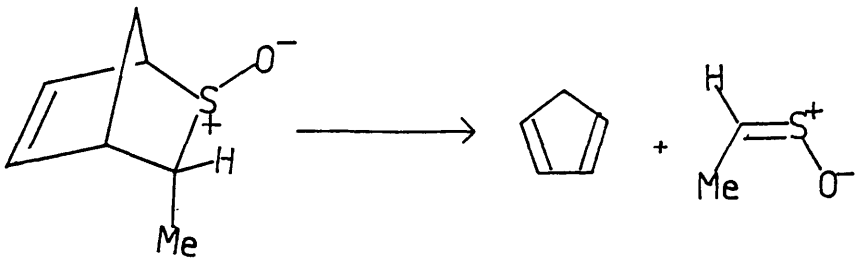
Fluorodesilylation of (trimethylsilyl)methanesulphinyl chloride (46), prepared as shown (Scheme 9), in the presence of cyclopentadiene at -20 °C (gave a 9:1 mixture of the unstable endo-(47) and the stable exo-S-oxide (48)[44].



Scheme 9

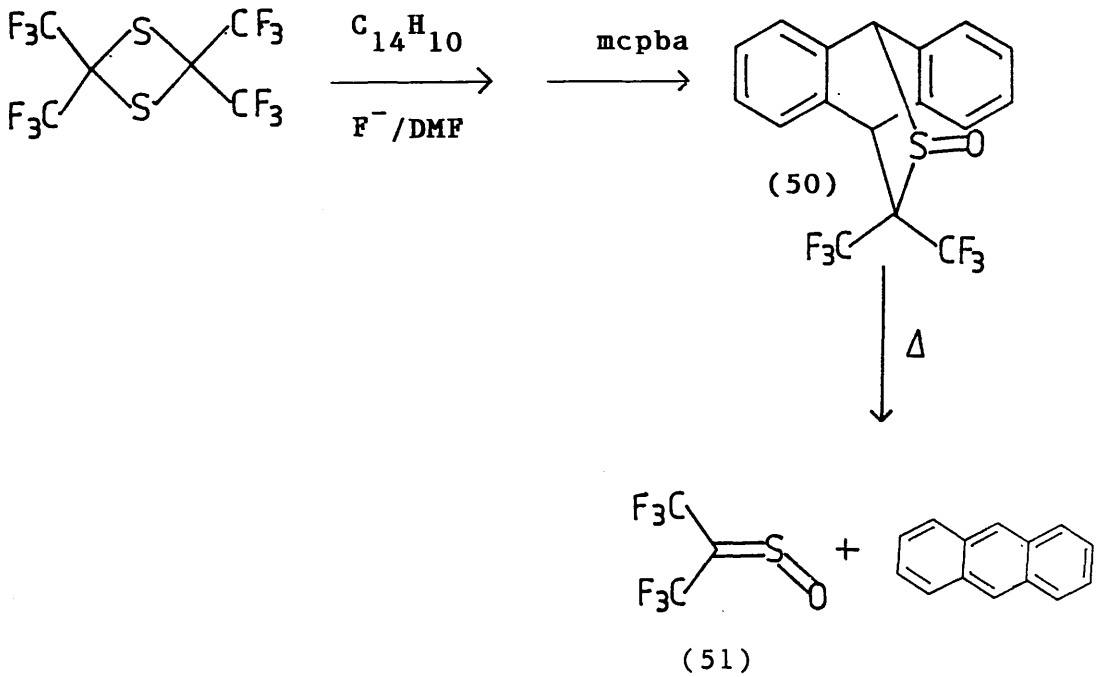
1.3.9 Retro-Diels-Alder Reactions

The purpose of this project was to generate sulphines by the Retro-Diels-Alder cleavage of suitable cycloadducts. The aim was to oxidise certain thioaldehyde Diels-Alder adducts to the corresponding sulphoxides. A thioaldehyde S-oxide might then be generated on thermolysis of the adduct. At the outset of this work no examples of this methods were known, but three examples have since come to light. In an unpublished report[10], flash-vacuum pyrolysis (FVP) of the trans-adduct (49) unexpectedly gave the thermodynamically more stable (Z)-methanethial S-oxide (17).



(49) (17)

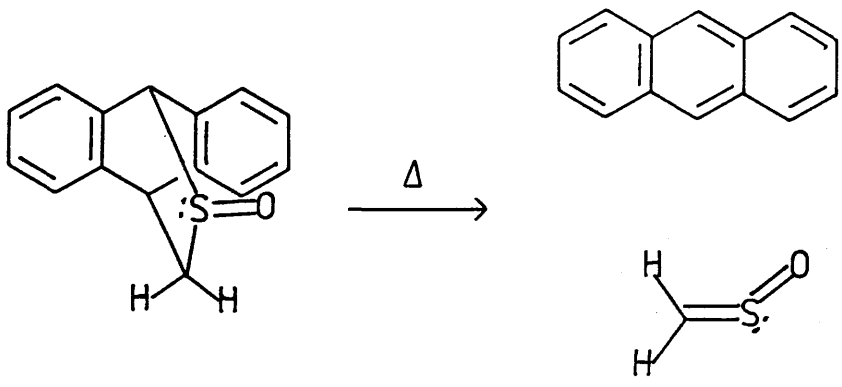
Similarly hexafluorothioacetone was trapped with anthracene and the adduct oxidised with mcpba to give the sulphoxide (50). Pyrolysis of this oxide at 180 °C gave the stable crystalline bis(trifluoromethyl)sulphine (51) in 91% yield [45]. In a similar manner, the anthracene adduct



(51)

(52) prepared in two steps from thiophosgene and anthracene was subjected to FVP at an oven temperature of 925 K. The

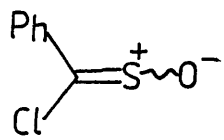
generated methanethial S-oxide (34) was trapped at -196 °C and characterised by ^{1}H, ^{13}C n.m.r. and photoelectron spectroscopy [46].



(52) (34)

1.3.10 Cycloaddition Reactions

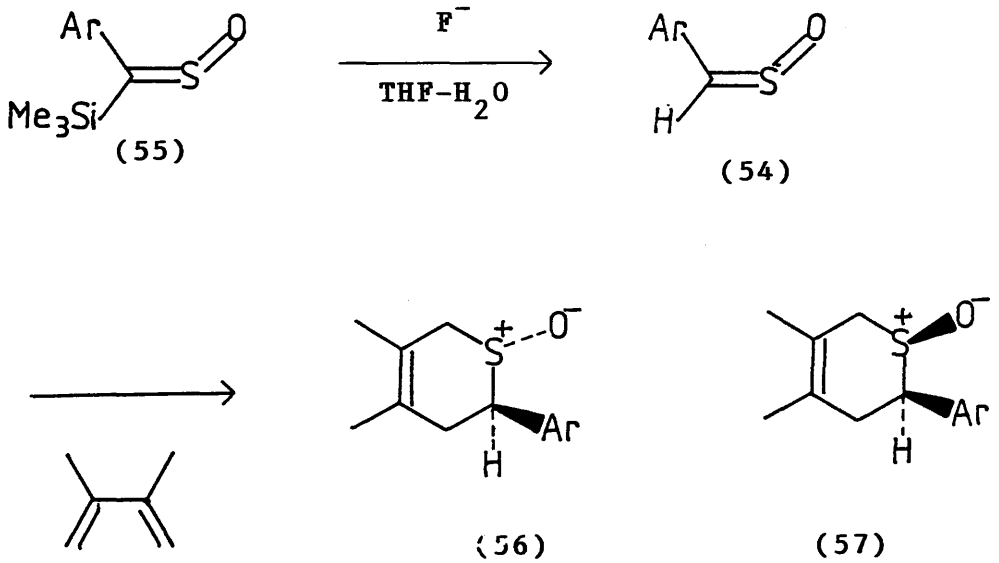
The most commonly reported reactions of sulphines are their cycloadditions, especially with conjugated dienes; many examples have been described previously. There is much interest in whether these reactions are concerted. It was found that when both isomers of the chlorophenylsulphine



(53)

(53) were treated with 2,3-dimethyl-1,3-butadiene, the stereochemistry of the sulphine was predominantly retained in the corresponding adduct. Thus the (E)-sulphine gave 67% of the trans and 12% of the cis product whereas the (Z)-sulphine gave 70% of the cis and 18% of the trans product.

Kinetics studies afforded negative $\Delta S^{\neq}$ values, suggestive of a concerted process [47]. In another investigation, (Z)-thiobenzaldehyde S-oxides (54) were prepared by protodesilylation of aryl trimethylsilyl thioketone (E)-S-oxides (55) (Scheme 10). When the (Z)-thioaldehyde S-oxides were allowed to react with 2,3-dimethylbuta-1,3-diene at room temperature a mixture of the diastereomeric S-oxides (56) and (57) was obtained. Compound (55a) gave trans and cis adducts in the ratio 3.3:1 after 48h, while (55b) gave a ratio of 8:1 after 5 days[48]. Neither the sulphine (54) nor the adducts (56) and (57) isomerised under the reaction conditions. The authors suggested that cis-trans isomerisation might take place during cyclisation. Clearly the concertedness of the



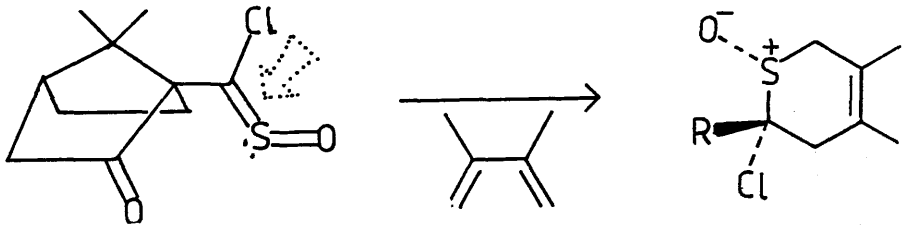
a; Ar = Ph
b; Ar = p-Tolyl

Scheme 10

cycloaddition reaction of sulphines merits further study.

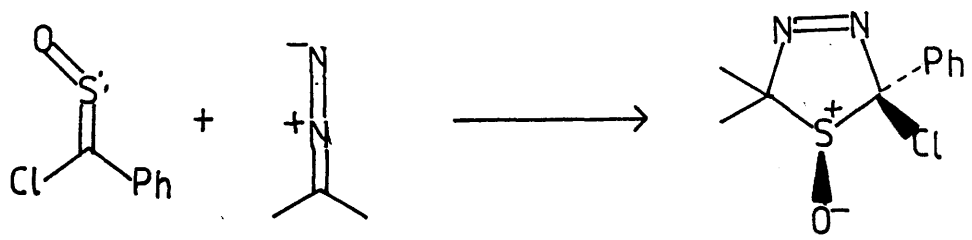
Several attempts have been made to achieve asymmetric

induction in Diels-Alder reactions using chiral sulphines. The first stable sulphine (1) gave complete asymmetric induction on reaction with 2,3-dimethylbuta-1,3-diene. Attack must come from the fact opposite to that of the carbonyl group [49].



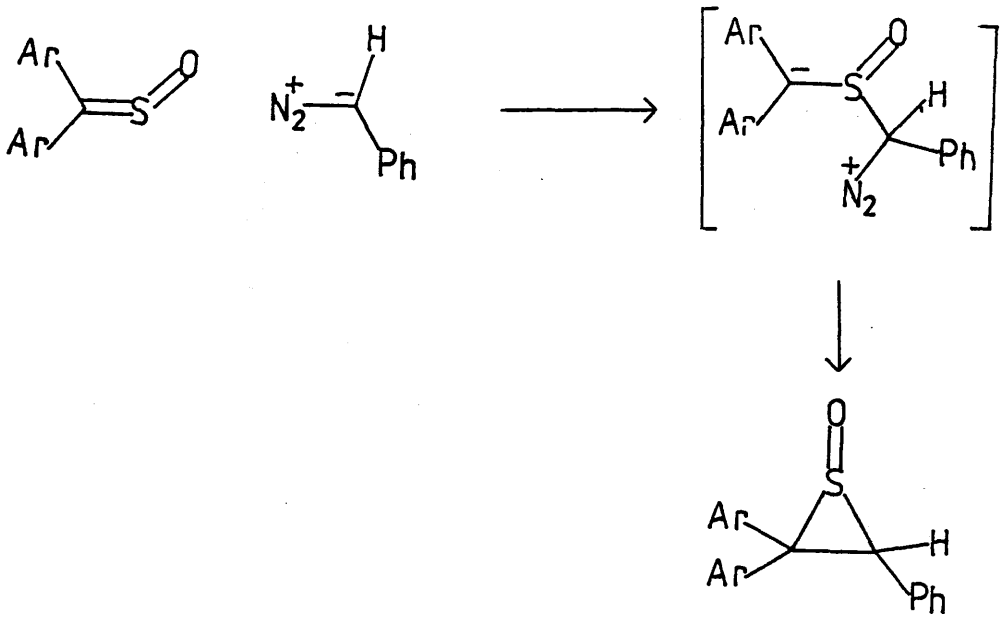
(1)

Sulphines also react as 1,3-dipolarophiles towards diazo compounds, nitrile ylids, azomethine ylids, nitrilimines and nitrile oxides [11]. To take one example, the (Z)-phenylchlorosulphine (58) reacted with diazopropane to give a single diastereometric product (59) [50]. It appears that aromatic and aliphatic sulphines react with diazopropane in a regio- and stereo-selective manner which is indicative of a synchronous reaction.



(58) (59)

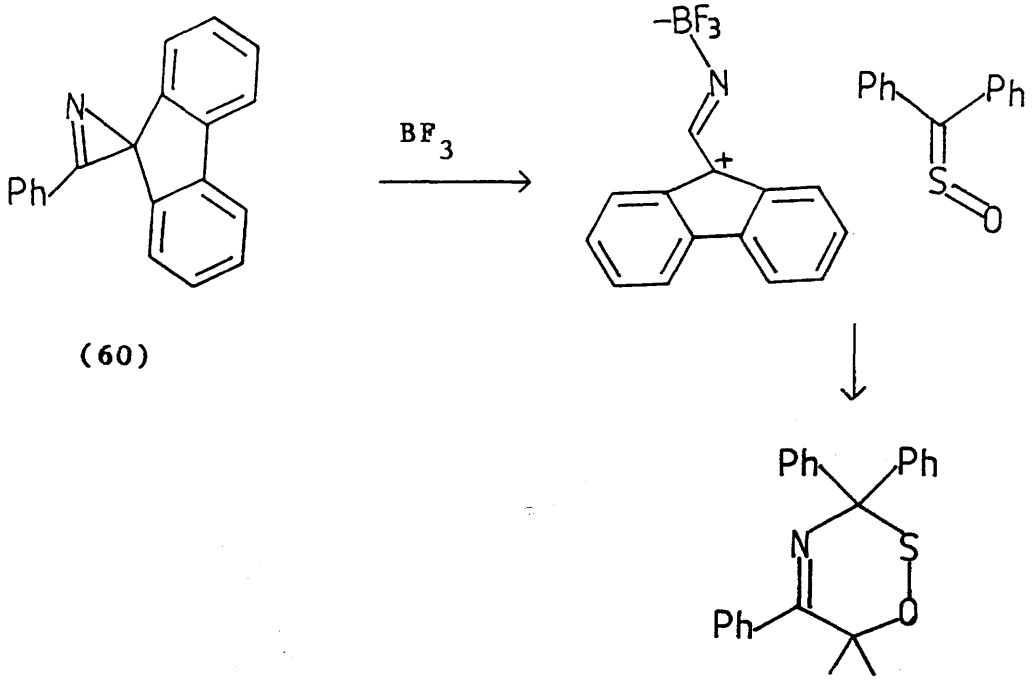
In some cases diazo compounds produce episulphoxides[51]. This could be explained by the extrusion of nitrogen from thiadiazoline S-oxides (59), but diastereomeric mixtures are obtained. It is proposed that episulphoxides arise from a two step process involving nucleophilic attack at sulphur followed by displacement of nitrogen to form the three membered ring (Scheme 11).



2:3 mixture of diastereomers

Scheme 11

An unusual [3,3] cycloaddition has been observed[52]. Diphenyl thioketone S-oxide reacted with the 2H-azirine (60) (Scheme 12) in the presence of boron trifluoride to give the oxathiazine (61). Presumably an aza-allyl cation is generated which reacts via a 1,3-cyclization across the sulphine C-S-O bond.

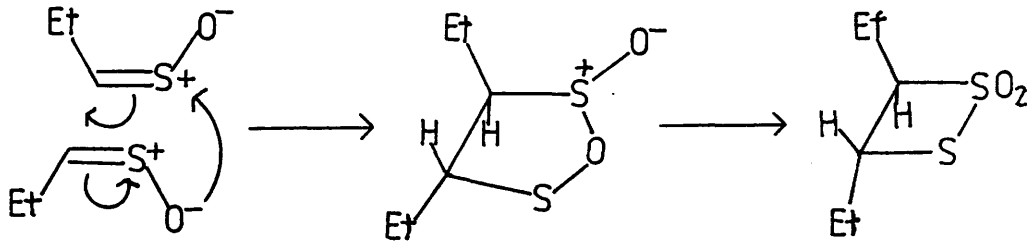


(60)

(61)

Scheme 12

This appears to be the only example of a simple sulphine acting as a 1,3-dipole itself, though a mechanism involving sulphine 1,3-dipolar addition was postulated for the formation of the known LF dimer (62) (Scheme 13)[53].



(62)

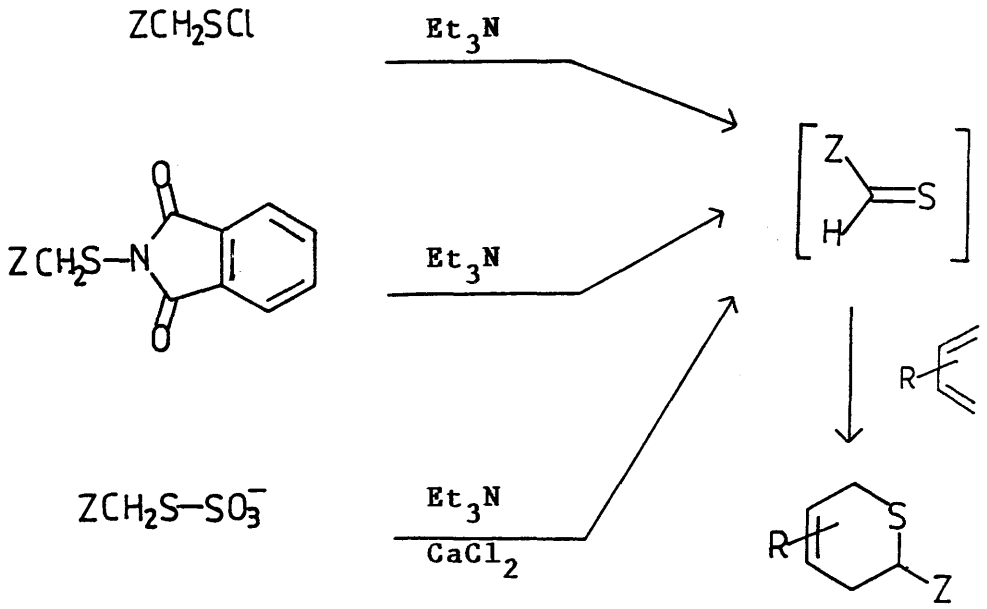
Scheme 13

1.4 Thioaldehydes

The main aim of this project was to generate thioaldehyde S-oxides by retro-Diels-Alder reactions of selected cylcloadducts. The oxidation of thioaldehyde cycloadducts which were know to dissociate on heating[54], would be expected to produce the required, thermally labile sulphine cycloadducts.

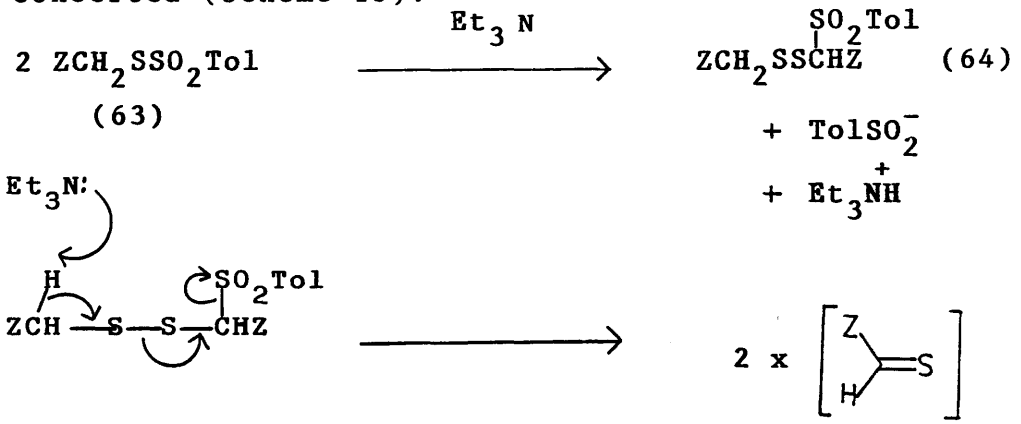
Thioaldehydes, having a weak CS π bond are reactive heterodienophiles. Work in this laboratory has developed methods for generating transient thioaldehydes by 1,2-elimination of HX from sulphenyl precursors, ZCH_2SX, where X is a good leaving group. Z is an electron withdrawing group, able to enhance both the rate of elimination and the reactivity of the dienophile.

With X=Cl or phthalimido, treatment of the precursor with base, usually triethylamine, gave an efficient production of the thioaldehyde, which was trapped *in situ* with conjugated dienes. Again, sulphite was readily eliminated from Bunte salts, ZCH_2SSO_3Na, although in this case the leaving group competed with the diene for the transient thioaldehyde and required to be removed as insoluble calcium sulphite (Scheme 14).



Scheme 14

The toluene-p-thiosulphonates ZCH_2SSO_2Tol (63) did not react so simply. Treatment of the precursor with triethylamine gave the α-sulphonyldisulphides (64) which in turn, were efficient generators of two thioaldehyde molecules by a fragmentation-elimination process, possibly concerted (Scheme 15).

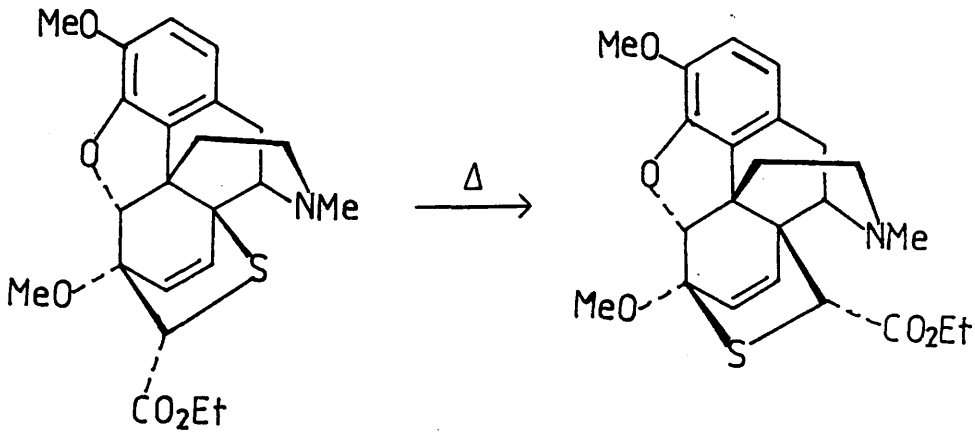


Scheme 15

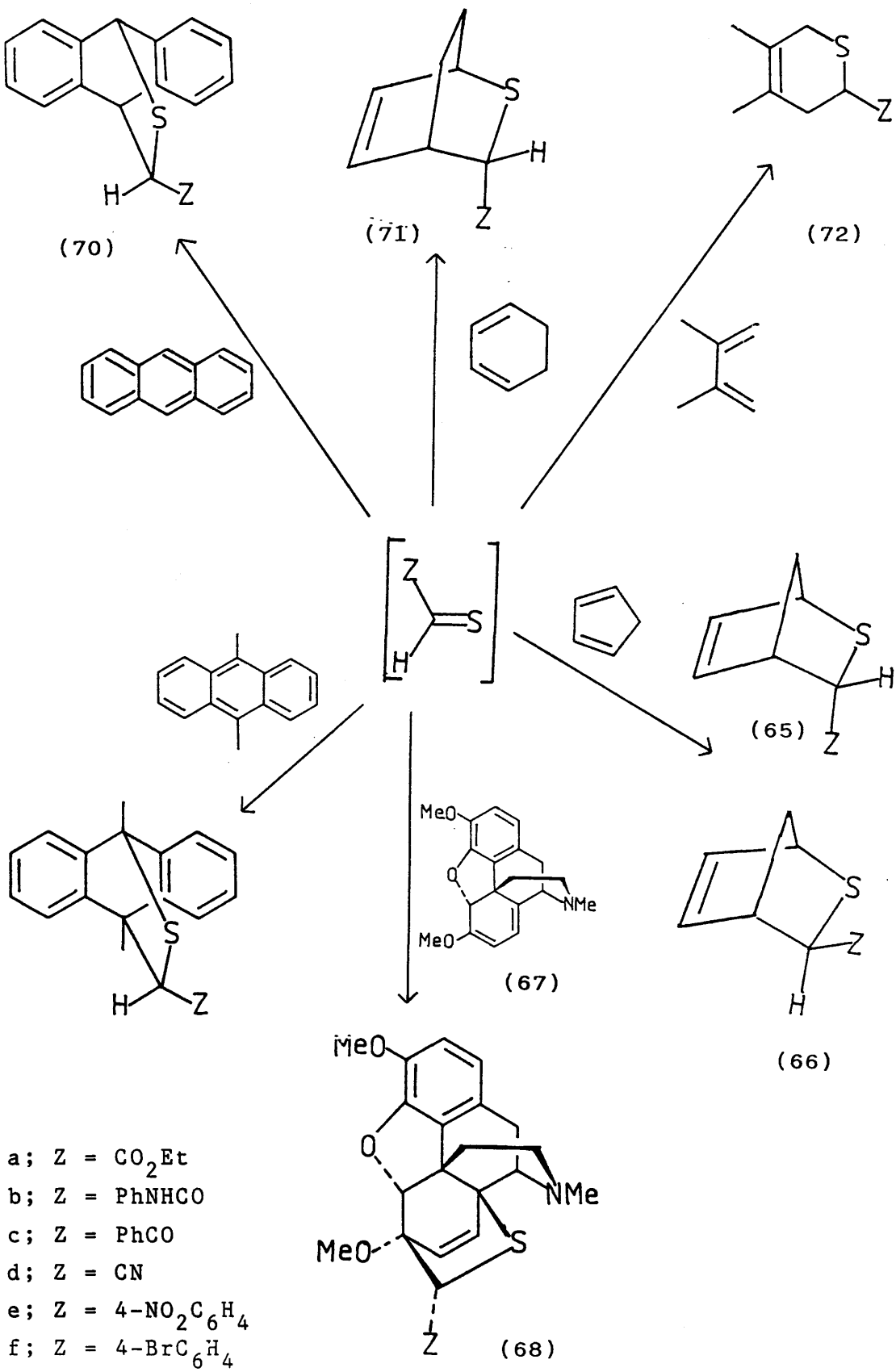
The thioaldehydes were generated in the presence of a variety of conjugated dienes (Scheme 16). The conditions and the yields of adducts mirrored the reactivity of the diene used. Less reactive anthracene gave moderate yields (30-40%) and required elevated temperatures for the most efficient trapping. Reaction with reactive dienes, 2,3-dimethylbuta-1,3-diene, cyclohexadiene, the alkaloid thebaine, and especially cyclopentadiene, proceeded in high yield and were performed at room temperature in polar solvents.

The cyclopentadiene adducts (65,66) were formed with a marked endo-preference, the ethyl esters being obtained in the ratio endo-(65) (70%) to exo-(66) (30%). Heating this kinetically determined mixture at 111 °C for 7h gave an equilibrium ratio of (65) (30%) and (66) (75%).

Ethyl thioxoacetate, EtO_2CCHS, was trapped with the unsymmetrical diene thebaine (67), under kinetic control to give predominantly the regioisomer (68). When the adduct was heated in refluxing toluene the thermodynamically more stable regioisomer (69) was obtained.



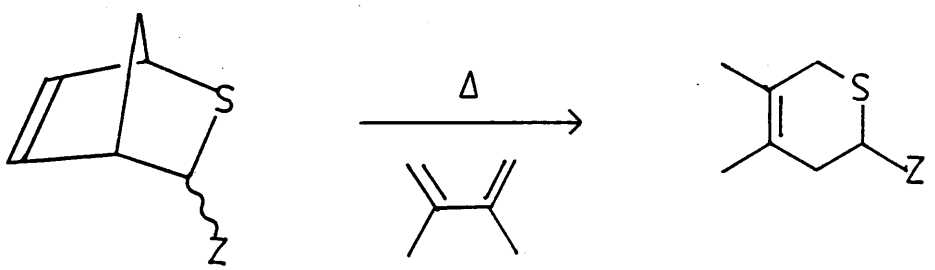
(68) (69)


(70)
(71)
(72)
(65)
(66)
(67)
(68)
MeO
NMe
a; Z = CO_2Et
b; Z = PhNHCO
c; Z = PhCO
d; Z = CN
e; Z = 4-$NO_2C_6H_4$
f; Z = 4-BrC_6H_4
g; Z = Ph

Scheme 16

Thermal isomerisation of the cyclopentadiene and thebaine cycloadducts must occur by dissociation and recombination of the two components. It was then shown that suitable adducts could act as thioaldehyde transfer agents. The anthracene adduct (70) reacted in refluxing toluene with thebaine to give the 'thermodynamic' adduct (69). Ethyl thioxoacetate was also transferred to 2,3-dimethylbuta-1,3-diene from anthracene in a sealed tube at 120 °C. When the same thioaldehyde was transferred from anthracene to cyclohexadiene in refluxing toluene the endo-adduct (71) was obtained. Similar transfers took place from dimethylanthracene.

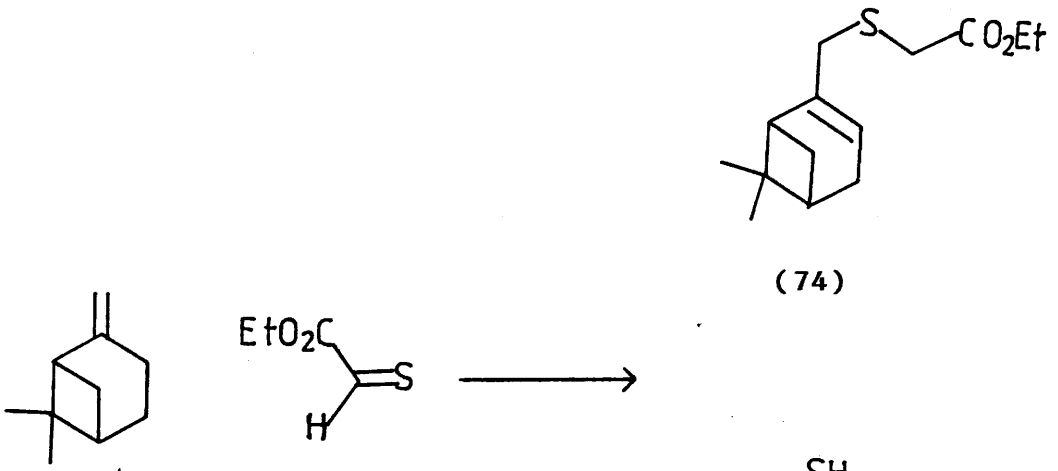
The cyclopentadiene adducts (65,66), when heated in a sealed tube with 2,3-dimethylbuta-1,3-diene, gave high yields of the corresponding dihydrothiin (72).



(65,66) (72)

The thebaine adduct (68) was shown also to be a clean, if expensive, source of ethyl thioxoacetate; when heated in refluxing toluene with 2,3-dimethylbuta-1,3-diene it decomposed to give thebaine and the adduct (72).

Ethyl thioxoacetate generated from the anthracene cycloadduct (70) at 111°C underwent an 'ene' reaction with β-pinene (73) to give two products (74) (78%) and (75) (21%).

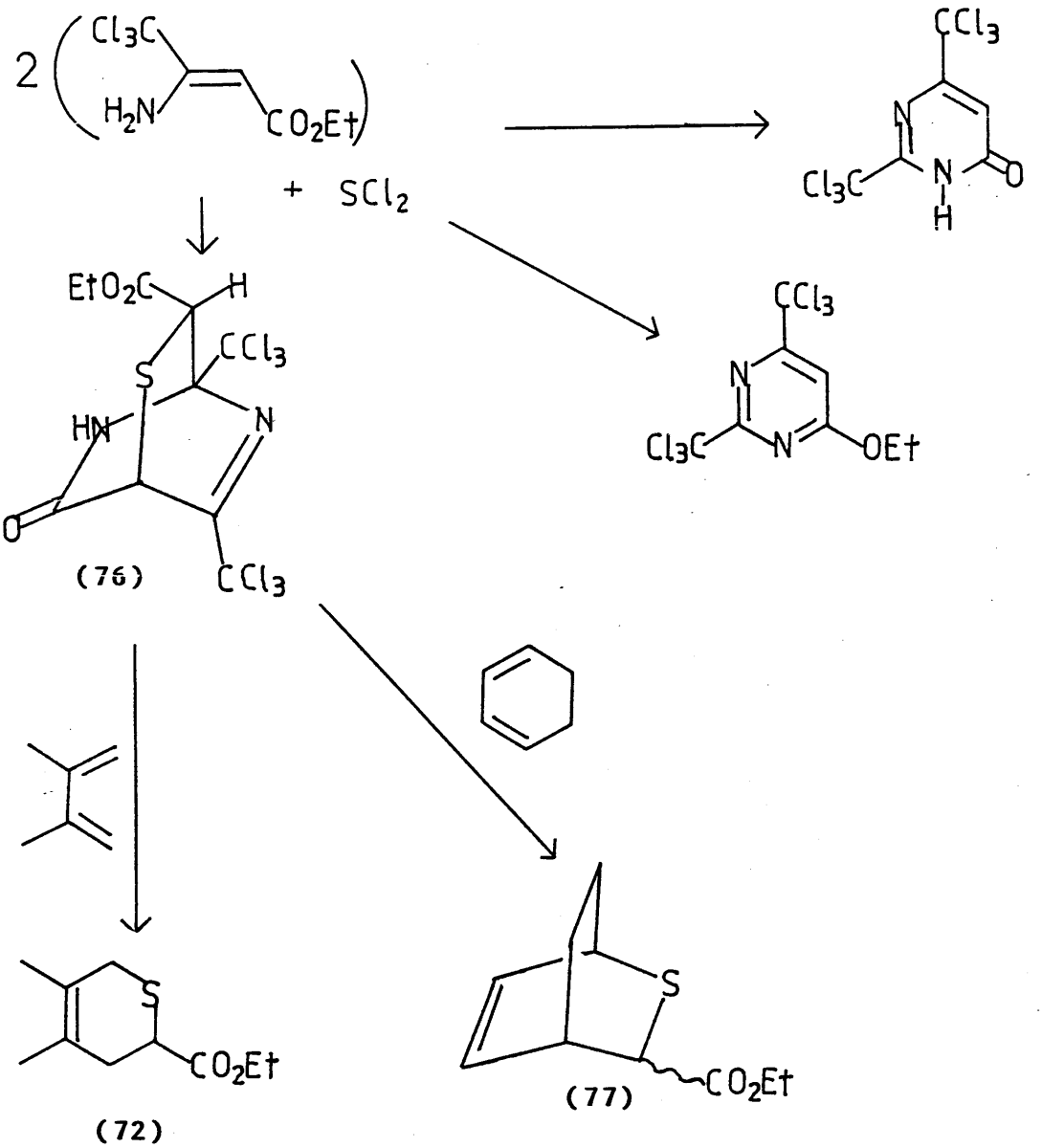


(74)

(73)

(75)

Another precursor of ethyl thioxoacetate was discovered by chance [55]. The reaction sequence shown in Scheme 17 yielded the bicyclic compound (76) as one of the products. When the compound (76) was heated in chlorobenzene at 80 ^{o}C in the presence of cyclohexadiene or 2,3-dimethylbuta-1,3-diene, the adducts (72) and (77) were obtained in 48% and 85% yield respectively.

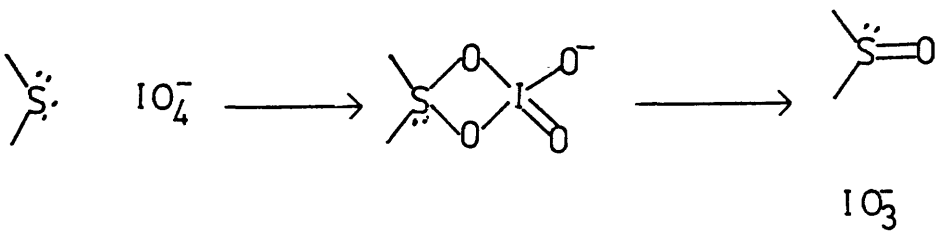

2
Cl3C
H2N
CO2Et
+ SCl2
CCl3
N
Cl3C
N
H
O
EtO2C
H
CCl3
S
HN
N
O
(76)
CCl3
CCl3
N
Cl3C
N
OEt
S
CO2Et
(72)
S
CO2Et
(77)

Scheme 17

Oxidation of Sulphides, and Sulphoxide Stereochemistry

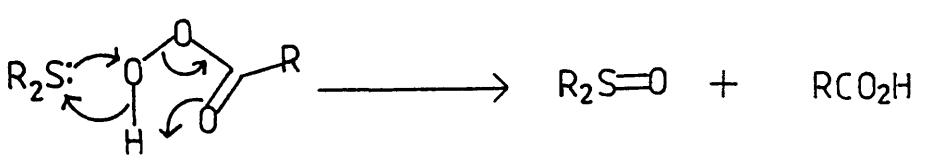
The oxidation of sulphides to sulphoxides has recently been reviewed[56]. The most commonly used reagents are hydrogen peroxide and peracids such as mcpba. Several others have been employed successfully ^{t}butyl hydroperoxide, peracetic acid, sodium metaperiodate, ^{t}butyl hypochlorite, sodium hypochlorite, halogens, iodosobenzene and its derivatives, oxygen, ozone, and dinitrogen tetroxide. More recently the cheap and stable reagent, sodium perborate was shown to give sulphoxides in high yield[57].

The stereochemical outcome of such oxidations depends on the reagents of choice. Periodate oxidation is believed to proceed *via* a cyclic intermediate (Scheme 18)[58], thus thermodynamic control may be operating .



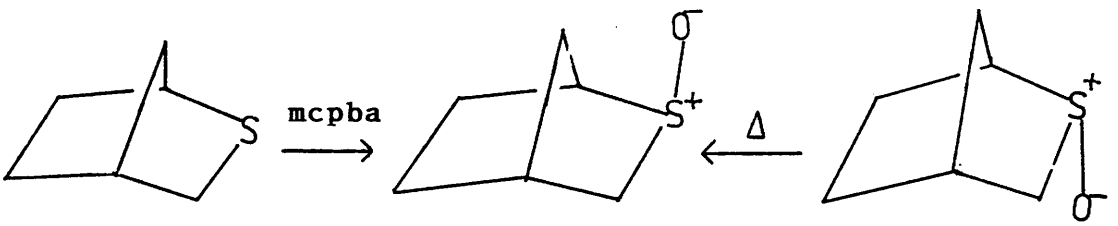
Scheme 18

The accepted mechanism for peroxy acid oxidation involves nucleophilic attack by sulphur on the electrophilic oxygen atom, possibly with concerted proton transfer (Scheme 19). If this is so, then steric approach, *i.e.*, kinetic control prevails[58]. Oxidation of 2-thiabicyclo-



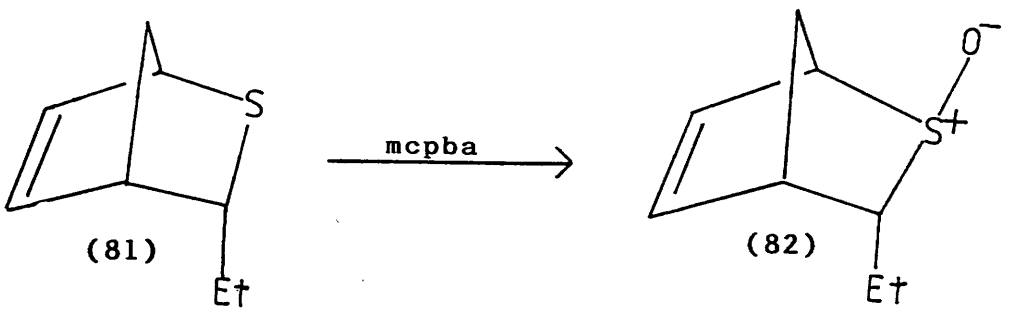
Scheme 19

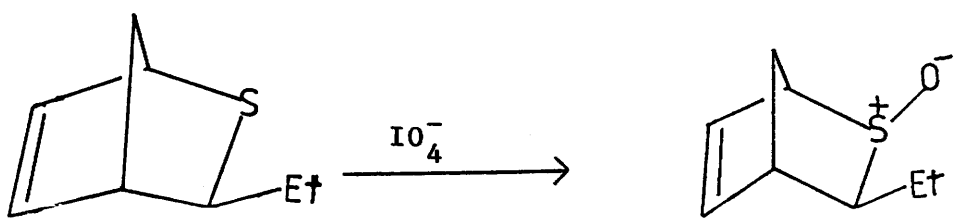
[2.2.1]heptane (78) with mcpba gave exo-(79) and endo-sulphoxides (80) in the ratio 77 : 23. The isomers were equilibrated under a variety of conditions: hydrochloric acid-dioxan, dinitrogen tetroxide, and thermally. Heating a mixture of isomers at 190 °C gave only the exo-compound (79)[59]. With other sulphides only exo-oxidation was



(78) (79) (80)

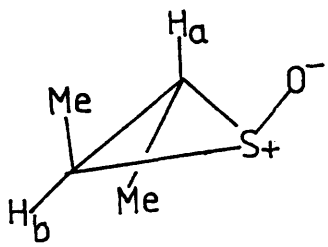
observed. The endo-sulphide (81) gave only the endo-ethyl exo-sulphoxide (82). Significantly, the exo-ethyl exo-sulphoxide (83) was the only product from periodate oxidation of the exo-ethyl sulphide (84)[42].





(84) (83)

The stereochemistry of cyclic sulphoxides has previously been assigned on the basis of i.r. spectral evidence and chromatographic behaviour [58,59]. More recently ^1H n.m.r. spectroscopic techniques were employed [60]. The SO bond has an acetylenic type anisotropy. It was observed that a β-hydrogen syn to the lone pair of the sulphinyl sulphur suffers a shielding effect, whereas a β-hydrogen syn to the S-O bond suffers a deshielding effect. For α-hydrogens the situation is less clear. A study on a series of episulphides and their corresponding sulphoxides produced the expected upfield shift of 0.32 p.p.m. for the hydrogen H_b syn to the lone pair of the sulphoxide (85). The proton H_a syn to the S-O bond, however, also gave an upfield shift, albeit minute, of -0.02 p.p.m.



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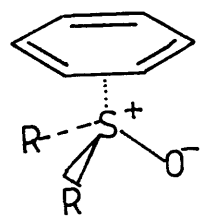
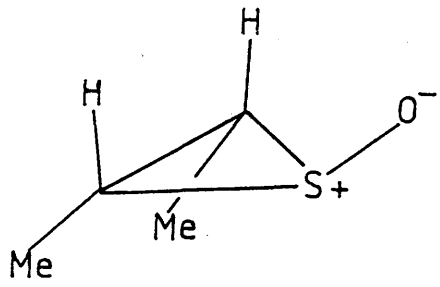


(88)

The assignment of H_a and H_b were made by comparison of the n.m.r. spectra with those of other isomers. Sulphoxides (86) and (87) were the sole products on oxidising the corresponding sulphides. Assuming the generally accepted premise that oxidation occurs from the least hindered side then their spectra could be assigned unambiguously.



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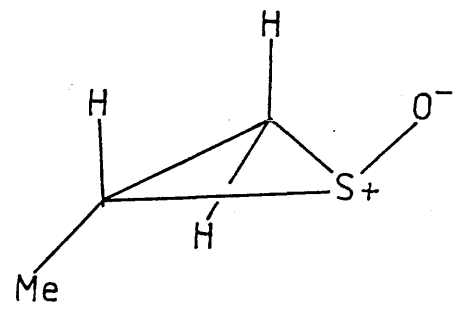
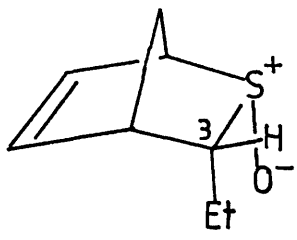
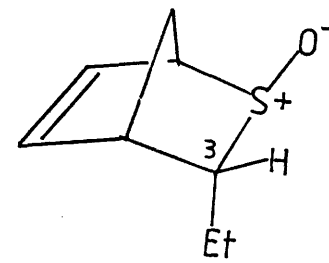


(87)

More reliable evidence came from aromatic solvent induced shifts (ASIS) and lanthanide induced shifts (LIS). If a sulphoxide is dissolved in benzene, for example, the aromatic ring tends to reside near the positive end of the sulphoxide dipole (88). Thus hydrogens anti to the S-O bond will experience a larger upfield shift than the corresponding syn-protons. In the sulphoxide (85) the benzene-induced shift of the anti-proton, H_b was -0.80 p.p.m. compared to -0.56 p.p.m. for the syn-proton, H_a. Lanthanides such as europium coordinate to the negative oxygen causing downfield shifts in protons syn to the S-O bond. ASIS and LIS data were recorded on the sulphoxides (89) and (90)[42]. A larger downfield LIS shift would be



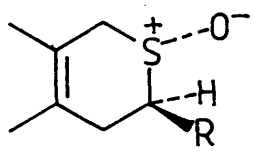
(89)



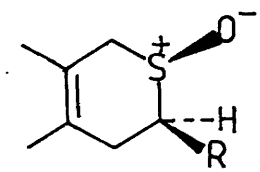
(90)

expected for 3-H of the sulphoxide (90) than of its isomer (89). With 0.33 mol equivalents ot $Eu(fod)_3$, shifts ($\Delta\delta$) of 3.7 and 1.5 were observed for 3-H of (90) and (89) respectively. For a spectrum recorded in C_6D_6 a larger upfield shift (relative to the value in $CDCl_3$) would be expected for 3-H of (89) than of (90). Indeed, shifts of $\Delta\delta$ -0.5 and -0.2 were observed for (89) and (90) respectively.

The conformationally more flexible sulphine cycloadducts of dimethylbutadiene have been studied recently. The ^1H n.m.r. signal for 2-H of the trans-sulphoxide (56a) was shifted 0.14 p.p.m. downfield with respect to the signal of the corresponding sulphide. A 0.25 p.p.m. upfield shift was observed for 2-H of the cis-adduct (57a)[48]. Similarly in a series of substituted benzoyl compounds 2-H of the cis-isomer (57b) resonated at lower field than that of the trans-isomer (56b). Model studies and LIS experiments were employed to confirm the assignments[43].



(56)



(57)

a; R = Ph
b; R = ArCO

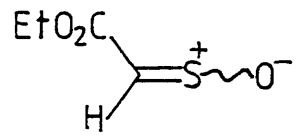
In summary, sulphines have been prepared by a variety of means. All reacted as dienophiles, particularly so when electron-withdrawing groups were present. Z-E isomerisation was not observed at ambient temperatures; however, in

supposedly concerted cycloadditions the configuration of the sulphine was not always retained in the product. Recent work has shown that certain sulphines can be generated by retro-Diels-Alder reactions at elevated temperatures. Moreover, unstable thioaldehydes such as ethyl thioxoacetate can be generated at moderate temperatures by dissociation of various adducts. As the following Chapter will describe, we have shown that S-oxides (sulphines), $RO_2C.CHSO$ of the electrophilic thioxoacetate esters, $RO_2C.CHS$, can be generated under mild, 'clean' conditions by retro-Diels-Alder reactions.

Chapter 2 Discussion

2.1 Anthracene Cycloadducts

The Introduction (Section 1.3) detailed the numerous methods that have been used to prepare sulphines. The aim of the present work was to generate thioaldehyde S-oxides by retro-Diels-Alder reactions. No examples of this approach had been published when the project began. The sulphine chosen was ethyl thioxoacetate S-oxide (91) since the corresponding transient thioaldehyde had already been generated successfully by dissociation of several

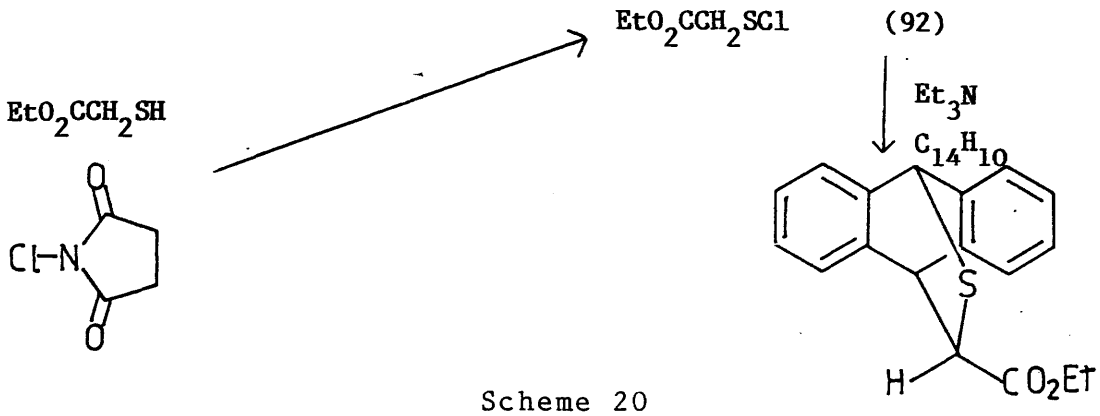


(91)

cycloadducts. Of these the anthracene adduct was one of the more convenient sources; anthracene adducts generally are crystalline and cycloreversion is aided thermodynamically by the formation of a stable aromatic compound, anthracene[54].

Thus the thioaldehyde anthracene adduct (70a) was prepared by the known sulphenyl chloride route (Scheme 20)[54e]. N-Chlorosuccinimide when added to a benzene solution of ethyl mercaptoacetate gave a yellow solution of the sulphenyl chloride (92) and a precipitate of succinimide. The unpurified sulphenyl chloride was added slowly to a solution of anthracene (1 mol. equivalent) and triethylamine (1.1 mol. equivalent) in refluxing chloroform to give the adduct (70a) in 35% yield after chromatography. The moderate yield was primarily due to the low reactivity of anthracene. Thus, anthracene reacts with maleic

anhydride at a rate only ca. 22% that of 2,3-dimethylbuta-1,3-diene, according to a previous study [61]. Ethyl thioxoacetate is generated immediately the precursor enters the solution of triethylamine and will polymerise unless rapidly trapped. To increase the efficiency of trapping, 5 mol. equivalents excess of anthracene was employed. In this way, a 61% yield of the adduct (70a) was obtained after purification.



Scheme 20

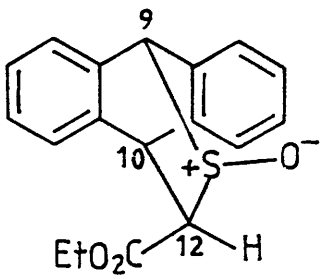
(70a)

To form the sulphoxide, mcpba was chosen as a convenient reagent. The outcome of reactions with this oxidising agent, is governed by steric approach control, so the trans-sulphoxide (93) was expected as the major product. Accordingly, addition of mcpba in dichloromethane to the sulphide in the same solvent gave a mixture of the trans-(93) and cis-sulphoxide (94) in the ratio ca. 2.5 : 1 determined by ^{1}H n.m.r. spectroscopy. Monitoring the reaction by t.l.c. or ^{1}H n.m.r. spectroscopy showed oxidation to be an almost instantaneous process. Exhaustive attempts to separate the isomers by t.l.c. were unsuccessful. Later efforts to separate by t.l.c. other adducts which differed only in sulphoxide geometry met with a similar lack of success.

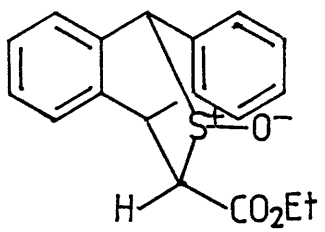
Encouragement for the success of subsequent retro-Diels-Alder reactions was provided by the product's melting point behaviour. The small needles appeared to melt sharply at 120 ^{o}C, but the melt crystallised as hexagonal plates which themselves melted at over 200 ^{o}C, close to that of

anthracene. The mass spectrum of the sulphoxide mixture showed a highest mass peak corresponding to that of anthracene; apparently, the sulphine (m/z, 134) had decomposed, perhaps thermally, before reaching the detector.

Treatment of the mixture of sulphoxides in hydrogen chloride-dioxane for 20 min increased the proportion of the trans-isomer (93) to ca. 10 : 1. The possibility that acid-catalysed epimerisation was occurring at the 12-



(93)



(94)

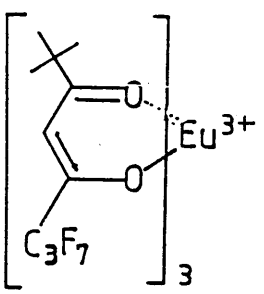
position was discounted by repeating the equilibration in DCl-D_2O. No deuterium exchange of 12-H was observed. The same isomeric ratio (10 : 1) was obtained by base catalysis, initially using sodium ethoxide or potassium ^{t}butoxide. Monitoring the rate of epimerisation by n.m.r. spectroscopy was more conveniently achieved with 0.2M triethylamine in [$^2H_6$]- benzene; epimerisation was completed in 12h.

As discussed in the Introduction (Section 1.5) a variety of techniques have been applied in assisting the assignment of sulphoxide stereochemistry. I.r. spectroscopy showed no differences in the S-O stretching frequencies. The n.m.r. technique of ASIS and LIS, and sulphoxide anisotropic shielding or deshielding effects were employed and the most significant observations are given in Table 1. From the previous discussion, where a proton is synperiplanar to the sulphinyl lone pair as is 12-H of the cis-isomer (94), an upfield shift relative to the

corresponding sulphide signal was expected, and observed ($\Delta\delta$ -0.19). A downfield shift was expected for 12-H of the trans-isomer (93). Surprisingly, a very large shift to higher field ($\Delta\delta$ -0.99) was observed. A deshielding effect did occur at 9-H of both isomers; assuming the adducts are not twisted significantly, 9-H bisects the lone pair and the S-O bond. Thus the anisotropic shielding cone of the sulphoxide group is so directed that its effect is greatly affected by bond angles and distances.

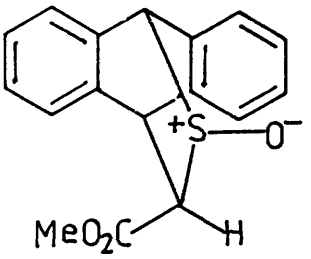
The n.m.r. spectra of the sulphoxides (93) and (94) were run in [$^2H_6$] -benzene and compared to the spectra obtained in $CDCl_3$ (Table 1 ASIS). The aromatic ring should coordinate to the positively charged sulphur, resulting in a ring current shielding of 12-H for the cis-oxide (94). Accordingly an upfield shift of 0.44 p.p.m. was observed for the cis-isomer (94) and no change in chemical shift was seen for 12-H of the trans-oxide (93).

For the LIS experiment $Eu(fod)_3$ (95) was employed, unfortunately, the signals arising from the diastereotopic methylene group of the ethyl ester overlapped with other important peaks, and made interpretation difficult. Thus,



(95)

the methyl ester (96) was prepared from methoxycarbonylmethanesulphenyl chloride (97) and anthracene. Mcpba oxidation gave the trans-(98) and cis-sulphoxides (99) in the same ratio (2.5 : 1) as before.



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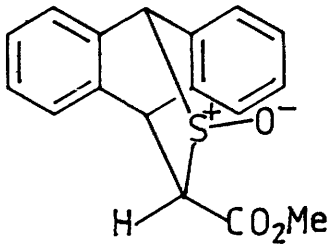


(99)

Aliquots of $Eu(fod)_3$ were added to the mixture of sulphoxides and the downfield shift was measured. The shift reagent should complex reversibly (Scheme 21) to the

$$R_2S^{+}—O^{-} + Eu(fod)_3 \rightleftharpoons R_2S^{+}—O^{-}\cdots\cdots Eu(fod)_3$$

Scheme 21

sulphoxide oxygen causing larger downfield shifts to the proton *syn* to the S-O bond, *i.e.*, 12-H of the *trans*-oxide (98). The equilibrium (Scheme 21) is fast on the n.m.r. timescale and a time averaged signal for the free sulphoxide and the complex is observed. Increased amounts of shift reagent 'push' the equilibrium to the right and the chemical shifts, for small amounts of added reagent, are directly proportional to the molar ratios of substrate to $Eu(fod)_3$. It is conventional to define a value, ΔM as the shift obtained for the 1 : 1 mol ratio of sulphoxide to $Eu(fod)_3$. This was calculated by extrapolation of the linear plot of amount (mol equivalent) of $Eu(fod)_3$ added versus the downfield shift (p.p.m.) to obtain the shift at 1 mol equivalent (Table 1). For a high equilibrium constant:

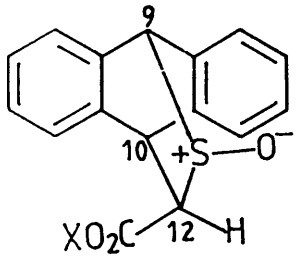
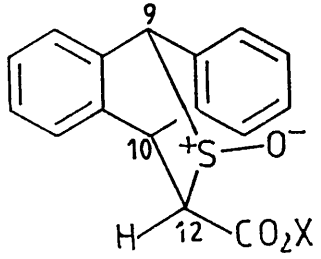
Compound	Proton	Δδ (SO-S)[a] X=Et	Δδ (SO-S)[a] X=H	Δδ (ASIS)[b] X=Et	ΔM[c] X=Me
	9-H	+0.45	+0.64	-0.23	7.72
	10-H	-0.13	-0.12	-0.21	3.41
	12-H	-0.99	-0.99	-0.00	10.96
trans					
cis					
	9-H	+0.56	+0.76	-0.35	6.50
	10-H	-0.10	-0.04	-0.31	4.78
	12-H	-0.19	-0.10	-0.44	5.75

Table 1 Chemical shift data for sulphoxide stereochemistry

(a) Chemical shift difference between sulphoxide and sulphide.
(b) Sulphoxide chemical shift differences observed in D_6-benzene.
(c) Eu(fod)$_3$ induced shifts.

$$\Delta M = \delta (SL) - \delta (S)$$

where SL is the sulphoxide-europium complex and S is the free substrate.

The results provided the most convincing evidence for the assignment of the correct stereochemistry. A large downfield shift, $\Delta M = 10.96$ occurred for the proton, 12-H, synperiplanar to the S-O bond and for 9-H ($\Delta M = 7.72$) of the trans-sulphoxide (98). This is consistent with a complex of the type shown in Figure 1. For the cis-isomer, in which 12-H is anti to the S-O bond a much smaller shift ($\Delta M = 5.75$) was observed. The values for 9-H and 10-H were of a similar magnitude, which suggested that europium was sited symetrically between the sulphoxide and the ester group, complexing to both groups (Figure 1). This latter

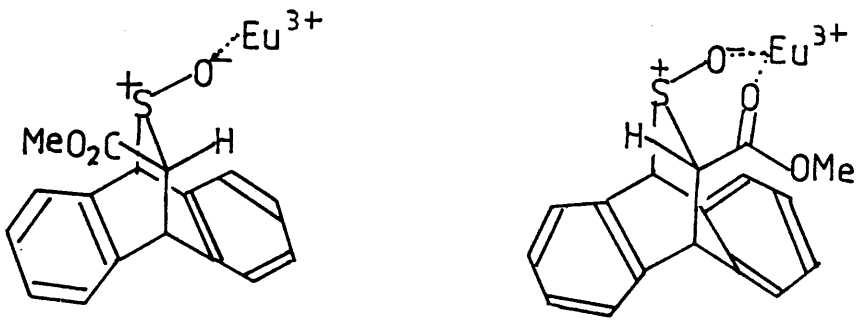
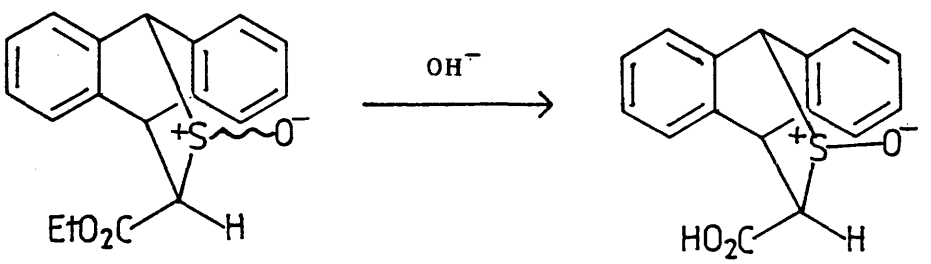


Figure 1

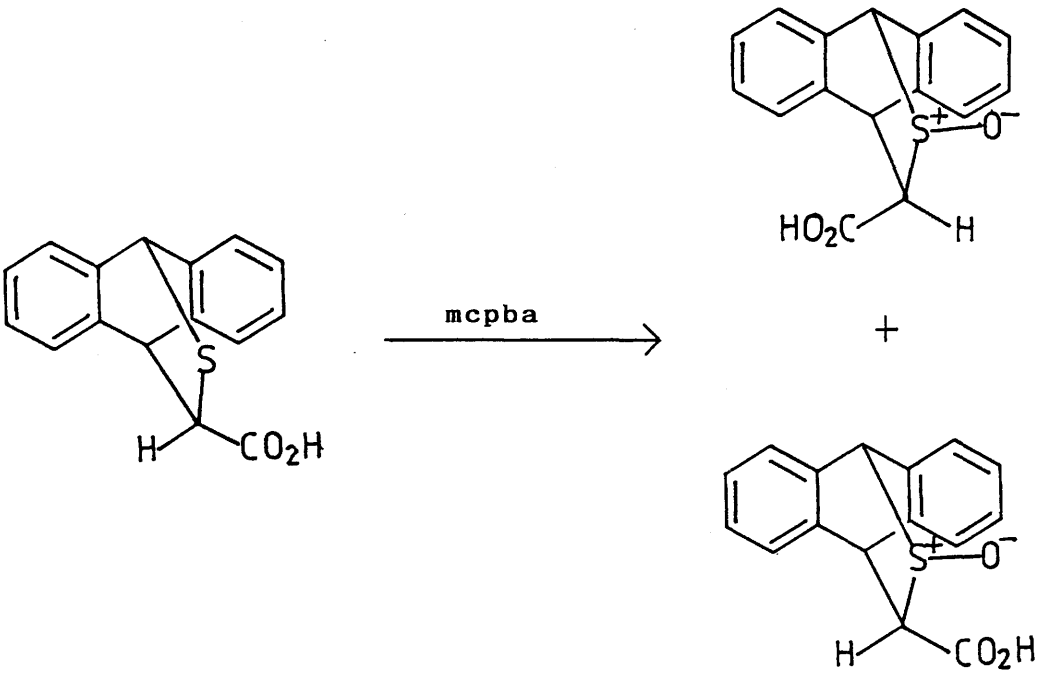
observation suggested that the equilibrium constant for the cis-complex may be greater than that of the trans-complex. Data obtained from a mixture of isomers may give spurious results. The experiment was therefore repeated on the equilibrated mixture (trans : cis ratio, 10 : 1) with almost identical results. The reversibility of the lanthanide complex formation was easily demonstrated by the addition of an excess of $[^2H_4]$ methanol, which returned the signals to their original positions.

Base-catalysed hydrolysis of the mixture of ethyl esters (93) and (94) gave a single isomeric acid S-oxide presumably the trans-acid (100), resulting in part from epimerisation of the cis-isomer. The main incentive in preparing acids was to observe the presence or absence of intramolecular hydrogen bonding in the infrared specra as an additional aid to assigning stereochemistry. The acid (100) was not sufficiently soluble in carbon tetrachloride or deuterochloroform to allow this. The ^{1}H n.m.r. spectrum [in $(CD_3)_2SO$] was compared to that of the sulphide acid[54e] [in $(CD_3)_2SO$] (Table 1). The results mirror very closely the shifts observed for the trans-ester (93), notably the large upfield shift for the proton 12-H syn to the SO bond ($\Delta\delta$ -0.99). For comparison the cis-sulphoxide acid (101)



(93) and (94) (100)

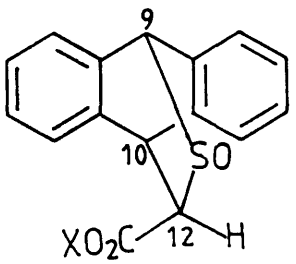
was also prepared. Mcpba oxidation of the sulphide (102) gave the trans-(100) and cis-oxide (101) in the ratio 1.1 : 1, plus 3-chlorobenzoic acid. The poor solubility of the sulphoxide acids proved useful as the benzoic acid was easily removed by washing the solid with ether. The chemical shift differences between the cis-acid (101) and the corresponding sulphide (102) were also in close agreement with the trends observed for cis-ester (94), i.e. a small upfield shift for 12-H and a larger downfield shift for 9-H (Table 1). Finally, esterification of trans-acid (100). obtained by hydrolysis, with diazomethane gave the trans-methyl ester (98) with no epimerisation.



(102) (101)

2.1.1 ^{13}C n.m.r. Spectra

^{13}C N.m.r. spectra were recorded for the sulphide and sulphoxide esters (ethyl and methyl). Only slight differences in chemical shift were observed between trans- and cis-oxides, except for the carbonyl groups. The carbonyl carbon occupies a position similar to that of 12-H; hence similar anisotropic shifts might be observed, but for the opposite isomer. The shifts are displayed in Table 2 and reflect the same trends observed in the proton n.m.r. spectra, namely a small upfield shift for the carbonyl carbon syn to the lone pair and a much larger upfield shift for the carbonyl carbon syn to the S-O bond.



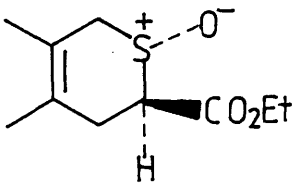
	C=O	Δδ (SO-S)
X	trans	cis
Et	-2.8	-5.5
Me	-2.8	-5.7

Δδ (SO-S) values for the carbonyl carbon.

Table 2

2.1.2 Retro-Diels-Alder reactions of Anthracene Adducts

In the first experiment, the mixture of trans-(93) and cis-(94) oxides (ca. 2.5 : 1) was heated with 2,3-dimethylbuta-1,3-diene (1.2 equivalents) in refluxing benzene. After 10h all the anthracene adduct had dissociated. The product was chromatographed to remove anthracene and the oily product distilled to give a mixture of trans-(103) and cis-dihydrothiapyran S-oxide (104). The ratio of isomers obtained from 360MHz spectrum run some time afterwards was almost unchanged at 2.4:1.



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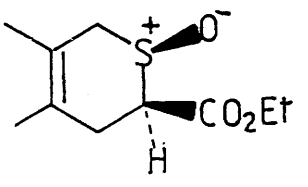
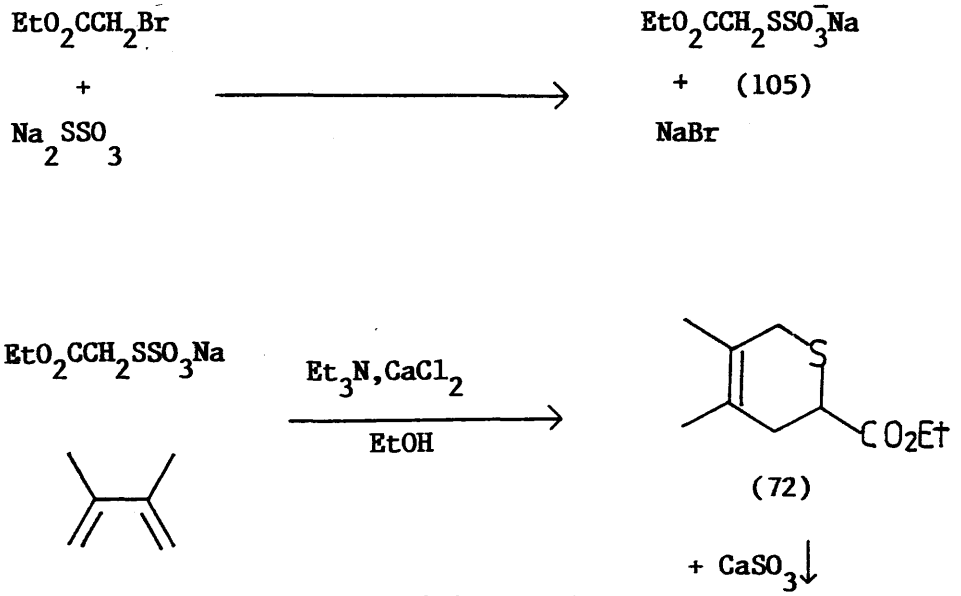


(104)

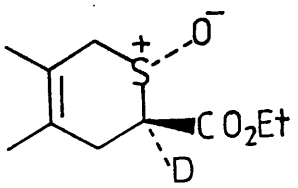
To provide structural confirmation, the sulphoxides were prepared from the known [54e] sulphide (72). This sulphide was first prepared from ethoxycarbonylmethanesulphenyl chloride (92) and 2,3-dimethylbuta-1,3-diene in 63% yield. Later the Bunte salt route [54c] was employed, which avoids the handling of evil smelling mercaptans. Samuel Choi, in this laboratory, developed an easy method of synthesising the known Bunte salts. Shaking an acetone solution of ethyl bromoacetate with an aqueous solution of sodium thiosulphate followed by evaporation gave a mixture of salts. The Bunte salt (105)[62] was extracted with hot ethanol. The sulphide (72) was then prepared (45%) by addition of triethylamine to a stirring suspension of 2,3-dimethylbuta-1,3-diene, the Bunte salt and calcium chloride in ethanol (Scheme 22).



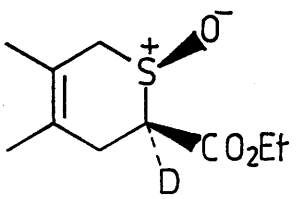
Scheme 22

Oxidation of (72) with mcpba gave a mixture of the trans-(103) and cis-oxide (104) in an increased ratio (ca. 4 : 1 by n.m.r. spectroscopy). Separation by t.l.c. was not possible but capillary g.c. gave a separation in retention times of 2.08 min after ca. 30 min. (90-150 °C, 2 °C/min). The ratio of the isomers was 4.0 : 1.0, in agreement with the spectroscopic value. The nature of the two g.c. signals was confirmed by g.c-m.s. using a packed column.

Equilibration of the mixture of oxides in concentrated hydrochloric acid-dioxan gave the isomers with a trans:cis ratio of ca. 1 : 1, but with considerable decomposition. Base-catalysed epimerisation proceeded more cleanly in a 0.2M solution of triethylamine in [$^2H_6$]-benzene - [$^2H_4$]-methanol during 24h, monitored by n.m.r. spectroscopy. Capilliary g.c. analysis gave a trans:cis ratio of 1.2 : 1 for the deuteriated sulphoxides (106) and (107). This was later confirmed by g.c-m.s. The ratio of 1.2 : 1 corrects our previously published [63] value of ca. 2 : 1.

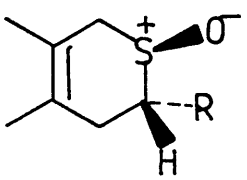


(106)

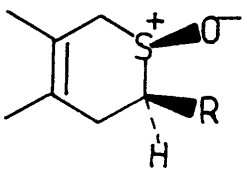


(107)

The ^{1}H n.m.r. spectrum of similar dihydrothiapyran S-oxides (56,57) was discussed in the Introduction (Section 1.5). The 2-H signal for the trans-oxide (103), δ 3.75, was shifted downfield (Δδ 0.21) from that of the corresponding sulphide (72). An upfield shift (Δδ-0·21) was observed for 2-H of the cis-isomer. These shifts are in agreement with those observed for the similar adducts (56,57). The stereochemistry at the 2- and 3- positions can be deduced from the vicinal coupling constants. In the trans-isomer



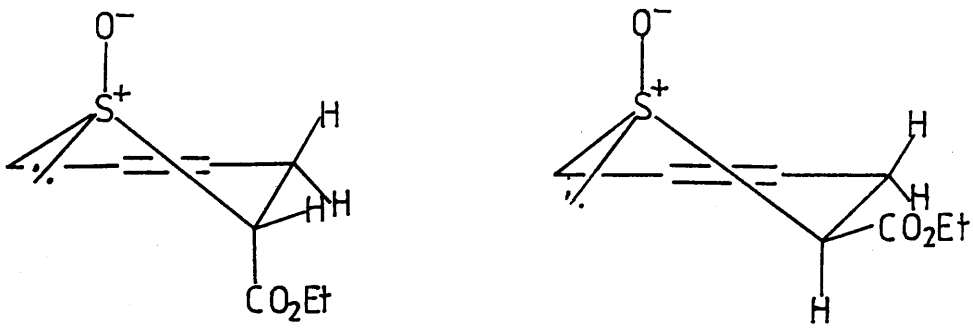
(103) R = CO_2Et
(56a) R = Ar
(56b) R = COR



(104) R = CO_2Et
(57a) R = Ar
(57b) R = COR

(103) the vicinal coupling constants for the $COCHCH_2$ group were similar (J 8.0 and J 5.2 Hz). These are in agreement with those observed for the trans-oxide (56b)[43] where inspection of molecular models revealed similar dihedral angles (ca. 60°). A conformation which accounts for these data is shown in Figure 2. As previously stated the

deshielding at 2-H ($\Delta\delta$ 0.21) agrees with that previously observed. The conformation shown in Figure 2 has the proton at C-2 bisecting the sulphoxide bond and the sulphinyl lone pair. The proton (9-H) in the anthracene S-oxides (93,94) is similarly disposed and deshielding was also observed. This is in contrast to the upfield shift normally observed.

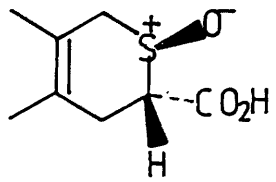


trans cis

Figure 2

For the cis-isomer (104) the vicinal coupling constants for the $COCHCH_2$ group were unequal (J 12.1 and J 4.7 Hz) which corresponds to unequal dihedral angles (ca 150° and 60°) and a conformation consistant with these observations is shown in Figure 2. In this instance the proton (2-H) is only influenced by the adjacent sulphinyl lone pair and the signal is shifted upfield from that of the sulphide ($\Delta\delta$ -0.21). The chemical shift difference observed for 12-H, syn-periplanar to the sulphinyl lone pair of the cis-anthracene S-oxide (104) was -0.19 p.p.m.

Hydrolysis of the mixture of S-oxides (103) and (104) gave a single isomeric acid. The 2-H n.m.r. signal (δ4.01) was shifted downfield ($\Delta\delta$ +0.35) from that of the corresponding sulphide [54e] and appeared as a triplet (J 7 Hz). Comparison of the data with that of the esters suggested a trans-configuration (108). The white foam



(108)

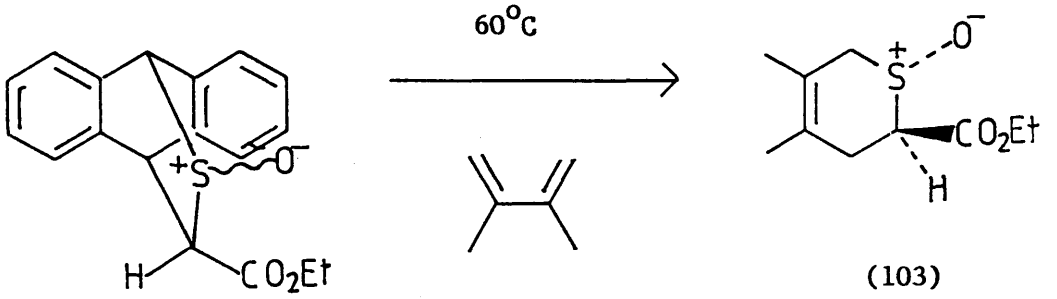
decomposed to a green, unidentified solid on attempted crystallisation and so the acid could not be fully characterised.

2.1.3. Thermal Transfer of (E)- and (Z)-Sulphines

Thus far, a mixture of the (E)- and (Z)- sulphines, $EtO_2C.CHSO$ has been liberated at 80 °C from the anthracene adducts (93) and (94) and trapped with dimethylbutadiene without change in the isomer ratio. Unfortunately, the anthracene adducts could not be separated by any physical method. What was hoped for was a method for generating the (E)- and (Z)- sulphines separately; a chance observation made this possible. A solution of the anthracene S-oxides in di-isopropyl ether (b.p. 68 °C) was being concentrated in an attempt to crystallise them. A precipitate which turned out to be anthracene, appeared while the solution was still boiling. The supernatant liquid was decanted off and evaporated when, surprisingly, the residue was found to contain only the minor, cis-adduct (94). Thus, by suitable choice of temperature, it appeared possible to generate the (E)-sulphine, even from a mixture of isomeric precursors. This was achieved as follows:

The equilibrated S-oxide , ca. 95% trans, was heated with 2,3-dimethyl-1,3-butadiene (1.1 equivalent) in benzene at 60 °C for 5 h to give only the trans-adduct (103). A

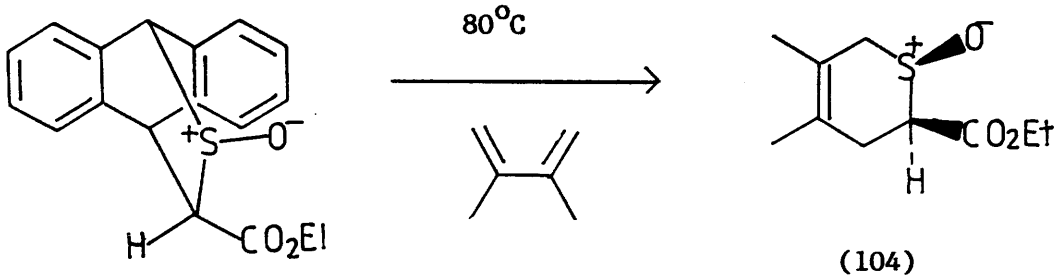
similar experiment with the S-oxides in the ratio ca. 2.5 : 1 showed that none of the anthracene cis-S-oxide (94) dissociated at this temperature.



(93) + (94) +(94)

trans cis

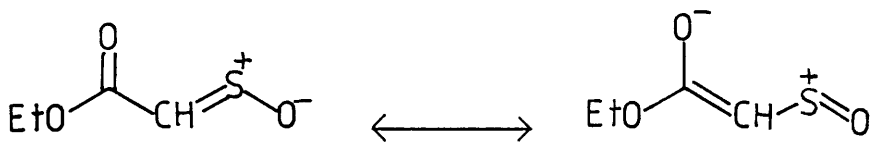
The last experiment was repeated to give a mixture containing the cis-adduct (94), the dihydrothiapyran (103), and anthracene. Chromatography, to remove the trans-dimethylbutadiene adduct (103) often led to partial or sometimes complete epimerisation of the required cis-anthracene adduct (94). To avoid chromatography, the mixture of (94), (103), and anthracene was washed with cold ether, which removed the more soluble dihydrothiapyran adduct (103). The cis-oxide (94), still mixed with anthracene, was heated with 2,3-dimethylbuta-1,3-diene in benzene under reflux (80 °C). Transfer of the (Z)-sulphine to dimethylbutadiene was complete within 10 h and with no detectable isomerisation.



(94) (104)

$+ C_{14}H_{10}$ $+C_{14}H_{10}$

The preceeding experiments offered an apparent contradiction; the more stable trans-S-oxide dissociated more readily (at 60 °C) than the less stable cis-oxide (94) (at 80 °C). It seems likely (see Section 2.1.4) that dissociation of the anthracene adduct is the rate determining step in the overall transfer reactions. If a 'late' transition exists then conjugation (109) stabilising



(109)

the planar sulphine (Figure 3) would also lower the energy of the transition state. This would pose no steric problem for the trans-oxide (Figure 3). However, a correspondingly planar transition state for the cis-oxide would result in serious steric repulsion between the oxygen of the sulphoxide and ethoxy (or carbonyl) groups. Of course, there is steric crowding in the ground state of the cis-oxide (94), which results in its being thermodynamically less stable than the trans-adduct (93). However, in the ground state of (94) the ethoxycarbonyl group can rotate to relieve this effect. It is the increased steric effects in the 'planar' transition state that may account for the

relative kinetic stability of the cis-adduct. The energetic situation is summarised in Figure 3.

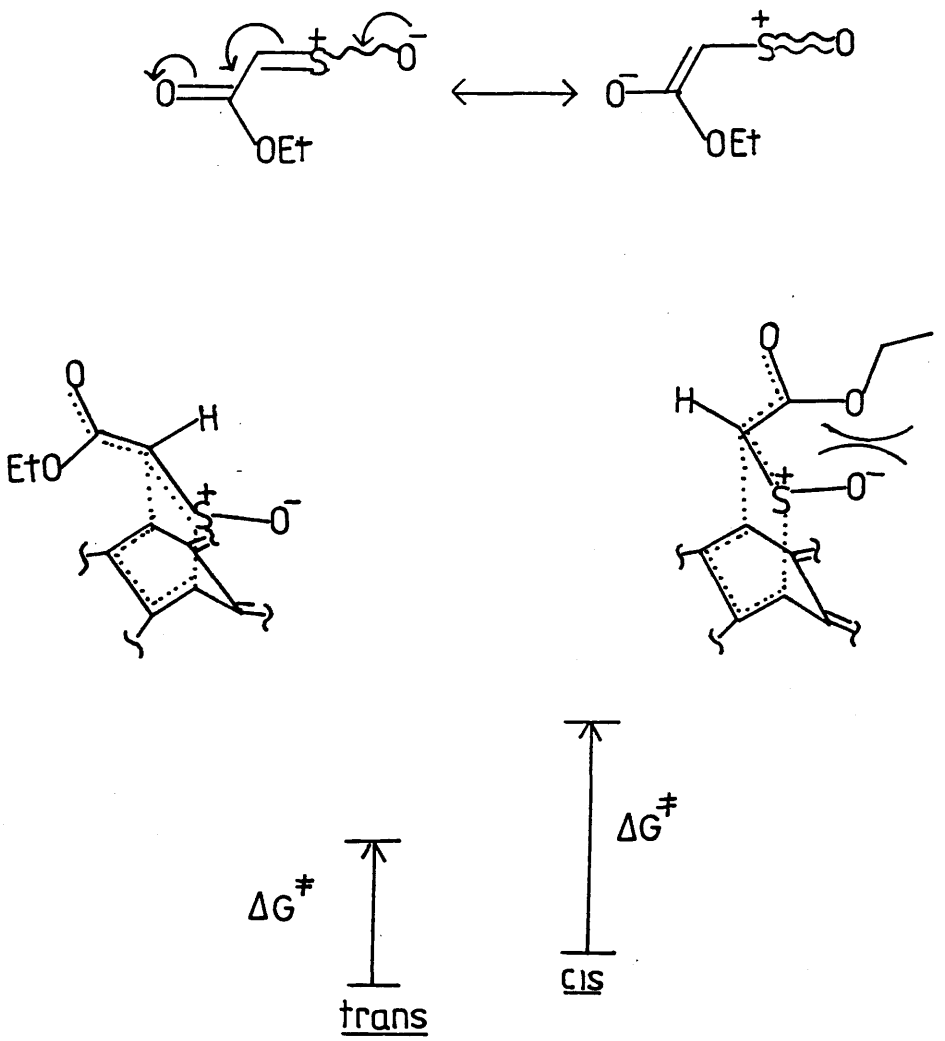
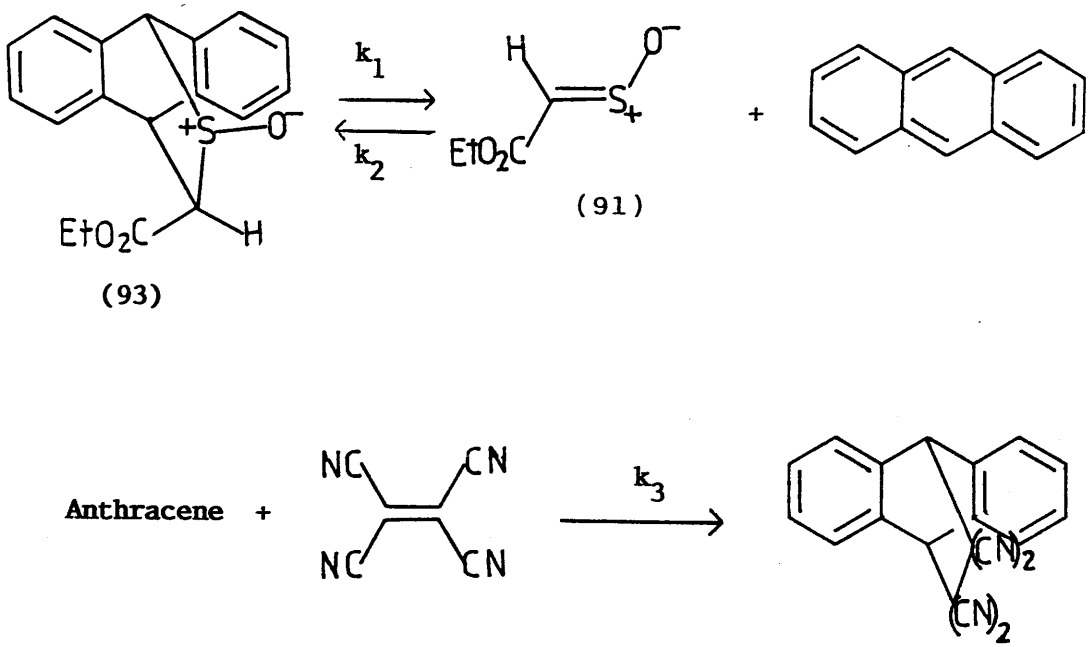


Figure 3

2.1.4 Rate of Formation of the (E)-Sulphine (91)

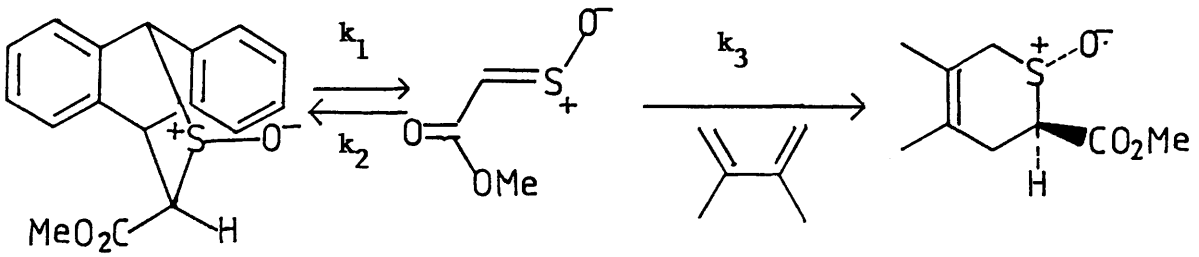
The first attempt to obtain quantitative data on the dissociation rate of the adduct (93) of the (E)-sulphine (91) involved the use of tetracyanoethylene (TCNE) (Scheme 23).



Scheme 23

It was hoped that, if k_3 was significantly large, the reverse reaction (k_2) would be insignificant and thus the reaction would follow first order kinetics. The rate was monitored by the disappearance of the TCNE-benzene charge transfer complex ($\lambda_{max.}$ 384 nm). Dissociation of the epimerised oxide mixture [ca. 95% trans-(93)] at 60 °C took almost 12 h and did not follow first order kinetics. Thus the sulphine and TCNE probably had comparable dienophilicities ($k_3 \simeq k_2$).

In a second, and successful, experiment the sulphine was trapped with 2,3-dimethylbuta-1,3-diene (Scheme 24). The methyl ester (98), being the only material immediately



(98)

+

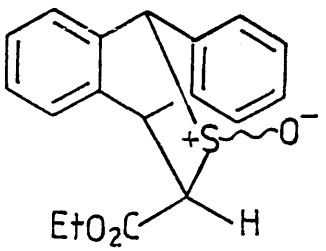
Anthracene

Scheme 24

available, was used. A mixture of the trans-(98) and cis-(99) S-oxide, in the ratio 2.2 : 1, was heated with the diene (2 mol. eq) in benzene at 60 °C and the formation of anthracene was monitored by u.v. spectroscopy ($\lambda_{max.}$ 277 nm). First order kinetics was observed giving $k_1 = 1.53 \times 10^{-4} s^{-1}$ ($t_{\frac{1}{2}}$ = 76 min).

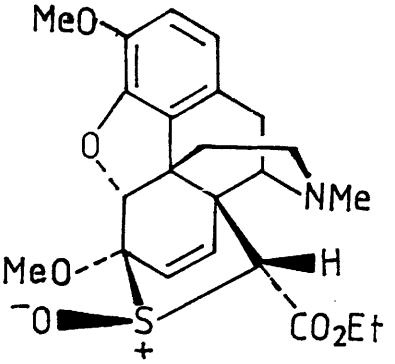
2.1.5 Transfer of the Sulphine from Anthracene to Thebaine

A mixture of the trans-(93) and cis-(94) anthracene adducts in the ratio 2.5 : 1 was heated at 60 °C in benzene with the alkaloid thebaine (67). After 6 h, both the anthracene adducts had disappeared and a single thebaine adduct isomer (109) was formed. Presumably, the unreactive cis-oxide (94) was converted into the reactive trans-oxide (93) by the tertiary amine thebaine.

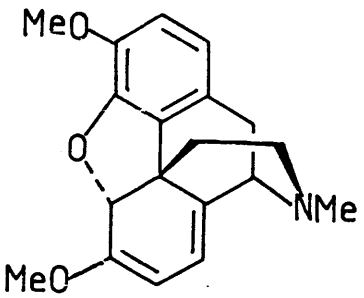


(93,94)

60°C

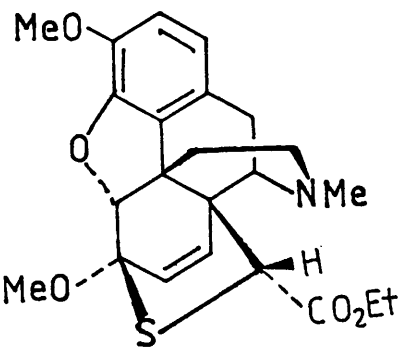


(109)



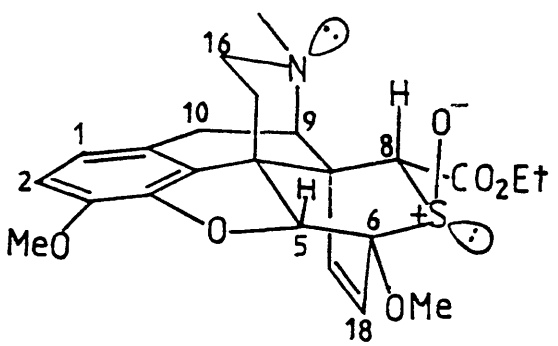
(67)

Chemical evidence for the structure (109) was provided by reduction with the mild reagent phosphorus pentasulphide, which yielded the known thioaldehyde adduct (69)[54e]. Mcpba



(69)

oxidation of the sulphide (69) was expected to give mainly the trans-oxide (109), but it was hoped with some cis-isomer for spectroscopic comparison. The 'thermodynamic' adduct (69) was obtained in quantity, via the sulphenyl chloride route, by heating the 'kinetic' adduct (68a)[54e]. Treatment with mcpba gave the trans-S-oxide (109) as the only product. Acid catalysed epimerisation resulted in decomposition. Further chemical evidence for the trans-stereochemistry about the 7,8-bond was obtained by heating this thebaine adduct with 2,3-dimethylbuta-1,3-diene in refluxing benzene. After 10 h, transfer of the sulphine was complete to afford the trans-dimethylbutadiene adduct (103) containing a trace of cis-adduct (104), presumably arising from thebaine-catalysed epimerisation. When the thebaine S-oxide (109) was heated alone at 80 °C, no other isomers appeared, only increasing amounts of thebaine. Thus (109) may be the thermodynamically favoured isomer but this cannot be proved without comparison with the other possible isomers. Whether (109) is also the kinetically favoured product or arises from a labile intermediate requires experimentation.



(110)

The n.m.r. spectra for the known sulphide (69) obtained by reduction, has been discussed previously in some detail [54e]. ^{13}C and ^{1}H n.m.r. spectra of the sulphoxide (110) were assigned by comparison with those of (69) and other known

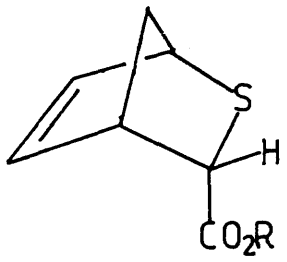
thebaine adducts [54e]. The only points which require mention are the significant changes caused by replacing sulphide with sulphoxide. In the ^{1}H n.m.r. spectrum, the signal for 8-H (δ 4.73) syn to the S-O bond, was shifted upfield (Δδ -0.50) from that of the sulphide (69). The ^{13}C n.m.r. signal of the carbonyl carbon (δ 168.5) syn to the sulphinyl lone pair was shifted slightly upfield (Δδ -2.7). The magnitude was almost identical to that observed in the trans-anthracene adduct (93). Other observations were not so predictable. For example, the signal for 5-H resonated to lower field (Δδ -0.54). Thus assignment of sulphoxide stereochemistry from anisotropic effects alone is not an exact science and is better supported by chemical or other spectroscopic methods (ASIS and LIS).

2.2 Cyclopentadiene Adducts

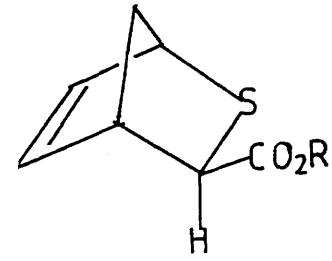
As explained in the Introduction the thioaldehyde ethyl thioxoacetate had been generated from the kinetically determined mixture of cyclopentadiene adducts (endo: exo, 7 : 3) at 120 °C over 24 h and trapped in situ with dimethylbutadiene[54c]. When simple (Z)-alkanethial S-oxides were trapped with cyclopentadiene the resulting endo-adducts underwent rapid rearrangement to sultenes (Scheme 7). The corresponding exo-sulphoxides, obtained by oxidation of the sulphides, were thermally stable[42]. With these observations in mind, a study of the thermal decomposition of cyclopentadiene adduct sulphoxides as a route to sulphines was undertaken.

The adducts (65) and (66) were prepared by the Bunte salt route. Triethylamine was added to the Bunte salt in ethanol [methanol for the methyl ester (105a)] containing cyclopentadiene and calcium chloride. The endo-adduct (65) was separated by double elution preparative t.l.c. To obtain the slower running exo-isomer (66) it was more convenient first to increase the proportion of this isomer

by heating the mixture, before preparative t.l.c.

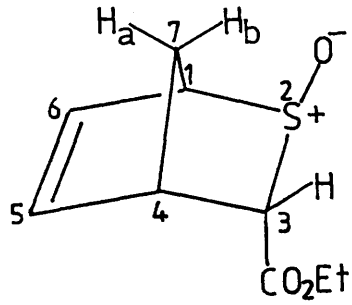


R= Et (65a)
R= Me (65h)

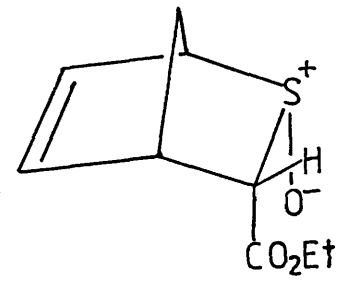


R= Et (66a)
R= Me (66h)

Mcpba oxidation of the endo-adduct (65a) gave a mixture of the exo-(111) and endo-(112) S-oxide in the ratio (ca. 4 : 1). When the mixture was crystallised from di-isopropyl ether the proportion of endo S-oxide in the crystalline product increased (exo:endo ratio ca. 2 : 1). This mixture was characterised by ^{1}H and ^{13}C 200 MHz n.m.r. spectroscopy. The mixture of S-oxides kept in $CDCl_3$ solution for some time was re-examined by ^{1}H n.m.r. spectroscopy and found to contain only the exo-S-oxide (111). Surprisingly, the endo-S-oxide (112) had not undergone the sigmatropic



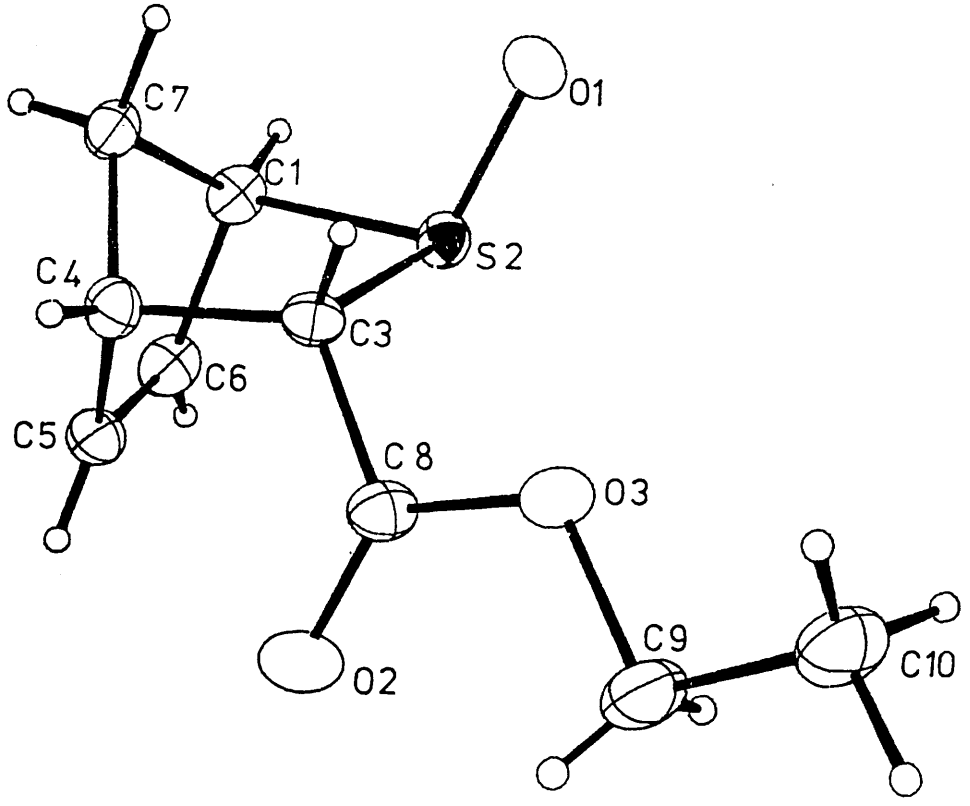
(111)



(112)

rearrangement previously observed by Block *et al.*[42] for simple *Z*-alkyl derivatives, but had isomerised to give the more stable *exo*-oxide (111). This process was presumably catalysed by traces of acid in solution.

Repetition of the oxidation sometimes gave the two isomers (111) and (112) in the ratio *ca.* 4 : 1. These mixtures again isomerised to give the single *exo*-oxide (111) during crystallisation. More often, only the 2-*exo*-3*endo* isomer (111) was obtained after the usual work up. Oxidation with sodium metaperiodate afforded only the *exo*-oxide (111) and later repetitive attempts to obtain mixtures of the isomers (111) and (112) were unsuccessful. However, isolation of a single crystalline isomer was fortunate in some ways and allowed structural confirmation by X-ray crystallography (Figure 4). Attempted acid-epimerisation of the *exo*-*S*-oxide (111) produced no effect and heating the adduct in benzene under reflux resulted in gradual decomposition, but not isomerisation.



(Figure 4)

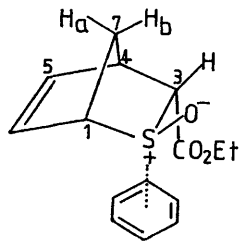
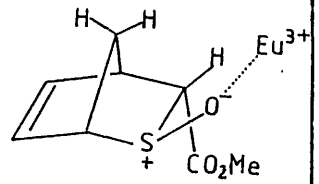
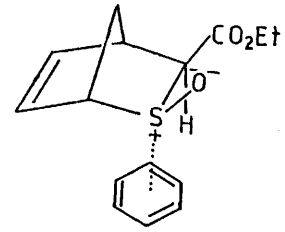
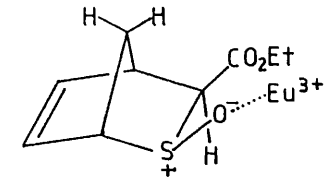
The anisotropic shielding effects in the n.m.r. spectra of the oxides were most obvious at the 3-position. The signal for 3-H, syn to the S-O bond of the exo-oxide (111) δ3.32, appeared substantially upfield (Δδ -1.09) from that for 3-H in the sulphide (65a). For the endo-S-oxide (112), where 3-H, δ3.78, was syn to the lone pair, a smaller upfield shift (Δδ -0.63) was observed. ^{13}C N.m.r. spectra gave similar results; the carbonyl carbon of (112), δ164.9 resonated at a considerably higher field from that of the exo-S-oxide (111), δ168.8.

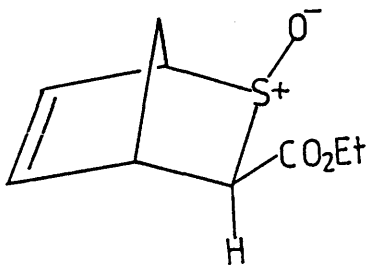
The exo-S-oxide (111) was subjected to ASIS and LIS studies, the corresponding methyl ester (113) being employed for the latter (Table 3). The shifts of 3-H were again the most significant; a negligible upfield shift arose from the ASIS experiment whereas europium caused a large shift to lower field. The LIS experiment also made it possible to assign the ABX system of the bridging methylene group 7_a-H and 7_b-H.

Oxidation of the 3-exo sulphide (66a) with mcpba showed again the strong preference for exo attack. The 2-exo-3-exo sulphoxide (114) was the only isomer produced. The ^1H

Table 3

Aromatic solvent induced shifts (ASIS) and europium shifts (ΔM) for the cyclopentadiene cycloadducts (111), (113) and (114)

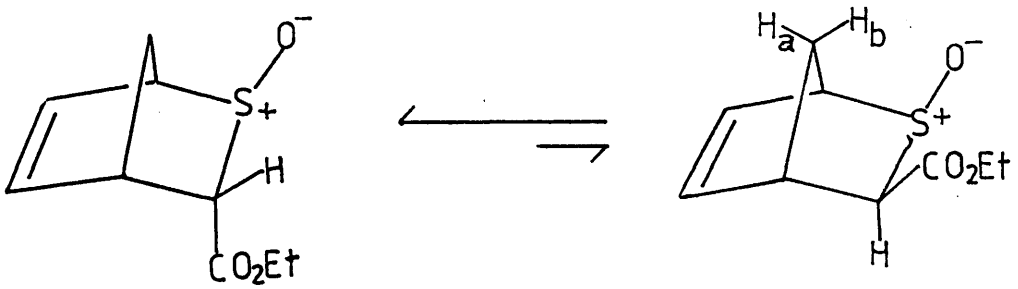
Proton	Δδ(ASIS)	ΔM	Δδ(ASIS)	ΔM
1-H	-0.46	8.1	-0.42	3.0
3-H	-0.07	13.1	-0.32	5.3
4-H	-0.45	3.7	-0.32	3.4
7_b-H	-0.12	10.3	-0.33	8.2
7_a-H	-0.39	4.0	-0.29	2.8
	 (111)	 (113)	 (114)	 (114)



(114)

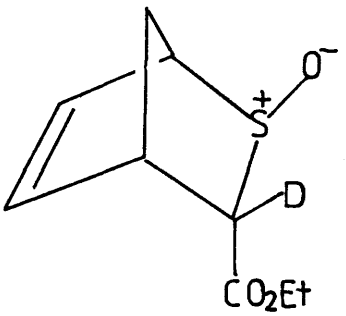
n.m.r. signal, δ3.56, for 3-H, syn to the lone pair, was expected to be shifted slightly upfield from that of the sulphide. A small movement to lower field (Δδ+0.27) was observed, which suggests that some twisting of the crowded cis-geometry may have occurred. The 3-H, 4-H vicinal coupling (J 1.7 Hz) is indicative of an exo-ethoxycarbonyl group and a long range coupling was detected as has been observed for the exo sulphide. ASIS and LIS data are given in Table 3; the values for 3-H are in agreement with the expected trends. Assignment of the ^{1}H n.m.r. signals of 7_a-H and 7_b-H were made from the LIS data.

Base-catalysed epimerisation of the sulphoxides (111) and (114) under the usual conditions (0.2M Et_3N in C_6D_6) produced no change. The same conditions at 60 °C converted both isomers into the same mixture of the 3-endo (111) and 3-exo (114) isomers in the ratio 7 : 3, after 48 h. Clearly the preference for an exo stereochemistry at both



(111) (114)

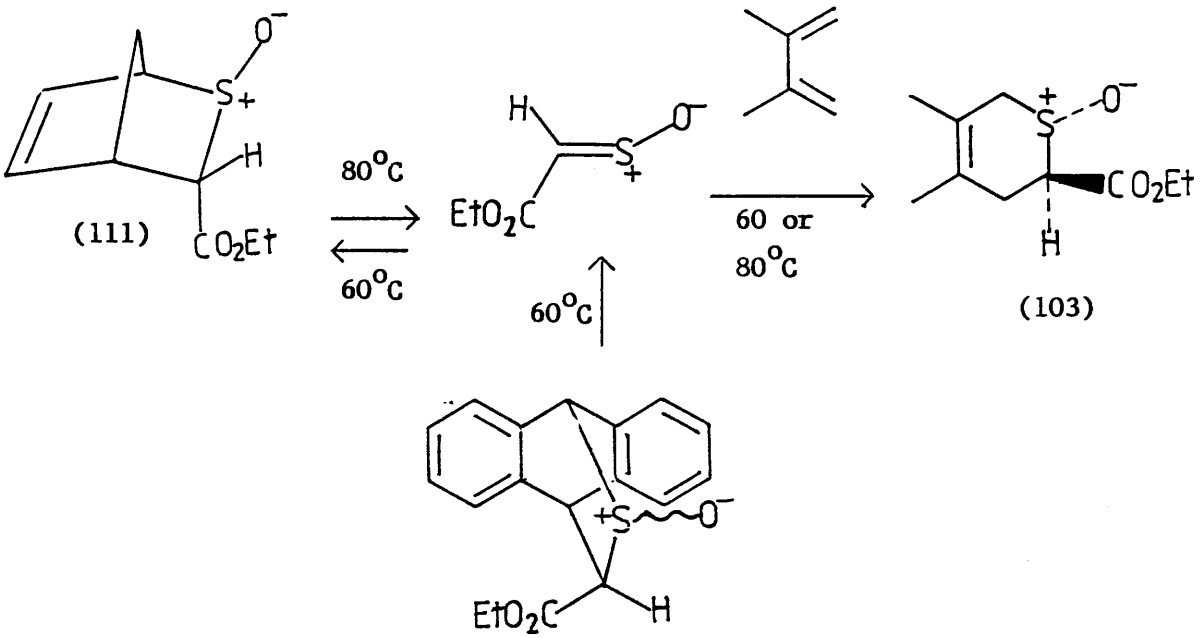
centres was outweighted by a preference for trans geometry. The 2-exo-3-endo oxide (111) was also treated with triethylamine in [$^2H_6$]benzene - [$^2H_4$]methanol for 36h at room temperature. The deuteriated compound (115) was obtained without any detectable epimerisation. Presumably the rate of exo-protonation is much greater than that of endo-protonation.



(115)

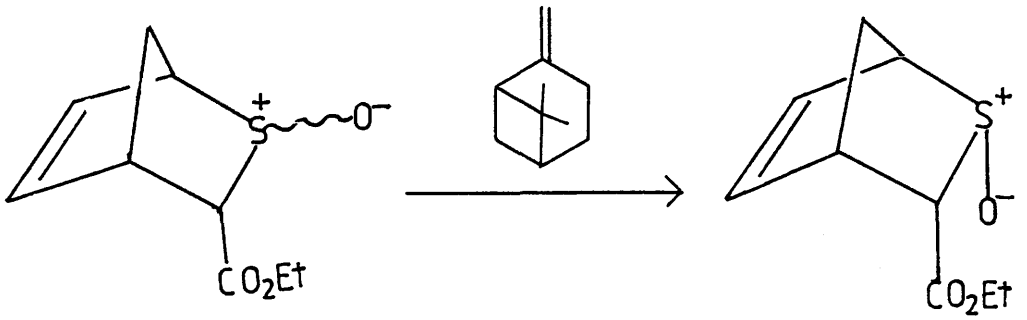
2.2.1 Retro-Diels-Alder reactions

The trans-oxide (111) and dimethylbutadiene were heated in benzene under reflux for 13 h. Transfer of (E)-sulphine took place without isomerisation to give the trans-adduct (103). Transfer of the (E)-sulphine from its anthracene adduct (ca. 95% trans) to cyclopentadiene was achieved at 60 °C during 5 h, the product being exclusively the trans-sulphoxide (111) (Scheme 25).



Scheme 25

Ethyl thioxoacetate was known to undergo 'ene' reactions with β-pinene[54e]. Similar experiments with the corresponding sulphine, although promising did not lead to the isolation of 'ene' products. On one occasion, a mixture of the 2-exo-3-endo (111) and 2-endo-3-endo (112) oxide in the ratio ca. 4 : 1 was heated with β-pinene in benzene under reflux. After 13 h, the mixture was chromatographed and a fraction with an R_F value identical to that of the S-oxide mixture was found (^{1}H n.m.r.) to contain only the 2-endo-3-endo oxide (112). This observation indicates that, as for the anthracene adducts, the cis-S-oxide (112) is thermally more stable than the trans-oxide (111).

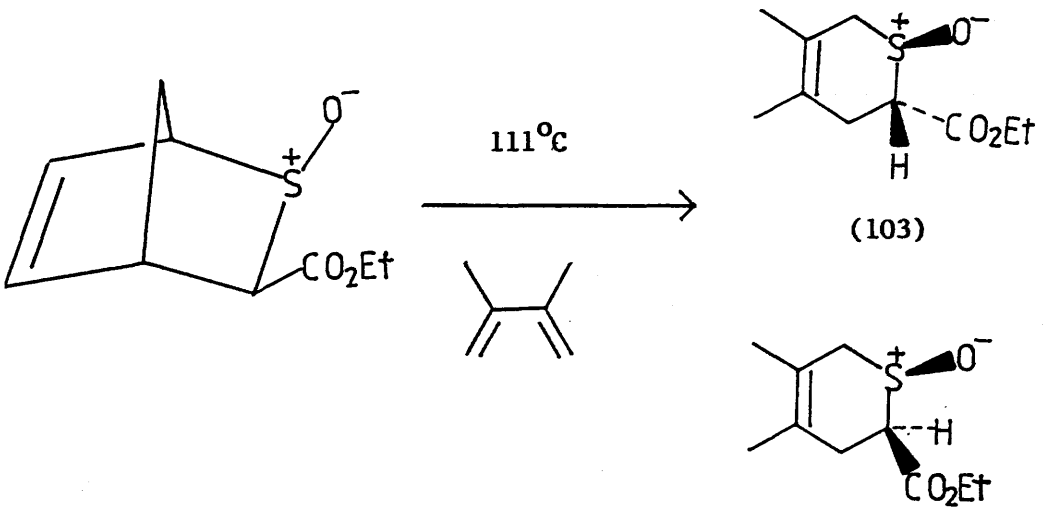


(112)

(111) and (112) + unidentified products

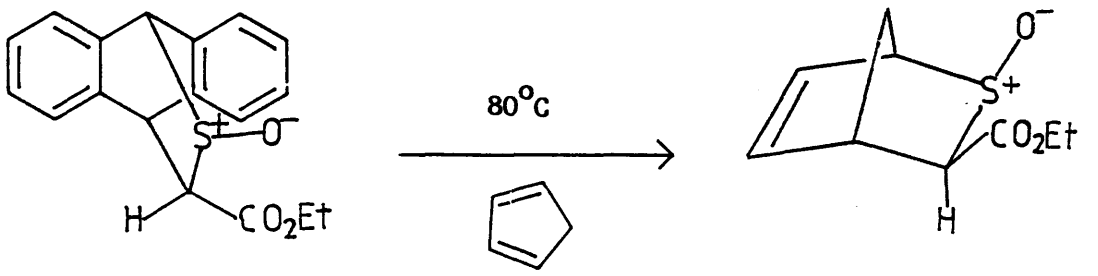
The 2-exo-3-exo adduct (114) required higher temperatures to dissociate. In the first experiment (114) was heated with dimethylbutadiene in toluene in a sealed tube at 110 $^{o}C^{\pm}$ 10 ^{o}C for 13 h. The product was a mixture of the trans-(103) and cis-(104) oxide in the 'thermodynamic' ratio ca. 1 : 1. When the experiment was repeated in toluene with heating under reflux, the trans-(103) and cis-(104) oxide were obtained in the ratio 1 : 2 (not 2 : 1 as previously reported[63]).

These last two transfer experiments were the first where isomerism was observed. Control experiments were carried out to discover at what point this process took place. The starting cis-exo-cyclopentadiene cycloadduct (114) was heated alone in refluxing toluene for 10 h; slow decomposition took place but no other cycloadducts, (111) or (112), were detected. In addition the cis-(104) and trans-(103) dimethylbutadiene products were heated separately in refluxing toluene for 10 h. No epimerisation was evident in either case. As no isomerisation was detectable in either the starting material or product then the most reasonable asumption is, that the transient cis-sulphine generated at 111^{o}C isomerises . (Scheme 26).



Scheme 26

The cis-anthracene oxide (94) successfully underwent thermal cycloreversion and the transient (Z)-sulphine was trapped with dimethylbutadiene. Trapping the (Z)-sulphine with cyclopentadiene might yield useful information. When the cis-adduct (94) was heated with cyclopentadiene in refluxing benzene, only the cis-exo (114) adduct was produced, although in moderate yield; none of the cis-endo-(112) or trans-(111) adduct was seen (Scheme 27). The previous attempted 'ene' reaction showed that the endo-cis

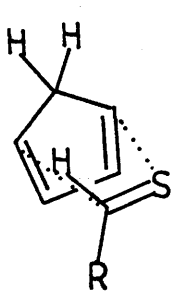


(94) (114)

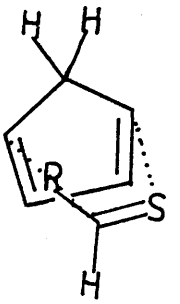
Scheme 27

adduct (112) was stable at 80 °C. Combining this result with that just obtained (Scheme 27) implies that the cis-exo-adduct (114) is the kinetically favoured product.

A theory to begin rationalising the endo or exo preference would be useful. In a recent study the Diels-Alder reaction of a large number of thioaldehydes with cyclopentadiene was examined[64]. All showed a marked kinetic endo selectivity, whereas the thermodynamically favoured product was the exo-isomer. Even simple alkane thials e.g. ^{i}PrCH=S gave an endo:exo ratio of 16 : 1. The secondary orbital overlap would be too small to explain the endo-preference here. The authors proposed a steric explanation in which the transition state is 'early' i.e. it resembles the reactants more than the products (Figure 5). The cyclopentadiene is nearly flat and in the exo-transition state there is an interaction between the R group of the thioaldehyde and the saturated CH_2 bridge. In the endo-transition state the interaction between the methylene group and thioaldehyde hydrogen is much less. As bonding proceeds



endo



exo

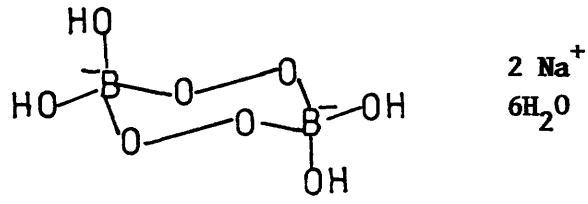
Figure 5

the separation between the methylene and R group increases until, in the product, the exo-isomer is slightly favoured energetically.

If this approach is applied directly to (Z)-ethyl thioxoacetate S-oxide (91), which reacted with cyclopentadiene to give the exo-cycloadduct (114) then a 'late' transition state would be envisaged, but an 'early' transition state would be expected for the reactive sulphine dienophile. However, at this stage there are many unknown factors. Electronic effects due to the S-oxide may strongly affect the stereochemical outcome of the Diels-Alder reaction; indeed a strong exo-oxide preference was observed at the S-oxide, whether formed by sulphide oxidation or Diels-Alder reaction. The reactions of a larger range of (Z)- and (E)- sulphines with cyclopentadiene requires investigation before firm conclusions can be drawn. However, this steric approach must be considered in future in addition to secondary orbital overlap effects.

2.3 Oxidation with Sodium Perborate

Oxidation of the dihydrothiin (72) might in principle occur at sulphur or the tetrasubstituted double bond. In fact, mcpba proved to be selective for sulphur. Initially however, sodium perborate was investigated as an oxidant since it was reported to oxidise sulphides to sulphoxides or sulphones while being unreactive towards olefins[57]. This reagent, often given the formula $NaBO_3.4H_2O$, actually has a dimeric structure (116) in the solid phase.



(116)

When the dihydrothiin (72) was treated with an excess of the perborate (116) of unknown purity in acetic acid at 55 °C the epoxy sulphone (117) was formed. The trans-relationship of the ester and epoxide were established by X-ray analysis (Figure 6). The same result was obtained when newly purchased perborate was employed.

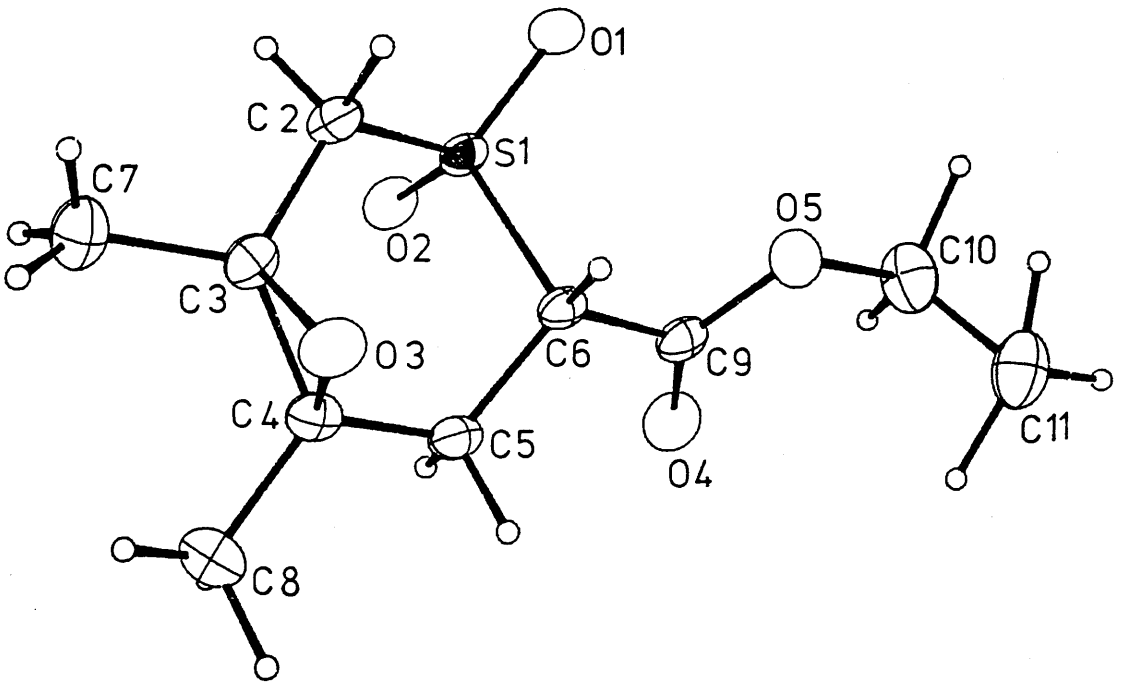
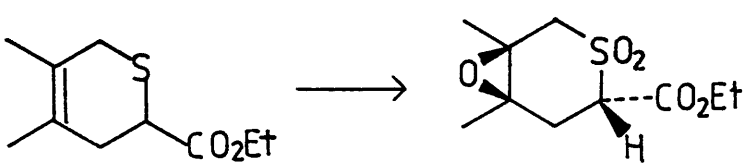


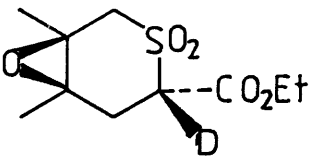
Figure 6



(72) (117)

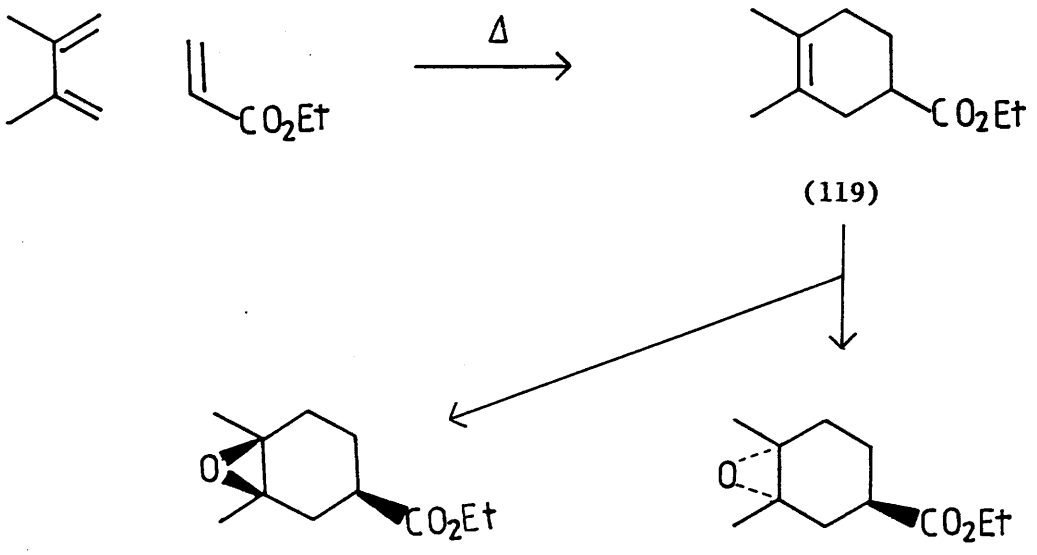
There was a possibility that the stereospecific outcome arose from kinetic control i.e. initial formation of a trans-sulphoxide which directed the epoxidation to the same face and finally oxidation to the sulphone.

To investigate this, the sulphone (117) was treated with 0.2M triethylamine in [$^2H_6$]benzene - [$^2H_4$]methanol overnight at room temperature. Complete exchange of 2-H occurred, but with no isomerisation to give the deuteriated sulphone (118). Thus it was much more likley that acid-catalysed epimerisation took place giving the less sterically kindered trans-sulphone (117). The epimerisation mechanism was further supported when the sulphide was treated with one equivalent of perborate. The reaction gave a mixture of trans-(103) and cis-(104) S-oxides in the ratio ca. 2 : 1 and not purely the trans-isomer as required.



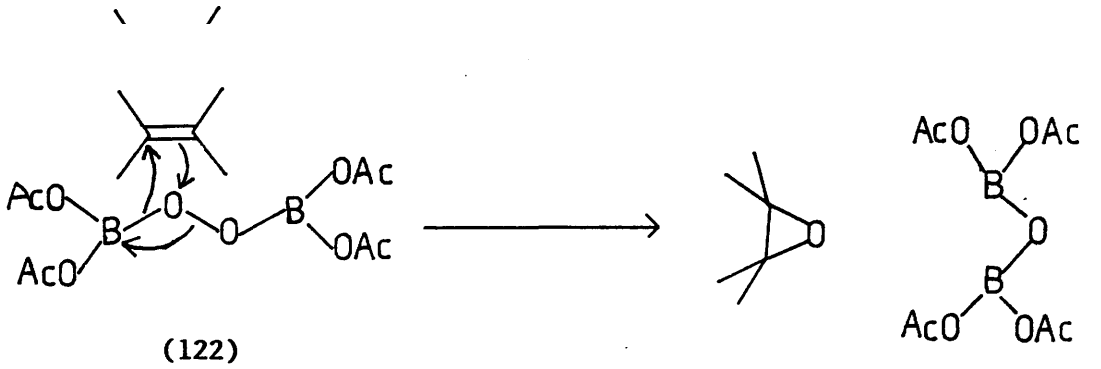
(118)

Although the trans-epoxide did not arise from kinetic control, epoxide formation was still unexpected. Oxidation of the double bond may yet have been assisted by the initially formed sulphoxide or was simply due to the reactive nature of the olefin. To investigate these possibilities the carbon analogue (119)[65] of the dihydrothiapyran (72), was prepared from ethyl acrylate and 2,3-dimethylbuta-1,3-diene. Treatment with mcpba afforded a mixture of epoxides in the ratio 1.35 : 1 (by g.c. and g.c-m.s. analysis). The major isomer was presumably the trans-oxirane.



(121) (120)

Treatment of (119) with an excess of sodium perborate in acetic acid at 55 °C gave a mixture of compounds. Examination of the mixture by capillary g.c. and packed column g.c-m.s. showed 6 components with retention times 18.4, 20.2, 27.8, 36.0 and 37.2 min. The first two components were tentatively identified from their mass spectra as the epoxides (120) and (121). However, the four less volatile, major components also showed fragments (m/e 198) corresponding in mass to the molecular ions of the epoxides (120,121). Perhaps these components were diols, or the corresponding borates, that dehydrated in the mass spectrometer. Whatever the composition of the mixture, it is clear that the clean epoxidation observed for the dihydrothiapyran (72) had not taken place. This suggests that epoxidation may indeed be assisted by a sulphoxide group. Very recently successful epoxidation of a tetrasubstituted double bond was accomplished using sodium perborate in acetic anhydride-dichloromethane (Scheme 28). The authors speculated that the species (122) was the active oxidising agent under these conditions[66].

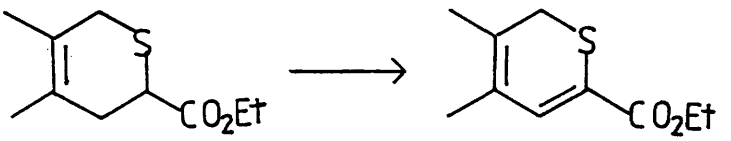


(122)

Scheme 28

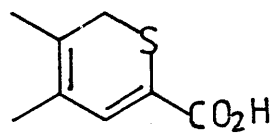
2.3.1 Thiapyran and Thiopyrylium Salt

The problems of controlling oil bath temperatures led to a novel discovery. Treatment of the dihydrothiin (72) with sodium perborate (1 equivalent) in acetic acid at 55 °C, followed by an increase in temperature to ca. 110 °C, gave the thiapyran (123) in 86% yield. This arose, presumably from a Pummerer rearrangement of the initially formed sulphoxide. Thiapyrans have been prepared before by



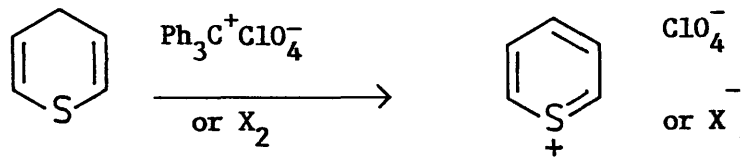
(72) (123)

acid -catalysed dehydration of the corresponding S-oxides[43]. Accordingly, when a mixture of the trans-(103) and cis-(104) oxides in toluene containing a few crystals of p-toluenesulphonic acid was heated under reflux, the thiapyran (123) was formed in 81% yield. Hydrolysis of the oil (123) with aqueous sodium hydroxide gave a yellow crystalline acid (124).



(124)

It was apparent that a further oxidation of the thiapyran might yield a novel thiopyrylium salt. The unsubstituted thiopyrylium salts have been prepared by treatment of thiapyran with trityl perchlorate[67] or halogens[68] (X_2) (Scheme 29). The chloride, bromide and iodide are white, yellow and orange respectively[69], due to



Scheme 29

increasing charge transfer. Extended Huckel calculations and ^{1}H.n.m.r. spectroscopy have shown that 3d-2p π bonding contributes to their high stability. The canonical forms shown in Figure 7 suggests that the β- and γ- positions have similar electron densities; this was supported by the identical chemical shift values for the attached protons.

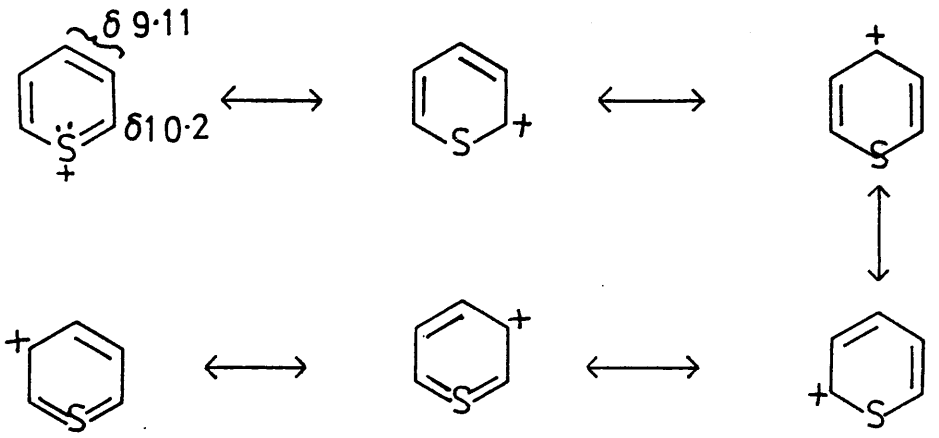
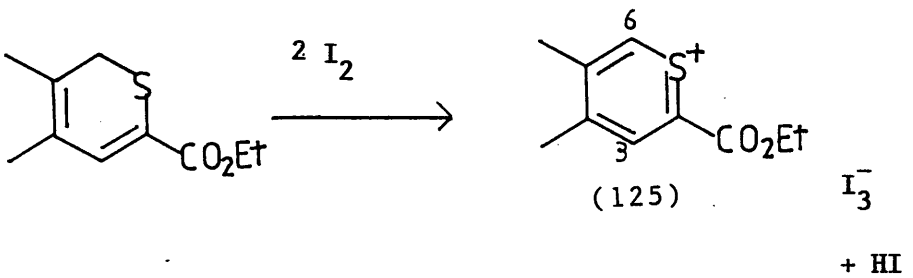


Figure 7

To the thiapyran ester (123) was added 1 mol. equivalent of iodine. Addition of ether precipitated a light red crystalline salt which was identified as the triiodide salt (125) by combustion analysis. Repeating the reaction with two equivalents of iodine gave the salt (125) in 69% yield. The ^{1}H n.m.r. [in $(CD_3)_2CO$] showed singlets δ 3.00 (3H) δ 3.09 (3H), δ 9.37 (1H) and δ 10.27 (1H), together with muliplicities expected for the ethoxy group. The singlets δ 9.37 and δ 10.27 are assigned to the aromatic protons 3-H and 6-H, respectively, the former showing additional deshielding by the adjacent ester group.



(125) I_3^-

+ HI

2.4 Conclusions

The main aim of this work was to demonstrate the retro-Diels-Alder reaction as a method for generating sulphines. This has been achieved for ethyl thioxoacetate S-oxide[63] which exists as independent (Z)- and (E)- isomers. The sulphine cycloadducts of anthracene, cyclopentadiene, and the alkaloid thebaine (67) dissociated on heating (60-111 °C), releasing the sulphine which was trapped with various conjugated dienes. In general the trans-sulphoxides, precursors of the (E)-sulphine, dissociated at lower temperatures than the cis precursors of the (Z)-sulphine. This is explained by a requirement for planarity in the sulphine unit in a late transition state.

A mixture of trans-(93) and cis-(94) anthracene S-oxides released ethyl thioxoacetate (E)-S-oxide at 60 °C and the (Z)-S-oxide at 80 °C, which were trapped with 2,3-dimethylbuta-1,3-diene. When thebaine was employed as the trapping diene the tertiary base isomerised the cis-precursor, releasing all the sulphine in the (E)-form. The thebaine adduct itself released the (E)-sulphine at 80 °C. It was not determined whether this adduct is the kinetically or thermodynamically favoured regioisomer.

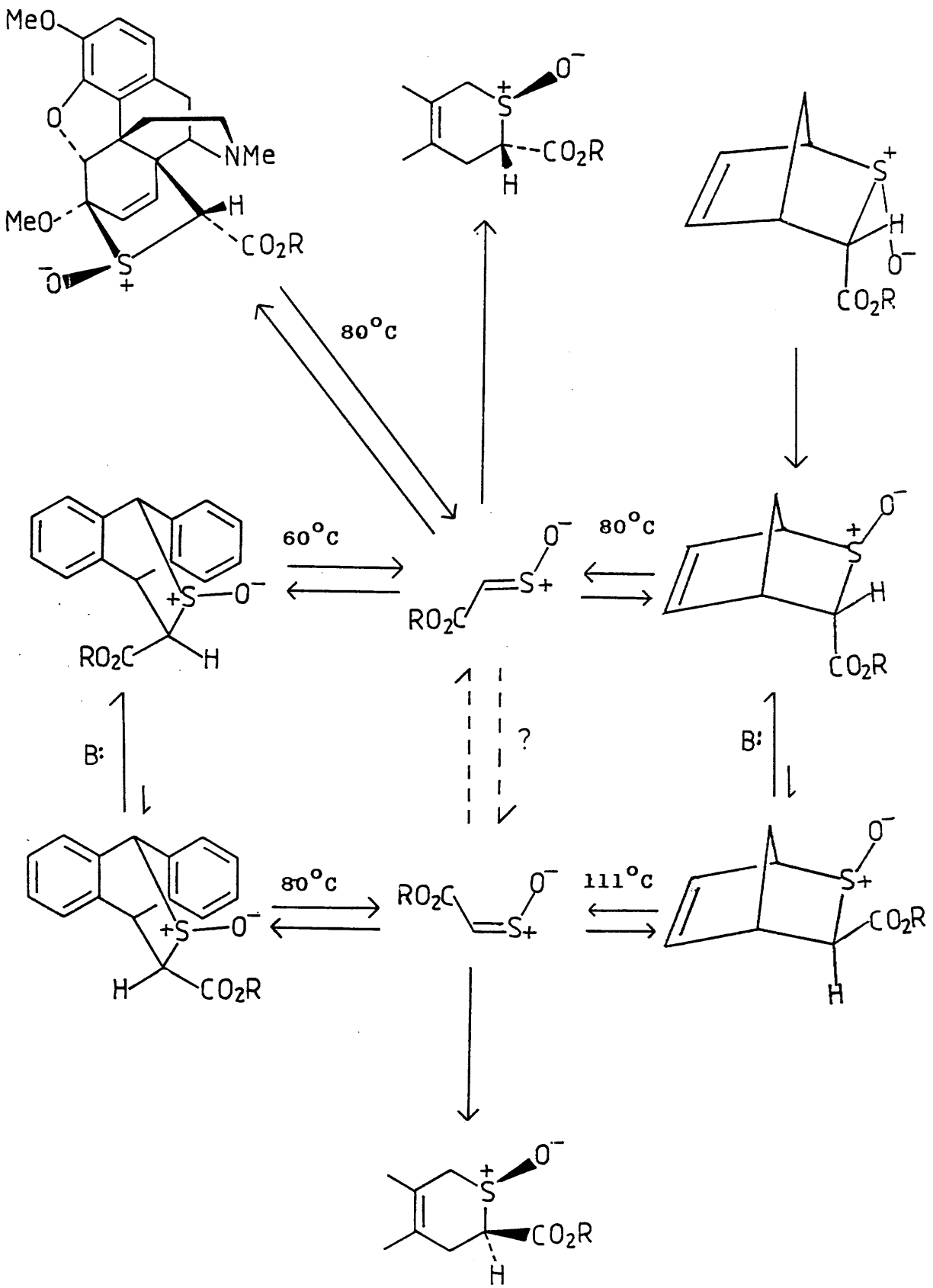
The trans-cyclopentadiene adduct (111) provided a clean source of the (E)-sulphine at 80 °C. The cis-exo-adduct (114) required a higher temperature, 111 °C, to dissociate and yielded a variable mixture of (Z)- and (E)-sulphine. Further work is required to examine the extent of (Z)-(E) sulphine isomerisation.

A remarkable difference in behaviour was observed between these α-oxo sulphines and the previously reported[42] (Z)-alkanethial S-oxides, which showed a strong endo-preference in cycloaddition to cyclopentadiene. Both the (E)- and (Z)-isomers of ethyl thioxoacetate S-oxide, when

trapped with cyclopentadiene, gave adducts in which the sulphoxide oxygen occupied the exo-position. The latter observation was particularly unexpected since both simple thioaldehyde esters and alkanethial (Z)-S-oxides favour endo addition.

Another difference was noted between the cyclopentadiene endo-sulphoxide adducts having either 3-endo alkyl groups or a 3-endo ethoxycarbonyl group (112). The 3-endo-alkyl substituted endo-sulphoxides rapidly undergo a sigmatropic rearrangement to give a sultene[42] while the 3-endo-ethoxycarbonyl substituted sulphoxide was stable at 80 °C; indeed crystallising solutions containing the endo-S-oxide epimerised, possibly catalysed by traces of acid, to give the exo-isomer. Although more investigations are needed to explain these substituent effects, it is likely that the electron withdrawing nature of the ester group, rather than any steric hinderance, retards the rearrangement.

During the work, much time was taken gathering n.m.r. spectroscopic evidence for assigning cis or trans configurations to sulphoxides. This experience suggests that the use of lanthanide shift reagents provides the most reliable and easily interpreted results. The sulphines themselves were unfortunately never directly observed. It would be pleasing to do so, possibly by the use of the popular FVP technique. A scheme summarising the various transformations is outlined below.


MeO
O
NMe
MeO
H
CO2R
O
S
+
80°C
60°C
80°C
RO2C
B:
?
B:
80°C
111°C
RO2C
H
CO2R

Chapter 3 Experimental Section

3.1 General Procedures

Melting points were recorded on a Reichert hot-stage apparatus, and are uncorrected.

Elemental analyses were recorded by Mrs. W. Harkness and her staff. Ultra-violet spectra were recorded on Pye-Unicam machines.

Infrared spectra were recorded on a Perkin-Elmer 580 spectrometer by Mrs. F.W. Lawrie and her staff. Routine, C.W. proton n.m.r. spectra were recorded on a Perkin-Elmer R32 (90MHz) spectrometer. High resolution F.T. proton n.m.r. spectra were obtained from various instruments: Bruker (200MHz) by Dr. D.S. Rycroft; Bruker (360MHz), by Dr. I. Sadler and his staff (University of Edinburgh); a spectrometer at Portsmouth Polytechnic (270 MHz), by arrangement with Dr. A.N. Trethewey.

Carbon-13 n.m.r. spectra were recorded on a Varian XL-100 spectrometer in the F.T. mode at 25.2MHz. Proton-noise and off-resonance decoupling were employed to assign carbon multiplicities. Other ^{13}C spectra were recorded at 50.3MHz on a Bruker instrument using the DEPT pulse sequence to assign multiplicities. All signals are given with reference to tetramethylsilane (δ 0).

Analytical t.l.c. were carried out on precoated Kieselgel GF_{254} plates of thickness 0.25 mm (Merck). Spots were located by u.v. light (254 nm) and developed in an iodine tank. Preparative t.l.c. separations were carried out on 20x20 cm plates coated with a 1 mm-thick layer of

silica gel GF_{254} (Merck).

Column chromatography was carried out using the 'flash' method or by short column. In the latter method t.l.c. grade silica was added, dry, to a sintered column under reduced pressure (water pump). The dry silica was tamped down and solvent passed through. After loading the column, fractions were collected under reduced pressure. The column could be sucked dry, without cracking to allow a change of solvent.

3-Chloroperbenzoic acid was purified by washing with pH 7.5 phosphate buffer and dried over P_4O_{10} *in vacuo*. Purity was determined iodimetrically by the method described by Vogel[70] for perbenzoic acid. Triethylamine was distilled from KOH or P_4O_{10} and stored over KOH pellets. Cyclopentadiene was obtained by thermal cracking of dicyclopentadiene, stored at 5 °C and used within 24 hours. Thebaine was recrystallised from ethanol prior to use.

All solvents used in reactions were Analar grade. Ether and benzene were dried over sodium wire. Di-isopropyl ether was passed through basic alumina.

Solutions of products in organic solvents were dried with anhydrous magnesium sulphate and evaporated under reduced pressure.

Gas chromatography was performed by Dr. W.J. Cole. Capillary g.c. was run on a Hewlett Packard 5880A containing a 25 m x 0.32 mm I.D. SE-54 fused silica capillary and detected by flame ionisation. G.c.-m.s. was carried out on an L.K.B. 9000 instrument *via* a packed column. Mass spectra were obtained by electron impact at an ionising voltage of 70eV.

N-Chlorosuccinimide was purified by washing with water, drying *in vacuo* over P_2O_5, and recrystallising from benzene.

Purified material was stored at 5 °C.

3.2 Preparation of Cycloadducts

Ethoxycarbonyl-(92) and Methoxycarbonylmethanesulphenyl Chloride (97)[54e]. - A few drops of ethyl or methyl mercaptoacetate were added to a stirred suspension of N-chlorosuccinimide at room temperature. After 5 min a yellow colour appeared, signifying the presence of the sulphenyl chloride. The rest of the thiol was then added at a rate, sufficient to keep the temperature below 40 °C. After 2h the solution was decanted off through glass wool, to remove succinimide, into a dropping funnel, prior to addition to a solution of the appropriate diene containing triethylamine.

11-Ethoxycarbonyl-9,10-dihydro-9,10-thiaethanoanthracene (70a)[54e]. - Ethoxycarbonylmethanesulphenyl chloride (92), prepared from ethyl mercaptoacetate (1.32 g, 11.0 mmol) and N-chlorosuccinimide (1.74 g, 13.0 mmol) in benzene (20 ml) was added dropwise with stirring to a solution of anthracene (9.79 g, 55 mmol) and triethylamine (1.31 g, 13.0 mmol) in chloroform (200ml) with heating under reflux. After 30 min the solution was cooled and excess anthracene filtered off. The filtrate was washed with dilute hydrochloric acid (2 x 50ml) and water, dried and concentrated in vacuo to 75 ml when more anthracene was filtered off. The solvent was evaporated and the residue was chromatographed using 30,60 and finally 100% chloroform-light petroleum to give the cycloadduct (70a) (61%), m.p. 136-137 °C (from di-isopropyl ether) [lit.[54e], 135-137 °C (from diethyl ether)]; δ_H(90MHz; $CDCl_3$) 1.15 (3H,t, J 7 Hz, Me), 4.09 (2H, q, J 7 Hz, CH_2), 4.12 (1H, d, J 3.5 Hz, $SC\underline{H}CO_2Et$), 5.07 (1H, d, J 3.5Hz, 10-H), 5.13 (1H, s, 9-H), and 7.1-7.55 (8H, m, Ar); δ_C (25MHz;$CDCl_3$) 14.0 (Me), 45.7, 47.4, and 52.0 (C-9, -10 and -12), 121.8, 122.2, 124.5, 126.0, 126.2, 126.7, and 126.8 (ArCH), 138.4, 142.0, 142.8, and 143.2 (ArC), and 170.5 (CO).

11-Methoxycarbonyl-9,10-dihydro-9,10-thiaethanoanthracene (96). - Methoxycarbonylmethanesulphenyl chloride (97), prepared from methyl mercaptoacetate (5 g, 47 mmol) and N-chlorosuccinimide (7.53 g, 56 mmol) in benzene (75 ml) was added dropwise with stirring to anthracene (41.8 g, 234 mmol) and triethylamine (5.66 g, 56 mmol) in chloroform (600 ml) with heating under reflux. After 30 min the solution was cooled and excess anthracene removed by filtration. The filtrate was washed with dilute hydrochloric acid (2 x 50ml) and water(50ml), and was dried and evaporated. The residue was chromatographed to give the methyl ester (96) (53%), m.p. 146-148 °C (from di-isopropyl ether) (Found: C, 72.32; H, 4.99; S, 11.35. $C_{17}H_{14}O_2S$ requires C, 72.59; H, 4.94; S, 10.96%); $\nu_{max.}$ (KBr) 1700 and 1740 (CO), 1468, 1454, and 1427 cm^{-1}; δ_H (90MHz; $CDCl_3$) 3.52 (3H, s, Me), 4.08 (1H, d, J 2 Hz, $SCHCO_2Me$), 5.02 (1H, d, J 2 Hz, 10-H), 5.07 (1H, s, 9-H) and 7.0-7.45 (8H, m, ArH); δ_c (25.2MHz; $CDCl_3$) 45.6, 47.3 and 51.7 (C-9, -10 and -12), 52.4 (OMe), 121.7, 122.1, 124.5, 126.1, 126.2 and 126.7 (ArCH), 138.3, 141.0, 142.6 and 143.2 (ArC) and 171.0 ($\underline{C}O_2Et$).

Oxidation of Sulphides with 3-Chloroperbenzoic Acid. - To a solution of sulphide (1 mmol) in dichloromethane (5 ml) was added dropwise, with stirring, a solution of 3-chloroperbenzoic acid (1 mmol), as determined by iodine titration) in dichloromethane (10 ml) at room temperature. After 3 min, the solution was washed with aqueous sodium sulphite to destroy excess peracid and twice with saturated sodium bicarbonate, and then water (or brine if an emulsion formed). The solution was dried and evaporated and the product was purified by crystallisation or distillation (Kugelrohr).

11-Ethoxycarbonyl-9,10-dihydro-9,10-thiaethanoanthracene S-Oxide (93) and (94). - The oxides (95%) were obtained as an inseparable mixture of trans (93) and cis (94) isomers (ratio 2.5:1, calculated from the ^{1}H n.m.r. spectrum) forming small white needles m.p. 120-121 °C (decomp. to form plates of anthracene) (Found: C, 69.10; H, 5.23; S, 10.48. $C_{18}H_{16}O_3S$ requires C, 69.21; H, 5.16; S, 10.26%); $\nu_{max.}$ (CCl_4) 1740 (CO) and 1070 cm^{-1} (SO); δ_H (200 MHz; $CDCl_3$) [trans-isomer (93)] 1.19 (3H, t, J 7.1 Hz, Me), 3.13 (1H, d, J 2.3 Hz, 12-H), 4.11 and 4.17 (2H, qABq, J 10.7 and 7.1 Hz, OCH_2), 4.94 (1H, d, J 2.3 Hz, 10-H), 5.69 (1H, s, 9-H) and 7.19-7.60 (8H, m, ArH); δ_c (25.2 MHz; $CDCl_3$) [trans-isomer (93)] 14.0 (Me), 45.9 (C-10), 62.1 (OCH_2), 67.1 and 76.0 (C-9 and -10), 124.5-139.2 (C-Ar), and 167.7 ($\underline{C}O_2Et$); δ_H (200 MHz, $CDCl_3$) [cis-isomer (94)] 1.20 (3H, t, J 7.1 Hz, Me), 3.93 (1H, dd, J 2.1 and 0.6 Hz, 12-H), 4.09 and 4.15 (2H, qABq, J 10.8 and 7.1 Hz, OCH_2), 4.97 (1H, d, J 2.1 Hz, 10-H), 5.69 (1H, S, 9-H), and 7.1-7.7 (8H, m, ArH); δ_c (25.2 MHz; $CDCl_3$) [cis-isomer (94)] 14.0 (Me), 45.4 (C-10), 61.7 (OCH_2), 67.1, and 76.0 (C-9 and -12), 124.5-139.2 (C-Ar), and 165.0 ($\underline{C}O_2Et$).

11-Methoxycarbonyl-9,10-dihydro-9,10-thiaethanoanthracene S-Oxide (98) and (99). - The corresponding sulphide (96) on oxidation gave (95%) an inseparable mixture of the trans (98) and cis (99) S-oxides in the ratio 2.5:1 respectively (obtained from the ^{1}H n.m.r. spectrum), m.p. 119-121 °C (decomp. to give hexagonal plates of anthracene) (Found: C, 68.41; H, 4.79; S, 10.45. $C_{17}H_{14}O_3S$ requires C, 68.43; H, 4.73; S, 10.75%); $\nu_{max.}$ ($CHCl_3$) 1743 (CO) and 1055cm^{-1} (SO); m/z 178 (anthracene, 100%), δ_H (90 MHz; $CDCl_3$) [trans-oxide (98)] 3.01 (1H, d, J 2 Hz, 12-H), 3.67 (3 H, s, Me), 4.91 (1H, d, J 2 Hz, 10-H), 5.66 (1H, s, 9-H), and 7.10-7.60 (8H, m, ArH); δ_c (25.2 MHz; $CDCl_3$) [trans-oxide (98)] 45.7 (C-10), 52.9 (OMe), 67.3, and 75.9 (C-9 and -12), 124.6-132.4 (C-Ar), 138.8 and 139.8 (ArC), and 168.3 $\underline{C}O_2Me$); δ_H (90MHz; $CDCl_3$) [cis-oxide (99)] 3.65 (3H, s, Me), 3.92 (1H,

d, J 2Hz, 12-H), 4.94 (1H, d, J 2Hz, 10-H), 5.75 (1H, s, 9-H), and 7.10-7.60 (8H, m, ArH); δ_c (25.2MHz; $CDCl_3$) [cis-oxide (99)] 45.4 (C-10), 52.5 (Me), 67.3 and 75.3 (C-9 and -12), 124.2-132.1 (C-Ar), 136.5 and 141.8 (ArC) and 165.3 ($\underline{C}O_2Me$).

trans-11-Carboxy-9, 10-dihydro-9,10-thiaethanoanthracene S-Oxide (100). - The mixture of cycloadducts (93) and (94) (0.35 g, 1.1 mmol) in ethanol was added, with stirring to 0.1 M sodium hydroxide (20 ml). After 14 h ethanol was evaporated under reduced pressure and the aqueous layer was washed with dichloromethane (2 x 5ml), acidified with dilute hydrochloric acid and cooled in an ice bath. A white precipitate was filtered off, washed with cold water, dried over P_4O_{10} and recrystallised from methanol to give the pure trans-acid (100) (92%), m.p. 184-186 °C (decomp. to plates of anthracene) (Found: C, 67.46; H, 3.95; S, 11.50. $C_{16}H_{12}O_3S$ requires C, 67.58; H, 4.26; S, 11.28%); $\nu_{max.}$ (KBr) 1720 (CO) and 1020 and 1009 cm^{-1} (SO); δ_H [90MHz; $(CD_3)_2SO$] 3.09 (1H, d, J 2Hz, 12-H), 4.98 (1H, d, J 2Hz, 10-H), 6.05 (1H, s, 9-H) and 7.13-7.68 (8H, m, ArH); m/z 284 (M^+, 0.25%), 179 (91), 106 ($HO_2C.CH{=}SO$, 22) and 189 (100).

cis-(101) and trans-(100) 11-Carboxy-9,10-dihydro-9,10-thiaethanoanthracene S-Oxide. - 3-Chloroperbenzoic acid (0.67 mmol) in dichloromethene (3 ml) was added dropwise with stirring to the sulphide acid (102)[54e] (181 mg, 0.67 mmol) in dichloromethane (3ml) at room temperature. 5 min after addition of all the peracid, the solution was evaporated and the residue washed with cold ether (75 ml) to remove 3-chlorobenzoic acid. The residue was recrystallised from methanol to give a mixture of acids (100) and (101) (88%) in the ratio (100):(101) 1.1:1, m.p. 184-186 °C (decomp. to give anthracene); $\nu_{max.}$ (KBr) 1725 (CO), 1471, 1208 and 1021 and 1010cm^{-1} (SO); δ_H [90MHz; $(CD_3)_2SO$] cis-

isomer (the spectrum of the trans-isomer is given above) 3.98 (1H, d, J 2Hz, 12-H), 5.06 (1H, d, J 2Hz, 10-H), 6.17 (1H, s, 9-H) and 7.10-7.75 (8H, m, ArH).

Esterification of trans-Acid (100) with Diazomethane. - Diazomethane (2 mmol) in ether (10 ml) was added dropwise with stirring to the acid (100) (45.6 mg, 0.16 mmol) in methanol (10 ml). After 10 min the yellow solution was evaporated and the residue dried in vacuo to leave the methyl ester (45 mg, >90% purity as judged by ^{1}H n.m.r. spectroscopy). The ^{1}H n.m.r. spectrum (90 MHz; $CDCl_3$) was identical to that of the trans-methyl ester (98) with no trace of cis-isomer (99) being present.

2-Ethoxycarbonyl-3,6-dihydro-4,5-dimethyl-2H-thiapyran 1-Oxide (103) and (104). - The anthracene adduct sulphoxides (93) and (94) (trans :cis = 2.5:1) (2.52 g, 8.07 mmol) and 2,3-dimethylbuta-1,3-diene (0.99 g, 12.1 mmol) were heated in benzene (15 ml) under reflux for 6 h. Solvent was evaporated and the product was purified by short column chromatography to give an inseparable mixture of dimethylbutadiene adducts (103) and (104) [trans:cis = 2.4:1] calculated from the ^{1}H n.m.r. (360MHz) spectrum (95%) (b.p. 130-135 ^{o}C 0.1 mmHg) (Found: C, 55.5; H, 7.6; S, 14.8. $C_{11}H_{16}O_3S$ requires C, 55.5; H, 7.5; S, 14.8%); $\nu_{max.}$ (CCl_4) 2980, 2917, 1740 (CO), and 1064cm^{-1} (SO); m/z 216 (M^+, 8%), 200 (M-O, 42), 183 (37), 168 (M-SO, 23), 153 (49), 125 (92), 111 (48) and 93 (100); trans-isomer (103): δ_H (360 MHz; $CDCl_3$) 1.23 (3H, t, J 7.2 Hz, CH_2Me), 1.66 and 1.67 (6H, s, 4- and 5-Me), 2.54 (1H, dd, J 17.5 and 8 Hz, 3-H) 2.67 (1H, br.dd J 17.5 and ca. 5Hz, 3-H), 3.45 (2H, ABq, J 16.0 Hz, $\Delta\delta$ 0.09, 6-H), 3.75 (1H, dd, J 8.0 and 5.2 Hz, 2-H) and 4.19 (2H, q, J 7.2 Hz, OCH_2); δ_C (25.2 MHz; $CDCl_3$) 14.0 (OCH_2Me), 19.3 and 19.8 (4- and 5-Me), 29.9 (C-3), 52.4 (C-6), 61.8 (C-2), 62.0 (OCH_2), 117.1

and 126.6 (C-4 and -5), and 168.4 ($\underline{C}O_2Et$); cis-isomer (104): δ_H 1.26 (3H, t, J 7.2 Hz, $OCH_2\underline{Me}$), 1.68 and 1.72 (6H, s, 4- and 5- Me), 2.33 (1H, dd, J 17.5 and 5Hz, 3-H), 2.87 (1H, br. dd, J 17.5 and 12Hz, 3-H), 3.25 (2H, m, 6-H), 3.33 (1H, dd, J 12.1 and 4.7 Hz, 2-H), and 4.23 (2H, q, J 7.2Hz, OCH_2); δ_c (25.2 MHz; $CDCl_3$) 14.0 ($OCH_2\underline{Me}$), 19.5 and 19.7 (4- and 5- Me), 24.9 (C-3), 51.9 (C-6), 57.2 (C-2), 62.0 (OCH_2), 115.2 and 126.8 (C-4 and -5), and 168.1 ($\underline{C}O_2Et$).

Sodium S-ethoxycarbonylmethyl thiosulphate (Bunte salt) (105)[62]. - Ethyl bromoacetate (8.03 g, 48.9 mmol) in acetone (25 ml) was added to sodium thiosulphate pentahydrate (12.13 g, 48.9 mmol) in water (25 ml). The mixture was shaken for 30 s, evaporated and dried in vacuo. The white solid was extracted with hot ethanol (75 ml); the extract was filtered, and the filtrate set aside to cool. The crystalline precipitate of the product was filtered off. The supernatant liquid was concentrated to 20 ml in vacuo. After cooling a precipitate was filtered off. The precipitate was extracted with hot ethanol (30ml) and the extract was set to cool. The crystalline precipitate of product was filtered off. The combined crops gave the Bunte salt (105) (82%).

Sodium S-Methoxycarbonylmethyl thiosulphate (Bunte salt) (105a). - Methyl bromoacetate (23.3 g, 0.15 mol) in acetone (75 ml) was added to sodium thiosulphate pentahydrate (37.7 g, 0.15 mol) in water (75 ml) and shaken for 30 s. The solution was evaporated and the residue was dried in vacuo over P_2O_5, and extracted with hot methanol (175 ml). The hot extracts were filtered. The filtrate, on cooling gave a first crop of the product (105a), which was filtered off. The filtrate was concentrated to 40 ml in vacuo and filtered. The residue was extracted with hot methanol (30ml). After cooling the extract gave a second

crop of the Bunte salt (105a) (86%) m.p.164-165 °C (from methanol) (Found: C, 17.2; H, 2.3; S, 30.4. $C_3H_5O_5S_2Na$ requires C, 17.3; H, 2.4; S, 30.8%); ν_{max} (KBr) 1719 (CO), 1250, 1215, 1046, and 650 cm^{-1}; δ_H (90MHz; D_2O; standard CH_3CN, δ 2.06) 3.68 (3H, s, Me), and 3.82 (2H, s, CH_2).

Ethyl 3,6-Dihydro-4,5-dimethyl-2H-thiin-2-carboxylate (72). - This compound was prepared by the method of Bladon et al.[54e] from ethyl mercaptoacetate (2.0 g, 16.6 mmol), N -chlorosuccinimide (2.64 g, 19.7 mmol) and 2,3-dimethylbuta-1,3-diene (1.24 g, 15.1 mmol) to give the ethyl ester (72) (14.0 g, 63%), b.p. 150-160 °C (0.05 mmHg) [lit.[54e], 110-120 °C (0.02 mmHg)].

Preparation of the Cycloadduct (72) by the Bunte salt method. - Triethylamine (2.54 g, 25.1 mmol) was added slowly with stirring to sodium S-ethoxycarbonylmethyl thiosulphate (105) (5.6 g, 25.2 mmol) in ethanol (100 ml) containing calcium chloride dihydrate (3.70 g, 25.2 mmol) and 2,3-dimethylbuta-1,3-diene (2.27 g, 27.7 mmol) at room temperature. After 24 h the mixture was filtered and evaporated. The residue was washed with dichloromethane (150 ml) and added to the ethanolic filtrate. The combined organic solutions were washed successively with water, dilute hydrochloric acid, aqueous sodium bicarbonate and water. The solution was dried and evaporated to give a yellow oil, which on distillation (Kugelrohr, 150-160 °C, 0.05 mmHg) (lit.[54e], 110-120 °C, 0.02 mmHg) gave the ethyl ester (72) (2.28 g, 45%); $\lambda_{max.}$ (cyclohexane) 269 (ε 353), 244 (337), and 209 nm (3254); δ_H (360 MHz; $CDCl_3$) 1.21 (3H, t, J 7.2 Hz, $OCH_2\underline{Me}$), 1.63 (3H, s, 4- or 5-Me), 1.65 (3H, s, 4- or 5-Me), 2.38 (2H, m, 3-H), 2.98 and 3.05 (2H, ABq, J 16.6 Hz, 6-H), 3.54 (1H, t, J 6.4 Hz, 2-H), and 4.11 (2H, q, J 7.2 Hz, OCH_2); δ_c (25.2 MHz; $CDCl_3$) 14.0 ($OCH_2\underline{Me}$), 19.3 and 19.9 (4- and 5-Me), 30.5 (C-3) 34.6 (C-6), 41.0 (C-2), 61.1 (OCH_2), 123.0 and 125.6 (C-4 and -5) and 171.5 ($\underline{C}O_2Et$).

Oxidation of Dihydrothiin (72) with mcpba. - The sulphide (72) (164 mg, 0.82 mmol) was oxidised with 3-chloroperbenzoic acid (0.82 mmol) to give a yellow oil. Purification by distillation (Kugelrohr 130-135 °C, 0.1 mm Hg) gave a mixture (85%) of the S-oxides (103) and (104) in the ratio trans (103): cis (104) = 4.0 : 1.0 (measured by capillary g.c. and confirmed by g.c.-m.s.).

Hydrolysis of the trans-and cis-Dimethylbutadiene Sulphoxide Adducts (103) and (104). - The sulphoxides (103) and (104) (206 mg, 0.95 mmol) in methanol (2 ml) were added to 0.1 M sodium hydroxide (10 ml) with stirring at room temperature. After 36 h, the solution was acidified with dilute hydrochloric acid and extracted with ethyl acetate (2 x 20ml). The extract was washed with water (5 ml), dried and evaporated to give the acid (108) as a white foam (150 mg, 80%), δ_H (90 MHz; $CDCl_3$) 1.73 (6H, br. s, 4- and 5-Me), 2.73 (2H, m, 3-H) 3.50 (2H, m, 6-H), 4.01 (1H, t, J 7 Hz, 2-H) and 8.71 (1H, br. s, CO_2H). The sample decomposed to an unidentified green solid on attempted crystallisation from di-isopropyl ether.

Thermal Transfer of the E-Sulphine from Anthracene to Dimethylbutadiene. - The anthracene adduct (93, 94) (62 mg, 0.19 mmol) (95% trans) and dimethylbutadiene (17.9 mg, 0.22 mmol) was heated in benzene (5 ml) for 5 h at 60 °C. Short column chromatography (ether) and distillation (Kugelrohr, b.p. 140 °C, 0.1 mm Hg) gave the trans-S-oxide (103) (36.3 mg, 88%) [identified by ^1H n.m.r. spectroscopy (90 MHz; $CDCl_3$)]

Thermal Cleavage of the Anthracene Adducts (93) and (94) at 60 °C in the Presence of Dimethylbutadiene. - The anthracene adducts (93) and (94) (trans: cis = 2.5 : 1) (150 mg, 0.48 mmol) and 2,3-dimethylbuta-1,3-diene (39 mg,

0.48 mmol) were heated in benzene (11 ml) at 60 °C for 5 h. After evaporation of the solvent and excess diene the mixture was purified by short column chromatography [ether-light petroleum (b.p. 40-60 °C) (7:3)] to give successively anthracene and cis-11-ethoxycarbonyl-9,10-dihydro-9,10-thiaethanoanthracene S-oxide (39 mg, 0.12 mmol) The ^{1}H n.m.r. spectrum showed that only the cis isomer (94) remained.

Preparation of the cis-Anthracene Adduct Sulphoxide (94) and Thermal Transfer of the cis-Sulphine (91). - The anthracene adduct sulphoxides (93) and (94) (trans : cis = 2.5 : 1) (150 mg, 0.48 mmol) and 2,3-dimethylbuta-1,3 diene (39 mg, 0.48 mmol) were heated in benzene at 60 °C for 5 h. The solvent was evaporated and the residue was triturated with cold ether (4 x 4 ml) to remove the soluble trans-dimethylbutadiene adduct leaving a white solid (79 mg). The ^{1}H n.m.r. spectrum showed the sample to contain anthracene, the cis-S-oxide (94) and ca. 3% of the trans-S-oxide (93). This solid was heated with 2,3-dimethylbuta-1,3-diene (30 mg) in benzene under reflux for 10 h. After evaporation of solvent, the ^{1}H n.m.r. spectrum of the residue showed complete disappearance of the anthracene adducts (93) and (94) to give the cis-dimethylbutadiene adduct (104) (containing 5% of the trans-isomer).

KINETICS EXPERIMENTS

Dissociation Rate of the trans-Anthracene Adduct Sulphoxide (93) at 60 °C using Tetracyanoethylene to Trap Anthracene. - The epimerised adducts (93) and (94) (32.8 mg, 0.1 mmol of trans-oxide) and tetracyanoethylene (12.8 mg, 0.1 mmol) in benzene (25 ml) were heated at 60 $\pm$ 1 °C for 500 min. Aliquots (1 ml) were taken and made up to 25 ml in benzene. U.v. spectra were recorded of the tetracyanoethylene-benzene

charge transfer complex [$\lambda_{max.}$ (benzene) 384 nm (ε 3570)]. Nine such readings were taken. A plot of log $[A_o]/[A]$ versus time showed first order behaviour over 200 min when the straight line began to flatten off. Calculations from the straight line portion gave $k_{diss.} = 3.25 \times 10^{-5}\ s^{-1}$ ($t_{\frac{1}{2}}$ = 5 h 56 min). A repeat experiment produced similar behaviour to give $k_{diss.} = 3.22 \times 10^{-5}\ s^{-1}$ ($t_{\frac{1}{2}}$ = 5 h 58 min).

Dissociation of trans-Anthracene Adduct Sulphoxide and Trapping of the Sulphine with Dimethylbutadiene. - The methyl ester (98, 99) (28.1 mg, 0.094 mmol) was employed as a mixture of isomers in the ratio trans:cis = 2.2:1 (^{1}H n.m.r. assay), equivalent to 0.064 mmol of the trans-adduct. Thus, the adduct mixture (0.064 mmol) and 2,3-dimethylbuta-1,3-diene (10.6 mg, 0.13 mmol) in benzene (25 ml) were heated at 60 $\pm$1 ^{o}C for 7 h in a flask fitted with a lightly stoppered condenser. Aliquots (0.5 ml) were diluted to 10 ml and u.v. spectra recorded at regular intervals to monitor the formatiton of anthracene (A). A graph of absorption (277 nm) versus time was extrapolated to give the quantity of anthracene at t_∞ (A_∞ = 0.68 mmol). A plot of log ($[A_\infty] - [A]$) versus time showed good first order behaviour to give $k_{diss.} = 1.53 \times 10^{-4} s^{-1}$ ($t_{\frac{1}{2}}$=76 min.). After benzene was evaporated the ^{1}H n.m.r. spectrum showed that only the trans-isomer had dissociated.

8α-Ethoxycarbonyl-6,7,8,14-tetrahydro-7-thia-6α,14α-ethenothebaine (69). - This compound was prepared from the appropriate sulphenyl chloride and thebaine to give the 8-thia adduct (68) which, on heating gave the 7-thia adduct (99), as described by Bladon et al.[54e]

8α-Ethoxycarbonyl-6,7,8,14-tetrahydro-7-thia-6α,14α-ethenothebaine 7β-Oxide (109). - The thebaine adduct (69) (0.6 g, 1.39 mmol) was oxidised with 3-chloroperbenzoic acid (1.5 mmol). The reaction time was 0.5 h. After evaporation of solvent, t.l.c. and the ^{1}H n.m.r. spectrum indicated formation of a single isomer. Purification by flash chromatography (ether) gave the trans-S-oxide (109) (80%), m.p. 124-126 °C (from isopropanol) (Found: C, 62.1; H, 6.1; N, 2.95; S, 7.4; m/z 445.1551. $C_{23}H_{27}NO_6S$ requires C,62.0; H, 6.1; S, 7.2%; M, 445.1543); $\nu_{max.}$ (CCl_4) 1730 (CO), and 1164 and 1040 cm^{-1} (SO); δ_H (200MHz; $CDCl_3$) 1.31 (3H, t, J 7.1 Hz , $OCH_2\underline{Me}$), 1.85 (1H, m, $15_{eq.}$-H), 2.29 (3H, s, NMe), 2.3-2.6 (4H, m, 10α-, $15_{ax.}$- and 16-H), 3.16 (1H, d, J 18.7 Hz, 10β-H), 3.53 (1H, d, J 6.5 Hz, 9-H), 3.81 (6H, s, OMe), 4.24 (2H, qABq, J_{AB} 10.5 Hz, $J_{vic.}$ 7.1 Hz, Δδ 0.015, OCH_2), 4.73 (1H, s, 8-H), 5.51 (1H, d, J 1.5 Hz, 5-H), 5.67 (1H, dd, J 9.0 and 1.5 Hz, 18-H), 5.97 (1H, d, J 9.0 Hz, 17-H), 6.57 (1H, d, J 8.0 Hz, 1-H), and 6.64 (1H, d, J 8.0 Hz, 2-H); δ_c (50.4MHz; $CDCl_3$) 14.2 ($OCH_2\underline{Me}$), 21.95 (C-10), 31.1 (C-15), 43.4 (NMe), 45.0 (C-13 or -14), 45.2 (C-16), 47.0 (C-13 or -14), 54.6 (6-OMe), 56.4 (3-OMe), 58.5 (C-9), 61.9 (OCH_2), 73.3 (C-8), 86.4 (C-5), 99.0 (C-6), 113.5 (C-2), 119.7 (C-1), 120.8 (C-17), 126.7 (C-11 or -12), 132.0 (C-11 or -12), 140.4 (C-18), 142.5 (C-3) 146.7 (C-4) and 168.5 ($\underline{C}O_2Et$); m/z 445 (M^+, 0.4%), 311 (thebaine, 100), 296 and 255.

Thermal Transfer of the E-Sulphine (91) from Anthracene to Thebaine at 60 °C. - The anthracene adducts (93) and (94) (trans:cis=2.5:1) (150 mg, 0.48 mmol) and thebaine (149 mg, 0.48 mmol) were heated in benzene at 60 °C. After 6 h, the solvent was evaporated. Short column chromatography with chloroform-light petroleum (b.p. 60-80 °C (1:1)), to remove anthracene, and then with ether gave the trans-thebaine adduct (109) (190 mg, 89%) m.p. 124-136 °C (from isopropanol). The ^{1}H n.m.r. spectrum was identical to that

of the trans-adduct (109) obtained from oxidation of the sulphide (69).

Reduction of the Thebaine Adduct Sulphoxide (109). - Phosphorus pentasulphide (200mg, 0.9mmol) was added to a stirring solution of the sulphoxide (109) (55 mg, 0.12 mmol) in dichloromethane (3 ml) at room temperature. After 4 h the suspension was filtered and the residue was washed with dichloromethane (15 ml). The combined organic solutions were washed with water, dried and evaporated to give a yellow solid. Purification by t.l.c. (ether) gave the sulphide (69) (90%). The ^{1}H n.m.r. and i.r. spectra, t.l.c., and mass spectra were identical with those of the known 7-thia adduct (69).

Thermal Transfer of the E-Sulphine (91) from Thebaine to Dimethylbutadiene. - The thebaine adduct sulphoxide (109) (123 mg, 0.276 mmol) and 2,3-dimethylbuta-1,3-diene (45 mg, 0.55 mmol) in benzene (10 ml) were heated under reflux for 10 h. The solvent was evaporated and purification of the residue by short column chromatography (ether) gave the trans-adduct (103) (57 mg, 86%). The ^{1}H n.m.r. spectrum showed that less than 5% of the cis-adduct (104) was present. This might arise through thebaine induced epimerisation.

Ethyl 2-Thiabicyclo[2.2.1]hept-5-ene-3-carboxylate (65a) and (66a) and Isolation of the endo Isomer (65a)[54c]. - Prepared from the Bunte salt (105) and cyclopentadiene, as described for the dimethylbutadiene adduct (72), the cycloadducts (65a) and (66a) were obtained as an oily mixture (72%) b.p. 100-130 ^{o}C (0.05 mmHg), endo (65a): exo (66a) ratio 7:3. The mixture was separated on 1 mm silica t.l.c. plates by double elution with 5% ether in light petroleum (b.p. 40-60 ^{o}C). The fraction R_F 0.4 gave the

endo-isomer (65a), b.p. 110 °C (Kugelrohr distillation, 0.4 mmHg) (lit.[54c], 95 °C, 0.02 mmHg); δ (90 MHz; $CDCl_3$) 1.21 (3H, t, J 7 Hz, Me), 1.63 (2H, m, 7-H), 3.64 (1H, m, 4-H), 4.1 (1H, m, 1-H), 4.12 (2H, q, J 7 Hz, OCH_2), 4.41 (1H, d, J 4 Hz, 3-H), 5.87 (1H, dd, J 6 and 2 Hz, 5-H), and 6.46 (1H, dd, J 6 and 2 Hz, 6-H).

Ethyl exo-2-Thiabicyclo[2.2.1]hept-5-ene-carboxylate (66a). - The 'kinetic' mixture (endo:exo, 7:3) of adducts (65a) and (66a) was converted into the 'thermodynamic' mixture (endo:exo, 3:7) by heating for 7 h in toluene under reflux. The slower running exo-adduct was obtained by double elution [5% ether in light petroleum (b.p. 40-60 °C)] of t.l.c. plates and was distilled (Kugelrohr, b.p. 110 °C, 0.04 mmHg) (lit.[54c] 95 °C, 0.02 mmHg); δ (90 MHz; $CDCl_3$) 1.27 (3H, t, J 7 Hz, Me), 1.59 (1H, d, J 9 Hz, $7\text{-}H_a$), 1.89 (1H, d, J 9 Hz, $7\text{-}H_b$), 3.29 (1H, s, 3-H), 3.55 (1H, m, 4-H), 4.15 (1H, m, 1-H), 4.22 (2H, q, J 7 Hz, OCH_2), 5.94 (1H, dd, J 5 and 3 Hz, 5-H), and 6.38 (1H, dd, J 5 and 3 Hz, 6-H).

Methyl 2-Thiabicyclo[2.2.1]hept-5-ene-3-carboxylate (65h, 66h). - This compound was prepared from the appropriate Bunte salt (105a) as described for the ethyl ester, substituting methanol for ethanol. The product (54%) b.p. 125 °C (0.03 mmHg), was obtained as a mixture of isomers in the ratio endo (65h) : exo (66h) = 7.3 (Found: C, 56.2; H, 5.7; S, 19.1; m/z 170.0406 (11%). $C_8H_{10}O_2S$ requires C, 56.4; H, 5.9; S, 18.85%; M, 170.0401); $\nu_{max.}$ (CCl_4) 1740 (CO), 1437, 1322, 1267 and 1175 cm^{-1}. The mixture was separated by double elution [5% ether in light petroleum (b.p. 40-60 °C)] of t.l.c. plates. δ_H (90 MHz; $CDCl_3$) [endo-isomer (65h), R_F 0.4] 1.64 (2H, m, 7-H), 3.68 (2H, s, Me), 3.77 (1H, m, 4-H), 4.11 (1H, m, 1-H), 4.45 (1H, d, J 4 Hz, 3-H), 5.90 (1H, dd, J 5 and 3 Hz, 5- or 6-H) and 6.49 (1H, dd, J 5 and 3 Hz, 5- or 6-H); [exo-isomer (66h), R_F 0.35] 1.68 (1H, d, J 8 Hz, $7\text{-}H_a$), 1.90 (1H, d, J 8 Hz, 7-

H_b), 3.32 (1H, S, 3-H), 3.56 (1H, m, 4-H), 3.77 (3H, s, Me) 4.12 (1H, m, 1-H), 5.96 (1H, dd, J 5 and 3 Hz, 5- or 6-H), and 6.39 (1H, dd, J 5 and 3Hz, 5- or 6-H).

endo-3-Ethoxycarbonyl-2-thiabicyclo[2.2.1]hept-5-ene exo-2-Oxide (111). - The endo-sulphide (65a) (680 mg, 3.4 mmol) oxidised with 3-chloroperbenzoic acid (3.4 mmol) gave the 3-endo-2-exo S-oxide (111) (85%); m.p. 63-64 °C (from di-isopropyl ether) [Found: C, 53.90; H, 5.85; S, 16.09; m/z 200.0507 (7%). $C_9H_{12}O_3S$ requires C, 53.98; H, 6.04; S, 16.01%; M, 200.0507]; $\nu_{max.}$ (CCl_4) 1735 (CO), 1185, and 1156cm^{-1} (SO); δ_H (200 MHz; $CDCl_3$) 1.24 (3H, t, J 7.2 Hz, Me), 2.29 (1H, dt, J 10.8 and 2.3 Hz, 7-H_a), 2.56 (1H, d, J 10.8 Hz, 7-H_b), 3.32 (1H, d, J 3.2 Hz, 3-H), 3.41 (1H, m, 4-H), 4.17 (2H, q, J 7.1 Hz, OCH_2), ca. 4.2 (1H, m, 1-H), 5.79 (1H, dd, J 5.6 and 3.3 Hz, 5-or 6-H) and 6.55 (1H, dd, J 5.6 and 2.9 Hz, 5- or 6-H); δ_c (50.3 MHz; $CDCl_3$) 14.0 (Me), 43.4 (C-4), 44.2 (C-7), 61.7 (OCH_2), 69.1 and 76.1 (C-1 and -3), 125.5 and 143.6 (C-5 and -6) and 168.7 ($\underline{C}O_2$ Et); m/z 200 (M^+, 7%), 184 (M^+-O, 1.4), 152 (M^+-SO, 98), 123(45), 105(30), 66(C_5H_6, 78) and 29 (100).

Repeated Oxidation of the endo Sulphide(65a). - The endo sulphide (65a) (188 mg, 0.94 mmol) was oxidised with 3-chloroperbenzoic acid (0.94 mmol) to give a crude mixture (191 mg) of sulphoxide isomers, exo : endo S-oxide ratio, ca. 4:1 (obtained from the ^1H n.m.r. spectrum). The crude product was recrystallised to give rhombic plates (101mg, 54%), m.p. 63-64 °C (from di-isopropyl ether). The ratio of the two isomers was now 2.4:1 (exo : endo S-oxide).

endo-3-Ethoxycarbonyl-2-thiabicyclo[2.2.1]hept-5-ene endo-2-Oxide (112). - δ_H (200 MHz; $CDCl_3$) 1.21 (3H, t, J 7.1 Hz, Me), 2.35 (2H, ABq, J 12 Hz, $\Delta\delta$ 0.06, 7-H), 3.48 (1H, m, 4-H), 3.78 (1H, d, J 3.7 Hz, 3-H), 3.98 (1H, m, 1-H), 4.19 (2H, m, OCH_2), 6.17 (1H, dd, J 5.6 and 3.3 Hz, 5-

or 6-H) and 6.78 (1H, dd, J 5.6 and 2.8 Hz, 5- or 6-H); δ_c (50.3 MHz; $CDCl_3$) 14.0 (Me), 43.2 (C-7), 43.4 (C-4), 61.6 (C-1 or -3), 62.2 (OCH_2), 66.3 (C-1 or -3), 127.5 and 140.5 (C-5 and -6) and 164.9 ($\underline{C}O_2Et$).

exo-3-Ethoxycarbonyl-2-thiabicyclo[2.2.1]hept-5-ene exo-2-Oxide (114). - The exo-sulphide (66a) (2.42 g, 1.21 mmol) was oxidised with 3-chloroperbenzoic acid (1.21 mmol) to give a yellow oil (2.40 g, ca. 94%) as crude product. Distillation in small aliquots (Kugelrohr, 150 °C, 0.05 mm Hg) avoided decomposition to give the exo-S-oxide (114) as a pale yellow oil (72%). (Found m/z, 200.0505 $C_9H_{12}O_3S$ requires M, 200.0507); $\nu_{max.}$ (CCl_4) 1740 (CO), 1170 and 1068 cm^{-1} (SO); δ_H (200 MHz; $CDCl_3$) 1.21 (3H, t, J 7.1 Hz, Me), 2.30 (1H, ddd, J 11.0, 4.1 and 1.9 Hz, 7-H_a), 2.88 (1H, d, J 11.0 Hz, 7-H_b), 3.48 (1H, m, 4-H), 3.56 (1H, dt, J 1.7 and 0.8 Hz, 3-H), ca. 4.1 (1H, m, 1-H), 4.13 (2H, qABq, J 10.8 and 7.1 Hz, $\Delta\delta$ 0.03, OCH_2), 5.84 (1H, dd, J 5.7 and 3.1 Hz, 5-H) and 6.28 (1H, dd, J 5.7 and 3.0 Hz, 6-H); δ_c (50 MHz; $CDCl_3$) 14.1 (Me), 43.6 (C-4), 43.9 (C-7), 61.6 (OCH_2), 64.3 and 67.6 (C-1 and -3), 128.3 and 144.1 (C-5 and -6) and 166.0 (CO_2Et); m/z 200 (M^+, 2%), 180 (M^+-O, 2), 152 (M-SO, 92), 123 (39), 78 (C_6H_6, 41) and 61 (100).

endo-3-Methoxycarbonyl-2-thiabicyclo[2.2.1]hept-5-ene-exo-2-Oxide (113). - The endo-sulphide methyl ester (65h) (251 mg, 1.35 mmol) was oxidised with 3-chloroperbenzoic acid (1.35 mmol) to give a single isomer, the exo-S-oxide (113) (223 mg, 89%), m.p. 89-90 °C (from di-isopropyl ether) (Found: C, 51.6; H, 5.4; S, 17.6; m/z 186.0369. $C_8H_{10}O_3S$ requires C, 51.6; H, 5.4; S, 17.2%; M, 186.0351); $\nu_{max.}$ ($CHCl_3$) 1735 (CO), 1438, 1263 and 1174 and 1037cm^{-1} (SO); δ_H (90 MHz; $CDCl_3$) 2.36 (1H, dt, J 10 and 2 Hz, 7-H_a), 2.61 (1H, d, J 10 Hz, 7-H_b), 3.36 (1H, d, J 3Hz, 3-H), 3.47 (1H, m, 4-H), 3.77 (1H, m, 1-H), 3.77 (3H, s, Me), 4.27 (1H, m, 1-H), 5.85 (1H, dd, J 6 and 3 Hz, 5- or 6-H) and 6.59 (1H,

dd, J 6 and 3 Hz, 5- or 6-H); δ_C (25.2 MHz; $CDCl_3$) 43.5 (C-4), 44.2 (C-7), 52.6 (Me), 69.2 and 76.0 (C-1 and -3), 125.7 (C-6), 143.6 (C-5) and 169.2 ($\underline{C}O_2Et$); m/z 186 (M^+, 1%), 185 (3), 138 (100), 106 (33), 105 (75), 79 (59), 78 (37), 77 (61) and 66 (52).

Thermal Transfer of the E-Sulphine (91) from Cyclopentadiene to Dimethylbutadiene. - The trans-cyclopentadiene adduct (111) (298 mg, 1.49 mmol) and dimethylbutadiene (245 mg, 3.0 mmol) were heated in benzene (7ml) under reflux. After 13 h, the solvent was evaporated Distillation (Kugelrohr, 150 °C, 0.2 mm Hg) of the residue gave the trans-dimethylbutadiene adduct (11) (262 mg, 88%) only (from the ^1H n.m.r. spectrum).

Thermal Transfer of the E-Sulphine (91) from Anthracene to Cyclopentadiene. - The anthracene adduct (93) (150 mg, 0.48 mmol) (ca. 95% trans-isomer) and cyclopentadiene (33 mg, 0.53 mmol) in benzene (5 ml) were heated at 60 °C for 5 h. Evaporation of the solvent and short column chromatography [ether-light petroleum (b.p. 40-60 °C) (1:1)] of the residue gave the endo-ethoxycarbonyl-exo-S-oxide (111) (87 mg, 91%).

Heating the 2-exo-3-exo-Cyclopentadiene Adduct Sulphoxide(114)and Dimethylbutadiene at 111 °C. - A solution of the adduct (114) (110 mg, 0.55 mmol) and 2,3-dimethylbuta-1,3-diene (90.2 mg, 1.1 mmol) in toluene (10 ml), previously purged with nitrogen, was heated at ca. 111 °C in a sealed tube for 13 h. During the reaction the pale yellow solution darkened considerably. After evaporation of the solvent, purification by short column chromatography (ether) gave the trans-and-cis-dimethylbutadiene adducts (103) and (104) in the ratio ca. 1:1 (similar to the ratio obtained upon base catalysed

epimerisation). The isomer ratio was obtained from the peak heights of the ester quartet (CH_2) and triplet (CH_3) in the ^1H n.m.r. spectrum.

Heating the 2-exo-3-exo-Cyclopentadiene Adduct Sulphoxide (114) and Dimethylbutadiene in Refluxing Toluene. - A solution of the adduct (114) (93 mg, 0.46 mmol) and 2,3-dimethylbuta-1,3-diene (76 mg, 0.93 mmol) was heated in toluene under reflux for 12 h. Darkening of the solution was markedly less than observed in the sealed tube. The solvent was evaporated and the ^1H n.m.r. spectrum of the crude product indicated an unequal mixture of isomers. Further purification by short column chromatography (ether) gave the dimethylbutadiene adducts (103) and (104) (82 mg, 83%) in the ratio cis : trans = ca. 2:1.

Thermal Transfer of the Z-Sulphine (91) from Anthracene to Cyclopentadiene. - The anthracene adducts (93) and (94) (170 mg, 0.55 mmol) (trans : cis = 2.5 : 1) and 2,3-dimethylbuta-1,3-diene (46 mg, 0.56 mmol) in benzene (5 ml) were heated at 60 °C for 5 h. The solvent was evaporated and the residue triturated with cold ether (4x4 ml) to leave the cis-anthracene adduct (94) and anthracene (identified from the ^1H n.m.r. spectrum) Cyclopentadiene (72 mg, 1.1 mmol) in benzene (5 ml) was added and the solution was heated under reflux for 10 h. Evaporation of the solvent and short column chromatography (ether) gave the exo-ester-exo-S-oxide (114) (16 mg, 51%). The ^1H n.m.r. spectrum of the crude product showed that none of the other known isomers (111) and (112) were present.

Thermal Stability of the 2-endo-3endo S-Oxide at 80 °C. - The cyclopentadiene adducts (111) and (112) (2-exo-3-endo : 2-endo-3-endo, ca. 4 : 1) (194 mg, 0.97 mmol) and β-pinene (263 mg, 1.94 mmol) in benzene (10 ml) were heated under

reflux for 13 h. The solvent was evaporated and purification by short column chromatography yielded a mixture of products. The fraction with R_F identical to that of starting material was found to be cis-endo-oxide (112) (35 mg, 0.175 mmol); δ_H (90 MHz; $CDCl_3$) 1.30 (3H, t, J 7Hz, Me), 2.39 (2H, distorted t, J ca. 2 Hz, 7-H), 3.54 (1H, m, 4-H), 3.83 (1H, d, J 3.5 Hz, 3-H), 4.04 (1H, m, 4-H), 4.27 (2H, q, J 7 Hz, OCH_2), 6.22 (1H, dd, J 6 and 4 Hz, 5- or 6-H) and 6.82 (1H, dd, J 6 and 2 Hz, 5- or 6-H). On crystallising from di-isopropyl ether rearrangement to the 2-exo-isomer (111) occurred.

Thermal Stability of the trans- and cis-Dimethylbutadiene Adducts (103) and (104). - The trans-S-oxide (103) (35 mg) was heated under reflux in both benzene and toluene; each for 10 h. The ^{1}H n.m.r. spectrum showed that no epimerisation had occurred. Similarly, the cis-isomer (104), containing 5% trans-isomer (103) was heated under reflux in toluene for 10 h; no increase in the proportion of the trans-isomer was observed.

Thermal Stability of other Adducts. - The thebaine adduct S-oxide (109) was heated in benzene under reflux. Monitoring by t.l.c. (ether) showed increasing amounts of thebaine (baseline) and decomposition products.

The 2-exo-3-endo oxide (111) afforded some starting material and decomposition products after 10 h in refluxing benzene. The 2-exo-3-exo oxide (114) was unchanged after 24 h in refluxing benzene, but partially decomposed after 10 h in refluxing toluene.

3.3 Epimerisation Reactions

Acid Catalysed Epimerisation of Sulphoxides. - The method of C.R. Johnson et al.[58] was employed in which the sulphoxide was added to a 1:2 (v/v) mixture of 36% hydrochloric acid and dioxane and stirred for 20 min. The solution was diluted with water (10 ml), extracted with dichloromethane (3x10 ml) and the combined extracts were dried and evaporated. The products were examined by ^{1}H n.m.r. spectroscopy.

The anthracene adducts (93) and (94) initially in the ratio trans: cis = 2.5 : 1 gave an equilibrium ratio of trans : cis = ca. 19 : 1.

The 2-exo-3-endo cyclopentadiene adduct (111) was recovered unchanged after the same treatment.

The 2-exo-3-exo-cyclopentadiene adduct (114) was also recovered unchanged.

The dimethylbutadiene adducts (103) and (104) obtained by oxidation of the sulphide in the ratio trans : cis = 4 : 1 gave an equilibrium ratio of trans : cis = ca. 1 : 1 but with some decomposition.

The thebaine adduct (109) showed no change after 20 min and considerable decomposition after 24 h.

Base catalysed Epimerisation with Triethylamine. - The anthracene adducts (93) and (94) (trans : cis = 2.5 : 1) in $[^{2}H_{6}]$-benzene at room temperature containing 0.2M triethylamine gave an equilibrium ratio, trans : cis = ca. 10:1 after 12 h.

Dimethylbutadiene Adducts. - The peracid oxidation mixture containing the adducts (103) and (104) (trans : cis = 4 : 1) (37.6 mg, 0.17 mmol) in [$^2H_6$]-benzene (0.45 ml)-[$^2H_4$]-methanol (0.5 ml) containing 0.2 M triethylamine was monitored by ^1H n.m.r. spectroscopy. After 24 h at room temperature the signal δ 3.70 (dd, 2-H) had disappeared and the isomer ratio remained constant. The solvent and triethylamine were evaporated to give 2-ethoxycarbonyl-3,6-dihydro-4,5-dimethyl-[2-^2H]-thiapyran 1-oxide(106) and (107) (38.5 mg, 100%) as a mixture of isomers. Analysis by capillary gas chromatography gave the equilibrium ratio (trans : cis = 1.2 : 1.0), the trans-isomer being eluted first; g.c.-m.s. analysis of the trans fraction gave m/z 217 (M^+, 17%), 198, 183, 169, 154, 126 and 95 (100) and of the cis fraction, m/z 217 (M^+), 200, 183, 171, 169, 154, 140, 112 and 95 (100%); δ_H (90MHz; CCl_4) 1.28 (t, J 7 Hz, cis-OCH_2Me), 1.32 (t, trans-OCH_2Me), 1.72 and 1.75 (s, trans- and cis-4- and 5- Me), 2.0 - 3.4 (m, trans and cis 6-H), 4.20 (q, J 7 Hz, cis-OCH_2) and 4.23 (q, J 7 Hz, trans-OCH_2). Spectra of solutions in CCl_4 gave better resolution of the isomers than did those in $CDCl_3$. Peak heights for the methyl triplets gave a ratio of trans : cis, 1.2 : 1.0.

The Cyclopentadiene Adducts (111) and (114). - The trans-adduct (111) (51 mg, 0.25 mmol) or cis-adduct (114) (54.2 mg, 0.27 mmol) in [$^2H_6$]-benzene (0.5 ml) containing 2M triethylamine was monitored by ^1H n.m.r. spectroscopy over 24 h. No change occurred. The solutions were then heated at 60 °C and after 48 h both solutions had attained the same ratio, trans : cis = 7 : 3. A control experiment at 60 °C in which no base was present produced no isomerisation.

endo-3-Ethoxycarbonyl-[3-^{2}H]-2-thiabicyclo[2.2.1]hept-5-ene exo-2-Oxide (115). - The trans-adduct (111) (42 mg, 0.21 mmol) in [$^2H_6$]-benzene (0.45 ml) - [$^2H_4$]-methanol (0.5 ml) containing 0.2M triethylamine at room temperature was monitored by ^{1}H n.m.r. spectroscopy. After 48 h the 3-H doublet had exchanged with no isomerisation. After evaporation of the solvent, crystallisation of the residue from di-isopropyl ether gave the deuteriated adduct (115) (86%), m.p. 63-64 °C; δ_H (90 MHz; CCl_4) 1.32 (3H, t, J 7 Hz, Me), 2.29) (1H, dt, J 10 and 2 Hz, 7-H_a), 2.55 (1H, d, J 10Hz, 7-H_b), 3.38 (1H, m, 4-H), 4.2 (1H, m, 1-H), 4.21 (2H, q, J 7Hz, (OCH_2), 5.79 (1H, dd, J 5 and 3 Hz, 5- or 6-H) and 6.65 (1H, dd, J 5 and 2 Hz, 5- or 6-H), m/z 200 (M^+-1, 3.4%), 153 (M-SO, 88), 124 (36), 106 (29) and 78 (100), No M^+ peak was detected.

trans-4,5-Epoxy-2-ethoxycarbonyl-3,6,dihydro-4,5-dimethyl-2H-thiapyran 1,1-Dioxide (117). - To a stirred suspension of sodium perborate tetrahydrate (Na BO_3. $4H_2O$) (639 mg, 4.15 mmol) in acetic acid (20 ml) was added the sulphide (72) (166 mg, 0.83 mmol) at 55 °C. After 3 h at 55 °C the solution was cooled and filtered to remove inorganic solids. The solution was added to water (150 ml) and the mixture extracted with dichloromethane (3x20 ml). The organic extract was washed with water, saturated sodium bicarbonate and water and was dried and evaporated. The pure epoxy-sulphone (117) was obtained as white needles from ethanol (189 mg, 92%), m.p. 108-110 °C (Found: C, 48.3; H, 6.3; S, 13.1. $C_{10}H_{16}O_5S$ requires C, 48.4; H, 6.5; S, 12.9%); $\nu_{max.}$ (KBr) 1740 (CO) and 1315 and 1120 cm^{-1} (SO_2 symmetric and asymmetric stretch); δ_H (270 MHz; $CDCl_3$) 1.31 (3H, t, J 6.9 Hz, OCH_2 Me), 1.41 and 1.43 (6H, s, 4- and 5-Me), 2.62 (1H, dd, J 15.6 and 3.6 Hz, 3-H), 2.87 (1H, dd, J 15.6 and 11.2 Hz, 3-H), 3.46 (2H, ABq, J 15.0 Hz, $\Delta\delta$ 0.31, 6-H), 3.93 (1H, dd, J 11.5 and 3.7Hz, 2-H) and 4.26 (2H, q, J 6.3 Hz, OCH_2); δ_C (25.2 MHz; $CDCl_3$) 14.0 (OCH_2Me), 19.3 and 21.6 (4- and 5- Me), 33.9 (C-3), 57.7 (C-6) 59.5 (C-2),

60.2 and 60.7 (C-4 and -5), 62.8 (OCH_2) and 163.4 ($\underline{C}O_2Et$); m/z 203.0383 (M-OEt, 10%), 184.1110 (M-SO_2, 5) and 111.0819 ($C_7H_{11}O_2$, 100). No molecular ion was observed with low or high resolution mass spectrometry.

trans-2-Ethoxycarbonyl-3,6-dihydro-4,5-dimethyl-4,5-epoxy-[2-^{2}H]-thiapyran 1,1-Dioxide (118). - The sulphone (117) (43 mg, 0.21 mmol) in [$^2H_6$]-benzene (0.45 ml)/[$^2H_4$]-methanol (0.5 ml) containing 0.2M triethylamine was left at room temperature overnight. Complete disappearance of the signal δ 3.9 (dd, 2-H) had taken place, but no signals for new isomers had appeared. Evaporation of solvent gave the deuteriated sulphone (118) (77%), m.p. 106-107 °C (from ethanol), δ_H (90 MHz; $CDCl_3$) 1.33 (3H, t, J 7 Hz, OCH_2 Me), 1.45 (6H, 2, 4- and 5-Me), 2.57 (2H, ABq, J 16 Hz, Δδ 0.59, 3-H), 3.58 (2H, ABq, J 16Hz, Δδ 0.06, 6-H) and 4.33 (2H, q, J 7 Hz, OCH_2); m/z 247 (M^+-2H, 0.5%), 185 (M-SO_2, 0.5), 100 (11), 95 (12), 79 (21) and 43 (100). No molecular ion was found.

3.4 Synthesis of the Thiapyrans and Thiopyrylium Salt

2-Ethoxycarbonyl-4,5-dimethyl- 6-thiapyran (123). - To a stirred suspension of sodium perborate ($NaBO_3.4H_2O$) (174 mg, 1.13 mmol) in acetic acid (10ml) was added the sulphide (72) (226 mg, 1.13 mmol) at 50-60 °C. After 30 min the oil bath temperature was increased to 110-115 °C and the mixture was stirred for 2.5 h with a condenser fitted. The dark solution was cooled, added to water (100 ml), extracted with dichloromethane (3x20 ml). The organic layer was washed with water, saturated sodium bicarbonate and finally water. After drying and evaporating the extract, the residual brown oil (195 mg) was purified by flash chromatography [ether-light petroleum (b.p. 40-60 °C) (1 : 1)] to remove a strongly coloured red impurity and distilled (Kugelrohr, 115 °C, 0.1 mm Hg) to give the pure

thiapyran (123) as a yellow oil (86%) (Found: C, 60.3; H, 7.3; S, 16.2; m/z, 198.0710. $C_{10}H_{14}O_2S$ requires C, 60.6; H, 2.1; S, 16.2%; M, 198.0705); $\lambda_{max.}$ (cyclohexane) 351 (ε3916) and 276 nm (3170); $\nu_{max.}$ (CCl_4) 1713 (CO), 1255 and 1220 cm^{-1}; δ_H (90 MHz; $CDCl_3$) 1.27 (3H, t, J 7 Hz, OCH_2Me), 1.81 and 1.89 (6H, s, 4- and 5-Me), 3.20 (2H, br. s, 6-H), 4.25 (2H, q, J 7Hz, OCH_2) and 7.06 (1H, s, 3-H); m/z 198 (M^+, 64%), 183 (100), 169, 155, 125 and 91.

Alternative Preparation of the Thiapyran (123). - A solution of the sulphoxide isomers (103) and (104) (102 mg, 0.52 mmol) in xylene (10 ml) containing two crystals of p-toluenesulphonic acid monohydrate was heated under reflux for 1 h. After evaporation of solvent the yellow oil was distilled (Kugelrohr 120 °C, 0.15 mmHg) to give the thiapyran (123) (83 mg, 81%).

2-Carboxy-4,5-dimethyl-6H-thiapyran (124). - A solution of the thiapyran ether (123) (162 mg, 0.82 mmol) in 1M sodium hydroxide (2 ml) and ethanol (2 ml) was stirred at room temperature for 10 h. Water (25 ml) was added and the mixture was washed with dichloromethane, acidified with dilute hydrochloric acid and extracted with dichloromethane. The organic extract was washed with water, dried and evaporated to give an orange solid. Chromatography [ether-light petroleum (b.p. 40-60 °C) (1 : 1)] afforded the thiapyran acid (124) (79%) as yellow needles, m.p. 83-85 °C (from cyclohexane) (Found: C, 56.4; H, 5.9; S, 18.8; m/z 170.0401. $C_8H_{10}O_2S$ requires C, 56.4; H, 6.1; S, 19.1%; M, 170.0402); $\lambda_{max.}$ (cyclohexane) 358 (ε 2208) and 282nm (1719); $\nu_{max.}$ (KBr) 1667 (CO) and 1279cm^{-1}; δ_H (90 MHz; $CDCl_3$) 1.84 and 1.93 (6H, s, 4- and 5-Me), 3.24 (2H, br. s, 6-H), 7.18 (1H, s, 3-H) and 11.67 (1H, br. s, CO_2H, exchangeable with D_2O); m/z 170 (M^+, 30%), 155 (100), 125 (19), 111 and 91.

2-Ethoxycarbonyl-4,5-dimethythiopyrylium tri-iodide (125). - To a solution of iodine (1.6 g, 6.2 mmol) in acetonitrile (10 ml) was added the thiapyran (123) (0.61 g, 3.1 mmol). After 5 min at room temperature, ether was added until no more red precipitate appeared. The solid was filtered off, washed with ether, redissolved in a small amount of acetonitrile and precipitated once more with ether to give pure thiopyrylium tri-iodide (125) as red needles (1.25 g , 69%) m.p. 107-108°C (Found: C, 21.0; H, 2.1; I, 67.1; S, 5.5; m/z 197.0614. $C_{10}H_{13}I_3O_2S$ requires C, 20.8; H, 2.3; I, 66.9; S, 5.5%; M 197.0636) $\nu_{max.}$ (KBr) 1733 (CO), 1369, 1265, 1256 and 1016cm^{-1}; $\lambda_{max.}$ (CH_3CN) 359 (ε 10998), 292 (25447) and 244nm (32564) (all absorptions for I_3 and I_2); δ_H [90MHz; $(CD_3)_2CO$] 1.49 (3H, t, J 7 Hz, OCH_2Me), 3.00 and 3.09 (6H, s, 4- and 5-Me), 4.65 (2H, q, J 7 Hz, OCH_2), 9.37 (1H, s, 3-H) and 10.27 (1H, s, 6-H); δ_c [25.2MHz; $(CD_3)_2CO$] 14.4 (OCH_2Me), 22.9 and 25.5 (4- and 5- Me), 65.6 (OCH_2), 141.2 (C-3), 152.6 and 153.8 (C-4 and -5), 156.7 (C-6), 100.2 (C-2) and 168.5 (CO_2Et); m/z 254 (I_2, 16%), 197 ($C_{10}H_{13}O_2S$, 19), 168 ($M-CH_2CH_3$, 100), 128 (85), 127 (I, 53), 121 (23), 91 (25) and 99 (26).

Perborate Oxidation of Sulphide (72) at 55 °C. - The sulphide (72) (80 mg, 0.4 mmol) and sodium perborate tetrahydrate ($NaBO_3.4H_2O$) (62 mg, 0.4 mmol) in acetic acid (10 ml) was stirred at 55 °C for 3 h. After cooling, the mixture was added to water (50 ml), extracted with dichloromethane (2x10 ml) and the organic solution was washed with water and saturated sodium bicarbonate. The solution was dried and evaporated to leave a yellow oil (83mg, 96%) of the sulphoxides (103) and (104). The ^1H n.m.r. spectrum in CCl_4 showed a mixture of isomers in the ratio trans : cis, ca. 2 : 1, measured from the peak heights of the ester triplet at δ 1.28.

3,4-Dimethylcyclohex-3-enecarboxylic Acid Ethyl Ester (119). - This was prepared by the method of Monnin[65], δ_H (90 MHz; $CDCl_3$) 1.26 (3H, t, J 7 Hz, OCH_2Me), 1.62 (6H, s, 3- and 4-Me), 1.7-2.7 (7H, m, 1-, 2-, 4- and 5-H). The compound was pure by capillary g.c. analysis.

3,3-Dimethyl-3,4-epoxycyclo-hexene-carboxylic Acid Ethyl Ester (120) and (121). - The ester (119) (81 mg, 0.44 mmol) and 3-chloroperbenzoic acid (0.67 mmol) in dichloromethane (10 ml) was left to stand at room temperature for 13 h. The solution was made up to 20 ml, washed with saturated sodium sulphite, saturated sodium bicarbonate (3x10 ml), water and dried and evaporated to leave an oil. Distillation (Kugelrohr 150-160 °C, 0.1 mm Hg) gave the epoxides (120) and (121) as a clear oil (122 mg, 92%) (Found: M^+, 198.1258. $C_{11}H_{18}O_3$ requires M, 198.1256); $\nu_{max.}$ (CCl_4) 1735 cm^{-1}; δ_H (90 MHz; CCl_4) 1.25 (3H, t, J 7 Hz, OCH_2Me), 1.28 (6H, br. s, 3- and 4-Me), 1.4-2.6 (7H, m) and 4.06 (2H,q, J 7 Hz, (OCH_2); capillary g.c. analysis showed a mixture of two isomers (120) and (121) in the ratio 1.35 : 1; the major product being the faster eluting fraction. G.c-.m.s. analysis of the mixture gave similar spectra for both isomers; m/z 198 (M^+), 180, 169, 153, 141, 128 and 125 (100).

Reaction of Cyclohexene Ethyl Ester with Sodium Perborate. - The ester (119) (121 mg, 0.67 mmol) was added, with stirring to acetic acid (10 ml) containing sodium perborate tetrahydrate ($NaBO_3.4H_2O$) (1.0 g, 6.7 mmol) and the mixture was stirred at 55 °C for 3 h. After cooling the white inorganic solid was filtered off and the solution was taken up in water (100 ml). The solution was extracted with dichloromethane (3x10 ml) and the organic portion was washed with water, saturated sodium bicarbonate, water, and dried and evaporated. Short column chromatography [ether-light-petroleum (b.p. 40-60 °C) (1 : 1)] gave a colourless oil

which appeared as one spot on t.l.c. Distillation (Kugelrohr 135 ^{o}C, 0.1 mmHg) gave a clear liquid (97 mg). Capillary g.c. analysis yielded 5 components, all at higher retention time than the epoxides (120) and (121). G.c.–m.s. analysis of the mixture showed the products to have higher molecular weights than the epoxide ($\underline{M}^{+}$, 198) but all the mass spectra had significant fragments at 198.

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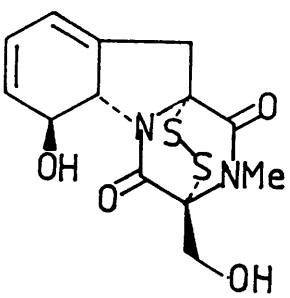
PART 2 FLUORINATED DERIVATIVES AS BIOSYNTHETIC PRECURSORS OF GLIOTOXIN ANALOGUES

Chapter 4 Introduction

4.1 General

Gliotoxin (1) was first isolated in 1936 by Weindling and Emerson[1] from Gliocladium fimbriatum. This culture has also been called Trichoderma viride and Gliocladium deliquescens but now has been defined as Gliocladium virens. It was not until 1958 that the structure (1), containing a sulphur-bridged dioxopiperazine moiety was elucidated[2]. There is now a large family of compounds containing either the sulphur-bridged ring (2) or the related cis-di(methylthio)dioxopiperazine ring system (3).



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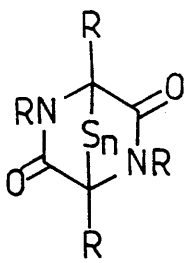

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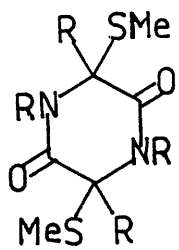

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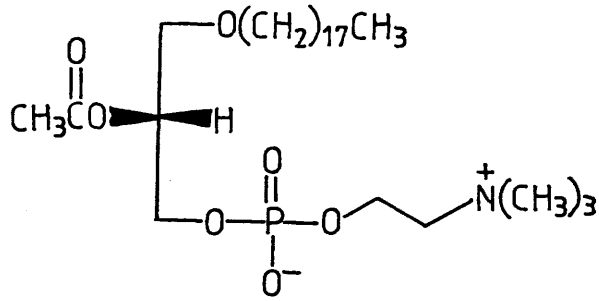
Several reviews have been published concerning the structure, chemistry, biosynthesis, and biological activity of these compounds [3,4,5]. The most recent review by Kirby and Robins covers the biosynthestic studies up to 1980[6].

The toxicity of the sulphur-bridged compounds has been reviewed by Taylor[7]. Gliotoxin causes inhibition of polio and influenza virus replication by specific inhibition of virus-induced RNA-polymerase; no effect was observed in E-coli B RNA-polymerase[8]. Pronounced toxicity against mammals precluded its use as an anti-viral drug. More recently gliotoxin, isolated from Aspergillus fumigatus, showed immunosuppressive activity[9].

The di(methylthio) compounds show none of the above activity, but recently were shown to be potent antagonists of PAF, the platelet activating factor (4). The corresponding sulphur-bridged compound showed little anti-PAF activity[10].



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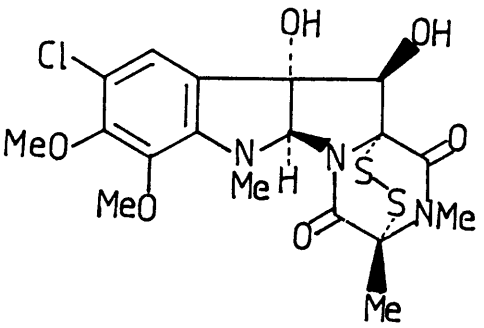
4.2 Biosynthesis of Gliotoxin

The biosynthesis of gliotoxin and the closely related aranotins will be briefly reviewed. Discussion of the other main structural types can be found in several reviews and papers : sporidesmin (5)[6], sirodesmin PL (6)[8] hyalodendrin (7), and its bisdethiodi(methylthio)-analogue[(8)].



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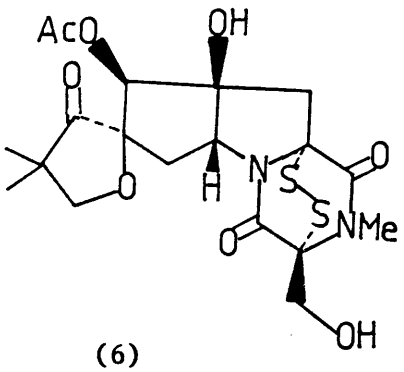

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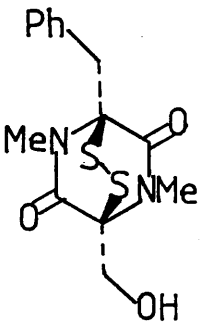

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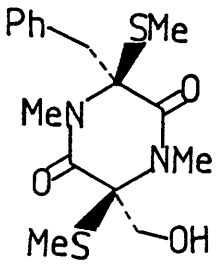
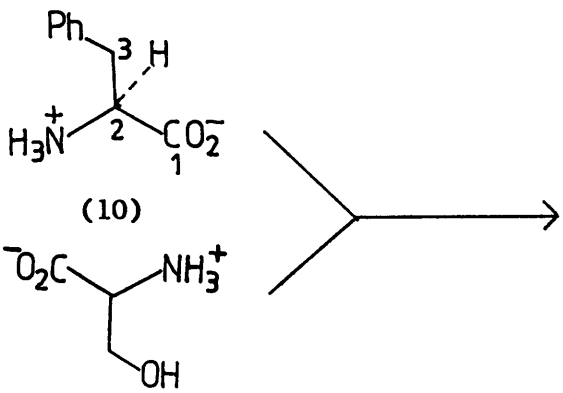


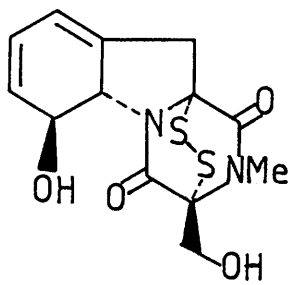
(8)

Examination of the structure of gliotoxin (1) suggests serine (9) as a source for the lower portion and an aromatic amino-acid such as phenylalanine (10) or m-tyrosine for the



(10)

(9)

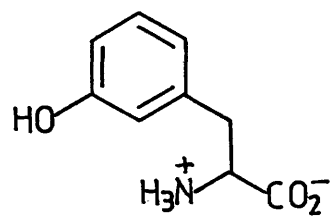


upper half. Accordingly DL-[1-^{14}C]phenylalanine and DL-[2-^{14}C]phenylalanine fed to Gliocladium virens were incorporated efficently (4-12%) into gliotoxin and degradation showed the amino acid was incorporated intact.

When doubly labelled [DL]-[1-^{14}C, ^{15}N]phenylalanine was fed the ^{15}N was diluted more than the ^{14}C label and D-[1-^{14}C]- and L-[1-^{14}C]- phenylalanine were incorporated with equal efficiency[12]. Thus it appears inversion of the chiral centre of phenylalanine readily occurs, presumably by deamination and reamination.

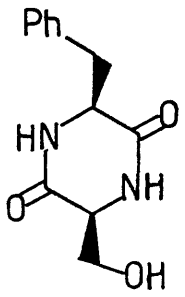
Feeding experiments with serine gave complicated results. DL-[3-^{14}C]serine gave gliotoxin with 25% of its activity in the N-methyl group due to transfer of the methylene group into the C-1 pool However, incorporation of DL-[1-^{14}C]serine was simpler with intact incorporation (1.9%) into gliotoxin[13].

A high incorporation (44%) of DL-[U-^{3}H]-m-tyrosine (11) was reported[13] but several attempts have failed to repeat this result and m-tyrosine (11) is not now considered to be a precursor.



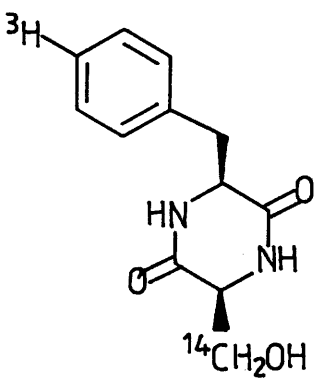
(11)

From these results it followed that cyclo-(L-phenylanalnyl-L-seryl) (12) would appear to be an attractive candidate as the next intermediate in the biosynthetic



(12)

pathway. Initial experiments were discouraging; cyclo-(L-[1-^{14}C]Phe-L-Ser) fed to Penicillium terlikowski[14] was incorporated very inefficiently into gliotoxin. Since then incorporation of 21% has been observed in G.virens[15] and the low value first observed was explained by the large amount of material fed. Kirby et al. fed all four stereoisomers of [^{14}C]-cyclo-(Phe-Ser) separately to G.virens[16] and observed high incorporation (48%) only for the LL-isomer. Feeding cyclo -([4'-^{3}H] Phe-L-[3-^{14}C]Ser) (13) gave gliotoxin with

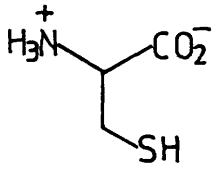


(13)

no alteration in the ^{3}H : ^{14}C ratio and there was no activity found in the N-methyl group. Thus, it appears that cyclo-(L-Phe-L-Ser) is metabolised to gliotoxin without separation of the amino-acid residues. A further experiment[16] confirmed that the cyclic dipeptide is indeed a free intermediate. Unlabelled cyclo-(L-Phe-L-Ser) was administered to a G.virens culture followed by L-[U-^{14}C] phenylalanine. The reisolated cyclo-(Phe-Ser) contained 1.3% of the activity of the labelled amino-acid.

Three processes are required to transform cyclo-(L-Phe-L-Ser) into gliotoxin : oxidative cyclisation of the phenyl ring, N-methylation and introduction of the sulphur bridge.

To date there has been little investigation of the mechanism of sulphur introduction. Cysteine (14) appears to be a better source of sulphur than methionine[17]. L-[^{35}S]-



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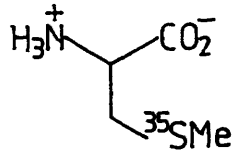
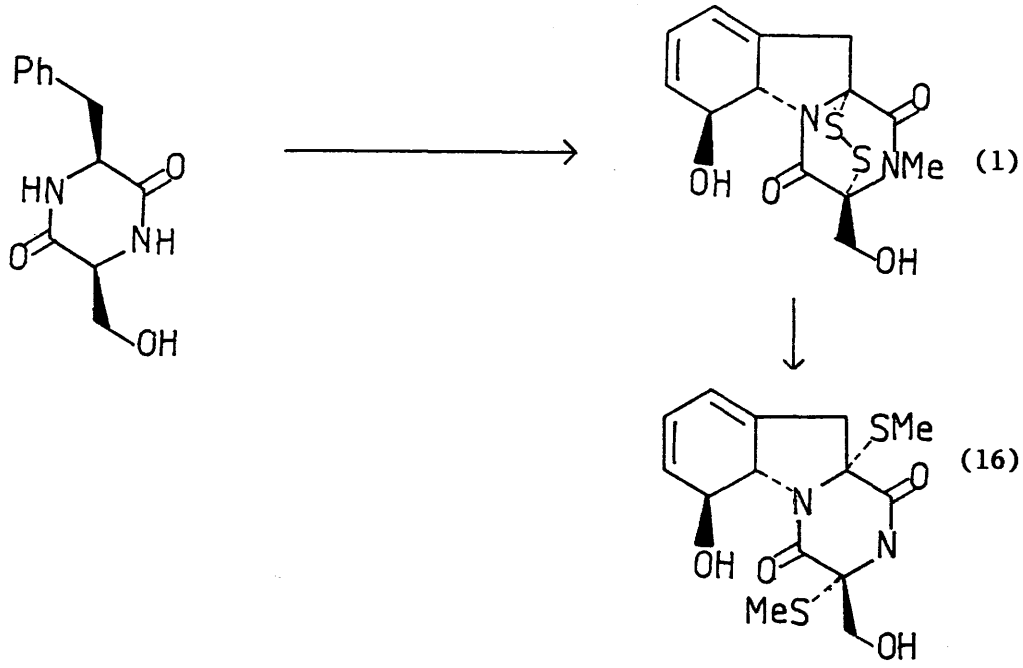


(15)

Methionine (15) was incorporated into G.virens (3.3%). The experiment was repeated with the addition of non-radioactive diluents. Notably L-cysteine (14) caused an increase (2.3-fold) in the yield of gliotoxin together with a marked reduction (to 0.1%) in the incorporation of ^{35}S from methionine.

As an aid to the detection of sulphur-containing metabolites G.virens[18] was grown in a medium containing [^{35}S]sulphate (14.5 μCi mmol^{-1}). The gliotoxin obtained had a specific activity of 29.6 μCi mmol^{-1}), twice that of sulphate. Thus all the sulphur in gliotoxin was derived from sulphate.

In G.virens as in other dioxopiperazine producing fungi, the epidisulphide co-occurs with the bisdethiodi-(methylthio) derivative (16)[19]. The biosynthetic relationship between the two has been studied[19]. [^{14}C]-Gliotoxin (derived biosynthetically from [U-^{14}C]-phenylalanine) was fed to G.virens. A significant incorporation (8.6%) into bisdethiodi(methylthio)gliotoxin (16) was observed and 27.4% was recovered as gliotoxin. The isolated [^{14}C] bisdethiodi(methylthio)gliotoxin was fed to G.virens and only poorly incorporated (0.2%) into gliotoxin; 58% of the activity was recovered as unchanged precursor.



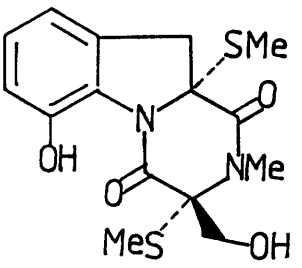
Scheme 1

Thus in one culture irreversible reductive methylation of the epidisulphide gave the methylthio metabolite (16) (Scheme 1). This may well apply to similar metabolites in other fungi.

The isolation of minor metabolites is often a useful adjunct to feeding experiments in determining a biosynthetic pathway. Figure 1 shows some metabolites isolated from G.virens[18].

The bis-S-methyl derivative of cyclo-(Phe-Ser) (17) shows that G.virens can introduce sulphur without prior N-methylation and oxidative cyclisation. Indeed, to date, no metabolites containing the reduced indole unit but no sulphur have been isolated. Thus it seems likely that sulphur introduction is an obligatory step prior to cyclisation.

A popular hypothesis for the formation of the cyclohexadienol ring involves the formation of an arene-oxide (24) or (25) followed by nucleophilic ring attack by the amide nitrogen. This would provide the trans-stereochemistry of gliotoxin. Two possibilities have been considered. A 2',3'-epoxide (24) would also provide a pathway in aranotin biosynthesis[12], via (26), and attack by nitrogen is 'allowed' by Baldwin's rules[20] (5- exo-tet).



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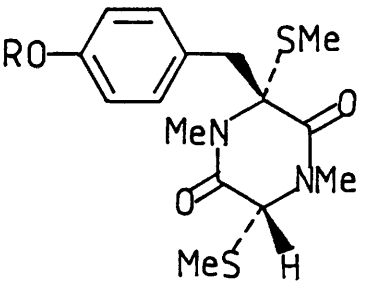
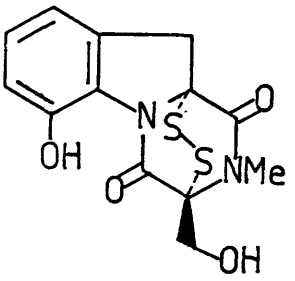
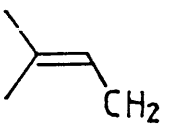


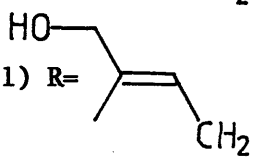
(19)R= H

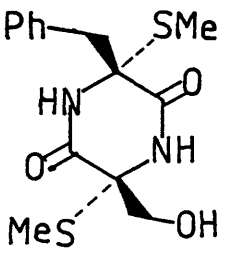


(22)

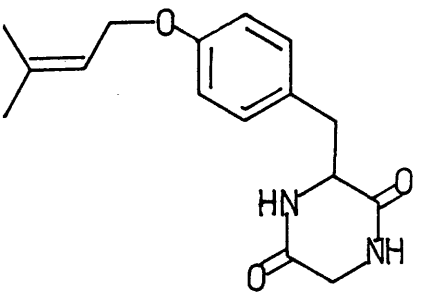
(20) R=







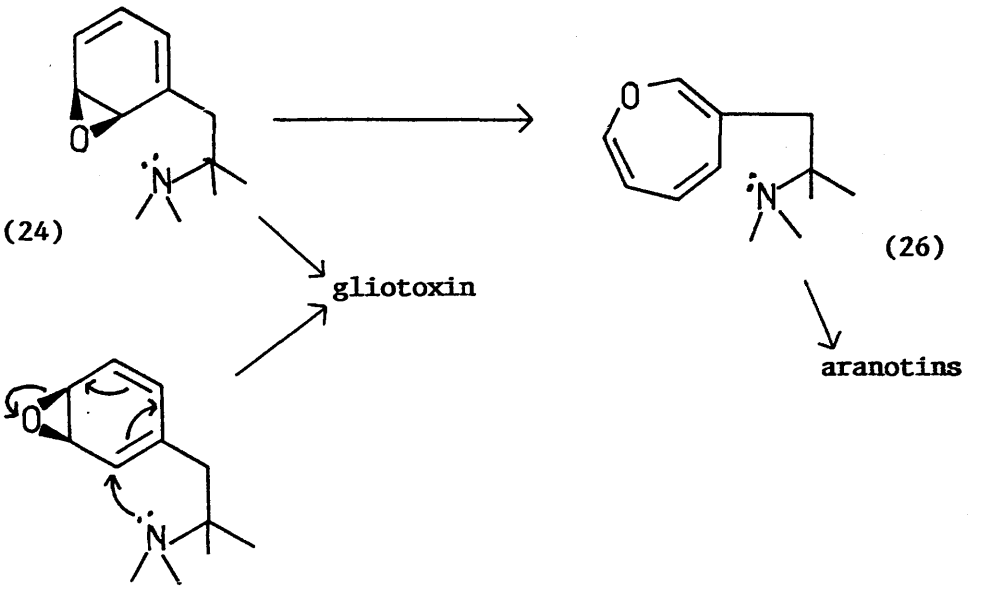
(17)



(23)

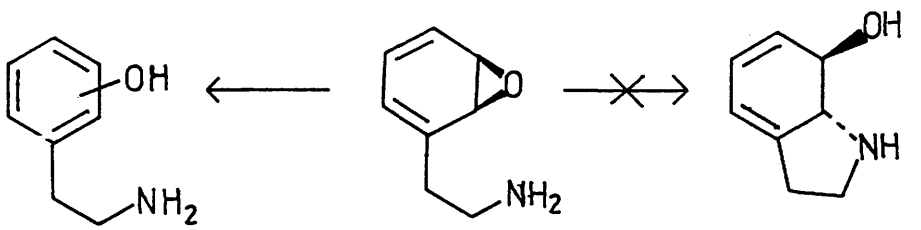
Figure 1

In contrast, the 3',4' epoxide (25) would not serve as an intermediate in aranotin biosynthesis, and is 'disallowed' by Baldwin's rules (5-endo-trig)



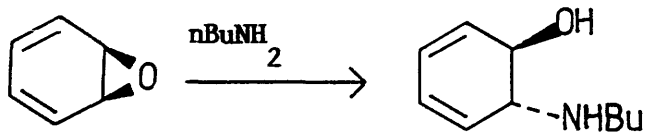
(25)

The lability of arene-oxides would make *in vivo* testing of these proposed intermediates impracticable, but model *in vitro* studies have been undertaken[21]. The model compound (27) did not undergo intramolecular ring closure, but rearranged to the phenol (28). Surprisingly, the inter-



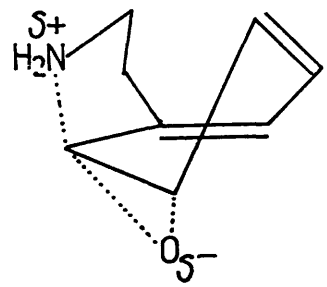
(28) (27)

molecular ring opening of benzene oxide (29) was successful.



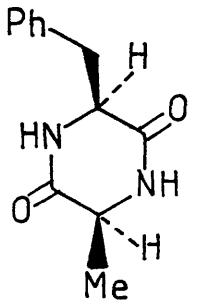
(29)

The failure of the intramolecular case was explained[21] by the development of strain in the transition state in which leaving group and nucleophile are held trans-diaxial (27a). A Dreiding model showed that, in the case of gliotoxin, the preferred antiperiplanar relation was maintained during ring closure without the development of strain.



(27a)

Other studies have shown that G.virens will readily perform the late-stage biosynthetic transformations on analogues of cyclo-(L-Phe-L-Ser). cyclo-(L-Phe-L-Ala) (30) was incorporated with high efficiency (40%) into the gliotoxin analogue (31)[22]. Surprisingly, the hydroxy group



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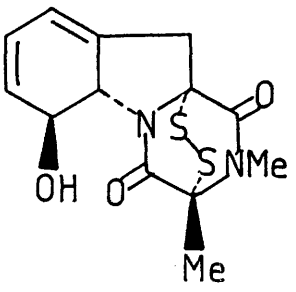
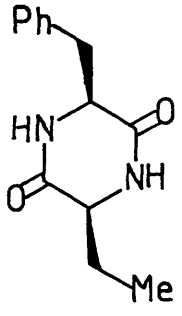


(31)

of the serine residue is not essential for binding substrates to any of the enzymes. Similarly cyclo-(L-Phe-L-α-aminobutryl) (32) was incorporated into three analogues of the normal metabolites (33), (34) and (35)[23].



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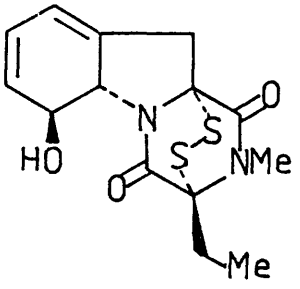

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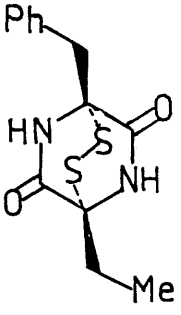

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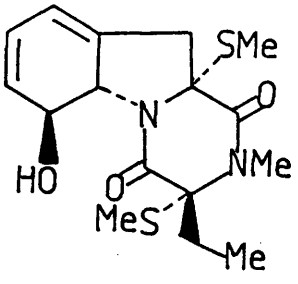

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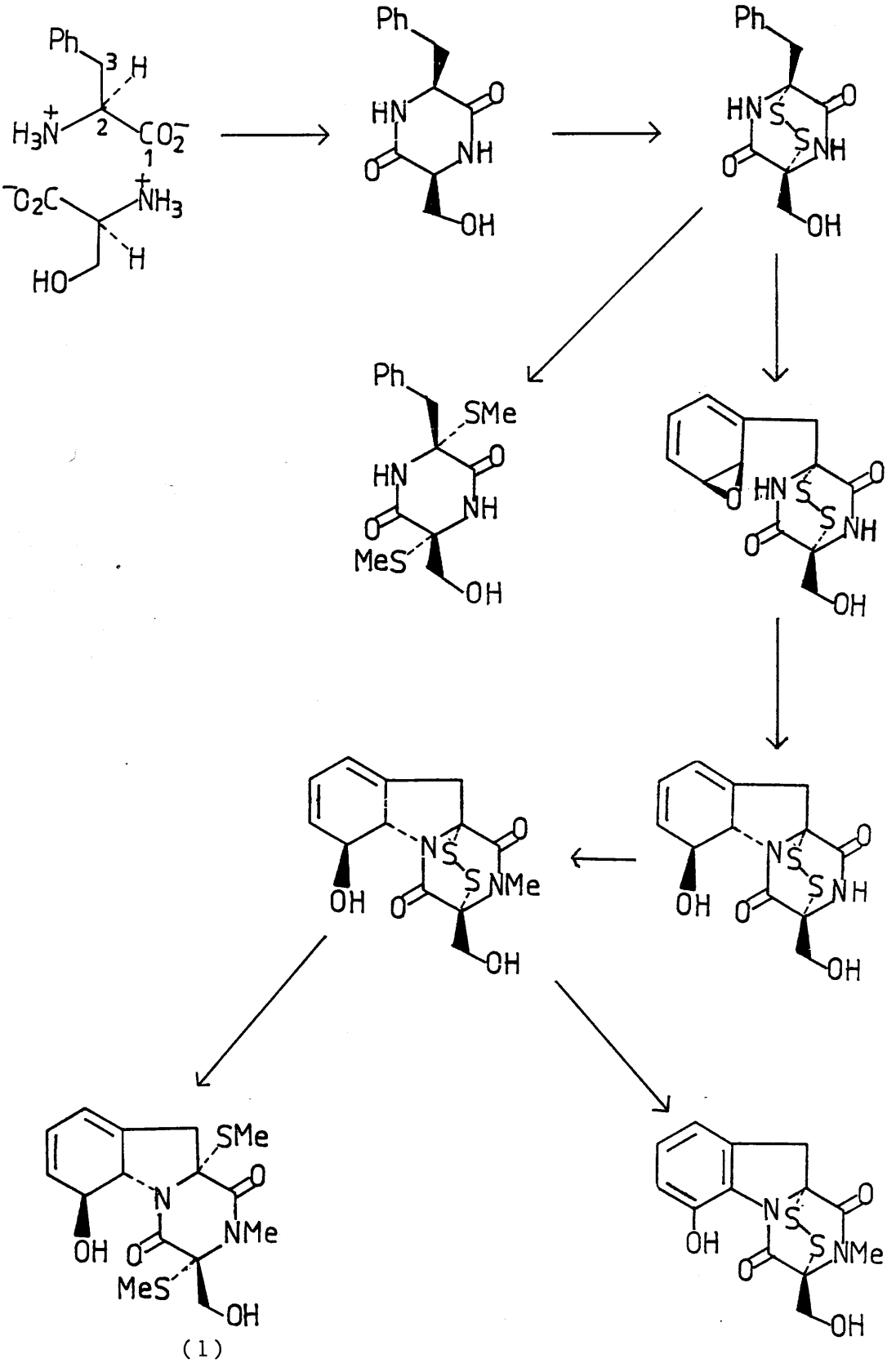


(1)

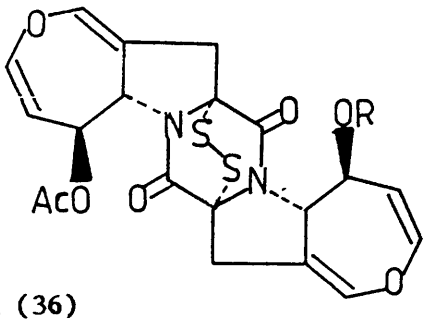
Scheme 2

A biosynthetic pathway agreeing with the experimental evidence available to date is given in Scheme 2. However, the timing of N-methylation is not defined and the mechanism for oxidative cyclisation via an arene oxide must be regarded as tentative, especially in view of the failure of the model experiments[21].

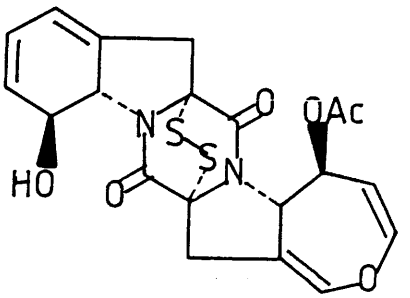
4.3 Biosynthesis of aranotins

In 1968[24,25] five new metabolites were isolated from cultures of Arachniotus aureus (Eidam) Shroeter. These were aranotin (36), acetylaranotin (37) and apoaranotin (38), containing an epidisulphide moiety and two S-methyl metabolites, bisdethiodi(methylthio)acetylaranotin (BDA) (39) and bisdethiodi(methylthio)acetylapoaranotin (BDAA) (40). BDA (39) and acetylaranotin (37) were also isolated from Aspergillus terreus[26]; a culture which more recently was shown to produce asteroxepin (41) and cis-di(methylthio)-cyclo-(Phe-Phe) (42).

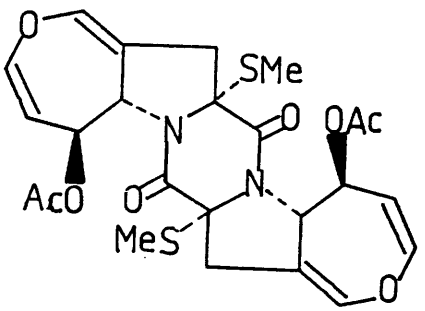
Initial feeding experiments[17] showed that as for gliotoxin phenylalanine (6.6% incorporation) and not m-tyrosine (0.46%) was the amino-acid source. Further



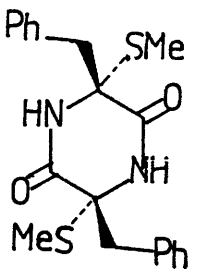
R = H (36)
R = Ac (37)



(38)



(39)



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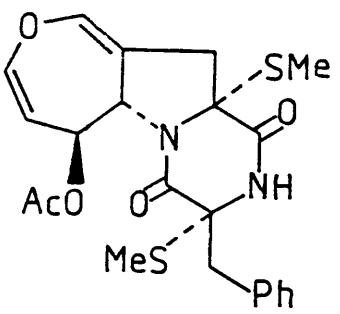
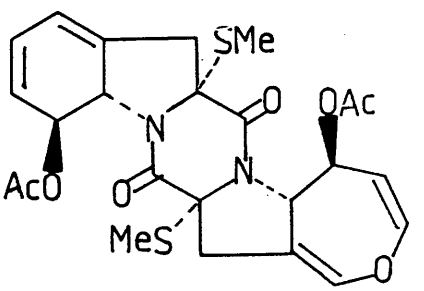
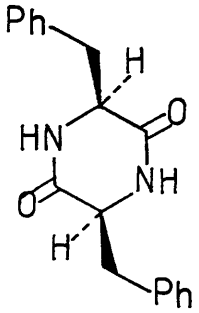


asteroxepin
(41)



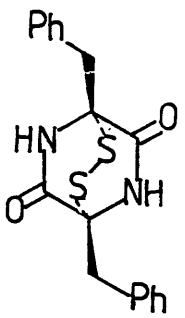
(40)

comparison with gliotoxin (1) suggested cyclo-(L-Phe-L-Phe) (43) as the next key intermediate in the biosynthesis of the aranotins. Accordingly, only the ^{14}C-labelled LL-isomer[27] was incorporated efficiently (20%) into BDA (39) (the major metabolite of A.terreus under their growth conditions).

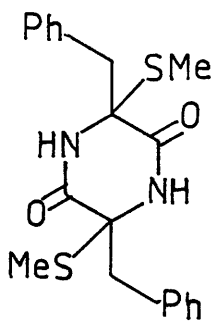


(43)

The disulphide (44) was then put forward as the precursor to the sulphur containing metabolites. In accord with this, the racemic disulphide (44) labelled with ^{35}S was



(44)

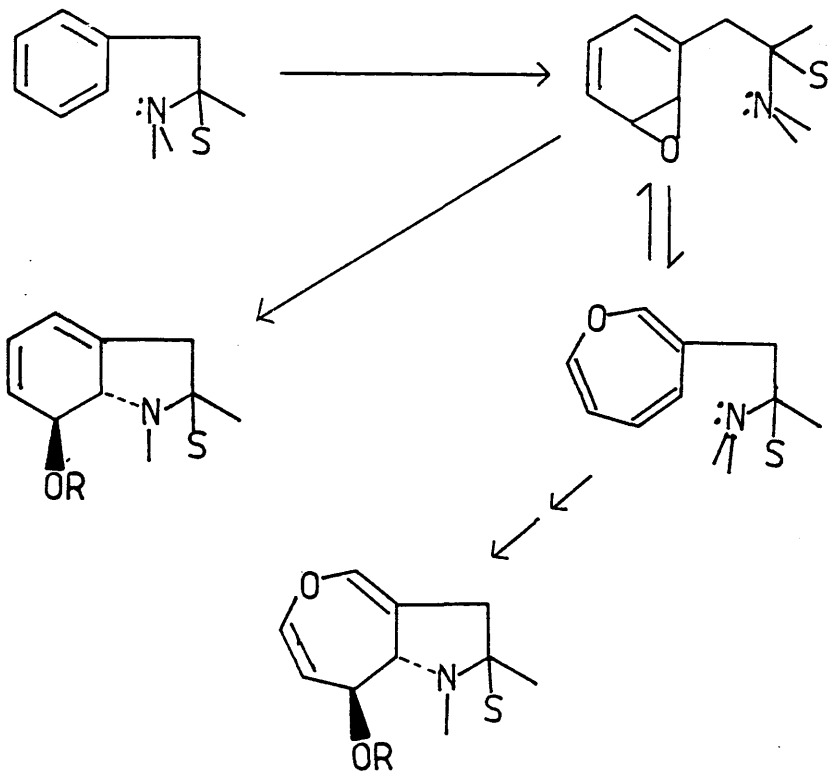


(45)

incorporated (4.4%) with low dilution by endogenous material (3.40) into BDA (39)[28,29]. Di(methylthio)-cyclo-(Phe-Phe) (45) was almost entirely derived from the disulphide, as shown by the very low dilution figures (1.06). In contrast [^{35}S]-cis-di(methylthio)-cyclo-(Phe-Phe) (45) fed to A.terreus was not incorporated well into BDA (0.26%) and the dilution was high (306).

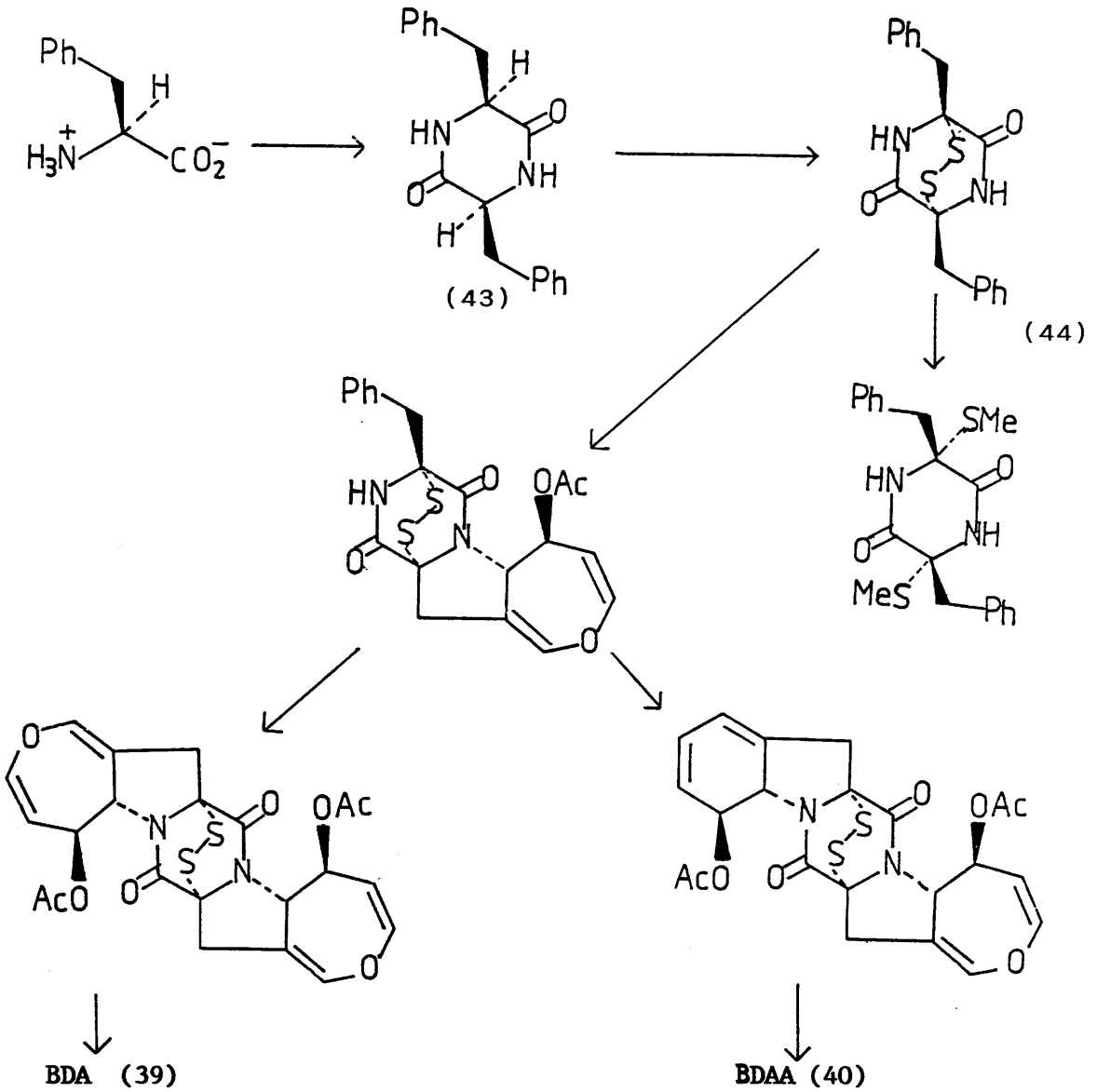
These results suggest that, as with gliotoxin, cyclisation occurs after introduction of a disulphide bridge. Reductive methylation to the dimethylthio-metabolites is irreversible and appears to stop further biosynthetic transformations.

The transformation of cyclic dipeptides to form oxepin rings has not been studied, but the existence of aranotins containing a cyclohexadienol ring, BDAA (40) and apoaranotin (38) suggest that an arene oxide mechanism may be involved (Scheme 3).



Scheme 3

The results obtained to date, including some not discussed here, suggest a probable pathway to the aranotin metabolites, as outlined in Scheme 4.



Scheme 4

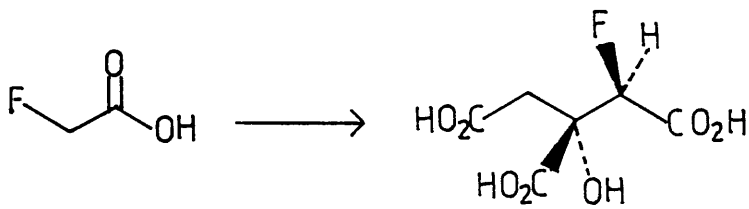
4.4 Fluoro-organic biochemistry

Experiments with structurally modified unnatural derivatives of known precursors, 'precursor analogues' can be of use in biosynthetic studies. The ability or inability of an organism to accept the substrate can provide information on enzyme specificty. The precursor analogue

may be metabolised normally to give the corresponding analogue of the natural product, or in an abnormal fashion giving rise to novel structures. Further, the metabolism of the analogue precursor may be inhibited at a particular step on the biosynthetic pathway producing isolable amounts of an analogue of an important intermediate.

Fluorine has many attractive properties as a replacement for hydrogen in biosynthetic studies, indeed it may almost be regarded as a third 'isotope': the carbon-fluorine bond is strong (ca. 460 kJ mol^{-1}) and so fluoride is not normally lost easily in chemical reactions; fluorine's Van-der-Waals radii (135 nm) is similar to that of hydrogen (120 nm); fluorine, the most electronegative element, alters electronic effects and thereby reactivity; its introduction greatly increases lipid solubility and it is rare in vivo and so can be easily followed by the sensitive technique of ^{19}F n.m.r. spectroscopy.

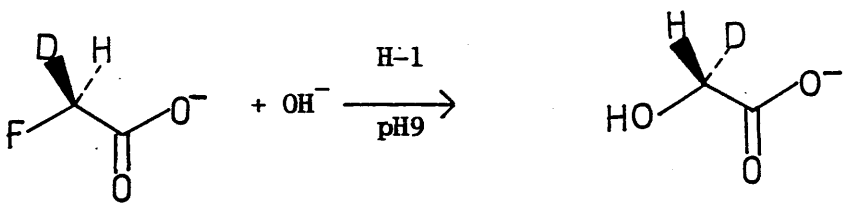
Early work on fluorinated compounds followed the isolation of naturally occurring, fluoroacetate (46)[30], from the plant Dichapetulum cymosium which is associated with cattle poisoning. Toxicity stems from metabolic conversion to (2R, 3R)-2-fluorocitrate (47) which was shown to be a



(46) (47)

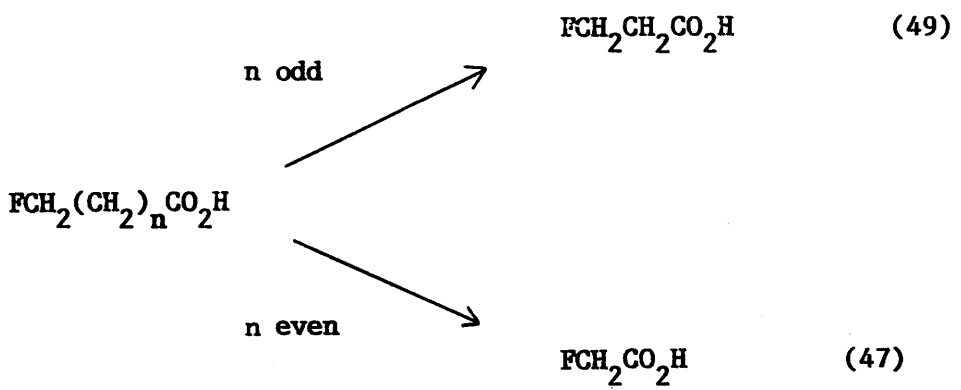
competitive inhibitor of the enzyme aconitase. Inhibition did not account for all the biological activity. Later

work[31] showed that the site of fluorocitrate action is at the mitochondrial membrane where the compound potently inhibits transport of citrate across the barrier. A strain of Pseudomonads has been identified with resistance to flouroacetate[32]. The species contains a plasmid encoded halohydrolase (H-1) enzyme; studies with (S)-deuteriofluoroacetate (48) showed that hydrolysis takes place with inversion.



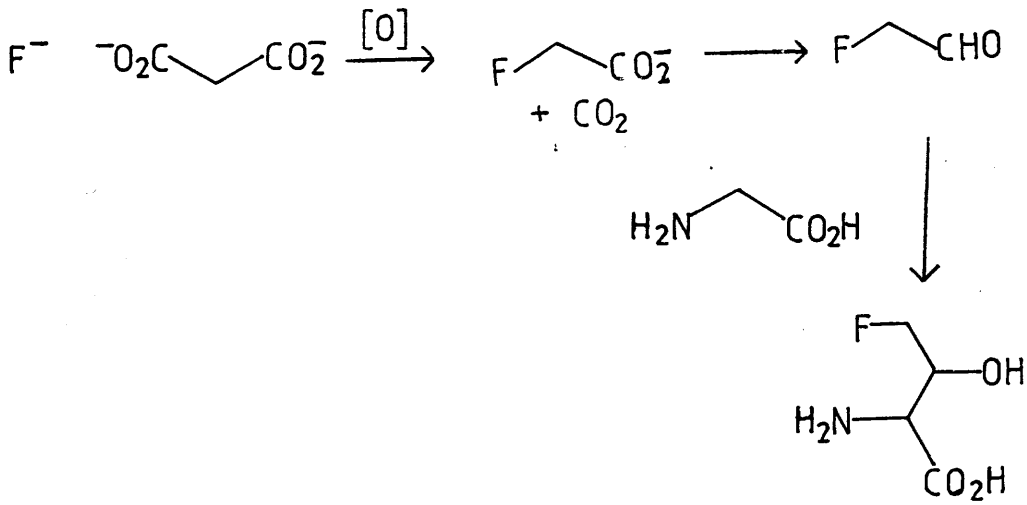
(S) (48) (R)

In drug design, careful attention must therefore be paid to metabolism, so that fluoroacetate is not a product. This principle was shown clearly with fluorinated fatty acids[30]. β-Oxidation by successive loss of acetyl-CoA units leads to toxic fluoroacetate for even carbon systems or non-toxic fluoropropionate (49) for odd carbon systems (Scheme 5).



Scheme 5

Recently, 4-fluorothreonine (50) (Scheme 6) was isolated from a strain of Streptomyces[32]. The only source of fluorine was inorganic fluoride, so this was the first report of a microorganism able to produce a fluoro amino-acid. Fluoroacetate was also produced, thus a biosynthetic pathway was proposed condensing glycine with flouroacetaldehyde. The chemical feasibility of

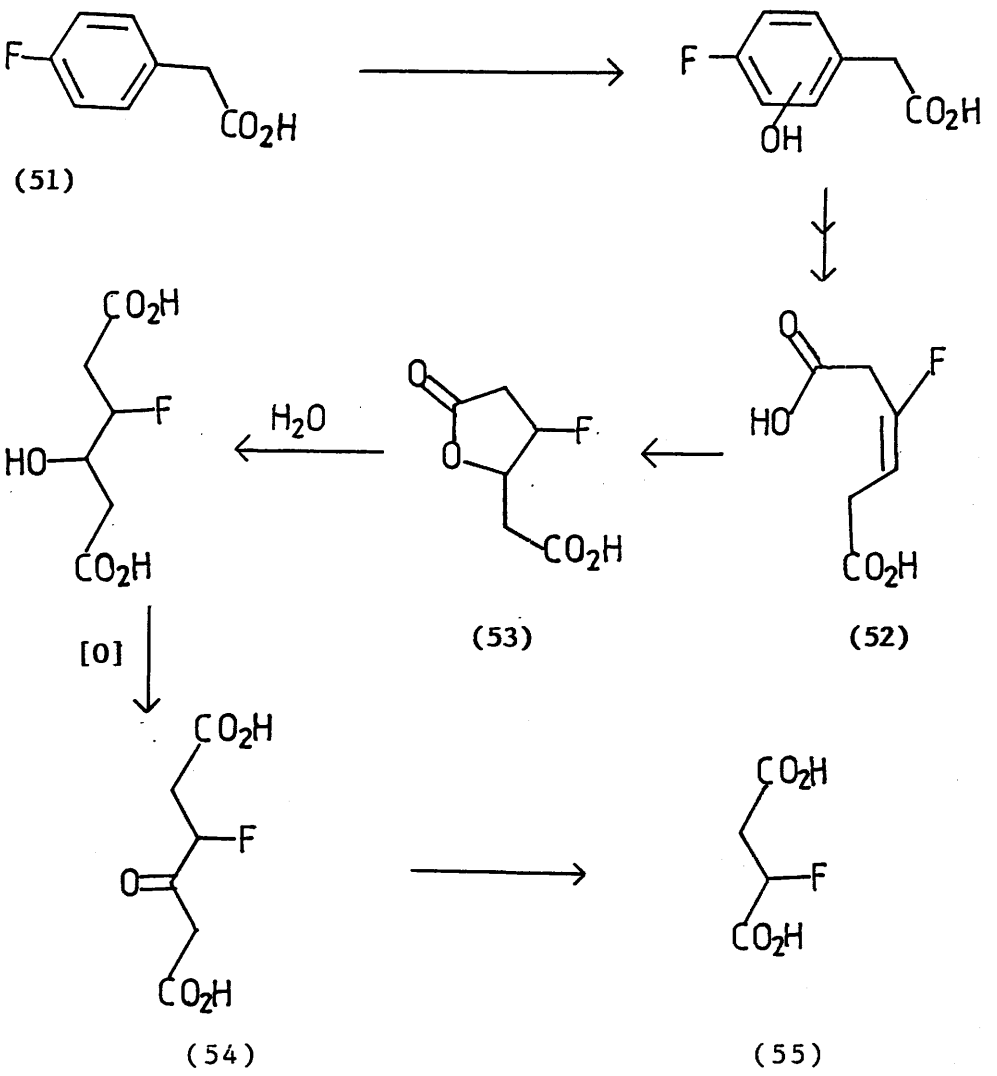


Scheme 6

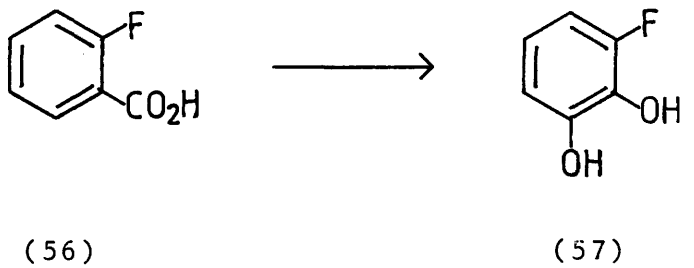
(50)

fluorodecarboxylation was shown in vitro[34], when malonic acid was incubated with sodium fluoride and sodium chlorite over 4 days small amounts of fluoroacetate were formed.

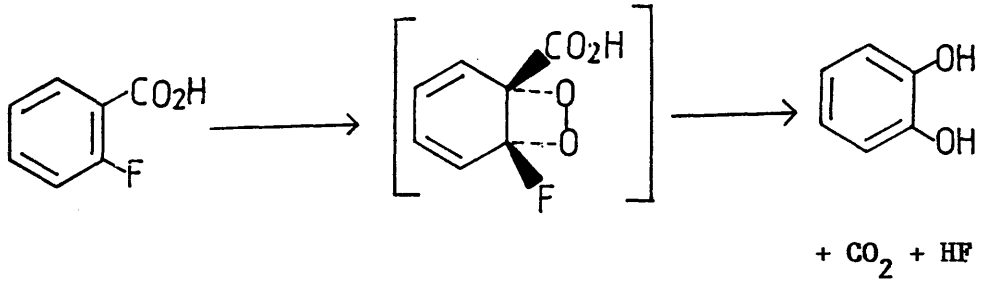
The fate of fluoroaromatic compounds has been studied in a wide spectrum of organisms. When resting cells of a Pseudomonas species were incubated with p-fluorophenylacetic acid (51) a number of fluorinated metabolites (52)-(55) were isolated. Cleaveage of the aromatic ring after hydroxylation would give rise to 3-fluoro-3-hexenedioic acid (52) which would cyclise to give butenolide (53) and thence 4-fluoro-3-oxoadipic acid (54) and fluorosuccinate (55)[35].



Another Pseudomonas species converted o-fluorobenzoic acid (56) into 3-fluorocatechol (57)[36].

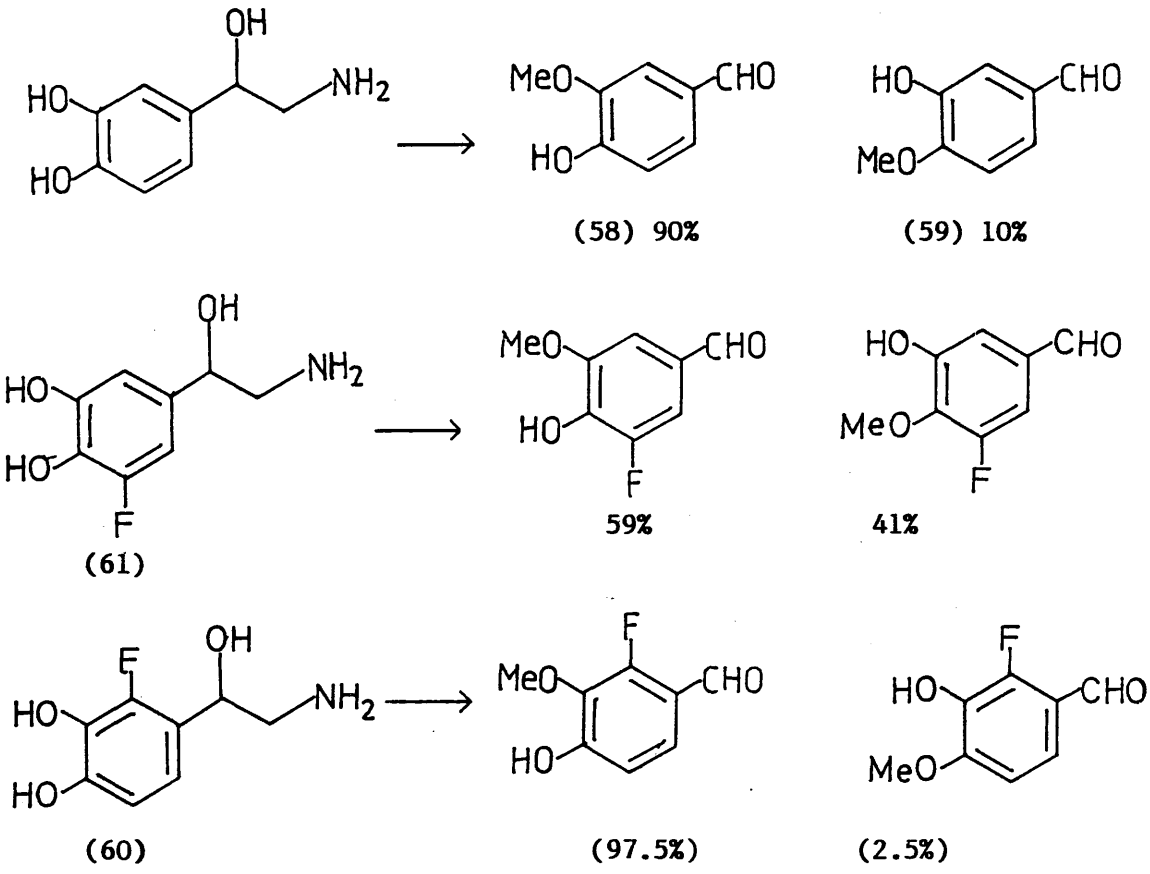


In some cases aromatic flourine is replaced by a hydroxyl group. A Pseudomonas employs molecular oxygen to snythesis catechol itself from o-fluorobenzoic acid[36]. When incubation of the cells was carried out in an atmosphere containing 50% $^{18}O_2$ and 50% $^{16}O_2$ both oxygens in the catechol were derived from a single molecule of oxygen. This suggests (Scheme 7) a cyclic peroxide as an intermediate.



Scheme 7

The effect of fluorine's electronegativity in biological processes was demonstrated in a study of the site of O-methylation of flourinated norepinephrines[37]. Substrates were methylated using S-adenosylmethionine (SAM) catalysed by catechol-O-methyl transferase (COMT) at pH7. The ratio of methylated norepinephrines was determined by periodate cleavage to vanillin (58) or isovanillin (59) (Scheme 8).

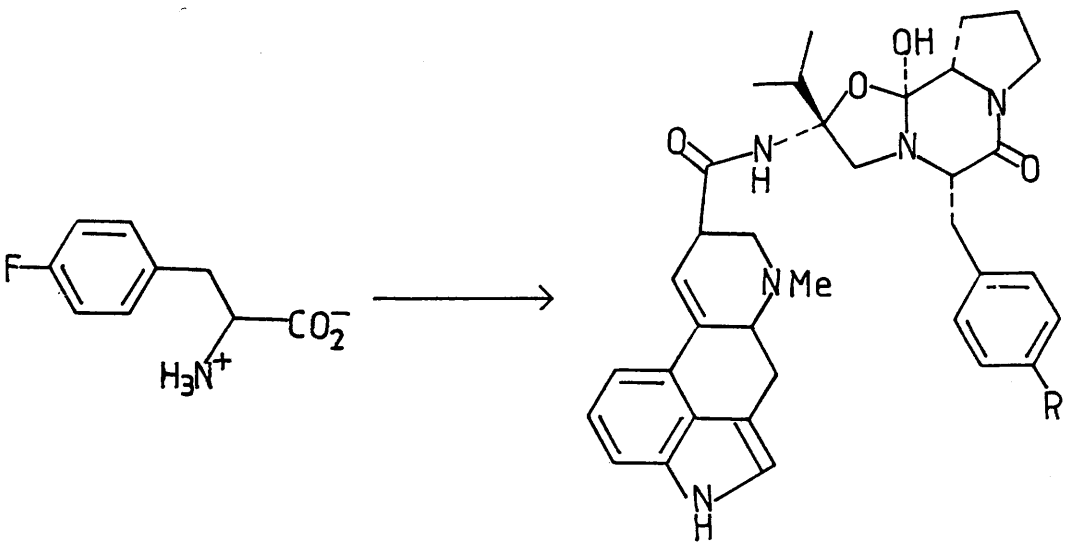


Scheme 8

It is generally agreed that the site at which COMT acts is governed by the orientation and acidity of the hydroxyl group rather than its nucleophilicity. The results (Scheme 8) show the consequence of fluorine increasing ionisation at the o-hydroxyl groups. The fluorine substituent in 2-fluoronorepinephrine (60) enhances the normal preferential methylation at the 3-hydroxyl. Conversely, that in 5-fluoronorepinephrine (61) is able to substantially reverse the normal pattern.

The ergot alkaloid ergocryptine (62) obtained from Claviceps purpurea[38] is an amide of lysergic acid containing a cyclic dipeptide moiety derived from proline, phenylalanine and α-hydroxyvaline. Mutant strains dependent upon phenylalanine were developed which incorporated p-fluorophenylalanine into the fluoro analogue (63) with high

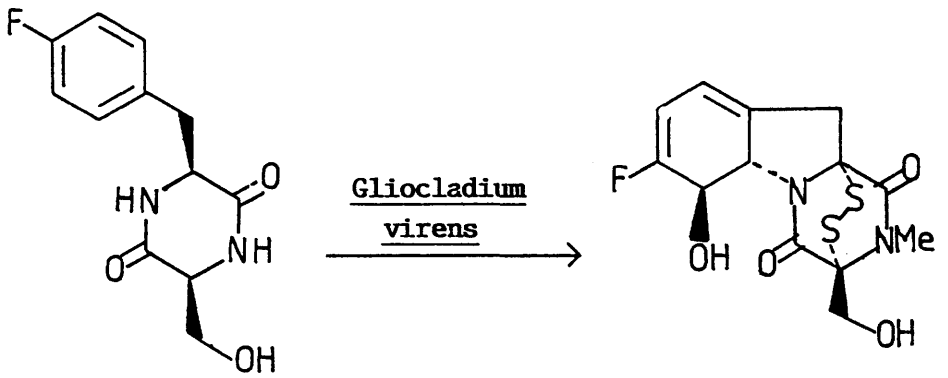
efficiency (80%).



(62) R=H

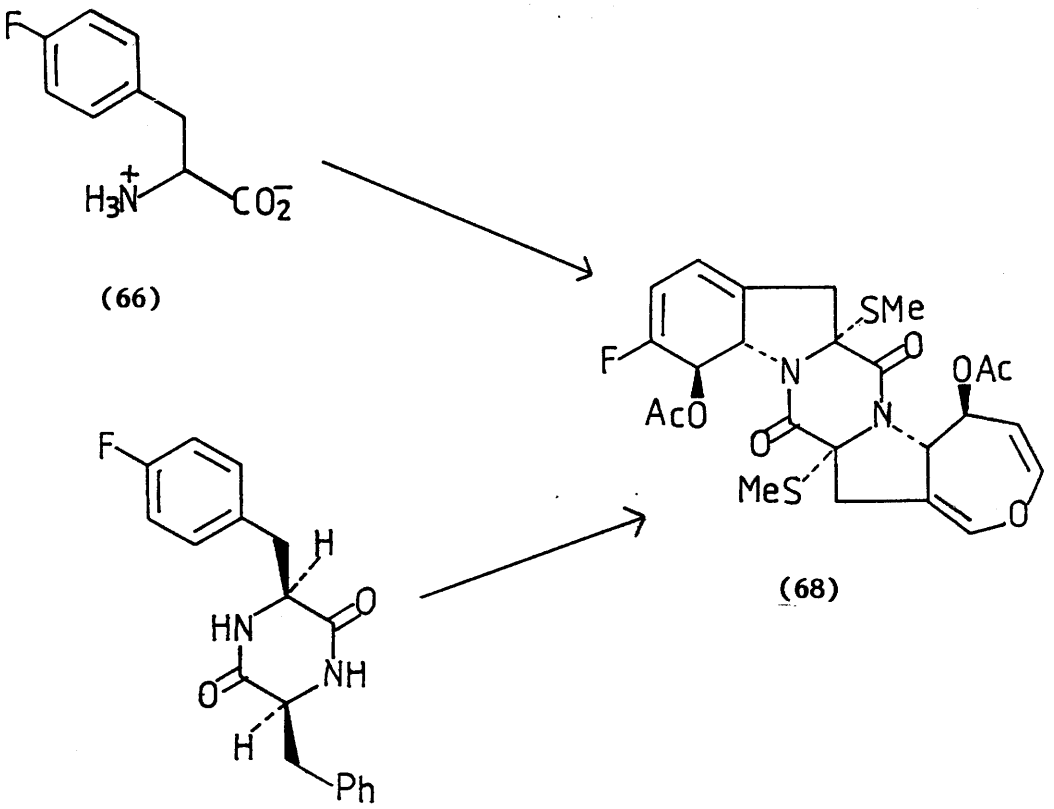
(63) R=F

In this laboratory, p-fluorophenylalanine and cyclo-(L-p-Fluoro-Phe-L-Ser) (64) was incorporated into fluorogliotoxin (65)[39]. The proton decoupled ^{19}F n.m.r.



(64) (65)

spectrum gave a signal at δ -122 relative to $CFCl_3$ (δ 0). In parallel studies of aranotin biosynthesis, p-fluorophenylalanine (66) and cyclo-(L-Phe-L-p-fluoro-Phe) (67) were incorporated into fluoro-BDAA (68)[29]. Remarkably

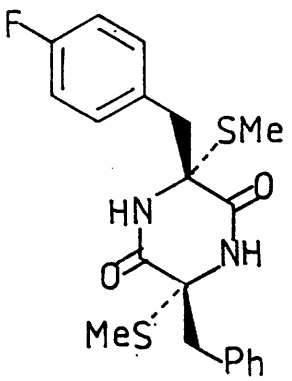


(66)

(68)

(67)

no compounds having fluorine in the oxepin ring were detected. The fluorinated analogue of di(methylthio)-cyclo-(Phe-Phe) (69) was also isolated from these feedings as a mixture with the normal metabolite. Similar analogues were



(69)

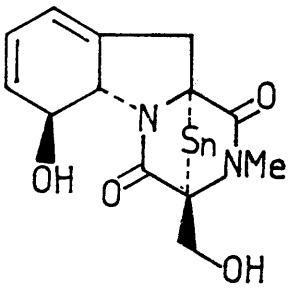
isolated from feeding of the o- and m-fluorinated dipeptides.

In summary, fluorinated analogues of natural precursors are metabolised by a variety of organisms into analogues of the normal metabolites. A few organisms have the ability to replace fluorine with hydroxyl, which would appear, energetically, to be a difficult proposition. Although the steric requirements of fluorine appear to pose little problem to enzymes, fluorine's high electronegativity can alter the course of biosynthetic processes.

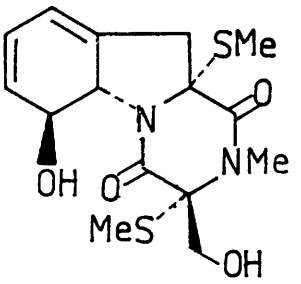
Chapter 5 Discussion

5.1 Summary of Earlier Biosynthetic Results

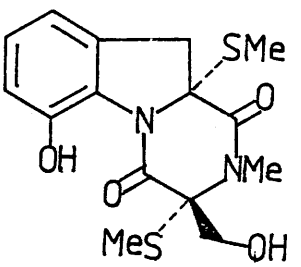
The research detailed in the Introduction (Section 4.2) has unravelled the early stages in the biosynthesis of sulphur-containing dioxopiperazines to a large extent. In the case of gliotoxin serine and phenylalanine were identified as the amino-acid precursors[12,13]. cyclo-(L-Phe-L-Ser)[10] was shown to be the next key intermediate. The steps still to be investigated are the introduction of the disulphide bridge, the cyclisation of the aromatic ring onto the amide nitrogen with accompanying oxygenation, and the N-methylation. At the time this work began the order of events was still unclear, but it now appears that sulphur introduction must occur before cyclisation. The only experimental evidence to shed light on the mechanism of ring closure was that, in gliotoxin and aranotin biosynthesis, no loss or migration of aromatic hydrogens took place[40]. This and other evidence conclusively ruled out phenolic intermediates. The observed trans stereochemistry in these metabolites, combined with experimental observations, led to the proposal of arene oxide intermediates (Introduction Section 4.2). The instability of such compounds deems it unlikely that conventional feeding experiments could be carried out. The possible use of analogue precursors remains. In addition to being metabolised normally they might be metabolised abnormally to novel structural types which, in turn, may provide information on the mechanisms of their formation. Finally, a late stage process might be sufficiently inhibited by structural changes to produce isolable quantities of analogues of previously ephemeral intermediates.



(1) n = 2
(71) n = 3



(16)



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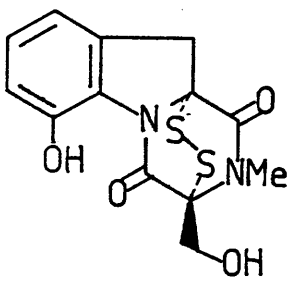

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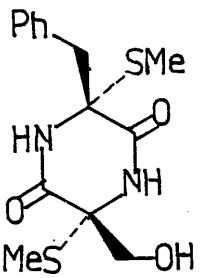

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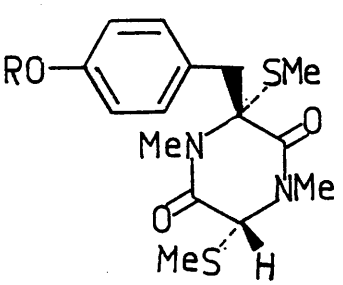
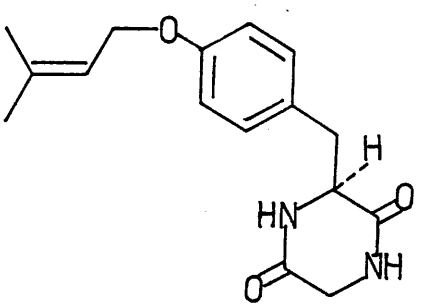


(19) R = H
(20) R = $Me_2C{=}CHCH_2$
(21) R = $HOCH_2MeC{=}CHCH_2$



(23)

FIGURE 2

5.1.1 Gliocladium virens

In all feeding experiments the strain of G.virens used was IMI 101525. The growth conditions of Johnson, Bruce and Dutcher[41] were employed at 27 °C. Gliotoxin (1), the major product of this fungus, is easily crystallised from the crude dichloromethane extract using methanol. Other metabolites produced by this strain (Figure 2) are bisdethidi(methylthio)gliotoxin (16), the phenol (19), its dimethylallyl ether (20), and the dihydroindole (70). Since this project was completed a feeding experiment with [^{35}S] sulphate [18] led to the detection of the epitrisulphide gliotoxin E (71), the hydroxymethylbut-2-enyl ether (21), didehydrogliotoxin (22), bis-N-norgliovictin (17) and the 3-methylbut-2-enyl ether of cyclo-(glycyl-L-tyrosyl) (23).

5.2 Synthesis of Fluorinated Analogues

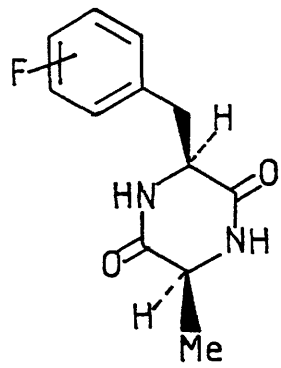
The choice of fluorinated analogues to prepare and feed was made by considering previous results. When p-fluoro-Phe (66) and cyclo-(L-p-fluoro-Phe-L-Ser) (64) were fed to G.virens it was hoped that fluorine would be accepted sterically and therefore allow the introduction of sulphur, but then its electronegativity might influence and thus shed light on the oxidative cyclisation. In fact S_2 was incorporated but the oxygenation also proceeded as normal. However, in the aranotin case no fluorinated oxepin ring formation was detected, showing that fluorine did produce notable changes from the norm.

The decision was made to prepare o-, m-, and p-cyclo-(L-Ala-L-fluoro-Phe) (72, 73, 74) for several reasons: the electronic effects on the metabolites produced might shed more light on the existence and/or the mechanism of arene oxide formation; deoxy analogues differ sufficiently in their chromatographic behaviour from the normal metabolites

to facilitate easy isolation. The easy detection by ^{19}F n.m.r. spectroscopy and fluorine's other attributes were discussed earlier.

5.2.1 Preparation of o,m, and p substituted cyclo-(L-Ala-L-fluoro-Phe)

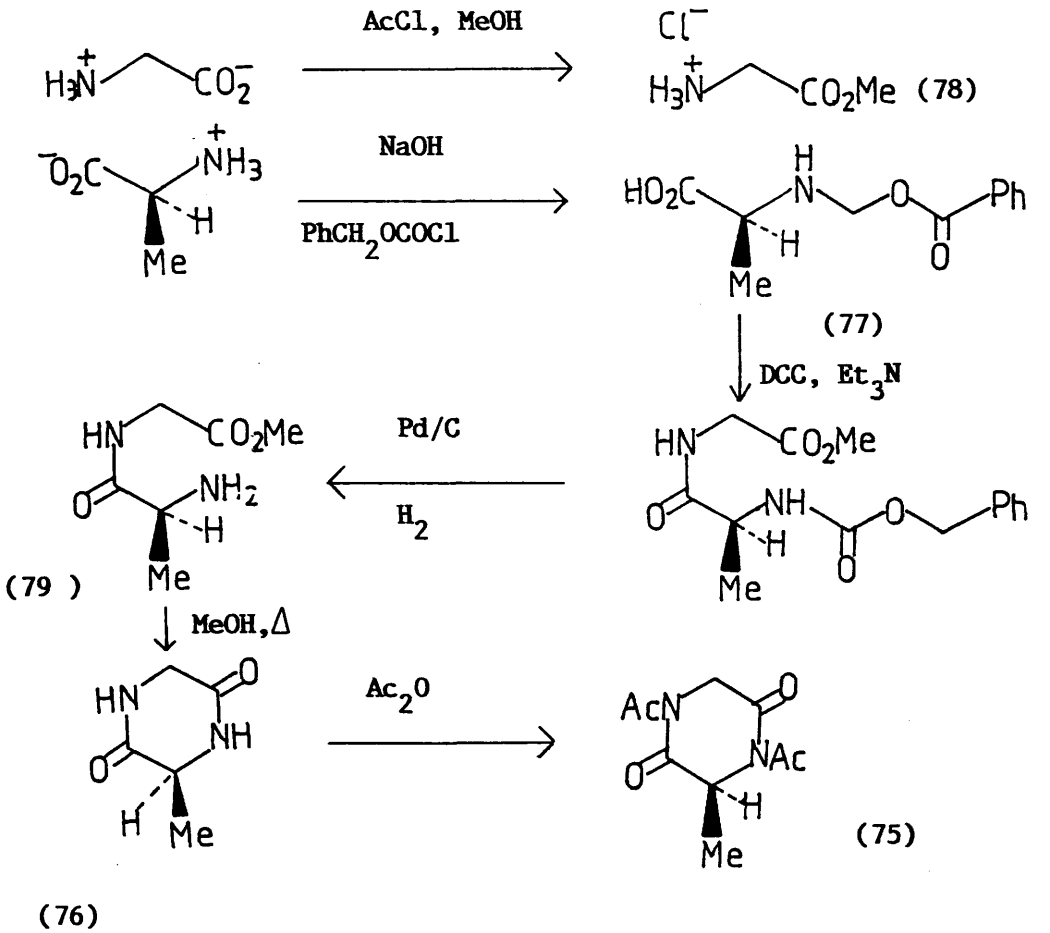
The choice of synthetic route to o (72), m-(73) and p-(74) substituted cyclo-(L-Ala-L-fluoro-Phe) was governed by certain constraints. Ultimately, ^{14}C-labelled fluorinated dipeptides would be required to allow detection and eventually isolation of metabolites. Glycine was chosen (Scheme 9) as a suitable precursor, since it was available in labelled form.



(72) o-F
(73) m-F
(74) p-F

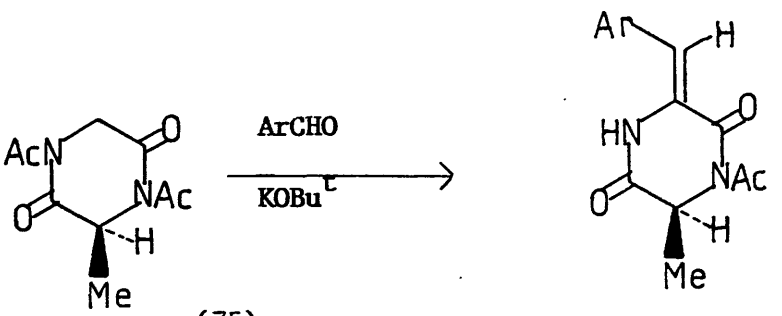
Kanmera et al.[42] condensed aromatic aldehydes with N,N'-diacetyl-cyclo-(L-Ala-Gly) (75) to give arylidene compounds. Asymmetric hydrogenation and deacetylation gave the cis-(LL) dipeptides with high chiral induction. This route was selected.

The preparation of cyclo-(L-Ala-Gly) (76)[42] was achieved by standard peptide synthesis. N-Benzyloxycarbonyl-L-alanine (Z-L-Ala) (77) was condensed with glycine methyl ester hydrochloride (78) using dicyclohexylcarbodiimide (DCC) and triethylamine. After hydrogenolysis of the protected dipeptide, cyclisation of the crude L-alanylglycine methyl ester (79) was accomplished in methanolic ammonia. This afforded cyclo-(L-Ala-Gly) (76), but with substantial racemization, m.p. 214-217 °C, $[\alpha]_D$-10.8° [N,N,-dimethylformamide (DMF)] [lit.[41] m.p. 228-230 °C, $[\alpha]_D$-21.3 ° (DMF)]. Kanmera et al.[42] overcame the same problem by heating the linear dipeptide (79) in methanol under reflux. This method afforded cyclo-(L-Ala-Gly) (76) with little loss of optical activity, $[\alpha]_D$-19.0° (DMF), m.p. 228-230 °C. Treatment of (76) with acetic anhydride under reflux gave N,N'-diacetyl-cyclo-(L-Ala-Gly) (75)[42] (Scheme 9).



Scheme 9

Following the method of Gallina and Liberatori[43] the diacetylated cyclic dipeptide (75) was condensed with 2-, 3- or 4-fluorobenzaldehyde using potassium ^{t}butoxide to yield the N-acetyl-arylidene derivatives (80, 81, 82). Slower



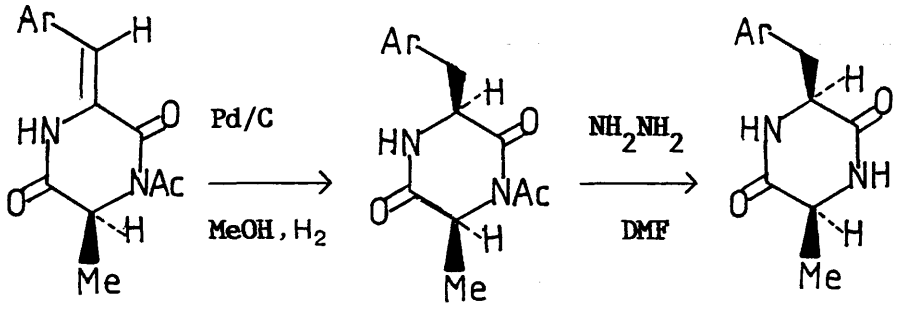
(75)

(80) Ar= o-FC_6H_4
(81) Ar= m-FC_6H_4
(82) Ar= p-FC_6H_4

reactions involving triethylamine as base have been found to effect more racemisation than these more rigorous conditions[29]. Because of the small scale of these preparations, the DMF used by Liberatori was replaced with an excess of the liquid aldehydes to achieve homogenicity. Z-Stereochemistry and the loss of an acetyl group from the glycyl residue is assumed from the mechanism proposed by Gallina and Liberatori[43]; t.l.c. and ^{1}H n.m.r. spectroscopy of the crude products indicated a single isomer.

Kanmera et al. deacetylated and then hydrogenated their products to give almost exclusively the LL-cyclic dipeptides. It proved more convenient to hydrogenate the more soluble acetyl-arylidene derivative itself; methanol being a suitable solvent. Indeed, whenever deacetylation occurred during reduction, substantial quantities of products were precipitated and lost when the mixtures were filtered through Celite to remove the catalyst. The oily hydrogenation products were not purified, but deacetylated directly by hydrazine to give the easily recrystallised

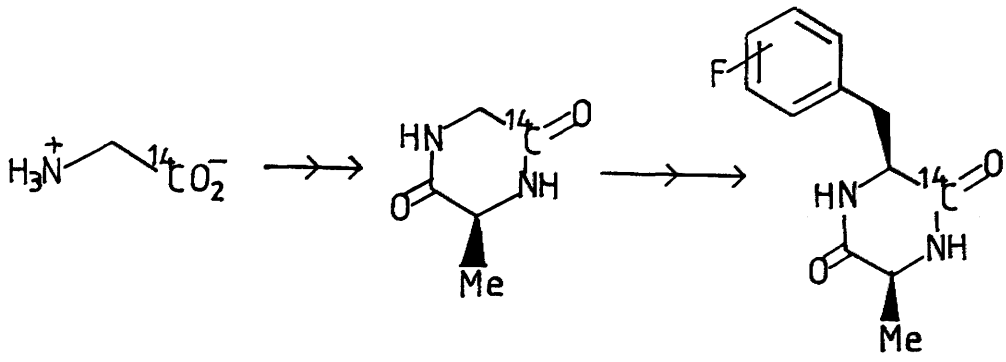
cyclic dipeptides (72, 73, 74). ^{19}F and ^{1}H n.m.r. and



	Ar
	(72) o-FC_6H_4
	(73) m-FC_6H_4
(80-82)	(74) p-FC_6H_4
and Ar=Ph	(83) Ph

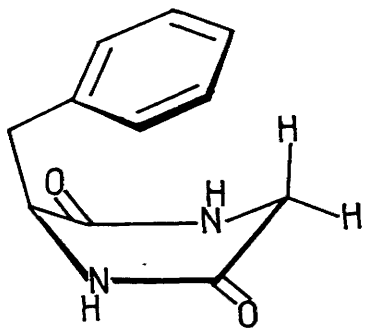
t.l.c. showed the presence of a single diastereomer in each product. Thus hydrogen was delivred stereospecifically to the face opposite to the alanyl methyl group. As a further test of stereochemical purity cyclo-(L-Ala-L-Phe) (83)[44,45] was prepared by the same route. T.l.c. analysis of the crude product indicated a single diketopiperazine. Two recrysallisations from methanol gave the LL-cyclodipeptide (83), m.p. 271-273 °C, $[\alpha]_D$+68 °(c 1.1 in AcOH). These data are in good agreement with those for the compound prepared from L-Phe and L-Ala derivatives [44,45].

The radio-labelled compounds (84,85,86) were prepared using the same sequence. [1-^{14}C] Glycine (250 µCi) was used to prepare the intermediate cyclo-(L-Ala-[1-^{14}C]Gly), which thus gave flourinated dipeptides labelled at C-1 of the fluorophenylalanyl residue.



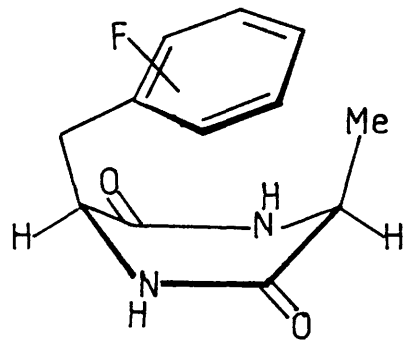
(84) o-FC_6H_4
(85) m-FC_6H_4
(86) p-FC_6H_4

The n.m.r. spectra of these aromatic cyclic dipeptides deserve a brief mention. In compounds such as cyclo-(Gly-Phe) (87)[46], the n.m.r. signal of one proton of the glycyl



(87)

methylene group is 1 p.p.m. upfield from that of the other at ca. δ 4.0. This is explained by the conformation adopted, whereby the benzene ring resides over the dioxopiperazine ring (87); the aromatic ring thus causes ring current shielding of the cis-glycyl proton. The methyl

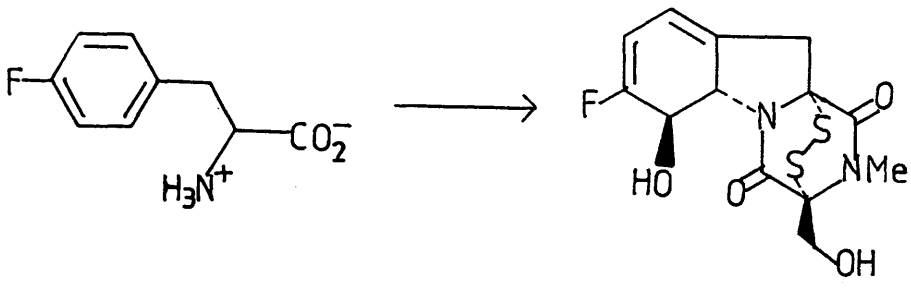


(72-74)

doublet of the cyclo-(L-Ala-L-fluoro-Phe) derivatives (72-74) was also at significantly higher field (δ ca. 0.9) than that of the same signal for cyclo-(L-Ala-Gly) (δ 1.88). Thus the derivatives (72-84) must adopt a similar conformation; this observation confirms the LL-configuration.

5.3 Feedings of Fluorinated Analogues to Gliocladium Virens

As a preliminary experiment, DL-p-fluorophenylalanine, an analogue of the natural precursor was fed. After five days incubation, the broth was extracted with dichloromethane and the resulting organic residue was triturated with $CDCl_3$. The majority of the gliotoxin crystallised out and was filtered off. The proton-decoupled ^{19}F n.m.r. spectrum of the filtrate showed a single signal at δ -122.5. Integration of a spectrum of the sample containing p-fluorobenzoic acid as an internal standard showed an incorporation of 5.8% of fluorophenylalanine into the compound giving this signal. The same chemical shift was observed for fluorogliotoxin (65)[39] isolated from a feeding of the p-fluorinated dipeptide (64) and for fluoro-BDAA (66)[29] obtained from Aspergillus terreus. Thus the δ -122.5 signal arises from fluorogliotoxin (65). No attempt

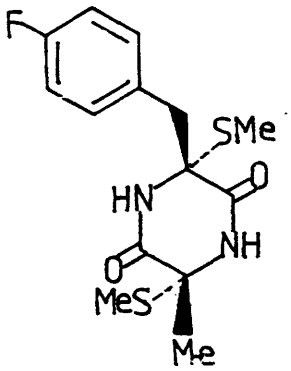


(66) (65)

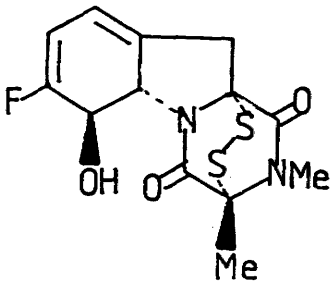
was made to isolate this metabolite. In contrast to the spectrum of the product mixture from A. terreus no signals at δ -115 due to cyclic dipeptide analogues were present.

Several feedings with unlabelled cyclo-(L-Ala-L-fluoro-Phe) (72-74) were conducted. To obtain n.m.r. spectra with good signal-to-noise ratios, the entire extracts were triturated with $CDCl_3$ and filtered; the proton decoupled spectrum signals of the filtrate and residues are given in Table 1 with approximate incorporation values. Spectra were recorded on representative samples of undissolved material in $(CD_3)_2SO$. Essentially all the fluorinated metabolites that were produced, were present in the chloroform soluble samples.

Feeding cyclo-(L-Ala-L-p-fluoro-Phe) (74) gave rise to two sets of signals, at ca. δ- 115.5 and ca. δ 122.5. On most occasions two very closely spaced signals were observed at δ -115.5. One of these may be unaltered precursor and the other may be speculatively assigned to an analogue (88) of bis-N-norgliovictim (17). From previous results



(88)



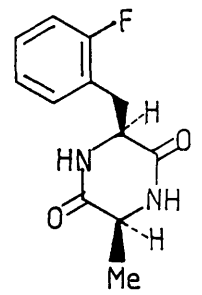
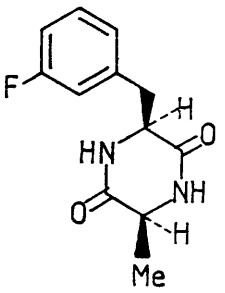
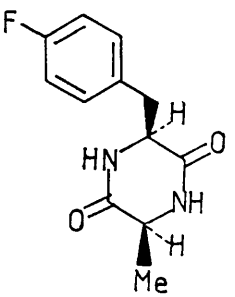
(89)

the signal at ca. δ -122.5 might well be due to the presence of fluorodeoxygliotoxin (89).

cyclo-(L-Ala-L-o-fluoro-Phe) (72) produced the most promising results, being incorporated into a wide range of fluorinated metabolites other than slightly altered dipeptide precursor. By analogy with the p-derivative (89)

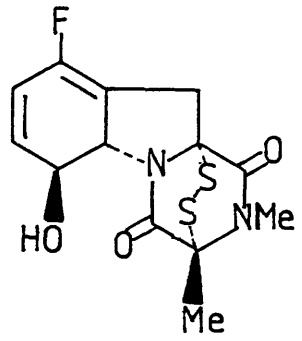
Table 1

Fluorine n.m.r. data for unlabelled feedings

Experiment Number	$CDCl_3$ - soluble fraction	Insoluble fraction in $(CD_3)_2SO$
 (72)	-117.4	-116.0
1.	-116.4(0.4) -117.3(1.3) -119.9(1.5) -120.2(1.1) -130.1(0.4)	-115.95(0.5) -119.4(w) -120.5(1.2)
2.	-116.0(0.2) -116.3(0.2) -117.2(0.8) -119.9(1.3) -120.2(0.5) -120.8(0.9) -121.0(0.1) -130.1(0.4)	-116.0(w) -120.8(1.0) -120.8(w)
 (73)	-112.4	-113.5
3.	-113.4(10.5) -113.5(6.7) -113.7(1.1) -114.0(2.0) -116.7(1.3)	
4.	-113.5(2.5) -114.0(1.9) -114.3(0.3) -114.6(0.4)	
5.	-112.9(1.3) -113.7(0.5)	-113.2(0.5) -113.4(1.0)
6.	-113.0(1.4) -113.8(w)	-113.4(2.0)
	115.1	-115.9
7.	-115.5(1.0) -116.1(0.9) -112.4(0.6) -122.8(1.8) -112.8(0.7) -148.7(0.1)	-115.0(0.6) -115.7(0.5) -112.3(1.3)
8.	-115.6(2.0) -122.4(0.6)	-115.7(0.1)
9.	-115.4(2.0) -116.0(2.5) -122.7(1.8)	-115.7(0.1)
10.	-114.8(0.17) -115.5(2.4) -122.4(0.5)	-115.8(1.9) -122.3(0.6)

^{19}F $\{^1H\}$ N.m.r. signals observed for the metabolites of the unlabelled precursors (72-74). The approximate % incorporations in parenthesis (w=weak) were obtained by the addition of p-fluorobenzoic acid as standard (δ -106). The figures underlined are the chemical shifts of the precursor in $CDCl_3$ or $(CD_3)_2SO$. No signals were observed for the $CDCl_3$ insoluble fractions from Experiments 3 and 4.

one of the two prominent peaks at ca. δ -120 may be due to the gliotoxin analogue (90). In addition to the signal



(90)

corresponding to the unchanged precursor (δ -117.4), there was a cluster of weak signals centred at ca. δ -116.3. The latter signals may arise from sulphur-containing but non-cyclised metabolites.

In contrast to the o and p case, cyclo-(L-Ala-L-m-fluoro-Phe) (73) was not incorporated into gliotoxin-like metabolites. Only signals from unchanged or slightly modified precursor were detected.

In the next set of experiments, the ^{14}C-labelled fluorinated dipeptides (84-86) were fed at three times the concentration normally fed. This was to ensure a sufficiently high level of activity for the detection of minor metabolites. Dichloromethane and ethyl acetate extracts of the culture medium were examined by radioscanning (Figure 3) and autoradiography (Figure 4) using a standard solvent system. It is clear from the radioscan and autoradiogram that dichloromethane

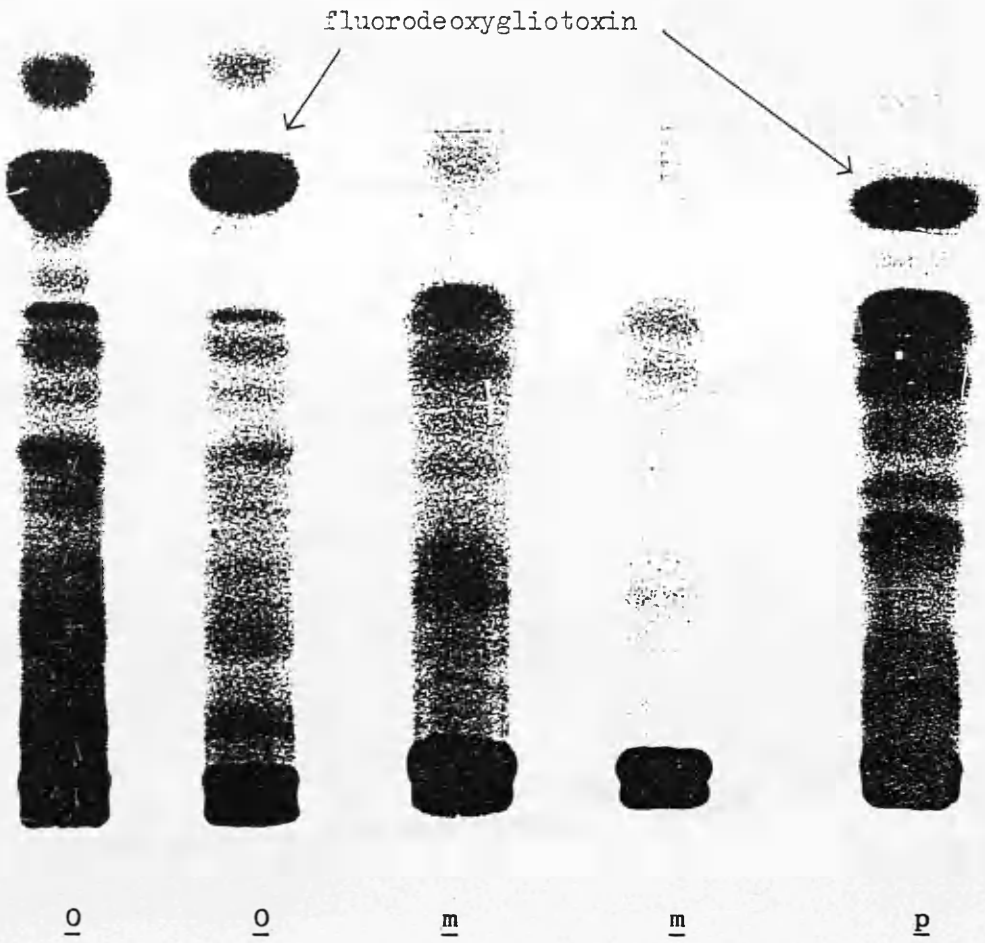


Figure 4

T.l.c. autoradiogram (toluene-acetone 2 : 1) of dichloromethane extract fed o, m, and p fluorinated cyclodipeptides (72), (73) and (74).

extraction is sufficient to obtain the metabolites of interest. There is high activity (Table 2) in the later ethyl acetate extract, but this is due to polar, slow running compounds (Figure 3, 4). These are either precursor or other simple diketopiperazines and as such, are of little interest. Another general observation can be made from the high activity (Table 2) in the dichloromethane extract of cyclo-(L-Ala-L-[1-^{14}C]-o-fluoro-Phe) (84) feeding. It is nearly double that of the meta and para precursors, confirming the more efficient conversion of this isomer by the organism.

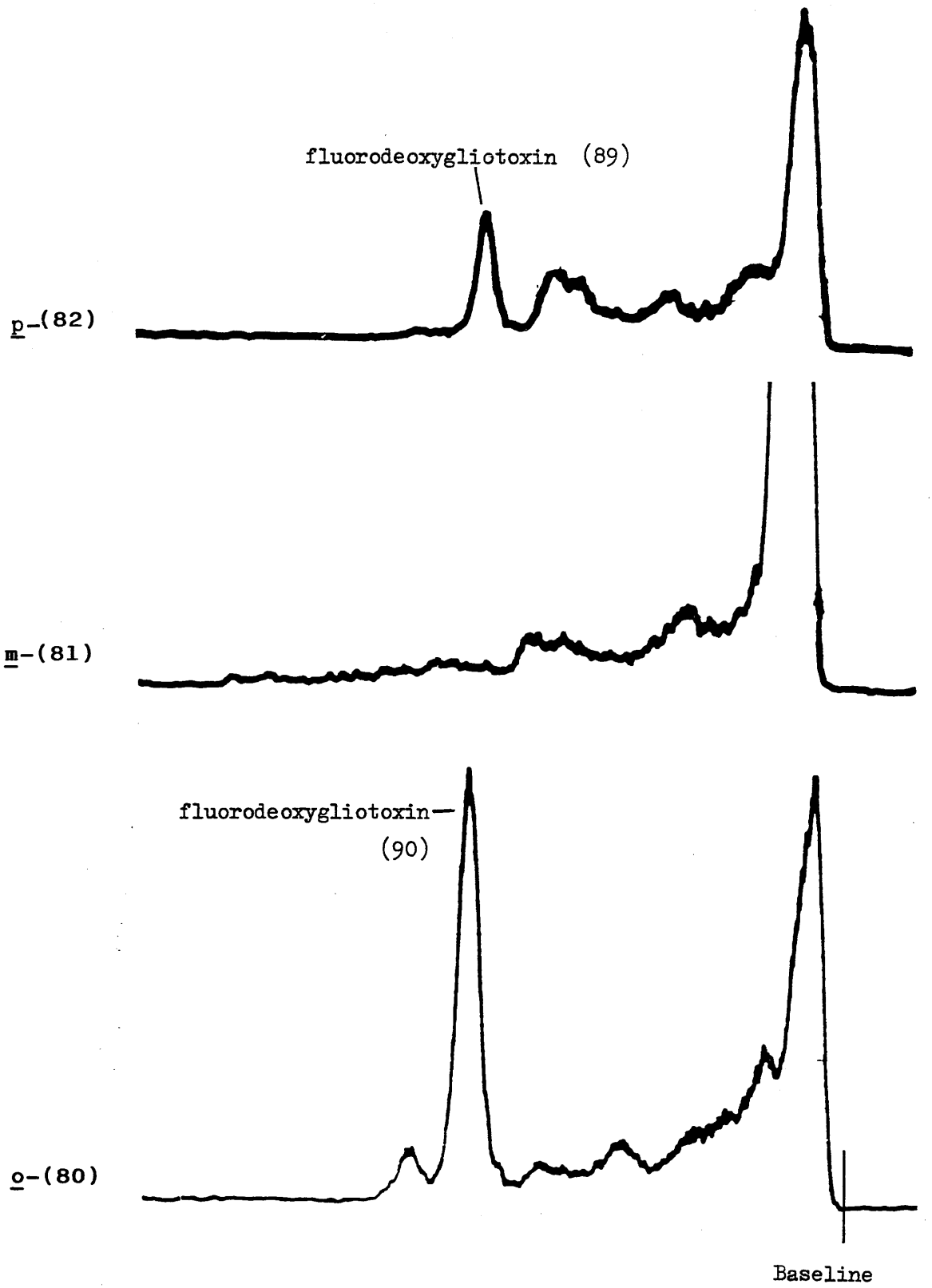
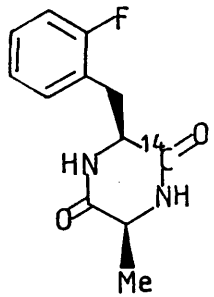
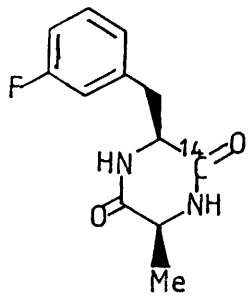
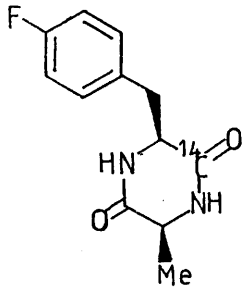


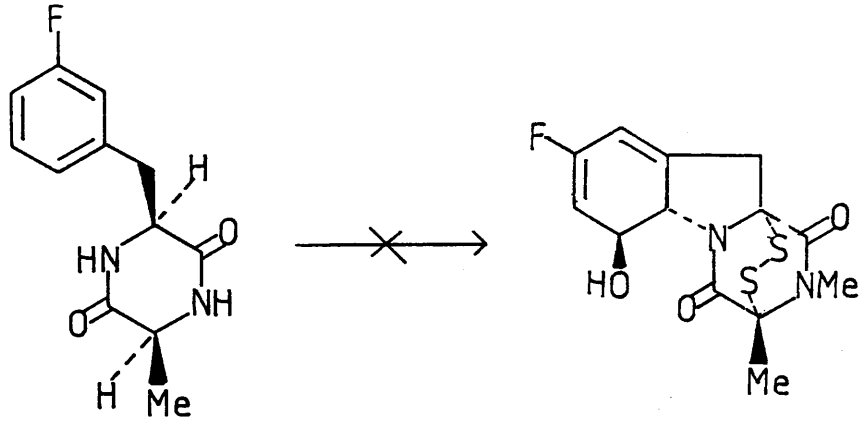
Figure 3

T.l.c. radioscan of dichloromethane extract fed o, m and p-fluorinated cyclopeptides (72), (73) and (74).

Table 2 Experimental data for feeding labelled precursors

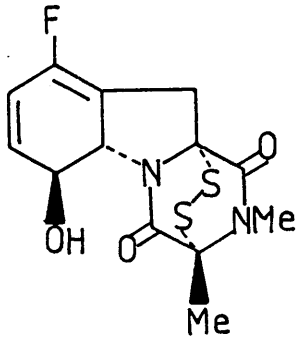
Compound fed	Weight fed (mg)	Activity fed (μCi)	CH_2Cl_2 Yield[2] (mg)	Extract Activity (μCi)	EtoAc Yield (mg)	Extract Activity (μCi)	Broth Activity (μCi)
(80) 	30.5	5.14	311.9	1.98	81.2	1.06	0.83
(81) 	30.7	5.56	275.4	1.06	68.2	1.35	-
(82) 	30.0	5.30	213.5	0.98	89.1	1.63	0.88

^{19}F N.m.r. data (Table 1) showed poor assimilation of m-fluoro-dipeptide (73) into gliotoxin-like metabolites δ -120. This was confirmed from the autoradiogram and radioscan of a t.l.c. plate of the dichloromethane extract. The scan shown (Figure 3) was run at a much higher sensitivity than for ortho or para extracts. A scan obtained at the same sensitivity and the same loading of the plate produced a nearly horizontal line above the baseline. It was clear that very little, if any, of the expected fluorodeoxygliotoxin (91) was present.

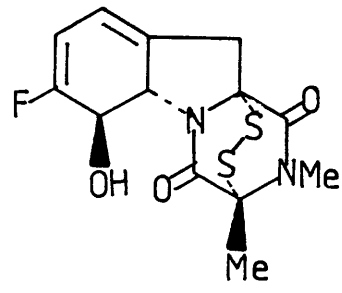


(73) (91)

Both ortho and para feedings gave rise to t.l.c. spots of high activity (Figure 3, 4), which ran slightly faster than gliotoxin itself and thus may possibly be the fluorodeoxygliotoxin analogues (90, 89) previously speculated from the ^{19}F n.m.r. data. A qualitative

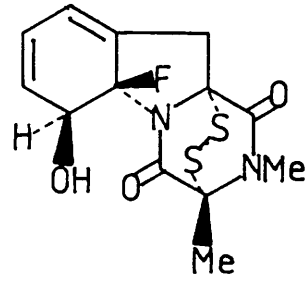


(90)



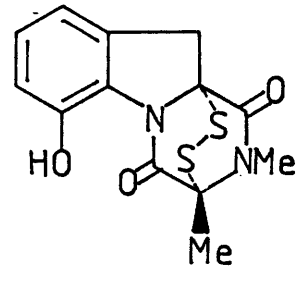
(89)

assessment of the radioscans indicates more efficient conversion of the o-fluoro than the p-fluoro cyclodipeptide into its gliotoxin analogue. The o-fluoro precursor might, in principle give rise to the alternative gliotoxin analogue (92). However, if this were formed, it would readily eliminate HF to give didehydrodeoxygliotoxin (93).

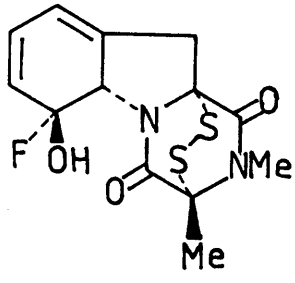


(92)

−HF



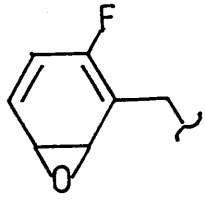
(93)



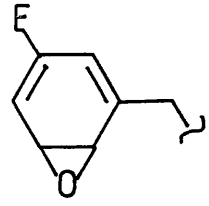
(94)

Similarly, the m-fluoro precursor might form the intermediate (94), which again would be converted into the didehydro compound (93). No spots on the autoradiogram were confidently assigned to this latter derivative.

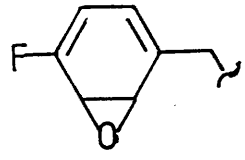
The foregoing experiments provide sufficient evidence to suggest that G.virens is able to epoxidise the p-fluoro precursor (74) and the o-fluoro precursor (72) on the side of the ring opposite to the fluorine, but not the m-fluoro precursor (73). Thus we need to explain the formation of arene oxides of the type (95) and (97) but not (96).



(95)



(96)



(97)

A possible explanation arises from a study[46] by Burka et al. on the mechanism of arene hydroxylation by cytochrome P-450. Iodo-, bromo-, chloro- and fluoro-benzene and benzene itself were incubated in vitro with phenobarbital-induced rat liver microsomes. The kinetics of hydroxylation and product ratios were determined. Possible mechanisms were discussed in the light of the data. The p-phenols were found to be the major products (Table 3), the proportion of

Table 3

Hydroxylation of PhX by rat liver microsomes

X	$V_{max.}$*	Km*	V_{max}/Km	Hydroxylation ratio p/o	σ^+
H	3.3	3.0	1.1	1	0.00
F	11	6.4	1.7	1.4	-0.07
Cl	6.7	1.9	3.5	1.8	+0.11
Br	6.4	0.41	16	6.4	+0.15
I	6.3	0.30	21	large	+0.13

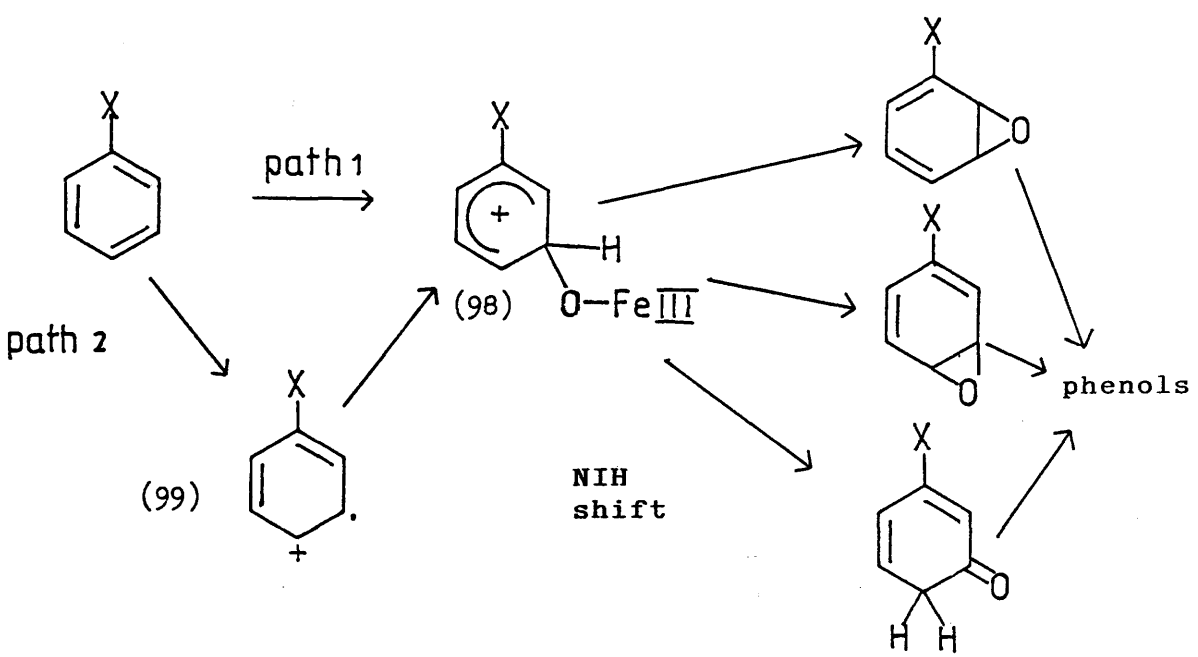
*Units: $V_{max.}$/nmol min^{-1} (mg. protein)$^{-1}$, Km/mM

o-phenol decreased with increasing atomic radius of the halogen. m-Phenols were found in trace amounts. At low substrate concentration, [S], the Michaelis-Menton expression simplifies to $V=V_{max.}/Kmx[S]$. Thus, $V_{max.}/Km$ becomes the rate constant for the catalytic step, giving the observed rate order of hydroxylation : PhI>PhBr>PhCl>PhF>PhH. It was surprising that benzene itself had the smallest rate constant since halogens are deactivitating in electrophilic aromatic substitution.

An 'excellent' free energy correlation was observed for the total hydroxylation (o and p) from a Hammett plot of log $V_{max.}$ versus σ^+. However, although the authors did not point this out, a good straight line plot was possible only if values for benzene itself were omitted. This is clear at once from Table 3 since $V_{max.}$ for benzene ($\sigma^+=0$) is less than those for all the halogenobenzenes. Also, the values for chloro-, bromo- and iodo-benzene are very similar and effectively constitute only 'one point' on the graph.

Nevertheless, this plot (excluding benzene) gave a ρ value of -1.1 which implied a positively charged transition state or intermediate and the correlation with σ^+, rather than σ, implied extended conjugation in the transition state. A poor correlation was obtained from Hammett plots for separate o and p hydroxylation, i.e. the p:o ratio varied markedly with the halogen. The authors deduced from this that hydroxylation involved a rate determining formation of a single intermediate which then led to products, i.e. the product forming step was not rate determining.

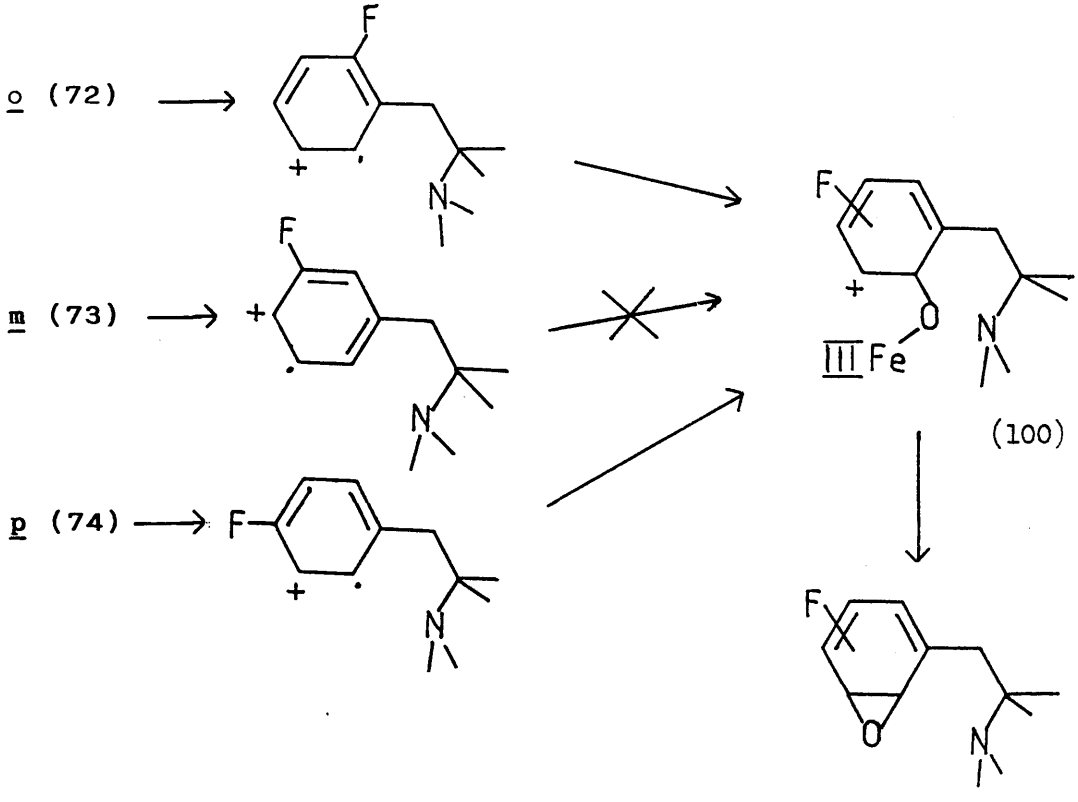
Several mechanisms were considered. A concerted epoxidation step was discounted since little charge separation would occur and a significant ρ value was observed. The second possibility discussed (Scheme 10, path 1) involved electrophilic attack to give the required single intermediate (98) and then the o and p phenols via epoxides. This would require the halogens to be m-

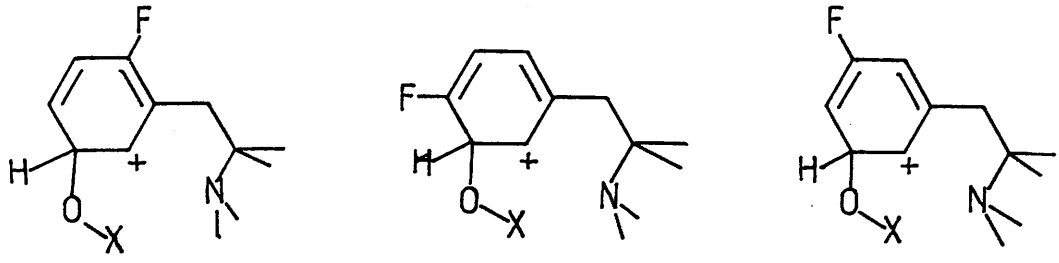


Scheme 10

directing and activating (see Table 3). However, halogens are known to be o, p-directing and deactivating. The third possibility they considered involved an initial, rate determining, one-electron oxidation to a radical cation (99) (Scheme 10, path 2). Huckel calculations showed the highest density of unpaired electrons to be at the m-position. Recombination of this radical with the [Fe IV=O] radical cation would give the positively charged intermediate (98). Conversion of the cation (98) into the epoxides, or a direct NIH shift, would provide a route to the phenols. This final mechanism was chosen as the most likely since it agreed with the Hammett correlation.

To employ the same mechanism to explain the results of the gliotoxin feeding experiments we must postulate formation of the o-intermediate (100) from the corresponding radical cation. According to the ideas of Burka et al., radical cations derived from the o- and p- fluoro dipeptides (72) and (74) would have maximum unpaired electron density





disfavoured

Scheme 11

at the required position o to the side chain but the radical cation from the m precursor (73) would not. This is in agreement with the successful incorporation of o and p precursors and the failure of the m precursor.

Arene oxide formation need not be mediated by cytochrome P-450 or a similar enzyme. A simple electrophilic aromatic substitution mechanism giving the usual Wheland intermediates (Scheme 11) would also agree with the feeding results following the normal o , p direction of fluorine. Cyclisation by attack of nitrogen on the ring would follow directly. Alternatively, the intermediate could collapse to give an arene oxide.

The preparation and feeding of the labelled cyclic dipeptides (84-86) and the isolation and preliminary examination of the metabolite mixtures, took longer than anticipated. It was decided that work on the project would stop, after the initial autoradiogramy and radioscanning was complete. The isolation and characterisation of individual metabolites was then studied by G.V. Rao. His results will for completeness, be summarised following the Conclusions. The present authors research was then directed to a study of thioaldehyde S-oxides (sulphines), which formed the first part of this Thesis.

5.4 Conclusions

At the outset it was hoped that analogues of the natural precursor, cyclo-(L-Phe-L-Ser) (12), lacking the serine hydroxyl group and and with fluorine substituents on the aromatic ring, would be acceptable as substrates to the enzymes of G.virens. The metabolites produced might also shed light on the arene oxide mechanism. cyclo-(L-Ala-L-p-fluoro-Phe) was converted into its gliotoxin analogue (65). cyclo-(L-Ala-L-o-fluoro-Phe) was accepted even more efficiently, producing a range of metabolites, one of which was thought likely to be the gliotoxin analogue (90), an idea confirmed later by Dr. Rao. The m-fluorinated diketopiperazine was not converted into any significant quantities of gliotoxin-like products.

Cell well impermeability cannot be the cause of lack of metabolism; the two very similar o- and p- dipeptides were metabolised, but the m-isomer was not. These three results, taken with an earlier study[46] of hydroxylation of halobenzenes, suggests that arene oxide formation is not a concerted process, but may involve a stepwise mechanism. Two mechanisms consistant with these results have been discussed: the first has a one electron oxidation of the benzene ring to give a radical cation as the initial, rate determining step; the second is a conventional electrophilic aromatic substitution. Both mechanisms could lead to the required arene oxide, although one could form gliotoxin without requiring an arene oxide. However, the mechanisms proposed must remain speculative until cell free systems are available for more detailed study.

5.5 Metabolites isolated by Dr. G.V. Rao (unpublished work)

The unlabelled and labelled extracts from previous and additional feedings led to the isolation of several fluorinated metabolites (Figure 5), which were characterised spectroscopically.

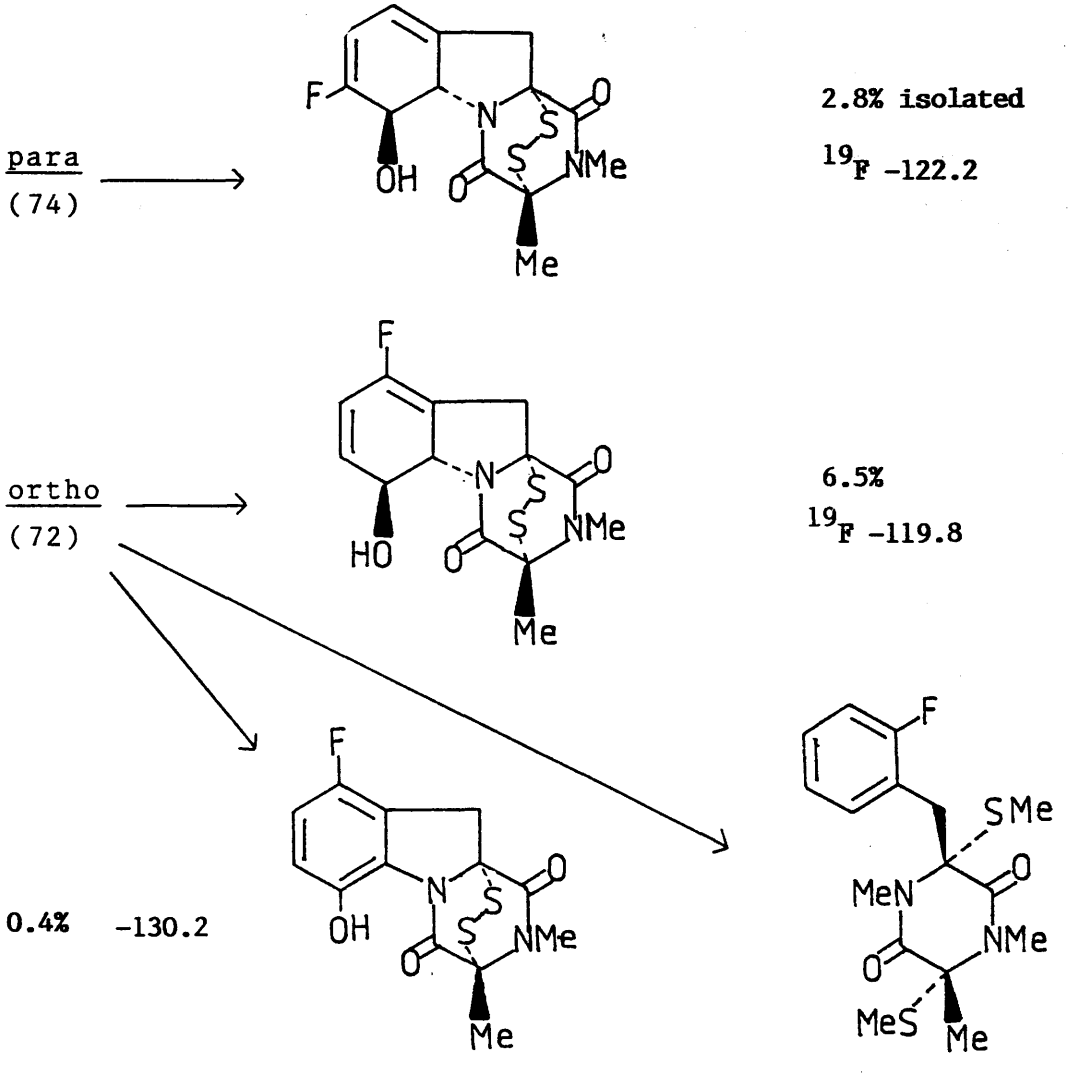


Figure 5

Chapter 6 Experimental Section

6.1 General Procedures

Melting points were recorded on a Reichert hot-stage apparatus and are uncorrected.

Optical rotations were measured at 589 nm using an Optical Activity Ltd. AA-100 polarimeter.

Elemental analysis were carried out by Mrs. W. Harkness and her staff.

Infrared spectra were recorded on a Perkin-Elmer 580 spectrometer by Mrs. F. Lawrie and her staff.

Proton n.m.r. spectra were recorded on a Perkin-Elmer R32 (90 MHz) spectrometer. Trifluoroacetic acid was the solvent used for cyclodipeptides with tetramethylsilane (TMS) as internal reference.

Fluorine n.m.r. spectra were recorded on a Varian XL-100 machine in $CDCl_3$ and $(CD_3)_2SO$. Signals were recorded in p.p.m. from fluorotrichloromethane, using a deuterium locking system. The offsets of $CFCl_3$ from the solvent deuterium signals were, 49661 Hz for $CDCl_3$ and 50019 Hz for $(CD_3)_2SO$. The ^{19}F n.m.r. spectra were recorded by Mr. J. Gall and Mr. R. Sharp.

Radioactive samples were counted on Phillips liquid scintillation counters, modesl PW4510 D649 and later PW4700, in toluene or, for aqueous samples, Ecoscint (National Diagnostics). Radioactive compounds were detected on t.l.c. plates with a Panax thin layer scanner or by autoradiography

on Kodak direct exposure X-ray film.

Analytical t.l.c. was carried out on precoated Kieselgel GF_{254} plates of thickness 0.25 mm (Merck). Cyclodipept ides (having NH groups) were detected by treatment of the dried plates with chlorine gas for 5 min followed by spraying with tetramethylbenzidine solution[48]. Cyclodipeptides with a secondary amide function give blue or yellow spots depending on concentration. Sulphur-containing compounds were detected by spraying with a 2% silver nitrate solution in aqueous acetone; compounds containing S-S bonds give brown spots after ca. 1 min.

Solutions in organic solvents were dried over magnesium sulphate and evaporated on a Buchi rotary film evaporator at water-pump pressure.

6.2 Preparation of Fluorinated Cyclodipeptides

Glycine methyl ester hydrochloride (78)[49].- Glycine (250 mg, 3.30 mmol) was added to anhydrous methanol (25 ml). Acetyl chloride (2 ml) was added dropwise down a condenser to the suspension with stirring. The resulting solution was heated under reflux for 30 min. After cooling, the solution was evaporated and the resulting brown solid was recrystallised from methanol-ether to give glycine methyl ester hydrochloride (78) (84%), m.p. 173 °C (lit.,[49] 175 °C).

N-Benzyloxycarbonyl-L-alanine (77).- The method employed was that described by Greenstein and Winitz[50] for glycine. A solution of L-alanine (8.91 g, 0.1 mol) in 4M sodium hydroxide (25 ml) was cooled to ca. 5°C. 4M Sodium hydroxide (0.12 mol) and benzyloxycarbonyl chloride (18.7 g, 0.12 mol) was added alternately in about 5 equal portions during 25 min with vigorous stirring and cooling in an ice bath. The reaction mixture was kept alkaline throughout. The solution was extracted with ether (20 ml) 10 min after the final addition; the aqueous layer was carefully acidified with 5M hydrochloric acid (Congo Red indicator). The organic layer was separated and further product was extracted from the aqueous layer with ether (50 ml). The organic products were combined and evaporated to give N-benzyloxycarbonyl-L-alanine (77) (87%), m.p. 81-85 °C (lit.[50], 82-84 °C), $[\alpha]_D$-13.3 ° (c 2 in CH_3CO_2H) (lit.[50], -13.9 °).

cyclo-(L-Ala-Gly) (76)[42].- To a suspension of glycine methyl ester hydrochloride (7.7 g, 62 mmol) in dichloromethane (200 ml) was added triethylamine (8.6 ml, 62 mmol), followed by benzyloxycarbonyl-L-alanine (13.9 g, 65 mmol). The solution was stirred at room temperature for 6 h and filtered. The filtrate was washed

with water, dilute hydrochloric acid, saturated sodium bicarbonate, water and brine, dried and evaporated. The resultant oil was dissolved in methanol (150 ml) and acetic acid (0.3 ml); 10% Pd/C catalyst (500 mg) was added and the mixture was hydrogenated at atmospheric pressure overnight. After the catalyst was filtered off through Celite the solution was evaporated; acetic acid was removed as an azeotrope with toluene. The resulting oil was dissolved in methanol (70 ml) and heated under reflux for 24 h. The solution was evaporated and the resulting white solid recrystallised from methanol to give cyclo-(L-Ala-Gly) (76) (7.1 g, 90%), m.p. 228-230 °C (lit.,[42] 228-230 °C), $[\alpha]_D$ -19.0 ° (c 0.42 in Me_2NCHO) (lit.,[42] -21.3 °) (Found: C, 46.8; H, 6.3; N, 22.0. Calc. for $C_5H_8N_2O_2$: C, 46.9; H, 6.3; N, 22.0%); $\nu_{max.}$ (KBr) 1670 cm^{-1}; δ_H (CF_3CO_2H) 1.8 (3H, d, J 8 Hz, Me), 4.43 (2H, br.s, CH_2N), 4.50 (1H, br. q, J 8 Hz, CHMe), 8.40 (1H, br.s, NH) and 8.60 (1H, br.s, NH); m/z 128 (M^+, 26%), 85 and 57.

N,N'-Diacetyl-cyclo-(L-Ala-Gly) (75)[42]. - cyclo-(L-Ala-Gly) (76) (400 mg, 5.12 mmol) in acetic anhydride (35 ml) was heated under reflux for 6 h. The solvent was evaporated and remaining traces of acetic anhydride were removed as an azeotrope with carbon tetrachloride to give diacetyl-cyclo-(L-Ala-Gly) (75) as a yellow oil (650 mg, ca. 98% pure as judged from the ^1H n.m.r. spectrum); $\nu_{max.}$ (CCl_4) 1720 cm^{-1}; δ ($CDCl_3$) 1.54 (3H, d, J 8 Hz, CH_3CH), 2.56 (3H, s, Ac), 2.58 (3H, s, Ac), 4.07 (1H, d, J 18 Hz, CH_2N), 5.11 (1H, d, J 18 Hz, CH_2N) and 5.26 (1H, q, J 8 Hz, $CHCH_3$); m/z 212 (M^+, 20%), 170 (100), 127, 99, 85, 70 and 56.

Modification of the method[42] for the preparation of the cyclodipeptides (72-74). - To a solution of diacetyl-cyclo-(L-Ala-Gly) (76) (212 mg, 1 mmol) and the appropriate, freshly distilled aromatic aldehyde (4 mmol) was added

slowly with stirring potassium ^{t}butoxide (1 mmol) in ^{t}butanol (1 ml). The mixture was stirred at 25 ^{o}C for 40 min and partitioned between chloroform (20 ml) and water (20 ml). The aqueous layer was extracted with chloroform (20 ml) and the combined organic portions were washed with saturated sodium carbonate and water, dried and evaporated. The residual oil containing the monoacetylarylidene derivative (80-82) was dissolved in methanol (20 ml) and acetic acid (2 drops); 10% Pd/C Catalyst (50 mg) was added and the mixture was hydrogenated at atmospheric pressure for 24 h. The catalyst was filtered off through Celite, the filtrate evaporated and the residue, containing the N-acetylcyclodipeptide, was dissolved in N,N-dimethylformamide (2 ml). Hydrazine hydrate (4 mmol) was added and the mixture was left at room temperature for 1 h and then acidified with acetic acid (0.2 ml). The product was filtered off*, washed with ether and crystallised from acetic acid-water or methanol.

*On some occasions the product did not precipitate. The product was then extracted with ethyl acetate. The extract was dried and evaporated and the residue was washed with ether and the cyclodipeptide crystallised from methanol.

cyclo-(L-Ala-L-o-fluoro-Phe) (72). - (111 mg, 47%) m.p. 252-255 ^{o}C (decomp.) (from AcOH-H_2O) (Found: C, 61.1; H, 5.6; F, 8.0; N, 11.9; m/z 236.0964. $C_{12}H_{13}FN_2O_2$ requires C, 61.0; H, 5.55; F, 8.0; N, 11.9%; M, 236.0958); $[\alpha]_D$ +51.1 ^{o} (c 1.07 in AcOH); $\nu_{max.}$ (KBr) 3440 (NH), 1680 (CO), 1670 (CO), 1580 and 1395 cm^{-1}; δ (CF_3CO_2H) 0.92 (3H, d, J 8Hz, CH_3), 3.20-3.65 (2H, m, CH_2Ar), 6.37 (1H, q, J 8 Hz, $CHCH_3$), 6.79 (1H, m, $CHCH_2Ar$), 7.0-7.50 (4H, m, Ar), 8.21 (1H, br.s, NH) and 8.41 (1H, br.s, NH); $\delta_F\{^1H\}$-116.0; m/z 236 (M^+, 47%), 127 (33), 109 (82), 99 (100) and 71 (24).

cyclo-(L-Ala-L-m-fluoro-Phe (73). - (129 mg, 55%), m.p. 254-256 °C (from AcOH-H_2O) (Found: C, 60.9; H, 5.45; F, 8.0; N, 11.7; m/z 236.0960. $C_{12}H_{13}FN_2O_2$ requires C, 61.0; H, 55.5; F, 8.0; N, 11.9%; M, 236.0961); $[\alpha]_D$ + 45.0 ° (c 0.94 in AcOH); $\nu_{max.}$ (KBr) 3440 (NH), 1680 (CO), 1670 (CO), 1605 and 1510 cm^{-1}; δ_H (CF_3CO_2H) 0.94 (3H, d, J 8 Hz, CH_3), 3.31 (2H, dABq, J 14 and 4 Hz, Δδ 0.12, CH_2Ar), 4.75 (1H, m, $CHCH_2Ar$), 6.33 (1H, q, J 8 Hz, $CHCH_3$), 6.85-7.55 (4H, m, Ar), 8.17 (1H, br. s, NH) and 8.32 (1H, br. s, NH); $\delta_F\{^1H\}$ ($CDCl_3$) -112.4; m/z 236 (M^+, 30%), 127 (46), 109 (45), 99 (100) and 71 (28).

cyclo-(L-Ala-L-p-fluoro-Phe) (74). - (153 mg, 65%), m.p. 249 °C (sublimed), 265-267 °C (decomp.) (from acetic acid-water) (Found: C, 60.9; H, 5.5; F, 7.8; N, 11.7; m/z 236.0959. $C_{12}H_{13}FN_2O_2$ requires C, 61.0; H, 5.55; F, 8.0; N, 11.9%; M, 236.0961); $[\alpha]_D$ + 41.0° (c 0.90 in AcOH); $\nu_{max.}$ (KBr) 1665 (CO), 1515, 1460 and 1335 cm^{-1}; δ_H (CF_3CO_2H) 0.94 (3H, d, J 8 Hz, CH_3CH), 3.23 (1H, dd, J 14 and 4 Hz, CH_2Ar), 3.39 (1H, dd, J 14 and 4 Hz, CH_2Ar), 4.34 (1H, q, J 8 Hz, $CHCH_3$), 6.74 (1H, m, $CHCH_2Ar$) and 6.95-7.35 (4H, m, Ar), 8.18 (1H, br. s, NH) and 8.32 (1H, br. s, NH); $\delta_F\{^1H\}$ [$(CD_3)_2SO$] -115.91; m/z 236 (M^+, 15%), 109 (100) and 99.

cyclo-(L-Ala-L-Phe) (83). - (54%), m.p. 271-273 °C (decomp.) (from AcOH) (lit., [44,45] m.p. 290-291 °C, 271 °C) (Found: C, 60.1; H, 6.45; N, 12.7. Calc. for $C_{12}H_{14}N_2O_2$: C, 66.0; H, 6.5; N, 12.8%); $[\alpha]_D$ +68.0 ° (c 1.1 in AcOH) [lit., [44,45] +67.1 ° (c 1.4), +66.3 °]; $\nu_{max.}$ (KBr) 1670 cm^{-1}; δ_H(CF_3CO_2H) 0.89 (3H, d, J 7 Hz, Me), 3.30 (2H, dABq, J 15 and 5Hz, Δδ 0.17, CH_2Ar), 4.26 (1H, q, J 7 Hz, CHMe), 4.75 (1H, m, $CHCH_2Ar$), 7.1-7.6 (5H, m, Ar), 8.17 (1H, br. s, NH), and 8.33 (1H, br. s, NH); m/z 218 (M^+, 21%), 127 (22), 99 (43), 91 (100), 88 (13) and 65 (20).

6.2.1 Preparation of ^{14}C-Labelled Cyclodipeptides

[1-^{14}C]Glycine methyl ester hydrochloride. - [1-^{14}C]-Glycine (250 μCi, 56mCi mmol^{-1}) was diluted with unlabelled glycine (309 mg, 4.12 mmol) giving a specific activity of 60.7 μCi mmol^{-1}. Esterification proceeded, as described for unlabelled material, in methanol (25 ml) with acetyl chloride (2 ml) to give the ester hydrochloride (464 mg, 89%) (52.8 μCi mmol^{-1}).

cyclo-(L-Ala-[1-^{14}C]Gly). - The cyclodipeptide was prepared in the same manner as the unlabelled material. [1-^{14}C]Glycine methyl ester hydrochloride (427 mg, 3.40 mmol) gave the cyclodipeptide (289 mg, 67%)(57.4 μCi mmol^{-1}).

cyclo-(L-Ala-L-[1-^{14}C]-o-fluoro-Phe) (84). - Labelled cyclo-(L-Ala-Gly) (97 mg, 0.76 mmol) was diluted with unlabelled material (48 mg). This cyclo-(L-Ala-[1-^{14}C]Gly) (144 mg, 1.12 mmol) (39.5 μCi mmol^{-1}) gave, by the usual procedure the o-fluorinated dipeptide (94) (60 mg, 23%) (39.5 μCi mmol^{-1}).

cyclo-(L-Ala-[1-^{14}C]-m-fluoro-Phe (85). - Labelled cyclo-(L-Ala-Gly) (104 mg) was diluted with unlabelled material (45 mg). This cyclo-(L-Ala-[1-^{14}C]Gly) (149 mg, 1.16 mmol)(40.9 μCi mmol^{-1}) gave the m-fluorinated dipeptide (95) (98 mg, 36%) (41.4 μCi mmol^{-1}).

cyclo- (L-Ala-[1-^{14}C]-p-fluoro-Phe) (86).- The cyclo-dipeptide was prepared as for the unlabelled material. cyclo-(L-Ala-[1-^{14}C]Gly) (104.5 mg) was diluted with unlabelled material (44.9 mg, 0.35 mmol) to give dipeptide (149 mg, 1.2 mmol) (40.8 μCi mmol^{-1}). The usual procedure gave the p-fluorinated dipeptide (86) (77 mg, 28%) (41.5 μCi mmol^{-1}).

6.3 Biosynthetic Experiments

Fermentation conditions. - Cultures of the fungus used throughout this study, obtained from the Commonwealth Mycological Institute, now the C.A.B. International Mycological Institute (Kew), were originally described as Gliocladium deliquescens (CMI 10525, NRRL 1828), formerly Trichoderma viride. Dr. M.A.J. Williams (Kew) has now confirmed that their isolate (IMI 101525) is properly described as Gliocladium virens J. Miller, Giddens and Foster.

Cultures of G.virens were started on potato dextrose agar slants and incubated for 10 days. The slants were used to inoculate 250 ml wide-necked conical flasks containing 100 ml each of J.D.M.[41] nutrient medium.

Sucrose	1.5 g
Ammonium sulphate	1.67 g
Dipotassium orthophosphate	1.1 g
Magnesium sulphate	0.83 g
Ferric chloride	8.3 mg
Peptone (Bacto)	16.7 mg
Deionised water	1 l

(pH adjusted to 3-3.5 with concentrated H_2SO_4).

Typically 3 l of medium (30 x 250 ml conical flasks) were inoculated and incubated for 7 d at 27 ^{o}C; feeding was carried out after 48 h. The culture flasks were agitated on an orbital shaker at 160 r.p.m. All operations concerning the growth and propagation of the fungus were carried out by Mrs. M. Tait and her staff.

Extraction procedure. - The cultures were harvested by filtering off the myceluim; the broth was extracted with

dichloromethane (4 x 10% broth volume). The organic extract usually formed an emulsion which was cleared by filtering through Celite. The extract was washed with water (2 x 50 ml) dried and evaporated to give an orange gummy solid. Typically, the fluorinated dipeptide (30 mg, 0.13 mmol) in dimethyl sulphoxide (6 ml) was fed to 30 flasks of the medium giving dipeptide and DMSO concentrations of 0.4 mM and 0.2% (v/v), respectively.

The broth (3 l) yielded between 200 and 600 mg of organic extract. The variation was due to the general state of the fungus and not due to poisoning by the precursors. This was evident from the concurrent variation in unfed control flasks.

After extraction, the solid was triturated with $CDCl_3$ and the partially crystalline mixture was filtered. $^{19}F\{^1H\}$ N.m.r. spectra were recorded for the filtrates. A known amount of p-fluorobenzoic acid was added to each solution to obtain approximate incorporation values by integration of the ^{19}F signals. A portion of the $CDCl_3$- insoluble residue was dissolved in $(CD_3)_2SO$) and a ^{19}F n.m.r. spectrum was obtained similarly. Several feedings of the unlabelled dipeptides were carried out and the chemical shifts of the ^{19}F n.m.r. signals, with appropriate incorporation values are given in Table 1.

Feeding Experiment with DL-p-Fluorophenylalanine (66). - The precursor (28 mg, 0.15 mmol) in DMSO-H_2O (8 ml) was fed to 2.6 l of G.virens culture broth yielding a mixture containing the fluorogliotoxin (65); $\delta_F\{^1H\}(CDCl_3)$ -122.47, incorporation 5.8%.

Feeding Experiments with Radio-Labelled Fluorinated Dipeptides. - To 1 l batches of the same culture of G.virens (31) were fed, separately, ortho, meta and para substituted cyclo-(L-Ala-L-[1-^{14}C]fluoro-Phe) (ca. 30 mg, ca. 5 uCi). After dichloromethane extraction, the remaining broth was extracted continuously using ethyl acetate. Aliquots of the two organic extracts and the remaining broth were counted for ^{14}C; the results are summarised in Table 2.

Analytical t.l.c. in toluene-acetone (2:1) of the organic extracts was carried out; ethyl acetate extraction produced negligible quantities of gliotoxin-like metabolites as judged by u.v. spectroscopy (gliotoxin shown $\lambda_{max.}$ 272 nm) and autoradiography of t.l.c. plates. The radioscan and autoradiogram of the dichloromethane extract are shown in Figures 3 and 4.

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