SOME SYNTHETIC APPROACHES TO BIOTIN

A thesis presented by

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ABSTRACT

The existing synthetic approaches to biotin and its analogues are reviewed. The inhibition of growth produced by antibiotins in micro-organisms is discussed on the basis of the currently accepted mechanism of biotin action.

A new route to biotin using 3-sulpholene as a starting material is investigated with special emphasis on the introduction of nitrogen functions at the 3- and 4-positions of the sulpholane ring.

The reaction of iodine isocyanate with olefinic double bonds is investigated as a possible starting point for the formation of an imidazolidone ring.

The reactions of amines with 3,4-dibromo-sulpholane are examined and the stereochemical course of the reaction discussed. The dibromide reacted with methylamine to give 3,4-bis(methyl-amino) sulpholane. Reaction with phosgene yielded a cyclic urea which was shown to have a trans-fused ring junction by X-ray analysis. Use of ammonia instead of methylamine gave a similar product.

The intramolecular Michael-type addition reactions of suitably substituted 2-sulpholenes are investigated. 3-Carbamido-2,3-dihydrothiophen-1,1-dioxides are shown to undergo a general base induced reaction to produce 4-amino2,3-dihydrothiophen-1,1-dioxides, via a cyclic carbamate followed by elimination and double bond migration. This reaction is utilised in the synthesis of symmetrically and unsymmetrically cis-fused cyclic ureas involving the cyclisation of 3-ureido-2,3-dihydrothiophen-1,1-dioxides with base.

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Acknowledgements.

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Lastly, my thanks to Barbara for her companionship over the past three years.

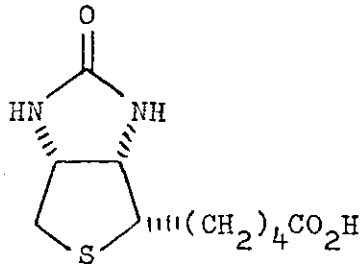
To my parents.

... with apologies to Charles M. Schulz.

REVIEW

Introduction

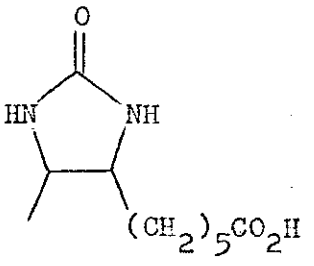
d-Biotin (1) has long been known as a necessary growth factor for yeast and it was only later that it was found to be identical to Vitamin H, the curative factor for egg white injury[1]. The structure was elucidated in 1942 by a series of degradative studies by du Vigneaud, Hofmann and Melville[2,3] and shown to be (+)-cis-hexahydrothieno[3,4-d]-imidazol-2-one-4-valeric acid. The relative configuration of each of the three asymmetric centres was confirmed by X-ray crystallography[4,5] and recently the absolute stereochemistry has been established as in (1).[6]



(1)

The structure determination by chemical methods.

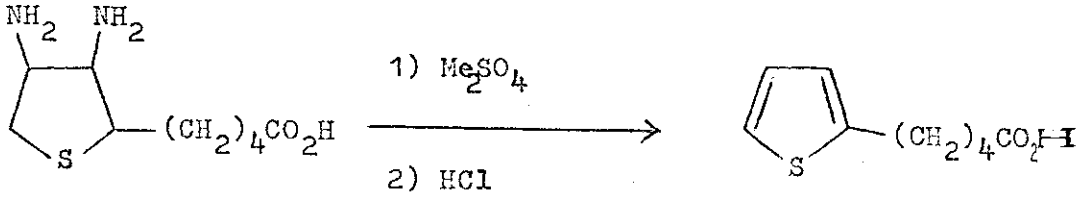
The work of du Vigneaud *et al*[2,3] showed that a 5-membered cyclic urea function was probably present in biotin since CO was lost, upon treatment with barium hydroxide at 140°, to give a diamino acid, from which biotin could be resynthesised with phosgene. The presence of a thio-ether linkage was shown by the formation of biotin sulphone by oxidation with hydrogen peroxide. Biotin was also desulphurised with Raney nickel to desthiobiotin (2).



(2)

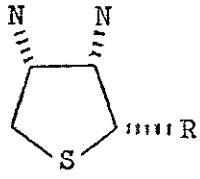
Oxidative degradation of biotin yielded adipic acid which led them to believe the molecule contained a valeric acid side chain.

This, and other data, led du Vigneaud to propose structure (1) for biotin. Final proof was obtained by the degradation of the diamino acid to δ-(2-thienyl)valeric acid which was compared with authentic material.

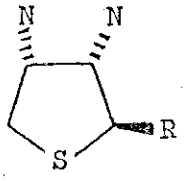


The isomers of biotin.

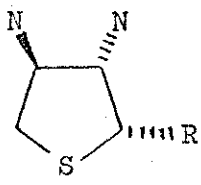
Biotin has three asymmetric centres thus giving rise to eight stereoisomeric forms or four racemic modifications - (±)-biotin, (±)-epibiotin, (±)-allobiotin and (±)-epiallobiotin. Biotin and epibiotin are epimeric at position-2 of the thiolan ring; allobiotin and epiallobiotin are analogous, but with a trans fusion of the two rings. Their inter-relationship is shown below.



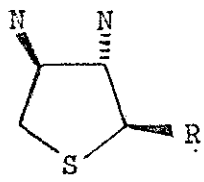
biotin



epi-



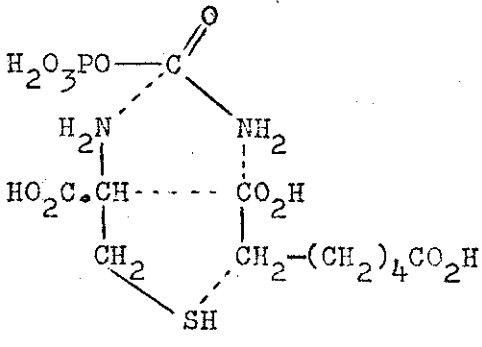
allo-



epiallo-

The biosynthesis of biotin.

The biosynthesis of biotin has been described in great detail by Lezius et al[7] based upon previous labelling results. Basically it is derived from pimelic acid, cysteine and carbamyl phosphate.



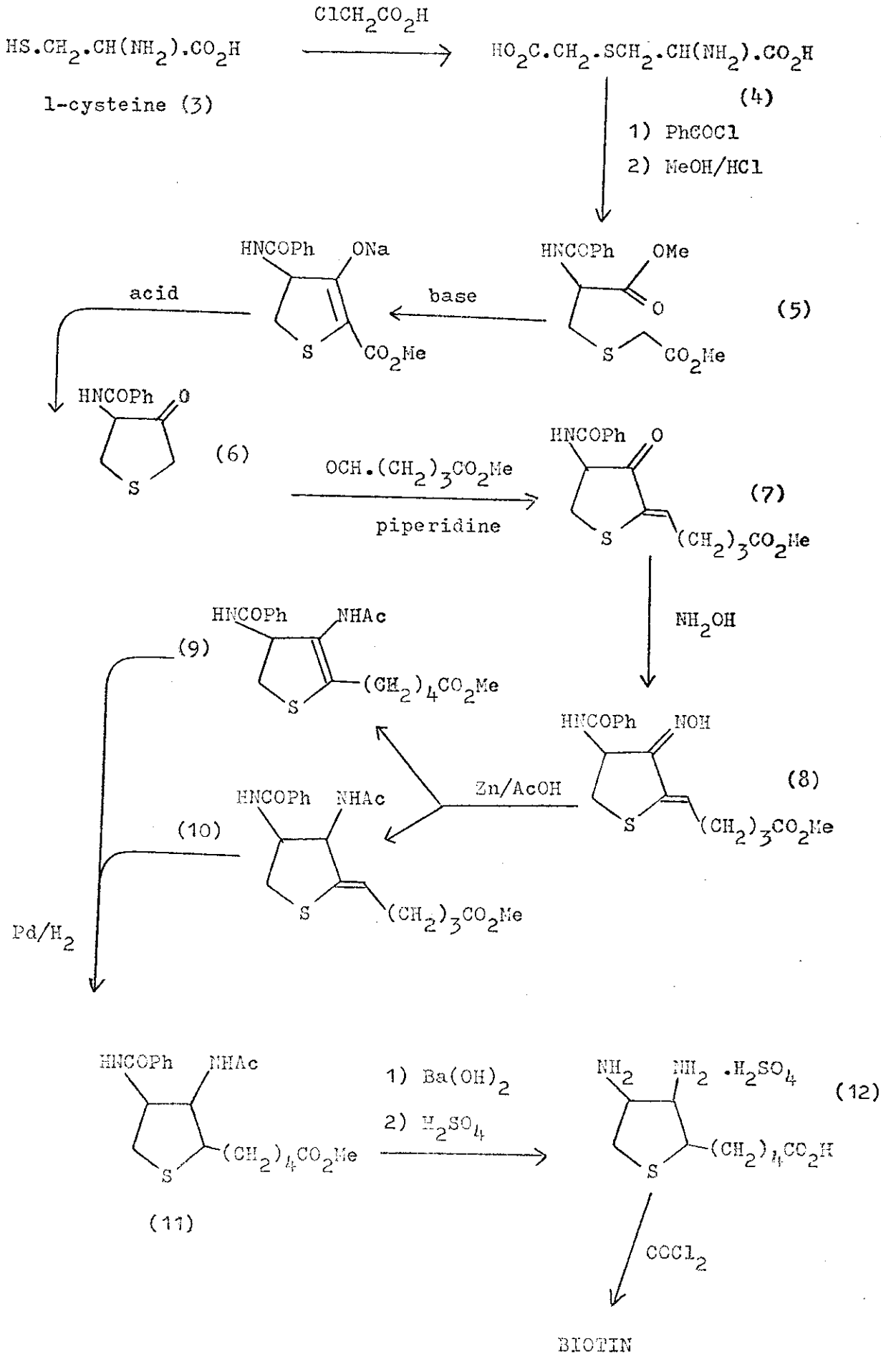
Synthesis of biotin.

The first reported synthesis of biotin.

The publication of the structure of biotin necessitated the chemical synthesis and this was first carried out successfully by Harris et al.[8-14] The key steps (Scheme 1) were the formation of a 4-amino-3-oxo-tetrahydrothiophen (6) by a Dieckmann reaction, condensation with an aldehyde to introduce the side chain in the 2-position (7), transformation to a diamino-tetrahydrothiophen (12) and ring closure with phosgene. The synthesis was not stereospecific and yielded 6 of the 8 possible isomers.

l-Cysteine (3) was treated with chloroacetic acid to give the S-substituted amino acid (4) which was benzoylated and esterified to (5). Dieckmann cyclisation of this product yielded 4-benzamido-3-oxo-tetrahydrothiophen (6) after acidification and

Scheme 1.


HS.CH2.CH(NH2).CO2H
l-cysteine (3)
ClCH2CO2H
HO2C.CH2.SCH2.CH(NH2).CO2H
(4)
1) PhCOCl
2) MeOH/HCl
HNCOPh OMe
O
S CO2Me
(5)
base
acid
HNCOPh ONa
S CO2Me
HNCOPh O
S
(6)
OCH.(CH2)3CO2Me
piperidine
HNCOPh O
S (CH2)3CO2Me
(7)
NH2OH
HNCOPh NOH
S (CH2)3CO2Me
(8)
Zn/AcOH
HNCOPh NHAc
S (CH2)4CO2Me
(9)
HNCOPh NHAc
S (CH2)3CO2Me
(10)
Pd/H2
HNCOPh NHAc
S (CH2)4CO2Me
(11)
1) Ba(OH)2
2) H2SO4
NH2 NH2 .H2SO4
S (CH2)4CO2H
(12)
COCl2
BIOTIN

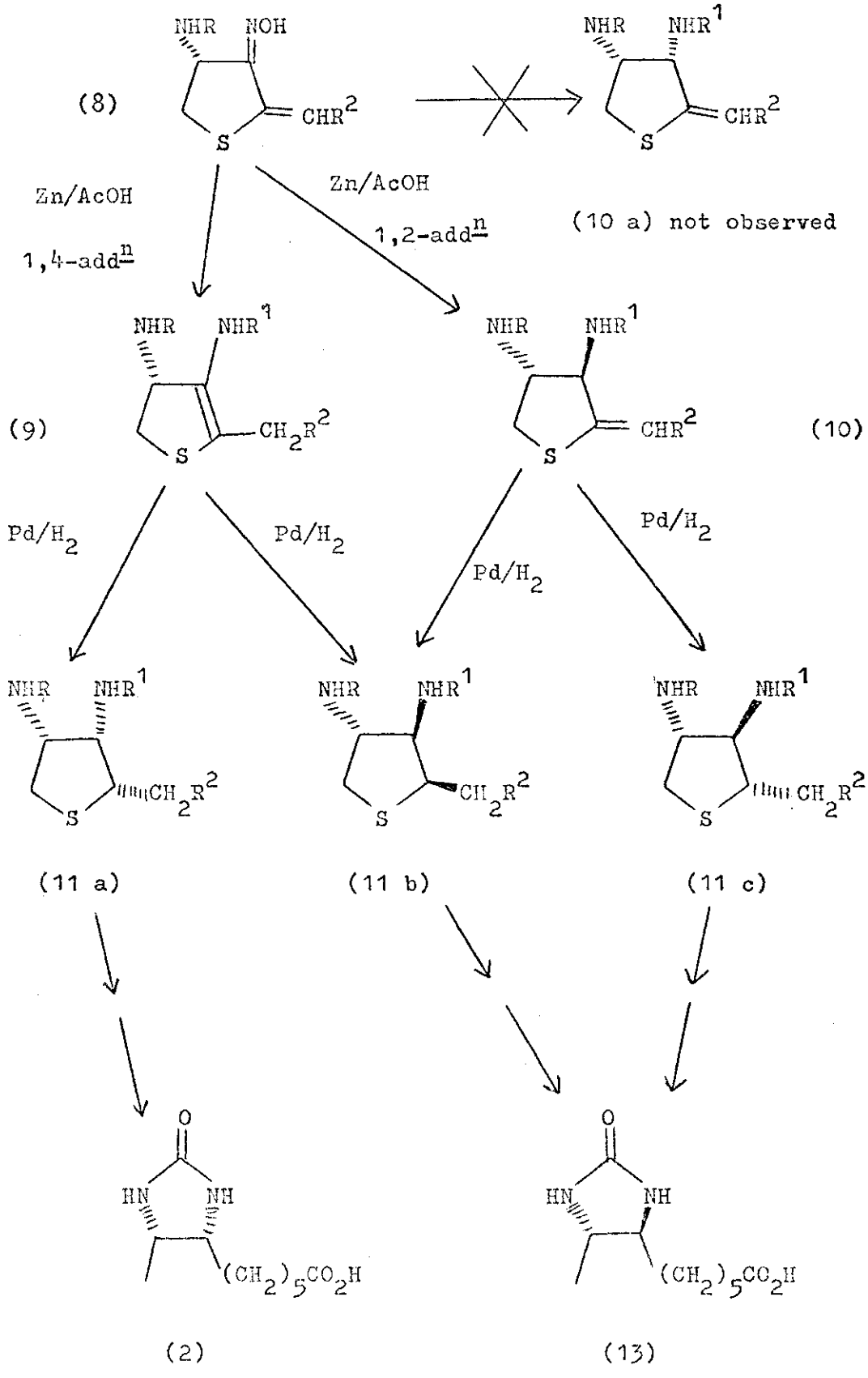
decarboxylation. This oxothiolan (6) was condensed with methyl γ-formylbutyrate (prepared in 3 simple steps from glutaric anhydride) to give the α,β-unsaturated ketone (7) which was converted to the oxime (8). Reduction of the oxime with zinc in acetic acid led to the formation of 2 isomeric products (9) and (10) which could be hydrogenated to the saturated diamine (11). Hydrolysis of the substituted amino groups with barium hydroxide followed by saponification led to the diamino acid (12), which was cyclised with phosgene to give dl-biotin.

The optical isomers were separated through their l-mandelic acid ester, though it was later found the separation was better using ℓ (+)-arginine. The d-biotin obtained by synthesis proved to have exactly the same growth promoting activity as natural material.

It was mentioned earlier that the synthesis was not stereospecific and consequently dl-allobiotin and dl-epiallobiotin were isolated as well as dl-biotin. The problem arises in the 2 reduction steps leading to the diamine (11) from the α,β-unsaturated oxime (8) (scheme 2.). The 2 electron reduction with zinc in acetic acid gives rise to 2 isomeric products; (9) being derived from 1,4-addition and (10) being derived from 1,2-addition. Although catalytic hydrogenation is known to proceed in a cis-fashion, the unsaturated amines (9) and (10) are not sufficiently hindered so as to direct the addition of hydrogen to the least hindered side of the molecule. This therefore leads to 3 saturated diamines; (11a) and (11b) being derived from (9), and (11b) and (11c) being derived from (10). It is these 3 diamines (11a), (11b) and (11c) which lead respectively to dl-biotin, dl-allobiotin and dl-epiallobiotin.

Desulphurisation of the diamines (11) with Raney nickel, and subsequent treatment with phosgene led to desthiobiotins. The dl-diamine (11a) gave dl-desthiobiotin (2). The dl-allodiamine (11b) and the dl-epiallodiamine (11c) gave the same allodesthiobiotin (13) showing

Scheme 2. (Only one enantiomorph is shown for clarity.)



that the dl-allo- and the dl-epiallodiamines were epimeric about carbon - 2.

The fact that epibiotin was not isolated in the synthesis reflects the tendency of the 2-electron reducing agent to give the least hindered trans product (10).

Although the route was not stereospecific, it was through this work that it was first realised that isomers of biotin did exist and it is to the authors' credit that they were able to deduce the correct stereorelationship of the isomers they obtained.

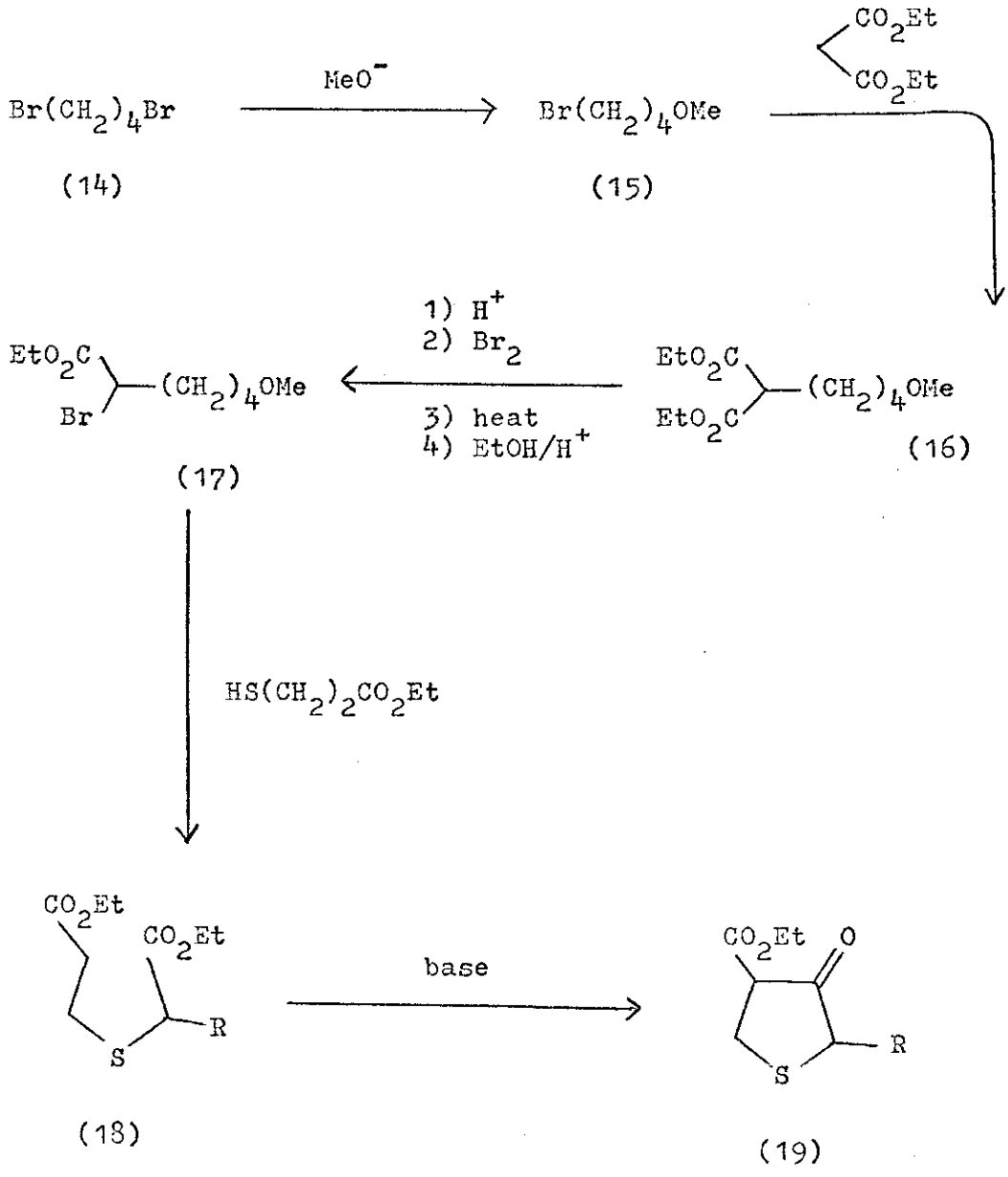
Biotin synthesis by Grussner.

The second synthesis, reported in 1946, was carried out by Grussner, Bourquin and Schnider [15,16] and again was based on a 3-oxothiolan (19) as the key intermediate. This was converted to a diester and thence by a Curtius reaction to a diamine which was cyclised with phosgene.

Their synthesis was in fact based upon the 3-oxothiolan (19) prepared in 1944 by Schmid,[17] whose route to this compound is shown in scheme 3. The synthesis started from 1,4-dibromobutane (14) which was treated with sodium methoxide to give the methoxybromobutane (15). Reaction with sodium diethylmalonate gave the methoxy-diester (16) which was saponified, brominated, decarboxylated and re-esterified to the α-bromo-ester (17). This was reacted with 3-mercaptopropionate to give (18) which was cyclised by a Dieckmann reaction to the 4-carbethoxy-3-oxothiolan (19) having a suitable side chain at the 2-position. Schmid made various attempts at converting this to a pre-biotin diamine but failed.

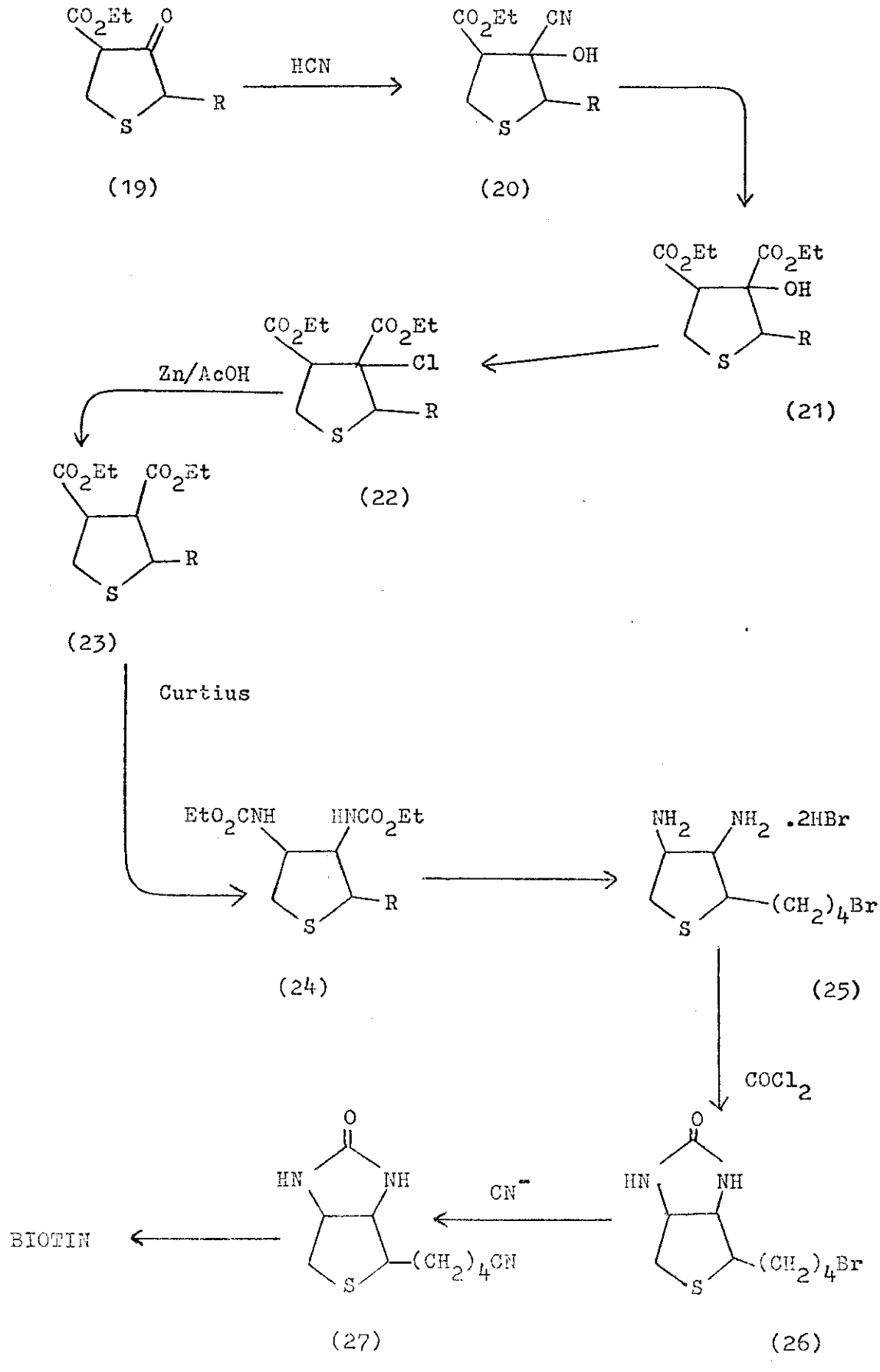
Grussner et al were more successful (scheme 4).[15,16] They treated Schmid's oxothiolan (19) with HCN to form the cyanohydrin (20) which was hydrolysed to the hydroxy-diester (21) and chlorinated (22).

Scheme 3.



R = $-(CH_2)_4OMe$

Scheme 4. R = $-(CH_2)_4OMe$



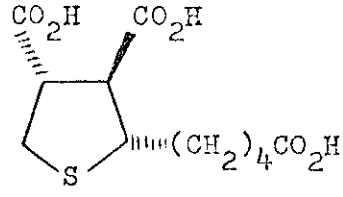
The halogen was reduced off with zinc in acetic acid and the diester (23) subjected to a Curtius reaction which yielded the urethane (24) from which the diamine (25) was formed upon hydrolysis. The diamine was ring closed with phosgene to the cyclic urea (26). The side chain was then modified through the cyanide (27) to biotin.

The biotin so obtained was obviously a mixture of isomers since desulphurisation with Raney nickel gave a mixture of dl-desthiobiotin and dl-desthioallobiotin. They also obtained other side products which were at first claimed to be isomers of biotin but later this was withdrawn because desulphurisation did not yield desthiobiotins.[16]

This synthesis suffers from very low yields and is complicated by the formation of unknown side products from the Curtius reaction and by lack of stereospecificity.

The synthesis of dl-biotin, -epiallobiotin and -epibiotin by Baker (1947).

In a long series of papers, Baker et al.[18-24] describe a stereoselective route to dl-biotin, dl-epibiotin (the first preparation of this isomer) and dl-epiallobiotin starting from the same intermediate, the tricarboxylic acid (27).[21]



(27)

The stereochemistry (only one enantiomorph shown) is implied from the preparation, which gives the staggered, least hindered product, and from the reactions which it undergoes. They found that the stereorelationship between the ring carboxyls, or any carbonyl substituent, could be varied under basic conditions, and with suitable substitutions

and epimerisations they could obtain all four isomers of biotin. As represented, the tricarboxylic acid (27) is in the epiallo-configuration. Epimerisation at C-3 gives the normal biotin configuration; change at C-4 gives the epi-configuration, and epimerisation at both C-3 and C-4 gives the allo-configuration.

Using this type of reasoning, Baker's group stereospecifically isolated normal,epiallo- and epi-biotin without resorting to fractional crystallisations of the products as Harris had to. (Baker did not attempt to synthesise the fourth, allo-isomer).

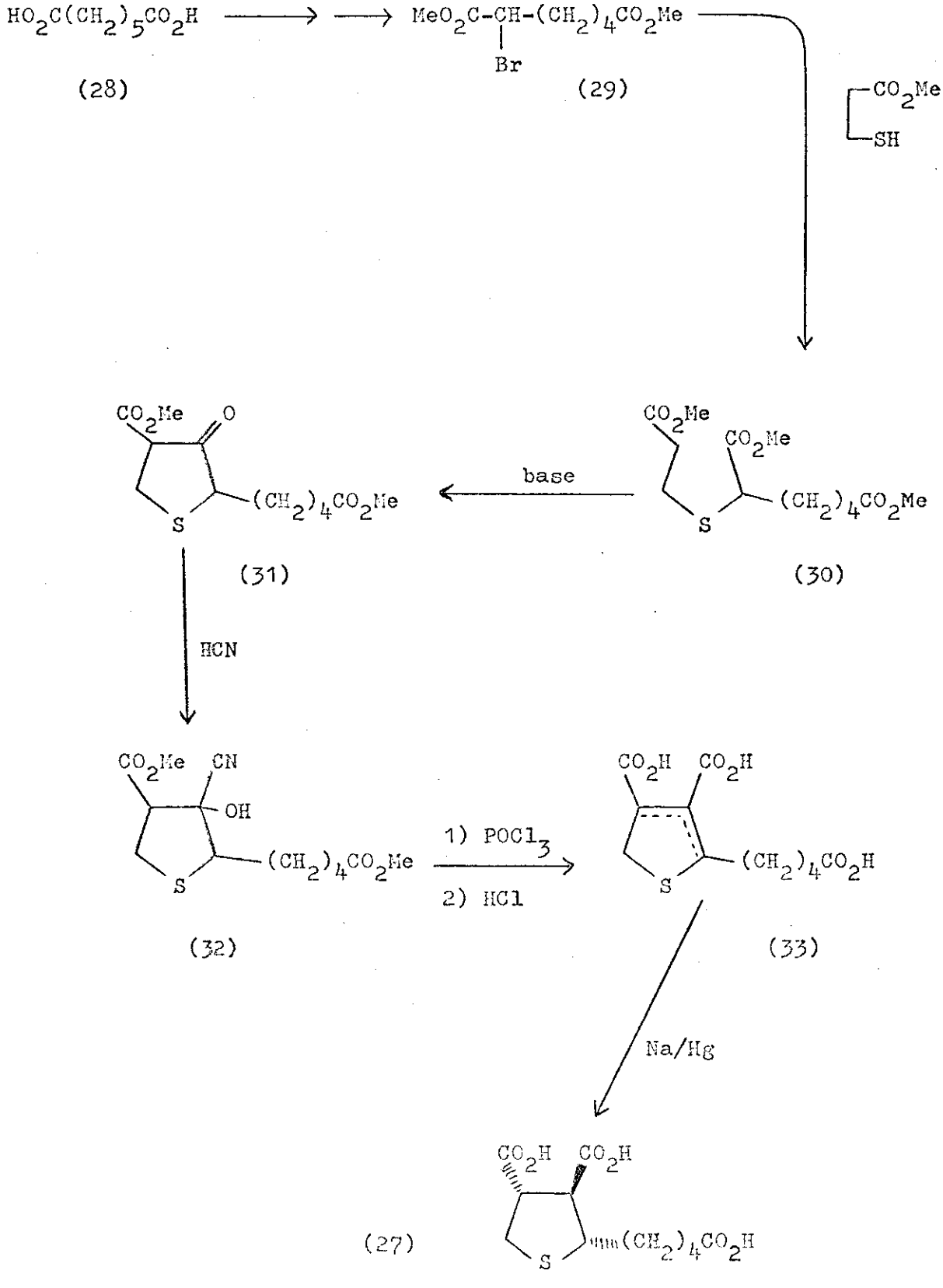
The tricarboxylic acid (27) was prepared [21] from a 3-oxothiolan (31) prepared earlier, in 1944, by Karner, Keller and Usteri,[25] (Scheme 5).

Pimelic acid (28) was methylated and monobrominated in the α-position (29). Treatment with 3-mercaptopropionate gave the triester (30) which was cyclised by a Dieckmann reaction to the 3-oxothiolan (31). Reaction with HCN gave the cyanohydrin (32) which was dehydrated and saponified to the unsaturated triacid (33). Reduction with sodium amalgam gave the required triacid (27) in the least hindered configuration.

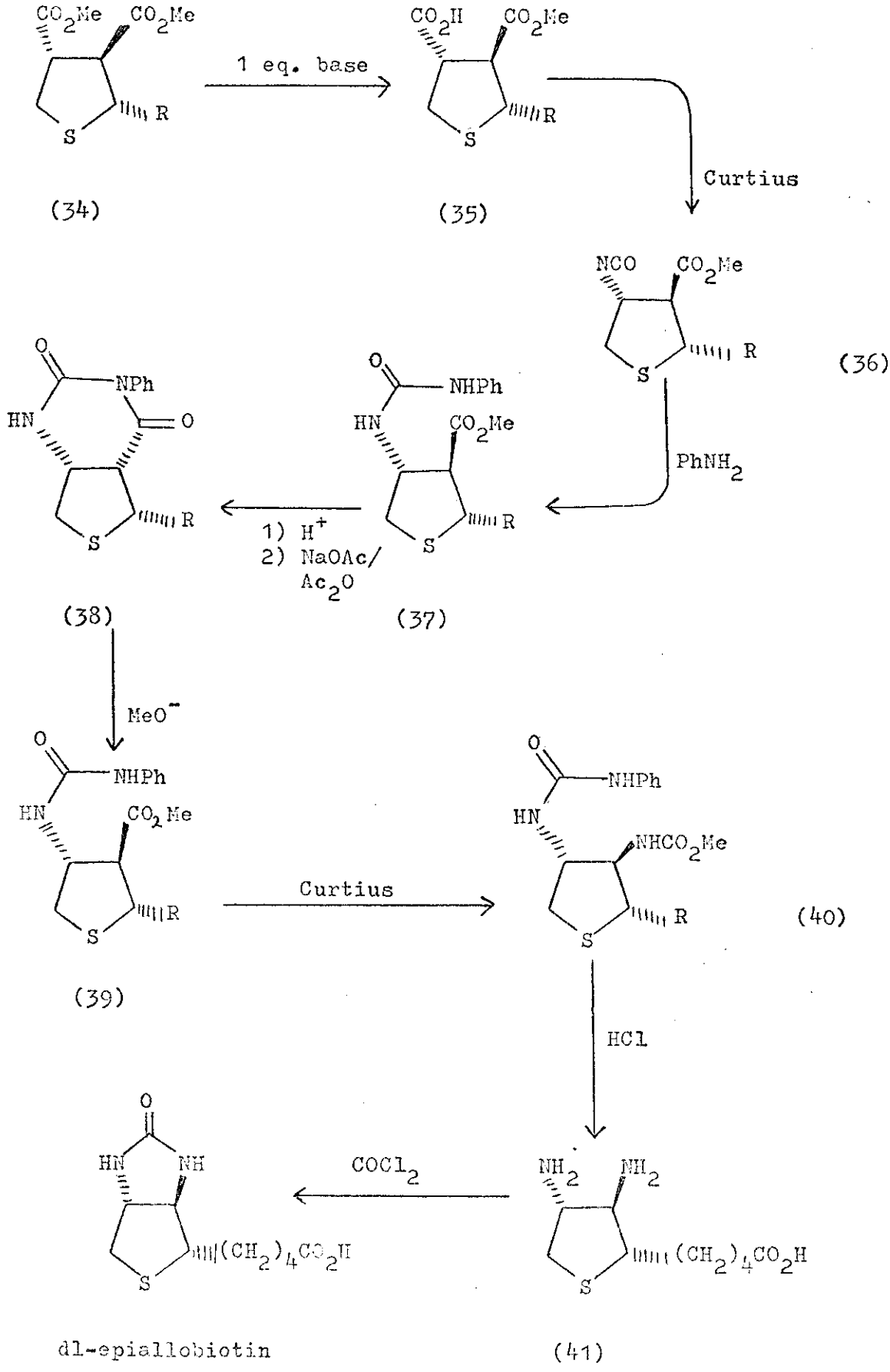
The conversion of the triacid (27) into the biotin isomers was complicated by the fact that the side chain contained a carboxyl group, and so each acid group had to be specifically blocked, or functionalised, at various stages in the synthesis.

In the route to epiallobiotin [23] (scheme 6), the triacid (27) was methylated (34) and treated with one equivalent of base to give the diester with the free acid group in the 4-position (35), a structure for which they had ample justification. [23] The free carboxyl group was subjected to a Curtius rearrangement to give the isocyanate (36) which was treated with aniline to yield the phenyl urea (37). The two diester functions were saponified followed by ring closure with sodium acetate in acetic anhydride to give the uracil (38) which was

Scheme 5.


HO2C(CH2)5CO2H
(28)
MeO2C-CH-(CH2)4CO2Me
Br
(29)
CO2Me
SH
CO2Me
O
(CH2)4CO2Me
S
(31)
base
CO2Me
CO2Me
(CH2)4CO2Me
S
(30)
HCN
CO2Me
CN
OH
(CH2)4CO2Me
S
(32)
1) POCl3
2) HCl
CO2H
CO2H
(CH2)4CO2H
S
(33)
Na/Hg
CO2H
CO2H
(CH2)4CO2H
S
(27)

Scheme 6. R = $-(CH_2)_4CO_2Me$

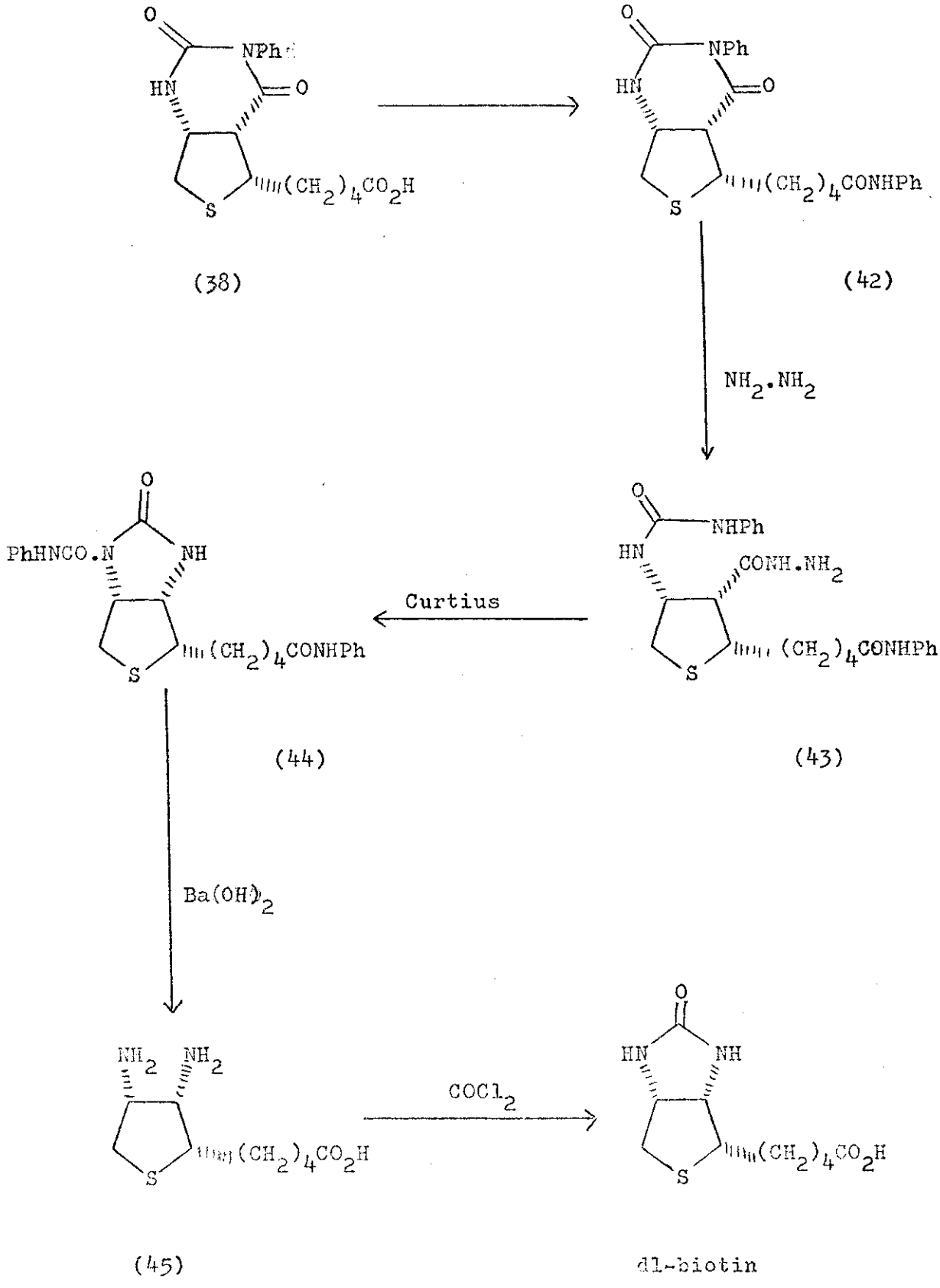


shown to have the cis-configuration.[23] Opening of the uracil with methoxide gave the trans-monoester (39) in which epimerisation had again occurred about the carbonyl bearing position-3. Treatment of the monoester (39) with hydrazine and subsequent Curtius rearrangement gave the carbamate (40) which was hydrolysed to the diamine (41) and cyclised with phosgene to dl-epiallobiotin.

To prepare normal dl-biotin[23] (scheme 7) the acid group of the all cis uracil (38) was protected as the anilide (42) and the ring opened with hydrazine to give the all cis hydrazide (43). (Hydrazine was not basic enough to cause epimerisation). This compound (43) was subjected to a Curtius rearrangement to give the isocyanate which reacted with the adjacent urea group to yield the imidazolone (44). Hydrolysis with barium hydroxide gave the diamine (45) which was converted with phosgene to dl-biotin.

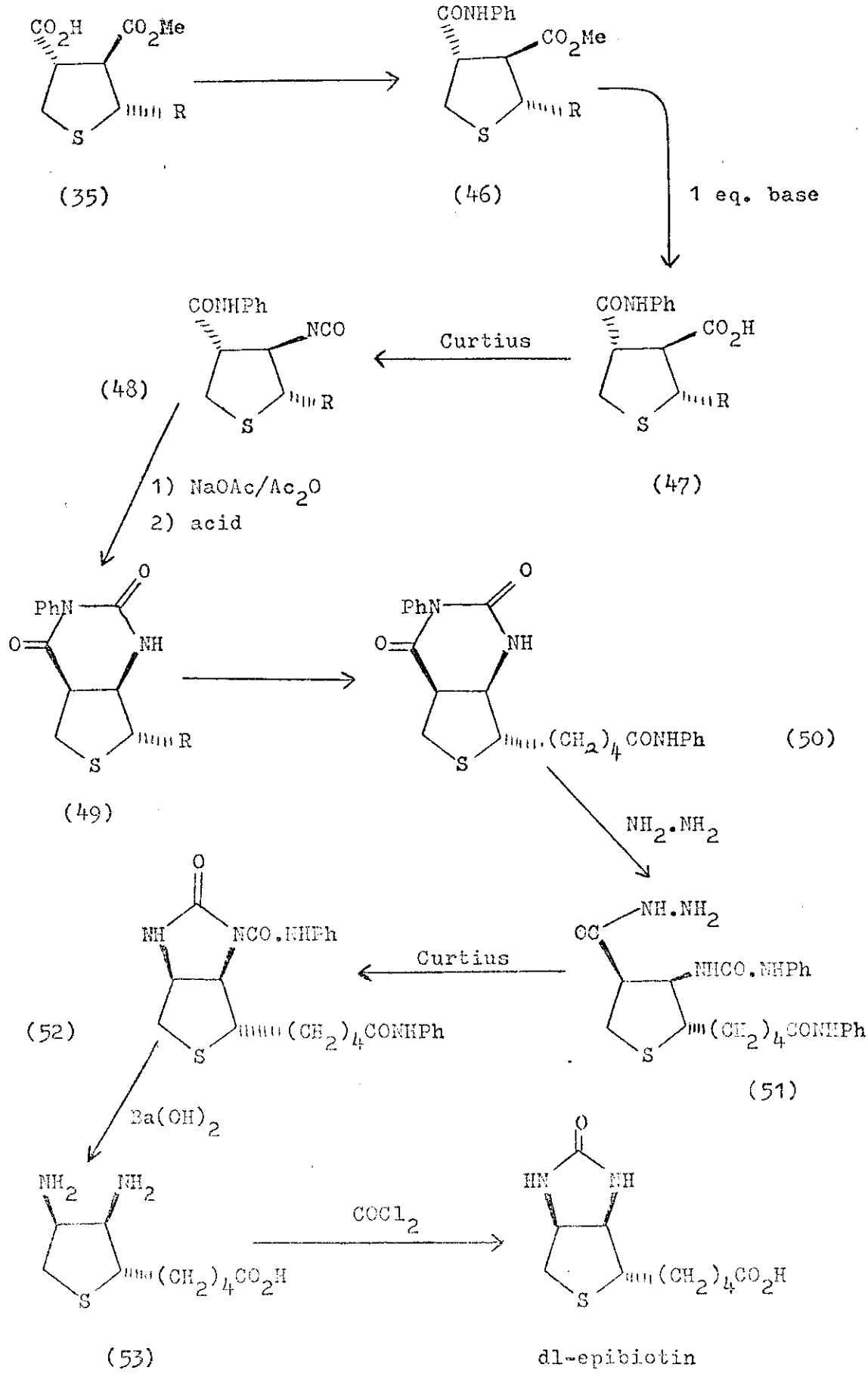
The route to dl-epibiotin[24] (scheme 8) commenced by converting the free acid group of the diester (35) to the anilide (46) which was saponified with one equivalent of base to the monoester (47) having the free carboxyl group at the 3-position.[24] Curtius rearrangement of the azide formed from this acid group gave the isocyanate intermediate (48) which cyclised to the cis uracil (49) on treatment with base. (N.B. it was the 4-position bearing the carbonyl group that was epimerised). The ester group of (49) was saponified and the free acid converted to the anilide (50). The uracil was opened with hydrazine, without epimerisation, to the hydrazide (51) which underwent Curtius rearrangement to the isocyanate with subsequent ring closure to the imidazolone (52). Hydrolysis of this compound with barium hydroxide gave the diamino acid (53) which was readily converted to dl-epibiotin with phosgene.

Scheme 7.


NPh
HN
O
S
$(CH_2)_4CO_2H$
(38)
$(CH_2)_4CONHPh$
(42)
$NH_2.NH_2$
NHPh
$CONH.NH_2$
(43)
Curtius
PhHNCO.N
NH
(44)
$Ba(OH)_2$
NH_2
(45)
$COCl_2$
dl-biotin

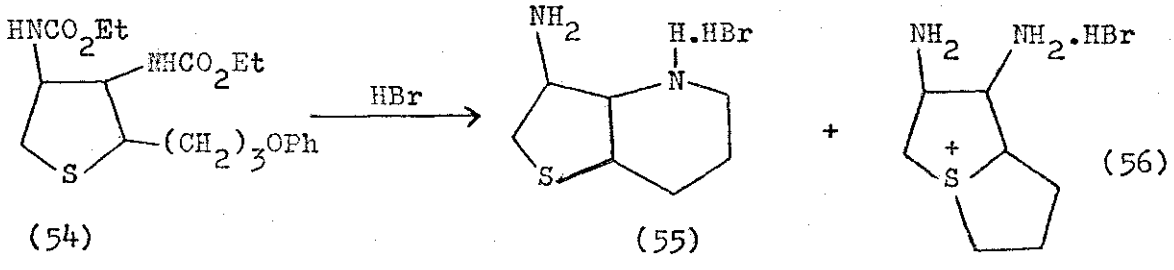
Scheme 8.

R = $-(CH_2)_4CO_2Me$

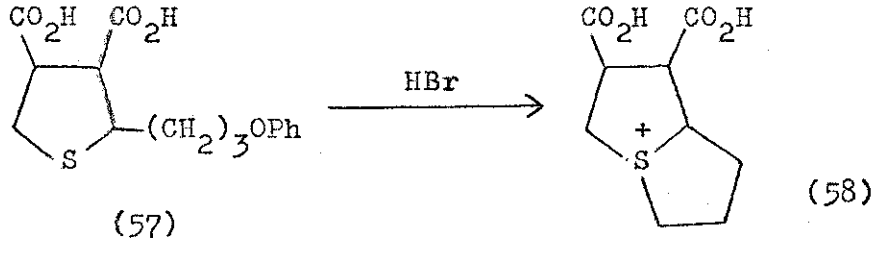


Baker's routes to the biotin isomers, whilst being rather lengthy, are highly stereospecific and go in quite good yields.

An interesting observation to come out of their background work[18] was the effect of hydrobromic acid on the dicarbamate (54) which they hoped would be hydrolysed to a pre-biotin type diamine. They in fact obtained a mixture of bicyclic compounds (55) and (56). Studies with the simpler compound (57) showed how facile the formation of the internal thiolanium salt (58) was.



(54) (55) (56)



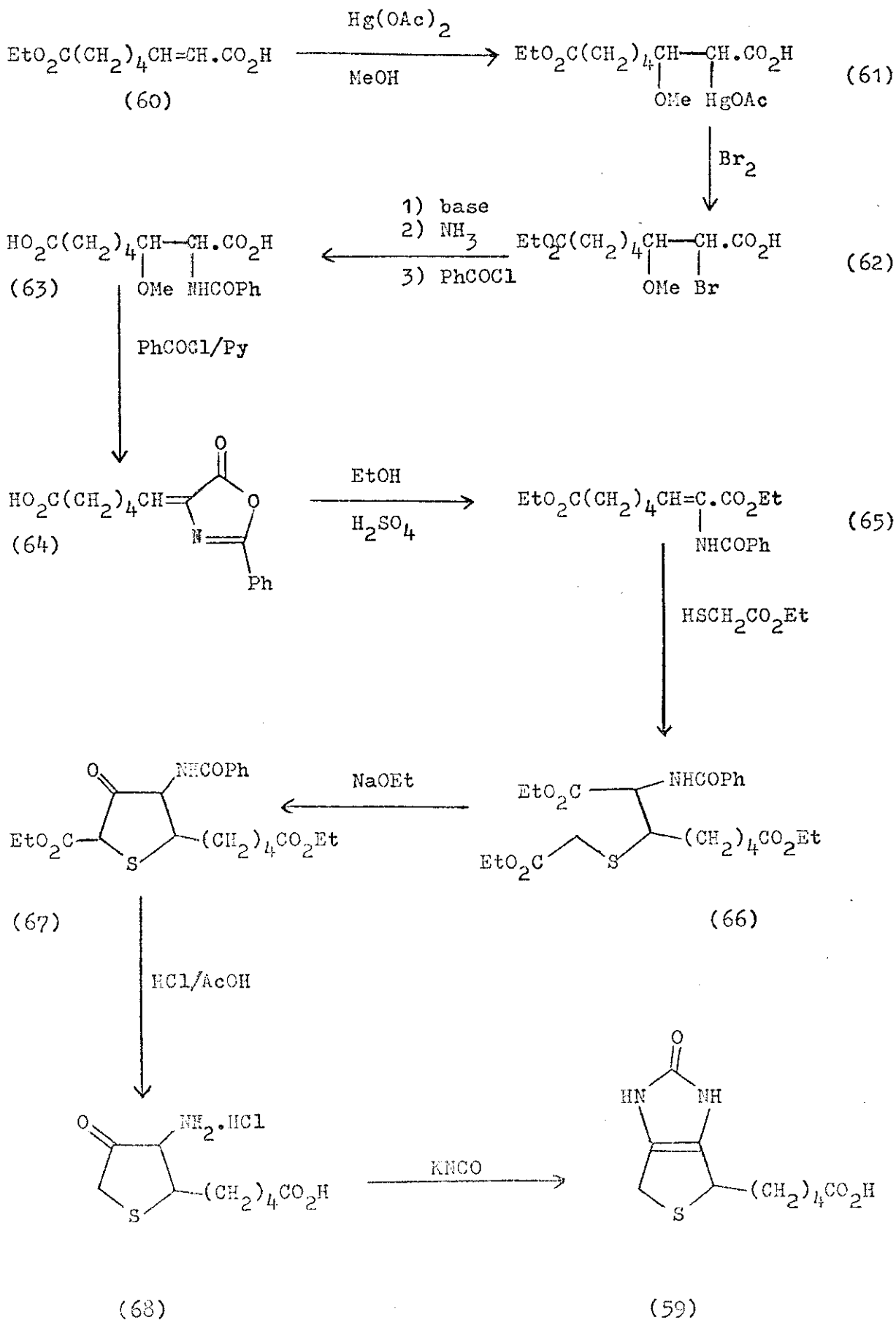
(57) (58)

Although Baker did no more work on these types of thiolanium salts they were utilised as a key step in the biotin synthesis of Goldberg and Sternbach a few years later.

Baker and his group also attempted a different route,[26] proposing to obtain biotin by hydrogenation of the unsaturated dehydrobiotin (59). They successfully synthesised the dehydrobiotin (59) (scheme 9) but did not report any attempts at the reduction.

7-Carbethoxy-2-heptenoic acid (60) was treated with mercuric acetate in methanol to give the acetoxymercuri-derivative (61). This gave the bromo-acid (62) upon treatment with bromine. The ester function was

Scheme 9.


Hg(OAc)2
MeOH
EtO2C(CH2)4CH=CH.CO2H
(60)
EtO2C(CH2)4CH—CH.CO2H
OMe HgOAc
(61)
Br2
1) base
2) NH3
3) PhCOCl
EtO2C(CH2)4CH—CH.CO2H
OMe Br
HO2C(CH2)4CH—CH.CO2H
OMe NHCOPh
(63)
(62)
PhCOCl/Py
HO2C(CH2)4CH
(64)
Ph
EtOH
H2SO4
EtO2C(CH2)4CH=C.CO2Et
NHCOPh
(65)
HSCH2CO2Et
NaOEt
EtO2C
NHCOPh
(CH2)4CO2Et
S
(66)
(67)
HCl/AcOH
NH2.HCl
(68)
KNCO
HN
NH
(59)

saponified to give the diacid which was reacted with ammonia and benzoyl chloride to yield the benzamide (63). Further treatment with benzoyl chloride in pyridine gave the azlactone (64) which gave the diester (65) upon treatment with sulphuric acid and ethanol. Reaction with mercaptoacetate, and subsequent Dieckmann cyclisation of the product (66) afforded the oxothiolan (67). Drastic hydrolysis of this stripped off the carbethoxy and benzoyl groups to give the amino-oxothiolan (68) which gave the dl-dehydrobiotin (59) on reaction with potassium isocyanate.

It is strange that they did not report the final hydrogenation step, since using a suitable metal catalyst they should have obtained, by cis donation of hydrogen, a mixture of dl-biotin and dl-epibiotin which could have been separated by fractional crystallisation.

They did test the biological activity of dehydrobiotin (59) though and found it to be biologically inert.

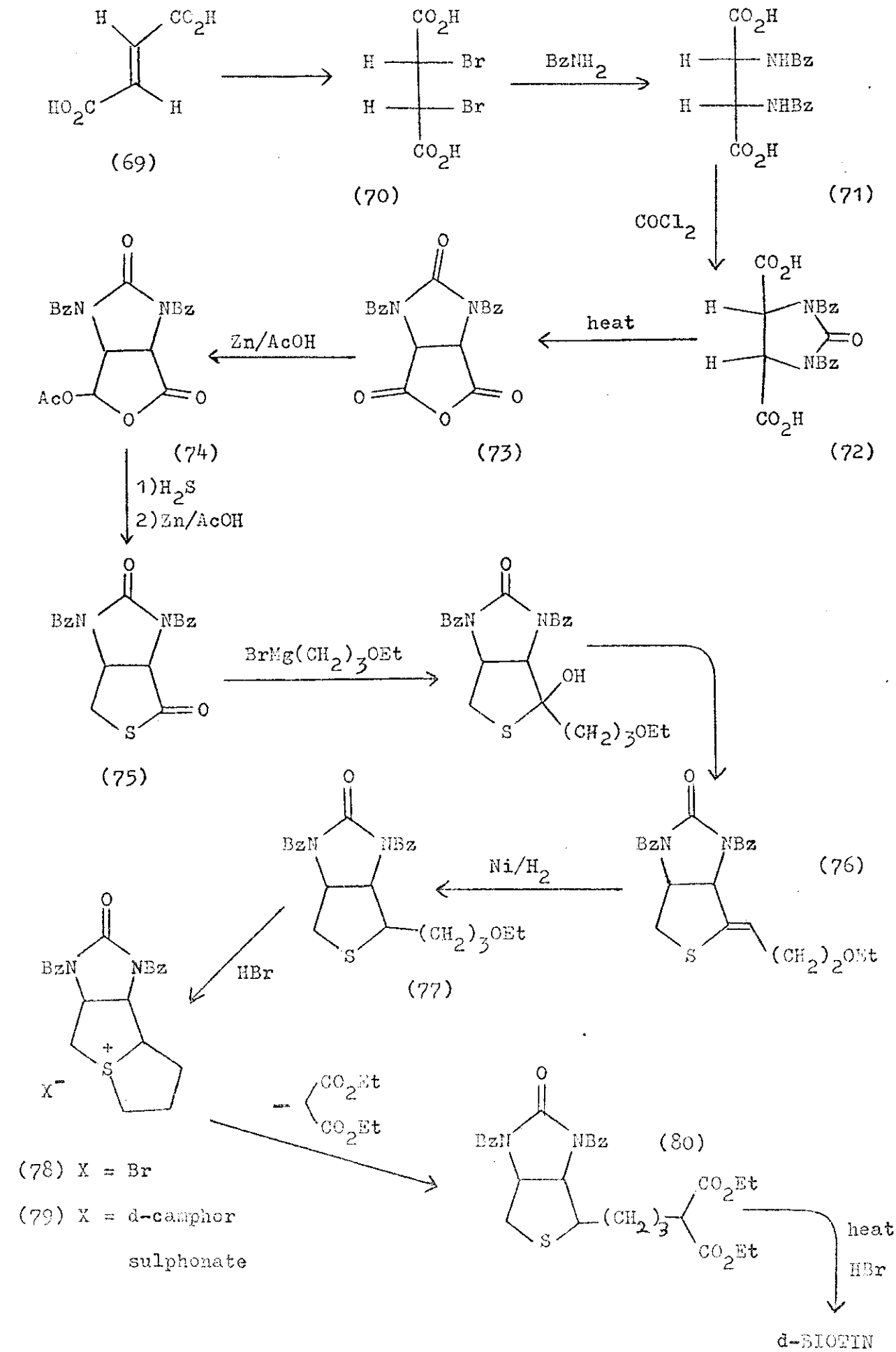
The Goldberg and Sternbach synthesis.

By far the best route to biotin is that of Goldberg and Sternbach which is described in a series of Hoffmann-La Roche patents.[27]

The route is highly stereospecific and is characterised by the fact that a meso-diaminosuccinic acid derivative was used as an intermediate which automatically led to the required cis-fused structure of the final product. Biotin was also isolated in an optically active form since resolution was carried out at an earlier stage utilizing a thiolanium salt.

Their now classic route (scheme 10) began by brominating fumaric acid (69) to mesodibromosuccinic acid (70) which was converted to meso-α,β-bisbenzylaminosuccinic acid (71). Treatment with phosgene gave the cyclic diacid (72) which was dehydrated to the anhydride (73). Reduction with zinc in acetic acid gave the acetoxy-lactone (74) which,

Scheme 10. (Bz = $C_6H_5.CH_2$-)



upon treatment with hydrogen sulphide followed by reduction gave the thio-lactone (75). This intermediate can be converted to biotin by various methods but the most efficient route involved the reaction of the thio-lactone (75) with 3-ethoxypropyl magnesium bromide followed by dehydration to (76) and catalytic hydrogenation to the ester (77). Treatment of (77) with HBr gave the thiolanium bromide (78) from which was made the d-camphor sulphonate salt (79). The salt was resolved and condensed with sodium diethylmalonate to give the diester (80). Acid hydrolysis of this with boiling HBr cleaved off the benzyl protecting groups as well as forming the valeric acid side chain to give d-biotin.

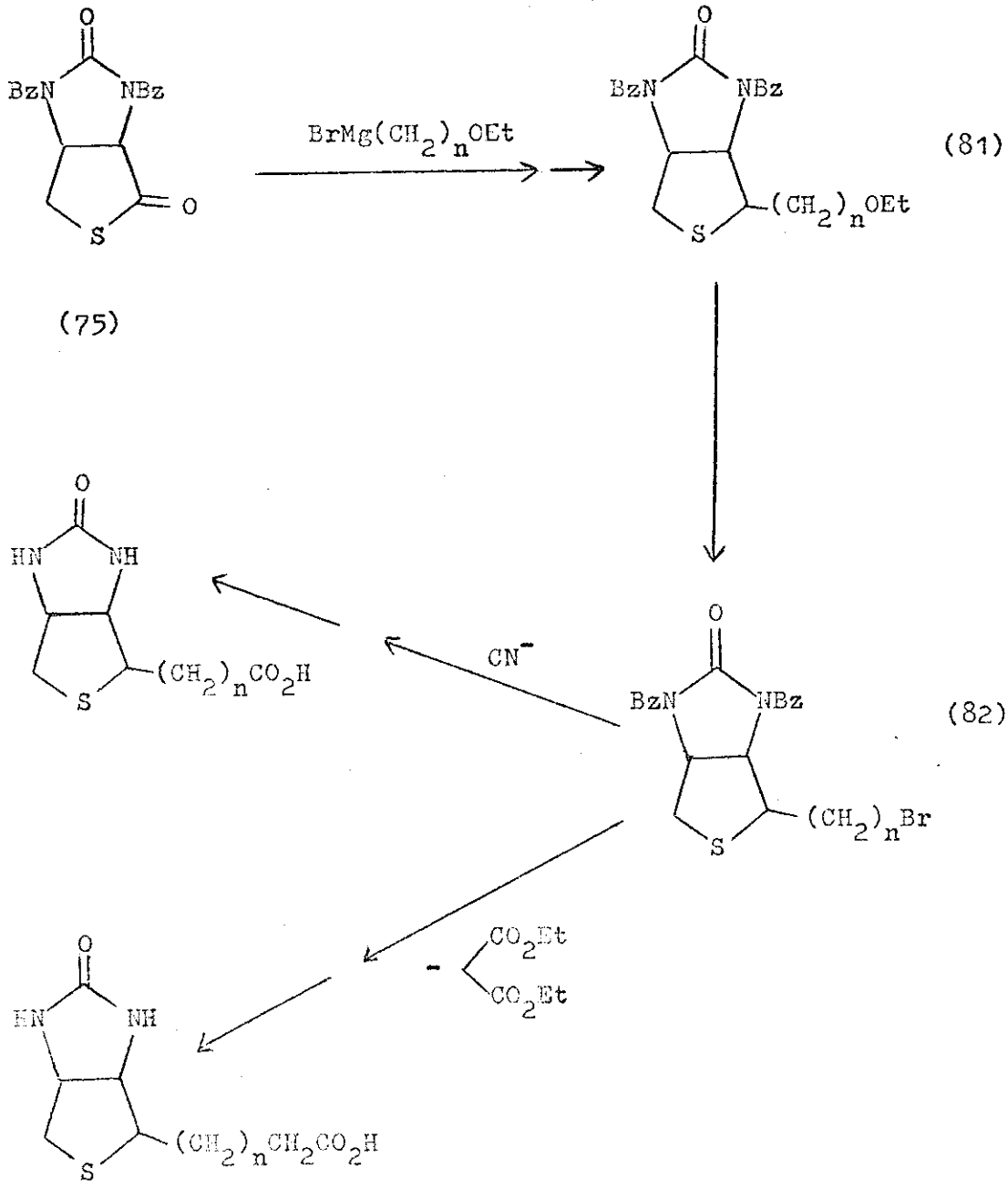
It should be noted that as the imidazolidone ring was formed before the thiophan ring, in such a manner that the amino groups were cis-orientated, the formation of allo- and epiallobiotin was prevented.

It is also interesting that no epibiotin was formed. The crucial stage was in the catalytic hydrogenations of the double bond from (76) to (77). The hydrogen was apparently donated totally to the least hindered side of the molecule to give the all cis product. This should be contrasted with the similar reactions of Harris et al, [cf.(9) to (11) and (10) to (11)], who found that with free amino groups present, the hydrogen was donated to both the most hindered and the least hindered sides of the molecule. The closure of the amino groups into the cyclic urea obviously has a strong stereo-directing effect upon the catalytic hydrogenation of the molecule.

The thiolactone (75) was found to be a useful intermediate for the introduction of various side chains into the biotin nucleus (scheme 11). Reaction with ω-ethoxyalkyl magnesium bromides, followed by dehydration and hydrogenation gave ethoxyalkyl compounds (81) which were converted to the bromides. The acid group was introduced in one of two ways;

1) by treatment of the bromide with cyanide and subsequent hydrolysis, or 2) by reaction with sodium diethylmalonate which, on work up, yielded the homologue.

Scheme 11.



The thiolanium salt (either bromide or camphor sulphonate) has also proved its worth as an intermediate as was demonstrated by the recent preparations of the antimetabolites α-dehydrobiotin[28] (83) and α-methylbiotin[29] (84).

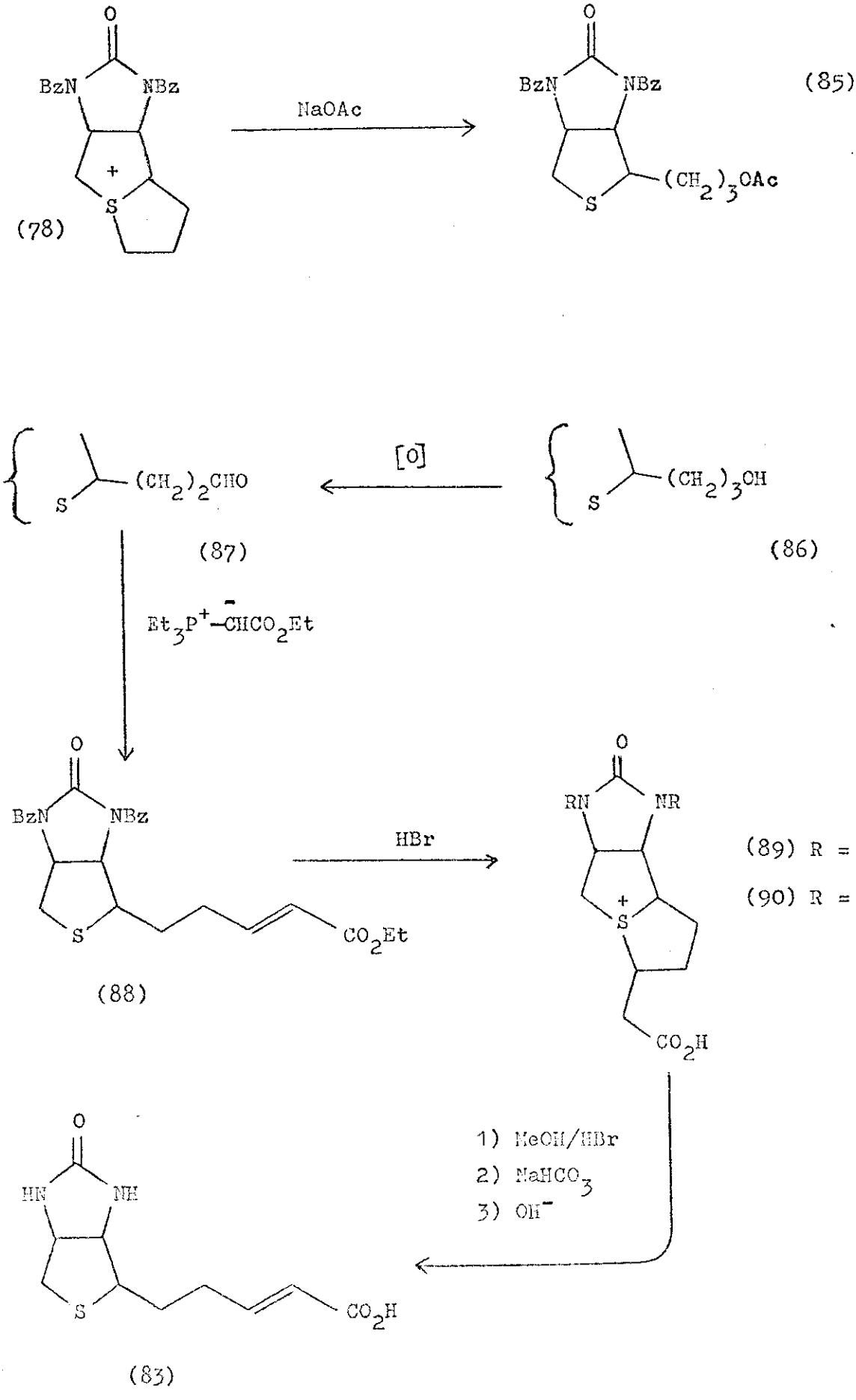
α-Dehydrobiotin (83) was prepared (scheme 12) by opening the thiolanium salt with sodium acetate and hydrolysing the acetoxy compound (85) to the alcohol (86). Oxidation gave the aldehyde (87) which was condensed with a triethylphosphono-acetate to give the α,β-unsaturated ester (88). The removal of the benzyl groups presented unexpected problems due to the juxtaposition of the double bond and the electron rich sulphur linkage. Heating (88) with HBr gave the cyclic thiolanium acid (89) which on further heating gave the debenzylated acid (90). To prevent possible decarboxylation the acid was re-esterified and the salt opened up with mild base. Saponification gave α-dehydrobiotin.(83).

To prepare α-methylbiotin (84) (scheme 13) the thiolanium salt was opened with the thallium salt of ethyl methylacetoacetate (the sodium salt gave mixtures) to give the acetyl ester (91). Alcoholysis[30] of the acetyl group followed by hydrolysis of the ester afforded α-methylbiotin in an overall yield from the thiolanium salt of 72%.

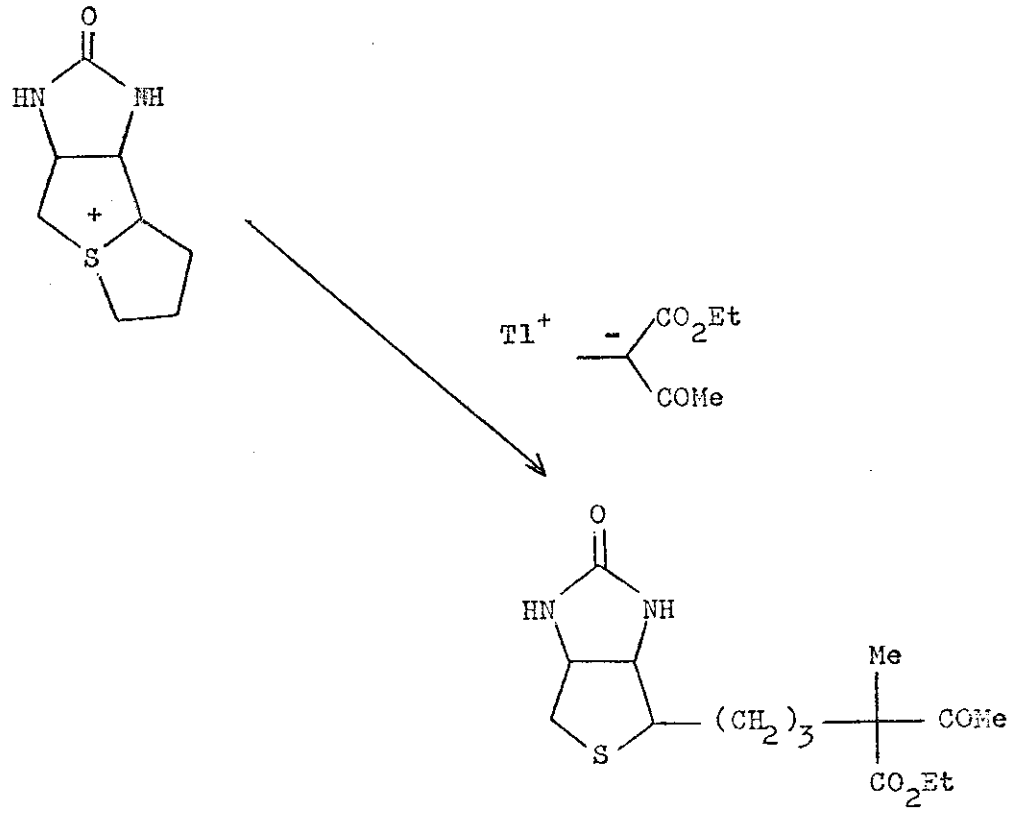
Recently the Goldberg and Sternbach synthesis has been improved by the Hoffmann-La Roche group by separating the optical isomers at a much earlier stage and recycling the unwanted one.[31]

The new modifications (scheme 14) converted the cyclic diacid (72) into the anhydride (73) as before. The anhydride was then treated with an optically active alcohol (menthol, borneol or cholesterol) to give the half esters (92a and b). The optically active antipodes were resolved and the unwanted one treated with base to regenerate the cyclic diacid (72) which was recycled. The other isomer was reduced with lithium borohydride to the optically active lactone (93) and

Scheme 12.


BzN
NBz
NaOAc
(78)
(85)
(CH2)3OAc
(CH2)2CHO
[O]
(CH2)3OH
(87)
(86)
Et3P+–CHCO2Et
HBr
CO2Et
(88)
RN
NR
(89) R = Bz
(90) R = H
CO2H
1) MeOH/HBr
2) NaHCO3
3) OH−
HN
NH
(83)

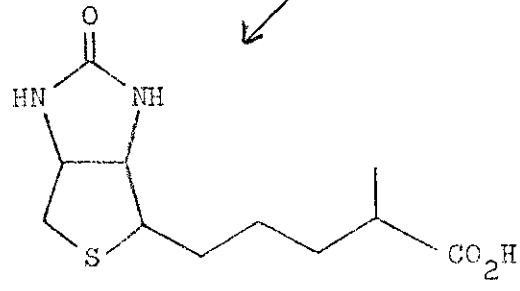
Scheme 13.



(91)

1) alcoholysis

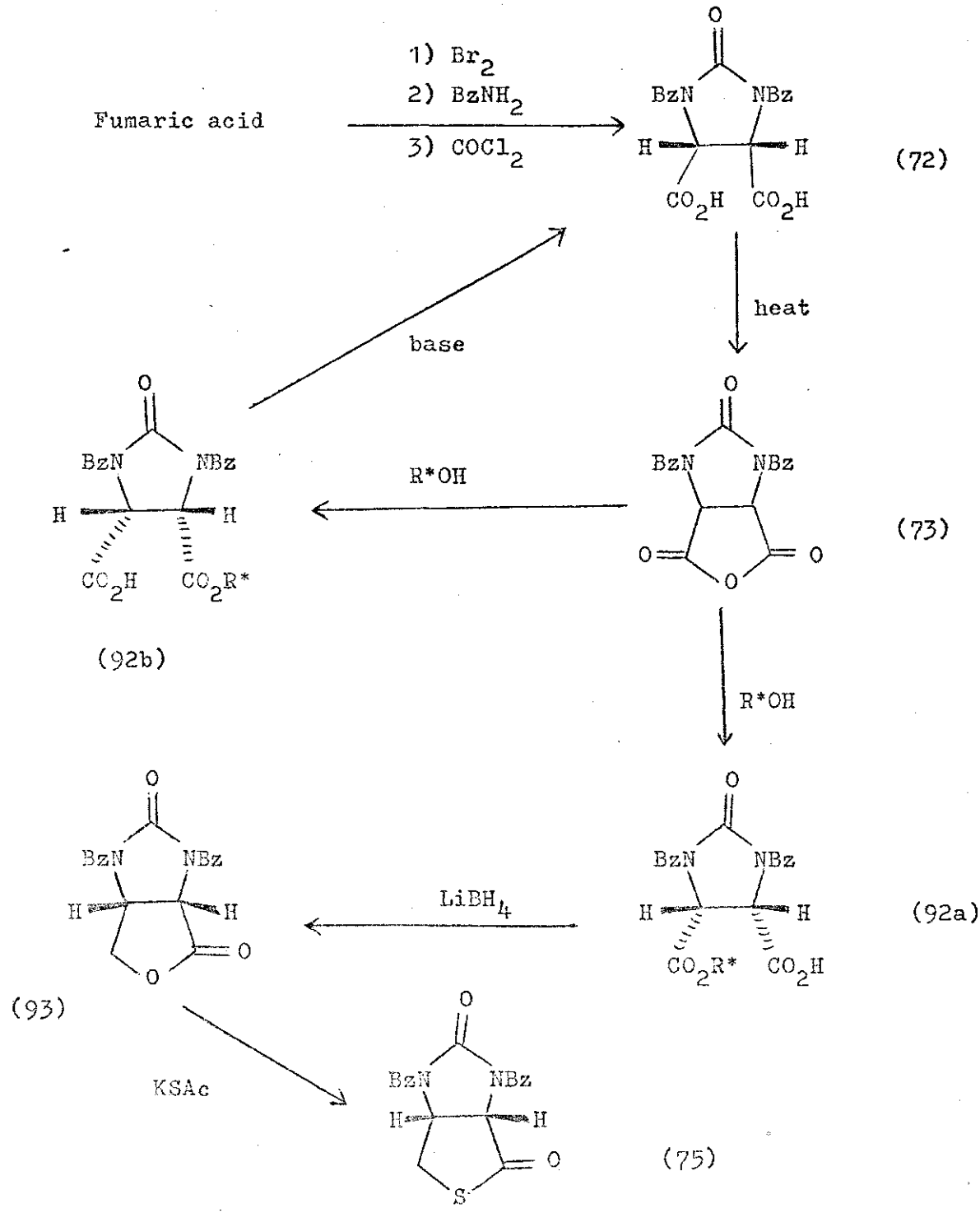
2) hydrolysis



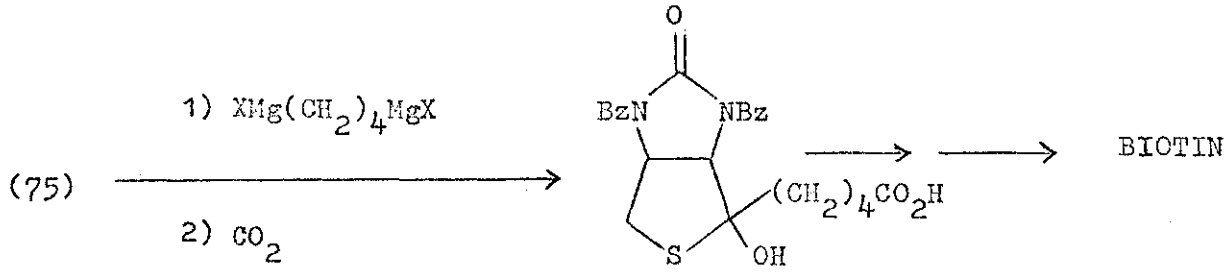
(84)

this was treated with potassium thioacetate under mild conditions, which did not cause racemisation, to give the thiolactone (75). This was converted into biotin as before (scheme 10) without the need for resolution at a later stage using the camphor sulphonate salt.

Scheme 14. (R*OH = menthol, borneol or cholesterol)



Japanese workers have reported a synthesis of biotin but it varies only slightly from Goldberg and Sternbach's method. The thiolactone (75) was treated with the double ended Grignard reagent, prepared from a 1,4-dihalobutane, and CO_2 added to the reaction mixture to give the valeric acid derivative (94). This was dehydrated, hydrogenated and hydrolysed with boiling HBr to biotin.



(94)

Other Japanese work which appeared in patents[33,34] converted Goldberg and Sternbach's thiolactone into biotin by using an ω-benzyloxybutyl Grignard reagent and modifying the side chain, via the cyanide, as described earlier (c.f. scheme 11).

Neither of these recent publications show any great improvements over the original method published 20 years earlier.

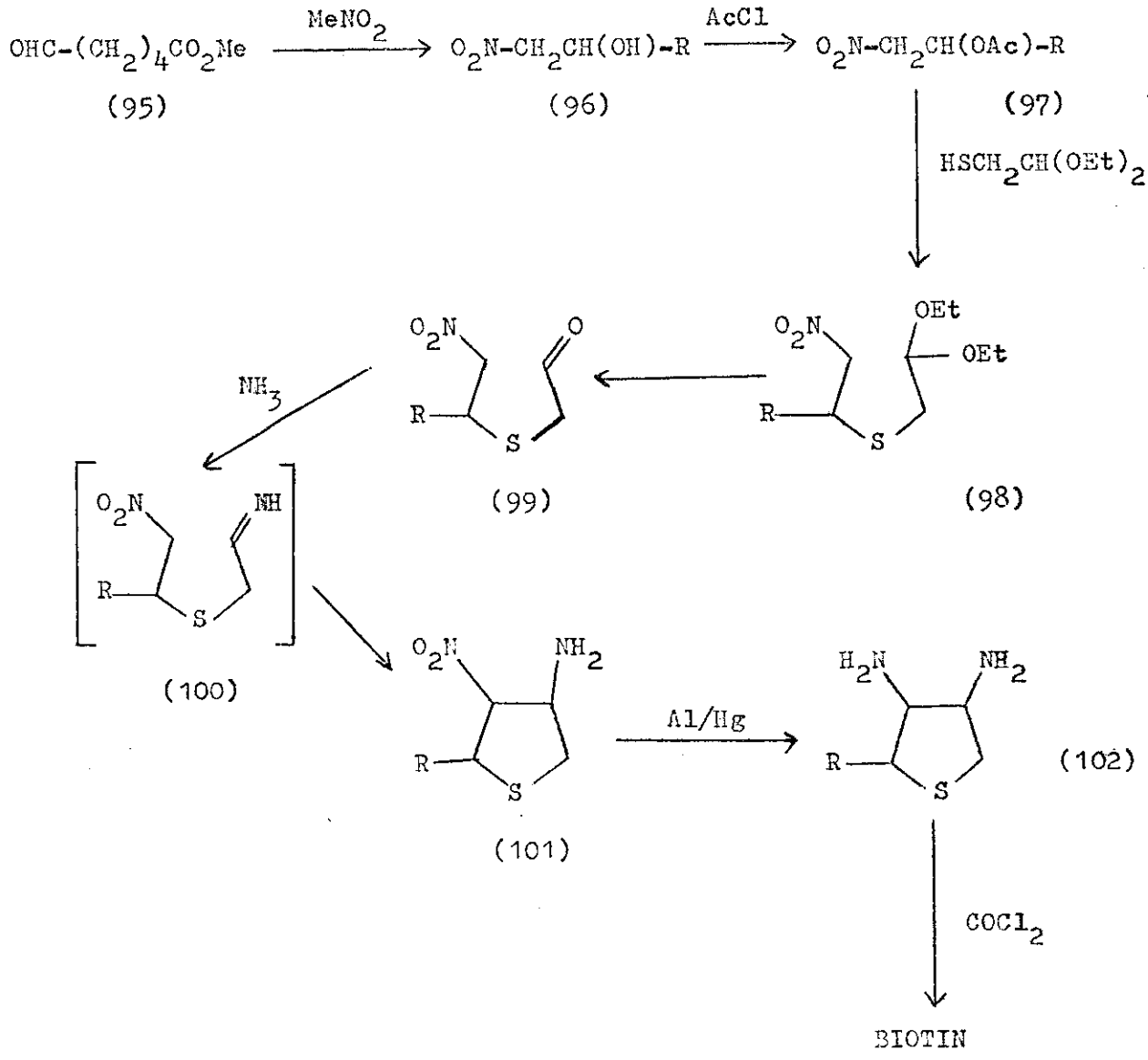
The unsuccessful route of Grob and Sprecher.

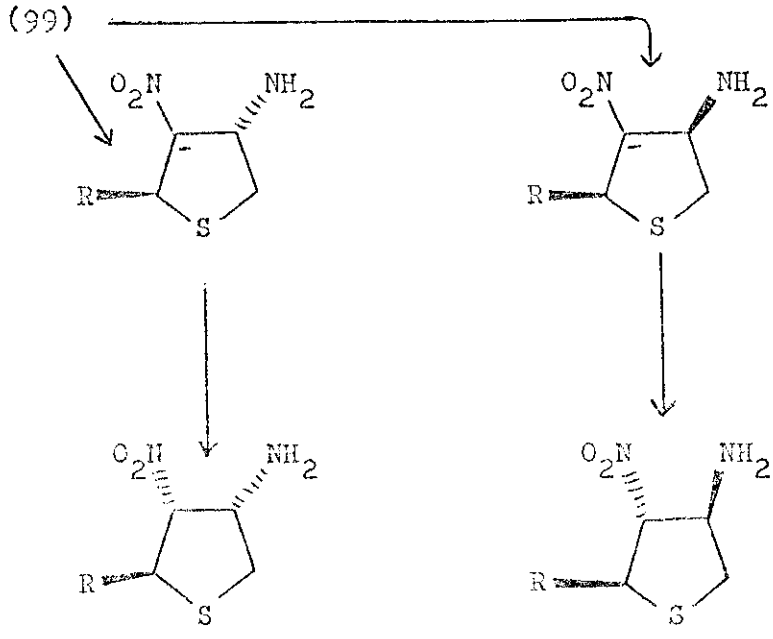
The work of Grob and Sprecher[35] was less friutful than other routes in that they only obtained a mixture of dl-epi- and dl-epiallobiotin.

The route (scheme 15) was characterised by forming the thiolan ring by a Dieckmann type cyclisation onto an imine. The product of this was saturated and so eliminated the need for a reduction step later on. The lack of the reduction step was in fact their downfall since catalytic hydrogenation does have a certain amount of stereospecificity and would have given rise to some dl-biotin.

Methyl formylvalerate (95) was treated with nitromethane and base to give the nitro-ester (96) which was acetylated (97) and reacted with the diethylacetal of mercaptoacetaldehyde to give (98). The acetal

Scheme 15. R = $-(CH_2)_4CO_2Me$



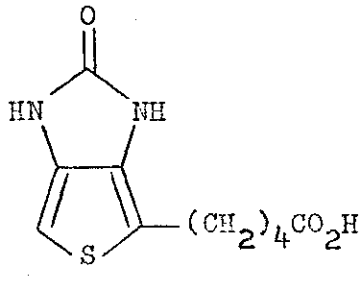


was hydrolysed with acid to the aldehyde (99) and treatment of this with liquid ammonia gave the thiolan (101) via the imine (100). Reduction of the nitro group gave the diamine (102) which was cyclised to biotin with phosgene.

The epi- and epiallo- configurations were formed during the cyclisation step from (100) to(101). The nitro group epimerised via the anion to the least hindered position with respect to the large alkyl side chain so giving rise to the two epi- isomers.

Aromatic biotin.

An alternative route to biotin has been suggested by the hydrogenation of 2,3,4,5-tetradehydro-biotin, so-called aromatic biotin (103).



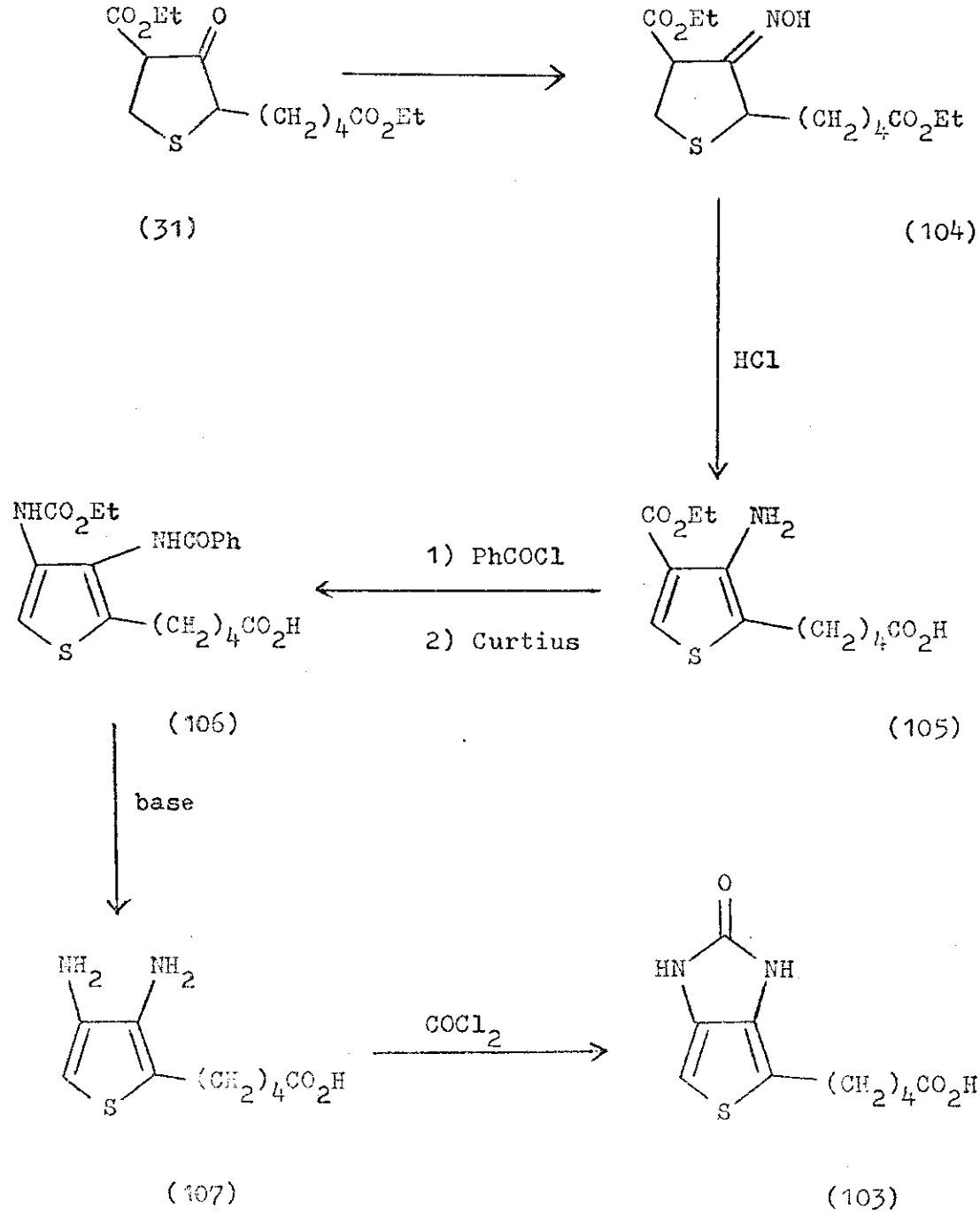
(103)

It might have been assumed that catalytic hydrogenation of this compound would have given the all cis biotin configuration but Cheney reported[36] that hydrogenation using molybdenum sulphide on alumina as catalyst gave a mixture of isomers. There are no other reports of the more usual catalysts being used.

Cheney's synthesis of aromatic biotin[37] (scheme 16) started from the 3-oxothiolan (31) of Keller et al[25] as used also by Baker. (Baker used the dimethyl ester whereas Cheney used the diethyl ester).

The ketone (31) was converted to the oxime (104) and treated with HCl to yield the aromatic amine (105). The amino group was protected with benzoyl chloride and the ester function converted to a urethane (106) via the hydrazide and Curtius rearrangement.

Scheme 16.


CO2Et
O
(CH2)4CO2Et
S
(31)
CO2Et
NOH
(CH2)4CO2Et
S
(104)
HCl
CO2Et
NH2
(CH2)4CO2H
S
(105)
1) PhCOCl
2) Curtius
NHCO2Et
NHCOPh
(CH2)4CO2H
S
(106)
base
NH2
NH2
(CH2)4CO2H
S
(107)
COCl2
O
HN
NH
(CH2)4CO2H
S
(103)

Hydrolysis of (106) gave the diamine (107) which was cyclised with phosgene to aromatic biotin.

Aromatic biotin was tested in biological systems but it was not found to have any growth promoting or inhibiting activities.

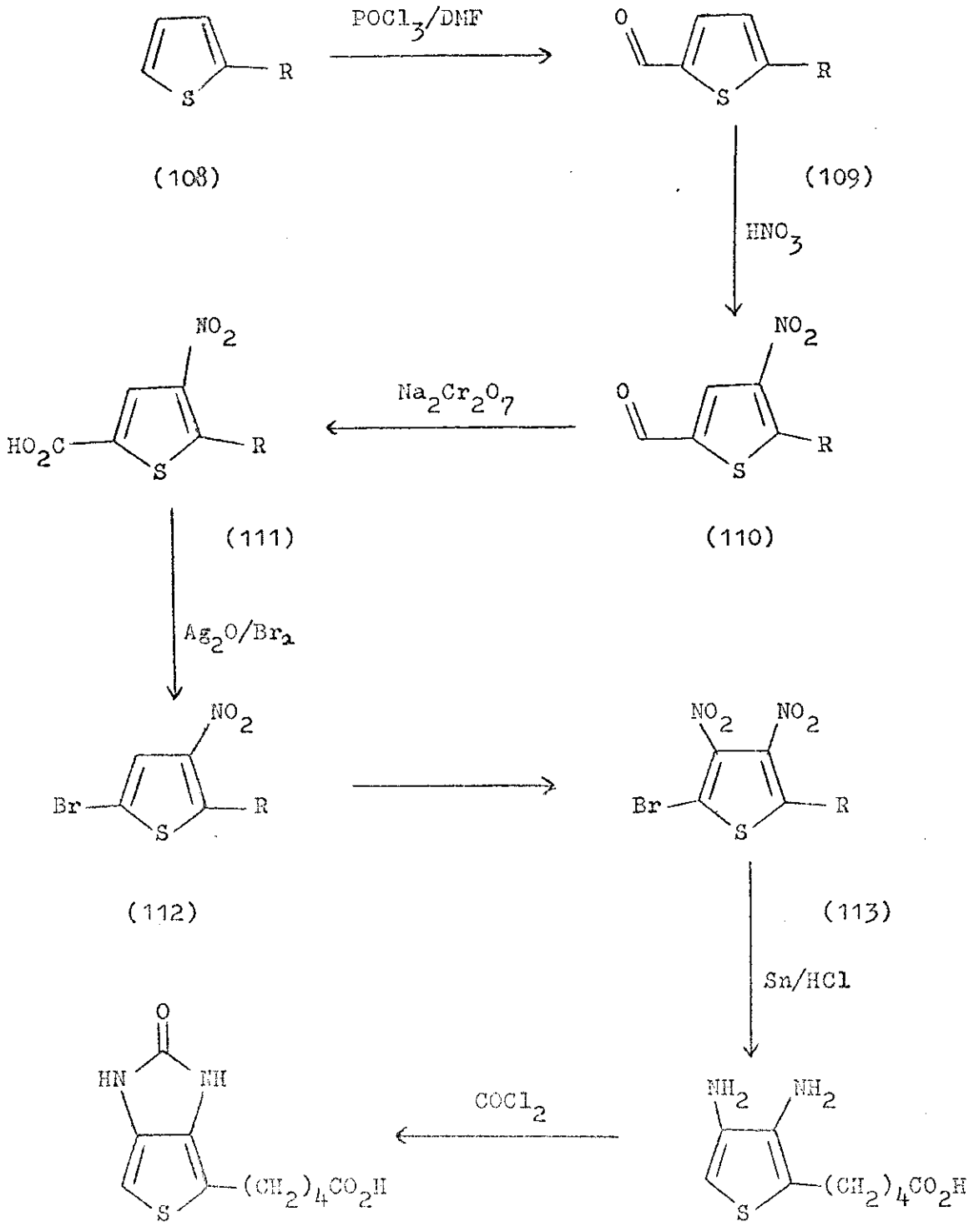
The next preparation of aromatic biotin was by Japanese workers[38] using a fairly-straight forward selective nitration of a substituted thiophen (scheme 17).

Methyl 4-(2-thienyl)butyrate (108) was formylated in the 5-position (109) and nitrated at the 3-position (110). The aldehyde function was oxidised to the acid (111) and this was subjected to a Hunsdiecker reaction with silver oxide and bromine to give the 5-bromo compound (112). Nitration of this in the 4-position gave the dinitro compound (113) which was reduced to the diamine (107) and cyclised with phosgene to aromatic biotin.

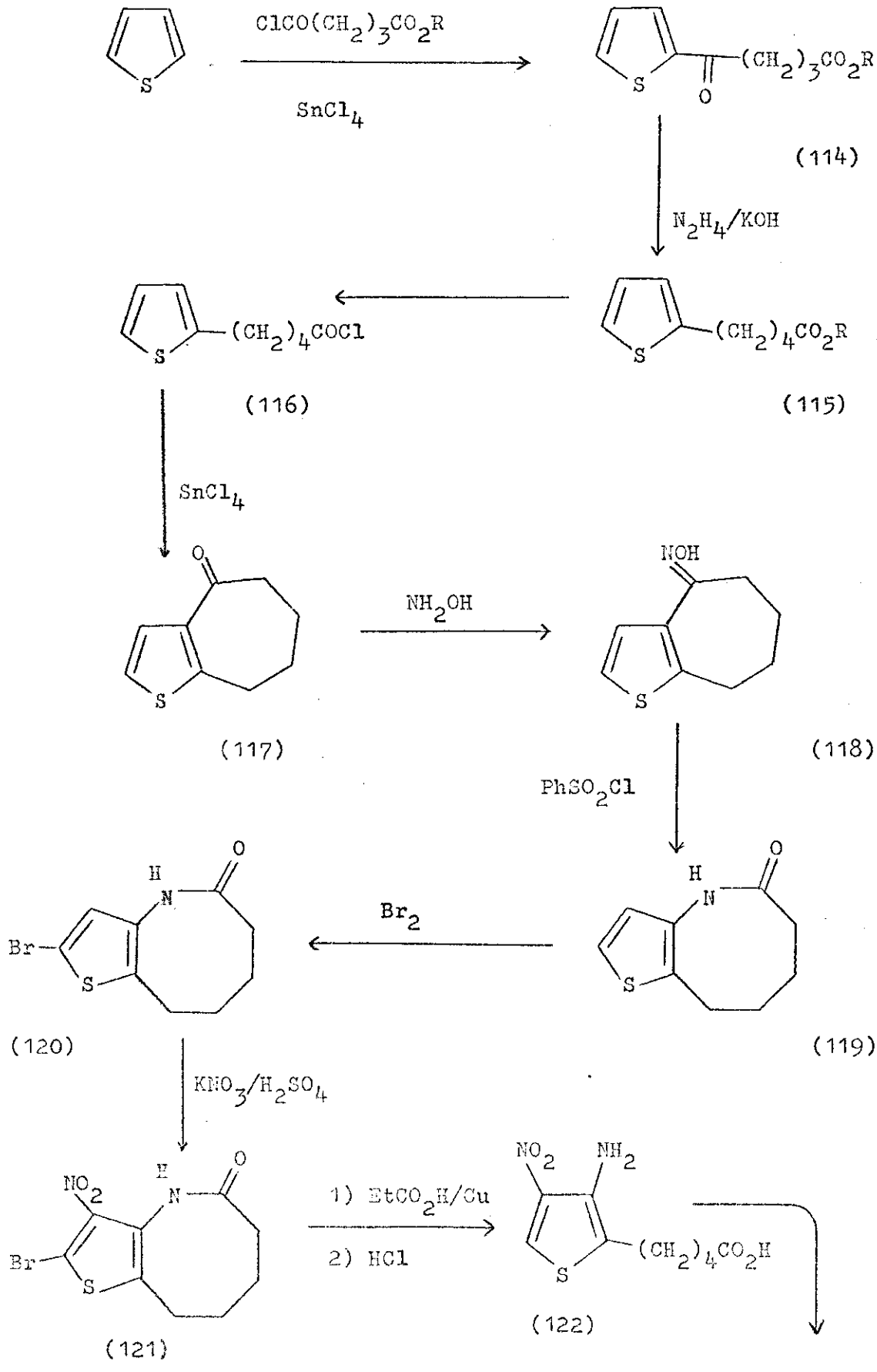
The third route to this compound by Russian workers[39,40] used quite a novel approach in that a Beckmann rearrangement was used to introduce one of the amino groups and also, indirectly, the carboxyl group (scheme 18).

Thiophen underwent a Friedel-Crafts reaction with the acid chloride of a half ester of glutaric acid to give (114) which was reduced under Wolff-Kishner conditions to the thienyl valerate (115). This was converted to the acid chloride (116) and subjected to an internal Friedel-Crafts acylation to the ketone (117) from which the oxime (118) was prepared. Beckmann rearrangement gave the lactam (119) which was brominated in the 5-position (120) and nitrated in the 4-position (121). Removal of the bromine, and acid hydrolysis of the lactam gave the nitro amine (122) which was reduced and treated with phosgene to give aromatic biotin.

Scheme 17. R = -$(CH_2)_4CO_2Me$



Scheme 18.


ClCO(CH2)3CO2R
SnCl4
(CH2)3CO2R
O
(114)
N2H4/KOH
(CH2)4CO2R
(115)
(CH2)4COCl
(116)
SnCl4
O
(117)
NH2OH
NOH
(118)
PhSO2Cl
H
N
O
Br2
Br
(120)
(119)
KNO3/H2SO4
NO2
1) EtCO2H/Cu
2) HCl
NH2
(CH2)4CO2H
(122)
(121)
AROMATIC BIOTIN

Biotin Analogues.

Paralleling all the work directed towards the synthesis of biotin, there has been a lot of effort put into investigations aimed at emulating the biotin structure in an attempt to find new growth promoting or inhibiting compounds.

There is particular interest in the preparation of good biotin inhibitors which will stop or reduce growth in microorganisms. The function of biotin is to perform carboxylations, and the basic concept is to synthesise biotin analogues which will either block the enzyme system or prevent the carboxylation step (c.f. α-dehydro- and α-methyl- biotin, compounds (83) and (84), which are good antimetabolites).

The efficiency of a biotin inhibitor (antibiotin) is measured by its 'Molar Inhibition Ratio (MIR)', defined[41] as the number of moles. of an antibiotin required to inhibit one mole. of biotin. The antibiotin activity is determined experimentally by finding the amount of an antibiotin which is able to reduce the growth obtained with 0.2 m μ g of biotin to a level equivalent to that obtained with 0.1 m μ g of biotin. The MIR can then be calculated after the conversion of these amounts to molar quantities. The smaller the MIR, the greater the antibiotin activity of a given compound.

Of the compounds already mentioned, most of the homologues of biotin and their sulphones show antibiotin activity.

Dittmer and du Vigneaud[41] were the first to show that biotin sulphone was a very potent antibiotin against L.casei (MIR 280) and others but for S.cerevisiae it was found to act as a growth stimulant in place of biotin, although its activity was considerably lower.

Goldberg and Sternbach[42] subjected their homologues of biotin (c.f. scheme 11) to biological evaluation and their results are summerised below. (N.B. Norbiotin has a four carbon side chain, homobiotin a six carbon side chain, bis-homobiotin a seven carbon side chain, etc.)

Table of 'Molar Inhibition Ratios' *

	S. cerevisiae	L.casei
dl-Norbiotin	1,000	13,000
dl-Homobiotin	700	130
dl-Bis-homobiotin	30,000	7,000
dl-Tris-homobiotin	50,000	3,000
dl-Homobiotin sulphone	40,000	400
dl-Bis-homobiotin sulphone	60,000	8,000
dl-Tris-homobiotin sulphone	60,000	6,000

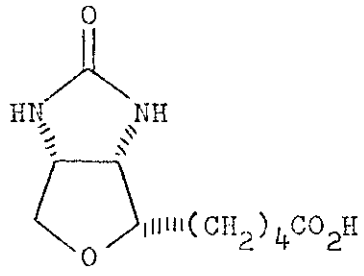
*The smaller the number, the better the inhibition; see text.

Using the d-isomers Briggs[43] has shown that the molar inhibition ratios are roughly half those of the corresponding dl-isomers showing that the l-isomers are biologically inert. For d-homobiotin the MIR's are 260 and 60 respectively for the two microorganisms.

As can be seen from the table, d-homobiotin is easily the best biotin inhibitor and it is also worth noting that of the sulphones, d-homobiotin sulphone is the best, especially against L. casei, (MIR 210; c.f. the MIR of biotin sulphone[41] against L. casei of 280).

Oxybiotin.

The first growth promoting compound to be prepared synthetically was d-oxybiotin (123), the tetrahydrofuran analogue.



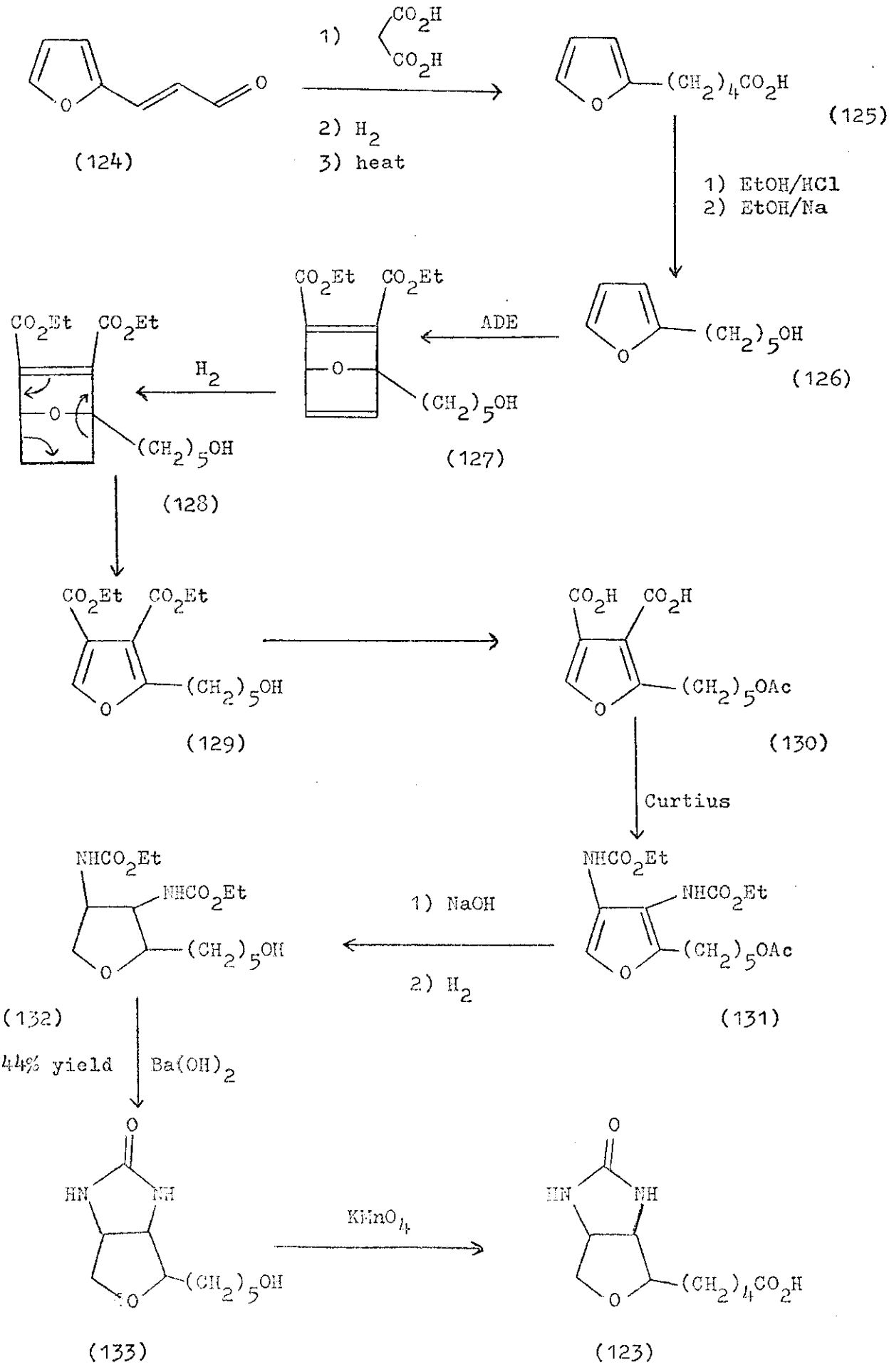
(123)

Hoffmann's route[44-49] was relatively straight forward (scheme 19). Thus,[44] furyl-acrolein (124) was reacted with malonic acid, the product hydrogenated and decarboxylated to the mono acid (125). The ester of this was reduced under Bouveault-Blanc conditions to the alcohol (126). Reaction with acetylene dicarboxylic ester gave (127)[45] which was hydrogenated (128) and decomposed by an Alder-Rickert procedure to the diester (129). Saponification and acetylation of the alcohol gave the diacid (130) which was subjected[48] to a Curtius rearrangement *via* the azide to the diurethane (131). The acetoxy group was hydrolysed and the furan ring hydrogenated (132). Heating the diurethane with barium hydroxide caused ring closure to the cyclic urea (133) which was oxidised to dl-oxybiotin (123).

It is interesting that no allo-orientated compounds were isolated. Reduction of the furan (131) to the tetrahydrofuran (132) would be expected to give a mixture of *cis*- and *trans*- orientated diurethanes. Only the *cis*- isomer would cyclise on treatment with barium hydroxide and this is reflected in the yield of only 44% on this step. Hence only *cis*-fused rings were formed.

The side chain homologues of oxybiotin were prepared in a similar manner.

Scheme 19.


1) CO2H CO2H
2) H2
3) heat
(124)
(CH2)4CO2H
(125)
1) EtOH/HCl
2) EtOH/Na
(CH2)5OH
(126)
ADE
CO2Et CO2Et
(127)
H2
(128)
(129)
(CH2)5OAc
(130)
Curtius
NHCO2Et
1) NaOH
2) H2
(131)
(132)
44% yield
Ba(OH)2
HN NH
KMnO4
(133)
(123)

The second synthesis was carried out by Duschinsky and Dolan[50,51] for the Hoffmann-La Roche company (scheme 20).

Diacetyl-4-methyl-5-(δ-carbethoxyvaleryl)-2-imidazolone (134) was treated with N-bromosuccinimide to give the allylic bromide (135) which was converted to the acetoxy compound (136). The protecting acetyl groups were removed and the compound hydrogenated to the hydroxy-ketone (137). Further hydrogenation and dehydration gave oxybiotin.

In tests with micro-organisms it was found that dl-oxybiotin had about 50% of the growth promoting activity of d-biotin for L. arabinasus and 40% for L. casei.[52,53] It is probably a valid assumption to say that d-oxybiotin has almost the same growth promoting activity as d-biotin since it has been shown[43] that in many cases the d-isomers of biotin analogues have twice the activity of the dl-isomers, i.e. implying that the l-isomers are inactive.

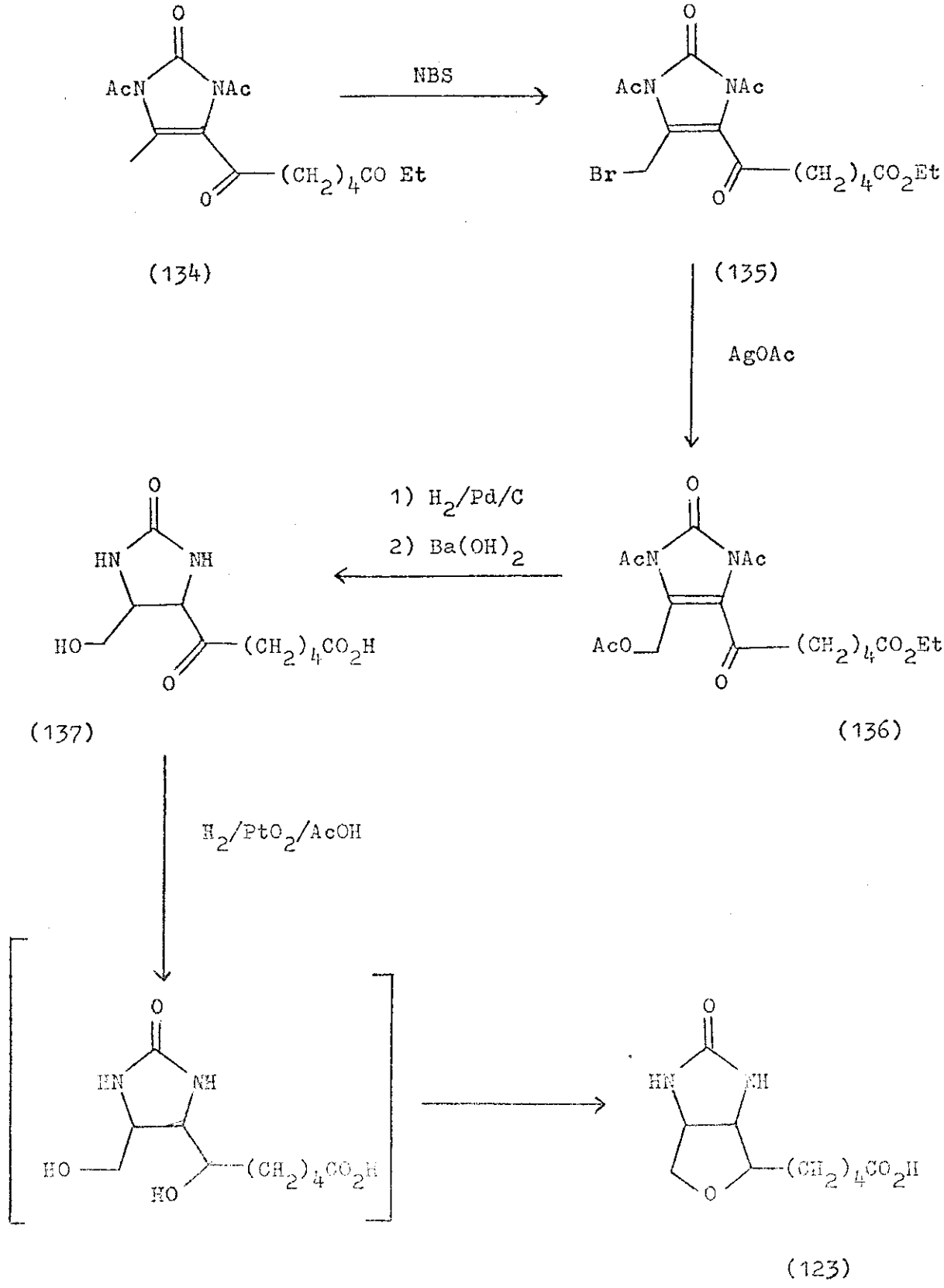
It has also been shown that yeast cells utilise oxybiotin as such without the prior conversion to biotin as occurs in the case of desthiobiotin, another good growth promoting compound.[54] In a very simple, but effective, experiment Hofmann added potassium permanganate to cells growing on a biotin medium and found the growth was stopped by oxidation to biotin sulphone, whereas yeast grown on oxybiotin was virtually unaffected by similar treatment.

It is especially noteworthy that the best antioxybiotin was homo-oxybiotin[49] just as homobiotin was the best antibiotin.

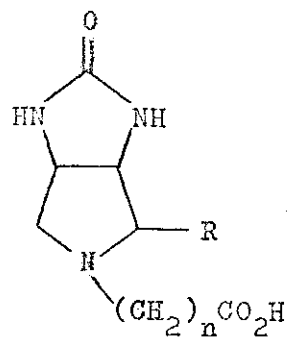
Azabiotin analogues.

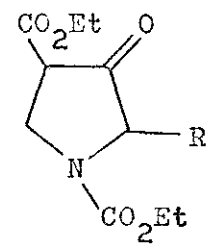
Azabiotin, the pyrrolo- analogue of biotin, has not been prepared as such, but Wormser[55,56] has developed a route to N-substituted analogues of azabiotin (138) by a relatively standard route from the ketone (139). No biological evaluations have been

Scheme 20.


AcN
NAc
NBS
Br
(CH2)4CO Et
(CH2)4CO2Et
(134)
(135)
AgOAc
1) H2/Pd/C
2) Ba(OH)2
AcO
HO
HN
NH
(CH2)4CO2H
(137)
(136)
H2/PtO2/AcOH
(123)

carried out on these compounds yet and probably a full route to azabiotin itself will be published in the near future.

(138) 

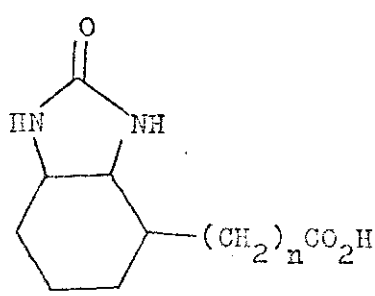
(139) 

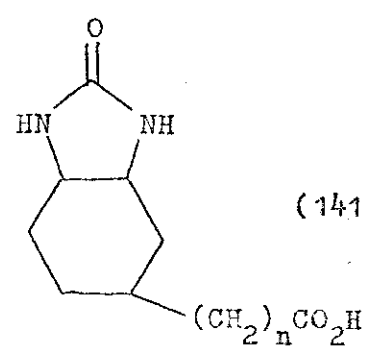
n = 3 or 4 R = Me or H

Carbocyclic derivatives

It is surprising that the carbocyclic derivative of biotin with a cyclopentyl ring has not yet been synthesised and biologically evaluated.

Various cyclohexyl derivatives were made in 1945 by English et al[57,58] with the side chain attached at the positions α or β to the cyclic urea (140,141).

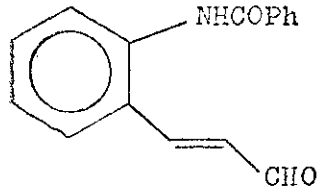
(140) 

(141) 

n = 0, 3, 4.

(140) was prepared from o-benzoylamino-cinnamaldehyde (142), which was readily available from quinoline, by extending the chain length, nitrating selectively in the 6-position, reducing the diamine,

treatment with phosgene, and hydrogenation of the ring.



(142)

The isomer (141) was prepared by Friedel-Crafts acylation of acetanilide in the 4-position and nitration in the 2-position. The conversion to (141) was then carried out in a similar fashion to that given above.

The compounds with n = 0 were inactive, and none of the compounds showed any growth promoting activity. The other four compounds showed good antibiotin activity but their relative activity varied with the organism. For L. casei the most potent was (141, n=3, MIR = 4,000) and the least was (140, n=3), whereas for yeast the most potent was (140, n=3, MIR=1,500) and the least (141, n=3).

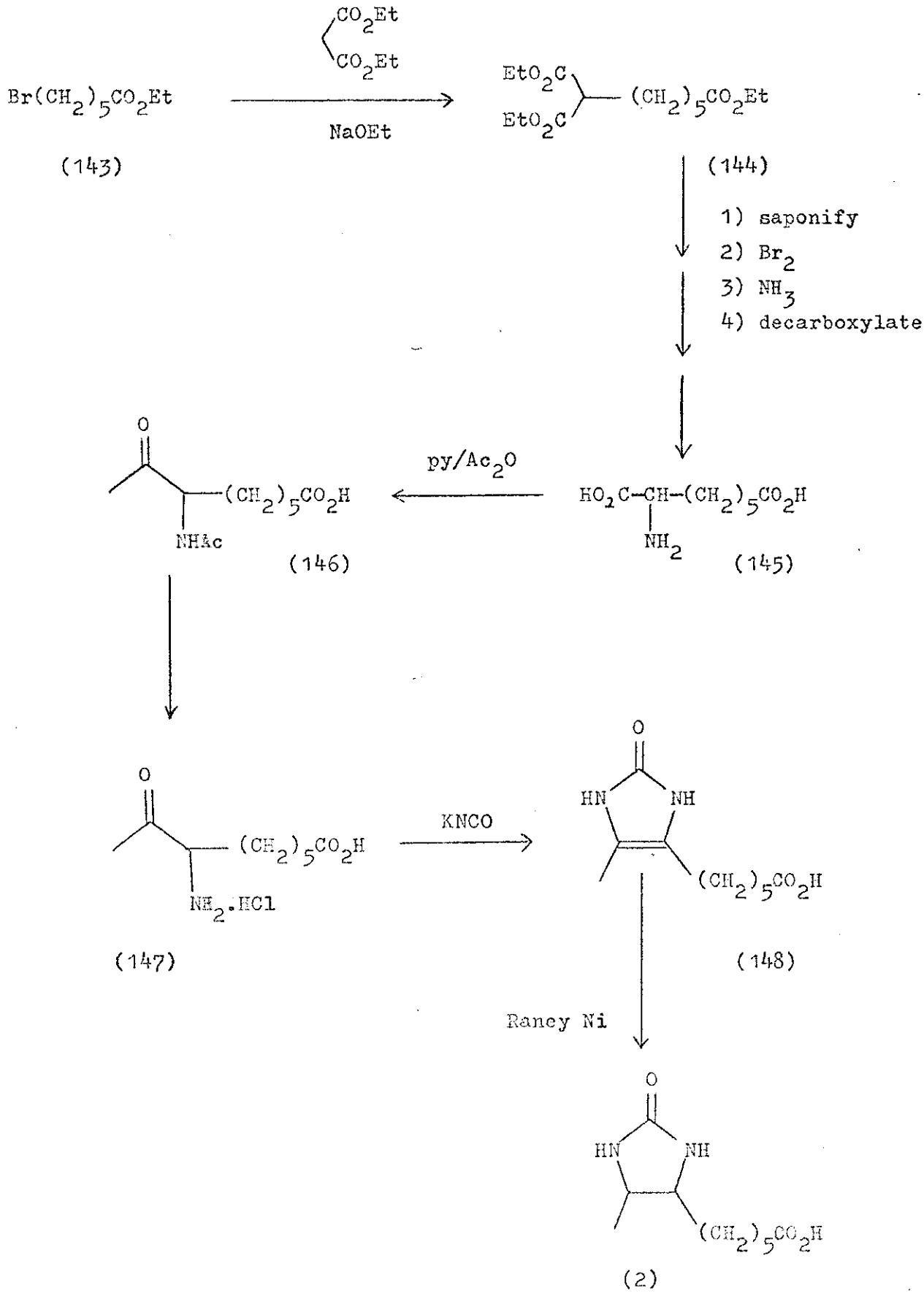
Desthiobiotin

Desthiobiotin (2), the compound produced by the desulphurisation of biotin with Raney nickel[3], acts as a growth promoter in certain micro-organisms[59,60] due to its prior conversion to biotin[61,62] or as an inhibitor in others, e.g. the MIR of d-desthiobiotin for d-biotin in L.casei is 9,000.[59,60]

The first reported synthesis was by Wood and du Vigneaud[63] from ethyl ε-bromocaproate (143) (scheme 21).

The bromide (143) was condensed with diethylmalonate and the diester (144) converted to α-aminosuberic acid (145) by saponification, bromination, amination and decarboxylation. A Dakin-West acylation[64]

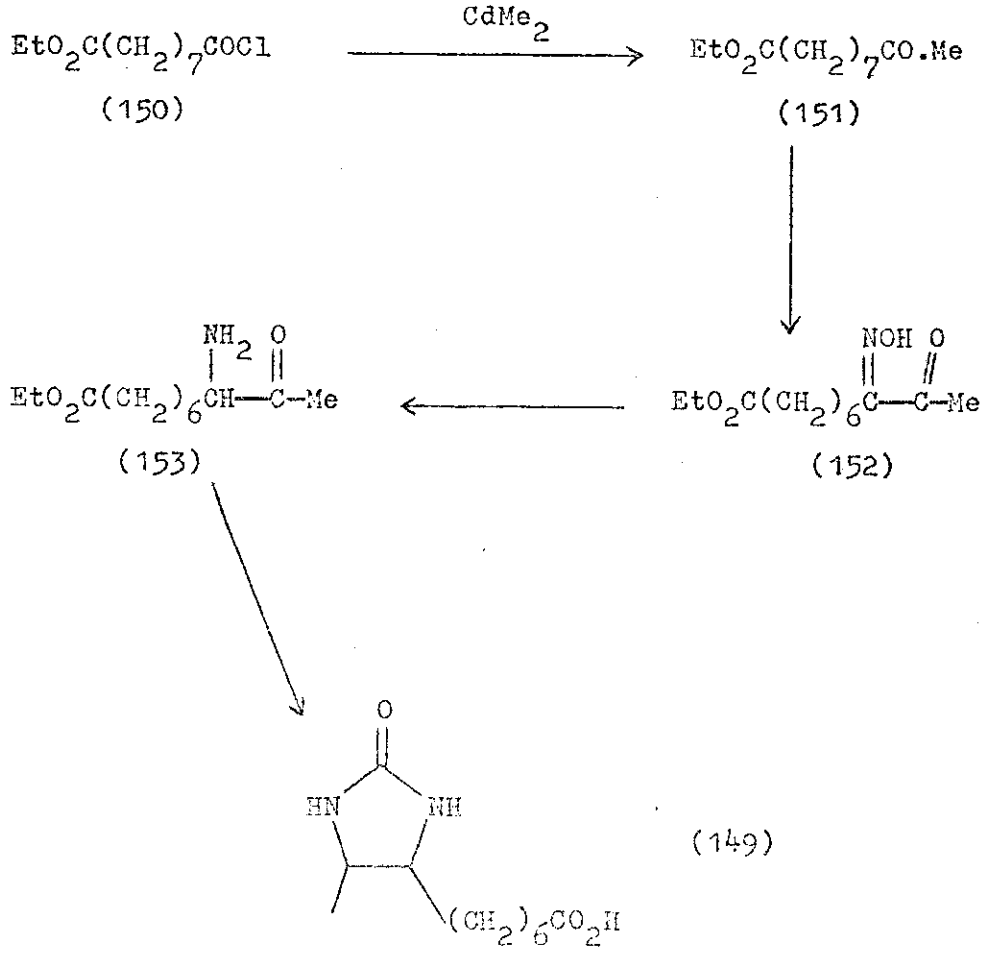
Scheme 21.


Br(CH2)5CO2Et
(143)
CO2Et
CO2Et
NaOEt
EtO2C
EtO2C
(CH2)5CO2Et
(144)
1) saponify
2) Br2
3) NH3
4) decarboxylate
HO2C-CH-(CH2)5CO2H
NH2
(145)
py/Ac2O
O
(CH2)5CO2H
NHAc
(146)
O
(CH2)5CO2H
NH2.HCl
(147)
KNCO
O
HN
NH
(CH2)5CO2H
(148)
Raney Ni
O
HN
NH
(CH2)5CO2H
(2)

of this amino acid gave the β-ketoamine (146) which was hydrolysed to (147). Treatment with potassium cyanate gave the cyclic compound (148) which was hydrogenated to a mixture of dl-desthiobiotin (2) and dl-allodesthiobiotin. They subsequently prepared related compounds to desthiobiotin by an alternative route[65], e.g. homodesthiobiotin (149) (scheme 22).

δ-Carbethoxycapryloyl chloride (150) was converted to the ketone (151) with dimethyl cadmium. Ethyl nitrite reacted to give the α-oximinoketone (152) which was reduced to the aminoketone (153). This was converted to homodesthiobiotin by the same reactions as before.

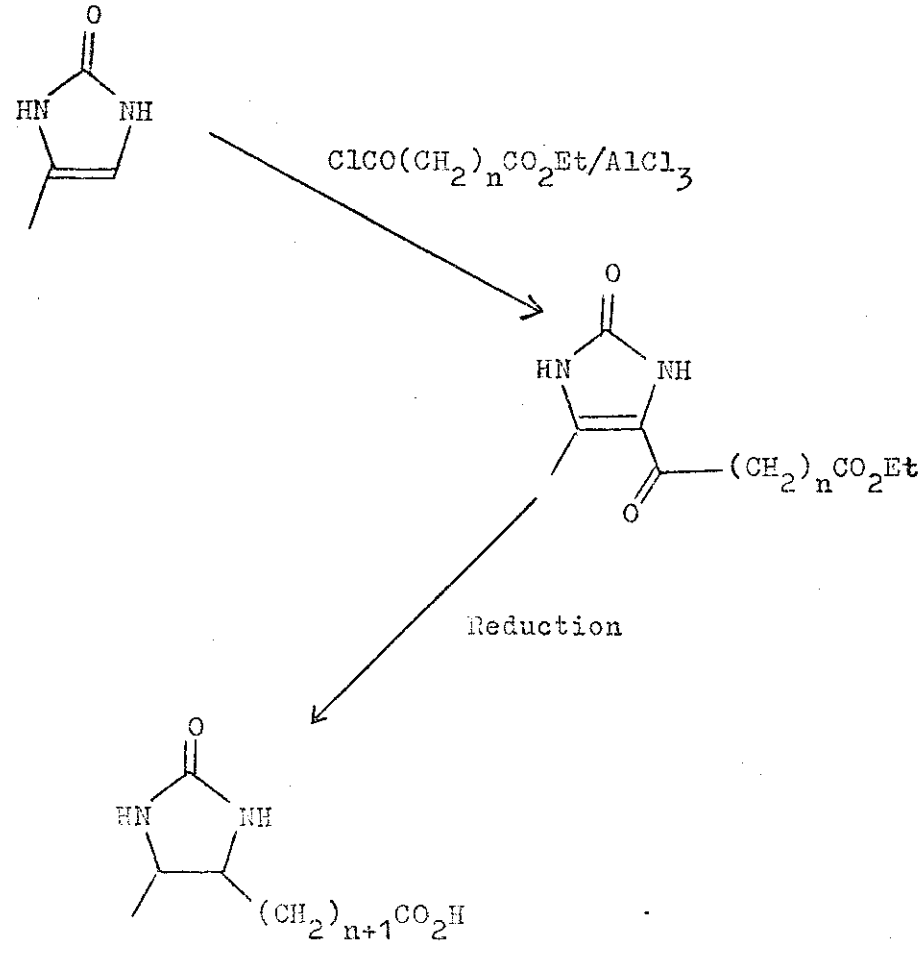
Scheme 22.



It was found that homodesthiobiotin inhibited the growth of yeast but no quantitative data was given.[65] This was probably due to its prior conversion to homobiotin.

An alternative route to dl-desthiobiotin and related compounds was carried out by Duschinsky and Dolan[50] (scheme 23) by a Friedel-Crafts type acylation of 4-methylimidazolone and subsequent reductions.

Scheme 23.



Unlike du Vigneaud et al they did not isolate any allodesthiobiotin suggesting that catalytic hydrogenation using platinum is more specific than Raney nickel.

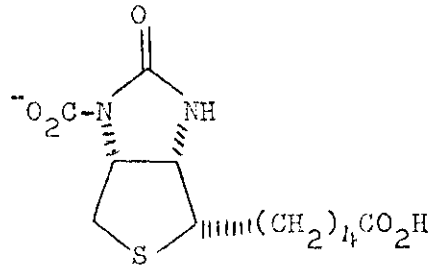
Filippov and his coworkers have investigated the desthiobiotin field in the last few years[66,67] and have published a different route (scheme 24)[66] to the dehydrodesthiobiotin (148) although it is basically a modification of du Vigneaud's original method.

Benzoyl alanine and the acid chloride of monomethyl pimelate were combined in a Dakin-West reaction to give the β-ketoamine (154) which was hydrolysed and treated with potassium cyanate to give (148).

They state,[68] like du Vigneaud, that hydrogenation of (148) with Raney nickel gave rise to a mixture of isomers but doing it in the presence of NaOH the all cis- isomer was obtained.

Variations in the imidazolone ring.

Lynen has shown[69] that carboxylations by biotin go through the N-carboxybiotin intermediate (155).

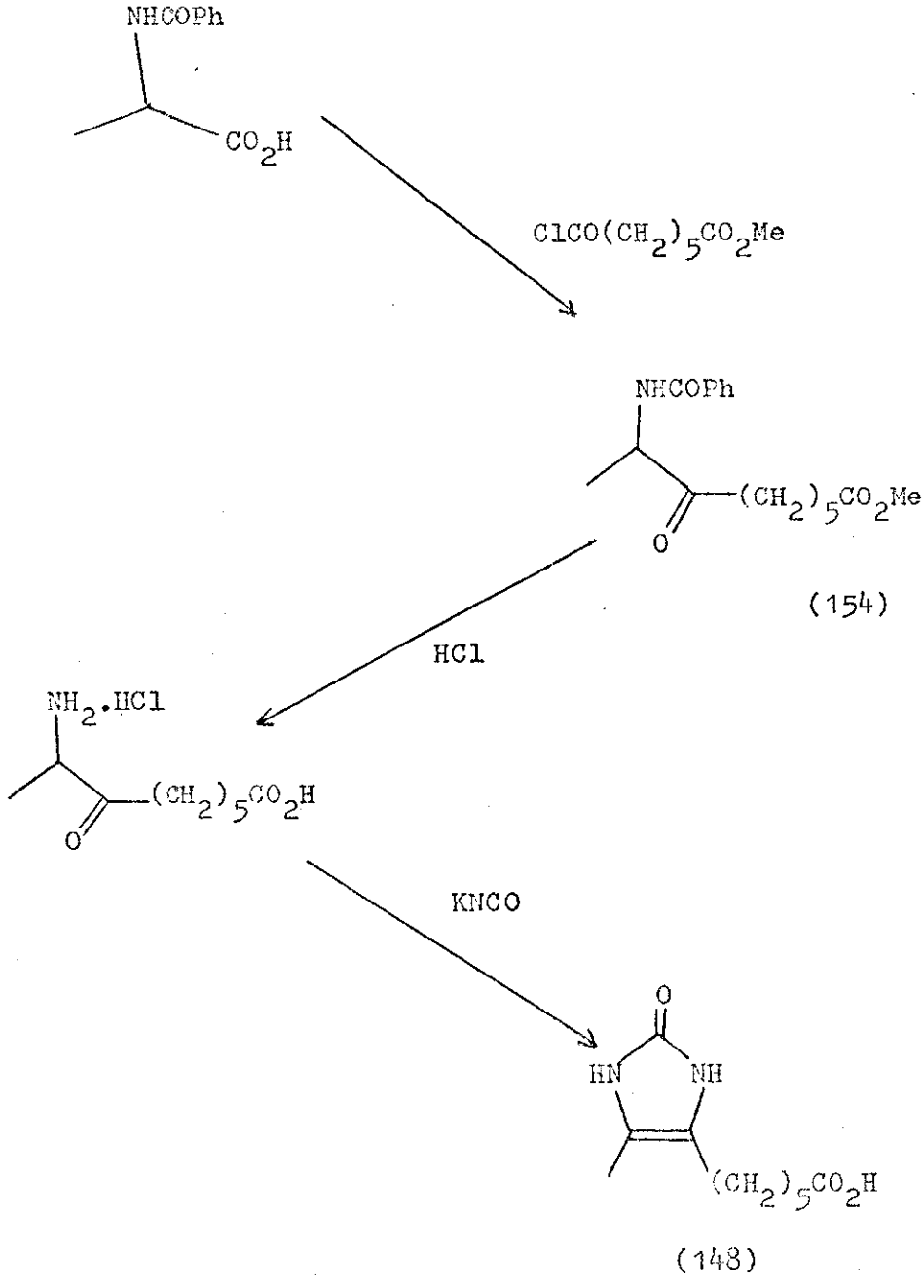


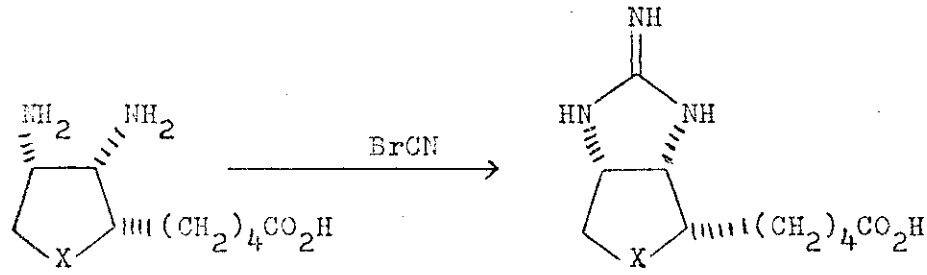
(155)

This therefore suggested to various workers that variations in the imidazolone ring should inhibit biotin action to a certain extent.

Hofmann and Axelrod[70] prepared the iminobiotins (157) by treatment of the corresponding diaminobiotins (156) with cyanogen bromide. They found that the imino compounds were devoid of any biological activity though they do not specifically give any details of antibiotin activity.

Scheme 24.


NHCOPh
CO2H
ClCO(CH2)5CO2Me
NHCOPh
(CH2)5CO2Me
O
(154)
HCl
NH2.HCl
(CH2)5CO2H
O
KNCO
O
HN
NH
(CH2)5CO2H
(148)

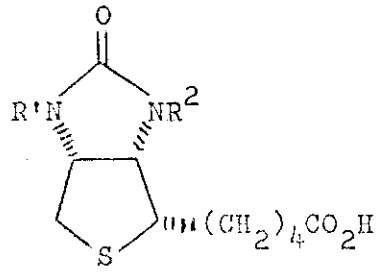


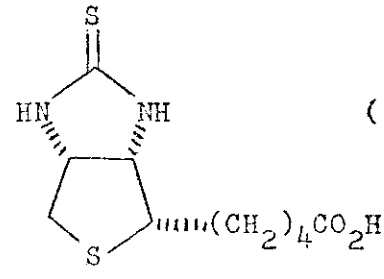
(156) (157)

X = S or O

Jansen and Stokes[71] made a series of N-substituted biotins (158). ~~Diamino~~ Biotin (158, $R^1=R^2=H$) reacted with formaldehyde in the presence of formic acid to give N,N'-dimethylbiotin (158, $R^1=R^2=Me$). ~~after cyclisation with phosgene~~. In the absence of formic acid they obtained N,N'- bishydroxymethylbiotin (158, $R^1=R^2=-CH_2OH$). The mono- and diacetylated compounds (158, R^1 = Ac, R^2 = Ac or H) were formed on treatment of biotin with acetic anhydride.

(158)





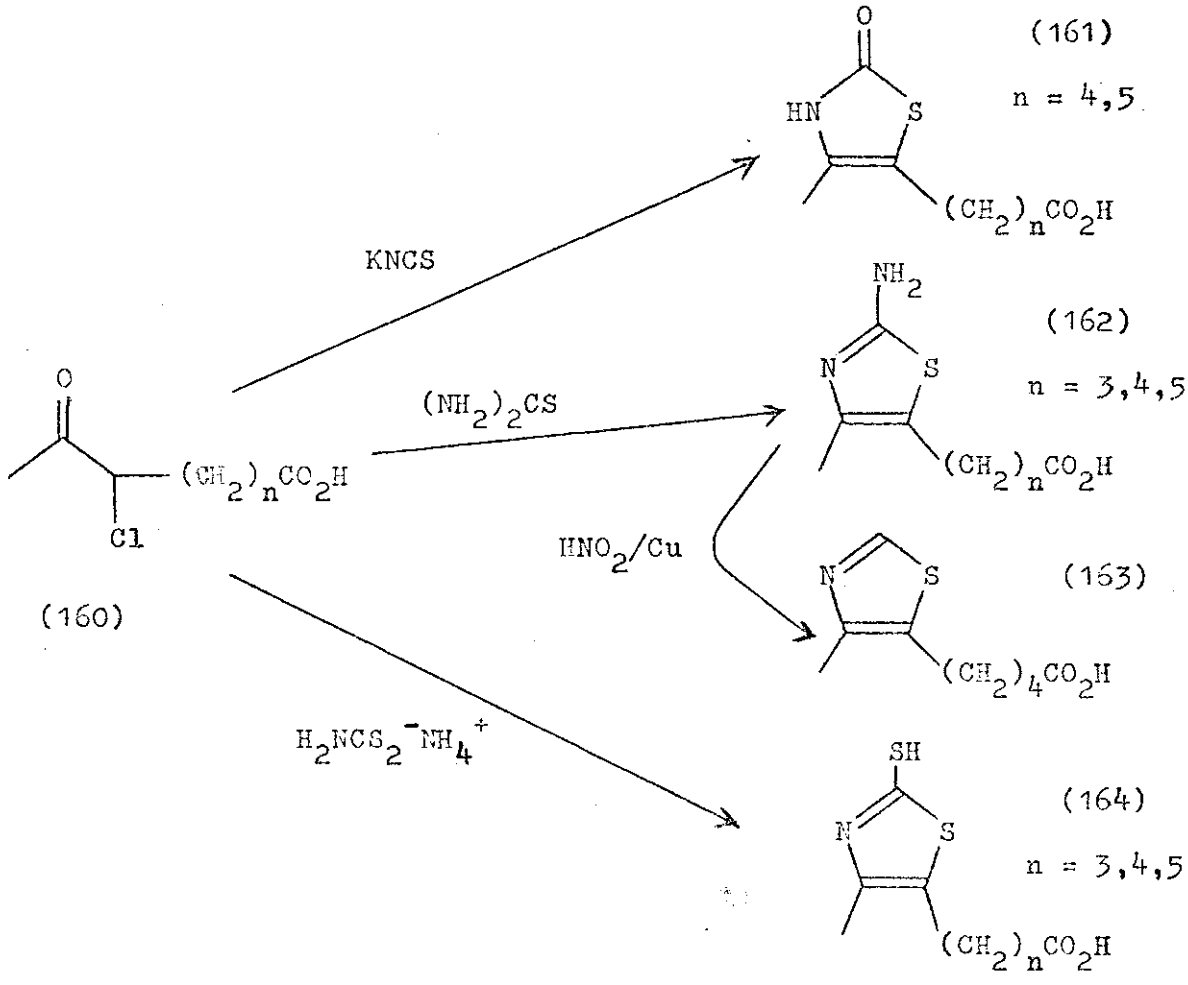
(159)

They also prepared the thione derivative (159) by treatment of the diamino biotin (156, X=S) with carbon disulphide and acid.

None of the compounds prepared showed any antibiotin activity against a number of micro-organisms although the N,N'-dimethylbiotin did support growth in L.arabinosus probably due to prior demethylation to biotin.

Some thiazole derivatives of dehydrodesthiobiotin have also been prepared from the α-chloroketones (160).[72]

Treatment of (160) with potassium thiocyanate gave the simple thiazolones (161), whilst thiourea gave the 2-aminothiazoles (162), which could be converted to the thiazoles (163), and ammonium dithiocarbamate gave the mercaptothiazoles (164).



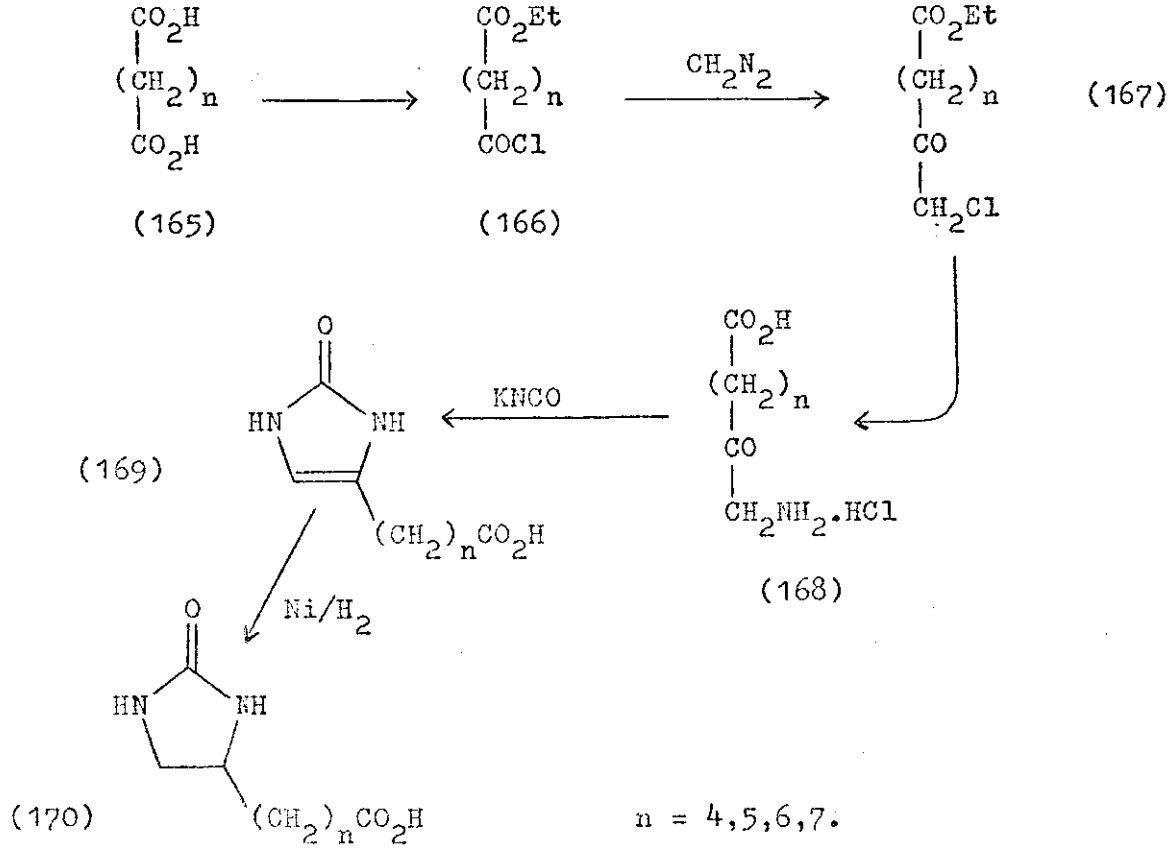
None of the compounds prepared inhibited growth in any of the microorganisms tested. One cannot help feeling that they may have had better results if the compounds had been hydrogenated.

Imidazolidone aliphatic acids.

Dittmer and du Vigneaud[73,74] prepared four imidazolidone aliphatic acids analogous to desthiobiotin but without the 5-methyl group.

The route (scheme 25) consisted of converting a dicarboxylic acid (165) to the acid chloride of the half ester (166). Treatment with diazomethane gave the α-chloroketone (167) which was converted to the α-aminoketone (168) via the phthalimide. Reaction with potassium isocyanate led to the cyclic compound (169) which was hydrogenated to the imidazolidone aliphatic acids (170).

Scheme 25.



In tests against S.cerevisiae and L. casei the dl-caproic acid (170, n=5) was the best antibiotin (MIR 8.5×10^5 and 10^5 respectively) and the dl-valeric acid (170, n=4) the worst. They showed that decreasing the side chain, n=5, by one carbon atom decreases the

activity drastically whereas increase caused the activity to decrease to a lesser extent. It should be noted that imidazolidone caproic acid has the same chain length as desthiobiotin (2).

The mechanism of biotin action.

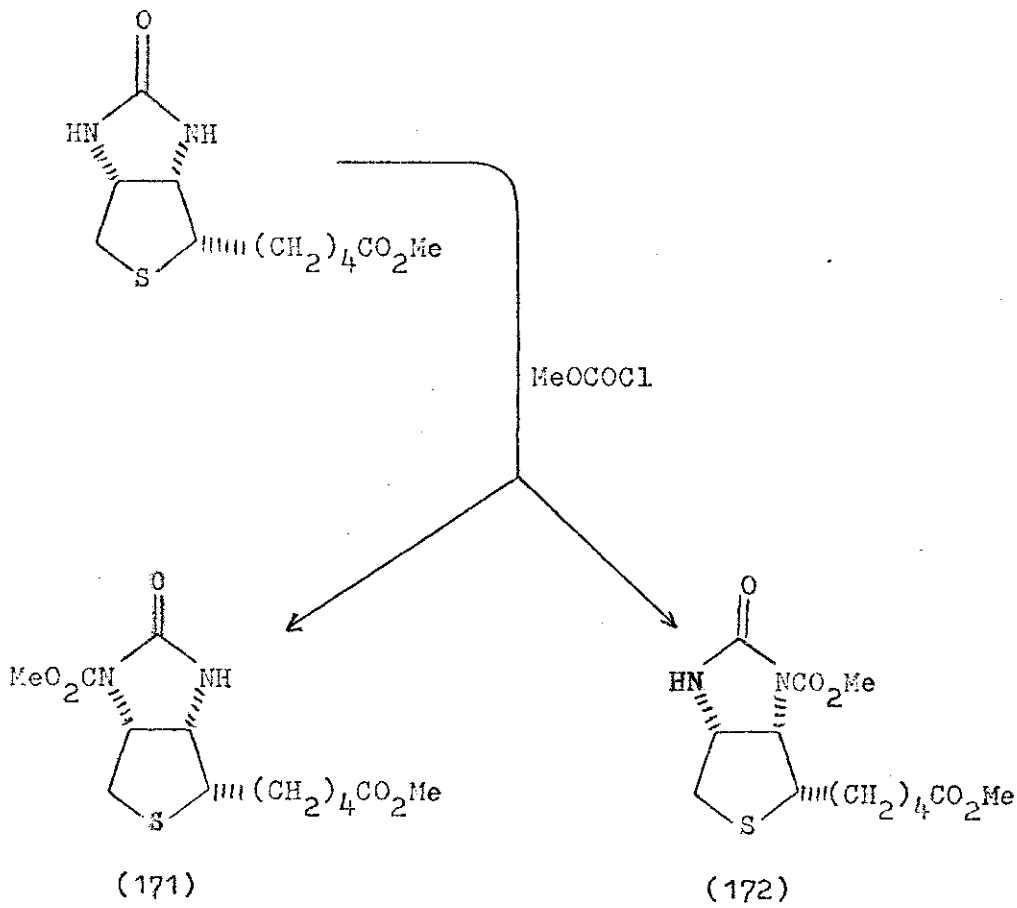
The mechanism of biotin action has been discussed in depth in recent articles.[75,76] This brief synopsis is intended to serve as a guide to the currently accepted mechanism of biotin action and to try to correlate this to the mode of inhibition produced by the antibiotins.

The principle role of biotin is in carboxylations and decarboxylations. The overall reaction catalysed by biotin-based enzymes involves two successive half-reactions, which can be expressed generally, for the case of carboxylases, as:-

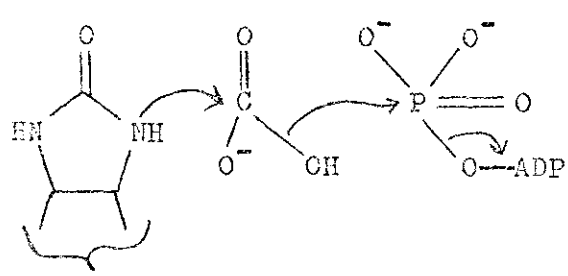
$$\text{E-biotin} + \text{ATP} + HCO_3^- \xrightleftharpoons{Mg^{2+},Mn^{2+}} \text{E-biotin}—CO_2^- + \text{ADP} + \text{Pi}$$

$$\text{E-biotin}—CO_2^- + \text{RH} \rightleftharpoons \text{E-biotin} + \text{R}—CO_2^-$$

The nature of the carboxy-biotin intermediate was first ascertained by Lynen et al.[69,77,78,79] In experiments with C^{14}-bicarbonate they succeeded in isolating carboxy-biotin. The adduct was very labile to acid and $^{14}CO_2$ was liberated even at low temperatures. This led them to consider it to be an N-carboxy species and it was subsequently stablilised by treatment with diazomethane to give the methyl ester and its structure proved by synthesis. Biotin methyl ester was treated with methyl chloroformate to give the two isomers (171) and (172) in the ratio of 100:7. The low yield of (172) is explained by the fact that N-3 is sterically hindered by the valeryl side chain. The enzymatically formed carboxy-biotin methyl ester was shown to be identical to (171) and it was also established that the enzymatic reaction gave rise exclusively to isomer (171). The structure was later conclusively proved by X-ray analysis of the di-p-bromoanilide derivative of the methyl ester.[80]



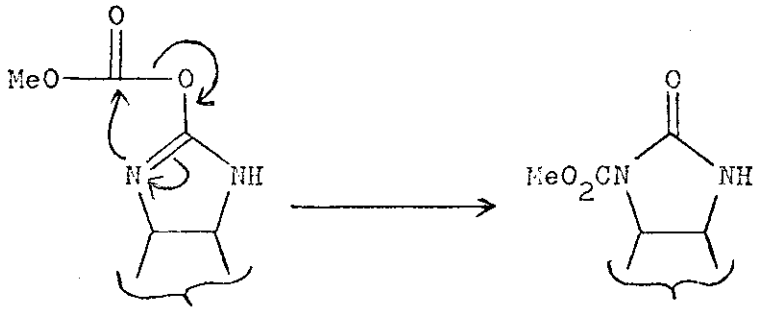
Lynen[78] proposed that the mechanism of the carboxylation of biotin by bicarbonate, which requires ATP, proceeded as follows to give ADP and inorganic phosphate.



Recently Hegarty and Bruice[81,82] have suggested that the initial site of carboxylation was the ureido oxygen atom instead of the nitrogen.

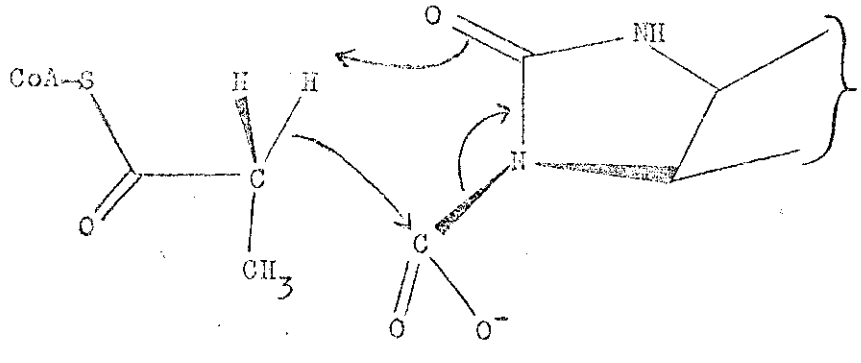
This was substantiated with some model studies[83] in showing that O-acylisoureas had a high acyl transfer potential, whereas the N-carboxy imidazolone system[84,85] was an indifferent carboxyl (and acyl) transfer reagent.

Hegarty and Bruice suggest that Lynen isolated the N-carboxy-biotin due to intramolecular transfer of the methoxycarbonyl group (after treatment with diazomethane) from oxygen to nitrogen, to give the thermodynamically more stable product.

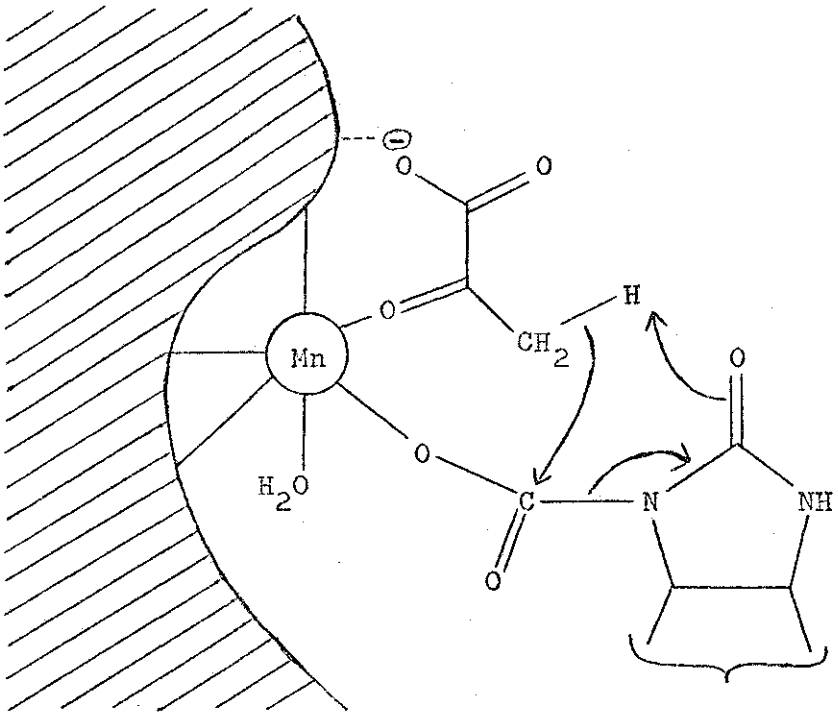


If this postulated nonenzymatic shift occurs, it is not apparent why the sole methylated product obtained by Lynen, from the enzymatic carboxylation of free biotin, was the 1-N-carboxybiotin rather than the 100:7 mixture of 1-N- to 3-N--carboxybiotin, as obtained by chemical synthesis.

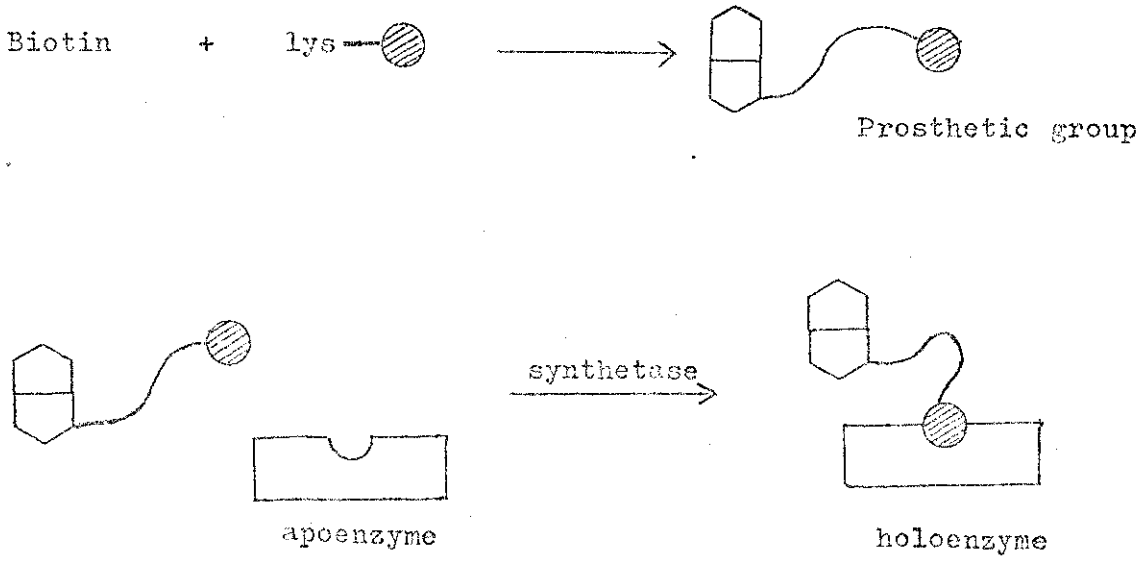
The exact nature of the carboxylation half reaction is not entirely clear but cyclic mechanisms are favoured. Lynen[86] has proposed a concerted cyclic mechanism for the carboxylation of propionyl - CoA with retention of configuration at C-2 as found by tritium labelling experiments.



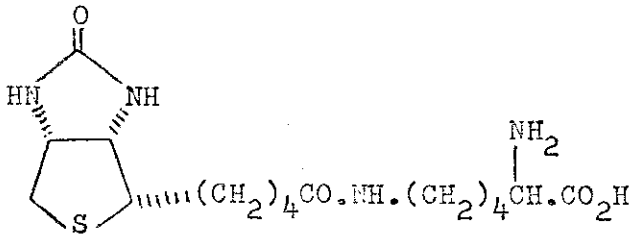
In the case of carboxyl transfers to α-ketoacids the participation of Mn^{2+} has been shown to be necessary, and Mildvan and Scrutton[87] have shown that there is a direct linkage between pyruvate and the enzyme bound manganese. The following transfer mechanism was proposed;-



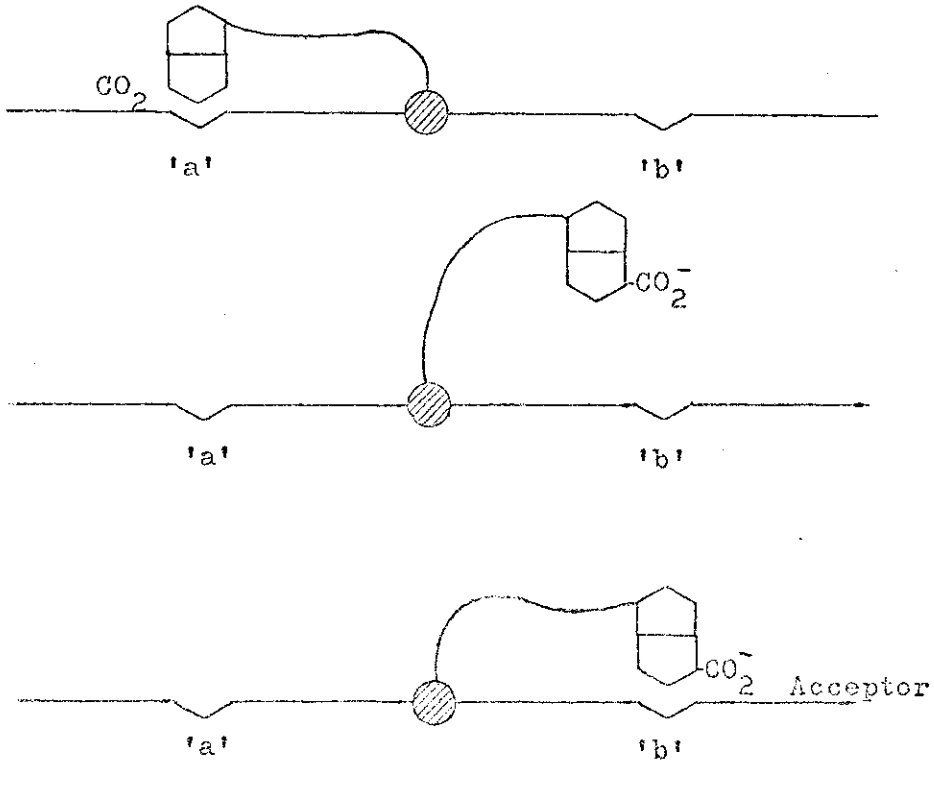
The final step in the formation of the biotin carboxylating enzyme is believed to be the covalent attachment of the prosthetic group to the apoenzyme to form an active holoenzyme.



Biotin is known to be joined to the prosthetic group via a lysine residue. The biotin-lysine conjugate was isolated and shown to be ε-N-biotinyl-L-lysine, known as biocytin.[88]



As a result of kinetic experiments the holoenzyme is believed[89] to have two distinct active sites; 'a', where biotin is carboxylated and 'b', where transcarboxylation to an appropriate acceptor takes place. The biotinyl moiety is assumed to take on a carboxyl group at site 'a', flip to a neighbouring site 'b' and transfer its activated CO_2 in a type of "ping-pong" mechanism.



It is unlikely that the sulphur atom takes any important part in the biotin action since oxybiotin[52,53] and biotin sulphone[41] both show growth promoting activity. The fused 5-membered ring system though is probably critical as imidazolidone caproic acid[73,74] and the cyclohexane analogue of biotin[57,58] are inactive as is desthiobiotin without prior conversion to biotin.[54] On the basis of this evidence it appears that a five membered ring is essential but the nature of the ring is not necessarily important. This probably indicates that the 5-membered ring exerts a certain amount of strain on the imidazolidone ring which influences the basicity of the ureido group. In this respect it is important that biological evaluations be carried out on azabiotin and upon the unknown carbocyclic analogue of biotin.

Considerations of the mechanism of biotin action would indicate that there are four main ways in which biotin activity might be inhibited.

1) By blocking the biosynthesis of biotin or the enzymes.
2) By blocking the synthetase which binds the prosthetic group to the apoenzyme.
3) By preventing the biotin nucleus from taking up CO_2.
4) By preventing the biotinyl — CO_2 moiety from transferring its CO_2 to the acceptor.

The first two methods are probably impractical and it is the latter two which are of interest.

The most obvious way of preventing CO_2 uptake would be to block the nitrogen atoms with, say, alkyl groups. In the simplest case the N,N'-dimethyl compound (158) was prepared[71] but proved to be demethylated back to biotin. The dibenzyl and diacetyl analogues were inactive.

Variation in the basicity of the ureido group should influence the uptake of CO_2. Only two specific examples of this, i.e. imino biotin (157)[70] and thiobiotin (159),[71] have been prepared and both appear to have no antibiotin activity. The cyclohexane analogues(140,141) of English et al.[57,58] fit

into this group as well, due to the lessening of the strain imposed on the cyclic urea function, and these did show some antibiotin activity. It may be of interest to prepare compounds in which the urea function is present in a 6-membered ring.

A third method of preventing CO_2 uptake would be to lengthen or shorten the biotin side-chain or hinder the approach of the biotin nucleus to the active site. From the good inhibition results given by homo- and nor-biotin and also by α-methylbiotin[29] (84) this hypothesis would appear to be valid.

Variation in the chain length, and hindering groups would also inhibit the transfer of the biotinyl-CO_2 moiety to the second active site.

An alternative method of preventing CO_2 transfer to the acceptor would be to stabilise the CO_2 complex but this is probably difficult whilst keeping the important parts of the molecule the same.

A third method of preventing flipping from sites 'a' to 'b' would be to bind the biotinyl moiety to site 'a' and it is in this way in which α-dehydrobiotin (83)[23] probably functions. The double bond in the side chain is likely to be bonded somehow to the holoenzyme, thus preventing 'flipping'.

Little work has been carried out on this latter type of antibiotin and compounds with suitably substituted or modified side chains merit attention in the future.

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DISCUSSION

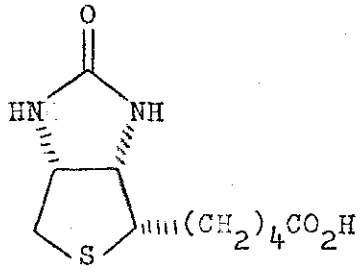
AND

RESULTS

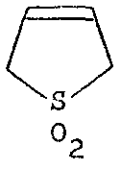
SYNTHETIC APPROACHES TO BIOTIN

Introduction

The prime objective of the work was to devise a synthesis of biotin (1) starting from 3-sulpholene (2). The route had to be stereospecific, fairly general and flexible so that various biotin analogues could be prepared and their growth inhibitory properties in micro-organisms studied.

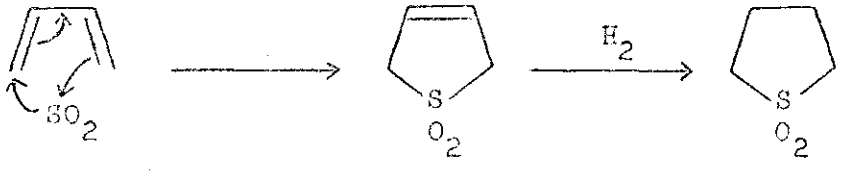


(1)



(2)

3-Sulpholene (butadiene sulphone)(2) is a cheap, readily available compound prepared industrially by the reaction between butadiene and sulphur dioxide under pressure[1] (scheme 1). The bulk of sulpholene commercially produced is not used as such but is hydrogenated to sulpholane (3), a very useful solvent. It was of interest to examine the possibility of using sulpholene and its derivatives in organic synthesis and to study the chemistry of substituted sulpholenes further.



(2) (3)

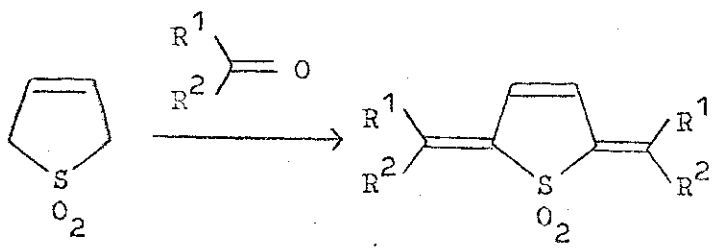
Scheme 1

A cursory look at the proposed starting material and the product shows the three main areas into which work had to be carried out.

These are 1) the introduction of the cis-cyclic urea function at the 3- and 4-positions, 2) the introduction of a valeric acid side chain at the 2-position cis to the urea ring, and 3) the reduction of the sulphone group to a thio-ether linkage.

The reduction step had sufficient literature precedent to suggest that the removal of the oxygen atoms might be fairly easy. Bordwell and McKellin[2] have published two reliable methods using either zinc and acid, or lithium aluminium hydride, for the reduction of simple sulphones, including sulpholane, to the respective sulphides. There are also other, more abstruse, methods recorded in the earlier literature using sulphur,[3] phosphorus pentachloride[4] or hydrogen sulphide[4] as reducing agents.

Similarly the alkylation at the 2-position did not seem to present too many problems since it was well known that sulpholene can form an anion at the 2-position as was demonstrated by its ready deuteration in weak $NaOD/D_2O$ solutions.[5] Condensations at the 4- and 2-positions have also been performed in basic solution with acetone and benzaldehyde[6] (scheme 2).

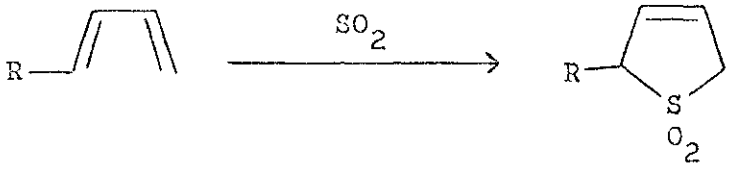


$R^1 = R^2 = Me$ or $R^1 = H$, $R^2 = Ph$

Scheme 2

There are also many reports of α-metalation of sulphones to give anions which will react with ketones etc.. Such reactions have been induced with ethyl magnesium bromide,[7,8] butyl lithium[8,9] and lithium amide[10,11] upon sulpholane and other systems.

A third possible route to 2-substituted sulpholenes is by the reaction of substituted butadienes with sulphur dioxide.[1]



The fusion of the cyclic urea group onto sulpholene had no literature precedent and work had not been done in this field. It was therefore decided to concentrate upon the introduction of nitrogen functions into the 3- and 4-positions of sulpholene before the other two problems were investigated in depth.

Iodine isocyanate and model compounds.

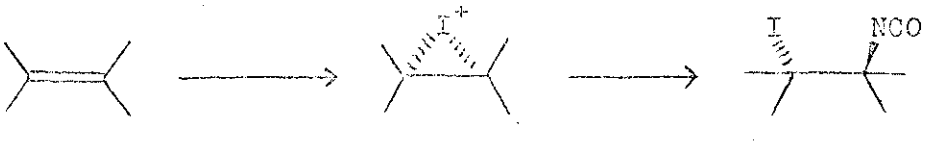
The first method of introducing the urea function to be considered was by the reaction of the double bond of sulpholene with iodine isocyanate.

Birckenbach and Linhard[12] were the first to show that iodine isocyanate adds to olefinic double bonds, though it was only recently that Hassner and Heathcock examined the synthetic utility of the reaction[13] and Gebelein and Swern studied the kinetics.[14,15]

Iodine isocyanate is prepared *in situ* from iodine and silver isocyanate in either an ether[13] or a dichloromethane[14] solution of the olefin.

$$AgNCO + I_2 \rightleftharpoons AgI + INCO$$

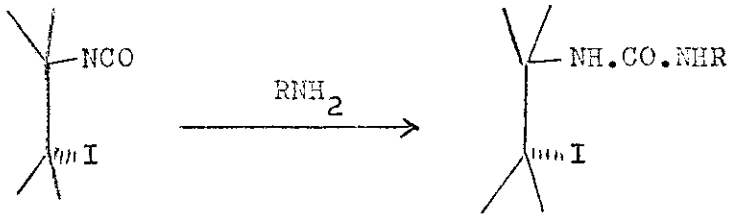
It has been shown that the addition occurs in a stereospecific *trans*- manner and it is suggested that an iodonium ion is involved as an intermediate[16,17] (scheme 3).



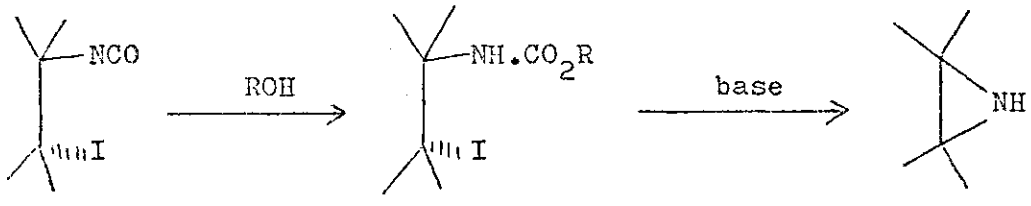
Scheme 3

The isocyanates so formed can then be treated in one of three ways;

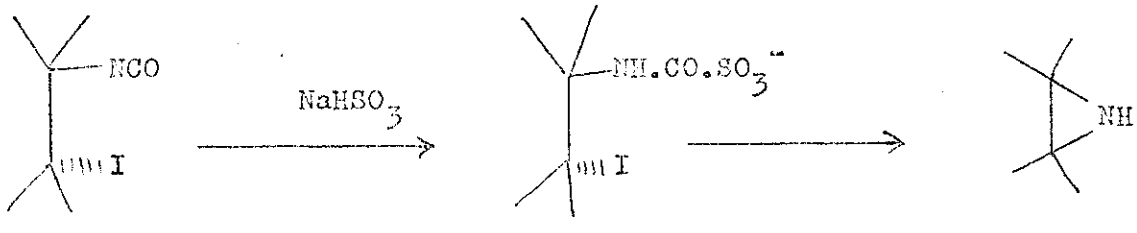
1) reaction with an amine (or ammonia) to give a substituted urea:-



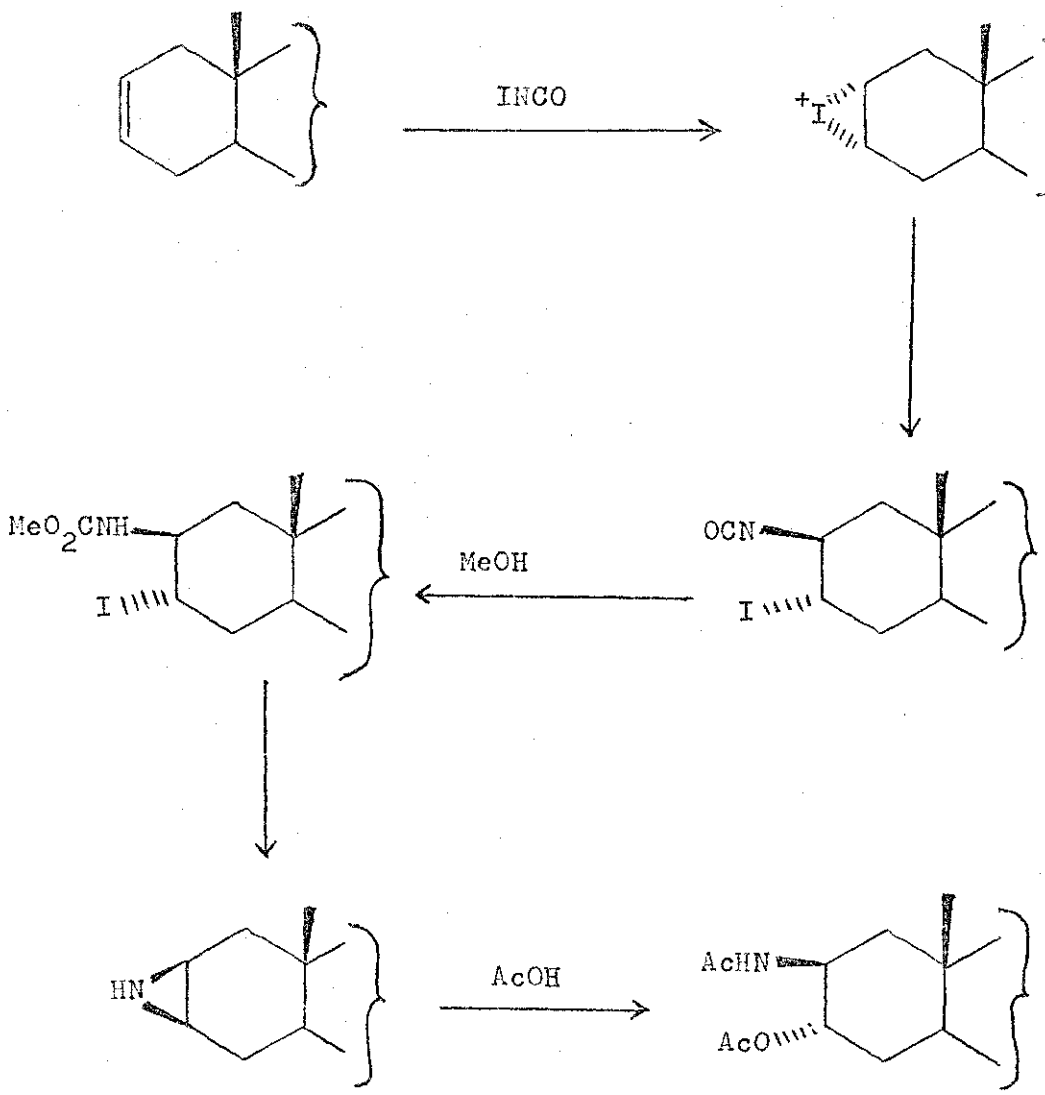
2) reaction with an alcohol to give a carbamate, and then with base to form an aziridine:-



3) reaction with sodium bisulphite to give a bisulphite adduct which can be converted to the aziridine with base:-



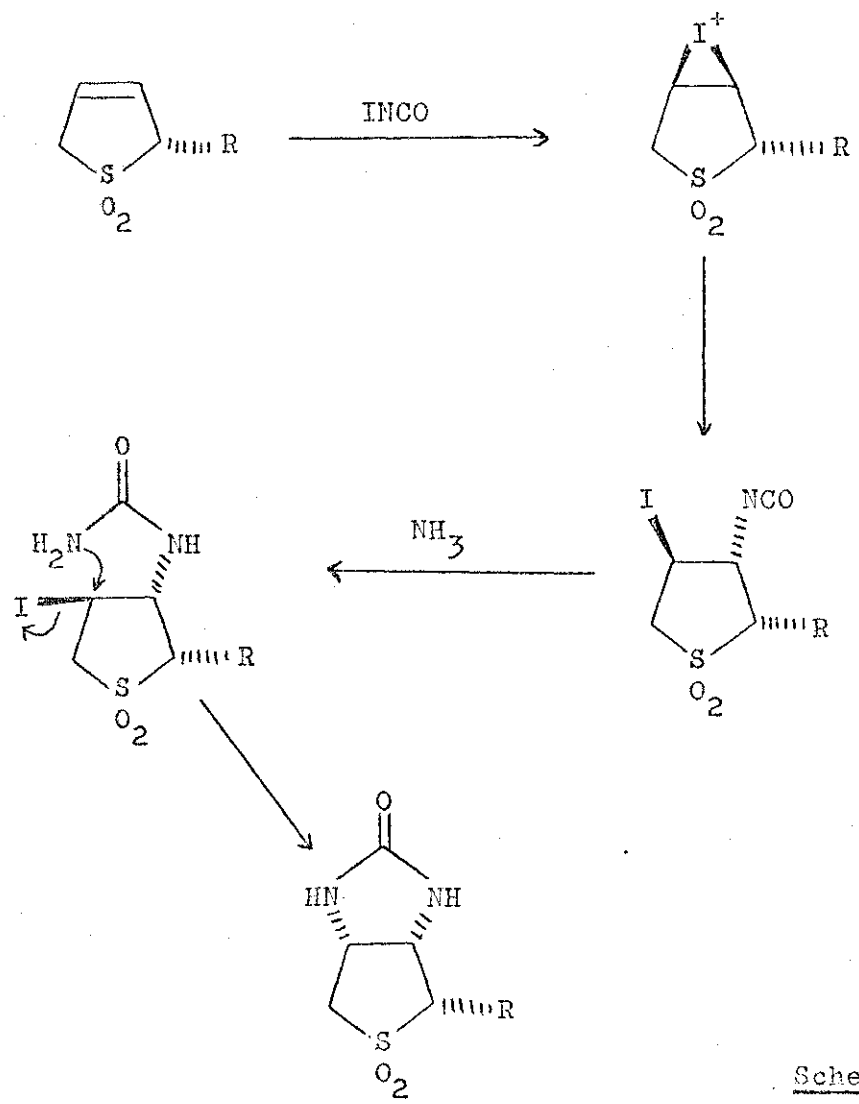
The stereochemical course of the formation of the aziridine ring was studied by the reaction with cholest-2-ene and shown to be as follows:-[17,18]



(4)

The stereochemistry of (4) was ascertained by comparison with an authentic sample prepared by an alternative route via the epoxide. Notice that the iodonium ion forms on the least hindered side of the molecule.

It was proposed that if iodine isocyanate reacted with a sulpholene, stereospecific addition would take place as shown, to give the isocyanate which could be converted to the urea with ammonia. Cyclisation of this should give the biotin configuration (scheme 4.).



Scheme 4

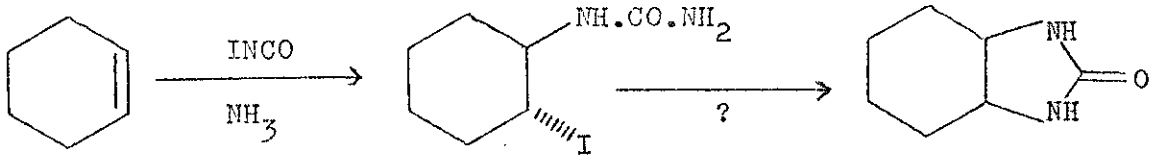
Unfortunately sulpholene could not be induced to react with iodine isocyanate under any conditions. This apparent lack of reaction was probably due to the deactivation of the olefinic double bond by the electron withdrawing effect of the sulphone group.

Although this seemed to preclude the use of iodine isocyanate for the introduction of the ureido-ring it was of interest to investigate the generality, if any, of using β-iodoureas to form cyclic ureas.

Cyclohexene was accordingly chosen as a model compound to test the feasibility of the reaction (scheme 5) since the expected products,

cis- (5a)[19,20] or trans- hexahydrobenzimidazolone[21,22] (5b) were known compounds.

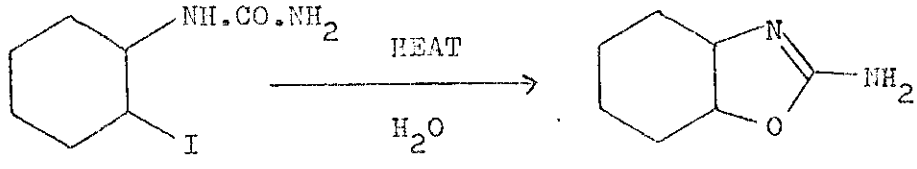
2- Iodocyclohexyl urea (6) was prepared by treatment of cyclohexene with silver isocyanate and iodine in dichloromethane solution with subsequent passage of ammonia through the filtered reaction mixture. (It was advisable to ensure that all molecular iodine was removed from the reaction before the addition of ammonia in order to prevent the formation of nitrogen tri-iodide).



(6) (5) a) cis b) trans

Scheme 5

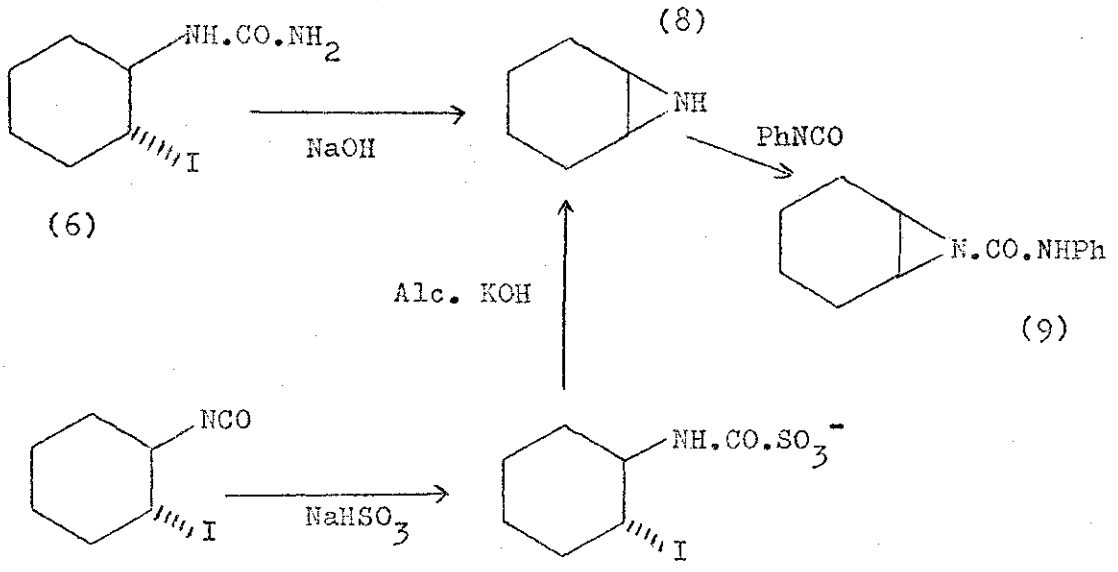
The iodourea is known to undergo cyclisation in boiling water to give the amino-oxazoline (7) (scheme6) as a viscous oil,[12,23] but its reaction with base has not been reported.



(6) (7) Scheme 6

It was found that ethoxide, t-butoxide or aqueous 2N sodium hydroxide had no effect on the iodo-urea at room temperature. However brief boiling with 2N sodium hydroxide, yielded an oil which was shown to be 1,2-iminocyclohexane (8). Reaction of the product (8) with

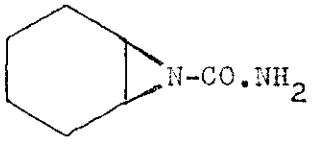
phenyl isocyanate gave the known N-phenylcarbamoyl-1,2-iminocyclohexane (9), previously prepared via the bisulphite adduct,[13] (scheme 7).



Scheme 7

The ring junction protons of (8) and (9) were observed in the ^{1}H n.m.r. at τ 7.8 and τ 7.25 respectively, indicating their presence in a three membered ring and the carbonyl of (9) was at 1690 cm^{-1} in the infrared.

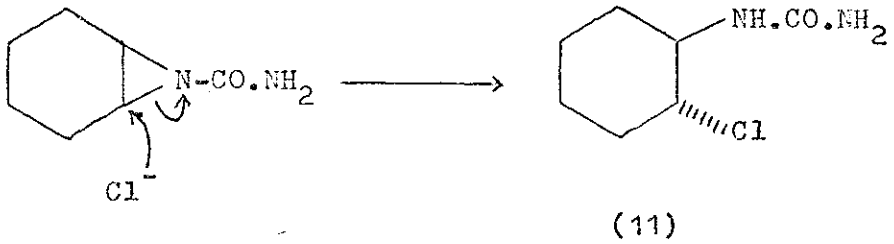
On stirring the iodo-urea (6) with strong base (10N KOH) at room temperature for 10 days, the water insoluble urea went into solution and a crystalline product was isolated which melted at 129°. The ^{1}H n.m.r. had τ 4.45 (2H, exch. with D_2O), 7.35 (2H,m), 8.15 (4H,m), 8.65 (4H,m), the infrared had ν max 3520,3425,1685 cm^{-1}, and the analysis indicated the loss of HI from the starting material. The melting point and the position of the protons at τ 7.35 precluded it from being cis- (5a; m.p. 148°)[19,20] or trans-hexahydrobenzimidazolone (5b; m.p. 233°)[21,22] and structure (10) was proposed.



(10)

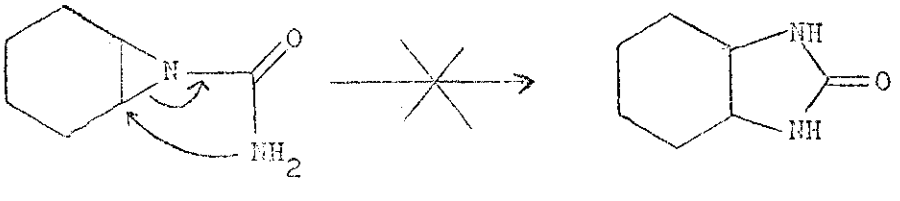
The infrared spectrum of (10) compared favourably with that of N,N-diphenylurea[24] ($Ph_2N.CO.NH_2$) which had ν max. 3530, 3410, 1690 cm^{-1}.

On boiling the aziridine (10) briefly with 5N hydrochloric acid a new compound was formed which had taken up the elements of HCl. The infrared spectrum showed no NH_3^+ peaks but was identical to the iodo-urea (6). It was concluded that the aziridine ring had opened to give the chloro-urea (11).



(11)

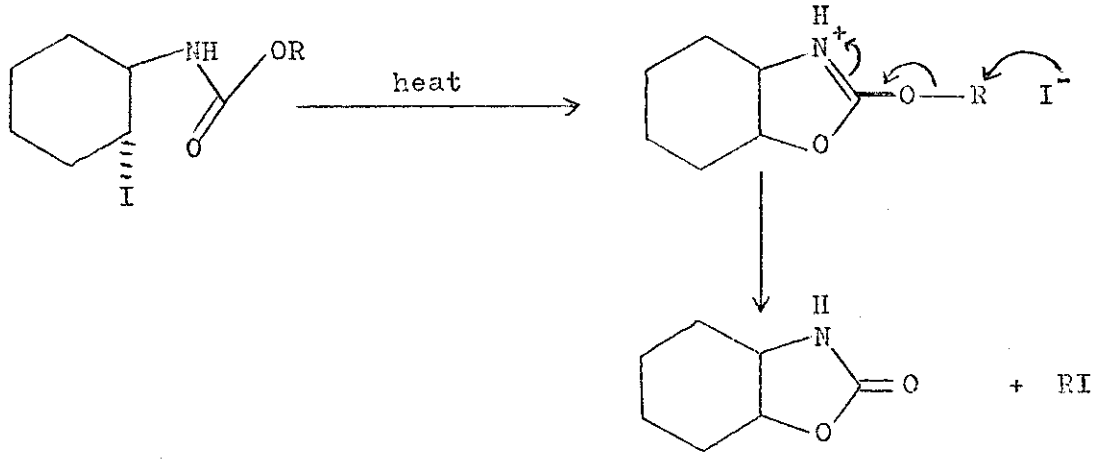
Attempts to thermally rearrange the aziridine (10) to the required imidazolone (5) failed, the aziridine being stable up to 200° (70° above its m.p.).



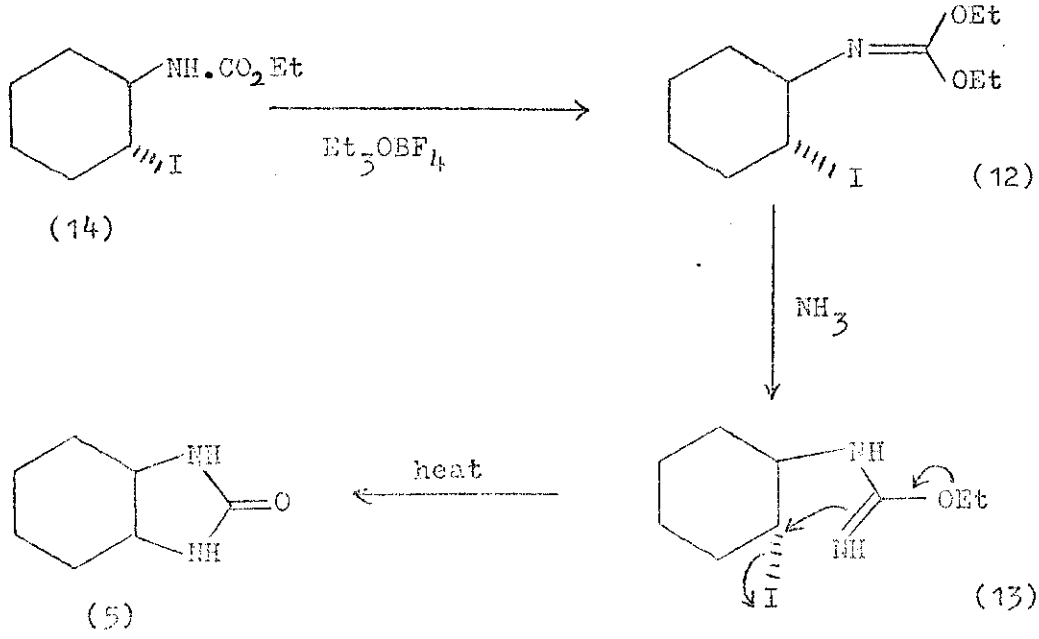
(10) (5)

The cyclisation of the iodo-urea to the aziridine, rather than to the cyclic urea, under the basic conditions of the reaction, must be a consequence of a kinetic effect due to the more favourable entropy associated with this process, viz, a dominant neighbouring group effect.

It is known that the iodo-carbamates formed from the iodo-isocyanates readily pyrolyse to 2-oxazolidones, the following mechanism being proposed,[13,25]



By analogy with this reaction the following route to the imidazolone (5) was attempted, using the iminocarbonate (12) and the iminocarbamate (13) (scheme 8).



Scheme 8

The ethyl carbamate (14) was prepared by the standard route from 2-iodocyclohexyl isocyanate and ethanol. Treatment of this carbamate with triethyloxoniumfluoroborate in boiling dichloromethane gave the iminocarbonate (12), as an oil with ν max. 1670 cm^{-1}. The ^1H n.m.r. was of interest in that the ethyl groups were observed as a double triplet centred at τ 8.7 and a double quartet centred at τ 5.9. The coupling constant between the methyl and methylene groups was 7Hz and the separation between the pairs of quartets and triplets was 2Hz. This splitting is doubtless due to the syn- and anti- positions of the ethoxy groups round the carbon-nitrogen double bond. This explanation was supported by the observation that the signals broadened when the sample was heated.

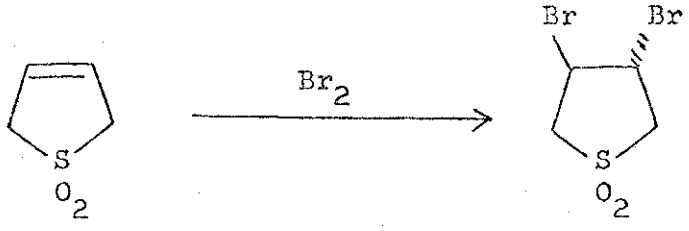
Unfortunately, the imino-carbonate (12) could not be induced to react with ammonia, or in fact any amines, to give the required imino-carbamate.

It was at this point that this type of approach was abandoned.

Substitution and elimination reactions of 3,4-dibromosulpholane.

The second attempt to introduce nitrogen substituents onto the sulpholane nucleus was by direct or indirect displacements on 3,4-dibromosulpholane (14) (3,4-dibromotetrahydrothiophen-1,1-dioxide) by nitrogen nucleophiles.

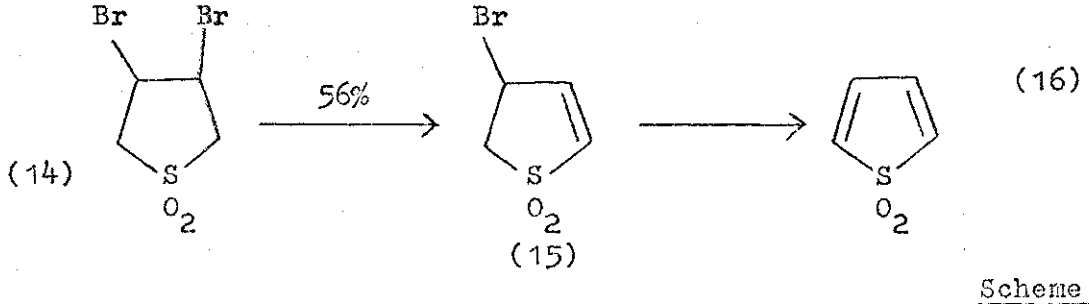
The dibromide (14) was readily prepared by treatment of 3-sulpholene with bromine (scheme9). Although it has never been proved conclusively, the orientation of the bromide atoms was assumed to be trans- by analogy with the mode of addition of bromine to cyclopentene.[26]



(14)

Scheme 9.

Early workers[27] found that the dibromide (14) eliminated HBr on treatment with an equivalent of pyridine to give the allylic bromide (15). This compound eliminated a second molecule of HBr when reacted with piperidine to give thiophen dioxide (16) (scheme 10).

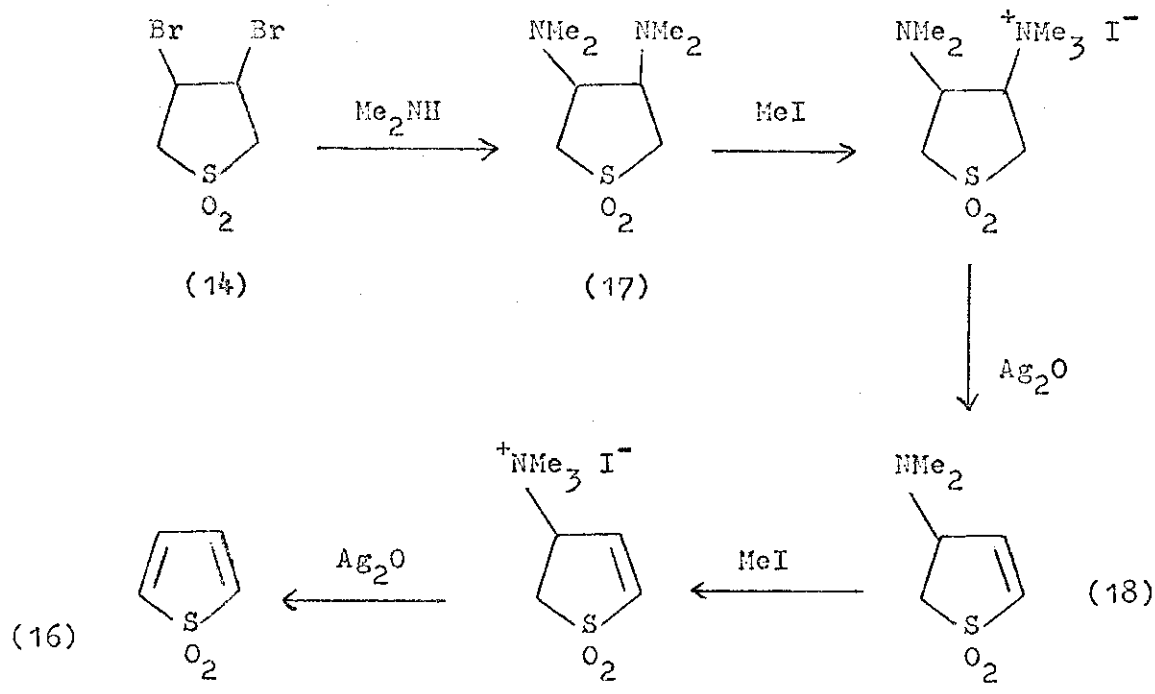


(16)

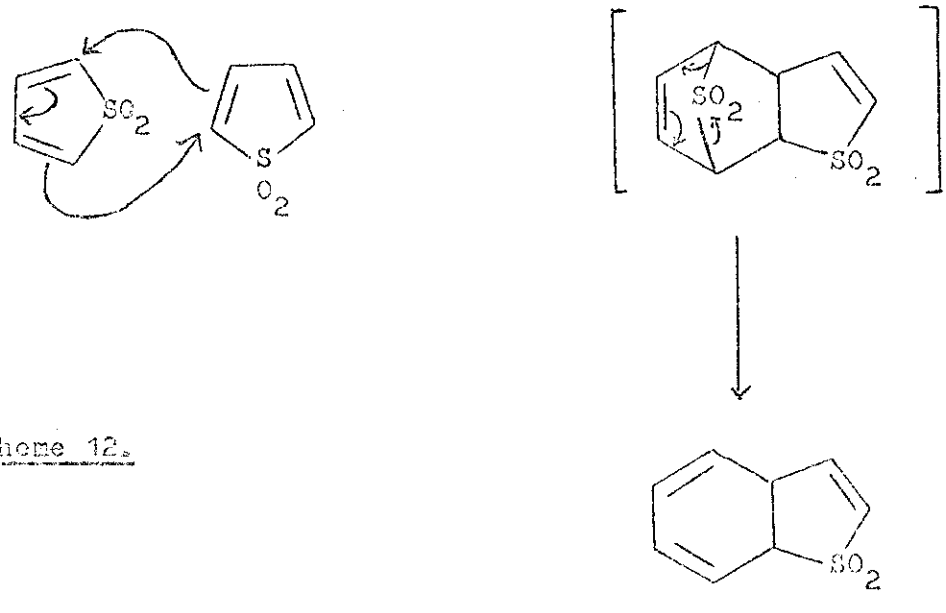
Scheme 10.

Almost concurrently, Bailey and Cummins[28] developed a preparation of thiophen dioxide to gain knowledge of its aromatic character (they in fact showed that it had none). Their five step synthesis involved the exhaustive methylation of 3,4-bis(dimethylamino)tetrahydrothiophen dioxide (17) (scheme 11) and went in an overall yield of 73%.

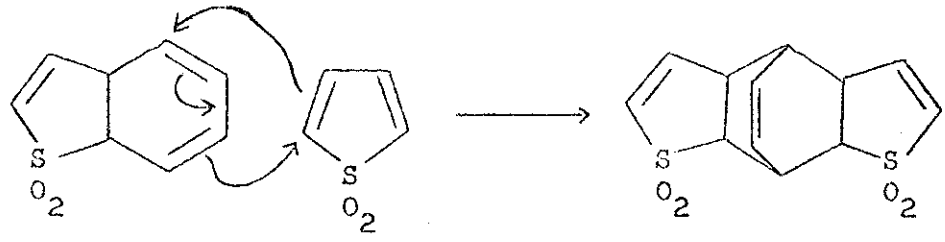
Scheme 11.



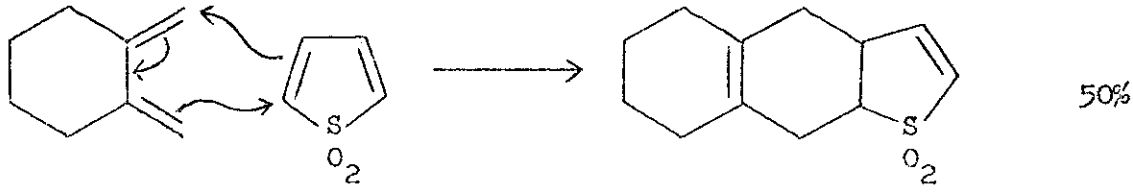
Thiophen dioxide reacts both as a diene, and as a dienophile, and dimerises[29] or trimerises[27] upon isolation with loss of SO_2 (scheme 12).



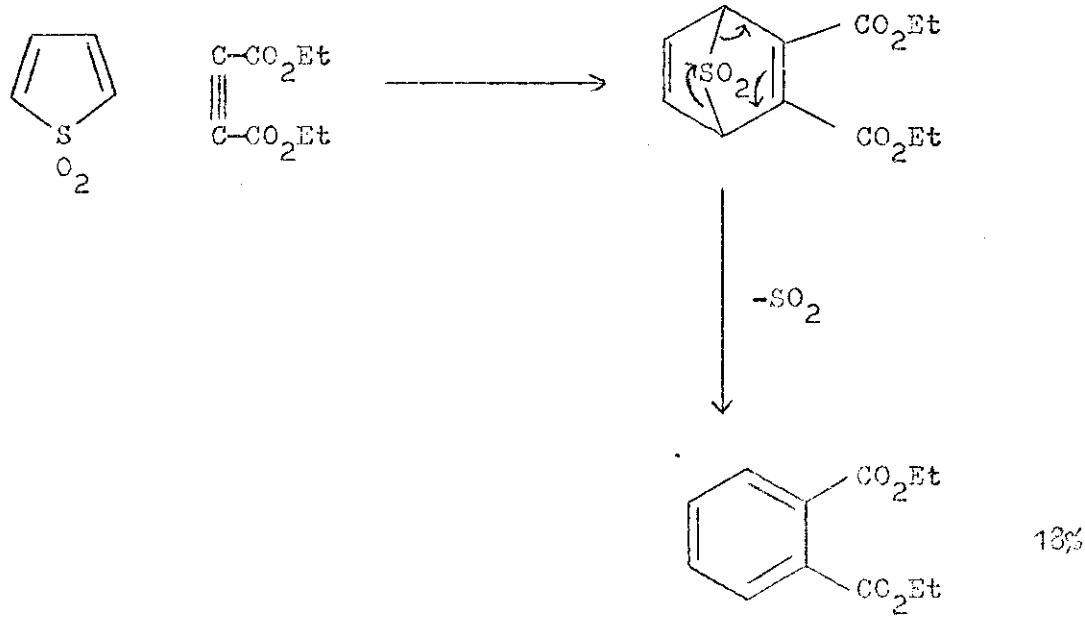
Scheme 12.



Bailey and Cummins[30] investigated the Diels-Alder reactions given by thiophen dioxide and quote the following results,

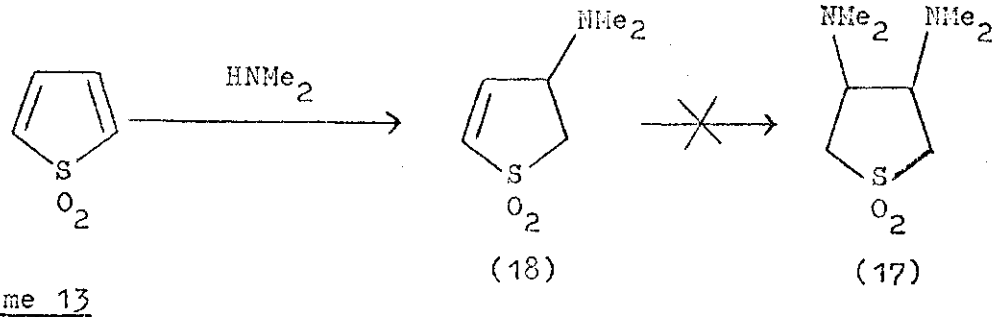


50%



18%

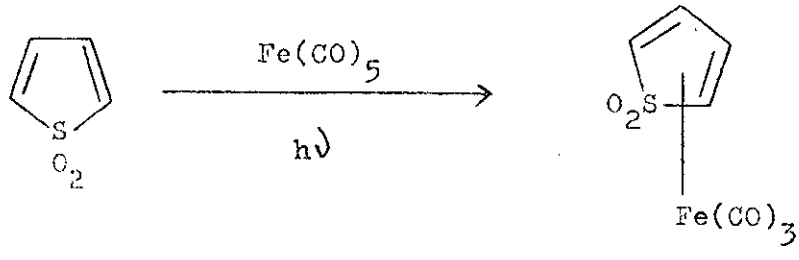
They also showed that thiophen dioxide reacted with dimethylamine to give the allylic amine (18) but that no further reaction occurred to give the diamine (17) (scheme 13).



(18) (17)

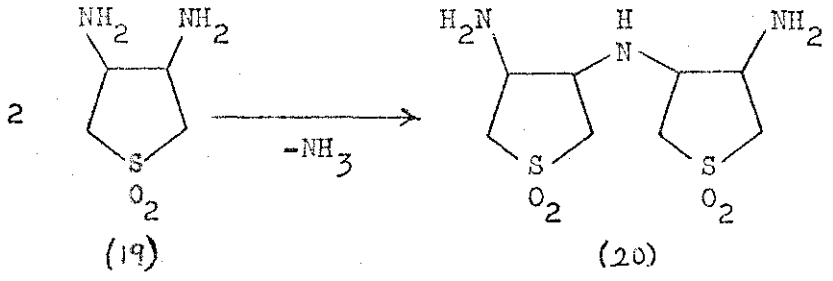
Scheme 13

Recently a very interesting reaction between thiophen dioxide and iron pentacarbonyl has been shown to give an iron tricarbonyl complex under the influence of u.v. light[31] (scheme 14).



Scheme 14

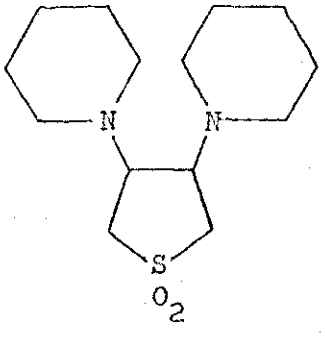
Prochazka and Horak[32] showed that the dichloride derivative of sulpholane reacted with ammonia under normal conditions to give thiophen dioxide, but treatment with liquid ammonia at room temperature for one month in an autoclave gave the diamine (19). This amine (19) readily self-condensed with loss of ammonia to give (20) (scheme 15), although it could be isolated as its hydrochloride salt.



(19) (20)

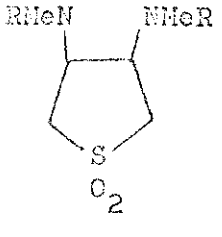
Scheme 15.

Our work demonstrated that piperidine reacted very readily with the dibromide (14) in aqueous solution to give crystals of 3,4-dipiperidinotetrahydrothiophen-1,1-dioxide (22) which was characterised as its monomethiodide derivative.



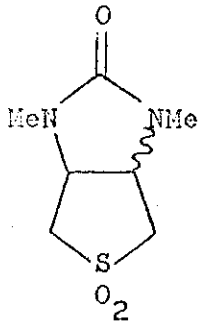
(22)

Treatment of the dibromide (14) with a cooled solution of methylamine in chloroform gave high yields of 3,4-bis(methylamino)-tetrahydrothiophen-1,1-dioxide (23, R = H). This diamine was a low melting solid, which formed a dihydrochloride salt and a di-N-acetyl derivative (23, R = Ac).



(23)

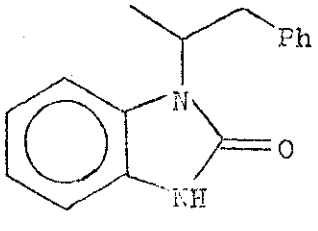
Reaction of the diamine (23, R=H) with phosgene in the presence of sodium carbonate gave the cyclic urea(24) in which the nature of the ring fusion was uncertain.



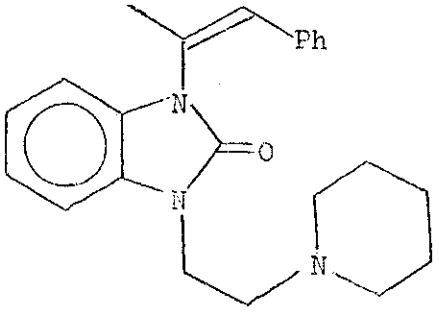
(24)

The cyclic structure (24) was assigned on the basis of analytical, infrared and ^{1}H n.m.r. data.

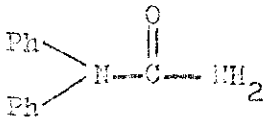
The infrared indicated sulphone bands and had a carbonyl absorption at 1710 cm^{-1}. This was higher than normal for urea carbonyls (ca. 1660 cm^{-1}). However, there are examples of highly substituted, and cyclic ureas which have absorptions in this region viz., (25)[33], (26)[33], (27)[24], (28)[34]:-



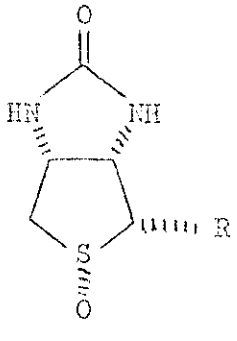
(25) 1701 cm^{-1}.



(26) 1709 cm^{-1}.



(27) 1690 cm^{-1}



(28) 1695 cm^{-1}.

The ^{1}H n.m.r. spectrum of (24) consisted of a methyl singlet at τ7.2 and a complex multiplet (due to the protons α- and β- to the sulphone) at τ6.2 - 7.0 in the ratio of 1:1. After heating the compound with 4N NaOD in D_2O for 78 hrs. at 75° the spectrum showed a singlet at τ7.2 and a singlet at τ6.75 in the ratio of 2.9:1. This showed that all four α protons had exchanged and were weakly acidic.

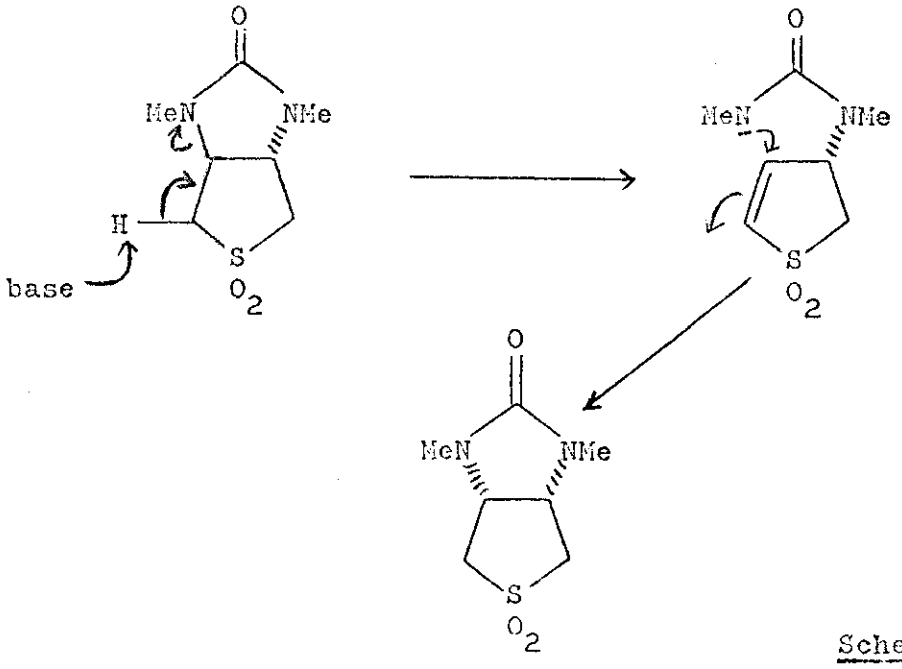
The weak acidity of the α protons of the parent compound, sulpholane, was demonstrated under similar conditions. The ^{1}H n.m.r. of sulpholane exhibits a multiplet at τ6.9 due to the α protons and a multiplet at τ7.8 due to the β protons; these signals being in the ratio 1:1. After heating with 4N NaOD in D_2O at 75° for 16 hrs. the multiplet at τ7.8 collapsed to a broad singlet and the one at τ6.9 decreased in size; the ratio being 4:1 showing that 75% of the α protons had exchanged after 16 hrs.

In contrast, the α protons in 3-sulpholene exchanged almost completely within 6 mins. at room temperature in 0.01N NaOD in D_2O.

The acidity of the α-protons therefore seems to be considerably enhanced by the presence of the double bond in the ring.

The trans- configuration of the bromine atoms in the dibromide (14) was assumed by analogy to the addition to cyclopentene,[26] but the configuration of the diamines derived from it were not necessarily the same. If the approach from the dibromide (14) was to be of any use in a synthesis of biotin, the ring junction of a cyclic urea, such as (24), derived from a diamine, had to be cis. It may be argued , that by whatever mechanism the bismethylamino compound (23, R=H) was formed, be it by direct substitution or by substitution-addition, the amino groups should be in the least hindered trans-configuration, and thus, the cyclic urea derived from it (e.g. 24) would be trans. Alternatively, two trans-fused five membered rings represent a highly strained system and as

basic conditions were utilised in the ring closure with phosgene, isomerisation could have taken place since the α protons are slightly acidic (see scheme 16).



Scheme 16.

This contrariety was resolved by an X-ray analysis on the cyclic urea (24).

The urea (24) crystallised from water as monoclinic prisms with unit cell dimensions a = 8.616(3), b = 15.280(5), c = 7.409(3) Å; β = 102.62(4)°, u = 951,85 Å^3, and with D_c = 1.423 g.cm^{-3}; D_m = 1.42(2) g.cm^{-3} (flotation). The unit cell contained four molecules.

The successful analysis in the space group C 2/c requires a two-fold axis in the molecule, indicative of a trans- fused structure; such trans-fused [3.3.0] systems are rare.[36] The structure was solved and refined using the heavy atom method (sulphur). The final refinement was to an R factor of 0.0619 for the observed reflections. Table 1. lists the final positional and thermal parameters. Figure 1 shows the (001) projection of a single molecule, whilst Figure 2 details the intramolecular bond lengths and angles. The trans- fused bicyclic system is clearly seen in Figure 3.

Table 1.

Final positional and thermal parameters, x 10^4. Standard deviations are in parentheses.

Atom	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
S	0000*	2312(1)	2500*	193(4)	32(1)	233(5)	000*	-69(3)	000*
N(1)	1302(6)	4782(3)	2432(7)	227(9)	43(2)	220(11)	-21(4)	-8(8)	-1(4)
O(1)	0593(6)	1823(2)	4179(6)	282(9)	42(2)	257(10)	1(4)	-87(7)	27(4)
O(2)	0000*	6113(4)	2500*	483(20)	33(3)	281(16)	000*	26(14)	000*
C(1)	1478(7)	3092(4)	2104(8)	140(9)	50(3)	258(14)	4(4)	22(9)	-6(5)
C(2)	0892(6)	3907(3)	2921(7)	190(9)	33(3)	204(13)	-10(4)	-33(8)	-1(4)
C(3)	2919(11)	5102(6)	3187(12)	294(17)	82(5)	294(21)	-81(8)	-19(14)	6(8)
C(4)	0000*	5304(6)	2500*	320(22)	51(5)	168(18)	000*	-11(15)	000*
H(11)	1537(81)	3127(43)	0381(108)	64(18) †					
H(21)	2451(69)	2879(34)	2679(77)	29(13)					
H(32)	0876(58)	3871(31)	4439(73)	24(11)					
H(43)	2930(96)	5215(50)	4766(134)	78(22)					
H(53)	3843(112)	4581(62)	2558(160)	109(34)					
H(63)	3193(89)	5465(48)	2569(110)	52(22)					

* Parameters fixed during the refinement.

† Isotropic thermal parameters (B's), in Å^2 x 10^2. The anisotropic thermal parameters are in the form: $f_i = f_i^o \exp-(\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + 2\beta_{12}hk + 2\beta_{13}h.l + 2\beta_{23}k.l)$.

The second digit in each hydrogen atom identification number refers to the carbon atom to which it is attached. The average carbon-hydrogen bond length is 1.07(8) Å.

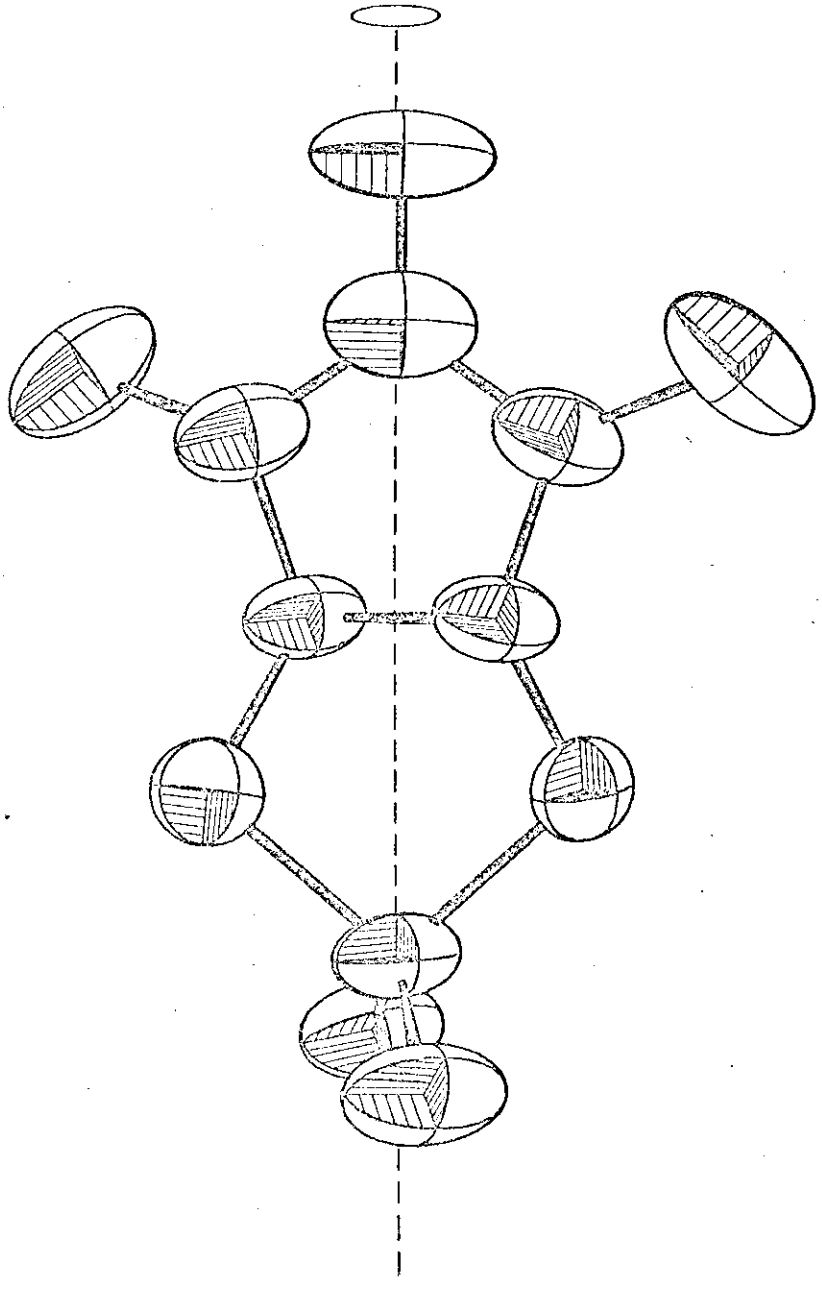


FIGURE 1. The (001)projection of the molecule. The thermal ellipsoids are scaled to include 50% probability.

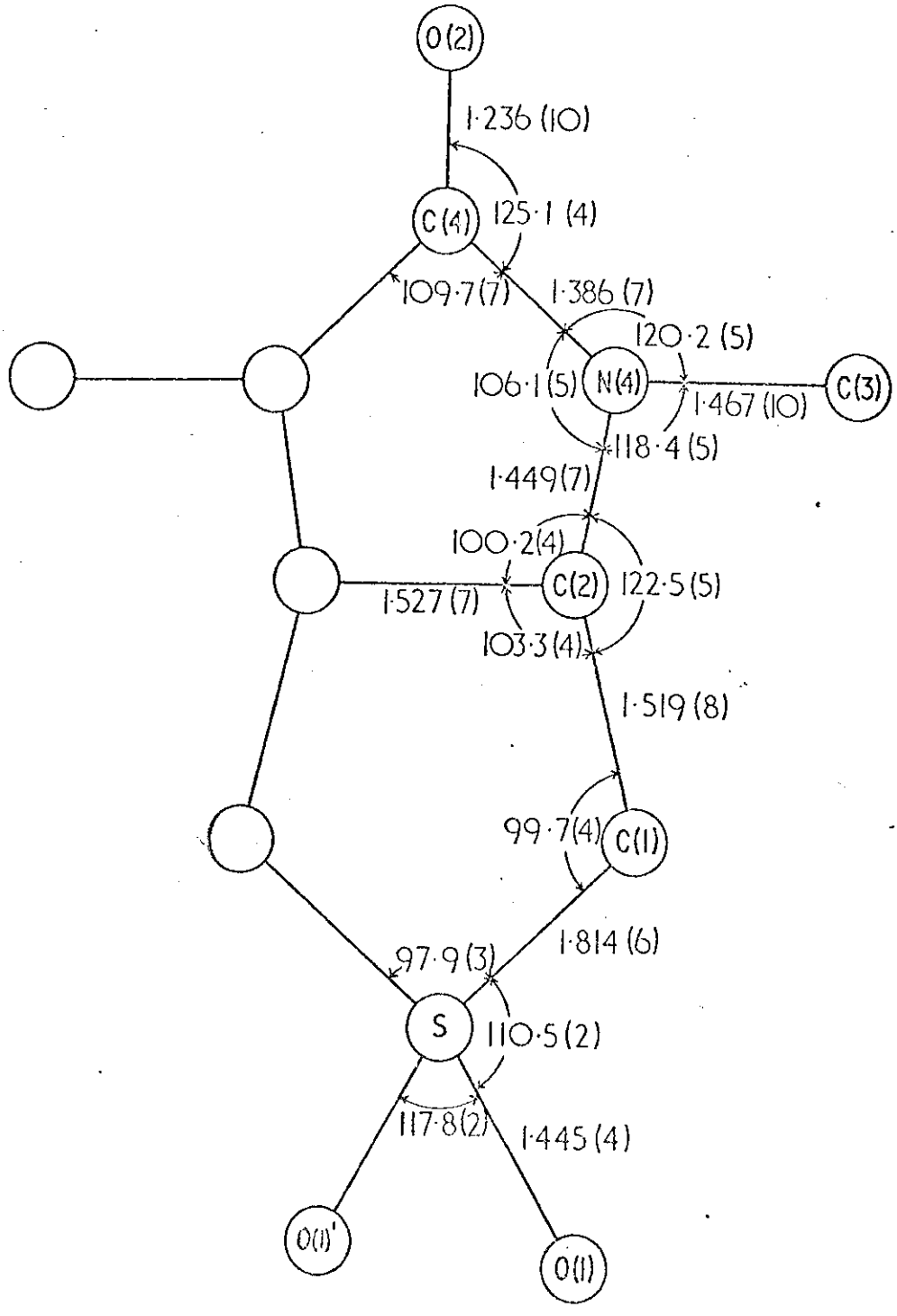


FIGURE 2. Intramolecular bond lengths and angles with standard deviations in parentheses. O(1)' refers to the two-fold axis atom related to O(1). The C(1)-(S)-O(1)' angle is 109.2(3)°.

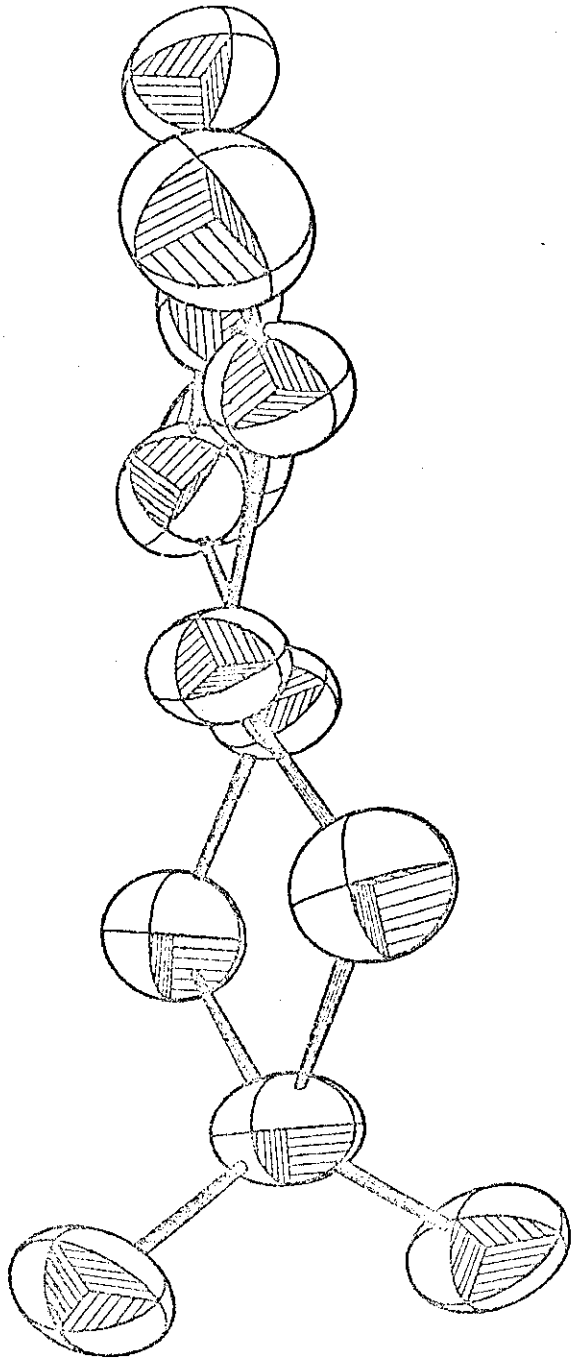
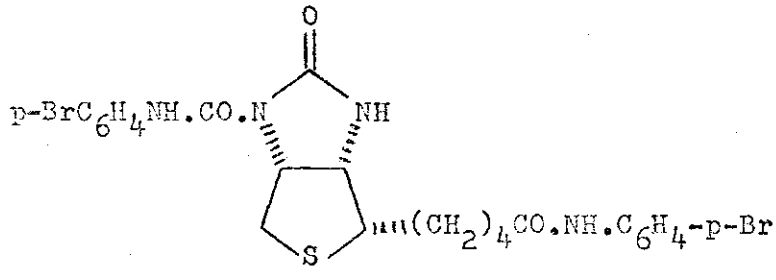


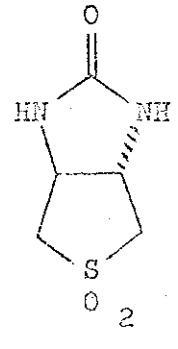
FIGURE 3. The (100) projection of the molecule.

Bond lengths compare well with the expected values. Thus the sulphur-oxygen length of 1.445(4) Å is in good agreement with that, for example, in dimethyl sulphone [37] (1.473 Å) and methane sulphonanilide[38] (1.438 Å). The ring strain of the urea (24) due to the trans- fusion causes a number of intramolecular angles to deviate markedly from the expected values. Both rings are highly twisted (Figure 3) and do not have the normal envelope conformation such as is associated, for example, with the cis- fused biotin derivative (29)[39].



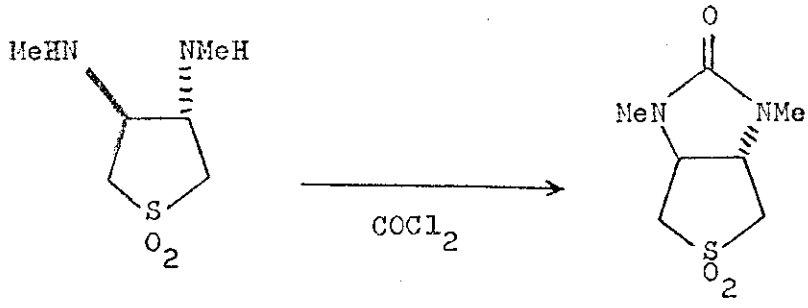
(29)

By analogy to the preparation of the dimethyl urea (24), the dibromide (14) was reacted with liquid ammonia for one month, following the method of Prochazka,[32] to give the diamine which was not isolated but cyclised with phosgene. Thespectral properties of the product were consistent with structure (30) and will be discussed in a later section.



(30)

The result that the cyclic urea (24) was trans- fused indicated that the diamine (23,R=H) from which it was derived must also be in the trans- form. This therefore gives some insight into the mechanism of the reaction between amines and the dibromide (14).

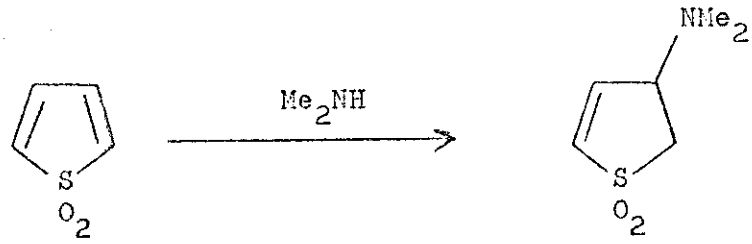


(23) (24)

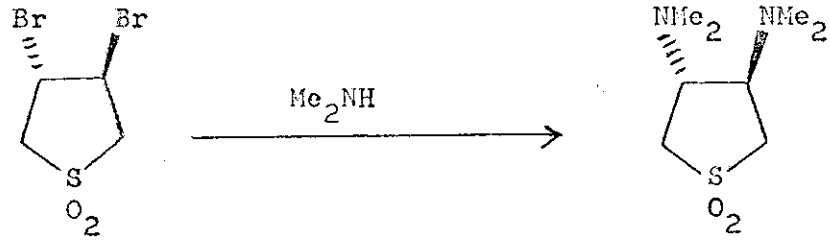
Bailey and Cummins[28] have shown that thiophen dioxide (16) reacts with dimethylamine to give the allylic amine (18) but not further to give the diamine (17). The diamine, however, was formed by the action of dimethylamine upon the dibromide (14) or the allylic bromide (15).

As mentioned previously the dibromide (14) was assigned the trans- configuration and the diamine (17) as trans- by analogy to the above result. These observations suggest that dimethylamine reacts with the dibromide via an intermediate (31) (scheme 17) which does not directly dehydrobrominate to the allylic amine (18) since further reaction would not take place. If direct substitution of the bromide of (31) occurred the cis-diamine would be formed, whereas reaction via an aziridinium species (32) would result in the trans-diamine.

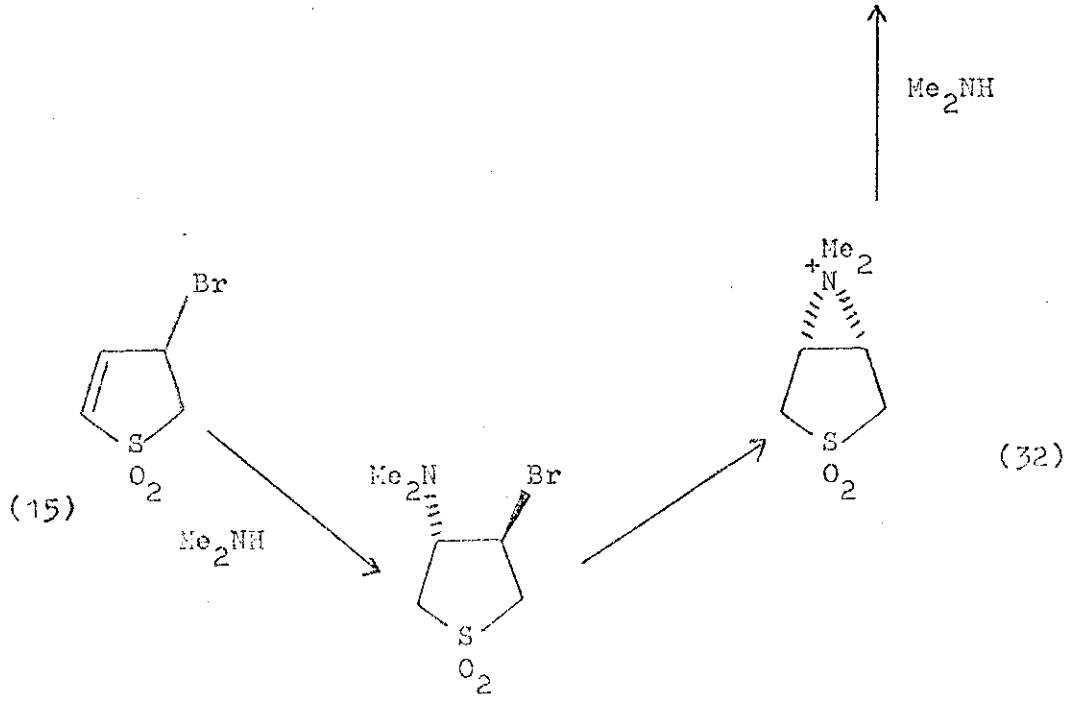
Scheme 17.



(16) (18)



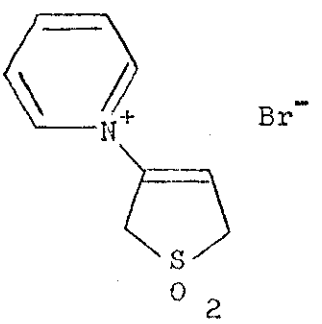
(14) (17)



(15) (31) (32)

An unusual reaction was observed between pyridine and the dibromide. Whereas a stoichiometric quantity of pyridine eliminated HBr from the dibromide[27] (cf. Scheme 10), it was found that the use of a large excess of pyridine afforded a purple crystalline salt. This was assigned the structure (33) on the basis of a) elemental analysis, b) the infrared spectrum with $\nu_{max.}$ 1134, 1320 and 1630 cm^{-1}, and c) the ^1H n.m.r. spectrum which showed τ0.7(2H,d,J6Hz), 1.25(1H,t,J8Hz), 1.75(2H,t,J7Hz), 2.9(1H, broad s, $W_{\frac{1}{2}}$ 7Hz), 5.3(2H,m), 5.7(2H,m).

The infrared confirmed the presence of a sulphone group and the n.m.r. indicated a pyridine ring bonded through nitrogen and one vinylic proton (τ2.9). The vinylic proton was broadened and showed fine splitting. The remaining ring protons (τ5.3 and τ5.7) resonated as narrow signals and showed fine structure inconsistent with adjacent methylene groups. These facts point to the following structure (33),



(33)

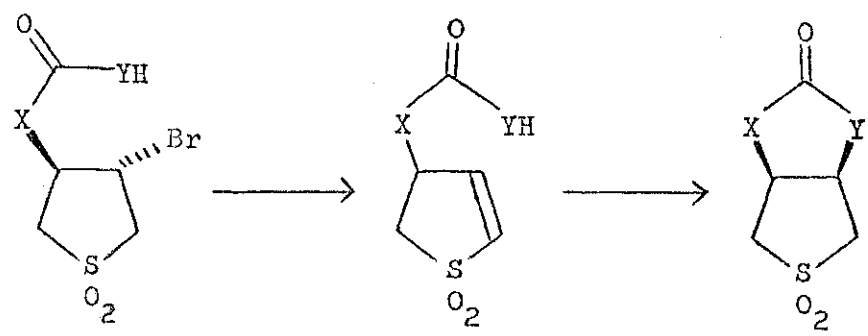
The salt probably formed by the reaction of pyridine with the allylic bromide (15) which was initially produced,[27] followed by base-catalysed migration of the remaining double bond to the 3(4)-position, a rearrangement with considerable precedent.[40]

Of interest was the purple colour of the salt. Repeated recrystallisations did not remove the colour, which was retained in solution. Measurement of its e.s.r. spectrum ruled out the presence of free radicals. The exact cause of the colour remains unexplained but may be due to an intramolecular charge-transfer interaction between the sulphone and pyridinium functions. With base a brilliant yellow colour formed which was attributed to the formation of an ylide.

The use of the dibromide as a precursor seemed to be ruled out by the trans- orientation of the groups which were introduced. All attempts to isomerise the trans- fused urea (24) to the cis- isomer with base failed and so a new approach was attempted.

Intramolecular addition reactions of 2-sulpholenes.

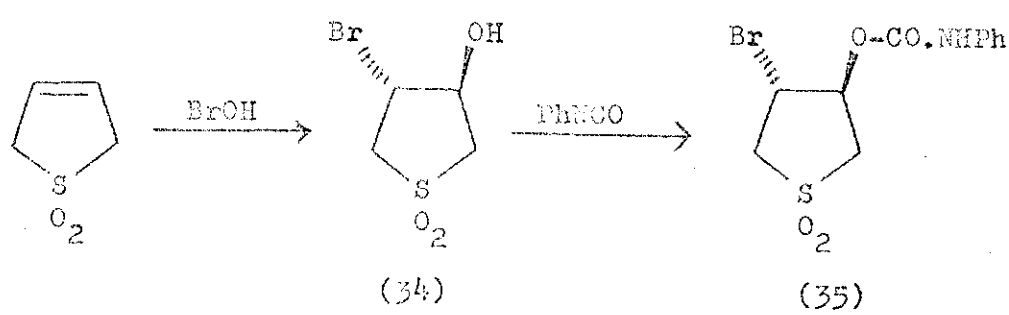
Since Michael-type addition reactions to 2-sulpholenes are well established,[41,42] intramolecular additions to appropriately substituted 2-sulpholenes were investigated, e.g. -



If X = Y = NH, the required cyclic urea would be formed, presumably with a cis- ring junction. Since the appropriate bromo-urea starting material was not available the reactions of bromo-carbamates (X = O, Y = NR) were studied to investigate the scope of the reaction.

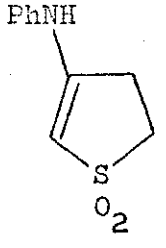
3-Sulpholene (2) was converted with bromine water into the known bromohydrin (34)[43] which on heating with phenylisocyanate gave the corresponding phenylcarbamate (35)(scheme 18),

Scheme 18.



(34) (35)

On treating the phenylcarbamate with an excess of sodium ethoxide in ethanol a new product formed which was assigned the enamine structure (36) on the basis of analytical, infrared and ^1H n.m.r. data.

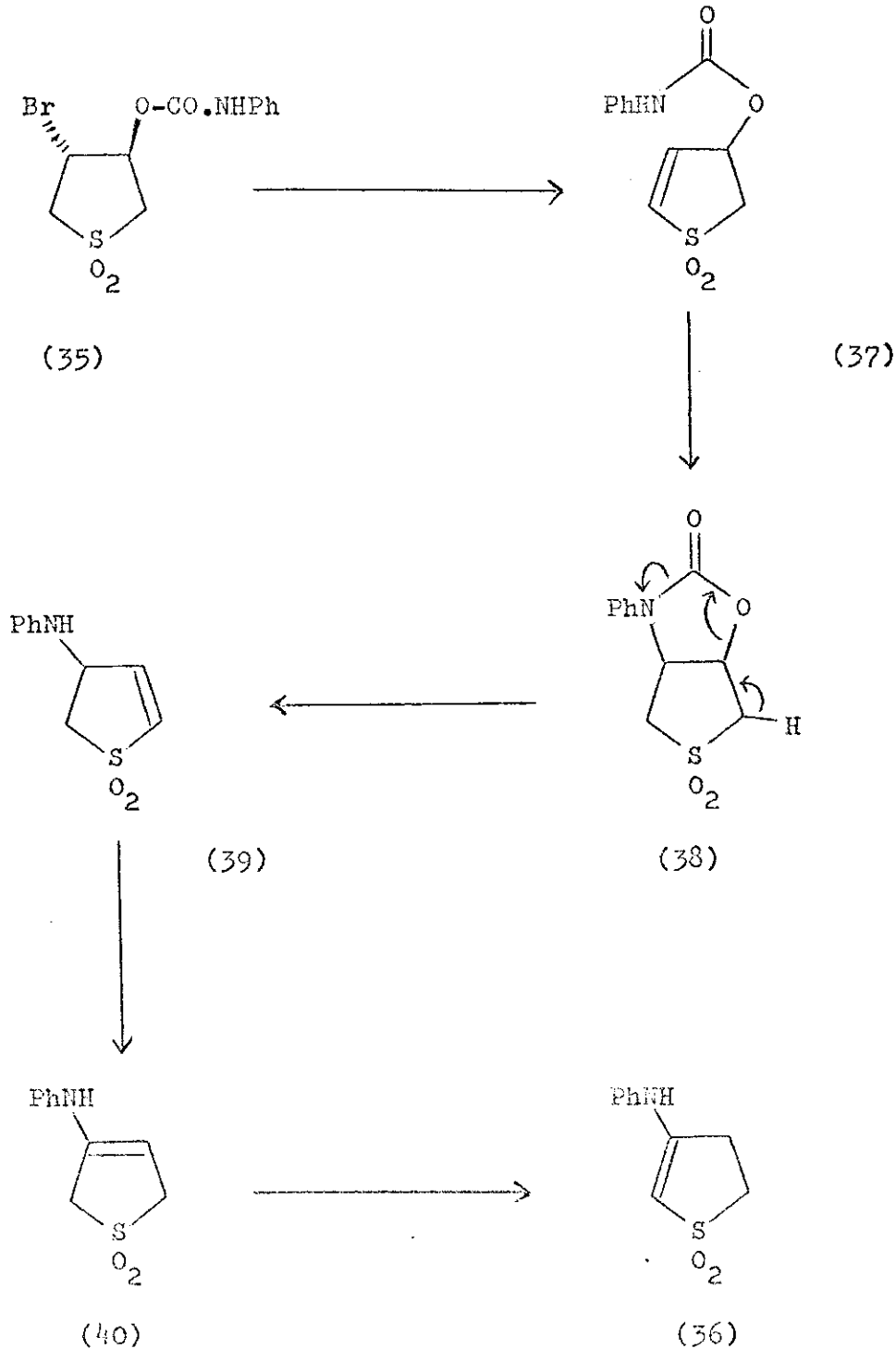


(36)

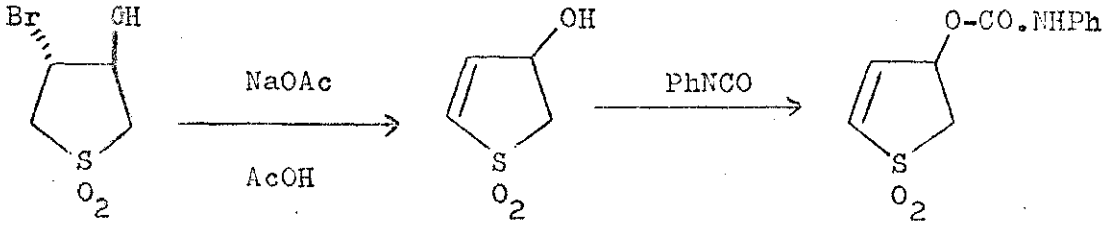
The infrared indicated -NH(3300 cm^{-1}); C-C double bond (1620 cm^{-1}), phenyl and sulphone (1290, 1100 cm^{-1}) functions. The ^1H n.m.r. showed phenyl protons and an exchangeable -NH, plus a singlet at τ4.2 (1H) and a multiplet at τ6.5-7.2 (4H).

The mechanism proposed for this conversion (scheme 19) involves an initial dehydrobromination to give the unsaturated sulphone (37) followed by formation of the cyclic carbamate (38). Further reaction with base abstracts a proton α to the sulphone group with subsequent elimination, by ring opening, of the carbamate group followed by loss of carbon dioxide. The conjugated olefin (39) initially formed can equilibrate by $\alpha\beta$-$\beta\gamma$ double bond isomerism[40] leading to migration of the double bond, via the unconjugated enamine,(40) to the more stable position to form the conjugated enamine(36).

Scheme 19.



That such a series of reactions was occurring was established by varying the base treatment of the starting carbamate (35). Slow addition of one equivalent of triethylamine to the carbamate eliminated hydrogen bromide to form the allylic phenylcarbamate (37). This was identified by comparison with authentic material prepared by reaction between the allylic alcohol (41)[44] and phenylisocyanate.



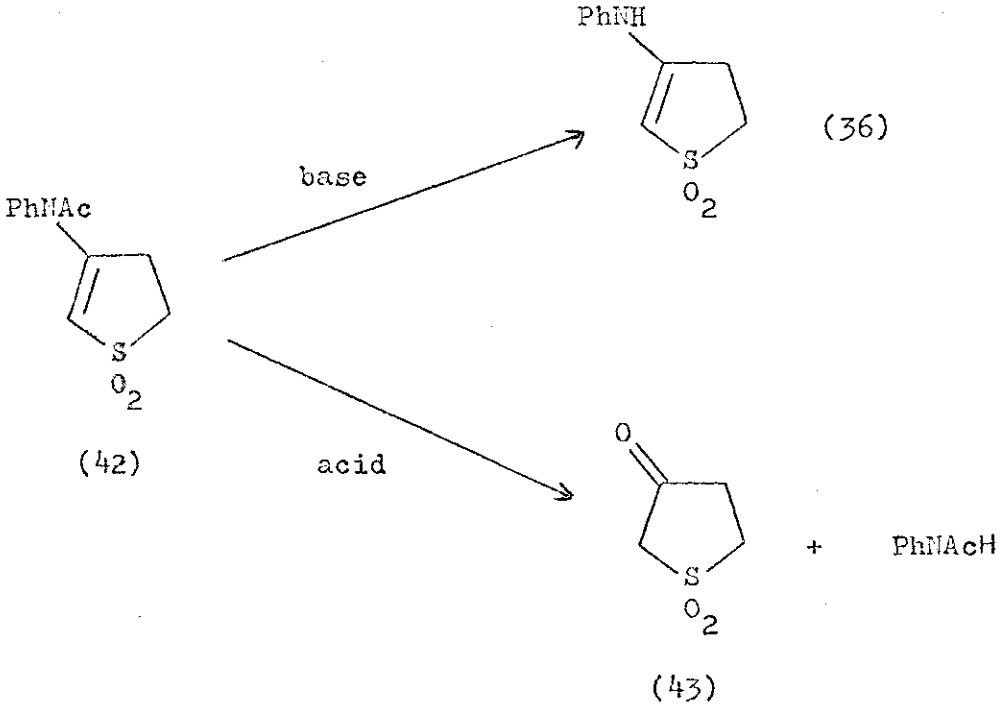
(41) (37)

The allylic carbamate readily cyclised into the cyclic carbamate (38) on treatment with an excess of triethylamine. Heating the cyclic carbamate with triethylamine in ethanol converted it into the allylic amine (39). All the intermediates in this sequence were converted by sodium ethoxide in ethanol into the enamine (36).

Although migration of the double bond from isomer (39) to isomer (36) must proceed via the βγ-unsaturated isomer (40), no evidence that the latter could exist as a stable intermediate was found.

The enamine (36) could be acetylated with acetyl chloride to give the N-acetyl derivative (42). Basic hydrolysis of this anilide (scheme 20) liberated the starting enamine whilst acid hydrolysis, with 2N hydrochloric acid, afforded acetanilide and the ketone (43)[45], identified by its 2,4-dinitrophenyl hydrazine derivative.

Scheme 20.

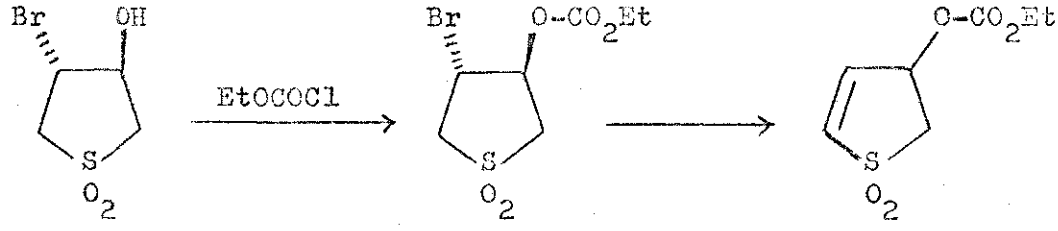


The concept of intramolecular Michael additions to 2-sulpholenes now seemed valid but it was necessary to prepare other bromo-carbamates to test the generality of the reaction.

The method of treating the bromohydrin (34) with isocyanates only seemed applicable to high boiling isocyanates since the low boiling methylisocyanate failed to give any reaction. A sealed tube reaction was not attempted.

The bromohydrin (34) reacted with ethyl chloroformate in the presence of one equivalent of triethylamine to give the ethyl carbonate (44) (scheme 21), but use of an excess of triethylamine afforded the allylic ester (45) by elimination of hydrogen bromide.

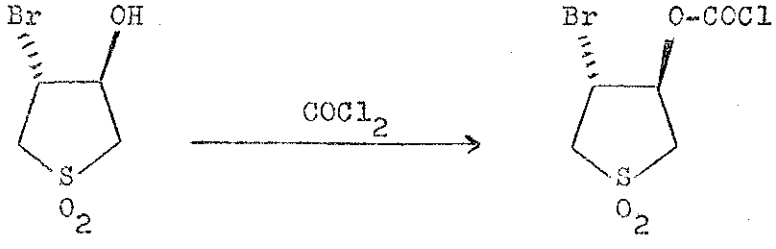
Scheme 21.



(34) (44) (45)

Neither of these carbonates reacted with ammonia, aniline or methylamine to give carbamates. Use of more vigorous conditions caused eliminations to occur.

A general preparation of the carbamates was achieved, however, by use of the chloroformate (46), prepared by reaction of the bromohydrin (34) with phosgene in the presence of quinoline.

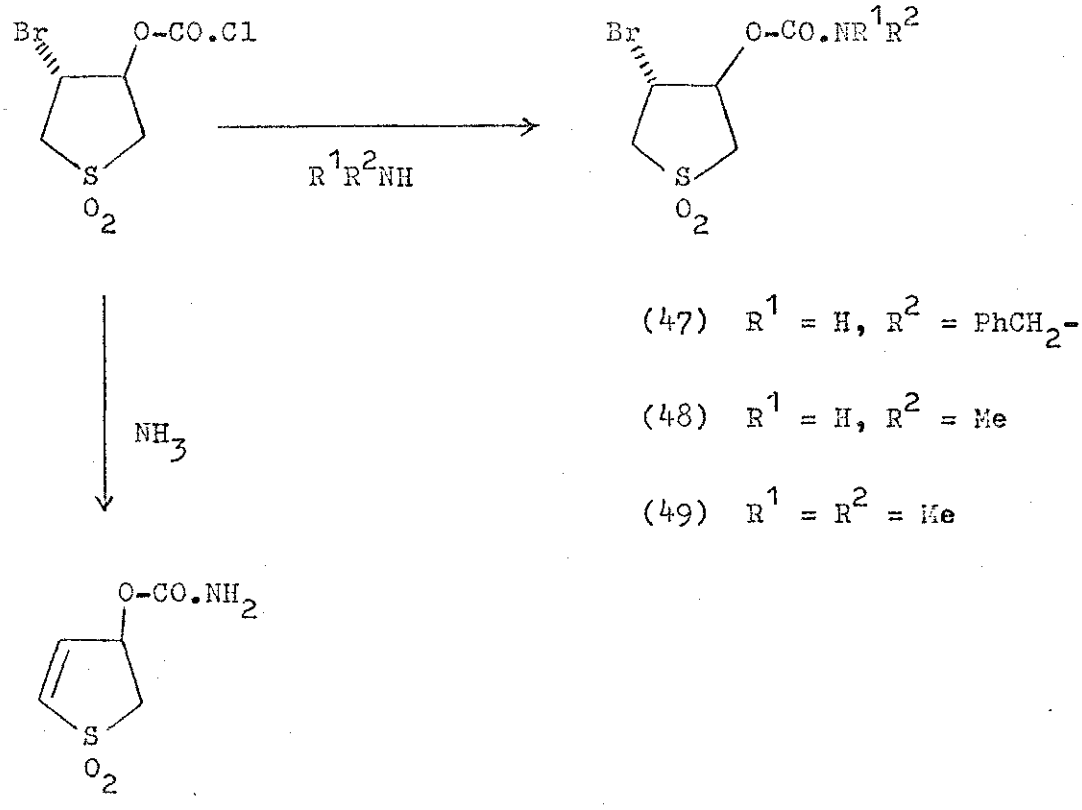


(34) (46)

The chloroformate reacted with aniline to give the same carbamate (35) as described above, and with ethanol to give the ethyl carbonate (44).

Benzylamine, methylamine and dimethylamine reacted similarly with the chloroformate to give the expected carbamates (47), (48) and (49), whereas an excess of ammonia gave the unsaturated carbamate (50) (scheme 22).

Scheme 22.



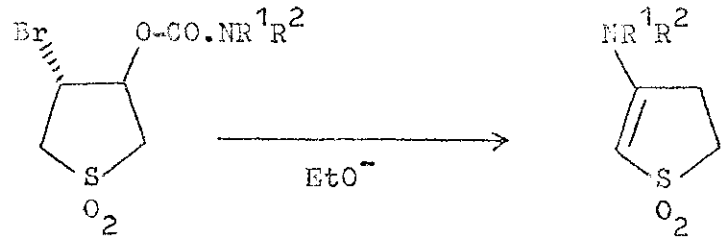
(47) R^1 = H, R^2 = $PhCH_2$-

(48) R^1 = H, R^2 = Me

(49) R^1 = R^2 = Me

(50)

On treatment of the secondary carbamates from benzylamine and methylamine, (47) and (48), with sodium ethoxide in ethanol, the corresponding enamines, (51) and (52), were produced in high yields.

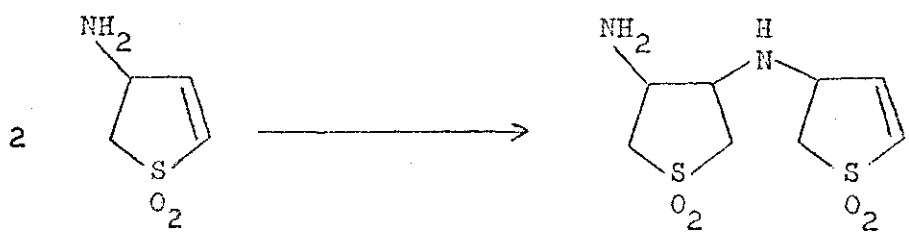


(47), (48)

(51) R^1 = H, R^2 = $PhCH_2$-

(52) R^1 = H, R^2 = Me

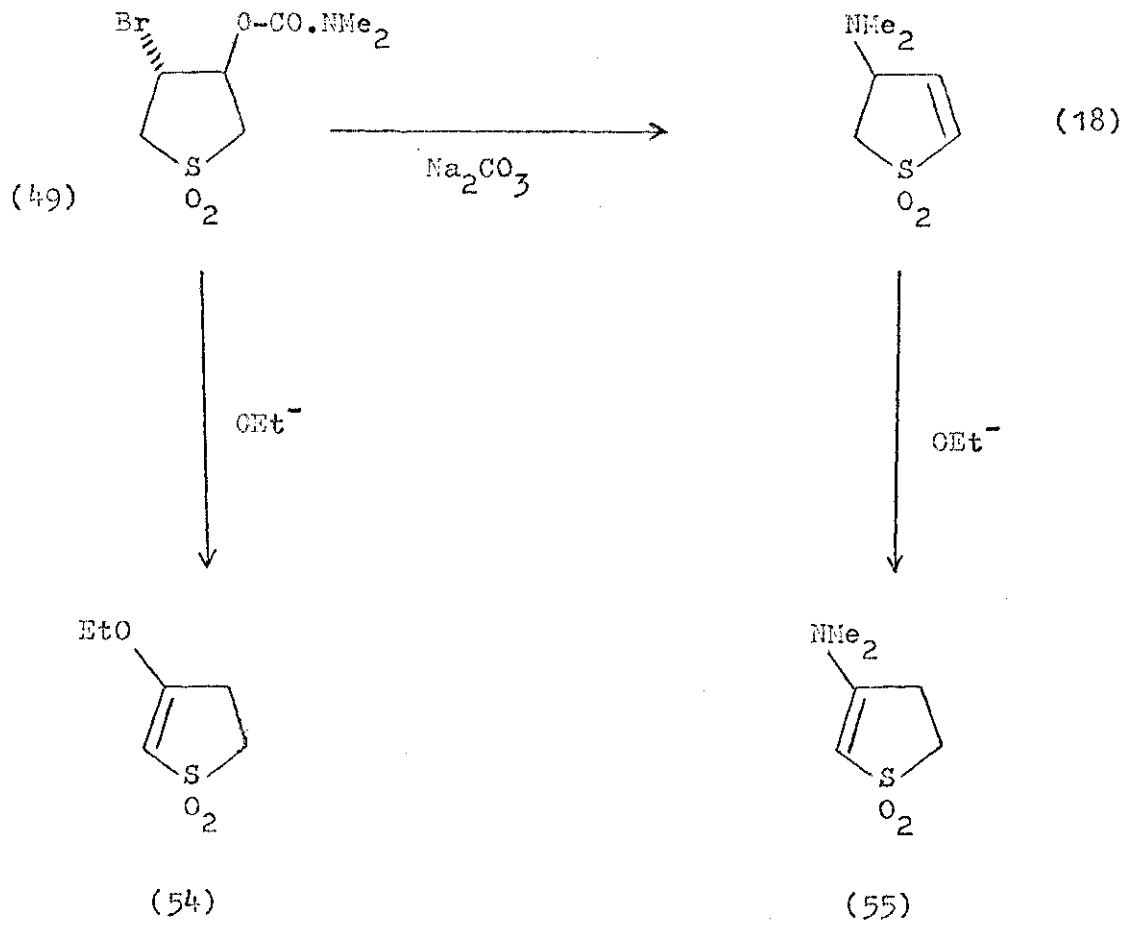
Similar treatment of the primary carbamate (50) did not produce the corresponding enamine but this was not surprising since the amine (53) is known to undergo dimerisation[32] -



(53)

An intramolecular Michael addition reaction was not possible with the tertiary carbamate (49) and treatment of this with ethoxide afforded the vinylic ether (54) (scheme 23). The reaction probably proceeded by an elimination-addition reaction. Using the milder conditions of aqueous sodium carbonate, the allylic amine (18) was produced which was identical to the compound prepared by Bailey and Cummins[28] from either thiophen dioxide, or from bis(dimethylamino)sulpholane (17) (cf. schemes 11 and 13). This amine (18) was readily isomerised into the enamine (55) with ethoxide.

Scheme 23.



The ^{1}H n.m.r. spectra of the five enamines (36), (42), (51), (52) and (55), and of the vinylic ether (54) were very similar with respect to the ring protons and were typified by that of the acetylated enamine (42) (Spectrum 1). They were characterised by having two sets of methylene protons resonating as overlapping multiplets between τ6.3-7.3 and a singlet due to the vinylic proton resonating between τ3.3 and τ4.9 (Table 2).

$NaOD/D_2O$ exchange studies indicated that the vinylic proton was in the position α- to the sulphone.

SPECTRUM 1

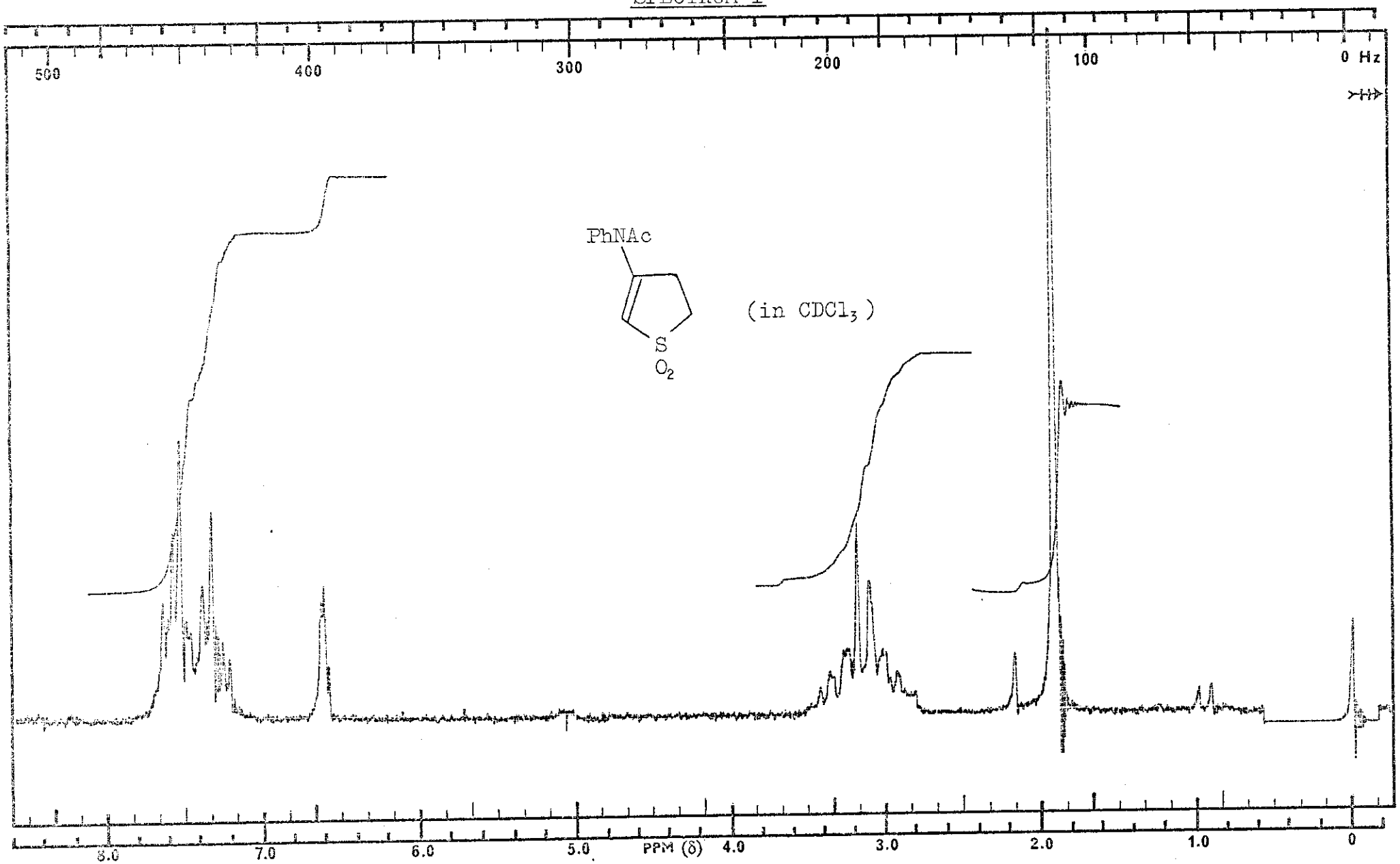
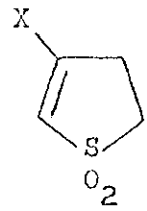

500
400
300
200
100
0 Hz
PhNAc
S
O2
(in CDCl3)
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0

Table 2. ^1H n.m.r. chemical shifts in compounds of the type:-

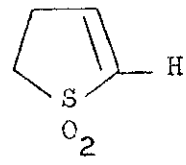


Shifts (τ) measured in $CDCl_3$, except where otherwise stated, on a Varian T60 instrument with TMS as internal reference.

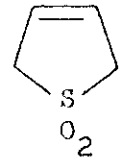
Compound	X	Methylene protons	vinylic proton
(36)	PhNH	6.5 - 7.2	4.2
(42)	PhNAc	6.5 - 7.2	3.3
(51)	$PhCH_2NH$	6.6 - 7.3	4.7
(52)*	MeNH	6.5 - 7.3	4.9
(55)	Me_2N	6.4 - 7.2	4.8
(54)	EtO	6.3 - 7.3	4.3

*measured in D_2O.

Broaddus[5] has shown that the 5-proton of 2,3-dihydrothiophen -1, 1-dioxide (56) (2-sulpholene) exchanged rapidly with $NaOD/D_2O$, as do the four allylic protons of 2,5-dihydrothiophen-1,1-dioxide (2) (3-sulpholene).

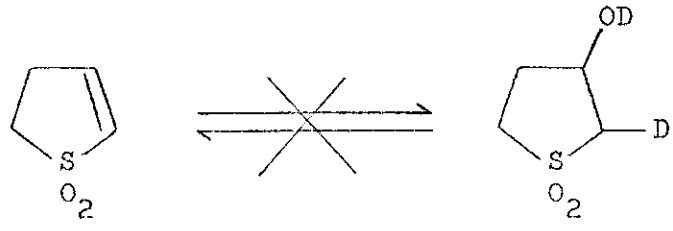


(56)



(2)

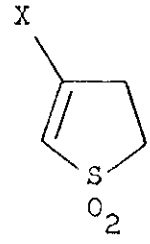
The rapid exchange of the 5-proton is attributed to the formation of an anion at this position, as opposed to a reversible Michael addition of OD^- to the double bond. This was proved by the fact that the n.m.r. of 3-hydroxysulpholane under exchange conditions showed no sign of any 2-sulpholene, viz. -



When the anilino-enamine (36) was treated with $NaOD/D_2O$ it was the vinylic proton which exchanged and not the methylene protons

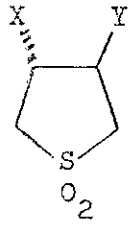
The conjugation of the nitrogen through the double bond to the sulphone group was confirmed by the ultraviolet spectra of these compounds, with strong absorptions ranging from 233 nm. to 271 nm. (Table 3). This strong absorption should be contrasted with the u.v. of 2-sulpholene (56) which showed no absorptions above 210 nm.

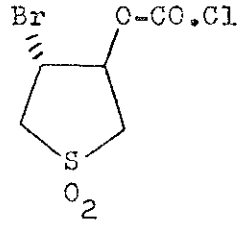
Table 3. Ultra violet absorptions of compounds of the type:-



Compound	X	λ max. nm.	ε
(36)	PhNH	271	17,800
(42)	PhNAc	243	14,200
(51)	$PhCH_2NH$	235	17,500
(52)	MeNH	233	14,700
(55)	Me_2N	242	16,350
(56)	H	210	520 (end absorption)

The ^{1}H n.m.r. spectra of the 3,4-disubstituted compounds of the type shown below were very similar and were typified by the chloroformate (46) (spectrum 2).

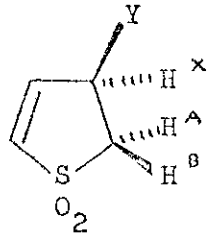


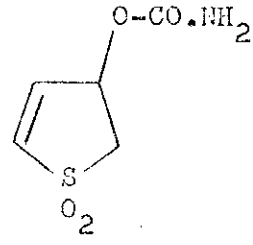


(46)

For cases where X = Br and Y = OCO-, the four methylene protons generally occurred as a multiplet at τ5.9 - 6.9, the methine next to bromine as a multiplet at about τ5.3 and the methine next to oxygen as a multiplet at about τ4.4.

The allylic substituted compounds also had similar ^{1}H n.m.r. spectra and a good example was the primary carbamate (50) (spectrum 3.).

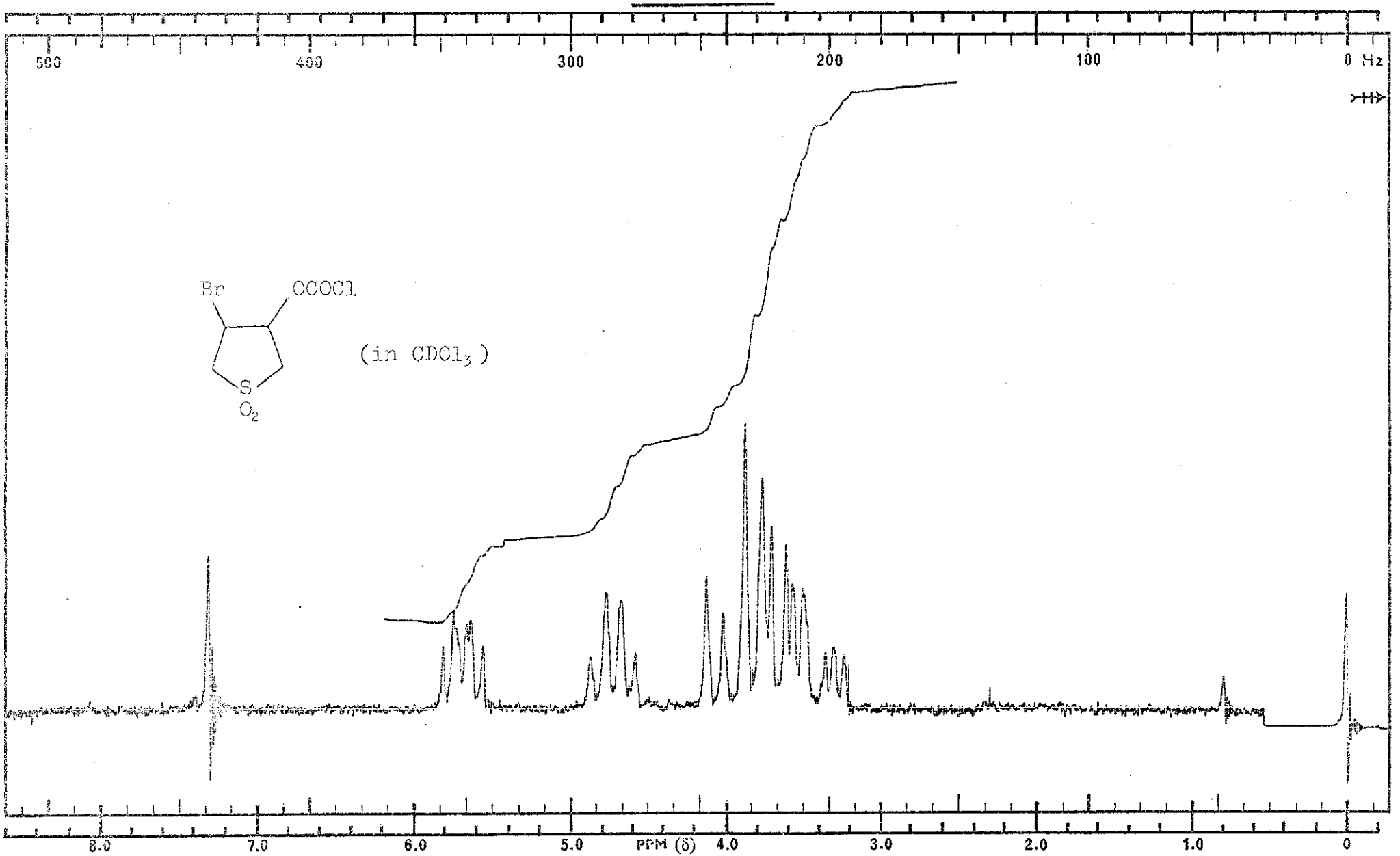




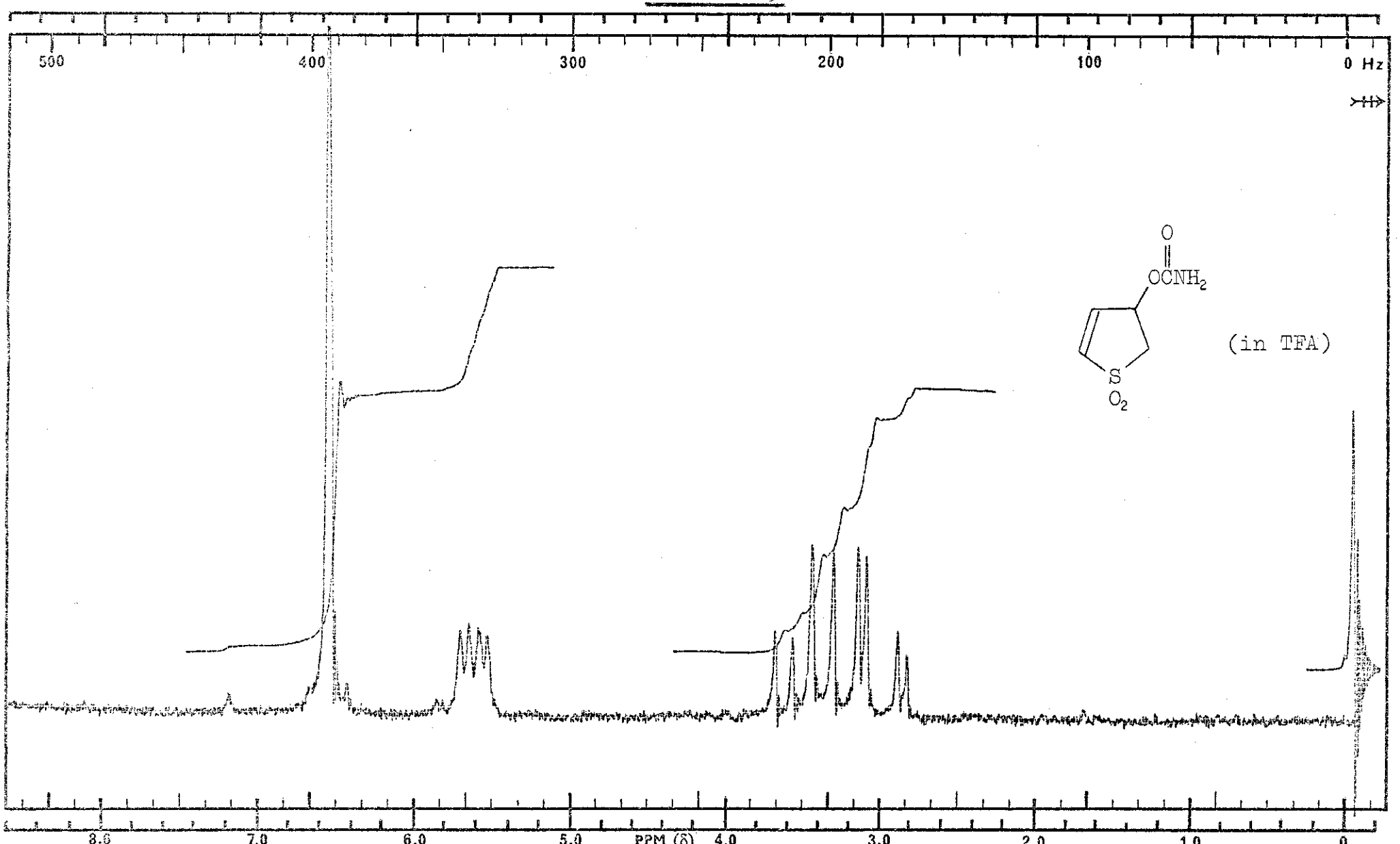
(50)

The vinylic protons were accidentally coincident and resonated at about τ3.2. The methine and methylene protons gave rise to an ABX pattern with the methine as a multiplet at about τ4.0 or 4.5 and the methylenes as a double quartet at τ6.0 - 7.0. The coupling constants for the system were J_{AB} 14 Hz, J_{AX} 7 Hz, J_{BX} 4 Hz.

SPECTRUM 2

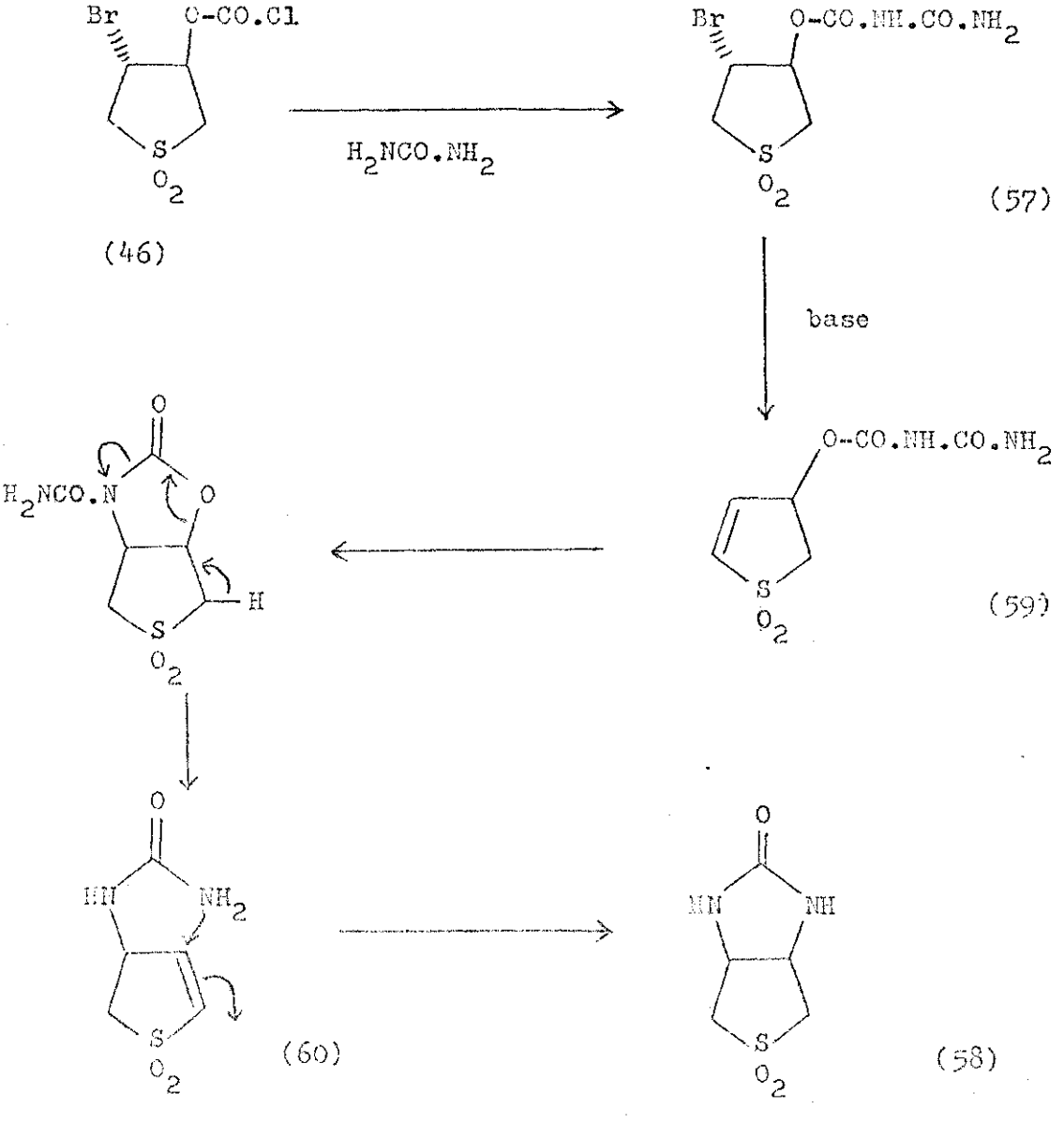

500
400
300
200
100
0 Hz
Br
OCOCl
S
O2
(in CDCl3)
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0

SPECTRUM 3


500
400
300
200
100
0 Hz
O
OCNH2
S
O2
(in TFA)
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0
0

The chloroformate (46) reacted with urea to give the allophanate (57) (scheme 24) which yielded the cyclic urea (58) on treatment with aqueous base. The mechanism proposed for this reaction involved an initial dehydrobromination to form the allylic allophanate (59) followed by cyclisation to a cyclic carbamate, with loss of CO_2, to give the allylic urea (60). Intra-molecular Michael addition would then give the product (58).

Scheme 24.

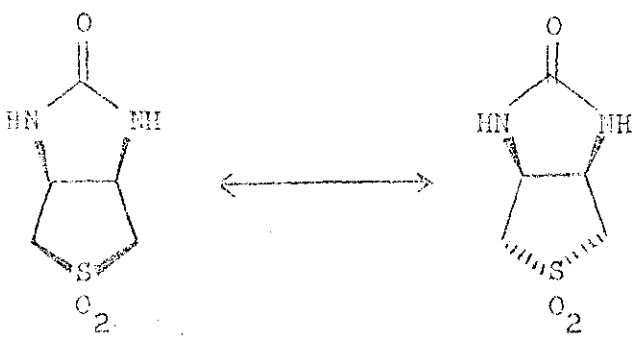


That such a series of reactions was taking place was shown by varying the base treatment of the starting allophanate. Dehydrobromination of (57) to the allylic allophanate (59) was carried out in aqueous quinoline, whilst warming the latter with aqueous sodium hydrogen carbonate afforded the allylic urea (60). The intermediate cyclic compound was not isolated but the conversion of (59) to (60) was accompanied by a fairly brisk evolution of carbon dioxide. On treatment with stronger base, such as aqueous sodium carbonate, cyclisation of the allylic urea into the isomer (58) occurred. The cyclic urea (58) was also obtained under the same conditions from the allylic allophanate (59).

The structure assigned to the cyclic urea (58) was consistent with analytical and spectral data.

From its mode of formation this compound (58) must have the cis-fused structure. Comparison with the known trans-compound (30), prepared from the trans-3,4-dibromotetrahydrothiophen dioxide with ammonia and phosgene, showed them to be isomeric. The former, cis-fused isomer showed ν max. 1710 cm^{-1} for its carbonyl absorption in the i.r., whereas the latter showed ν max. 1705 and 1690 cm^{-1}.

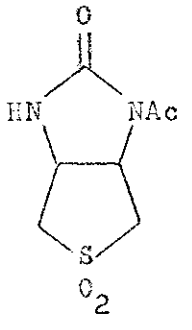
The ^1H n.m.r. spectrum of the trans-fused isomer showed a complex pattern for the ring protons with multiplets centred at τ 5.05 (2H) and τ 6.05 (4H). In contrast, the cis-fused urea showed two very broad peaks centred at τ 5.5 (2H) and τ 6.9 (4H). The lack of fine structure in the case of the cis-isomer was attributed to a conformational flipping of the sulphone groups between two extremes (58a $\leftrightarrow$ 58b) as illustrated by Dreiding models.

(58a)  (58b)

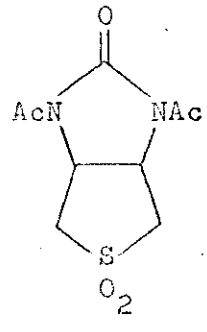
In contrast, the trans-fused isomer (30) was held completely rigid. The low solubility of the cis-fused isomer (58) in most solvents precluded low temperature n.m.r. studies.

The chemical difference between the cis- and the strained trans-fused ureas was demonstrated by the action of boiling 6N hydrochloric acid. The trans-urea was quantitatively hydrolysed in 24hr. to produce the trans-diamine (19), isolated as the hydrochloride salt. Under the same conditions the cis-compound (58) merely formed the urea hydrochloride, from which the starting material was regenerated by weak base. The stability of the cis-fused urea to hydrolysis is reminiscent of that recorded for biotin, which required treatment with barium hydroxide at 140° for hydrolysis[46].

The cis-fused urea (58) readily reacted with acetyl chloride to give both the mono- (61) and the di-acetyl (62) derivatives which were readily hydrolysed with dilute aqueous base to the parent urea (58).



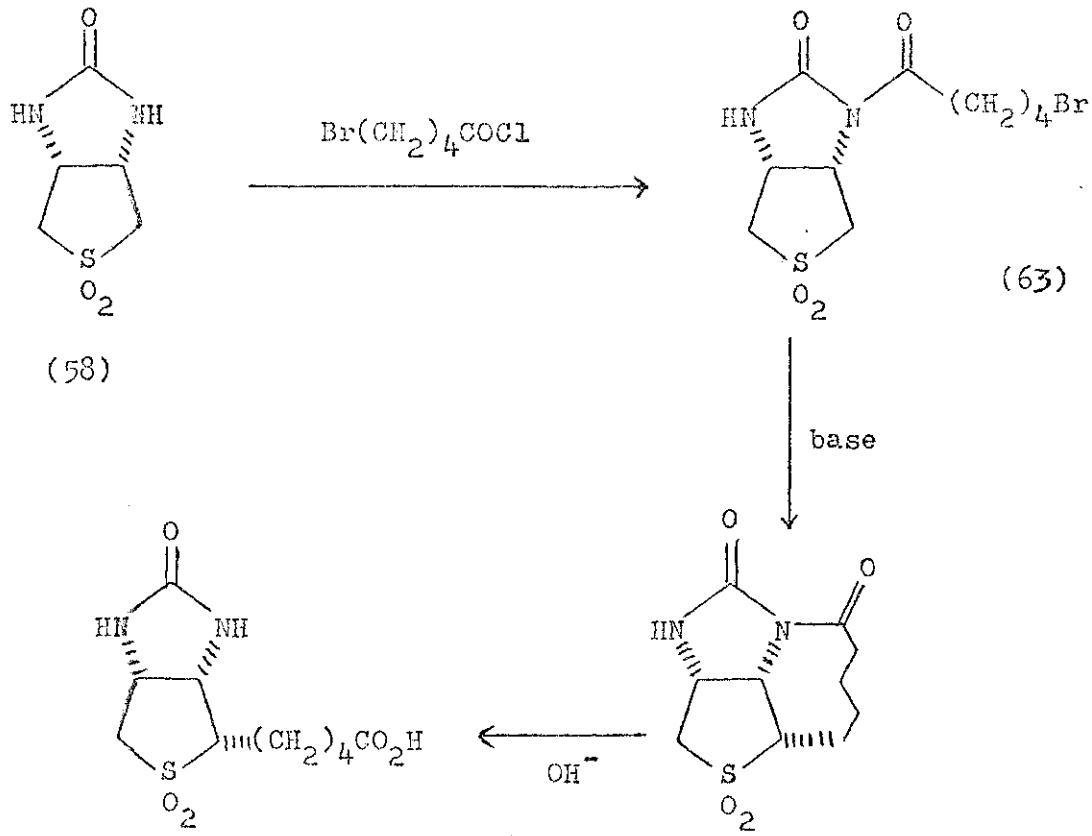
(61)



(62)

Having shown that the cis-urea was readily acylated and could be hydrolysed back to the starting material, the following route (scheme 25) for the introduction of the valeric acid side chain was investigated.

Scheme 25.



(58) (63) (64)

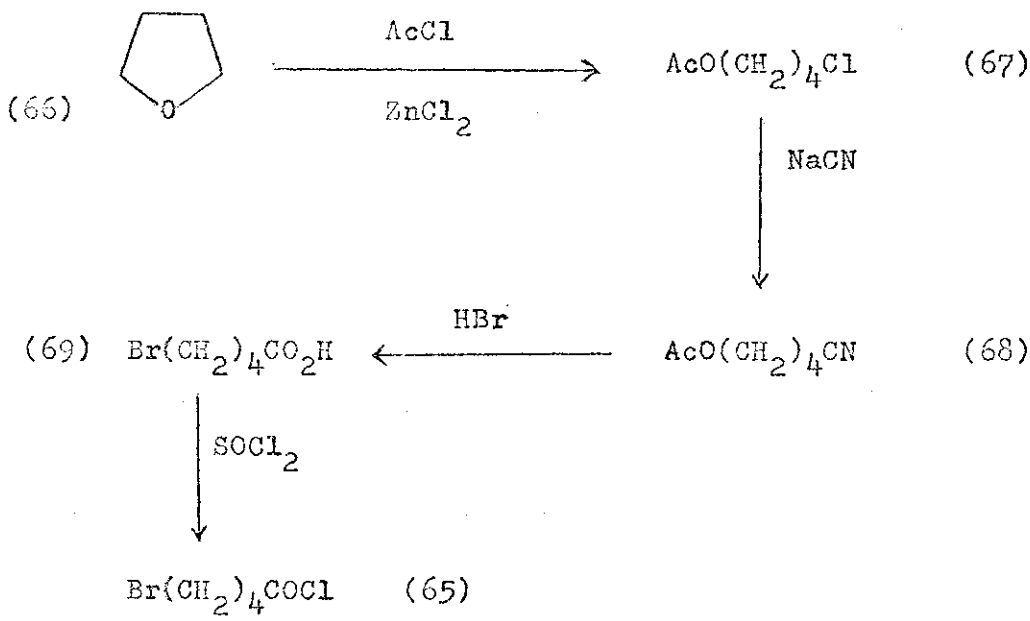
The cyclisation step of (63) to (64) was thought to be possible since the protons α to the sulphone group are known to be acidic. Models showed that the all cis form of (64) was unstrained whereas the cis, cis, trans- alternative had large proton-proton interactions and was therefore energetically less favourable.

The preparation of 5-bromovalerylchloride (65) required for the acylation was based upon Chrétien's preparation of 5-bromovaleric acid[47] followed by treatment with thionyl chloride[48] to give the acid chloride (scheme 26).

Thus, tetrahydrofuran (66) was treated with acetyl chloride and

a zinc chloride catalyst to give acetoxy-chlorobutane (67) which, with sodium cyanide, gave the acetoxy-cyanobutane (68). Refluxing the latter with hydrobromic acid afforded bromovaleric acid (69) which was converted to the acid chloride (65) with thionyl chloride.

Scheme 26.

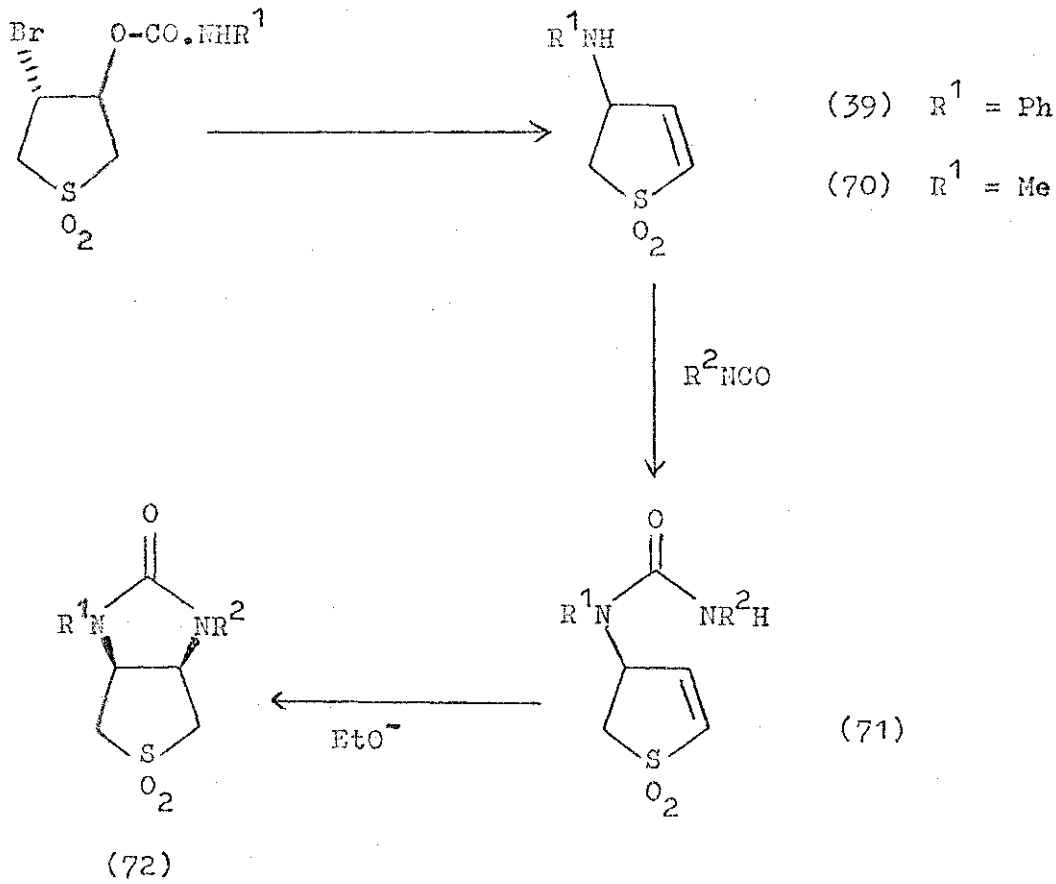


The N-acylation of the cyclic urea to the N-bromovaleryl compound (63) was carried out successfully in dioxan/DMF in the presence of sodium hydride. Although the first attempt at the cyclisation of this compound was unsuccessful further work is called for.

A general route to N-substituted cis-fused cyclic ureas was developed utilizing the allylic amines (39) and (70) prepared by controlled base treatment of the corresponding bromo-carbamates as described earlier (cf. scheme 19). Reaction of the allylic anilino compound (39) with phenylisocyanate or methylisocyanate gave the allylic ureas (71; $R^1=R^2=Ph$), (71; $R^1=Ph, R^2=Me$) (scheme 27). Similarly the methylamino compound (70) gave the dimethyl allylic urea (71, $R^1=R^2=Me$). Treatment of the allylic ureas with base isomerised them to the corresponding

cis-fused cyclic ureas (72;R^1=R^2=Ph; R'=Ph, R^2=Me; R'=R^2=Me).

Scheme 27.

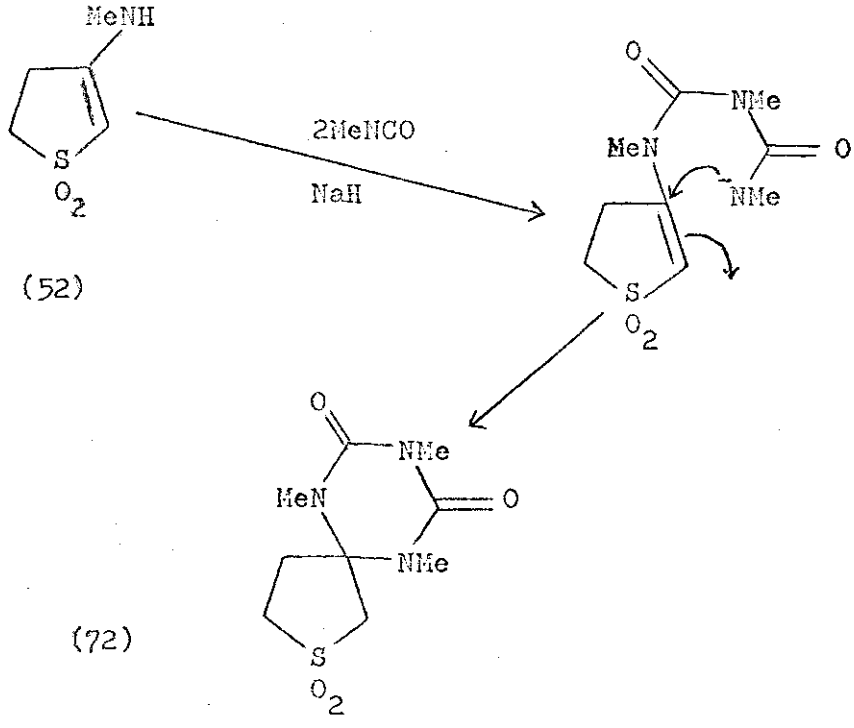


Comparison of the cis-fused dimethyl urea (71; R'=R^2=Me) with the known trans- compound (24), prepared from the trans-3,4-dibromotetra-hydrothiophen dioxide with methylamine and phosgene, showed them to be isomeric.

In contrast to the reaction of the allylic methylamino compound (70) with methylisocyanate to give the allylic urea (71; R'=R^2=Me), the methylamino enamine (52) did not react with the isocyanate on its own. However, in the presence of sodium hydride, two equivalents of methylisocyanate reacted to give the spiro-compound (73). The formation of this compound was envisaged as follows, (scheme 28), again

demonstrating the lability of the 2(3)-double bond of sulpholenes to Michael-type addition reactions.

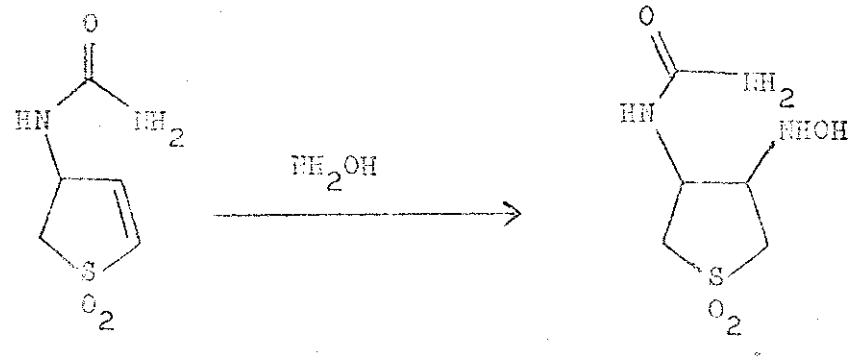
Scheme 28.



(52)

(72)

The allylic urea (60) was found to be a potentially useful compound since strong nucleophiles could add to the unsaturated sulphone. Hydroxylamine gave the Michael addition product (74) at the 4-position.

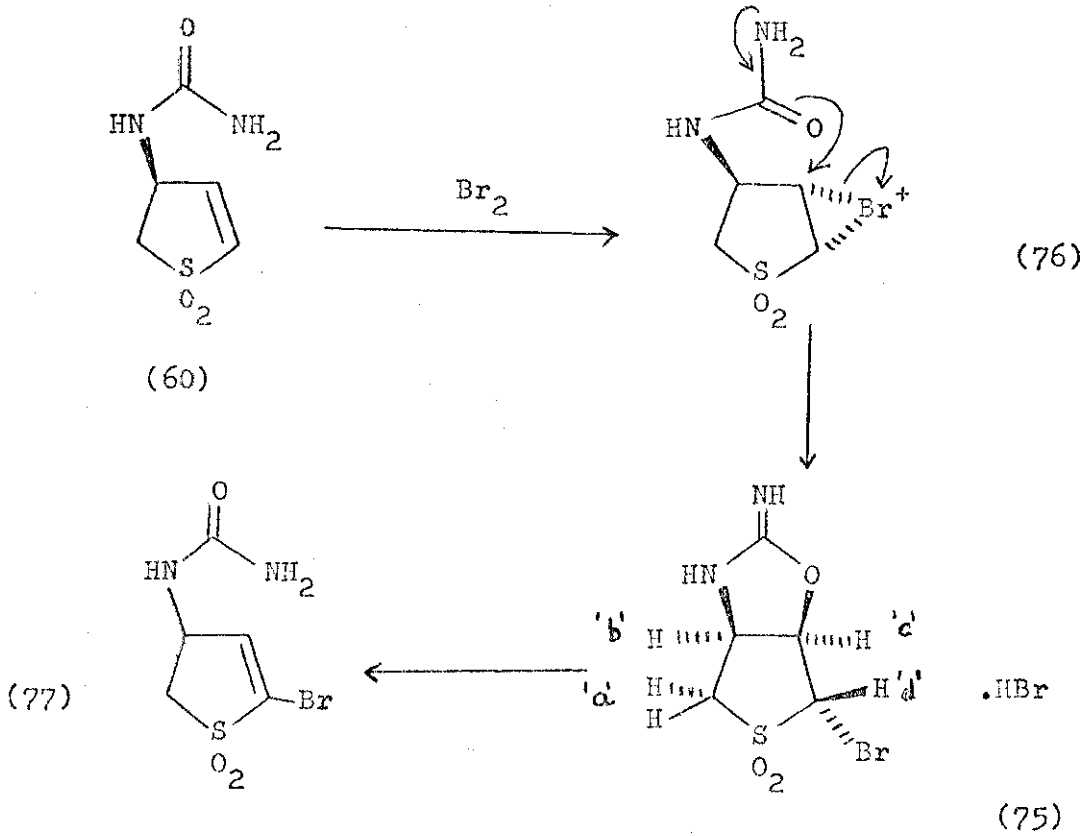


(60)

(74)

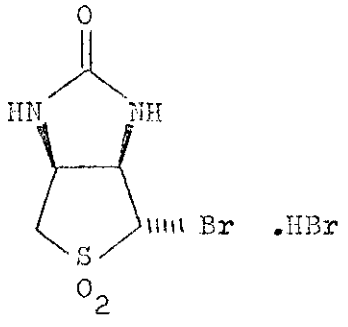
Bromine also reacted with the allylic urea (60) (in acetic acid at 60°) but the product was that of electrophilic addition to give the hydrobromide salt (75), probably via the intermediate (76) (scheme 29). With an equivalent of base the bromide salt (75) spontaneously underwent ring opening to give the brominated allylic urea (77).

Scheme 29.



The hydrobromide salt (75) was very water soluble and gave an ionic bromide test. The infrared showed no characteristic NH_2^+ bands around 2700 cm^{-1}. The spectrum was, in fact, very similar to that of the cis-urea (58) with a peak at 1710 cm^{-1} and a comparable NH region.

The possibility of the hydrobromide salt having structure (78), formed by ring closure through nitrogen, was discounted on the following grounds:-

(78) 

1), that compound (78) would not be expected to ring open to the allylic urea (77) with base, since the unbrominated cyclic urea (58) was quite stable to base. This should be contrasted to the facile ring opening (and subsequent loss of CO_2) that was observed in cyclic carbamates [e.g.(38)] with base (see scheme 19).

2), that thermally-induced ring closure of 2-iodocyclohexyl urea [(6); scheme 6] went through oxygen to give an oxazoline (7), whereas base-induced ring closures of allylic ureas (see schemes 24 and 27) went through nitrogen. There is therefore some justification in believing that ring closure to form the hydrobromide salt went through oxygen as it was formed thermally.

The ^1H n.m.r. spectra of the salt (75) in various solvents are summarised in Table 4. The protons are referred to by the letters in structure (75) and chemical shifts are expressed in τ. Coupling constants for protons 'c' and 'd' are given in Table 5 and are expressed in Hz.

The multiplicity of the signals was as expected for structure (75). The coupling constants for proton 'c' were consistent with the stereochemistry shown as the dihedral angle between 'b' and 'c' would approach 0° (J large) and that between 'c' and 'd' would be of the order

TABLE 4

Solvent	'a'	'b'	'c'	'd'
TFA	m 6.7τ	m 5.1	d 4.5	s 5.2
DMSO d_6	m 6.3	m.4.8	dd 4.0	d 3.9
D_2O	m 6.1	m 4.7	dd 3.9	d 4.3

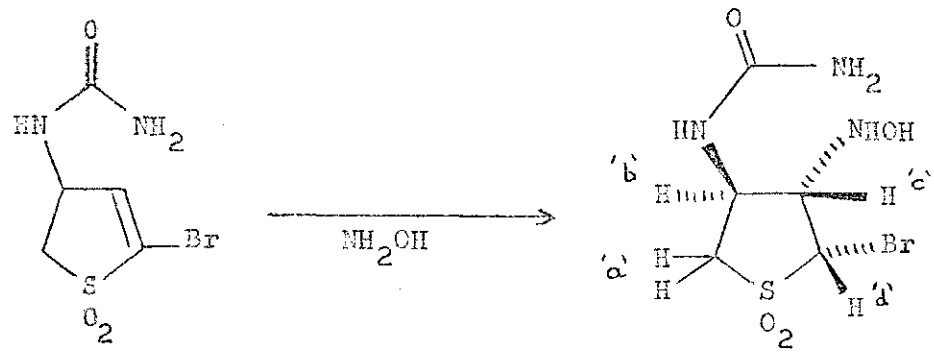
TABLE 5

Solvent	'c'	'd'
TFA	d J=10	s
DMSO d_6	dd J_1=11, J_2=2	d J=2
D_2O	dd J_1=10, J_2=1	d J=1

of 120° (J small). The resonance positions of the protons 'a', 'b' and 'c' varied little for the different solvents, whereas the position of 'd' was very solvent dependent, being downfield of 'c' in DMSO, upfield of both 'c' and 'b' in TFA and between the two in D_2O.

The infra-red spectrum of the of the brominated allylic urea (77) was very similar to that of the unbrominated material (60). The only difference in the ^1H n.m.r. spectra was that (60) had two vinylic protons (singlet and doublet) and (77) had one (doublet). Since the vinylic proton of the brominated material (77) was a doublet it indicated that the bromine was in the 2-position and not the 3-position.

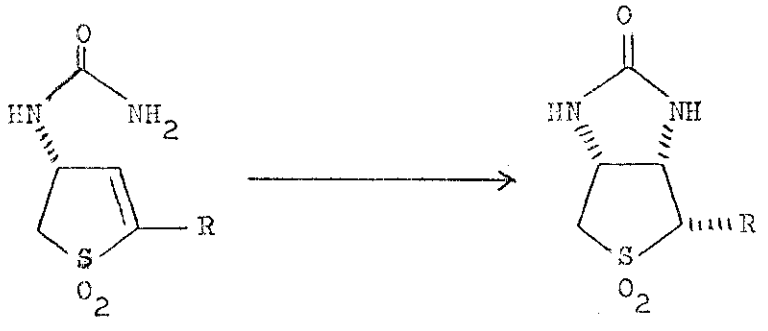
Further proof that the bromine was in the 2-position was obtained by treating the allylic urea (77) with hydroxylamine and isolating the addition product (79).



(77) (79)

The structure of the hydroxylamine adduct (79) was unquestionably proved by the ^1H n.m.r. spectrum comprising a double quartet at τ6.1-7.0 (2H, 'a'), a multiplet at τ5.3 (1H, 'b'), a multiplet at τ5.9 (1H, 'c') and a doublet at τ4.9 (1H, 'd', J = 9Hz). The fact that the coupling constant between 'c' and 'd' was 9Hz indicated a small dihedral angle, i.e. that protons 'c' and 'd' were cis. This proved that the addition had occurred specifically in a trans- manner. (The stereochemistry of 'b' and 'c' is pure conjecture based upon assumed attack from the least hindered side).

If trans- addition to 2-sulpholenes is a general rule it should be possible to cyclise compounds of the type (80) to give products with the biotin configuration,



(80)

"Science is a collection of successful recipes."

Paul Valéry.

EXPERIMENTAL

All melting points were determined on a Kofler-hot-stage and are uncorrected. Infrared spectra were recorded on a Unicam SP200 spectrophotometer for Nujol mulls unless otherwise stated. ^{1}H n.m.r. spectra were recorded on a Varian T60 or a Varian HA100 instrument for solutions in deuteriochloroform containing tetramethylsilane as internal reference unless otherwise stated. The following abbreviations are used in connection with n.m.r. spectra:

TFA	trifluoroacetic acid
s	singlet
d	doublet
t	triplet
q	quartet
dq	double quartet - the AB eight line pattern of an ABX system.
m	multiplet
b	broad(ened)
J	coupling constant

All solvents were G.P.R. grade. Benzene and ether were dried over sodium wire. Light petroleum refers to the fraction of boiling range 60-80°. All organic extracts were dried over anhydrous sodium sulphate before evaporation.

trans-2-Iodocyclohexylurea (6). - Silver isocyanate (0.75 g.) and iodine (1.08 g.) were stirred together in dichloromethane (15 ml.) at 0°. After 5 min. cyclohexene (0.35 g.) was added and the stirring continued for 2 hr. The inorganic salts were filtered off and ammonia gas passed through the colourless solution. After 1 hr. the white urea was filtered off and crystallised from ethanol, m.p. 152-3° (lit.[23] 151-2°), ν max. 3420, 3320, 3200, 1655 cm^{-1}.

N-Phenyl-carbamoyl-1,2-iminocyclohexane (9) - trans-2-Iodocyclohexylurea (1 g.) was heated under reflux with 2N sodium hydroxide solution (10 ml.) for 30 min. and cooled. The oily droplets were extracted with hexane. The extracts were dried and evaporated to about 5 ml. Phenyl isocyanate (0.5 ml.) was added and the mixture warmed. Cooling afforded the aziridine which was filtered off, m.p. (benzene) 149-51° (lit.[13] 149-50), ν max. ($CHCl_3$) 3410, 1690 cm^{-1}, τ 2.2-3.1 (5H,m), 7.3 (2H,s), 8.1 (4H, m), 8.6 (4H, m).

(Found: C, 72.12; H, 7.41; N, 12.95. $C_{13}H_{16}N_2O$ requires C, 72.16; H, 7.46; N, 12.95%).

Carbamoyl-1,2-iminocyclohexane (10). - trans-2-Iodocyclohexylurea (6 g.) was stirred with 10N potassium hydroxide solution (75 ml.) at room temperature for 10 days. The solution was diluted with water (75 ml.), saturated with salt and extracted with ether. Evaporation of the extracts afforded the urea (0.95 g.; 30%), m.p. (benzene) 128-30°, ν max. 3520, 3425, 1685 cm^{-1}, τ 4.45 exch. with D_2O (2H), 7.35 (2H,s), 8.15 (4H, m), 8.65 (4H, m).

(Found: C, 60.06; H, 8.40; N, 20.26. $C_7H_{12}N_2O$ requires C, 59.98; H, 8.63; N, 19.99%).

trans-2-Chlorocyclohexylurea (11). - Carbamoyl-1,2-iminocyclohexane (200 mg.) was heated at 100° for 2 hr. with 5N hydrochloric acid (3 ml.). The mixture was cooled and the urea filtered off and crystallised from ethanol (40 mg.), m.p. 173-5°, ν max. 3410, 3310, 3200, 1660 cm^{-1}.

(Found: C, 47.72; H, 7.44; N, 15.94; Cl, 20.03. $C_7H_{13}ClN_2O$ requires C, 47.60; H, 7.42; N, 15.87; Cl, 20.07%).

Ethyl-N-(trans-2-iodocyclohexyl) Carbamate (14). - Silver isocyanate (11.5 g.) and iodine (14.3 g.) were stirred together in dichloromethane (150 ml.) at 0°. After 5 min. cyclohexene (4.85 g.) was added and the stirring continued for 2 hr. The inorganic salts were filtered off and the colourless filtrate evaporated to half volume. Ethanol (60 ml.) was added to the solution and the mixture heated under reflux for 2 hr. The solution was concentrated to 20 ml. and cold water (100 ml.), containing a little sodium sulphite, added to produce a white precipitate. This was collected, dried at 50° in vacuo and crystallised from petrol/benzene (9:1) to give the yellow carbamate (14.8 g.; 87%), m.p. 122-3°, ν max. 3460, 1710 cm^{-1}, τ 5.0 (1H,m), 6.0 (1H, m), 5.9 (2H, q, J 7Hz), 7.3-8.7 (8H, m), 8.7 (3H, t, J 7Hz).

(Found: C, 36.55; H, 5.34; N, 4.66. $C_9H_{16}INO_2$ requires C, 36.39; H, 5.43; N, 4.72%).

Diethyl N-(2-Iodocyclohexyl) Isocarbamate (12). - Triethyloxoniumfluoroborate (8 g.) and ethyl-N-(2-iodocyclohexyl)carbamate (6 g.) were heated under reflux for 2 hr. in dichloromethane (100 ml.). The solution was cooled and washed with saturated sodium hydrogen carbonate solution and then water. The organic phase was evaporated to dryness to give an oil which was distilled under reduced pressure (167° at 55 mm.) to give the colourless isocarbamate, ν max. 1670 cm^{-1}, τ 5.3 (1H, bs), 6.3 (1H, bs), 7.3-9.2 (8H, m), 8.7 (6H, dt, J 7Hz), 5.9 (4H, dq, J 7Hz).

trans-3, 4-Dipiperidinotetrahydrothiophen-1,1-dioxide (22). - Freshly distilled piperdine (2.46 g.) was added to a suspension of 3,4-dibromo-tetrahydrothiophen dioxide (2 g.) in water (30 ml.) at room temperature with stirring. A thick, white suspension was formed. After 15 min. aqueous 2N sodium hydroxide (10 ml.) was added and the suspension cooled at 0° for 24 hr. and filtered. The solid was washed with cold water, and crystallised from ethanol to give colourless needles (1.75 g.), m.p. 122-3°, ν max. 1310, 1130 cm^{-1}. A sample (250 mg.) was warmed

with methyl iodide for 15 min. The excess of the reagent was evaporated off and the residue crystallised from acetonitrile to give colourless prisms of the monomethiodide, m.p. 177-9°,

(Found: C, 41.99; H, 6.64; N, 6.44; I, 29.61. $C_{15}H_{29}IN_2O_2S$ requires C, 42.07; H, 6.82; N, 6.54; I, 29.63%).

trans-3,4-Bis(monomethylamino)tetrahydrothiophen-1,1-dioxide (23, R=H). - Monomethylamine gas was slowly passed through a solution of 3,4-dibromotetrahydrothiophen dioxide (16.5 g.) in chloroform (325 ml.) with stirring at about 10°. After 1 hr. the reaction flask was sealed and the reaction mixture was then left to stir overnight at room temperature. The solution was evaporated to small volume and aqueous 1N sodium hydroxide (30 ml.) was added. The aqueous phase was continuously extracted with chloroform for 72 hr. The extracts were dried and evaporated to give an oil which slowly crystallised (9.5 g; 90%). The product was recrystallised from benzene-light petroleum to give the diamine, m.p. 67-9°, ν max. 3320, 1295, 1110 cm^{-1}, τ 6.1-7.1 (6H. m), 7.5; (6H, s), 8.5 exch. with D_2O (2H).

(Found: C, 40.31; H, 7.84; N, 15.71. $C_6H_{14}N_2O_2S$ requires C, 40.43; H, 7.92; N, 15.72%) With hydrochloric acid a bis-hydrochloride formed, m.p. (aqueous ethanol) 193-5°.

(Found: C, 28.82; H, 6.28; N, 11.13; Cl, 28.38. $C_6H_{16}N_2Cl_2O_2S$ requires C, 28.69; H, 6.42; N, 11.15; Cl, 28.23%).

The bis-methylamino compound was further characterised as its acetyl derivative, m.p. (water) 251-3°, ν max. 1645, 1360, 1115 cm^{-1}.

(Found: C, 45.86; H, 6.93; N, 10.85; $C_{10}H_{18}N_2O_4S$ requires C, 45.78; H, 6.92; N, 10.68%).

1,3-Dimethyl-trans-hexahydro[3,4-d]imidazol-2-one-5,5-dioxide (24) - trans-3,4-Bis(monomethylamino)tetrahydrothiophen-1,1-dioxide (6.25 g.) in 10% w/v aqueous sodium carbonate solution (100 ml.) was treated with

phosgene (17.4 g.) in toluene (12.6 g.) whilst stirring at room temperature. Ether (100 ml.) was added and stirring continued overnight so that most of the excess phosgene evaporated off. The aqueous phase was extracted with dichloromethane and the combined organic layers evaporated to dryness to yield a white solid (3.1 g; 44%). Recrystallisation from water gave the trans-urea, m.p. 198-9°, ν max. 1710, 1320, 1140 cm^{-1}, τ 7.2 (6H, s), 6.2-7.0 (6H, m). After heating the compound with 4N NaOD in D_2O for 78 hr. the n.m.r. spectrum showed τ 7.2 (6H, s), 6.75 (2H, s).

(Found: C, 41.34; H, 6.02; N, 13.59; S, 15.85. $C_7H_{12}N_2O_3S$ requires C, 41.16; H, 5.92; N, 13.72; S, 15,70%).

Details of the X-Ray Analysis. - Intensity data were collected for a crystal of the trans-urea (24) of approximate dimensions 0.10x0.15x0.60 mm, mounted about the C^* axis, using a General Electric XRD6 diffractometer equipped with a manual goniometer, pulse height analyser and scintillation counter. Nickel-filtered copper radiation was used (μ = 27,72 cm^{-1}. for Cu-Kα radiation; λ mean 1.54178 Å). The stationary crystal-stationary counter method of intensity estimation was used throughout, with a 4° take-off angle and a counting time of 10 sec. Individual backgrounds $(2\,\theta_{hKl} + 1)^\circ$ were measured for all reflections. The 204 and 112 reflections were used as reference reflections to check on crystal stability; in neither case was there any significant decline in the course of the data collection. The intensities of 700 reflections with $2\theta \leqslant 120^\circ$ were measured, of which 519 were considered to be statistically significant (net counts were $\geqslant 3\,\sigma\,(I)$, where σ, the standard deviation in the intensity, was taken as $[I + 2B + (0.03I)^2]^{\frac{1}{2}}$, and B is the background count[9]). The 181 'unobserved' reflections were excluded from any subsequent least squares calculations. No correction for absorption was applied. The intensity statistics were as expected for the space group C2/c. The position of the sulphur

atom was readily deduced from a 3-dimensional (E^2-1) sharpened Patterson synthesis. With the sulphur on the position (0,Y,¼), i.e. on the two-fold axis, a structure factor calculation gave an R-factor of 0.531. The resulting difference Fourier map revealed the complete structure. Full-matrix least squares refinement of the positional and isotropic thermal parameters for the atoms, all of which had been assigned their correct scattering factors, resulted in an R factor of 0.161 after four cycles. The x and z coordinates of the sulphur, carbon and oxygen atoms, assumed to lie on the two-fold axis were kept invariant. All bond lengths and angles had acceptable values after refinement. Conversion of the temperature factors to their anisotropic (β_{ij}) equivalents, followed by four more cycles of refinement, reduced R to 0.103. Inspection of a difference Fourier map at this stage revealed the presence of the 6 hydrogen atoms in the asymmetric unit. Further refinement with the positional and isotropic thermal parameters of the hydrogen atoms also being varied gave an R factor of 0.064. Inclusion of a Hughes-type weighting scheme[50] followed by four more cycles of refinement gave an R factor of 0.0619 for the 519 observed reflections (an R factor of 0.0929 for all 700 measured reflections). All parameter shifts were less than 0.1 of their corresponding standard deviations and refinement was judged to be complete.

All calculations were performed on the University of London CDC6600 computer using the XRAY 70 crystallographic computing system.[51] The scattering factors were taken from ref.[52] Figures 1 and 3 were drawn with the aid of ORTEP.[53]

trans-Hexahydro[3,4-d]imidazol-2-one-5,5-dioxide (30) - 3,4-Dibromotetrahydrothiophen-1,1-dioxide (30 g.) and liquid ammonia (500 ml.) were sealed in a stainless steel autoclave at room temperature for 1 month. The excess of ammonia was then evaporated off and the residue taken up in aqueous sodium carbonate solution, (90 g. in 500 ml.)

A solution of phosgene (30 g.) in benzene (100 ml.) was slowly added to the stirred ice-cooled solution. After leaving the mixture overnight, when the excess of phosgene evaporated off, the precipitated solid was filtered off and recrystallised from water to give needles of the urea (4.5 g.; 24%), m.p. (sealed tube) 298°, ν max. 3290, 1705, 1690, 1310, 1110 cm^{-1}, τ (TFA) 5.0 (2H,m), 5.6-6.4 (4H, m).

(Found: C, 34.21; H, 4.69; N, 15.81; S, 18.44. $C_5H_8N_2O_3S$ requires C, 34.09; H, 4.58; N, 15.91; S, 18.20%).

2,5-Dihydro-3(1'-pyridinium)thiophen-1,1-dioxide Bromide (33) - 3,4-Dibromotetrahydrothiophen dioxide (10 g.) was left at room temperature in dry pyridine (100 ml.) for48 hr. The purple crystals that formed were collected, washed with benzene and recrystallised from ethanol-methanol (3:2) to give purple needles of the salt (3.8 g., 38%), m.p. 177-9°, ν max. 1630, 1320, 1135 cm^{-1}, τ (DMSO-d_6) 0.7 (2H, d, J 6Hz), 1.25 (1H, t, J 8Hz), 1.75 (2H, t, J 7Hz), 2.9 (1H, bs, $W_{\frac{1}{2}}$ 7Hz), 5.3 (2H, m), 5.7 (2H, m).

(Found: C, 39.13; H, 3.65; N, 5.07; S, 11.61. $C_9H_{10}BrNO_2S$ requires C, 39.29; H, 4.03; N, 5.02; S, 11.68%).

3-Bromo-4-hydroxytetrahydrothiophen-1,1-dioxide (34). - Bromine (38 g.) in water (5 l.) and 3-sulpholene (24 g.) were reacted together at 5° for 3 days. The crystalline precipitate (34 g.; 7%) was collected and crystallised from methanol to give the bromohydrin, m.p. 189-90° (lit.[43] 189-90°), ν max. 3450, 1295, 1120 cm^{-1}.

N-Phenyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) Carbamate (35). - 3-Bromo-4-hydroxytetrahydro-thiophen-1,1-dioxide (4 g.) was heated with phenylisocyanate (3.3 g.) until all the solid dissolved. The mixture was cooled to give a white solid which was triturated with hot light petroleum to remove the excess of phenylisocyanate. The residue was crystallised from toluene (ca. 200 ml.) to give the carbamate (5.7 g.; 92%), m.p. 153-4° ν max. 3330, 1600, 1530, 1310, 1125 cm^{-1}, τ 2.7 (5H, m), 2.95 exch. with D_2O (1H), 4.4 (1H, m), 5.3 (1H, m), 5.9-6.9 (4H, m).

(Found: C, 39.61; H, 3.65; N, 4.23; $C_{11}H_{12}BrNO_4S$ requires C, 39.53; H, 3.62; N, 4.19%).

4-Anilino-2,3-dihydrothiophen-1,1-dioxide (36) - N-Phenyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) carbamate (2.3 g.) was stirred overnight with ethanol (80 ml.) in which sodium (0.65 g.) had been dissolved. The white suspension was evaporated to dryness, water added to the residue and the product extracted with chloroform. After evaporation of the solvent the amine (1.4 g.; 97%) was crystallised from ethanol, m.p. 162-3°, ν max. 3300, 1620, 1595, 1500, 1290, 1100 cm^{-1}, τ 2.7. (5H,m), 3.2 exch. with D_2O (1H), 4.2 (1H, s), 6.5-7.2 (4H, m).

(Found: C, 57.43; H, 5.40; N, 6.54; S, 15.31. $C_{10}H_{11}NO_2S$ requires C, 57.38; H, 5.30; N, 6.69; S, 15.32%).

N-Phenyl-O-(1,1-dioxy-2,3-dihydro-3-thienyl) Carbamate (37) - 3-Hydroxy-2,3-dihydrothiophen-1,1-dioxide[44] (1 g.) was heated with phenylisocyanate (1.5 g.) for 5 mins. under reflux. The mixture was cooled and triturated with light petroleum to remove phenylisocyanate. The residue was purified by precipitation from benzene with light petroleum to give the allylic carbamate (1.38 g.; 75%), m.p. 114-5°, ν max. 3370, 1725, 1655, 1600, 1300, 1150, cm^{-1}., τ 2.6 (5H, m), 2.7 (1H, bs), 3.15 (2H, s and d, J 2Hz), 3.95 (1H, m), 6.0-6.9 (2H, dq, J_{AB} 14Hz, J_{AX} 7Hz, J_{BX} 4Hz).

(Found: C, 52.37; H, 4.47; N, 5.37; $C_{11}H_{11}NO_4S$ requires C, 52.16; H, 4.38; N, 5.53%). The allylic carbamate (146 mg) was treated with sodium ethoxide in ethanol to yield 4-anilino-2,3-dihydro-thiophen-1,1-dioxide (108 mg.; 90%), m.p. 162-3°.

3-Phenyl-5,5-dioxyhexahydrothieno[3,4-d]oxazol-2-one (38) - To a solution of N-phenyl-O-(1,1-dioxy-4-bromo-tetrahydro-3-thienyl) carbamate (1.5 g.) in chloroform (20 ml.) was added triethylamine (0.94 g.). After 2 hr. the mixture was filtered and the residue washed with

chloroform to remove amine hydrobromide. The product was crystallised from acetonitrile to give the cyclic carbamate (107 g.; 94%), m.p. 191-3°, ν max. 1740, 1600, 1500, 1325, 1135 cm^{-1}, τ (DMSO-d_6) 2.3-3.0 (5H,m), 4.4-4.8(2H,m), 6.2-7.0 (4H, m).

(Found: C, 52.11; H, 4.34; N, 5.46; $C_{11}H_{11}NO_4S$ requires C, 52.16; H, 4.38; N, 5.53%). The cyclic carbamate (169 mg.) was treated with sodium ethoxide in ethanol (20 ml.) to yield 4-anilino-2,3-dihydrothiophen-1,1-dioxide (140 mg.; 99%). One equivalent of triethylamine in chloroform was added dropwise over a period of 2 hr. to a solution of N-phenyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) carbamate (one equivalent)in chloroform . Examination of the solution by t.l.c. (SiO_2; 5% MeOH/$CHCl_3$) showed it to be a mixture of N-phenyl-O-(1,1-dioxy-2,3-dihydro-3-thienyl) carbamate (37) and 3-phenyl-5,5-dioxyhexahydrothieno[3,4-d]oxazol-2-one (38).

3-Anilino-2,3-dihydrothiophen-1,1-dioxide (39). - N-Phenyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) carbamate (568 mg.) and triethylamine (361 mg.) were heated together under reflux in ethanol (20 ml.) for 3 hr. The solvent and excess of amine was evaporated off, water added to the residue and the product extracted with chloroform. After drying, the organic phase was evaporated to dryness and the solid (294 mg.; 85%) crystallised from benzene to give the allylic amine, m.p. 133-4°, ν max. 3375, 3100, 1605, 1505, 1290, 1120 cm^{-1}, τ 2.7 (2H, m), 3.2 (5H, m), 5.0 (1H, m), 6.0-7.0 (2H, dq, J_{AB} 14Hz, J_{AX} 7Hz, J_{BX} 4Hz), 6.1 exch. with D_2O (1H).

(Found: C, 57.60; H, 5.17; N, 6.62; S, 15.31; $C_{10}H_{11}NO_2S$ requires C, 57.38; H, 5.30; N, 6.69; S, 15.32%). Under similar conditions 3-phenyl-5,5-dioxyhexahydrothieno[3,4-d]oxazol-2-one gave the same product. On treatment with sodium ethoxide in ethanol the allylic amine was quantitatively isomerised to 4-anilino-2,3-dihydrothiophen-1,1-dioxide.

N-Acetyl-4-anilino-2,3-dihydrothiophen-1,1-dioxide (42) - 4-Anilino-2,3-dihydrothiophen-1,1-dioxide (703 mg.) was heated under reflux with acetyl chloride (4 ml.) for 2 hr. The acetyl chloride was evaporated off and the residue crystallised from a large volume of ethanol to give the N-acetyl derivative (705 mg; 84%), m.p. 171-3°, ν max. 3110(w), 1700, 1600, 1490, 1280, 1095 cm^{-1}, τ 2.2-2.8 (5H,m), 3.3 (1H,s), 6.5 -7.2 (4H,m,), 8.1 (3H,s).

(Found: C, 57.10; H, 5.25; N, 5.49. $C_{12}H_{13}NO_3S$ requires C, 57.35; H, 5.21; N, 5.58%). The N-acetyl derivative (96 mg.) was stirred overnight at room temperature with 1N sodium hydroxide solution (1 ml.) and ethanol (2 ml.). The solvents were evaporated off and water added to the residue. The aqueous phase was extracted with chloroform, which, upon evaporation, gave 4-anilino-2,3-dihydrothiophen-1,1-dioxide.

Treatment of the N-acetyl derivative (104 mg.) at room temperature with 2N hydrochloric acid (4 ml.) and ethanol (4 ml.) for 16 hr. afforded a sticky product, which t. l. c. (SiO_2; 5% MeOH/$CHCl_3$) showed to be a mixture of an unknown compound and acetanilide, the latter being isolated by recrystallisation from light petroleum. Upon addition of Brady's reagent to the residual mother liquid a yellow derivative formed which was crystallised from acetic acid, m.p. 208-9° (lit. m.p.s. of 2,4-DNP derivative of 3-oxotetrahydrothiophen-1,1-dioxide are 211-13° [54] and 205-7° [45]). The reaction mixture had ν max. 3300, 1660, 1600, 1560, 1500 (assigned to acetanilide), 1760, 1335, 1130 cm^{-1}, τ 1.8 (1H, bs), 2.3-3.1 (5H, m), 7.9 (3H,s) (acetanilide), 6.3 (2H,s), 6.4 (2H,m), 6.9 (2H,m).

1,1-Dioxy-4-bromotetrahydro-3-thienyl Ethyl Carbonate (44). - To a suspension of 3-bromo-4-hydroxytetrahydrothiophen-1,1-dioxide (350 mg.) in chloroform (6 ml.) containing ethyl chloroformate (180 mg.) cooled below 10° was slowly added, over a period of one hour, a solution of

triethylamine (166 mg.) in chloroform (6 ml.). The resulting solution was evaporated to dryness and the residue extracted with ethyl acetate. Evaporation yielded the product contaminated with bromohydrin, which was removed by dissolving the product in dichloromethane, filtering and evaporating to dryness. The product was crystallised from ethanol to give the ethyl carbonate, m.p. 108-9°, ν max. 1740, 1310, 1150 cm^{-1}, τ 4.5 (1H,m), 5.7 (2H,q,J 7Hz), 5.1-6.9 (5H,m), 8.6 (3H,t, J 7Hz). (Found: C, 29.09; H, 4.05; $C_7H_{11}BrO_5S$ requires C, 29.30; H, 3.86%).

1,1-Dioxy-2,3-dihydro-3-thienyl Ethyl Carbonate (45) - To an ice-cold suspension of 3-bromo-4-hydroxytetrahydrothiophen-1,1-dioxide (2.24 g.) in chloroform (100 ml.) was added triethylamine (2.1 g.) and ethyl chloroformate (1.13 g.). After stirring for 2hr. the solvent was evaporated off and the residue extracted with ethyl acetate. The extracts were evaporated to dryness to give an oil which was distilled under reduced pressure to give the allylic carbonate, b.p. 164° at 2mm., n^{24}_D 1.4843, ν max. (liq. film) 3080, 2980, 1750, 1615, 1310, 1260, 1150, 1100 cm^{-1}., τ 3.2 (2H, s and d, J 2Hz), 4.35 (1H,m), 5.75 (2H, q, J 7Hz), 6.0-6.95 (2H, dq, J_{AB} 14 Hz, J_{AX} 7Hz , J_{BX} 4 Hz), 8.65 (3H, t, J 7Hz). (Found: C, 40.68; H, 4.98; $C_7H_{10}O_5S$ requires C, 40.78; H, 4.89%).

1,1-Dioxy-4-bromotetrahydro-3-thienyl Chloroformate (46) - Finely powdered 3-bromo-4-hydroxytetrahydrothiophen-1,1-dioxide (20 g.) and a solution of quinoline (14 g.) in dry benzene (50 ml.) were simultaneously added over a period of two hours to a briskly sitrred, ice-cooled solution of phosgene (ca. 100 g.) in dry benzene (500 ml.). After addition was complete the suspension was stirred at room temperature for 3 hr. The mixture was washed twice with 5N hydrochloric acid (2x50 ml.), water (100 ml.), filtered to remove unreacted bromohydrin (2.05 g.) and dried (Na_2SO_4). The benzene solution was evaporated to dryness to give a

pale yellow solid (20.22 g.; 87% based on reacted material). The chloroformate was not further purified for reactions. A small sample was sublimed under reduced pressure (120° at 0.5 mm.) to give an analytical sample, m.p. 93-5°, ν max. 1760, 1320, 1165, 1130 cm^{-1}. τ 4.3 (1H, m), 5.3 (1H, m), 5.8-6.8 (4H, m).

(Found: C, 21.73; H, 2.13. $C_5H_6BrClO_4S$ requires C, 21.64; H, 2.18%).

N-Phenyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) Carbamate (35) - To a solution of 1,1-dioxy-4-bromotetrahydro-3-thienyl chloroformate (560 mg.) in benzene (21 ml.) was added a solution of distilled aniline (375 mg.) in benzene (9 ml.) After stirring briskly for 1 hr. the mixture was filtered and the residue washed well with hot ethyl acetate. The combined filtrates were evaporated to dryness to give a white solid (649 mg.; 96%).which was crystallised from toluene to give the N-phenyl carbamate identical to that prepared from phenylisocyanate.

N-Benzyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) Carbamate (47) - To a solution of 1,1-dioxy-4-bromotetrahydro-3-thienylchloroformate (2.77 g.) in benzene (70 ml.) was added a solution of benzylamine (2.14 g.) in benzene (30 ml.). A white precipitate formed and stirring was continued for one hr. The mixture was filtered and the residue washed with benzene. The filtrate was evaporated to dryness and the N-benzyl carbamate (3.33 g,; 96%) crystallised from benzene, m.p. 120-1°, ν max. 3350, 1700, 1550, 1340, 1150 cm^{-1}, τ 2.6 (5H, s), 4.0-4.6 (1H, b), 4.5 (1H, m), 5.4 exch. with D_2O (1H), 5.6 (2H, d), 6.0-7.0 (4H, m).

(Found: C, 41.42; H, 4.02; N, 4.00. $C_{12}H_{14}BrNO_4S$ requires C, 41.38; H, 4.05; N, 4.02 %).

N-Methyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) Carbamate (48). A solution of methylamine (0.31 g.) in cold benzene (20 ml.) was slowly added over a period of 20 mins. to an ice-cooled solution of 1,1-dioxy-4-bromotetrahydro-3-thienyl chloroformate (1.36 g.) in benzene (50ml).

After stirring for 1 hr. the mixture was filtered and the residue extracted with hot benzene. Evaporation of the extracts gave the N-methyl carbamate (1.18 g.; 88%) which was crystallised from ethanol, m.p. 96-7°, ν max. 3370, 1700, 1320, 1140 cm^{-1}, τ 4.3 (1H, m), 4.8 exch. with D_2O (1H), 5.2 (1H, m), 5.8-6.8 (4H, m), 7.0 (3H, d).

(Found: C, 26.78; H, 3.68; N, 5.08. $C_6H_{10}BrNO_4S$ requires C, 26.48; H, 3.70; N, 5.16%).

N,N-Dimethyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) Carbamate (49) - To an ice-cooled solution of 1,1-dioxy-4-bromotetrahydro-3-thienyl chloroformate (4.5 g.) in dry benzene (50 ml.) was added a cooled solution of dimethylamine (1.57 g.) in benzene (20 ml.) over a period of 1 hr. The mixture was stirred at room temperature for 16 hr. and filtered. The residue was washed well with dry benzene and the filtrate evaporated to dryness to give the N,N-dimethyl carbamate (3.68 g.; 80%), m.p. 128-9° (from benzene), ν max. 1705, 1320, 1130 cm^{-1}, τ 4.5 (1H, m), 5.3 (1H, m), 5.9-6.9 (4H, m), 7.1 (6H, s).

(Found: C, 29.50; H, 4.18; N, 4.78; Br, 27.89; S, 11.40. $C_7H_{12}NBrO_4S$ requires C, 29.38; H, 4.23; N, 4.90; Br, 27.92; S, 11.20%).

1,1-Dioxy-2,3-dihydro-3-thienyl Carbamate (50). - Ammonia gas was passed through a solution of 1,1-dioxy-4-bromotetrahydro-3-thienyl chloroformate (2.77 g.) in benzene (100 ml.) for 5 min. at room temperature. A white precipitate was formed and stirring continued for 2 hr. The precipitate was filtered off and the residue extracted with ethyl acetate which was evaporated to give the carbamate (1.72 g.; 97%) which was crystallised from acetonitrile, m.p. 148-50°, ν max. 3460, 3370, 3210 (w), 1710, 1615, 1300, 1150 cm^{-1}, τ (TFA) 3.4 (2H, s), 4.4 (1H, m), 6.3-7.1 (2H, dq, J_{AB} 14 Hz, J_{AX} 7Hz, J_{BX} J 4Hz).

(Found: C, 34.08; H, 3.97; N, 7.84; $C_5H_7NO_4S$ requires C, 33.89; H, 3.98; N, 7.91%).

4-Benzylamino-2,3-dihydrothiophen-1,1-dioxide (51). - N-Benzyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) carbamate (696 mg.) was stirred overnight at room temperature with ethanol (26 ml.) containing sodium (184 mg.). The mixture was evaporated to dryness, water added to the residue and the product extracted with chloroform. The extracts were evaporated to dryness to give the benzyl enamine (445 mg:, 97%) which was crystallised from methanol, m.p. 164-6°, ν max. 3350, 1615, 1550, 1365,1105 cm^{-1}, τ 2.7 (5H,m), 4.7 (1H, m)., 6.6-7.3 (4H,m).

(Found: C, 59.18; H, 5.78; N, 6.24. $C_{11}H_{13}NO_2S$ requires C,59.16; H, 5.86; N, 6.27%).

4-Methylamino-2,3-dihydrothiophen-1,1-dioxide (52). - N-Methyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) carbamate (0.54 g.) was stirred at room temperature overnight with ethanol (26 ml.) containing sodium (184 mg.). The mixture was evaporated to dryness, water added and the product extracted well with chloroform. Evaporation of the extracts afforded the methyl enamine (252 mg; 85%), which was crystallised from ethanol, m.p. 157-9°, ν max. 3380, 1615, 1240, 1100 cm^{-1}, τ (D_2O) 4.9 (1H, s), 6.5-6.9 (2H,m), 6.9-7.3 (2H, m), 7.4 (3H,s).

(Found: C, 40.67; H, 5.98; N, 9.48. $C_5H_9NO_2S$ requires C, 40.81; H, 6.16; N, 9.52%).

4-Ethoxy-2,3-dihydrothiophen-1,1-dioxide (54). - N,N-Dimethyl(1,1-dioxy-4-bromotetrahydro-3-thienyl) carbamate (426 mg.) was stirred overnight with ethanol (25 ml.) containing sodium (120 mg.). The mixture was evaporated to dryness, water added and the product extracted with chloroform. The extracts were evaporated to dryness to give an oil which slowly solidified. The ethyl ether had mp 65-6° (from CCl_4), ν max. 3100 (w), 1615, 1350, 1110 cm^{-1}, τ 4.3 (1H, s), 6.0 (2H,q,J 7Hz), 6.6 (2H, m), 7.1 (2H, m), 8.6 (3H, t, J 7Hz).

(Found: C, 44.25; H, 6.23; S, 19.77. $C_6H_{10}O_3S$ requires C, 44.43; H, 6.21; S, 19.77%).

3-Dimethylamino-2,3-dihydrothiophen-1,1-dioxide (18). - N,N-dimethyl-O-(1,1-dioxy-4-bromotetrahydro-3-thienyl) carbamate (98 mg.) was warmed at 60° with 10% sodium carbonate solution (5 ml.) until a clear solution formed. The solution was cooled and extracted with chloroform to yield an oil which slowly crystallised. The product m.p. 64-5°, was shown to be identical to the amine prepared by the method of Bailey and Cummins.[28]

4-Dimethylamino-2,3-dihydrothiophen-1,1-dioxide (55). - 3-Dimethylamino-2,3-dihydrothiophen-1,1-dioxide (303 mg.) was stirred overnight at room temperature with ethanol (20 ml.) containing sodium (87 mg.). The solvent was evaporated, water added, and the product extracted with chloroform. The extracts were evaporated to dryness to give the enamine (289 mg; 96%), m.p. 171-2° (from ethanol), ν max. 3100 (w), 1600, 1280, 1105 cm^{-1}, τ 4.8 (1H, s), 6.4-7.2 (4H, m), 7.1 (6H, s). (Found: C, 44.50; H, 6.70; N, 8.69. $C_6H_{11}NO_2S$ requires C, 44.70; H, 6.83; N, 8.69%).

1,1-Dioxy-4-bromotetrahydro-3-thienyl Allophanate (57) - An intimately ground mixture of 1,1-dioxy-4-bromo-tetrahydro-3-thienyl chloroformate (2.77 g.) and urea (1.2 g.) was heated at ca. 100° for 24 hr. The mixture fused, bubbled and finally solidified. After cooling the residue was washed well with hot acetone, cold water and again with acetone to give the white allophanate (1.96g.; 65%), m.p. 201-2° (from water), ν max. 3450, 3350, 1740, 1690, 1605, 1320, 1130 cm^{-1}, τ (TFA) 1.4 (1H, b), 2.0-4.0 (2H, b. hump), 4.8 (1H, m), 5.7 (1H, m), 6.1-7.1 (4H, m).

(Found: C, 24.31; H, 3.07; N, 9.47; $C_6H_9N_2BrO_5S$ requires C, 23.93; H, 3.01; N, 9.31%).

1,1-Dioxy-2,3-dihydro-3-thienyl Allophanate (59) -
1,1-Dioxy-4-bromotetrahydro-3-thienyl allophanate (934 mg.) was heated under reflux with quinoline (640 mg.) and water (10 ml.) for 2 hr. The mixture was cooled, 1N sodium hydroxide solution (3.1 ml.) added, and the water removed under reduced pressure. The residue was washed with ether to remove quinoline and the allophanate (497 mg; 73%) crystallised from water, m.p. 170-2°, ν max. 3400, 3310, 3240, 1760, 1710, 1600, 1320, 1170 cm^{-1}, τ (TFA) 3.5 (2H,m), 4.8 (1H, m), 4.3 exch. with D_2O (1H), 6.7 exch. with D_2O (2H), 6.7 (2H,dq, J_{AB} 14Hz, J_{AX} 7Hz, J_{BX} 4Hz).
(Found: C, 32.69; H, 3.74; N, 12.64; S, 14.53. $C_6H_8N_2O_5S$ requires C, 32.73; H, 3.66; N, 12.73; S, 14.55%).

1,1-Dioxy-2,3-dihydro-3-thienylurea (60) -
1,1-Dioxy-4-bromotetrahydro-3-thienyl allophanate (2 g.) was stirred in water (30 ml.) at 65°. Solid sodium hydrogen carbonate (560 mg.) was added over 30 min. and stirring continued for a further 4½ hr. to give a clear solution. The solvent was removed under reduced pressure and the residue crystallised from water to give the allylic urea (913 mg; 78%), m.p. 195-7°, ν max. 3480, 3380, 3300, 3080, 1660, 1560, 1295, 1120 cm^{-1}, τ (TFA) 3.3 (2H, s and s, J 1Hz), 5.0 (1H, m), 6.2-7.2 (2H, dq, J_{AB} 14 Hz, J_{AX} 7Hz, J_{BX} 4Hz).
(Found: C, 34.06; H, 4.63; N, 15.77; S, 18.35. $C_5H_8N_2O_3S$ requires C, 34.08; H, 4.58; N, 15.91; S, 18.21%).

Under similar conditions 1,1-dioxy-2,3-dihydro-3-thienyl allophanate (59) was converted into the allylic urea.

cis-Hexahydrothieno[3,4-d] imidazol-2-one-5,5-dioxide (58) -
1,1-Dioxy-4-bromotetrahydro-3-thienyl allophanate (3 g.) and anhydrous sodium carbonate (1.27 g.) were heated under reflux in water (20 ml.) for 2 hr. The solution was cooled, evaporated to dryness and the residue crystallised from water to give the cyclic urea (0.8 g.; 45%), m.p. 318-20° (sealed and evacuated tube), ν max. 3200, 3100, 1710, 1325, 1150 cm^{-1}, τ (TFA) 5.5 (2H,b), 6.9 (4H, b), m/e 176.

(Found: C, 34.05; H, 4.77; N, 16.02; S, 18.35. $C_5H_8N_2O_3S$ requires C, 34.09; H, 4.58; N, 15.91; S, 18.20%).

Under similar conditions both the allylic allophanate (59) and the allylic urea (60) were converted to the same cyclic urea.

Acid Hydrolysis of cis- and trans-Hexahydrothieno-[3,4-d]imidazol-2-one-5,5-dioxides. - The cyclic urea (176 mg.) was heated under reflux overnight with 6N hydrochloric acid (6 ml.). The acid was removed under reduced pressure to give a white solid. The product from the cis-urea (58) (213 mg.) was crystallised from water, ν max. 3500-2300, 1700, 1320, 1140 cm^{-1}. The analysis showed that the crystallised material was probably a mixture of the free urea and the hydrochloride. The carbon to nitrogen ratio was exactly 5:2, showing that the carbonyl had not been lost. When the product was suspended in dilute aqueous base the starting material was regenerated. The product from the trans-urea (30) (234 mg.) was crystallised from 2:3 aqueous ethanol, m.p. 302-4°, ν max. 3500-2400, 1310, 1180 cm^{-1}.

(Found: C, 21.60; H, 5.39; N, 12.51; Cl, 31.63. $C_4H_{12}N_2Cl_2O_2S$ requires C, 21.53; H, 5.42; N, 12.56; Cl, 31.78%).

1,3-Diacetylhexahydrothieno[3,4-d]imidazol-2-one-5,5-dioxide (62) - Hexahydrothieno[3,4-d]imidazol-2-one-5,5-dioxide (314 mg.) was boiled under reflux with acetyl chloride (5 ml.) in acetic acid (7.5 ml.) for 4 hr. The solvents were removed under reduced pressure and the residue washed free from acid with ether. The diacetyl derivative (300 mg.; 67%), was crystallised from methanol, m.p. 236-8°, ν max. 1745, 1695, 1330, 1310, 1150 cm^{-1}, τ (TFA) 5.3 (2H,m), 6.8 (4H,m), 7.8 (6H,s).

(Found: C, 41.53; H, 4.84; N, 10.88; S, 12.38. $C_9H_{12}N_2O_5S$ requires C, 41.53; H, 4.65; N, 10.76; S, 12.31%).

1-Acetylhexahydrothieno[3,4-d]imidazol-2-one-5,5-dioxide (61) - Hexahydrothieno[3,4-d]imidazol-2-one-5,5-dioxide (214 mg.) was boiled under reflux with acetyl chloride (5 ml.) in acetic acid (7.5 ml.) for 30 min to give a mixture of mono- and di-acetyl compounds. The less soluble

mono-acetyl derivative was isolated by crystallising the mixture from water, m.p. 260-2°, ν max. 3280, 1740, 1660, 1320, 1170 cm^{-1}., τ (TFA) 5.3 (2H, m), 6.8 (4H, m), 7.8 (3H, s).

(Found: C, 38.46; H, 4.53; N, 12.62; S, 14.78. $C_7H_{10}N_2O_4S$ requires C, 38.55; H, 4.62; N, 12.84; S, 14.69%).

Both acetyl derivatives were deacetylated by brief boiling with dilute aqueous base.

1-(5-Bromovaleryl)hexahydrothieno[3,4-d]-imidazol-2-one-5,5-dioxide (63) - cis- Hexahydrothieno[3,4-d]imidazol-2-one-5,5-dioxide (1 g.) and sodium hydride (278 mg. of 50% oil dispersion, pre-washed with dry ether) were heated at 100° in a mixture of dry dioxan (30 ml.) and dry DMF (5 ml.) for 2 hr. 5-Bromovalerylchloride (1.15 g.) was added in dioxan (10 ml.) and the heating continued for 3 hr. The mixture was allowed to cool and stirred at room temperature overnight. A little water was added, the mixture evaporated to dryness and the residue crystallised from 1:1 dioxan/water. The analysis indicated that the bromovaleryl derivative (0.78 g.; 40%) had probably co-crystallised with dioxan and this was confirmed by the n.m.r. with a spurious sharp singlet at τ 6.45. The impure product had m.p. 205-12°, ν max. 3250, 1720, 1690, 1320, 1160 cm^{-1}, τ (TFA) 2.2 (1H, bs, -NH), 5.25 (1H, m, =CH-N-CO), 5.6 (1H, m, =CH-NH-), 6.6-7.1 (4H, m, $-CH_2-SO_2-CH_2-$), 7.15 (2H, t, $Br-CH_2-$), 7.4 (2H, t, $-CH_2-CO-$), 8.65 (4H, m, $Br-CH_2-CH_2-CH_2-$).

N,N'-Diphenyl(1,1-dioxy-2,3-dihydro-3-thienyl)urea (71; R'=R^2=Ph). - 3-Anilino-2,3-dihydrothiophen-1,1-dioxide (32 mg.) and phenylisocyanate (0.5 ml.) were heated together until all the solid dissolved. The mixture was cooled and triturated with hot light petroleum to remove the excess of isocyanate. The residue was dissolved in a few drops of benzene and added to briskly-stirred light petroleum (15 ml.). The solid was collected and the allylic urea (43.2 mg.; 86%) crystallised from toluene, m.p. 132-4°, ν max. 3400, 1670, 1600, 1510, 1300, 1110 cm^{-1}., τ ($CDCl_3/D_2O$) 2.3-3.0 (10H, m), 3.3 (2H, m), 3.9 (1H, m), 6.0-6.9 (2H, dq,

J_{AB} 14 Hz, J_{AX} 8Hz, J_{BX} 4 Hz).

(Found: C, 62.29; H, 5.04; N, 8.50; S, 10.04. $C_{17}H_{16}N_2O_3S$ requires C, 62.18; H, 4.91; N, 8.53; S, 9.76%).

cis-1,3-Diphenylhexahydrothieno[3,4-d]imidazol-2-one-5,5-dioxide (72; $R'=R^2=Ph$). - N,N'-Diphenyl(1,1-dioxy-2,3-dihydro-3-thienyl)urea (67 mg.) was stirred at room temperature for 4 hr. with 0.5N sodium ethoxide in ethanol (10 ml.). The solution was evaporated to dryness, water added and the product extracted with chloroform. Evaporations yielded the cyclic urea (65.5 mg.), m.p. 176-7° (from methanol), ν max. 1695, 1600, 1500, 1295, 1160 cm^{-1}., τ (TFA) 2.9 (10H, bs), 4.9 (2H, bs), 6.9 (4H, bs).

(Found: C, 62.01; H, 4.97; N, 8.39; S, 9.71. $C_{17}H_{16}N_2O_3S$ requires C, 62.18; H, 4.91; N, 8.53; S, 9.76%).

1,3-Dimethylhexahydrothieno[3,4-d]imidazol-2-one-5,5-dioxide (72; $R'=R^2=Me$). N-Methyl(1,1-dioxy-4-bromotetrahydro-3-thienyl) carbamate (99 mg.) was heated under reflux with triethylamine (67 mg.) in ethanol (5 ml.) for 24 hr. The solvent was evaporated, water added to the residue and the mixture extracted with chloroform. Evaporation yielded an oil (46 mg.; 85%) which was assigned as 3-methylamino-2,3-dihydrothiophen-1,1-dioxide (70), (treatment with ethoxide isomerised the oil to 4-methylamino-2,3-dihydrothiophen-1,1-dioxide), ν max. (film) 3350, 1300, 1140 cm^{-1}., τ 3.2 (2H, s and d, J 2Hz), 5.8 (1H, m), 6.3-7.2 (2H, dq, J_{AB} 14Hz, J_{AX} 7 Hz., J_{BX} 4 Hz), 7.6 (3H,s).

The crude oil (46 mg.) was heated under reflux with methlisocyanate (0.5 ml.) for 30 min. The excess of reagent was evaporated off and the residue triturated with light petroleum and then extracted with hot benzene. Evaporation yielded an oil (45 mg.) which was assigned as N, N'-dimethyl-(1,1-dioxy-2,3-dihydro-3-thienyl)urea (71; $R'=R^2=Me$), ν max. (film) 3400, 1640, 1540, 1300, 1140 cm^{-1}., τ 3.2 (2H, m), 4.1 (1H, m), 4.8 (1H, m, -NH), 6.2-7.2 (2H, dq, J_{AB} 14Hz, J_{AX} 8Hz, J_{BX} 4Hz), 7.2 (6H).

The urea (72 mg.) was stirred with 0.5N sodium ethoxide in ethanol (5 ml.) at room temperature overnight. The solvent was evaporated, water added to the residue and the product extracted with chloroform. Evaporation yielded the cyclic urea, m.p. 178-9° (from ethanol), ν max. 1690, 1510, 1320, 1120 cm^{-1}, τ 5.6 (2H, bm), 6.7 (4H, bm), 7.1 (6H,s).
(Found: C, 41.18; H, 5.93; N, 13.96; S, 15.67. $C_7H_{12}N_2O_3S$ requires C, 41.16; H, 5.92; N, 13.72; S, 15.70%).

N-Methyl-N'-phenyl-N'-(1,1-dioxy-2,3-dihydro-3-thienyl)urea (71; R'=Ph, R^2=Me). - 3-Anilino-2,3-dihydrothiophen-1,1-dioxide (232 mg.) was heated under reflux with methylisocyanate (1 ml.) for 5 hr. The excess of reagent was evaporated off and the residue washed with hot light petroleum to give the allylic urea (171 mg.; 85%), m.p. 172-4° (from ethanol), ν max. 3400, 1655, 1600, 1520, 1300, 1140 cm^{-1}, τ ($CDCl_3$/TFA) 2.5 (5H,m), 3.3 (1H,m), 4.0 (1H,m), 5.9-6.8 (2H,dq, J_{AB} 14 Hz, J_{AX} 8Hz, J_{BX} 4Hz), 6.4 (1H,b), 7.2 (3H,s).
(Found: C, 54.01; H, 5.10; N, 10.37; S, 12.02. $C_{12}H_{14}N_2O_3S$ requires C, 54.12; H, 5.30; N, 10.52; S, 12.03%).

1-Phenyl-3-methylhexahydrothieno[3,4-d]imidazol-2-one-5,5-dioxide (72; R'=Ph, R^2=Me). - N-Methyl-N'-phenyl-N'-(1,1-dioxy-2,3-dihydro-3-thienyl)urea (84 mg.) was stirred with 0.5N sodium ethoxide in ethanol (5 ml.) at room temperature overnight. The suspension was evaporated to dryness, water added to the residue, and the product extracted with chloroform. Evaporation gave the cyclic urea, m.p. 191-2° (from 5% aqueous ethanol), ν max. 1695, 1600, 1510, 1315, 1120 cm^{-1}, τ (TFA) 3.0 (5H,m), 4.8-5.2 (1H,m), 5.2-5.6 (1H,m), 6.4-7.0 (4H,m), 7.3 (3H,s).
(Found: C, 54.13; H, 5.10; N, 10.43; S, 12.26. $C_{12}H_{14}N_2O_3S$ requires C, 54.12; H, 5.30; N, 10.52; S, 12.03%).

Bicyclo[spiro-6,3]-2,4-dioxo-1,3,5-trimethylperhydro-1,3,5-triazinyltetrahydrothiophen-1,1-dioxide (73). - Sodium hydride (85 mg. of 50% oil dispersion) was washed with ether. Dry dioxan (10 ml.) and

4-methylamino-2,3-dihydrothiophen-1,1-dioxide (251 mg.) was added. The mixture was heated under reflux for 3 hrs., cooled, and methylisocyanate (0.5 ml.) added. After stirring for 24 hr. at room temperature a few drops of water were added and the mixture evaporated to dryness. The residue was taken up in water and extracted with chloroform. Evaporation yielded the spiro compound, m.p. 179-80° (from 1:1 ethanol-chloroform), ν max. 1700, 1660, 1305, 1190 cm^{-1}, τ (TFA) 6.5 (2H,s), 6.8 (2H,t), 7.2 (9H,s), 7.4 (2H,t).

(Found: C, 41.44; H, 5.71; N, 16.23; S, 12.25. $C_9H_{15}N_3O_4S$ requires C, 41.37; H, 5.79; N, 16.08; S, 12.27%).

1,1-Dioxy-4-hydroxylaminotetrahydro-3-thienylurea (74). - 1,1-Dioxy-2,3-dihydro-3-thienylurea (528 mg.), hydroxylamine hydrochloride (417 mg.) and water were stirred together and a solution of sodium hydrogen carbonate (510 mg.) in water (10 ml.) added over a period of 2 min. The mixture was stirred at 60° for 2 hr. to give a clear solution which was evaporated to dryness. The residue was crystallised from water to give the hydroxylamine derivative (464 mg.; 75%), m.p. 185-6° (from 1:1 aqueous ethanol), ν max. 3440, 3390, 3270, 1655, 1610, 1540, 1305, 1130 cm^{-1}, τ (TFA) 3.7 (1H,b), 5.1-6.0 (2H,m), 6.3-7.1 (4H,m).

(Found: C, 29.09; H, 5.45; N, 20.05; S, 15.29. $C_5H_{11}N_3O_4S$ requires C, 28.70; H, 5.30; N, 20.09; S, 15.33%).

6-Bromo-5,5-dioxy-2-iminohexahydrothieno[3,4-d]oxazole Hydrobromide (75) - 1,1-Dioxy-2,3-dihydro-3-thienylurea (540 mg.) was stirred in acetic acid (25 ml.) at 65° with bromine (ca. 0.5 ml.). After 2 hr. the solid was filtered off and washed with ethanol and ether. Evaporation of the mother liquors and subsequent addition of ethanol produced more of the salt (990 mg; 96%), m.p. 190-2° (from 15% aqueous ethanol), ν max. 3200, 1705, 1325, 1115 cm^{-1}, n.m.r. - see discussion.

(Found: C, 18.00; H, 2.66; N, 8.19; Br, 46.76; S, 9.82. $C_5H_8N_2Br_2O_3S$ requires C, 17.87; H, 2.40; N, 8.34; Br, 47.57; S, 9.54%).

1,1-Dioxy-2,3-dihydro-5-bromo-3-thienylurea (77) -

6-Bromo-5,5-dioxy-2-iminohexahydrothieno[3,4-d]oxazole hydrobromide (336 mg.) was stirred in water (2 ml.) and 0.2N sodium hydroxide solution (5 ml.) added slowly to precipitate a white solid. The volume of solution was reduced by half and the urea (631 mg.; 86%), filtered off, m.p. 222-4° (from water), ν max. 3470, 3380, 3270, 3100, 1660, 1560, 1310, 1150 cm^{-1}, τ (TFA) 1.7 (2H,bs), 3.4 (1H,d, J 3Hz), 3.4 (1H,b), 5.0 (1H,m), 6.0-7.0 (2H, dq, J_{AB} 14Hz, J_{AX} 7Hz, J_{BX} 4Hz).

(Found: C, 23.78; H, 2.95; N, 10.84; Br, 30.94; S, 12.85. $C_5H_7N_2BrO_3S$ requires C, 23.54; H, 2.77; N, 10.98; Br, 31.34; S, 12.57%).

1,1-Dioxy-2-bromo-3-hydroxylaminotetrahydro-4-thienylurea (78) -

1,1-Dioxy-2,3-dihydro-5-bromo-3-thienylurea (105 mg.), hydroxylamine sulphate (134 mg.) and sodium hydrogen carbonate (140 mg.) were stirred together in water (10 ml.) at 65° for 4 hr. The solution was taken to dryness and the residue extracted well with hot methanol. Evaporation of the alcohol gave a solid which was crystallised from 20% aqueous methanol to give the hydroxylamine derivative m.p. 189-90°, ν max. 3490, 3390, 3240, 1660, 1560, 1315, 1125 cm^{-1}, τ (TFA) 3.8 (1H,b), 4.9 (1H,d, J 9Hz), 5.3 (1H,m), 5.9 (1H,m), 6.1-7.0 (2H,dq).

(Found: C, 21.28; H, 3.78; N, 14.69; Br, 27.85; S, 10.97. $C_5H_{10}N_3BrO_4S$ requires C, 20.84; H, 3.50; N, 14.59; Br, 27.74; S, 11.13%).

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