STUDIES ON PYRAZINES AND RELATED HETEROCYCLIC ~~COMPOUNDS~~ Systems.

A thesis presented by

JANET LOUISE MARKHAM

In partial fulfilment of the requirements for

the degree of

DOCTOR OF PHILOSOPHY

of

THE UNIVERSITY OF LONDON

Department of Chemistry
Imperial College
London

July, 1977

ABSTRACT

The literature pertaining to the biosynthesis of naturally occurring 2,5-dioxopiperazines is reviewed. Synthetic routes to the antibiotic bicyclomycin are discussed, and initial studies to test the feasibility of such routes have been performed. Methods and attempts to elaborate 2,5-dioxopiperazine and its derivatives and 2,5-dihydropyrazines are described. The latter can be alkylated and condensed readily with suitable reagents. Attempted hydrolyses of imino ethers to amides and ether cleavages to effect the same transformation are reported. Dichlorodicyanobenzoquinone oxidations of dihydropyrazines are extended to unsymmetrically substituted compounds. It has been found that singlet oxygen adds smoothly and efficiently to aromatic heterocycles, such as the substituted pyrimidines and pyrazines. The endoperoxides derived from these heterocycles are isolated and characterised, and their properties described. The conditions for the formation of endoperoxides are discussed.

ACKNOWLEDGEMENTS

My thanks are due to Professor P. G. Sammes for his guidance during this project and to Professor Sir Derek Barton for the privilege of working in his Department. I would also like to acknowledge the assistance of the technical staffs at both Imperial College and The City University; in particular, Mr. Jack Peppercorn and his staff.

I am indebted to my colleagues, especially Drs. Robert Watt and Nigel Walshe, Dr. and Mrs C. D. Bartlett, and Messrs Nigel Broom and Lyn Davies, for their valuable and stimulating discussions and distractions, and to my fiance, Michael, for his patience, and his assistance in the preparation of this thesis.

Lastly, my sincerest gratitude goes to my parents for their generosity, indulgence, and unfailing support.

Thinking the guy up ahead knows what he's doing
is the most dangerous religion there is.

Kurt Vonnegut Jr.

TABLE OF CONTENTS

	Page
ABSTRACT	I
ACKNOWLEDGEMENTS	II
REVIEW: Biosynthesis of 2,5-Dioxopiperazines	1
RESULTS AND DISCUSSION: Studies on Pyrazines and Related Heterocyclic Systems	45
I. Introductory remarks regarding bicyclomycin	45
II. Elaboration of the 6-membered ring : dioxopiperazines	51
III. Elaboration of the 6-membered ring : dihydropyrazines	60
IV. Oxygenation	74
V. Summary: Bicyclomycin synthesis to date	95
EXPERIMENTAL	97
REFERENCES	132

----------oOo----------

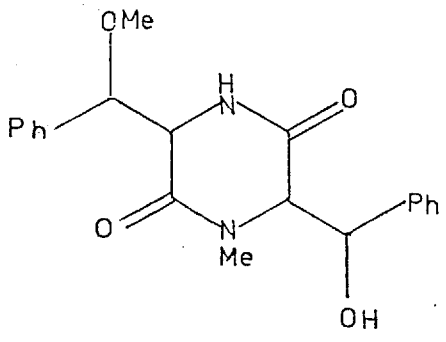
REVIEW

THE BIOSYNTHESIS OF 2,5-DIOXOPIPERAZINES

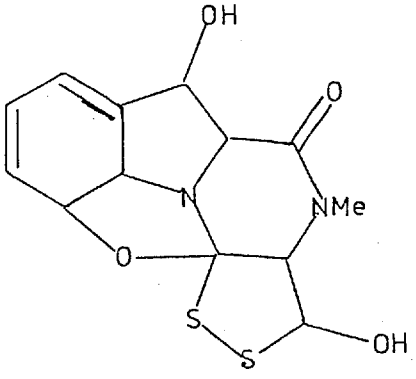
1. Introduction

Dioxopiperazines have been recognised as a distinct class of compounds since the end of the last century. Many of the simpler compounds were first synthesised by Emil Fischer[1], and the unsubstituted dioxopiperazine was prepared in 1888[2]. One of the earliest hints of the biological importance of this class came in 1922 with the determination of the structure of picrorocellin (1), isolated from Rocella fuciformis[3]. Subsequently, dioxopiperazines have been identified as natural products from a variety of fungi and plants, and have frequently shown biological activity. Their structures remained elusive in many cases and were only deduced long after the initial isolation. For instance, gliotoxin was isolated as the antibiotic principle of Penicillium brevicompactum in 1936[4]. A tentative structure was published in 1953 (2)[5], and a corrected structure in 1958 (3)[6]. The absolute configuration was determined by x-ray diffraction analysis[7]. Chetomin (4) was isolated in 1944[8], but its structure was not elucidated until 1976[9].

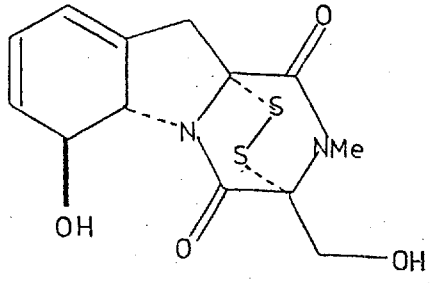
The realisation of the biological importance of the dioxopiperazines has no doubt contributed to the interest with which they have been pursued. Total synthesis of some of the simpler natural products, such as albournosin (Scheme 1)[10,11], as well as a few of the more complex molecules have been reported. In particular, dehydrogliotoxin (5)[12], sporidesmin (6)[13], and echinulin (7)[14] have been synthesised. New dioxopiperazine-based natural products continue to be reported. In recent years,


OMe
Ph
H
N
O
Ph
O
N
Me
OH

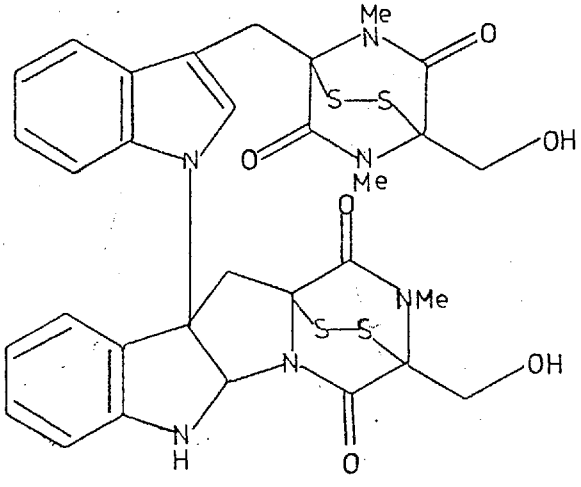
1


OH
O
N
NMe
O
S
S
OH

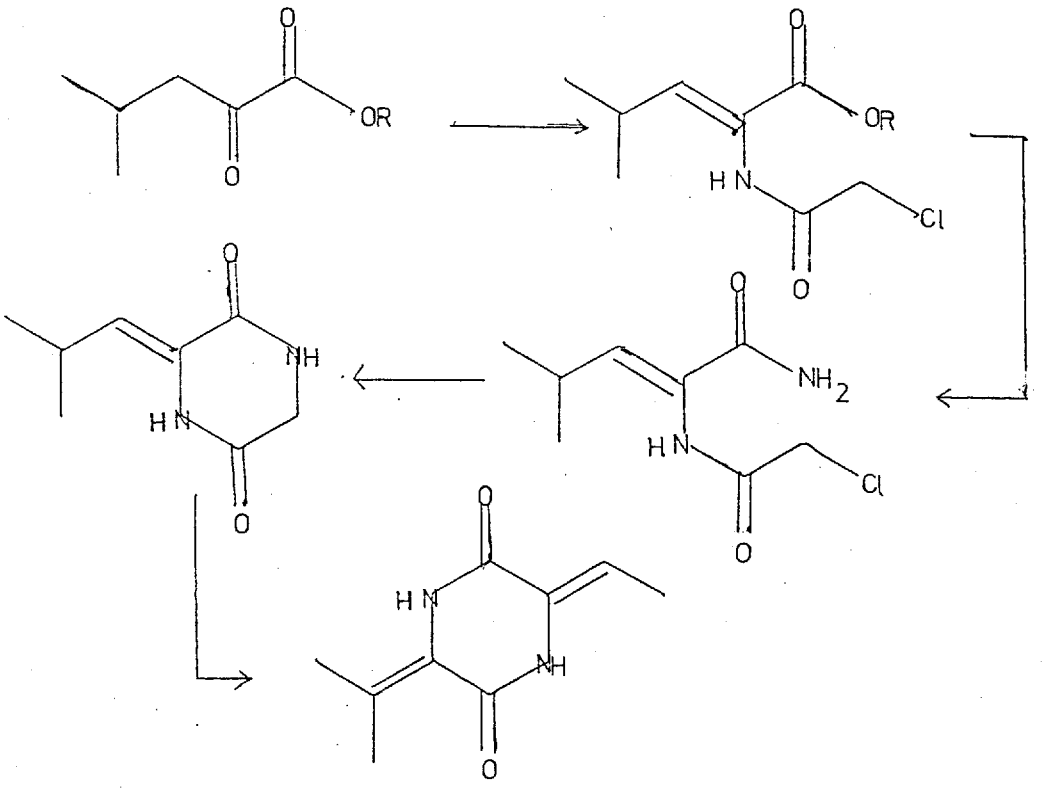
2


O
S
N
S
NMe
OH
O
OH

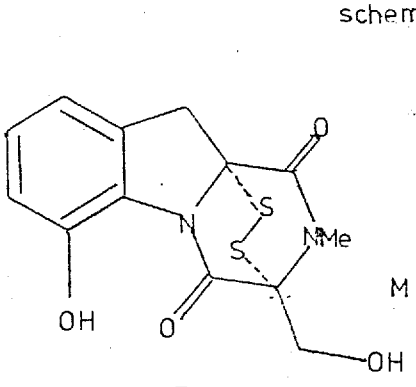
3


Me
N
O
S
S
OH
N
O
N
Me
O
NMe
S
S
OH
N
N
H
O

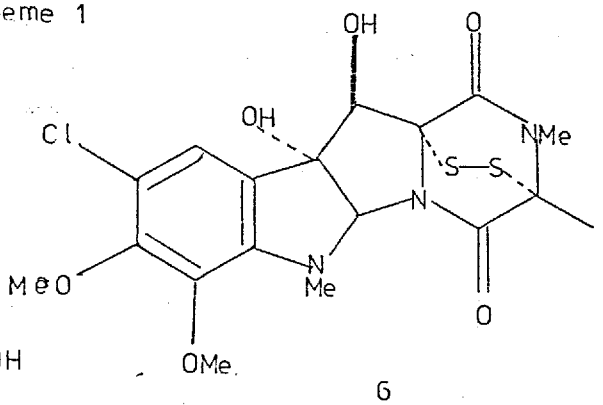
4



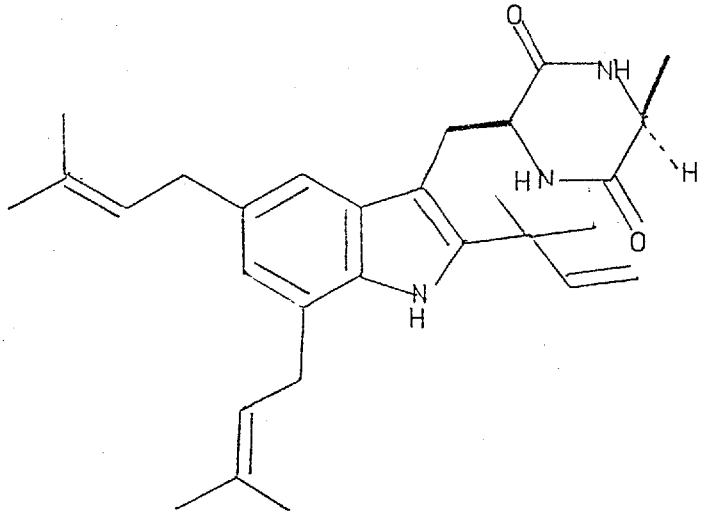
scheme 1



5



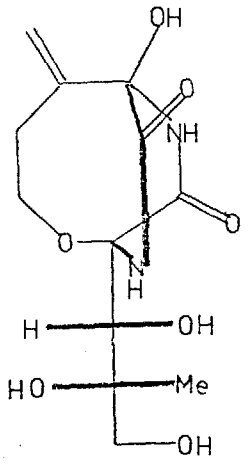
6



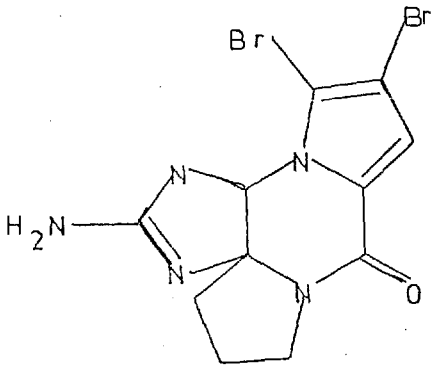
7

bicyclomycin (8)[15], dibromophakellin (9)[16], gliovictine (10)[17], hyalodendrin (11)[18], preechinulin (12)[19,20], the sirodesmins (13)[21], fumitremorgens (14a and b)[22,23,24], isoechinulins (15a, b and c[25]), and epicorazine (16)[26], have been isolated and characterised. The naturally-occurring dioxopiperazines have been reviewed[27].

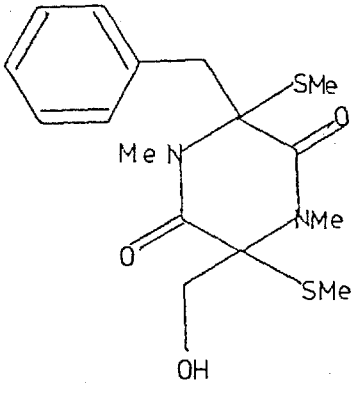
Biogenetic considerations have been useful in determining the structure of these natural products. Conversely, the biosynthesis of a compound of known structure is studied either directly, by the feeding of isotopically labelled potential precursors to co-operative organisms and the analysis of the metabolite of interest, or indirectly, by the isolation from the culture of intermediates on the proposed pathway. Both radioactive and non-radiative isotopes have been used as labels. The non-radiative isotopes have the advantage of being located by mass spectroscopy rather than by degradation. Precursors doubly labelled with isotopic atoms, most frequently ^{3}H and ^{14}C at nearby sites, provide reassurance that the prospective precursor has not been degraded by the organism to smaller units and then incorporated into the molecule. If the ratio of ^{3}H: ^{14}C remains unchanged in the metabolite or has changed in a predictable manner, then the precursor has been incorporated without scrambling (i.e. intact). More recently, labelling with ^{13}C at two adjacent sites has gained in popularity. The subsequent analysis is facilitated by the characteristic augmentation and splitting of signals from adjacent enriched atoms in the ^{13}C nuclear magnetic resonance spectrum. Lack of incorporation of a suspected precursor may result from its inability to be transported across the cell membrane, a possibility which can be checked by searching the organisms for cytoplasmic



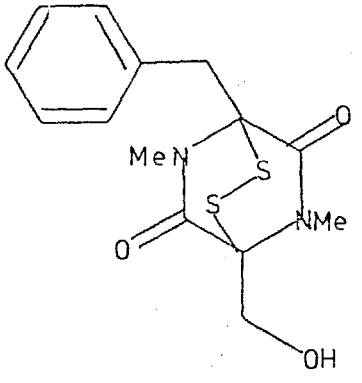
8



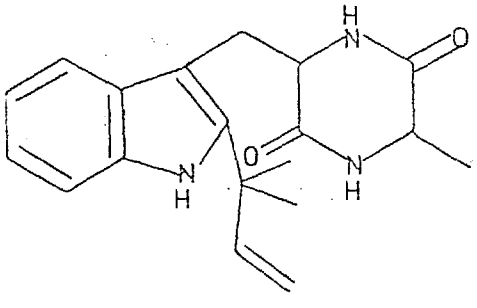
9



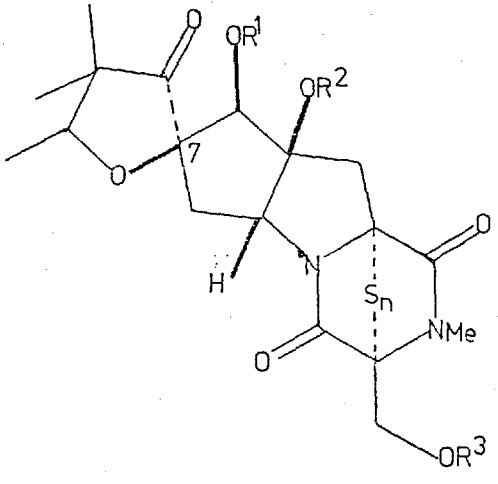
10



11



12



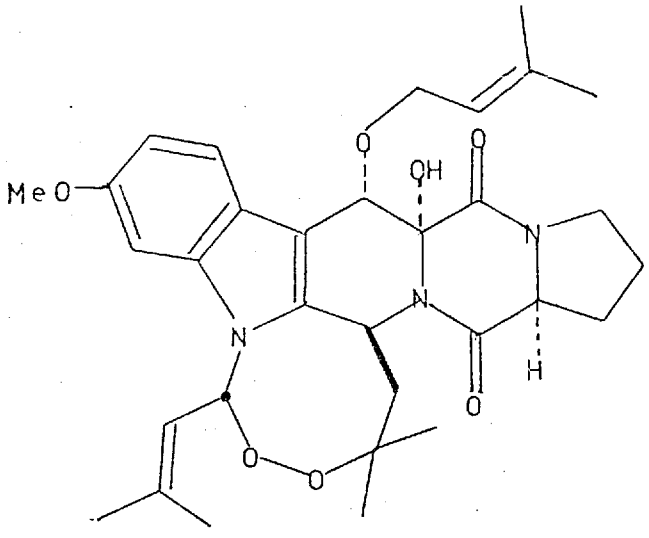
13

A: R^1 = Ac R^2 = R^3 = H n = 2

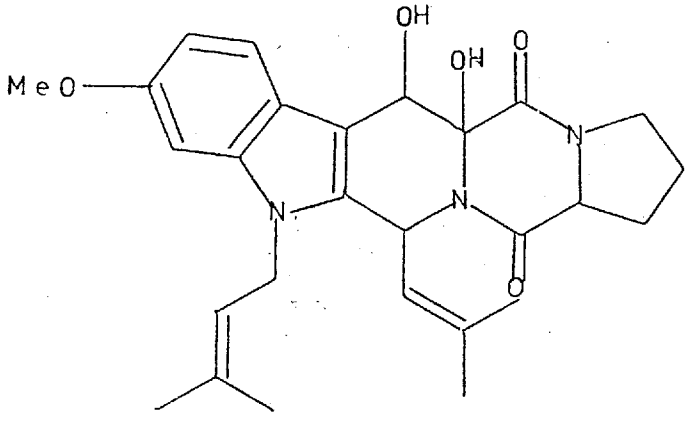
B: R^1 = Ac R^2 = R^3 = H n = 4

C.: R^1 = Ac R^2 = R^3 = H n = 3

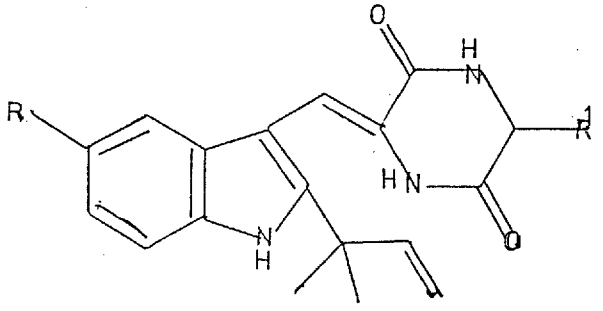
G: epimer of A at C-7


MeO
O
OH
O
N
N
N
H
O
O
O

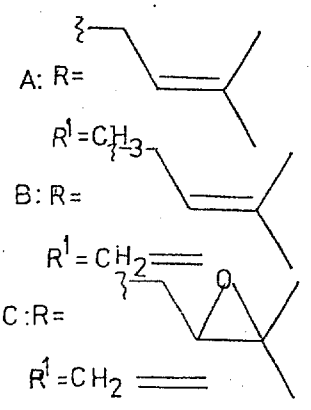
14a

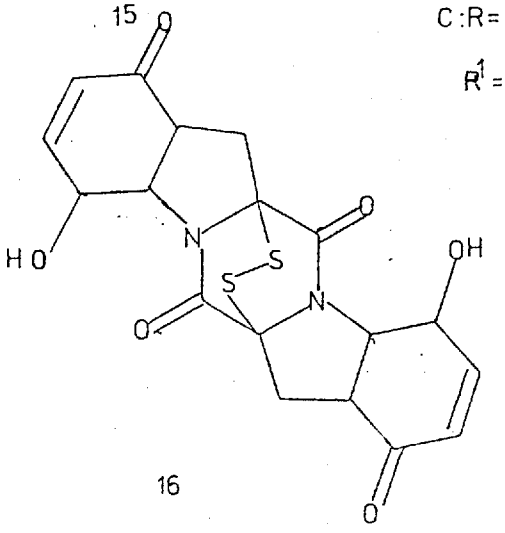

MeO
OH
OH
O
N
N
N
O

14b


R
O
H
N
R1
HN
O
N
H

15


A: R=
R1=CH3
B: R=
R1=CH2
C: R=
O
R1=CH2

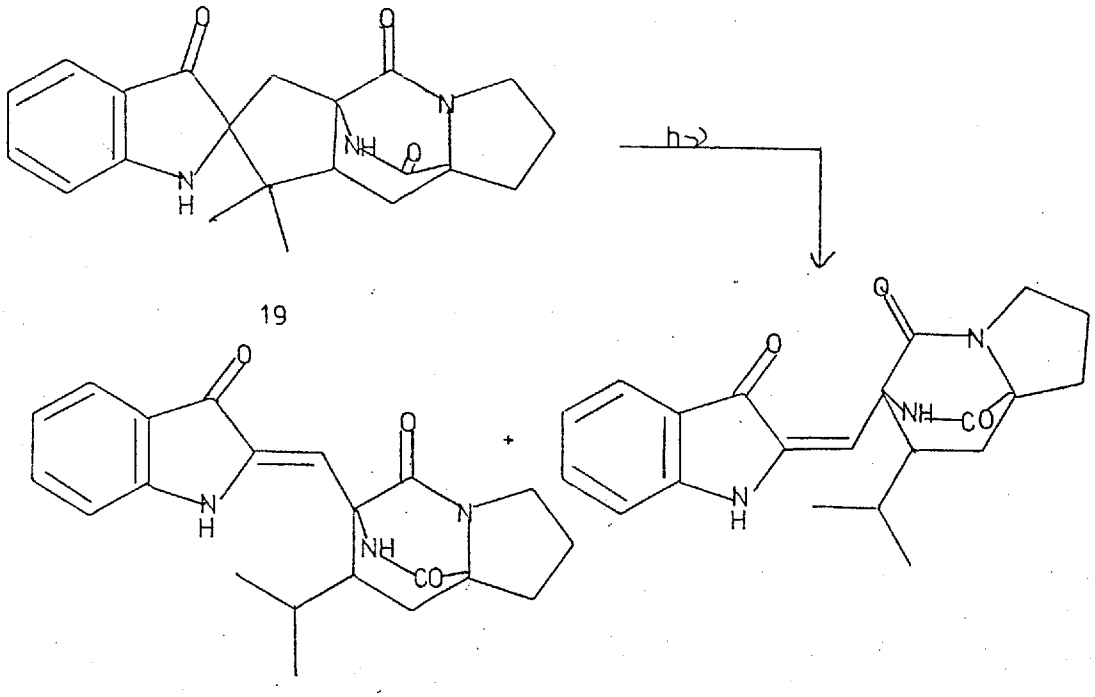

HO
O
N
S
S
O
N
OH
O
O

16

radioactivity or isotopic labelling. An absence of labelling indicates a failure to transport the precursor. The quantities of material fed are also important since these concentrations may interfere with the regulatory mechanisms of biosynthesis. In general, the further along the biosynthetic pathway the intermediate is, the more efficient will be its incorporation into the end metabolite. The isolation of proposed intermediates helps to confirm a pathway, but cannot be taken as proof. It also assists in the formulation of reaction sequences. However, some caution is necessary, as compounds may be artefacts of isolation rather than true metabolites, as in the case of brevianamides C and D (17 and 18) which were formed photolytically from brevianamides A and B (19)[28]. In addition, an isolated metabolite may not lie on the pathway to the end product, but may be derived from an intermediate of that pathway or from another route entirely.

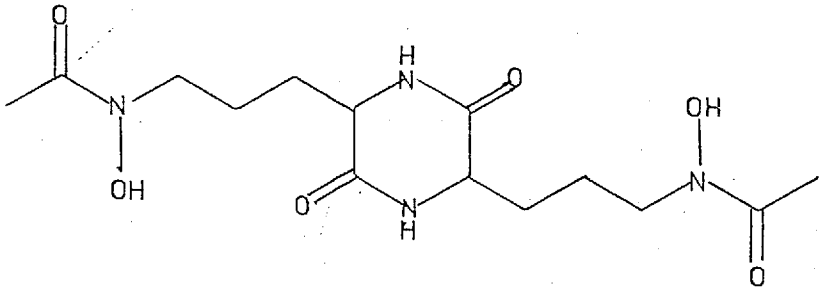
2. Simple dioxopiperazine derivatives: rhodotorulic acid and pulcherriminic acid

Some of the problems encountered can be illustrated by considering the biogenesis of a relatively simple dioxopiperazine. Rhodotorulic acid (20), an iron transport factor, was isolated from Rhodotorula pilimanae and characterised in 1968[29]. Feeding with ^{14}C-labelled amino acids indicated that glutamate, glutamine, proline, arginine, and ornithine were all incorporated rapidly into rhodotorulic acid, but as these are all interconvertible in vivo, no information on the precise precursor could be obtained



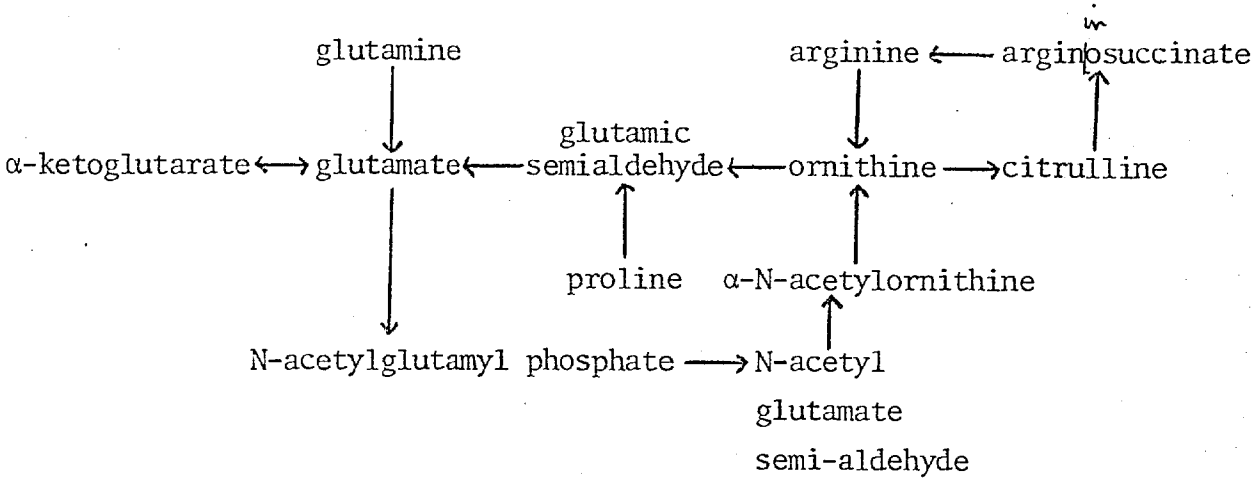
19

17 18



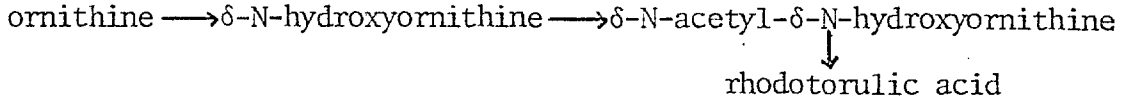
20

(Scheme 2).



Scheme 2

Using a D_2O medium and following retention of exogenous protons (i.e. those from the medium), it was determined that L-ornithine was the initial precursor and that δ-N-acetyl-L-δ-N-hydroxyornithine was an intermediate. By contrast, δ-N-acetyl-L-ornithine was not incorporated. These results lead to the conclusion that the formation of the hydroxamic acid precedes the cyclisation to the dioxopiperazine (Scheme 3). The use of labelled medium also provided indirect evidence for the source of the oxygen atom in the hydroxylamino functions, which arises from O_2 rather than from H_2O[30].

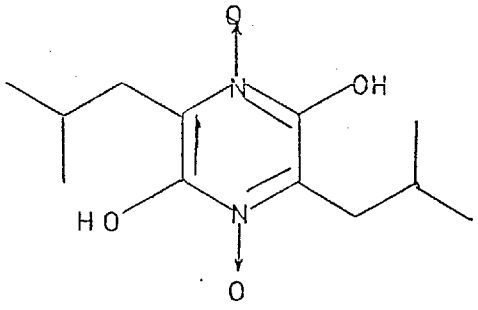


Scheme 3

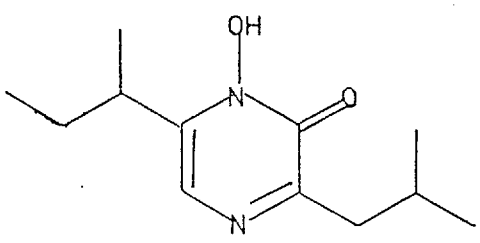
Another heterocyclic compound that appears to be derived

from a dioxopiperazine is pulcherriminic acid (21), a hydroxypyrazine. However, biosynthetic studies on other hydroxypyrazines, such as aspergillic acid (22), indicate that the related dioxopiperazines are not intermediates. Instead, the amino acids are the subunits which are modified before the hydroxypyrazines are synthesised[31,32]. Studies on aspergillic acid and hydroxyaspergillic acid (23) showed that both D- and L- leucine are incorporated by Aspergillus flavus. The studies further indicated that, because cyclo-isoleuleu is more efficiently incorporated into mycelial protein than into aspergillic acid, the dioxopiperazine is more rapidly hydrolysed to its constituent amino acids than it is incorporated into the hydroxypyrazine[33].

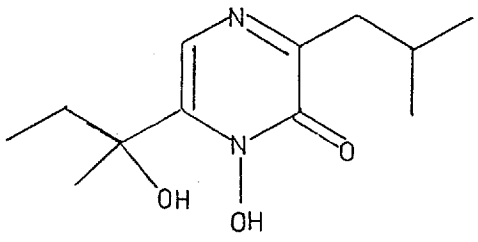
Feeding experiments with Candida pulcherrima, using L-^{14}C-leucine and cyclo-L-1-[^{14}C]-leu-L-1-[^{14}C]-leu, showed that the former is the precursor and the second an intermediate to pulcherriminic acid (Scheme 4). No scrambling of the label on L-leucine was detected, from which it was inferred that the amino acid was incorporated intact. Because the radioactivity of the pulcherriminic acid isolated was 1.7 times that of the leucine, two molecules of leucine were seen to be incorporated into one molecule of the pyrazine. This does not represent a great dilution of activity, but the experiments using the dioxopiperazine did show substantial dilution, possibly due to the incorporation of endogenous cyclo-L-leu-L-leu. That the dioxopiperazine was not first hydrolysed to the amino acid was determined by examining the hydrosylate of the mycelium for radioactivity and finding none. D-leucine was also incorporated but to a lesser extent, indicating



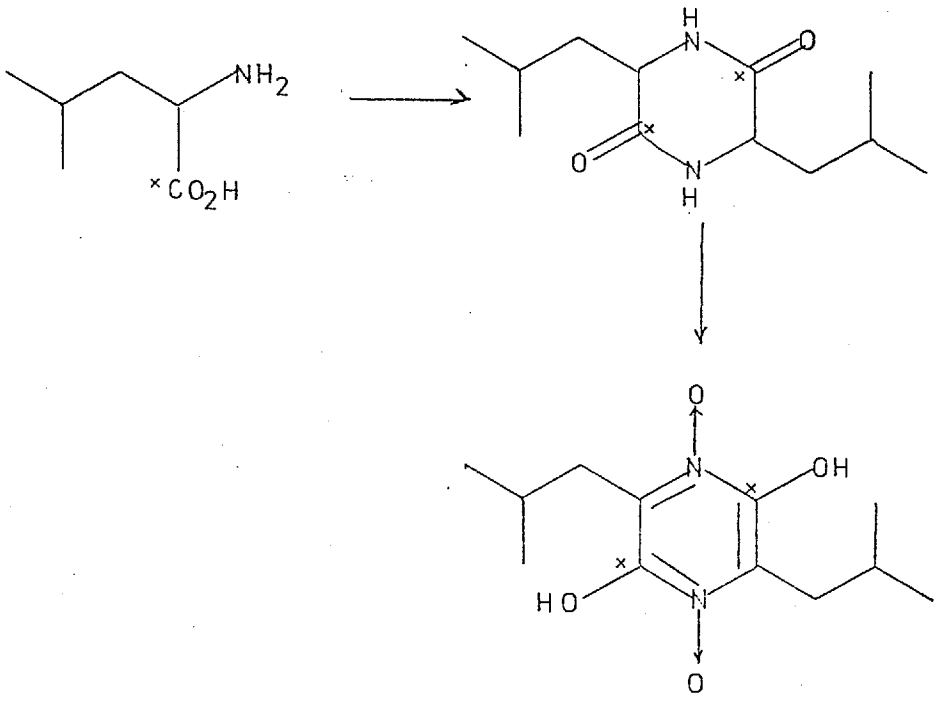
21



22



23



21

scheme 4

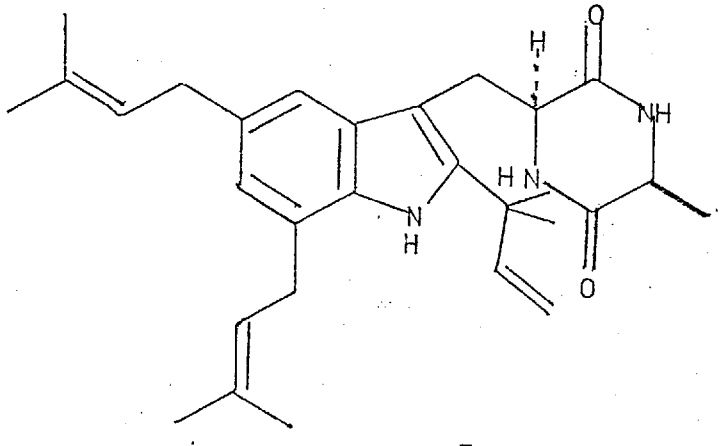
its probable conversion to the L-isomer prior to synthesis. Cyclo-D,D- or cyclo-D,L-leuleu were not incorporated, supporting this inference, and in fact, they inhibited the biosynthesis. The former nearly completely blocked the production of pulcherriminic acid and caused the accumulation of L,L-leuleu. This, together with the information that the dilution of activity in the cyclo-L,L-leuleu is comparable to that found in the natural product, suggests that the cyclo-L,L-leuleu isomer is the only one used, or generated, by the organism[34]. This is not surprising in that the L-isomer of amino acids is generally found to be the "natural" one.

3. Indole-isoprenylated tryptophyl dioxopiperazines

3.1. Echinulins

Of considerably greater complexity are the echinulins. Far more effort has been expended in the elucidation of the biosynthetic pathways leading to these and related compounds than on any other family of dioxopiperazines. Studies on the echinulins have, however, provided useful clues to the biogenesis of other compounds, such as the austamides and brevianamides.

Originally isolated in 1943[35], the structure of echinulin (7) remained a mystery until the early 1960's. It was known that a part structure contained a 5,7-disubstituted-2-dimethylallyl tryptophan unit, and after incorporation studies indicated the presence of three isopentenyl units, it was assumed that two of these were the 5,7-substituents[36]. The correct structure of echinulin was reported a year later[37]. Biosynthetic studies have,



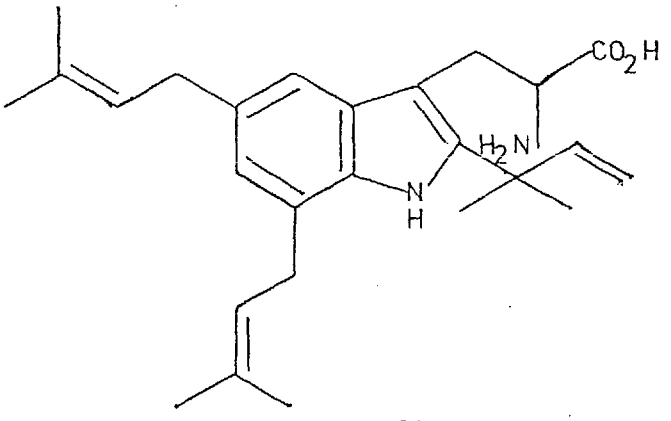
7

therefore, long played a role in echinulin chemistry, and were used first to elucidate the structure, rather than to explain it.

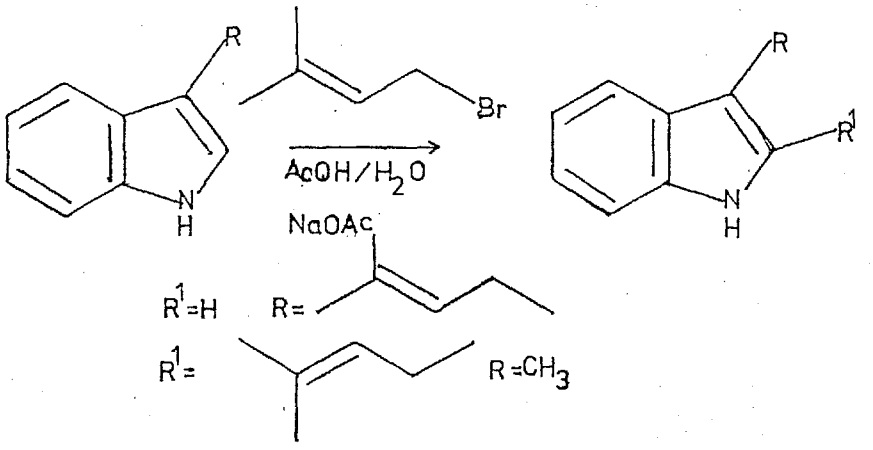
Subsequent feeding experiments demonstrated that L-tryptophan, L-alanine, mevalonic acid, and cyclo-L-ala-L-try are biosynthetic precursors of echinulin in Aspergillus amstelodami, [36,38,39,40]. It has been proposed that echinulin might arise by the cyclisation of an appropriately substituted amino acid, named echinin (24), with alanine, but the efficient incorporation makes it more probable that the dioxopiperazine is first cyclised and then alkylated[40].

The reversed isoprene unit at C-2 is an unusual feature, and its origin has been of considerable interest. Several mechanisms have been advanced to account for its orientation: direct substitution at C-2[41,46]; substitution at C-3 and subsequent rearrangement[42]; substitution at nitrogen followed by rearrangement[38]; and a rearrangement proceeding via enzymes bound by sulphur bridges to C-2[45].

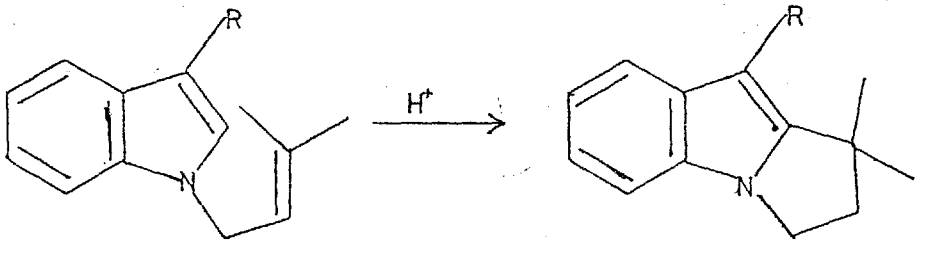
An attempt to alkylate directly at C-2 did indeed lead to substitution at that carbon atom with the 3-methylindole model compound. However, the dimethylallyl function was introduced in the normal fashion, and no reaction was observed using tryptophan, its N-acetyl derivative, or its cupric complex (Scheme 5). It seems unlikely that direct substitution is a reasonable mechanism on the basis of these experiments[41]. On the basis of more recent findings, direct alkylation at C-2 cannot be entirely dismissed. Acid-catalysed rearrangement of a 3-substituted-1-(3,3-dimethylallyl) indole gives a pyrrolo-indole as a major product (Scheme 6), presumably



24



scheme 5



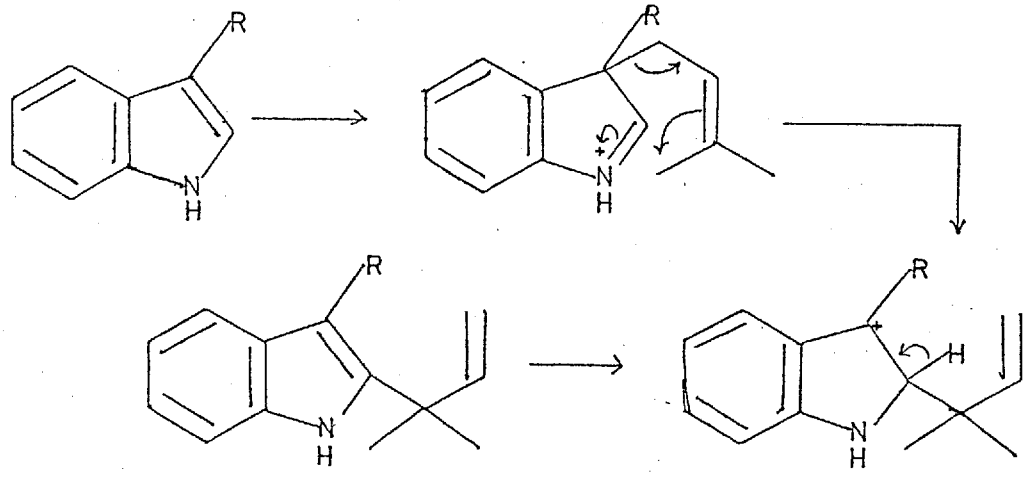
scheme 6

by a Friedel-Crafts mechanism. An enzyme capable of holding the indole and dimethylallyl pyrophosphate in the correct spacial relationship could catalyse direct alkylation at C-2 in vivo[43].

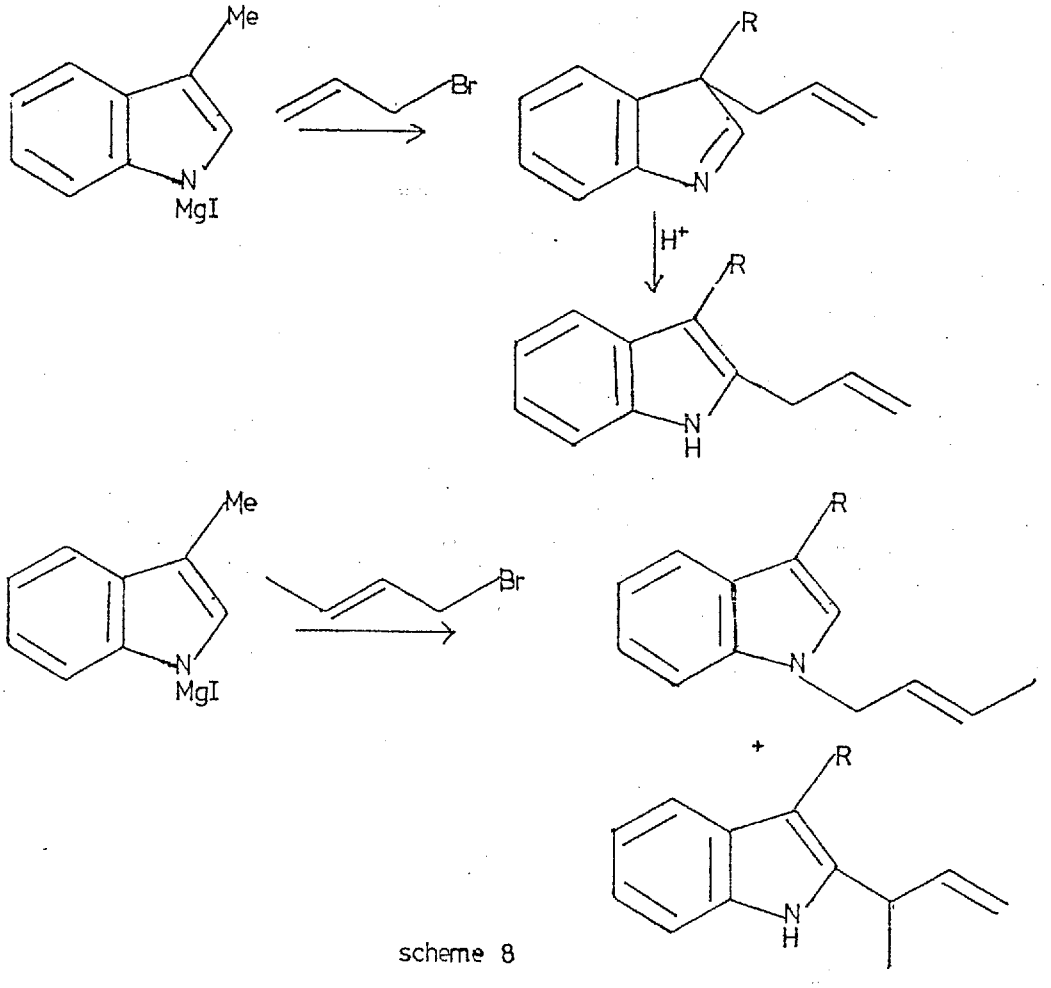
The 3 position in indoles is generally held to be the most activated to electrophilic attack[44]. Introduction of the dimethylallyl group at position 3 followed by Claisen rearrangement could account for the observed reversed isoprene unit at C-2 in the echinulin alkaloids (Scheme 7). Chemical studies on 3- methyl indoles show that reaction with allyl bromide occurs primarily at C-3. The alkylation product rearranges under acidic conditions to the 2-alkylated indole, evidently not in the manner proposed for echinulin. Similar experiments with crotyl bromide proceed with alkylation on the nitrogen atom. The 2-methylallyl indole is also isolated, possibly as a result of acid-catalysed rearrangement during work-up (Scheme 8). These results suggest that initial alkylation does not occur at C-3[42].

Using a thio-Claisen rearrangement as a model, a biosynthetic mechanism has been proposed that would account for the isoprenylation patterns in the ergot and ilamycin alkaloids, as well as in the echinulins (Scheme 9)[45].

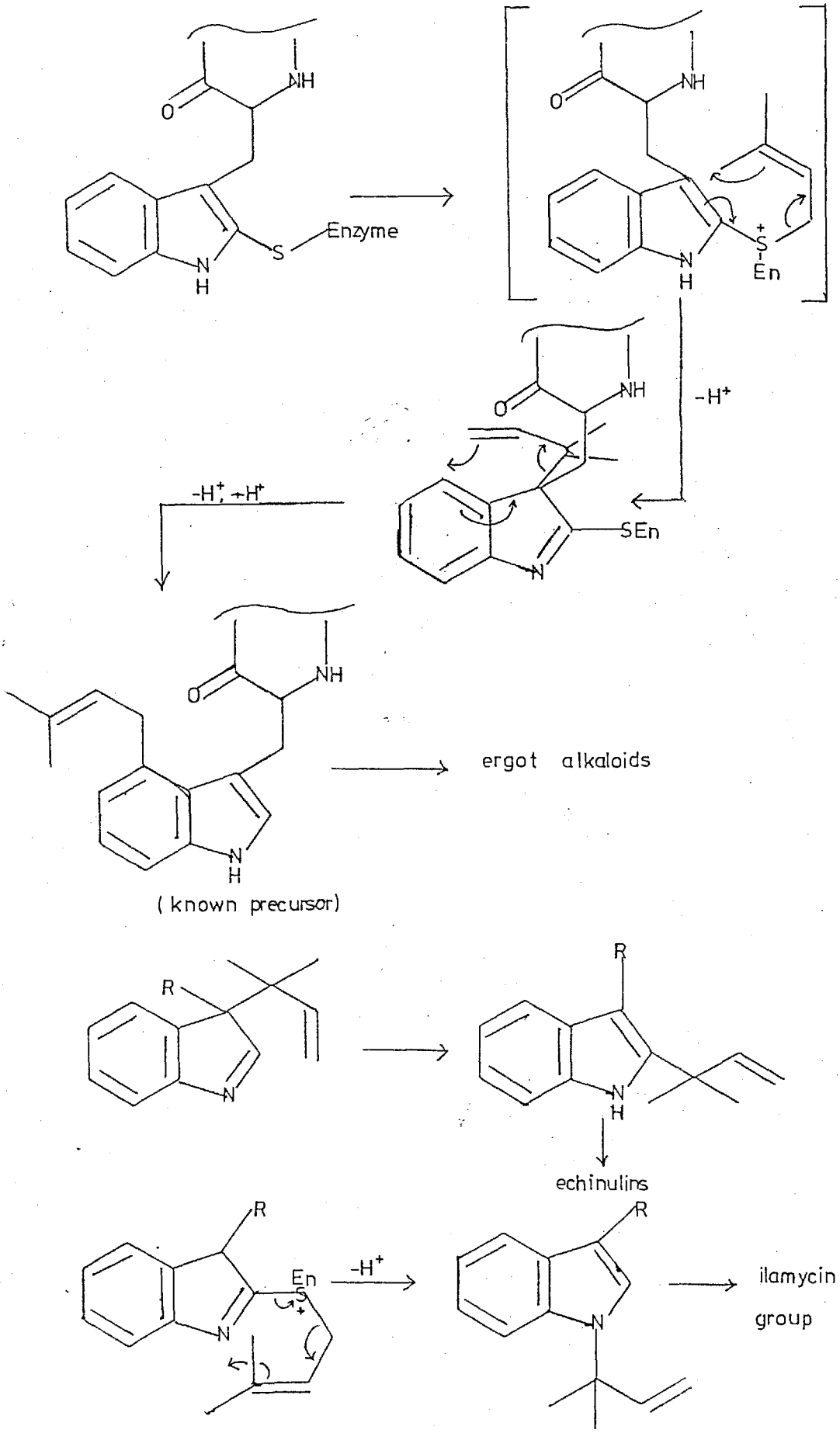
The strongest evidence, however, appears to favour the initial alkylation at nitrogen and subsequent rearrangement. Earlier studies on N-alkylated indoles indicated that the migration to the 2- position, in either the reversed or normal fashion, depended on conditions (Scheme 10)[46], although further work has thrown doubt on the generality of these findings. More recent evidence suggests that the alkenyl substituent may rearrange



scheme 7



scheme 8


NH
O
Enzyme
S
N
H
+
S
En
-H+
SEn
-H+, -H+
ergot alkaloids
(known precursor)
R
echinulins
-H+
ilamycin
group

scheme 9

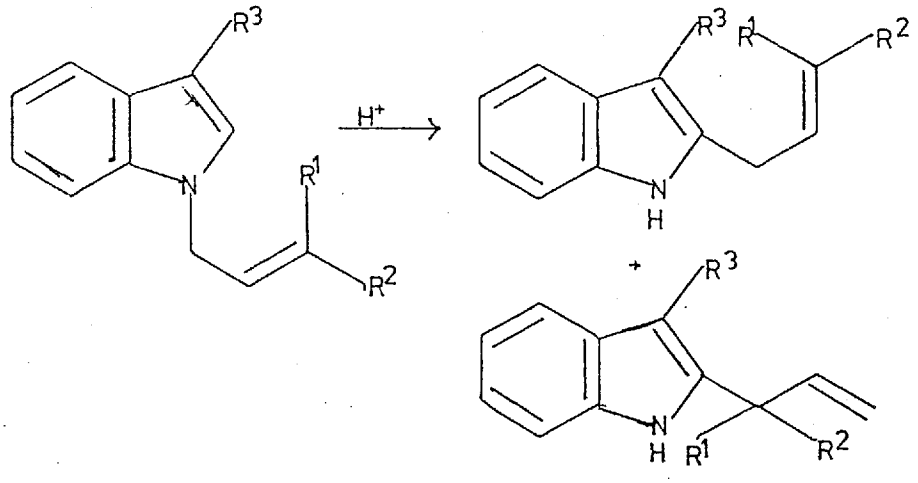
first to the 3 position and then to the 2 position. Acid-catalysed rearrangement of N-crotylindole affords the 3-(1-methylallyl) indole[47]. Thermal rearrangement of the same compound also gives the 3-alkyl indole which, at slightly longer reaction time, then rearranges to 2-(1-methylallyl) indole[48]. The N-alkylation hypothesis is further supported by the isolation of lanusolin (25), an N-isoprenylated metabolite[49].

It has also been suggested that the inverted isoprene unit may be transferred from the dioxopiperazine moiety (Scheme 11)[74].

To date, this problem of the mode of reversed incorporation of the isoprenyl group into position 2 remains unsolved.

In contrast to the echinulins, a small class of compounds containing a reversed isoprene unit at position 3 of an indole nucleus has been isolated. Of these, roquefortine (26)[50,51] is a dioxopiperazine, while oxaline (27)[52], and the metabolite of an unidentified species of Aspergillus (28)[53] are closely related.

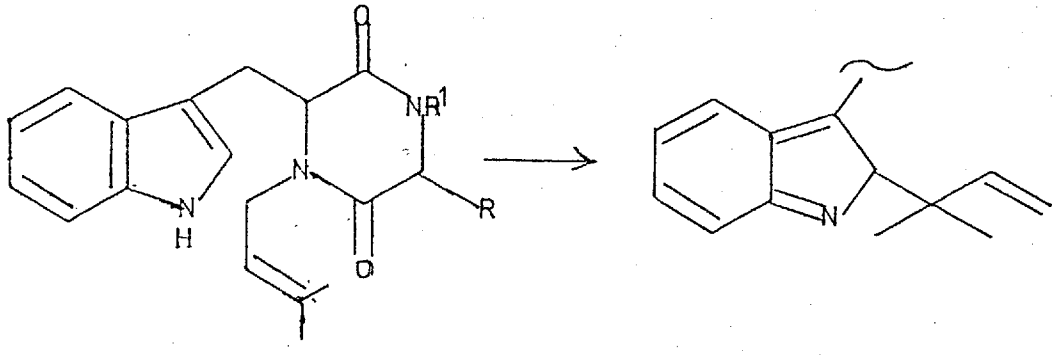
In addition to the C-2 dimethylallyl function, there are two other isoprene units in echinulin. Some attention has been given to the order in which the isoprene units are incorporated, and it has been found that in a cell-free extract of A. amstelodami only the 2-alkylated product is produced by reaction between dimethylallylpyrophosphate and cyclo-L-ala-L-try. Neither the free amino acids nor the dipeptides are substrates for this reaction, and no reaction was observed if isopentenyl pyrophosphate replaced isoprene pyrophosphate[54]. Isopentenyl pyrophosphate isomerase and the enzyme responsible for the alkylation of the dioxopiperazine moiety with dimethylallyl pyrophosphate (but not



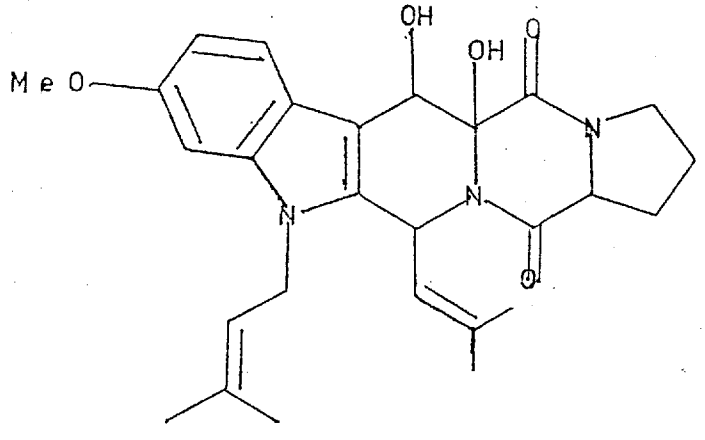
a : $R^1 = R^2 = H$ $R^3 = CH_3$ c: $R^1 = R^2 = R^3 = CH_3$

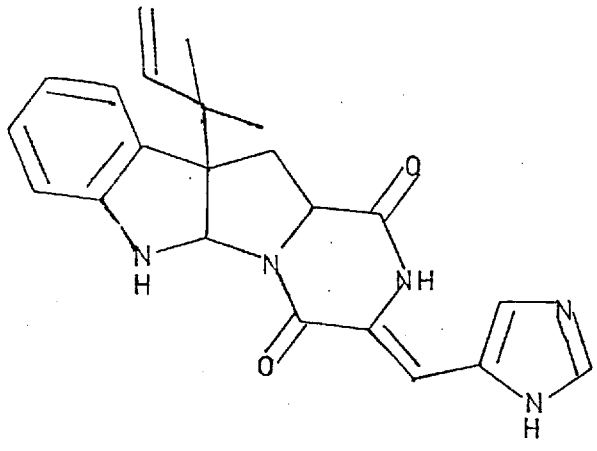
b : $R^1 = R^3 = CH_3$ $R^2 = H$ d: $R^1 = R^2 = CH_3$ $R^3 = Pr^n$

scheme 10

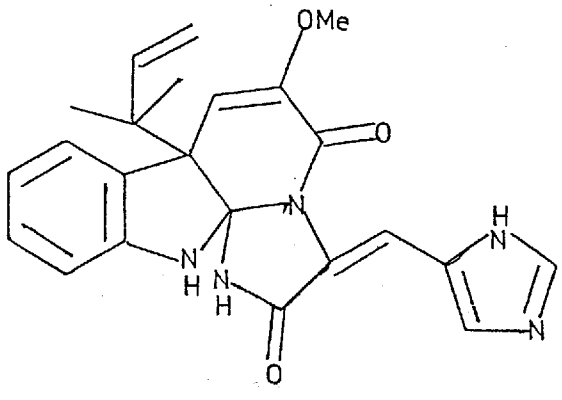


scheme 11

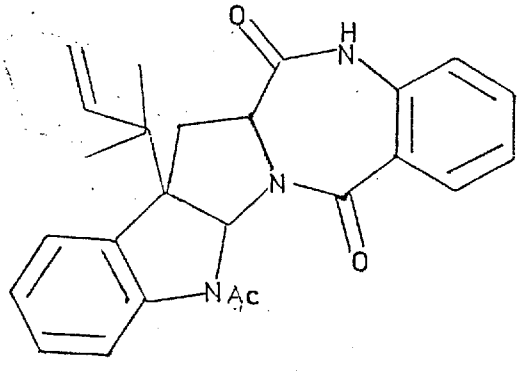




26



27



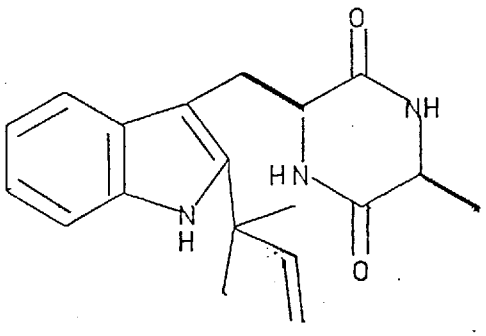
28

isopentenyl pyrophosphate) have been isolated[55,56]. The mono-isoprenylated dioxopiperazine (12), called preechinulin, is a good precursor to echinulin[56] and has subsequently been isolated from A. chevalieri[19,20]. These data suggest that the indole alkylation precedes the substitution of the aromatic ring. Comparative labelling studies with echinulin and neoechinulin demonstrated that the alkylation of the benzene ring proceeds with the loss of the proton at the site of alkylation[57].

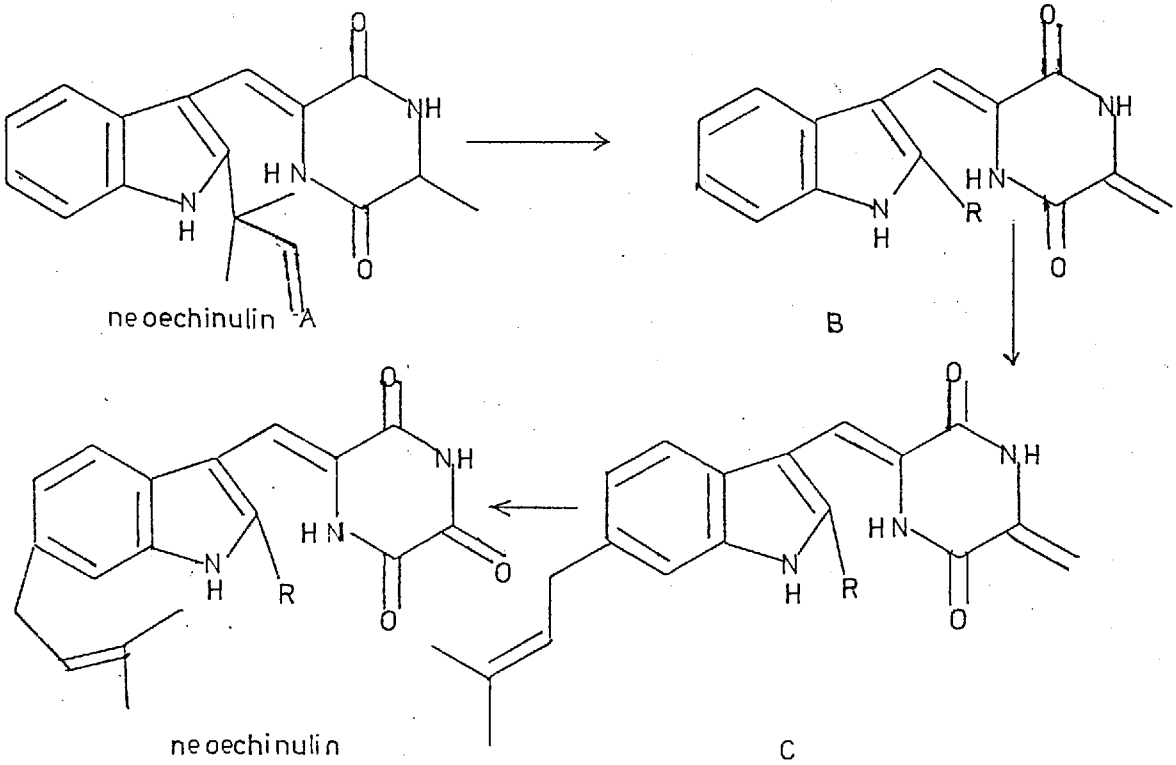
The neoechinulins are a closely related series of compounds. The isolation of neoechinulins A-C[58,59,60], after the discovery of neoechinulin itself[61,62], led to the formulation of the biosynthetic route as shown in Scheme 12. However, with the isolation of neochinulin D (29)[63], it appears that the pathway is not so straightforward. Neoechinulin D, in contrast with the proposed sequence, shows aromatic isoprenylation before the dehydrogenation of the alanine-derived methyl group. Crypto-echinulin C (30) is a monoalkylated variation of neoechinulin[64]. Isolation of these compounds further supports the hypothesis that the first alkylation site is C-2[58]. Feeding experiments have demonstrated that tryptophan and cyclo-L-ala-L-try are precursors to the neoechinulins[57,58,64,65]. Detailed labelling studies also revealed the exclusive removal of the pro-S proton in the biosynthetic α,β-dehydrogenation to neoechinulin (also called crypto-echinulin A)[64].

3.2. Austamides and brevianamides

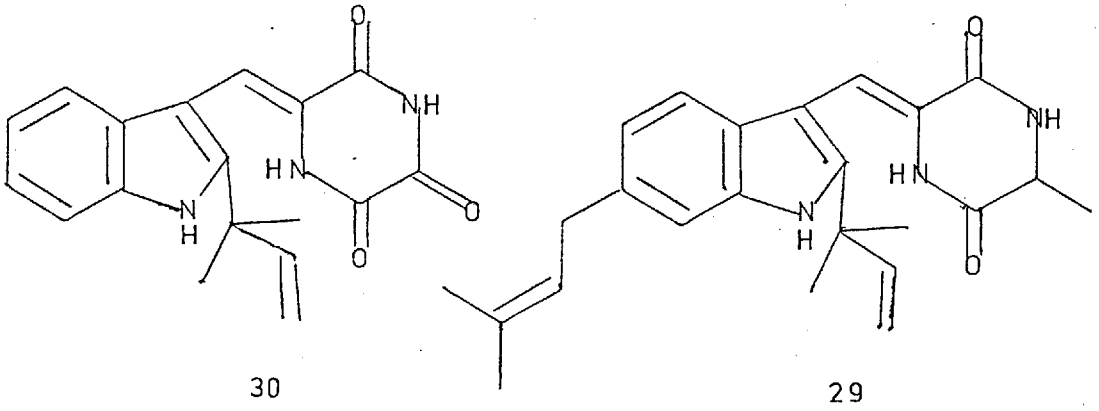
The austamides and brevianamides are similar to echinulin



12



scheme 12



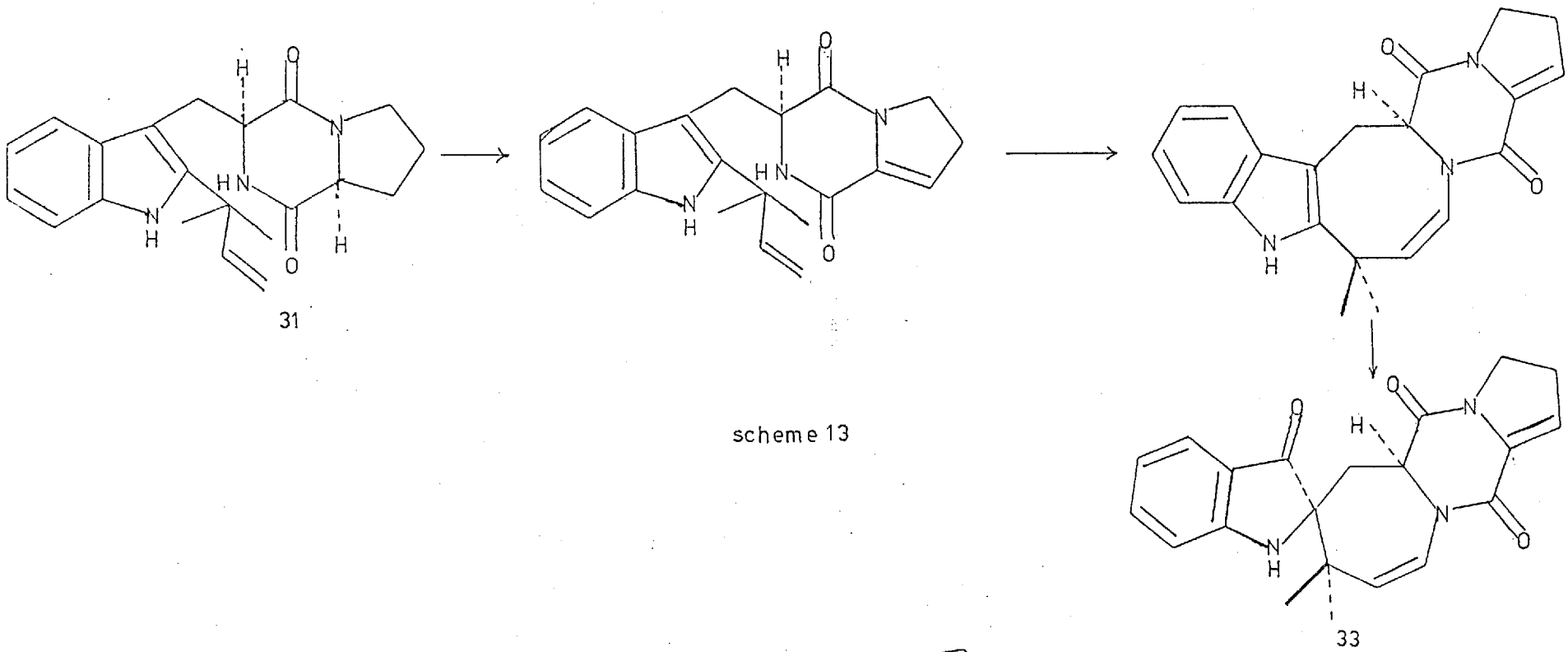
30 29

in the presence of the tryptophyl moiety and the 2-α,α-dimethyl-allyl unit; the alanyl section of the dioxopiperazine ring being replaced by a proline residue. The first group of compounds was isolated from Aspergillus ustus,[66,67,68] while the latter are metabolites of Penicillium brevicompactum[69,28]. The considerable structural similarities between the two have led to the proposal of a common intermediate. Deoxybrevianamide E (31) has been isolated from cultures of A. ustus[67] and, although it has not been found in P. brevicompactum, it is considered to be a biogenetic precursor of the brevianamides[70]. This is supported by the isolation of cyclo-L-pro-L-try(brevianamide F(32)) from P. brevicompactum[28] and, conversely, by its incorporation into brevianamide A,[70] and by the isolation of an analogous intermediate from A. amstelodami in the case of echinulin, as previously cited[40]. The discovery of several related metabolites from A. ustus suggests the adjacent sequence (Scheme 13) terminating in austamide (33)[67].

The 3,2-alkyl shift and ring contraction with concurrent oxidation has been performed in chemical systems (Scheme 14)[71].

Dehydropeptides have been synthesised by a variety of routes, including hydrolysis of an azlactone[72] and condensations of α-keto acids with amides or nitriles.[73] In close analogy with austamide, bis-dehydroproline anhydride has been prepared from the 3,6-diacetyldioxopiperazine (Scheme 15)[74,75]. Alternatively, oxidation can be effected by dehydrogenation using reagents such as dichlorodicyanobenzoquinone[74] or by anodic oxidation[76].

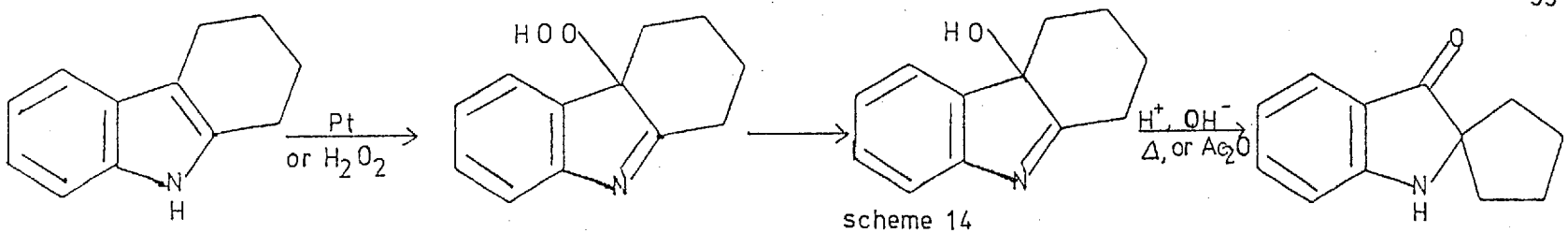
At first glance, the brevianamides (A-F) appear to be a confusing collection of similar molecules. Closer inspection



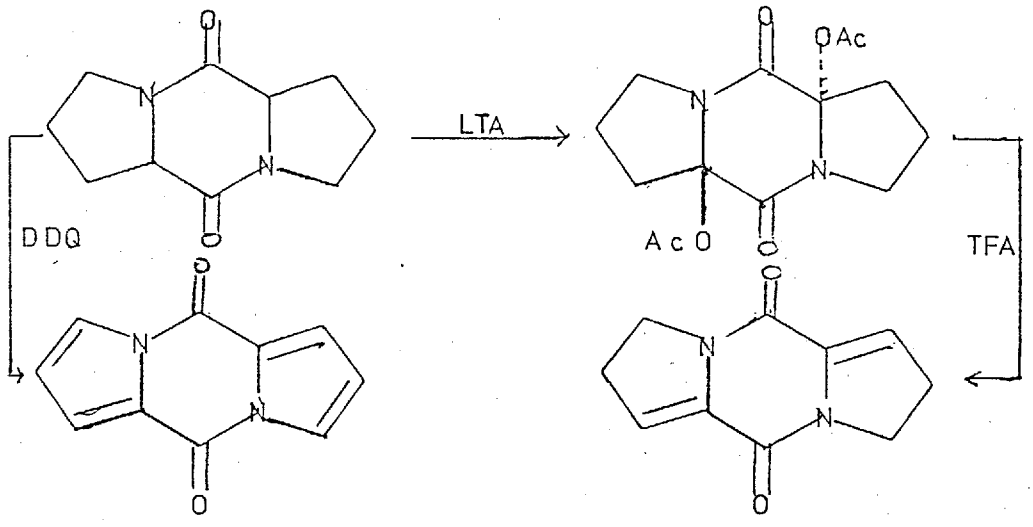
31

33

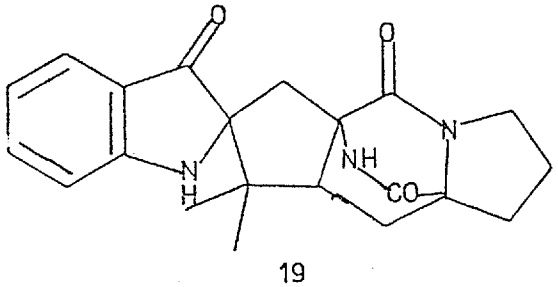
scheme 13



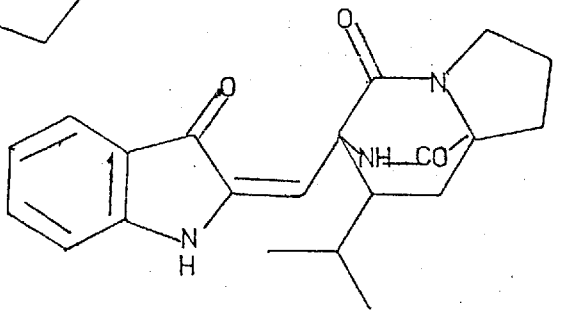
scheme 14



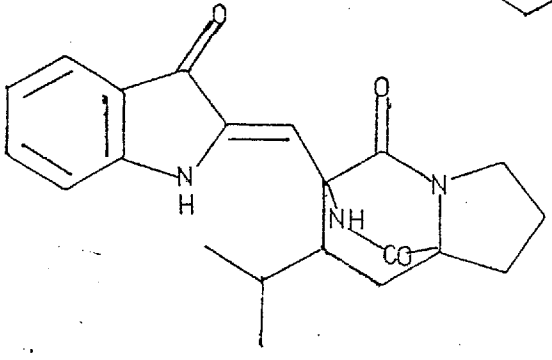
scheme 15



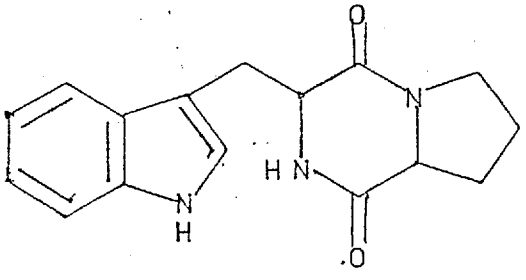
19



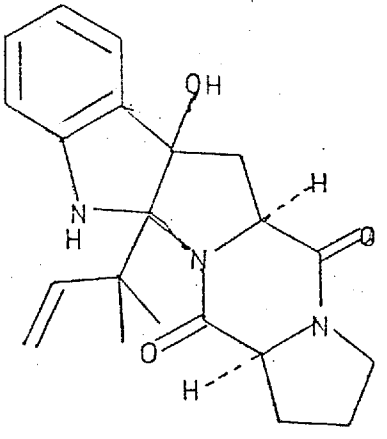
18



17



32



34

reveals that A and B (19) are stereoisomeric about position 2, and that F (32) is simply the dioxopiperazine derived from proline and tryptophan. As stated previously, C and D (17 and 18) are photochemical artefacts and not true metabolites[28]. Results from studies on austamide and echinulin together with mechanistic considerations allow the deduction of a reasonable biosynthetic pathway to brevianamides A and E (34) (Scheme 16)[27,70].

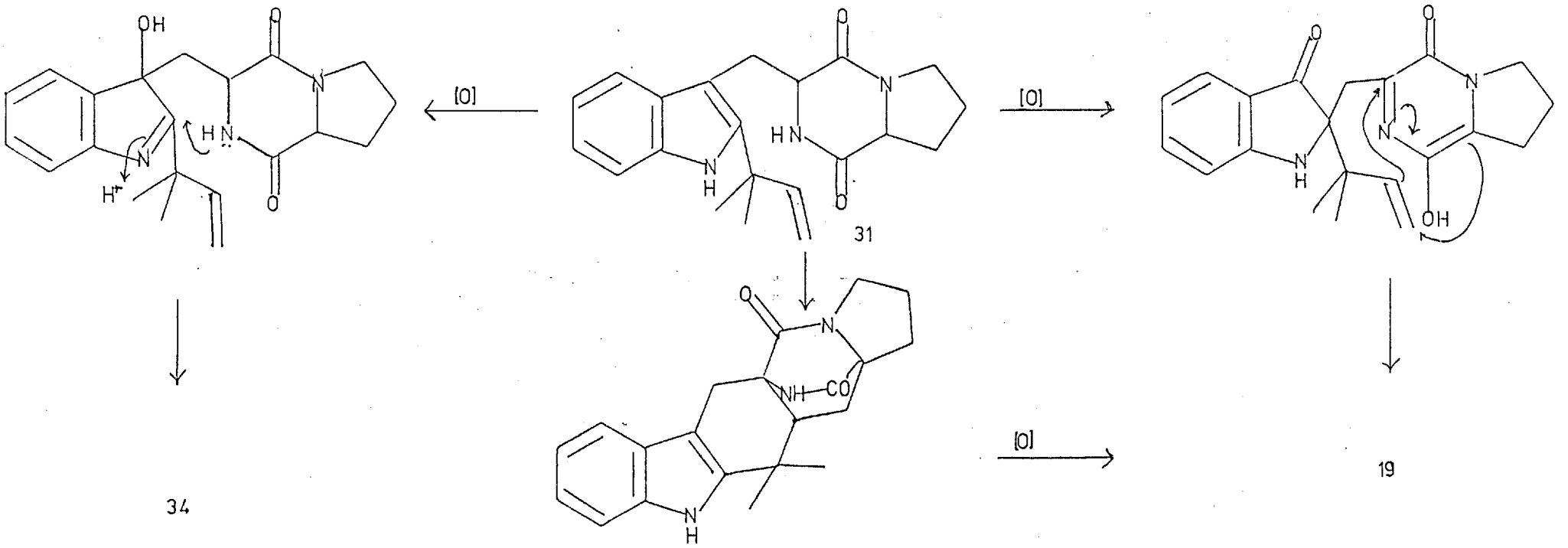
Model studies for cycloaddition reactions have been performed with pyrazines to establish the feasibility of the proposed biogenesis of the ring system in brevianamide A (Scheme 17)[77,78].

4. Epidithiodioxopiperazines

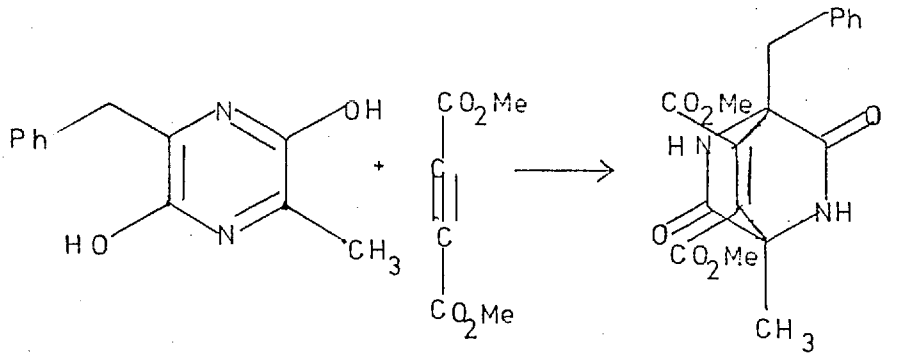
4.1 Gliotoxin

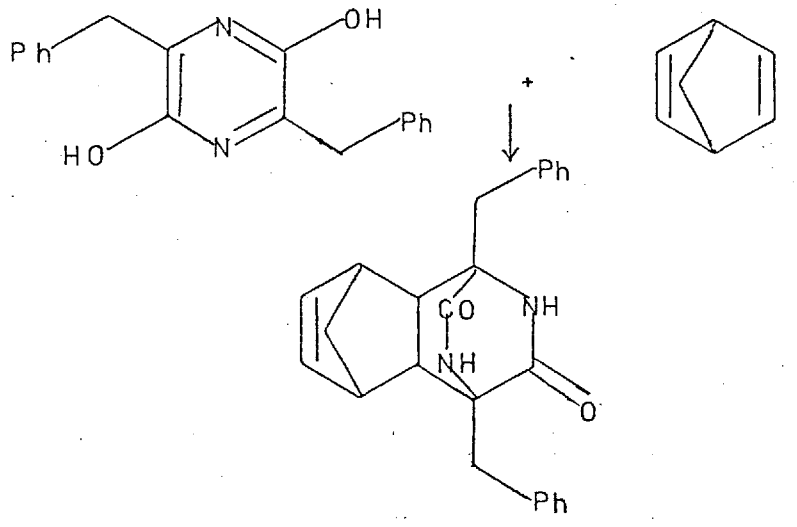
Another major category of naturally occurring antibiotics is characterised by the epidithiodioxopiperazine moiety. Within this class, gliotoxin (3) (from *Penicillium terlikowski*, *Gliocladium fimbreatum*, *Trichoderma viride*, or *Aspergillus terreus*)[4,79,80] and the aranotins (35-39) (from *Arachiotus aureus*)[81,82,83] are evidently derived from similar biosynthetic routes, while the sporidesmins (6)[84] and related compounds, such as chetomin (4)[8], derive from another pathway.

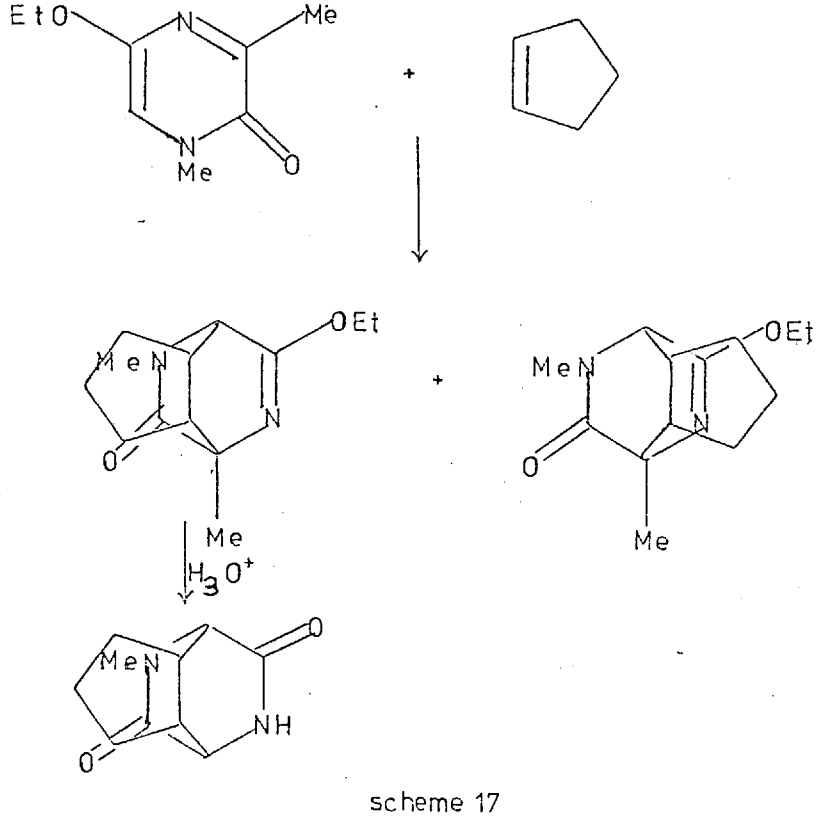
Efforts to elucidate the biogenesis of gliotoxin have been confused by conflicting reports and the isolation of similar compounds thought to be precursors. Early reports that *m*-tyrosine was incorporated more efficiently than phenylalanine led to the



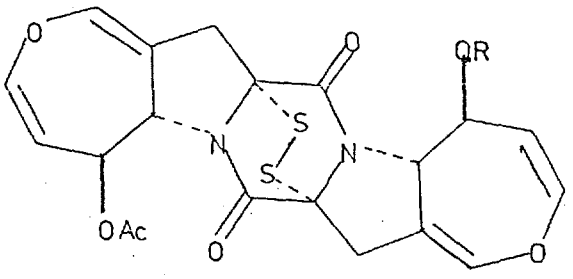
scheme 16





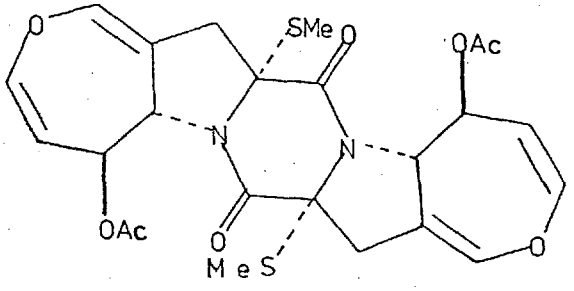


scheme 17

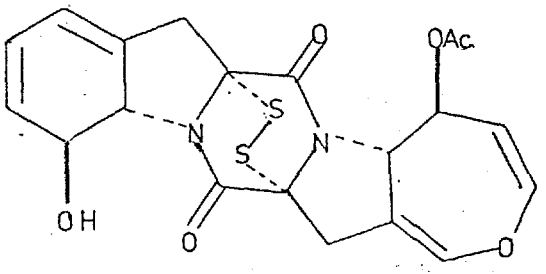


35 R=H aranotin

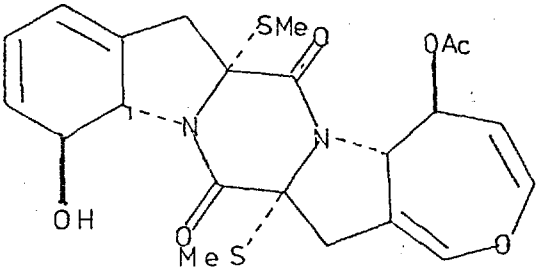
36 R=OAc acetylaranotin



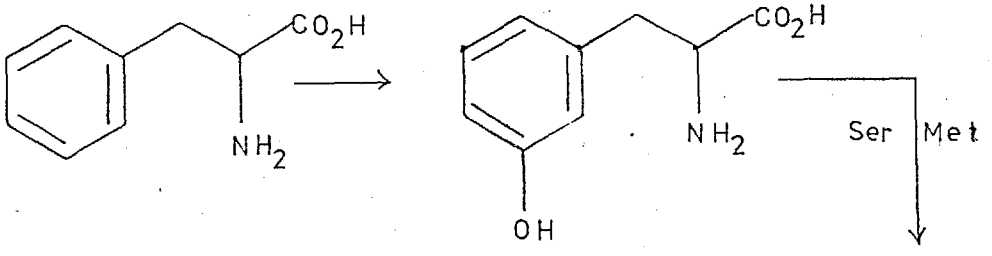
37 bisdethiodi(methylthio) - acetylaranotin

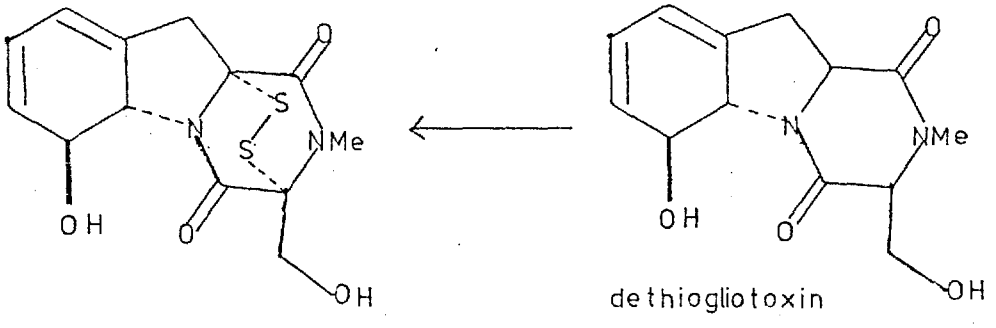


38 apoaranotin



39 bisdethiodi(methylthio)- apoaranotin





dethiogliotoxin

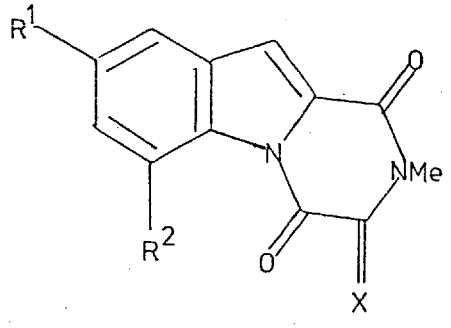
scheme 18

postulation of a route proceeding by hydroxylation of the benzene ring before closure to the reduced indole system (Scheme 18).[85] Subsequently, dehydrogliotoxin (5) was isolated from the same mold, P. terlikowski[85], and this, it was originally claimed, could be converted in vivo to gliotoxin[87]. These reports supported the original scheme.

Meanwhile, systems of the type (40) were isolated from the fungus. It was considered that the diene system might be a receptor for the disulphide bridge, and that these compounds were possible precursors to gliotoxin[88].

It has since been determined that phenylalanine, but not o- or m- tyrosine or dopa, is a precursor to gliotoxin[89,90,91]. Incorporation of phenylalanine-d_8 into acetylaranotin and phenylalanine-d_5 into gliotoxin with the retention of all deuterium atoms disproves both suggestions that a hydroxylated aromatic compound is an intermediate and that the diene system is an acceptor for the disulphide bridge[89,90]. Furthermore, recent attempts to add sulphur across a diene have been unsuccessful[92]. Cyclo-L-phe-L-ser has been shown to be an efficient precursor,[93,94] and the N-methyl group is probably derived from methionine[95]. Competitive labelling studies reveal that cysteine, methionine, and homocysteine are sources of sulphur in gliotoxin in decreasing order of efficiency[85].

Although these results are in conflict with the intermediacy of a hydroxylated benzene ring, they are not inconsistent with an arene-oxide mechanism. This mechanism, first proposed in 1968,[82,83] accounts for the formation and stereochemistry of

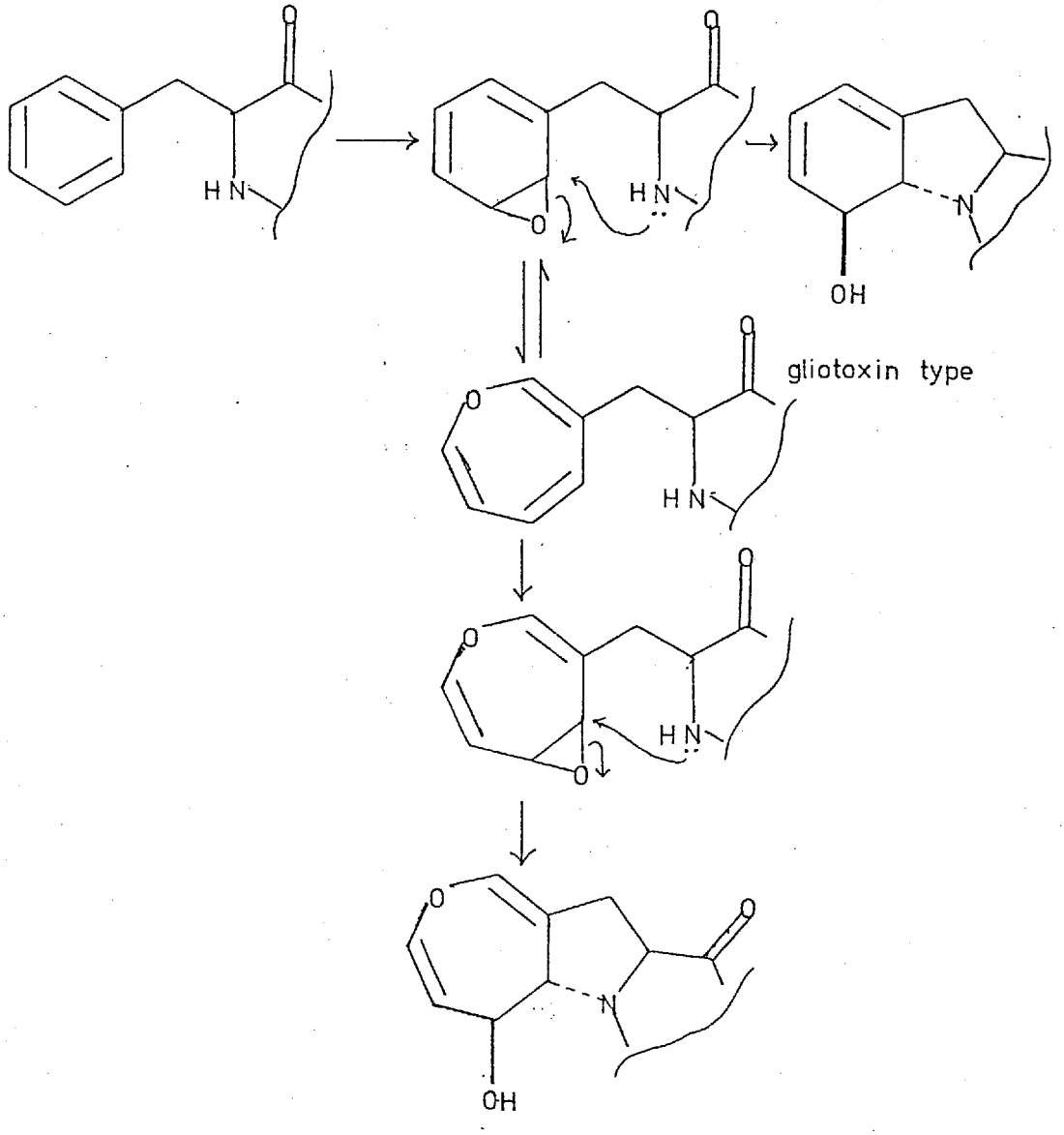


R^1=OH R^2=H X=CH_2

R^1=OH R^2=H X=O

R^1=R^2=H X=O

40



aranotin type

scheme 19

gliotoxin, as well as for the oxepin ring in the aranotins (Scheme 19).

4.2 Aranotins

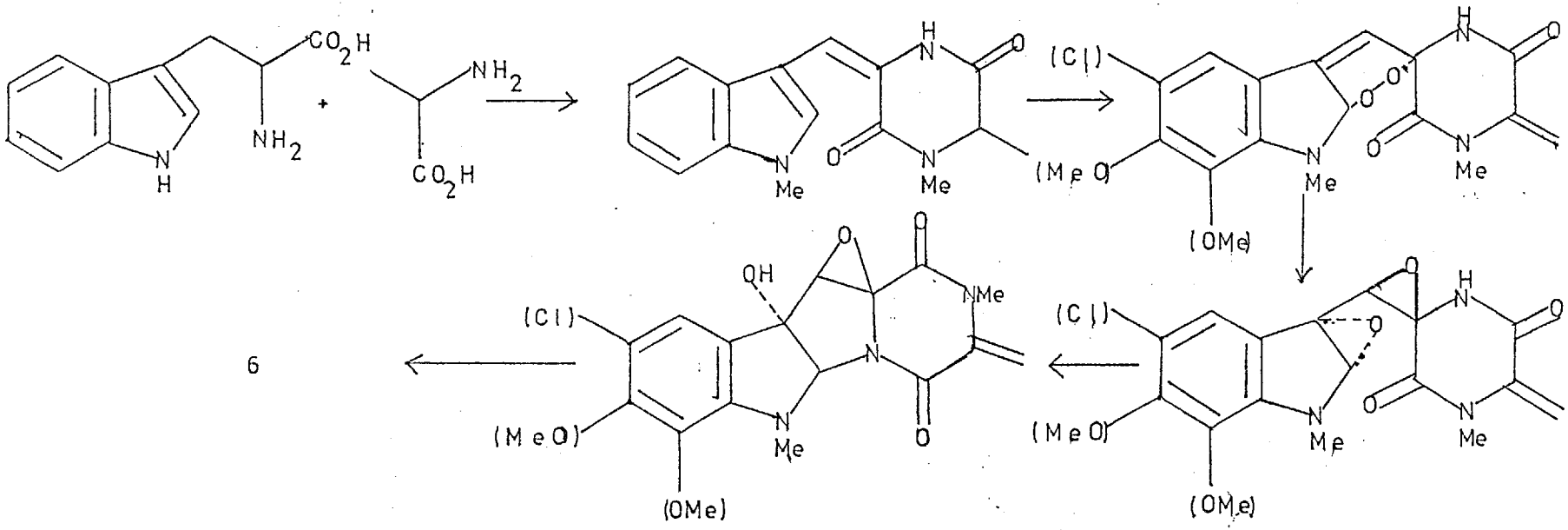
In other respects, the aranotins exhibit similarities to gliotoxin. Feeding experiments again indicate that phenylalanine but not o- or m- tyrosine is a precursor of the dioxopiperazine,[82,89] and feeding with phenylalanine-d_8 results in the retention of all eight deuterium atoms[89]. In the case of bis-dethiodi(thiomethyl) acetyl aranotin (from Arachiotus aureus and Aspergillus terreus), comparative feeding with $^{3}H_3C$-methionine and ^{35}S-methionine results in a greater incorporation of the labelled methyl group and suggests that the thiomethyl function is not added in a one-step process[82].

4.3 Sporidesmins

Although there are several conceivable routes to sporidesmin, little work has been conducted on its biosynthesis. The scheme opposite (Scheme 20) has been suggested as one of several possibilities[27]. Singlet oxygen addition to a related diene system (Scheme 21)[96], and closure to pyrrolo-indoles has been demonstrated (Scheme 22)[97,98].

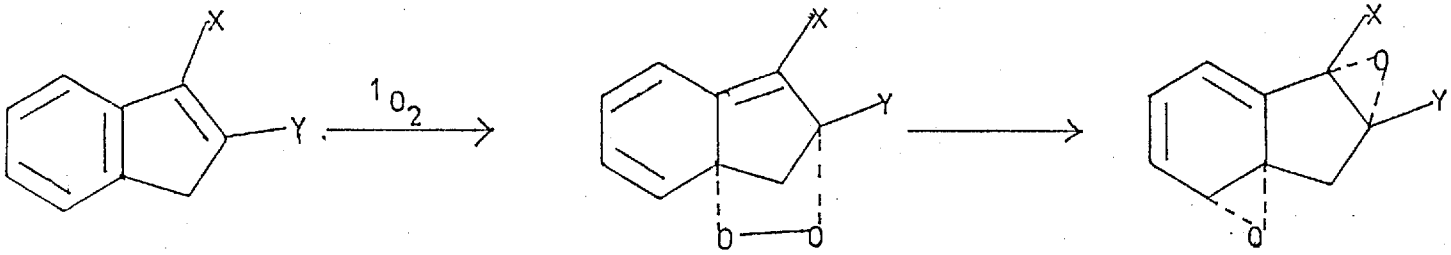
5. Mycelianamide

Mycelianamide (41)[99] is neither an isoprenylated alkaloid, an epidithiodioxopiperazine, nor a hydroxypyrazine derivative, but one of a growing class of naturally occurring hydroxamic acids. The heterocyclic ring is derived from tyrosine, the dehydrogenation

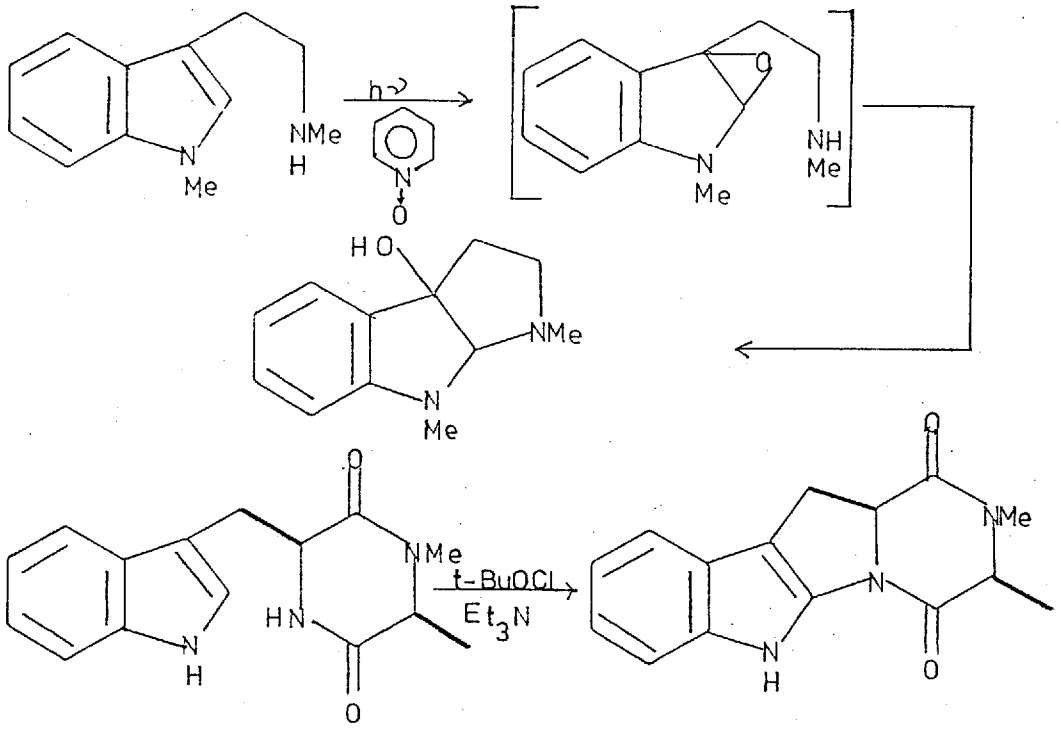


6

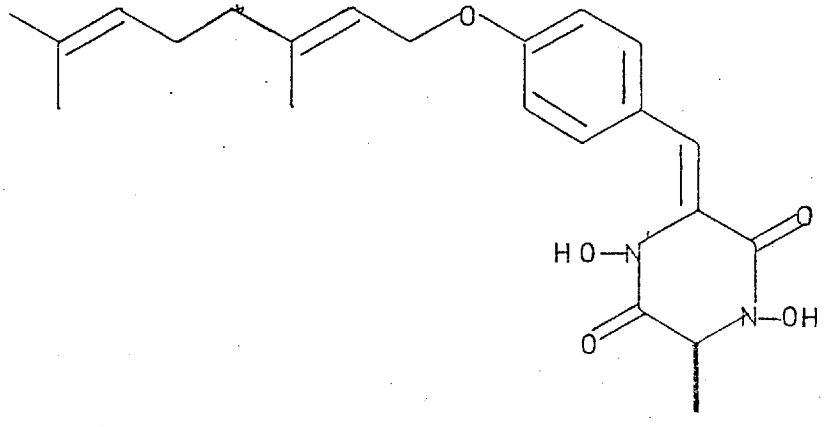
scheme 20



scheme 21


NMe
H
N
Me
h
N
O
O
NH
Me
N
Me
HO
NMe
N
Me
O
NMe
HN
N
H
O
t-BuOCl
Et3N
O
NMe
N
N
H
O

scheme 22


O
HO
N
O
N
OH
O

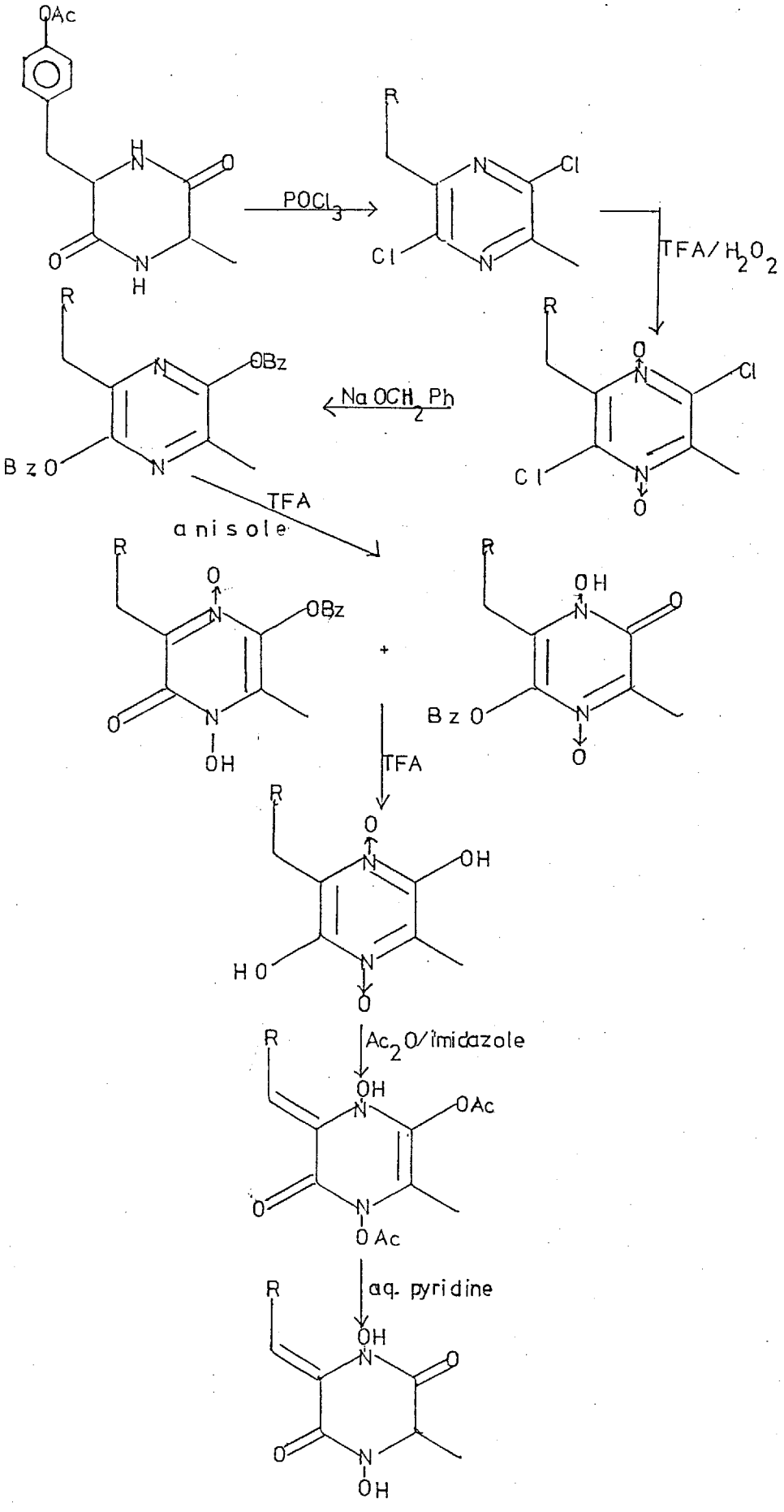
41

proceeding in a cis fashion[100]. Acetic acid, mevalonic lactone, or 3-methyl-butenoic acid are metabolised via the well-known route for terpene synthesis to give the geranyl side chain[101]. In studies directed toward the total synthesis of mycelianamide, it was found that the formation of the hydroxamic acid functions was more difficult than anticipated. Eventually, the conversion from the dioxopiperazine was accomplished by making the dichloropyrazine, oxidising the nitrogen atoms, exchanging the chlorine atoms with benzyl groups, and hydrolysing to the dihydroxamic acid (Scheme 23)[102,103,104]. This synthesis is similar to the one reported for pulcherrimin[105].

6. Summary

There are numerous other dioxopiperazines and related derivatives that have been isolated from fungi, but for which few biosynthetic studies have been undertaken. For instance, bicyclomycin (8) from Streptomyces sapporonensis, has generated some interest as a potential antibiotic due to its unusual selective activity against gram-negative bacteria, its low toxicity, the low natural resistance to it expressed by micro-organisms, its lack of cross-resistance with other antibiotics, and its stability in the bodies of test animals[106,107,108]. Its mode of action is thought to be interference with the synthesis of lipoprotein and its assembly to peptidoglycan[109].

Although few specific biosynthetic studies have been performed, it is possible to make some general observations


OAc
POCl3
R
Cl
TFA/H2O2
OBz
BzO
NaOCH2Ph
TFA
anisole
OH
HO
Ac2O/imidazole
aq. pyridine

scheme 23

concerning the biogenesis of dioxopiperazines. For the most part, they appear to be synthesised in a straightforward manner commencing with the dehydrative cyclisation of the appropriate amino acids to the dioxopiperazine, which is then elaborated. Broad classes of compounds can be delineated that share structural similarities and that may arise from a common precursor reacting by different mechanisms in different organisms. The isoprenylated tryptophan dioxopiperazines are one such group of compounds, and the epidithiodioxopiperazines from aromatic amino acids another. More specifically, austamide and brevianamide A are both descended from deoxybrevianamide E. The most basic difference between two of these natural products may be the substitution of one of the two amino acids. For instance, brevianamides are distinguished from echinulins by the incorporation of proline rather than alanine. Mechanistic considerations may reveal greater similarities than at first seem apparent. For example, the relationship between gliotoxin and aranotin may not be obvious until the formulation of an arene-oxide intermediate is considered. Model chemical studies can be involved to illustrate the feasibility of postulated biosynthetic processes, now that it is generally accepted that the same chemistry applies <u>in vivo</u> as <u>in vitro</u>.

REFERENCES

1. E. Fischer, Ber., 1906, 39, 530.
2. T. Curtius and Goebel, J. Prakt. Chem., 1888, 37, 173,
3. M. O. Foster and W. B. Saville, J. Chem. Soc., 1922, 121, 816.
4. R. Weindling and O. Emerson, Phytopathology, 1936, 26, 1068.
5. J. R. Johnson and J. B. Buchanan, J. Amer. Chem. Soc., 1953, 75, 2103.
6. M. R. Bell, J. R. Johnson, B. S. Wildi, and R. B. Woodward, J. Amer. Chem. Soc., 1958, 80, 1001.
7. A. F. Beecham, J. Fridricksons and A. McL . Mathieson, Tetrahedron Letters, 1956, 3131.
8. S. A. Waksman and E. Bergie, J. Bacteriol. 1944, 48, 527.
9. A. G. McInnes, A. Taylor and J. A. Walter, J. Amer. Chem. Soc., 1976, 98, 6741.
10. C. Shin, G. Chigera, M. Masaki, and A. Ohta, Tetrahedron Letters, 1967, 4601.
11. ----------, Bull. Chem. Soc. Jap., 1969, 42, 191.
12. Y. Kishi, T. Fukuyama, and S. Nakatsuka, J. Amer. Chem. Soc., 1973, 95, 6490.
13. Y. Kishi, S. Nakatsuka, T. Fukuyama and M. Havel, ibid., 6493.
14. N. Takamatsu, S. Inoue, and Y. Kishi, Tetrahedron Letters, 1971, 4665.
15. T. Kamiya, S. Maeno, M. Hashimoto and Y. Mine, J.Antibiotics, 1972, 25, 576.
16. G. M. Sharma and R. K. Burkholder, J.C.S. Chem. Commun., 1971, 151.
17. F. Dorn and D. Arigoni, Experientia, 1974, 30, 134.
18. G. M. Strunz, C. J. Hussner, M. Kakushima and M. A. Stillwell, Can. J. Chem., 1974, 52, 325.
19. T. Hamasaki, K. Nagayama, and Y. Hatsuda, Agric. Biol.Chem., 1976, 40, 203.
20. R. D. Stipanovic and H. W. Schroeder, Trans. Brit. Mycol. Soc., 1976, 66, part 1, 178.

21. P. J. Curtis, D. Greatbanks, B. Hesp, A. F. Cameron and A. A. Freer, J.C.S. Perkin I, 1977, 180.

22. E. Eickman, J. Clardy, R. J. Cole, and J. W. Kirksey, Tetrahedron Letters, 1975, 1051.

23. M. Yamazaki, H. Fujimoto, and T. Kawasaki, Tetrahedron Letters, 1975, 1241.

24. M. Yamazaki, K. Sasago, and K. Miyaki, J.C.S. Chem. Commun., 1974, 408.

25. H. Nagasawa, A. Isogai, A. Suzuki, and S. Tamura, Tetrahedron Letters, 1976, 1601.

26. R. Baute, G. Deffieux, M. A. Baute, M. J. Filleau and A. Neveu, Tetrahedron Letters, 1976, 3943.

27. P. G. Sammes, Fort. der. Chem. org. Naturstoffe, 1975, 32, 51.

28. A. J. Birch and R. A. Russell, Tetrahedron, 1972, 28, 2999.

29. C. L. Atkins and J. B. Nielands, Biochem., 1968, 7, 3734.

30. H. A. Akers, M. Llinas, and J. B. Nielands, Biochem., 1972, 11 2283.

31. J. C. MacDonald, J. Biol. Chem., 1961, 236, 512.

32. R. G. Micetick and J. C. MacDonald, J. Chem. Soc., 1964, 1507.

33. J. C. MacDonald, J. Biol. Chem., 1962, 237, 1977.

34. J. C. MacDonald, Biochem. J., 1965, 96, 533.

35. A. Quilico and L. Panizzi, Ber., 1943, 76, 348.

36. A. J. Birch, C. E. Blance, S. David and H. Smith, J. Chem. Soc., 1961, 3128.

37. G. Casnati, R. Cavalleri, F. Piozzi and A. Quilico, Gazz. Chim. Ital., 1962, 92, 105.

38. A. J. Birch and K. R. Farrar, J. Chem. Soc., 1963, 4277.

39. J. C. MacDonald and G. P. Slater, Can. J. Microbiol., 1966, 12, 455.

40. G. P. Slater, J. C. MacDonald, and R. Nakashima, Biochem., 1970, 9, 2886.

41. G. Casnati, M. Francioni, A. Guareschi and A. Pochini, Tetrahedron Letters, 1969, 2485.

42. A. H. Jackson and A. E. Smith, Tetrahedron, 1965, 21, 989.

43. A. C. Weedon, Ph.D. Thesis, University of London, 1976.

44. See, for example, Sumpter and Miller in "The Chemistry of Heterocyclic Compounds", Vol. 8, Chapter 50. New York: Interscience., 1958.

45. B. W. Bycroft and W. London, J.C.S. Chem. Commun., 1970, 967.

46. G. Casnati and A. Pochini, J.C.S. Chem. Commun., 1970, 1328.

47. S. Inada, K. Nagai, M. Okazaki and Y. Takayanagi, Bull. Chem. Soc. Jap., 1976, 49, 833.

48. C. S. Kook, J. M. Patterson, W. T. Smith and A. Wu, J. Org. Chem., 1974, 39, 486.

49. D. T. Dix, J. Martin, and C. E. Moppett, J.C.S. Chem. Commun. 1972, 1168.

50. M. A. Merrien, J. Polonsky and P. M. Scott, Experimentia, 1976, 32, 140.

51. M. Abe, S. Ohmono, T. Sato, and T. Utogawa, Agric. Biol. Chem., 1975, 39, 1333.

52. K. J. Kruger and D. W. Nagel, J.C.S. Chem. Commun., 1974, 1021.

53. G. A. Ellestad, M. P. Kunstmann, and P. Mirando, J. Org. Chem., 1973, 38, 4204.

54. C. M. Allen, Jr., Biochem. 1972, 11, 2154.

55. ----------, Can. J. Microbiol., 1972, 18, 1275.

56. ----------, J. Amer. Chem. Soc., 1973, 95, 2386.

57. G. Casnati, G. P. Gardini, G. Palla and C. Fuganti, J.C.S. Perkin I., 1974, 2397.

58. A. Dossena, R. Marchelli and A. Pochini, J.C.S. Chem. Commun., 1974, 771.

59. R. Cardillo, C. Fuganti, G. Gatti, D. Ghiringhelli and P. Grasselli, Tetrahedron Letters, 1974, 3163.

60. R. Marchelli, A. Dossena, and A. Pochini, J.C.S. Perkin I, 1977, 713.

61. M. Barbetta, G. Casnati, A. Pochini, and A. Silva, Tetrahedron Letters, 1969, 4457.

62. G. Casnati, A. Pochini, and R. Ungaro, Gazz. Chem. Ital., 1973, 103, 141.

63. A. Dossena, R. Marchelli, and A. Pochini, Experimentia, 1975, 31, 1249.

64. R. Cardillo, C. Fuganti, D. Ghiringhelli, P. Graselli, and G. Gatti, J.C.S. Chem. Commun., 1975, 778.

65. R. Marchelli, A. Dossena, and G. Casnati, J.C.S. Chem. Commun., 1975, 779.

66. P. Steyn, Tetrahedron Letters, 1971, 3331.

67. P. Steyn, Tetrahedron, 1973, 29, 107.

68. P. S. Steyn and R. Vleggaai, Phytochemistry, 1976, 15, 355.

69. A. J. Birch and J. J. Wright, Tetrahedron, 1970, 26, 2329.

70. J. Baldas, A. J. Birch, and R. A. Russell, J.C.S. Perkin I, 1974, 50.

71. B. Witkop and J. B. Patrick, J. Amer. Chem. Soc., 1951, 73, 2188.

72. E. Erlenmeyer, Annalen, 1893, 275, 1.

73. M. Bergmann and K. Grafe, Z. Physiol. Chem., 1930, 187, 187.

74. P. J. Machin, Ph.D. Thesis, University of London, 1974.

75. E. Ohler, F. Tataruch, and U. Schmidt, Chem. Ber. 1973, 106, 396.

76. L. A. Simmson and C. K. Mann, Tetrahedron Letters, 1970, 3303.

77. A. E. A. Porter and P. G. Sammes, J. C. S. Chem. Commun., 1970, 1103.

78. P. J. Machin, A. E. A. Porter, and P. G. Sammes, J.C.S. Perkin I, 1973, 404.

79. P. A. Miller, P. W. Trown, W. Fulmar, G. O. Morton and J. Karliner, Biochem. Biophys. Res. Comm., 1968, 33, 219.

80. J. R. Johnson, F. W. Bruce and J. D. Dutcher, J. Amer. Chem. Soc., 1943, 65, 2005.

81. R. Nagarajan, L. L. Huckstep, D. H. Lively, D. C. DeLong, M. M. Marsh, and N. Neuss, J. Amer. Chem. Soc., 1968, 90, 2980.

82. N. Neuss, et al., Antimicrob. Agents in Chemother., 1968, 213.

83. N. Neuss, P. Nagarajan, B. B. Molloy, and L. L. Huckstep, Tetrahedron Letters, 1968, 4467.

84. J. W. Ronaldson, A. Taylor, E. P. White and R. J. Abraham, J. Chem. Soc., 1963, 3172.

85. J. A. Winstead and R. J. Suhadolnik, J. Amer. Chem. Soc., 1960, 82, 1644.

86. G. Lowe, A. Taylor, and L. C. Vining, J. Chem. Soc. (C), 1966, 1799.

87. M. S. Ali, R. Rahman, and A. Taylor, Vth Intnl. Symposium on the Chem. of Natl. Prods. - Abstracts, IUPAC, London, 1968, p. 94.

88. M. S. Ali, J. S. Shannon, and A. Taylor, J. Chem. Soc. (C), 1968, 2044.

89. D. R. Brannon, J. A. Mabe, B. B. Molloy, and W. A. Day, Biochem.Biophys. Res. Comm., 1971, 43, 588.

90. J. D. Bu'Lock and A. P. Ryles, J.C.S. Chem. Commun., 1970, 1404.

91. N. Johns and G. W. Kirby, J.C.S. Chem. Commun., 1971, 163.

92. D. L. Coffen, D. A. Katonak, N. R. Nelson, and F. D. Sancilio, J. Org. Chem., 1977, 42, 948.

93. J. C. MacDonald and G. P. Slater, Can. J. Biochem., 1975, 53, 475.

94. J. D. Bu'Lock and C. Leigh, J.C.S. Chem. Commun., 1975, 628.

95. R. J. Suhadolnik and R. E. Chenowith, J. Amer. Chem. Soc., 1958, 80, 4391.

96. C. S. Foote, S. Mazur, P. A. Burns, and D. Lerdal, J. Amer. Chem. Soc., 1973, 95, 586.

97. M. Nakagawa, T. Kaneko, and H. Yamaguchi, J.C.S. Chem. Commun., 1972, 603.

98. M. Ohno, T. F. Spende, and B. Witkop, J. Amer. Chem. Soc., 1970, 92, 343.

99. A. E. Oxford and H. Raistrick, Biochem. J., 1948, 42, 323.

100. G. W. Kirby and S. Narayanaswami, J.C.S. Chem. Commun., 1973, 322.

101. A. J. Birch, R. J. English, R. A. Massey-Westropp, and H. Smith, J. Chem. Soc., 1958, 369.

102. A. K. Göktürk, Ph.D. Thesis, University of London, 1975.

103. K. W. Blake, Ph.D. Thesis, University of London, 1970.

104. A. E. A. Porter, Ph.D. Thesis, University of London, 1971.

105. A. Ohta, Chem. Pharm. Bull. (Japan), 1969, 12, 125.

106. T. Miyoshi, N. Miyairi, H. Aoki, M. Kohsaka, H. Sakai, and H. Imanaka, J. Antibiotics, 1972, 25, 569.

107. M. Nishida, Y. Mine, T. Matsubara, S. Goto, and S. Kuwahara, ibid, 582.

108. ----------, ibid., 594.

109. N. Tanaka, M. Iseki, T. Miyoshi, H. Aoki, and H. Imanaka, J. Antibiotics, 1976, 29, 155.

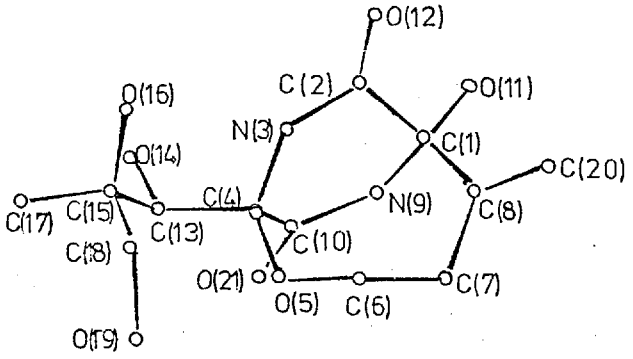
STUDIES ON PYRAZINES AND RELATED HETEROCYCLIC SYSTEMS

1. Introduction

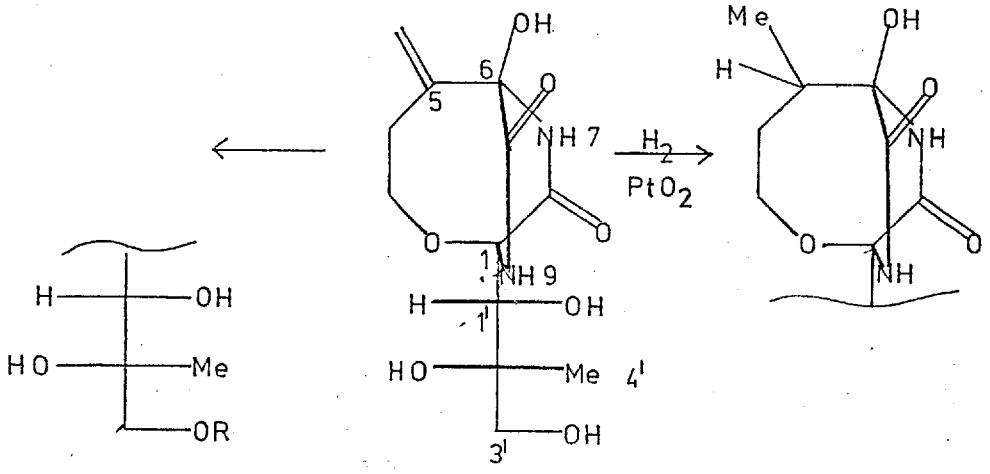
The object of this study was to explore possible synthetic routes to the antibiotic bicyclomycin. As mentioned previously (p. 36), bicyclomycin (1) is an antibiotic metabolite isolated from cultures of some Streptomyces strains, specifically S. sapporonensis[1], S. aizunensis[2], and S.griseoflavus[3]. Bicyclomycin is a solid that crystallises in both orthorhombic and monoclinic forms from water. The x-ray diffraction analysis shows that the dioxopiperazine ring exists in the boat form and that the conformations of the side chains are all gauche, to a first approximation. All of the oxygen and nitrogen atoms participate in two intramolecular and four intermolecular hydrogen bonds[4].

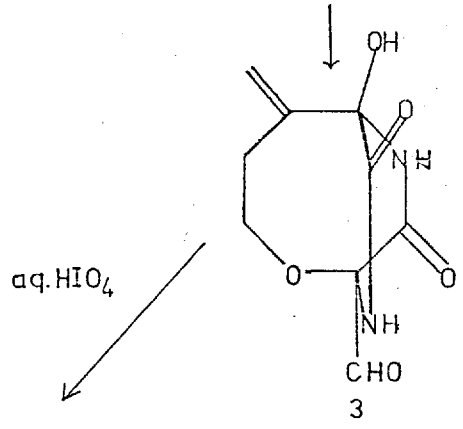
Of the work which has been published on bicyclomycin much concerns the formation of acyl derivatives. Acylation of (1) by treatment with an anhydride or acid chloride proceeds specifically to give the 3' ester[5]. The reaction is general, and a variety of derivatives has been prepared[5,6]. The acyl derivatives are of interest for their biological properties. They are absorbed into the body in higher concentration than the parent compound, and may be transformed in vivo to the antibiotic[5,7]. Recovery from the urine of the acyl derivatives is more efficient[7]. Monoacyl derivatives are readily hydrolysed by enzymes such as esterases, but their recovery increases with increasing lipophilicity of the derivative. Diacyl derivatives are recovered unmetabolised[8].

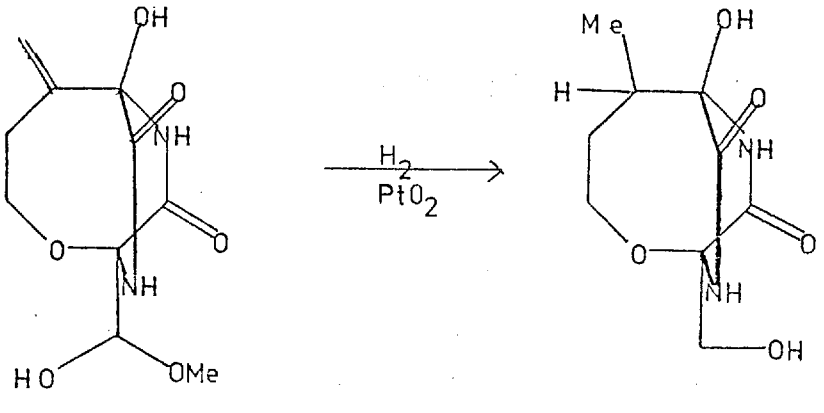
Hydrogenation of (1) affords the 5-methyl compound (2).



crystal structure of 1







scheme 1

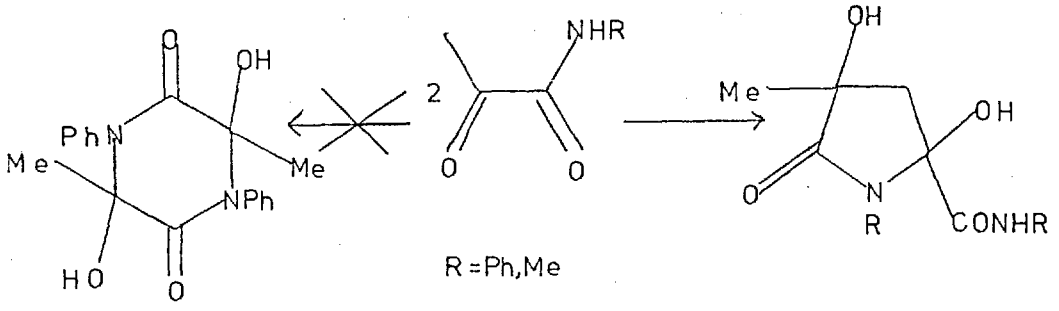
Reaction of bicyclomycin with periodic acid oxidatively cleaves the side chain at the 1 position to the aldehyde (3). Subsequent formation of the methyl hemiacetal (4) and hydrogenation yields the 1-hydroxymethyl-5-methyl derivative (5) (Scheme 1)[9].

There are two obvious approaches to the synthesis of bicyclomycin, these being functionalisation of an amino acid precursor followed by condensation to the dioxopiperazine and, secondly, elaboration of a preformed dioxopiperazine ring. The latter method was chosen as the more direct route, and by analogy with other naturally occurring dioxopiperazines, is more likely to parallel the biosynthetic pathway.

An examination of the bicyclomycin structure reveals a similarity to pyruvamide derivatives. In principle, cyclisation of pyruvamide would give the cyclol structure, solving directly the problem of oxidation at the 3,6-positions of the dioxopiperazine ring. In practice, however, pyruvamide cyclises to the pyrrole[10,11] rather than to the dioxopiperazine as previously reported[12] (Scheme 2).

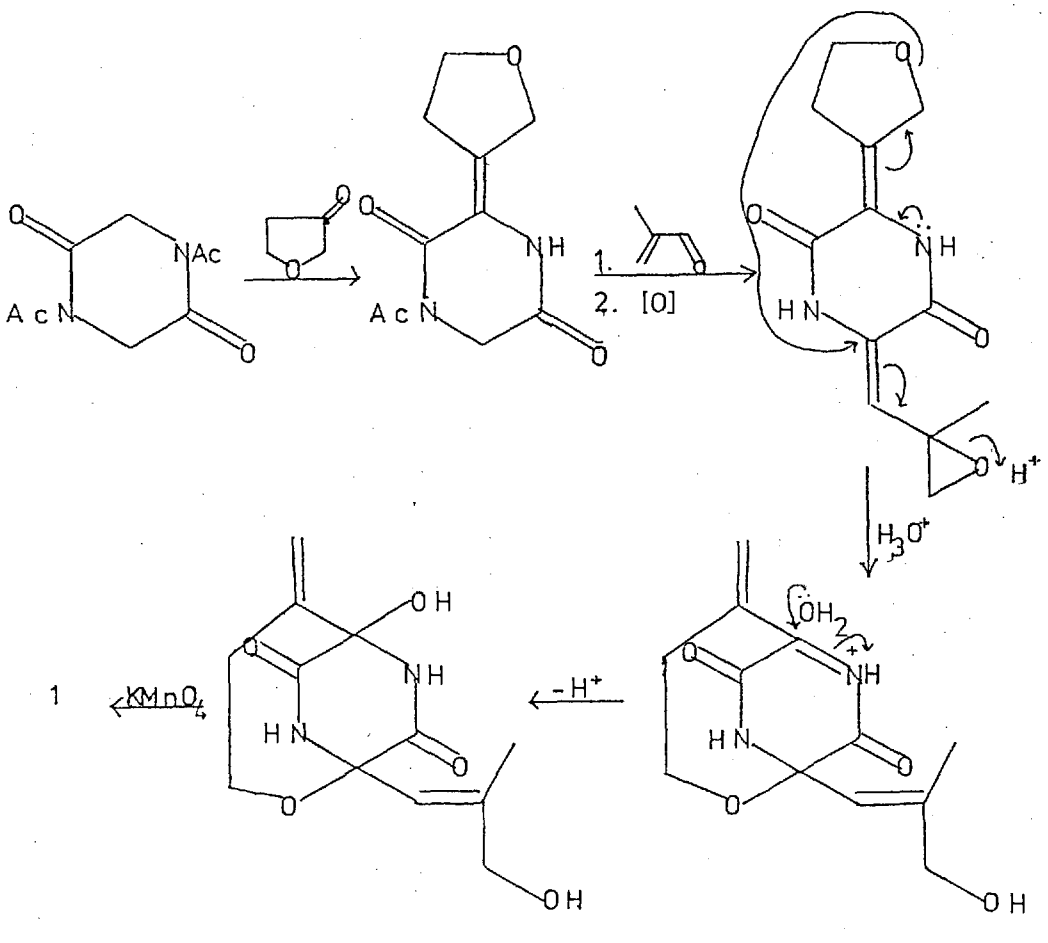
The initial pathway (Scheme 3) hinged upon the condensation of a dioxopiperazine with 3-tetrahydrofuranone (6, $R^1 = R^2 = R^3 = R^4 = H$) followed by acid catalysed nucleophilic addition of the oxygen atom to an exocyclic double bond, forming the bicyclic ring system.

3-Tetrahydrofuranone proved to possess a deceptively simple structure. Standard oxidative cyclisation of a 1,4-butynediol is ineffective in the unsubstituted case[13,14,15]. It has been reported that heating 1,4-butynediol with an organomercuric salt in an alcoholic solvent results in formation of the furanone[16].

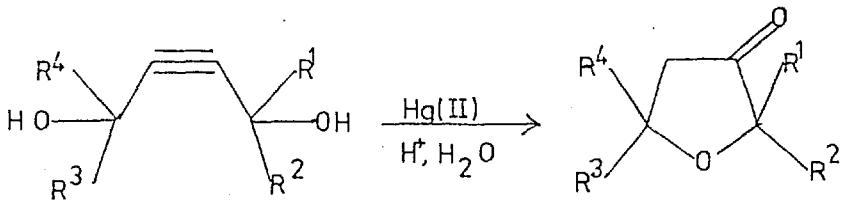


R=Ph,Me

scheme 2



scheme 3

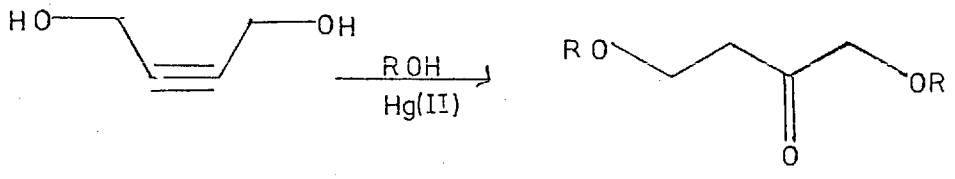


Attempts to repeat this procedure in a variety of solvents eventually resulted in the isolation from n-propanol, not of the furanone, but of 1,4-dipropoxy-2-butanone (7, R = Pr). Not surprisingly, these systems polymerise readily, and frequently the major products after removal of solvent were tars. A similar method has, in fact, been used to prepare 1,4-dihydroxy-2-butanone (7, R = H),[17,18] which, with the anhydrous conditions given for the synthesis of the furanone, casts some doubt on the reliability of that procedure.

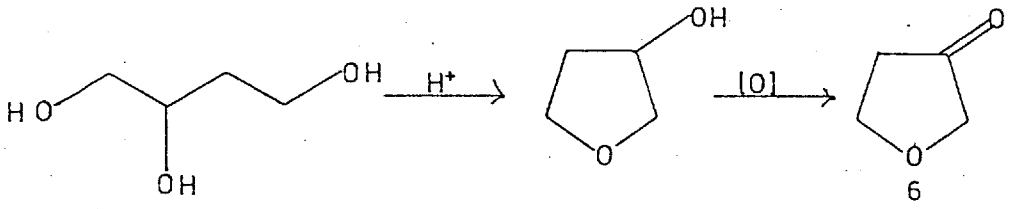
The two other published routes to 3-tetrahydrofuranone are less direct. One involves oxidation of 3-hydroxytetrahydrofuran (Scheme 4),[18,19,20] and the key intermediate in the other is 3-chloro-4-hydroxytetrahydrofuran (Scheme 5)[21]. The precursor (Scheme 4), 1,2,4-trihydroxybutane, is prepared by hydration of 1,4-butynediol followed by reduction to the triol.[17,18] The final oxidation to tetrahydrofuranone is not a facile one, requiring careful temperature control and proceeding only in low yield[19].

Recently, two syntheses have been published which might be applicable to the preparation of 3-tetrahydrofuranone. Oxidation of alkenes and cyclopropenes with thallium (III) nitrate affords the nitrate esters. Of particular relevance is the oxidation of 1-hydroxy-3-butene (Scheme 6).[22] Cyclic ketones can be prepared from dihaloalkanes or dihaloethers (Scheme 7).[23]

However, since it appeared that the 3-furanone was an unstable material, its successful application to elaborate the sec-butyl side chain of bicyclomycin was not envisaged and this

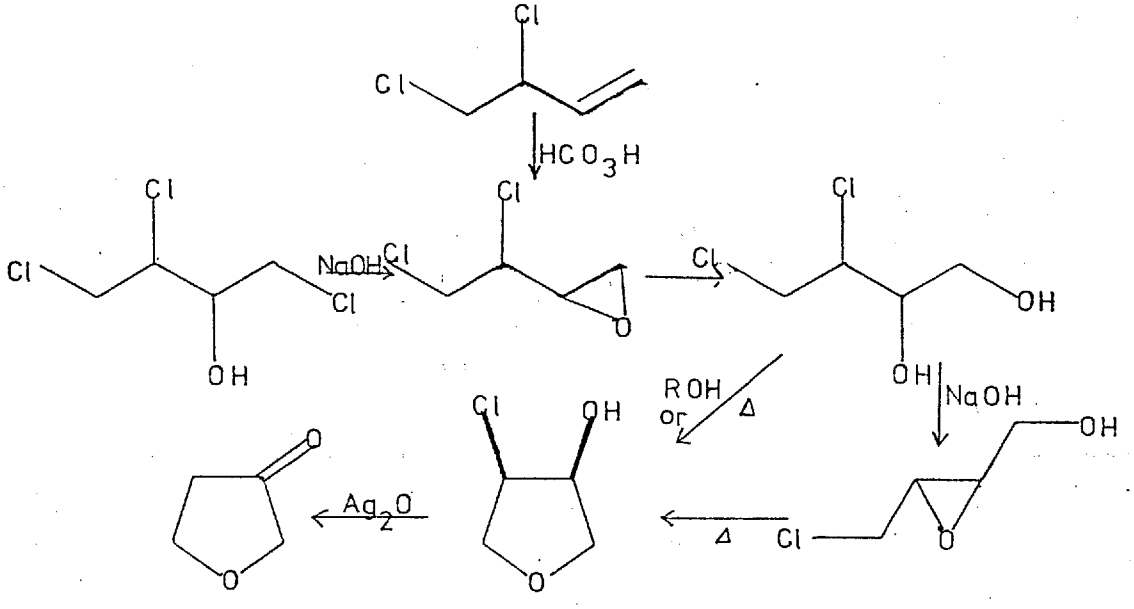


7

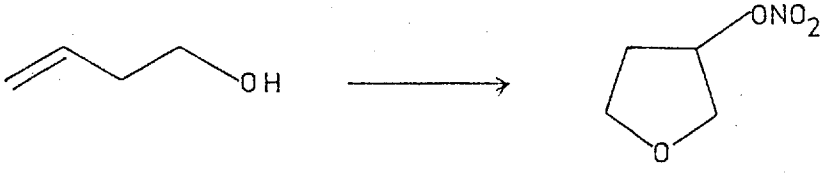


6

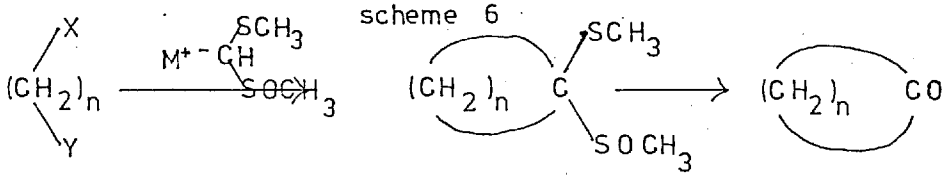
scheme 4

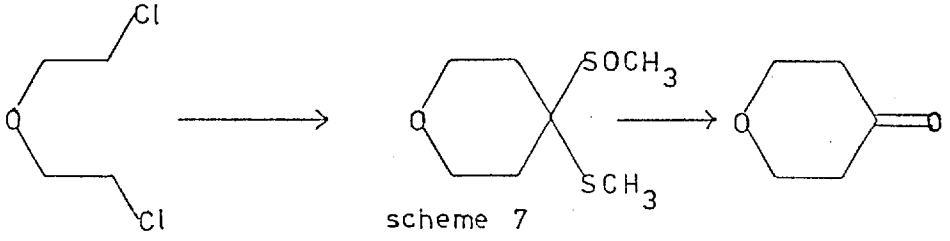


scheme 5



scheme 6



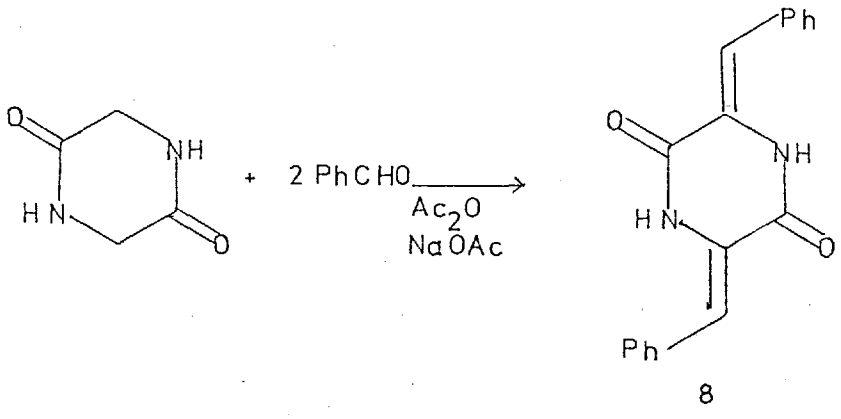


scheme 7

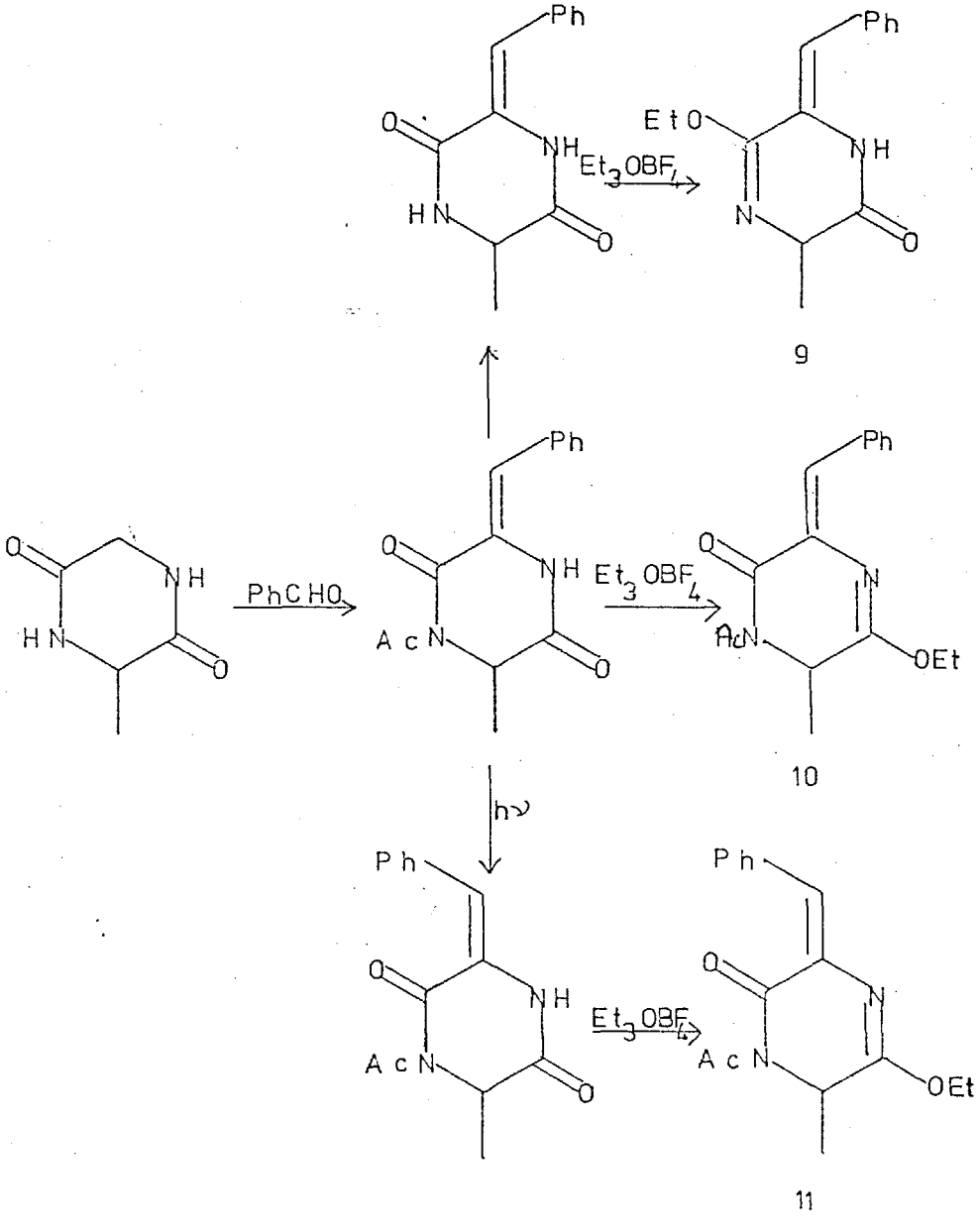
approach was abandoned. At this stage, it was evident that another pathway was necessary.

II. Elaboration of the six-membered ring : dioxopiperazines

Condensation of a carbonyl with an active methylene centre is one of the major means of creating new carbon-carbon bonds. Successful condensation of an aldehyde with 2,5-dioxopiperazine was first performed in 1921 by Sasaki.[24] By heating the dioxopiperazine in acetic anhydride with benzaldehyde and sodium acetate, he synthesised 3,6-dibenzylideny1-2,5-dioxopiperazine (8, Scheme 8). A later experiment condensing 3-methyl-2,5-dioxopiperazine with benzaldehyde (Scheme 9) allowed determination of the stereochemistry. The major project was assigned the trans-configuration shown on the basis of ^{1}H n.m.r. chemical shift data and reaction with triethyloxonium fluoroborate to form the mono-imidate (9). Irradiation of the trans isomer afforded the cis compound. The vinylic proton in the cis isomer was shielded relative to the comparable proton in the normal condensation product, as expected for a vinylic proton β to a carbonyl group. The assignment was confirmed by conversion of the deacetylated trans condensation product to the mono-imino ether with Meerwein salt. No bis-imino ether was observed. Irradiation gave the cis isomer which exhibited the expected shielding of the ethyl protons by the phenyl ring in the ^{1}H n.m.r. The trans-mono-acetylated product reacted only slowly with the same reagent to give low yields of the other mono-imidate (10) indicating the steric



scheme 8

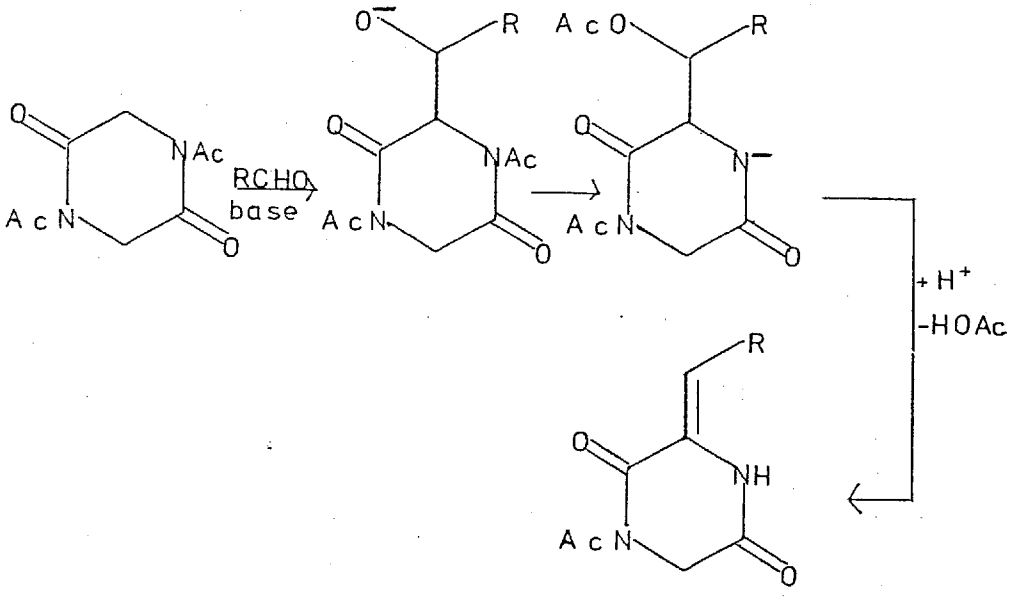


scheme 9

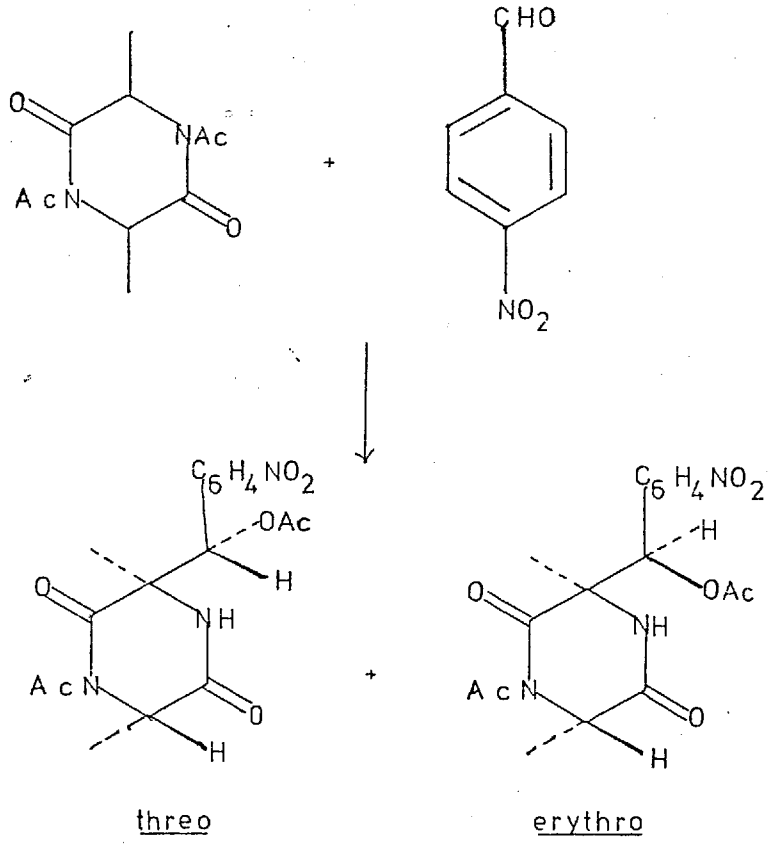
hindrance in the vicinity of the 4-amido function. By contrast, the acetylated photoisomer reacted efficiently to form the 4-imino ether (11).[25]

More recently, base catalysed condensations on 1,4-diacetyl-dioxopiperazines with a variety of aldehydes have been reported. Further condensation at position 6 is feasible with aryl aldehydes, but proceeds only in low yield, if at all, with alkyl aldehydes. Ketones are relatively inactive.[26] It has been postulated that the driving force for this reaction is transfer of the acetyl function from nitrogen to oxygen (Scheme 10). Further support for the necessity of the O-acylated intermediate was obtained by reaction of p-nitrobenzaldehyde with 1,4-diacetyl-3,6-dimethyl-2,5-dioxopiperazine. Elimination of acetic acid is not possible, and the O-acetylated intermediates were isolated. Careful spectral analysis allowed the assignment of the threo configuration to the major isomer (Scheme 11).[27] The O-acetyl intermediate is presumably involved in the sodium acetate catalysed condensation as well. The location of the acetyl protecting group in the product, deduced to be in the 1 position[28,29] was unambiguously confirmed by independent synthesis[30]. The location of the acetyl function was determined by cyclisation of sarcosyldehydrophenylalanine and (N-acetyldehydrophenylalanyl) sarcosine to the same compound, and substantiated by the chemical shift of the ring proton in the ^{1}H n.m.r., which is shifted downfield of the corresponding proton in the diacetylated compound.[25,29]

An attempt to condense benzaldehyde with 1,4-dimethyl-2,5-dioxopiperazine again afforded an isolable intermediate. In this



scheme 10



threo erythro

scheme 11

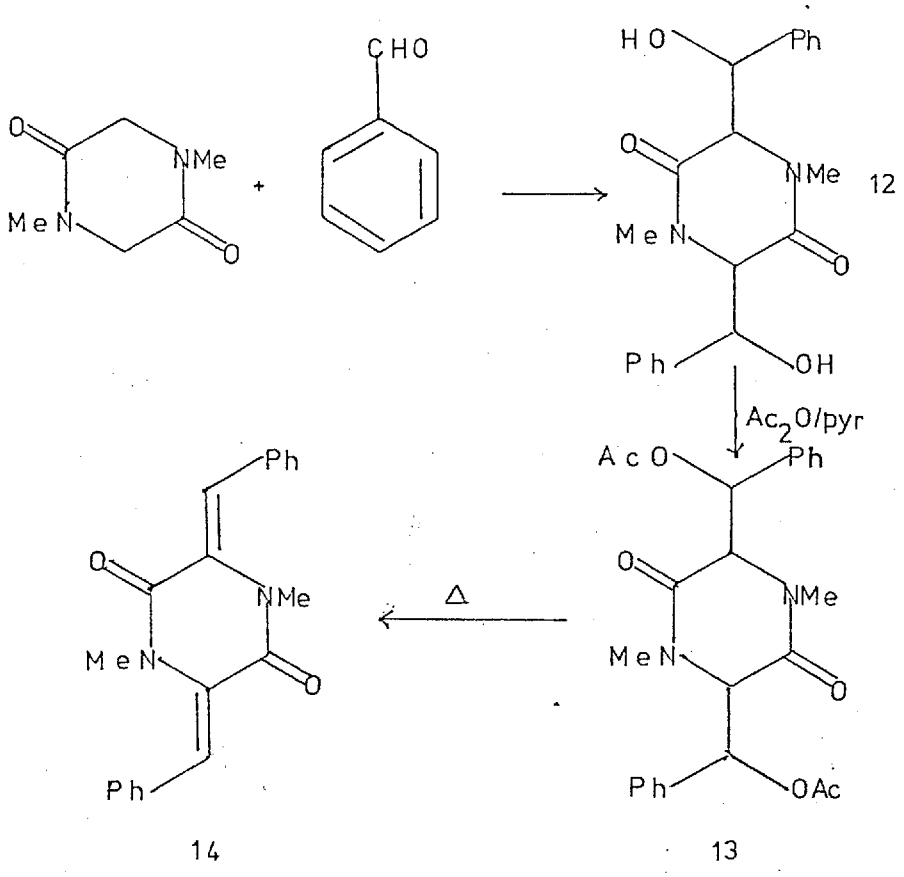
case, no transfer is possible and dehydration does not proceed. Instead, bis-condensation occurs. Dehydration was effected by acetylation in acetic anhydride and pyridine, followed by thermal elimination (Scheme 12). The structure of the product was confirmed by comparison with an authentic sample prepared by methylation of 3,6-dibenzyl-2,5-dioxopiperazine.[31]

Condensation of a dioxopiperazine with methacrolein was of interest due to the similarity of the product to a part structure of bicyclomycin. The expected product (15) was isolated in low yield (36%) and underwent decomposition on attempted purification on silica, and deacetylation to (16) on alumina (Scheme 13).

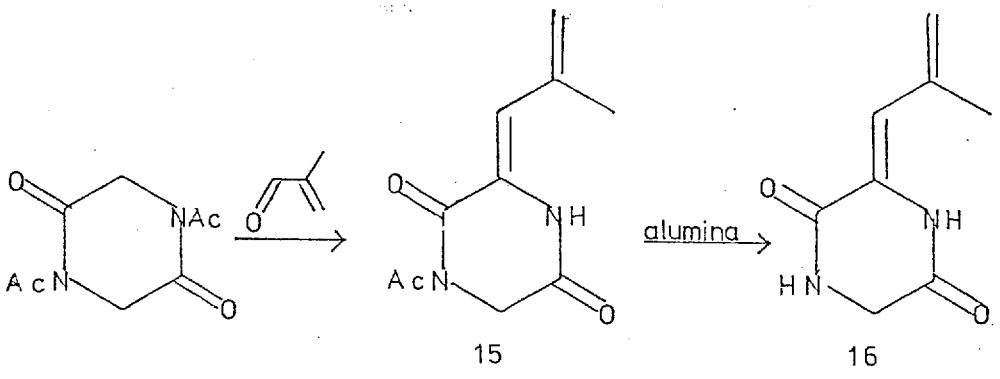
It has been reported in the literature that a steroidal vinyl ether adds formaldehyde to generate a new carbon-carbon bond.[38] Addition of formaldehyde to 1-acetyl-3-propylidenyl-2,5-piperazinedione gave the N-alkylated product rather than the enamine reaction (Scheme 14).

Reaction with a protected 3-hydroxy propionaldehyde would produce an analogue of the hydroxyalkyl side chain envisioned as a synthetic precursor to the bicyclic system of bicyclomycin. 3-Hydroxypropionaldehyde is a known compound, prepared by the aldol condensation of formaldehyde and acetaldehyde[32,33]. It is an unstable species which readily dimerises to 2 -hydroxy-6-hydroxyethyl-1,5-dioxan[34]. Attempts to trap the hydroxy aldehyde as the tetrahydropyranyl ether for further reaction were unsuccessful.

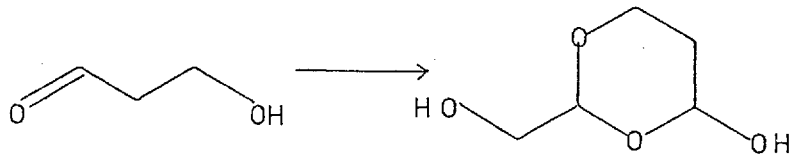
The same reagent could be generated by formation of the

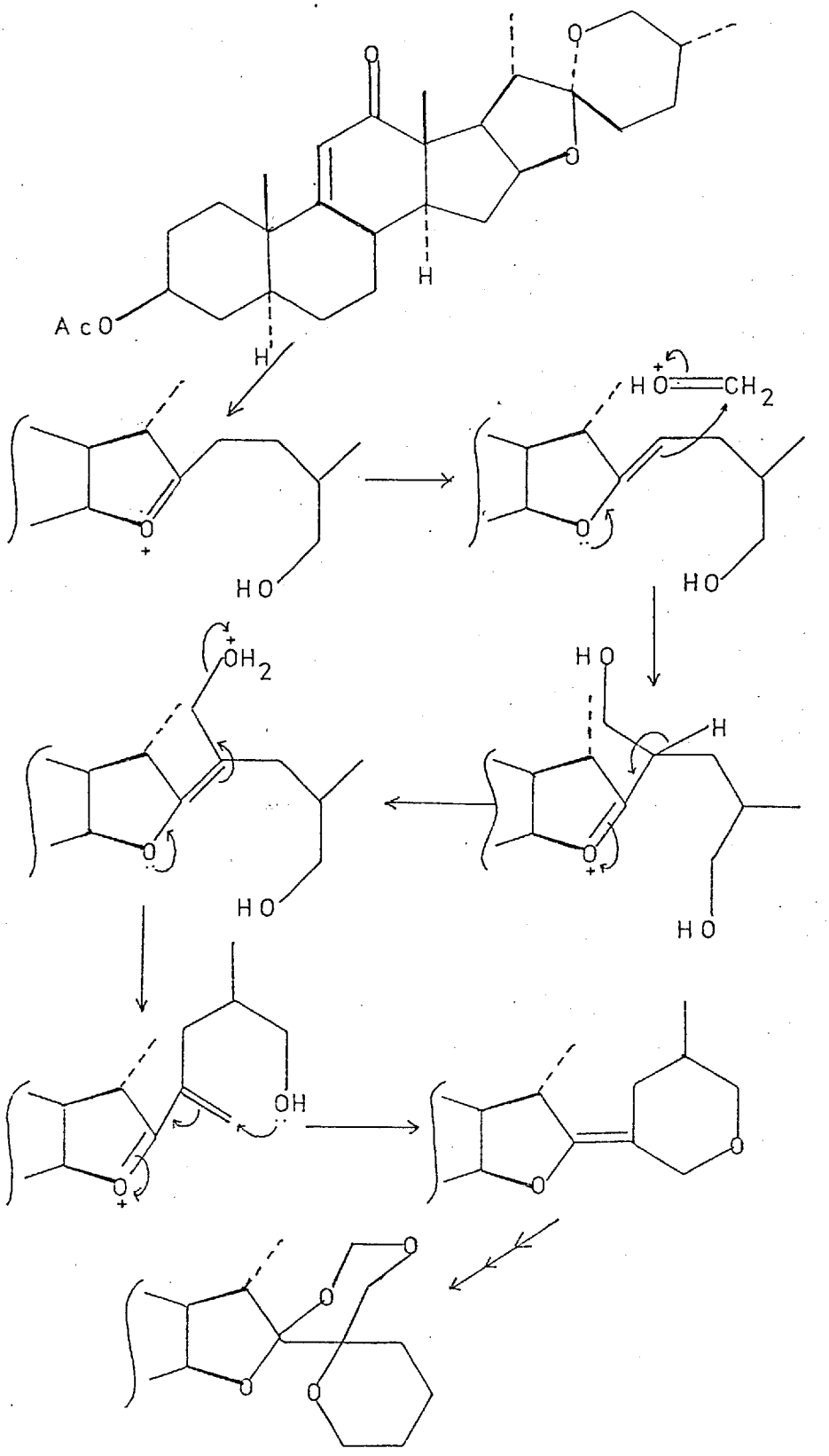


scheme 12



scheme 13





scheme 14

17

mono-tetrahydropyranyl ether of trimethylene glycol and subsequent oxidation of the remaining hydroxyl function under basic conditions. Although the chromium trioxide-pyridine complex consumed the starting material, the tetrahydropyranylaldehyde, if formed, was not isolable. Recently, 3-oxopropyl-1-tetrahydropyranyl ether has been reported as the ozonolysis product of 3-butenyl-1-tetrahydropyranyl ether, but no spectral or physical data were given[35]. Tetrahydropyranyl ethers have been the cause of serious explosions when treated with peroxidic oxidising agents,[36] so this seemed a somewhat hazardous approach.

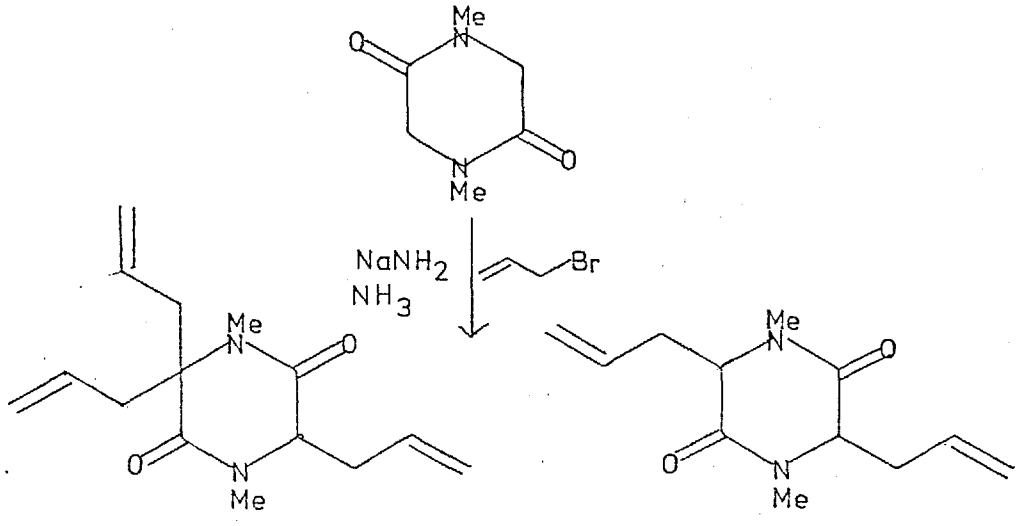
Alkylation presents an alternative to condensation in the extension of the carbon skeleton. No alkylations at carbon have been reported for 2,5-dioxopiperazines. Efforts to alkylate 1,4-diacetyldioxopiperazines with allyl bromide or 1-bromo-(3-tetrahydropyranyl)propanol under the standard condensation conditions[26] (potassium t-butoxide in dry DMF) gave inconsistent results. Traces of the alkylation products were occasionally observed spectrally but only in low yields (<< 10%). Use of another strong base, sodium hydride, resulted in decomposition of the dioxopiperazine ring. This observation was tentatively explained by the non-selectivity of the base, and other N-protecting groups were considered. Both 1,4-diformyl[37] and 1,4-di(trifluoro)-acetyl dioxopiperazines were intractable materials that were insoluble in organic solvents, unstable in protic ones, immobile by thin layer chromatography (silica, chloroform / 5-10% methanol), and, therefore, unsuitable as substrates.

In another approach, treatment of 1,6-dimethyldioxopiperazine

with the alkylating agent and two equivalents of n-butyl lithium, no reaction was observed and starting materials were recovered.

Some success was obtained using sodamide in liquid ammonia. In this fashion, 1,4-dimethyldioxopiperazine was alkylated with allyl bromide, the major products being those of polyalkylation.

Condensations are a useful method of extending the carbon skeleton of dioxopiperazines, but they are of limited application, both in the availability of particular aldehydes and in the functionalisation of both 3 and 6 positions. Alkylations, in general, were unreliable, and therefore, not a feasible synthetic procedure.



3%

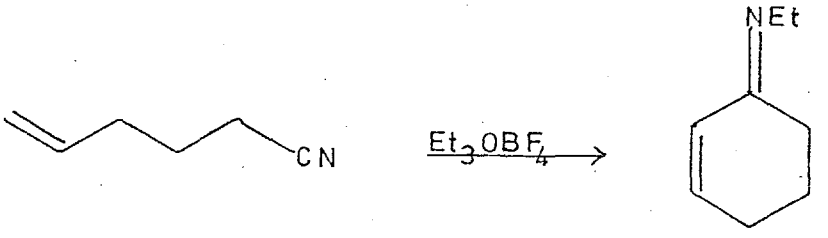
13 %

18

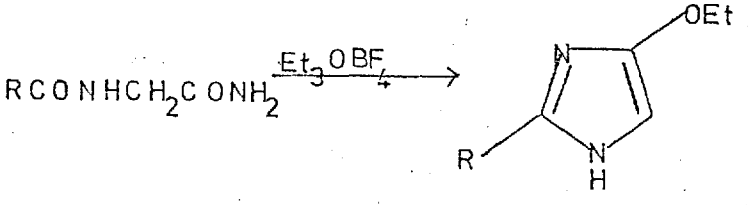
III. Elaboration of the six-membered ring:dihydropyrazines

Although trialkyloxonium tetrafluoroborates have been known for a considerable length of time, their use was restricted to polymerisation catalysts until H. Meerwein introduced the trimethyl and triethyl species into organic chemistry[39]. The tripropyl-[40] and triphenyl[41]-oxonium fluoroborates have also been prepared, but are not in common use synthetically. The straightforward synthesis of Meerwein salts from epichlorohydrin, boron trifluoride etherate, and an ether, leaves open the possibility of preparing many other species. In addition to widespread application in polymerisation catalysis, trialkyloxonium tetrafluoroborates are employed as anion exchange reagents, alkylating agents, and in the synthesis of heterocycles. The Meerwein salts are important reagents for the alkylation of nitrogen, oxygen, and sulphur, alkylation occurring preferentially at oxygen in the case of availability of both nitrogen and oxygen atoms[42]. The methyl Meerwein salt is more reactive, and also less easily hydrolysed, than the ethyl compound; however, the latter is more soluble in organic solvents[43]. The formation of heterocycles (e.g. Scheme 15)[44,45] seems to proceed first through alkylation and, therefore, activation of the heteroatom, followed by intramolecular addition.

Thus, the action of trialkyloxonium tetrafluoroborates on 2,5-dioxopiperazines produces the 3,6-dialkoxy-2,5-dihydropyrazines. Reactions of a variety of substituted dioxopiperazines with the ethyl Meerwein salt have been reported[46]. Treatment of 2,5-dioxopiperazine or 3,6-dimethyl-2,5-dioxopiperazine with the methyl



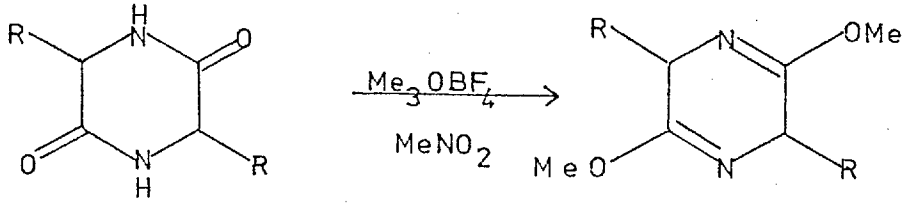
ref. 44



R= Ph, $PhCH_2$, p-MeC_6H_4, Me

ref. 45

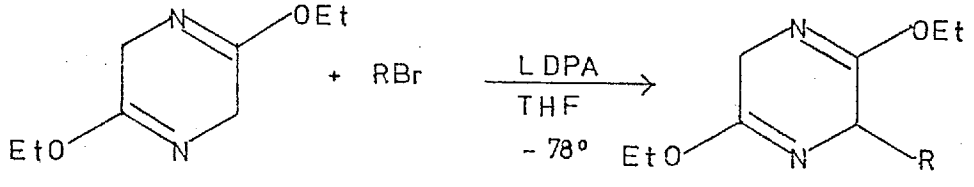
scheme 15



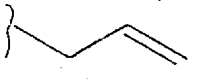
19

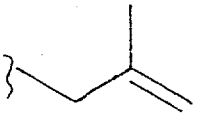
a. R = H

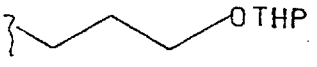
b. R = Me



20

a. R = 

b. R = 

c. R = 

derivative afforded water-soluble dihydropyrazines (19) which were difficult to separate from the quantities of inorganic residues present after workup. Dimethyl sulphate and methylfluorosulphonate were ineffective in this transformation.

An extensive study by A. I. Meyers determined that the most efficient conditions for generation of a carbanion α to an imino ether involves the use of a lithium base in tetrahydrofuran at low temperatures. No poly-alkylation products were observed[47-51]. This procedure was later adopted by B. Trost[52].

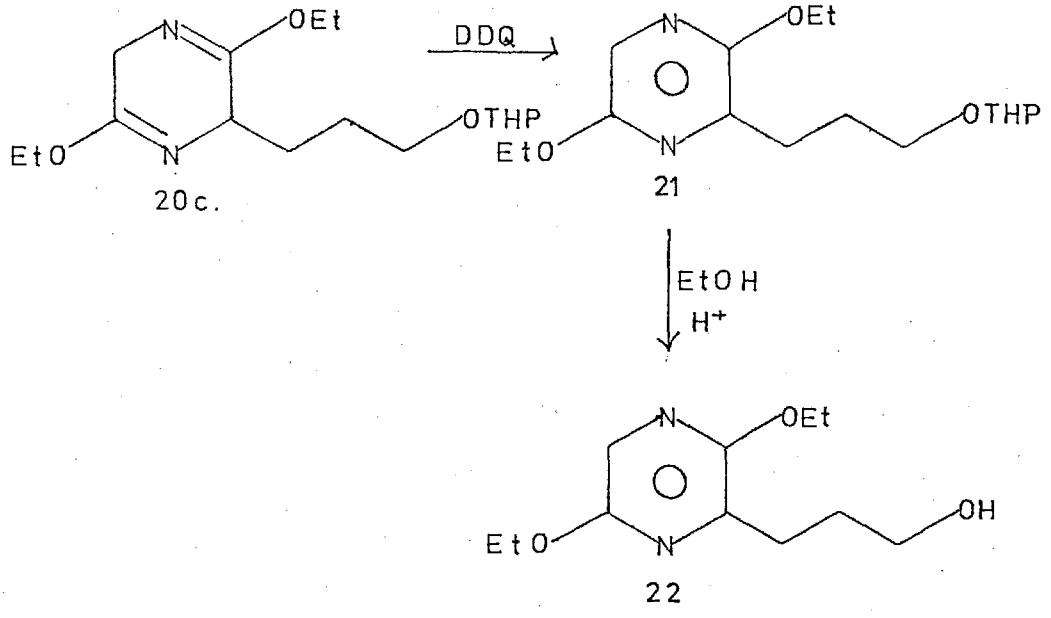
Application of these conditions to alkylate 3,6-diethoxy-2,5-dihydropyrazine with allyl bromide gave the corresponding allyl dihydropyrazine (20, R = $CH_2CH{=}CH_2$) as the major product. Mass spectral data revealed that the dihydropyrazine hydrates easily. The 2'-methylallyl side chain, a likely precursor to the isobutyl substituent of bicyclomycin possessing both the correct carbon skeleton and a useful level of oxidation, was introduced in a similar manner by reaction with methylallyl bromide (20, R = $CH_2C(CH_3){=}CH_2$).

A crucial factor in the proposed synthesis was the preparation of a dioxopiperazine or derivative with a hydroxyalkyl substituent, the hydroxyl function being essential to the formation of the cyclic ether. Alkylation of the bis-imino ether with 1-bromo-3-hydroxypropane, protected as the tetrahydropyranyl ether, afforded the tetrahydropyranyloxypropyldihydropyrazine (20, R = $CH_2CH_2CH_2OTHP$). After oxidation with dichlorodicyanoquinone (DDQ) to the aromatic system, as developed by Blake, Porter, and Sammes,[46] hydrolysis of the tetrahydropyranyl ether (THPE) was effected, albeit

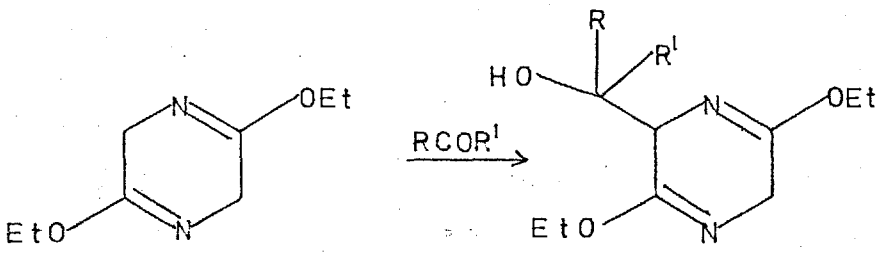
in low yield, by stirring in ethanol with acidic ion exchange resin at room temperature for several hours (Scheme 16).The acidic aqueous conditions normally used for the hydrolysis of THP ethers might also be expected to attack the imino ethers. The use of ethanol as solvent provided a safe alternative. Ethanol meets the requirement for a protic solvent, while the product of solvolysis is identical to the starting material.

These conditions for generating the imidate anion were also employed successfully in condensations of aldehydes and ketones with the dihydropyrazine. In no case was dehydration to the alkylidenyldihydropyrazine observed. Earlier work indicated that an exocyclic double bond, if formed, would rearrange readily to give the alkyl-substituted pyrazine.[53] Condensation with benzaldehyde afforded 3,6-diethoxy-2,5-dihydro-3-(1-hydroxybenzyl)pyrazine (23; R = Ph, R' = H). The major product after oxidation with DDQ was identified as the diethoxy(hydroxybenzyl)pyrazine (24). The related ketone (25) was isolated as a minor product, indicating that oxidation proceeds preferentially on the ring rather than on the exocyclic hydroxyl function (Scheme 17). A second molecule of benzaldehyde could be condensed onto the heterocyclic ring by repeating the reaction using the isolated benzaldehyde adduct and two molar equivalents of base (Scheme 17). A mixture of isomeric products (26) was obtained.

Further condensations on the dihydropyrazine included the use of methacrolein (23; R = $C(CH_3)=CH_2$, R' = H) and methyl vinyl ketone (23; R = $CH=CH_2$, R' = CH_3) as reagents and represents access to the desired isobutyl and sec-butyl substituents,



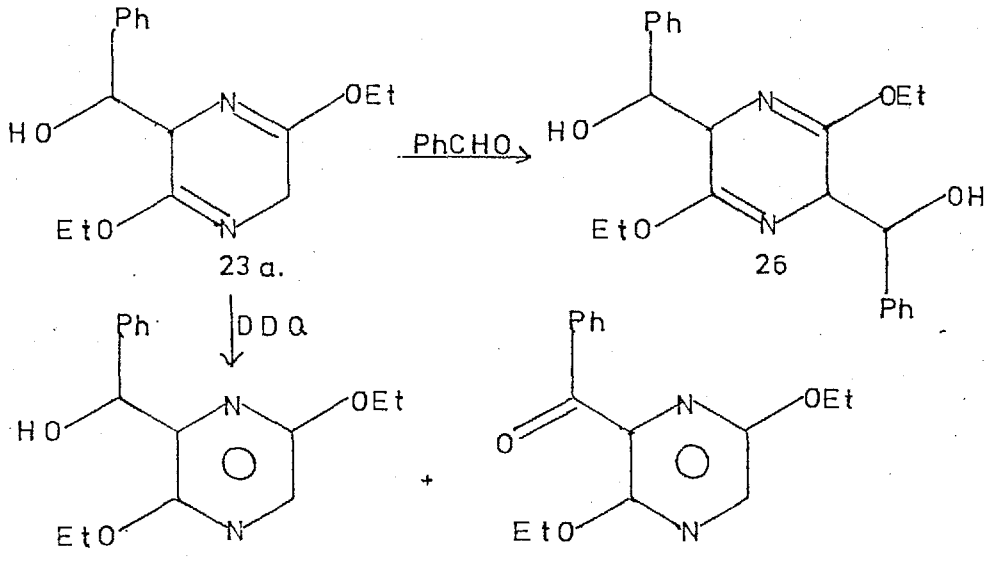
scheme 16



23 a. R=Ph, R^1=H

b. R= R^1=H

c. R= R^1=Me



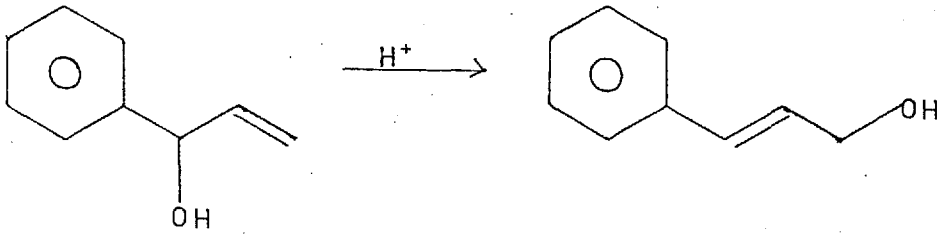
scheme 17

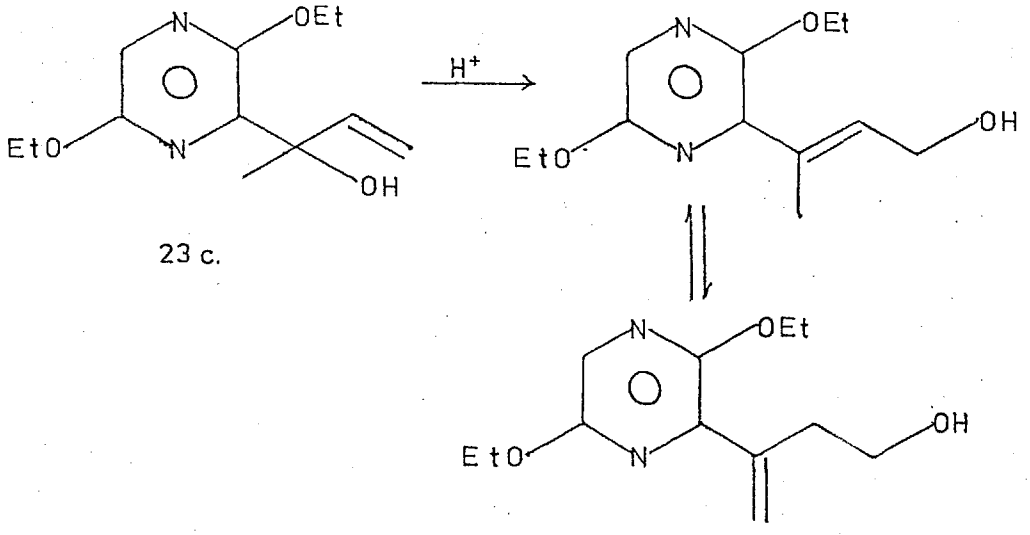
respectively. In the case of the α,β-unsaturated carbonyls, no evidence was seen for conjugate addition. The pattern characteristic of terminal methylene protons was clearly visible in the ^{1}H n.m.r. spectrum, and signals arising from alkyl protons were conspicuously absent.

The methyl vinyl ketone condensation product (23; R = CH= CH_2, R' = CH_3) may present an interesting route to the proposed hydroxy-alkenyl side chain precursor to the cyclic ether linkage in bicyclomycin. It is known that hydroxyallyl substituents on aromatic hydrocarbons undergo acid catalysed rearrangement resulting in conjugation of the exocyclic double bond with the aromatic ring and migration of the hydroxyl function (Scheme 18)[54]. A similar rearrangement could conceivably operate in the case of pyrazines. Compound (23c) would then isomerise to the (3-hydroxy-1-methyl-1-propenyl)pyrazine, possibly in equilibrium with the (2-hydroxyethylethylenyl)pyrazine (Scheme 18).

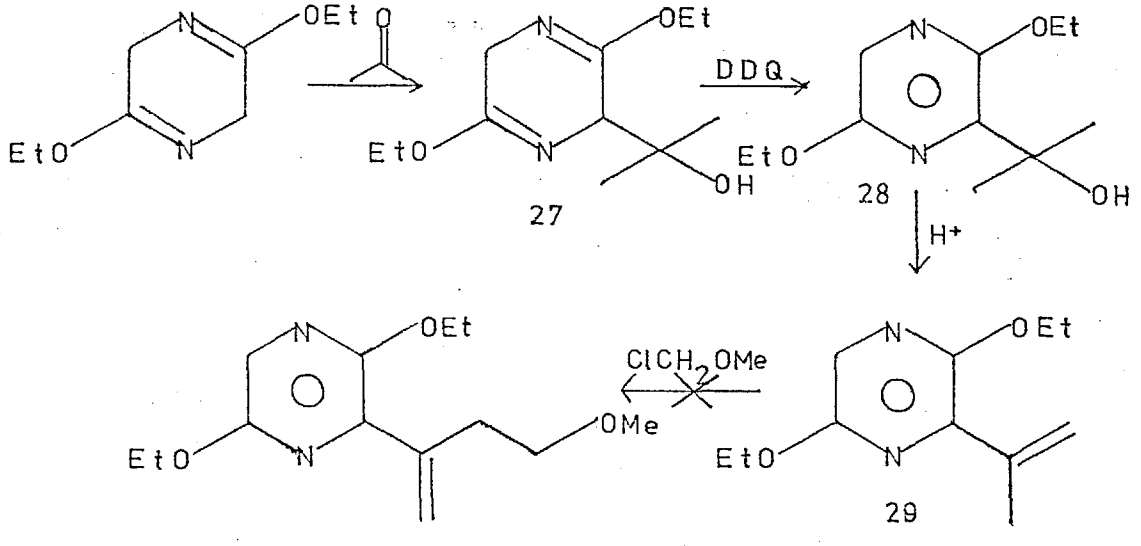
Another route to this difficult substituent was seen in the alkylation of an isopropenyl pyrazine with formaldehyde or chloro-methyl ether. Condensation of the diethoxydihydropyrazine with acetone afforded the expected pseudo-aldol intermediate. Oxidation with DDQ led to the pyrazine, and dehydration of the alcohol proceeded smoothly to give the desired isopropenyl pyrazine. Attempted alkylation with freshly distilled chloromethyl methyl ether failed repeatedly, and starting material was recovered (Scheme 19).

It has been reported in the literature that formaldehyde undergoes tin chloride catalysed addition to isoprene, generating 3-hydroxyethyl butadiene (Scheme 20)[55]. A similar addition to the

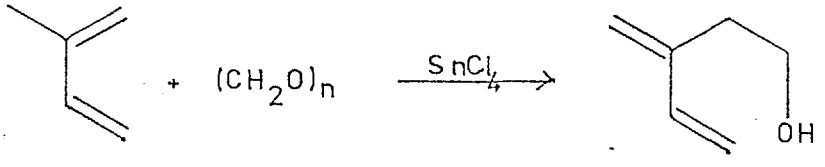


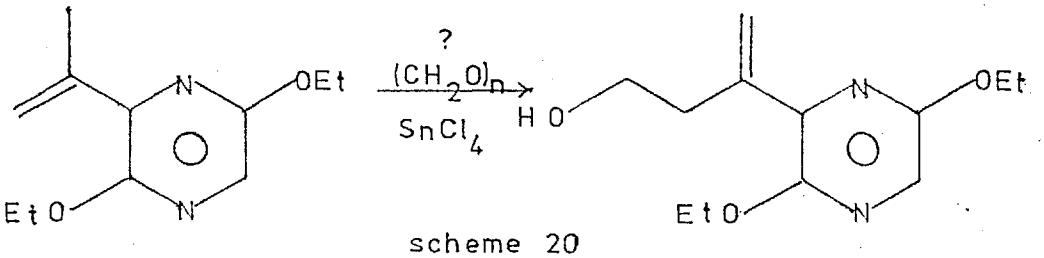


scheme 18



scheme 19



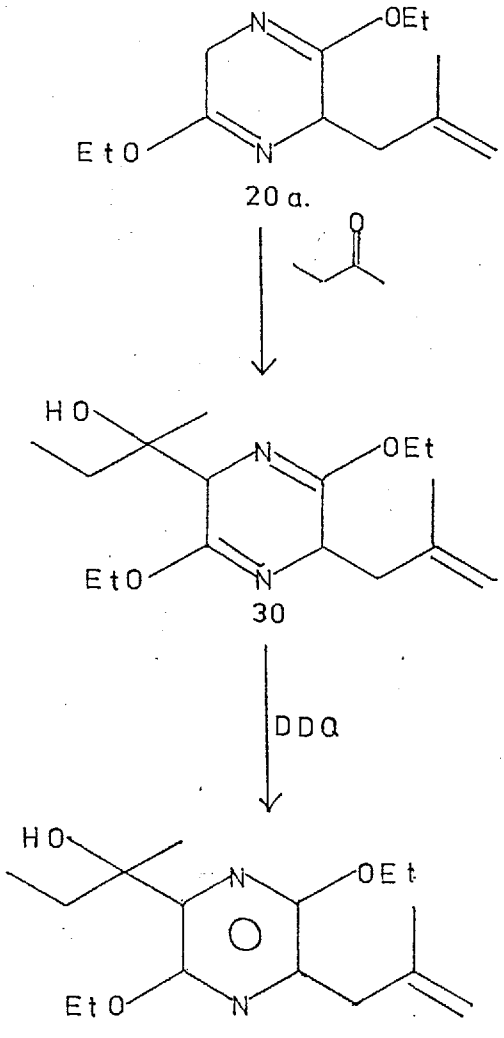


scheme 20

isopropenyl pyrazine would again, in principle, lead to the desired substitution pattern.

From Meyers' observation that no polyalkylation occurred on imino ethers under his conditions[51], the imino ether might be deactivated to alkylation after reaction with the first molecule of alkylating agent. However, it was found that a mono-substituted dihydropyrazine could be unsymmetrically p-disubstituted using this technique. Thus, 3,6-diethoxy-2,5-dihydro-2-methylallyl-pyrazine was condensed with ethyl methyl ketone to give 3,6-diethoxy-2,5-dihydro-2-(1-hydroxy-1-methylethyl)-5-(2-methylallyl)pyrazine (30). Again, oxidation with DDQ resulted in formation of the aromatic compound (31). With the preparation of these compounds, the synthesis of the carbon skeleton of bicyclomycin at a workable oxidation level was achieved.

The imino ethers had been selected as suitable protecting groups for the amide functionality of 2,5-dioxopiperazines due to their presumed facile hydrolysis. The conversion of the dioxopiperazines to dihydropyrazines improves the solubility of the compounds in organic solvents and provides access to a greater variety of structures than is available through dioxopiperazines. However, 3,6-diethoxy-2,5-dihydropyrazine proved to be resistant to hydrolysis over a range of conditions. Stirring the bis-imino ether in wet chloroform with silica, in aqueous tetrahydrofuran (THF) with copper metal, or in aqueous THF with 2-hydroxypyridine was ineffective, and starting materials were recovered. Decomposition resulted when the dihydropyrazine was refluxed in aqueous THF with copper metal, stirred at room temperature with ion exchange resin (H^+), treated

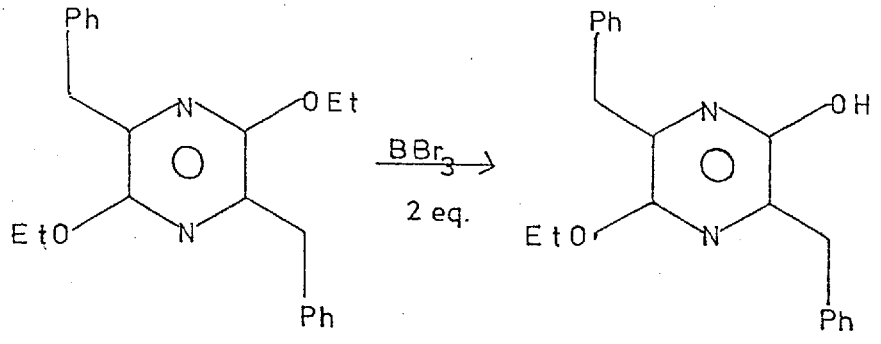


31

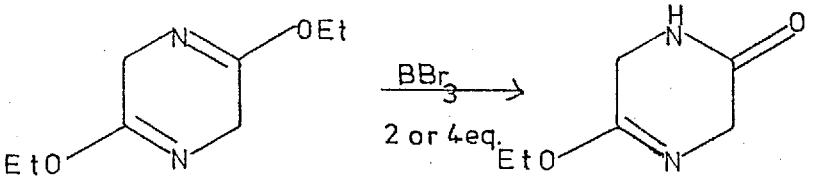
with a range of buffered solutions (pH 3,4,5,9), or stirred in aqueous acetone with potassium iodide. Eventually, it appeared that heating the dihydropyrazine in aqueous THF regenerated the dioxopiperazine (i.r. evidence). The difficulty of hydrolysing the bis-imino ether was perhaps indicated by the seeming stability of the dimethyldihydropyrazines (19) in aqueous fluoroboric acid solutions. On the other hand, methoxy imidates in the cephalosporin series are reputedly cleaved to amides by the action of trimethylsilyl chloride[56]. However, the dimethoxydihydropyrazines were found to be unsuitable for reactions in organic solvents, as previously discussed. The hydrolysis is further complicated by the fact that the resultant dioxopiperazine is readily hydrolysed to glycylglycine in aqueous acid or base.

Similarly, no evidence for exchange of the ethoxy groups with alcohols could be obtained. In particular, it was attempted to exchange the ethoxy groups with trichloroethanol which could then be reductively cleaved[57]. With ion exchange resin (H^+), trifluoroacetic acid, or citric acid catalysis, the imino ether was consumed but no exchange products were observed. Refluxing in trichloroethanol also caused decomposition of the dihydropyrazine, although O-methylhexanolactim has been shown to exchange thermally with allyl alcohol[58].

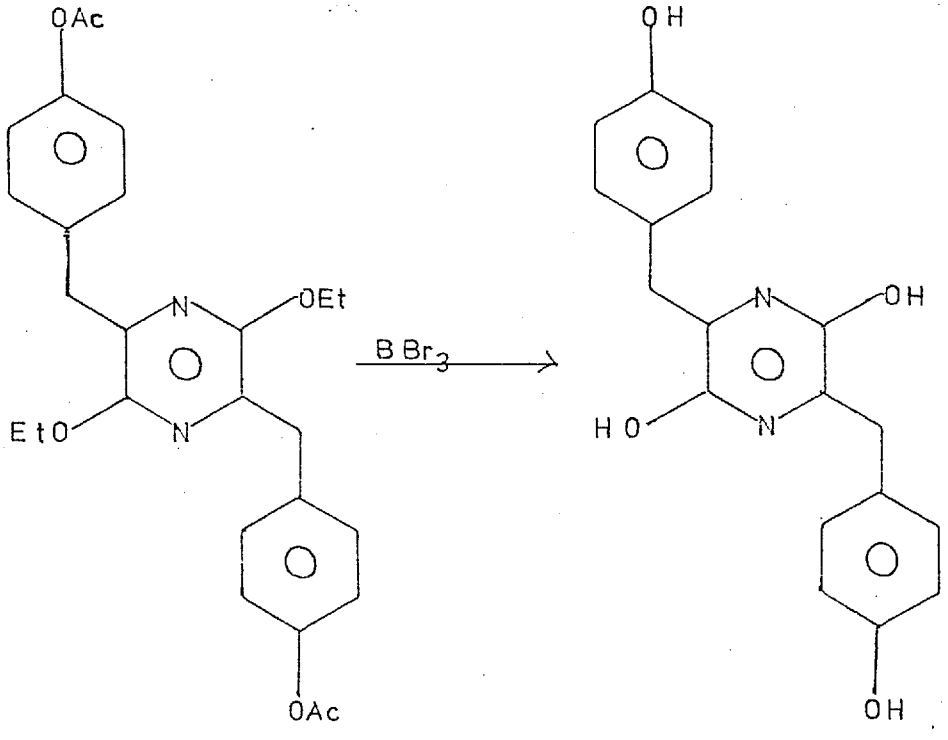
An alternative to hydrolysis in the regeneration of the dioxopiperazine exists in the cleavage of the ether. Reaction of 2,5-diethoxy-3,6-dibenzylpyrazine with two equivalents of boron tribromide produced the mono-hydroxy pyrazine (32) in low yield. Similar treatment of 3,6-diethoxy-2,5-dihydropyrazine also afforded


Ph
OEt
N
EtO
N
Ph
BBr3
2 eq.
Ph
OH
N
EtO
N
Ph

32


OEt
N
EtO
N
BBr3
2 or 4eq.
H
N
O
EtO
N

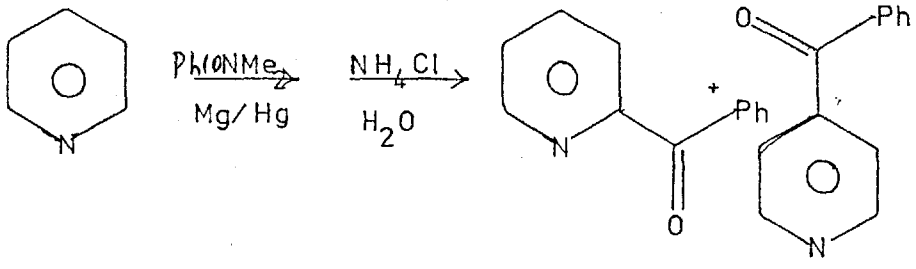
33


OAc
OEt
N
EtO
N
OAc
BBr3
OH
OH
N
HO
N
OH

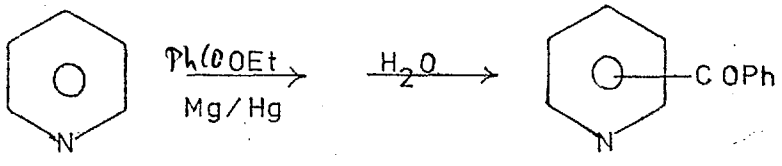
34

a mono-dealkylated product (33). Presumably, the low yields of mono-dealkylated compounds are due to complexation of the ring nitrogen atoms with the reagent, and use of four equivalents of boron tribromide should lead to the dihydroxypyrazine or dioxopiperazine, respectively. Repetition of the experiment involving the dihydropyrazine with double the quantity of boron tribromide again yielded the mono-imidate (33), possibly due to the insolubility of the borate ester produced in the reaction. Treatment of 3,6-diethoxy-2-methylallyl pyrazine, obtained from DDQ oxidation of the corresponding dihydropyrazine (20a) gave a complex mixture of products. In none of these reactions was any starting material recovered. Other workers have successfully applied this reaction to a related system, 3,6-di(p-acetoxybenzyl)-2,5-diethoxypyrazine (34)[59].

Acylation of the pyrazine ring appeared to be a tempting alternative to the alkylation, condensation, and oxidation of dihydropyrazines, particularly as the side chain introduced would be at a higher level of oxidation. However, little encouragement could be gleaned from the literature (Scheme 21). Pyridine is deactivated relative to aromatic carbocycles to Friedel-Crafts acylation, and pyrazines, with two nitrogen atoms in the ring, are even less reactive. Pyridines have been acylated by amides and esters in a magnesium/mercury amalgam[60,61]. Acylated pyrazines have been obtained by reaction of a Grignard reagent with cyanopyrazine[62]. Acylations proceed on exocyclic positions of pyrazines through nucleophilic substitutions on esters[63,64]. The only direct acylation reported for pyrazines involves a free radical process, and can be successfully applied to pyridine,

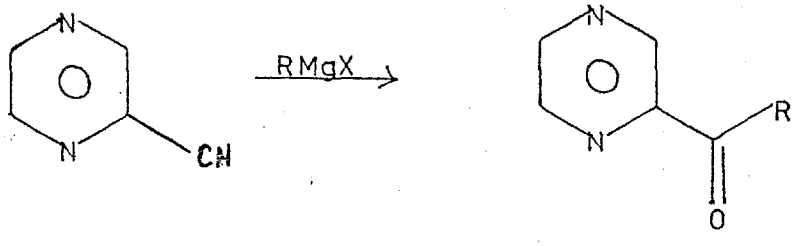


ref. 60

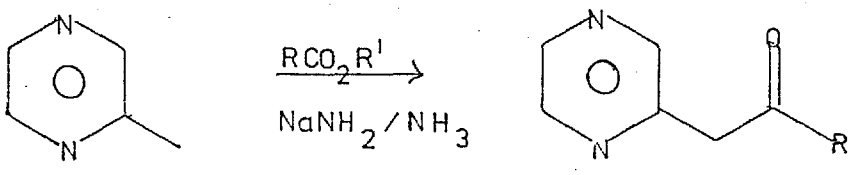


ref. 61

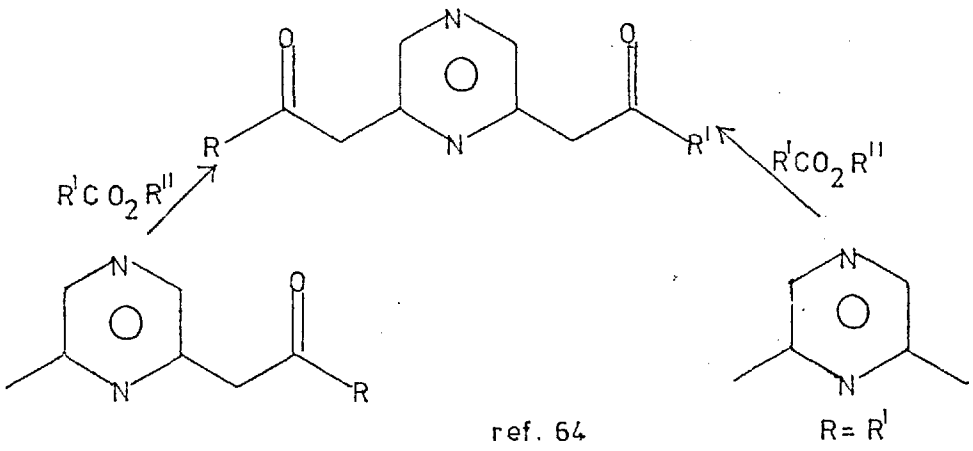
2 &4 isomers



ref. 62



ref. 63



ref. 64

$$RCHO + FeSO_4 + t\text{-}BuOOH \text{ or } H_2O_2 \xrightarrow[\text{aromatic N-heterocycle}]{H_3O^+} 2\&4 \text{ isomers}$$

ref. 65

scheme 21

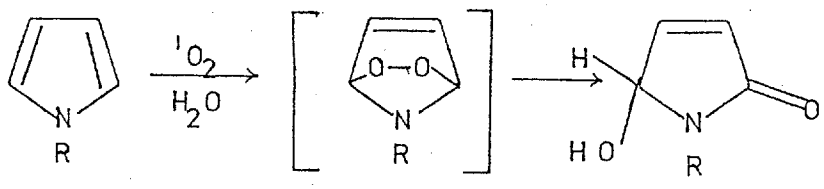
quinoline, isoquinoline, acridine, pyrimidine, quinoxoline sulphates, and benzene, in addition to pyrazine.[65]

The use of dihydropyrazines as surrogate dioxopiperazines is thus demonstrated to offer a convenient and general method for the construction of substituted heterocycles, although an efficient means of regenerating the dioxopiperazine has yet to be developed.

IV. Oxygenation

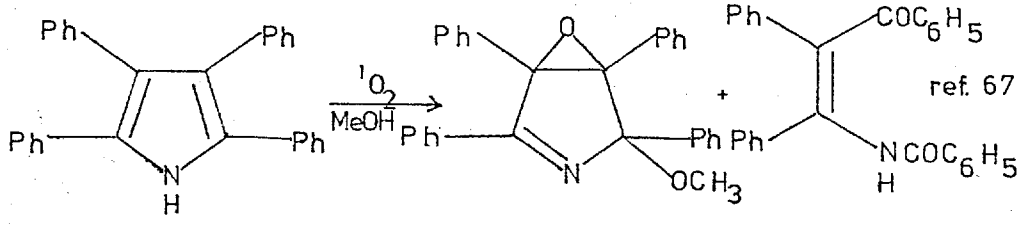
A second major problem in any proposed synthesis of bicyclomycin is the introduction of oxygen at the 3,6-positions of the dioxopiperazine ring. Attention was turned to singlet oxygen as a mild and selective agent for effecting this oxidation.

Singlet oxygen is known to react with heterocycles, most studies having been conducted on five-membered aromatic systems. Pyrrole and N-methylpyrrole, when irradiated in an aqueous solution, proceed to the corresponding 5-hydroxy-2-oxopyrroles[66]. Tetraphenylpyrrole, on photosensitised oxygenation in methanol, produces the epoxide (34) and the stilbene (35), the former being the major component[67]. In analogous fashion, tetraphenylfuran[67] and tetraphenylthiophen [68] are oxidised. Much of the earlier work on furans was published by Schenck[69]. 1,1-Dimethyltetraphenylsilole also reacts in the manner exhibited by pyrroles, furans, and thiophenes. In addition, a product is isolated from oxygenation of the silole in which the stilbene double bond has been reduced (36), presumably *via* hydrogen abstraction from the silylmethyl group[70]. 2,5-Dimethylthiophen affords a sulphine (37), which



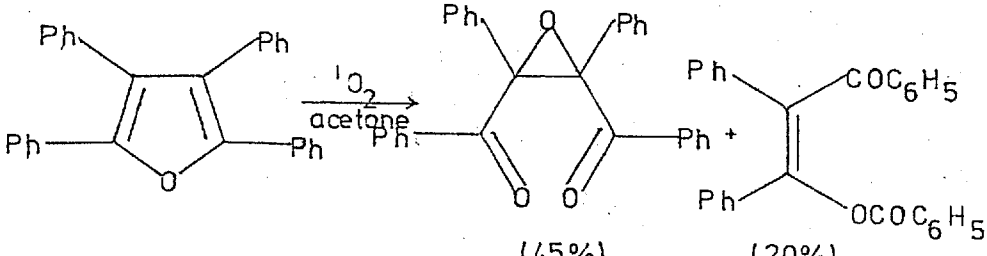
ref. 66

R = H, Me

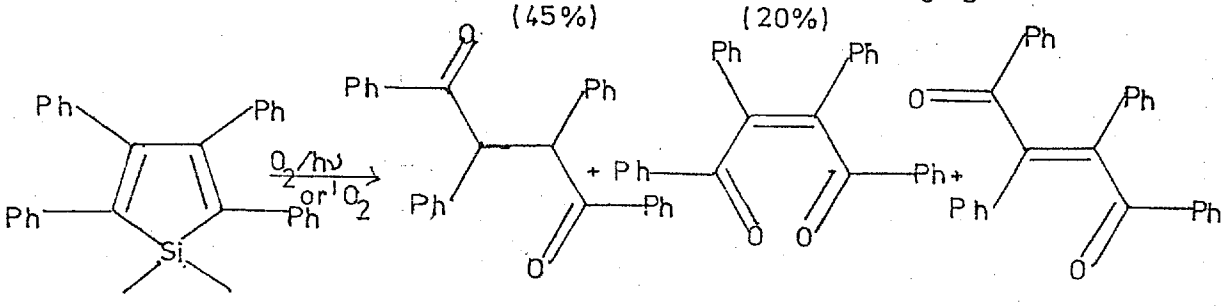


34 (55%) 35 (30%)

ref. 67

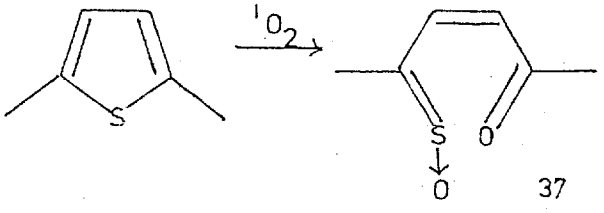


(45%) (20%)



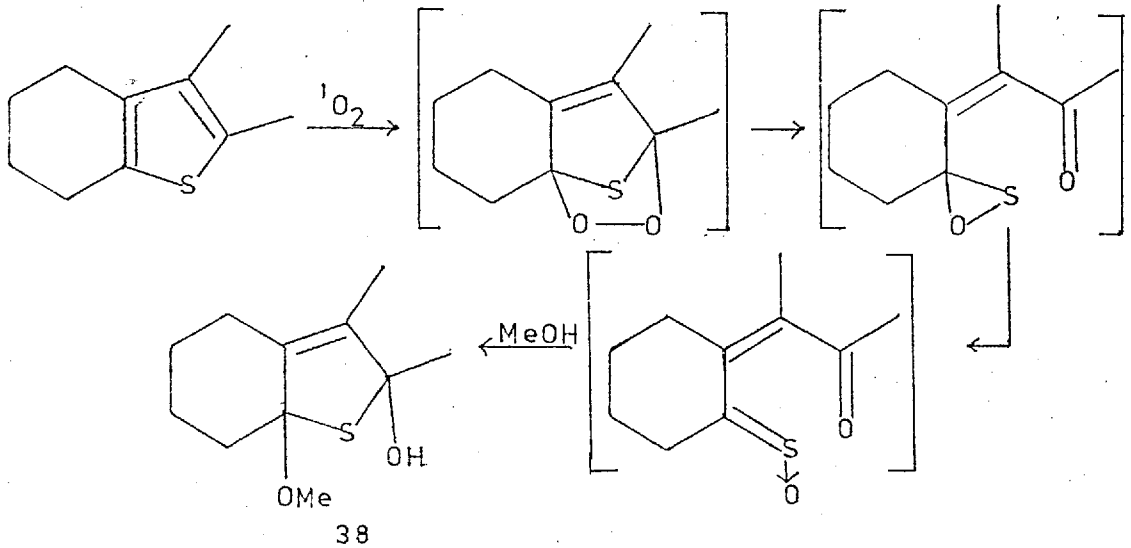
36 (31%) (6%) (2%)

ref. 70



37

ref. 71



38

reaction led to the postulation of a sulphine as an intermediate in the oxidative conversion of 2,3-dimethyl-4,5-tetramethylene-thiophen to the related 2-hydroxy-5-methoxy sulphoxide (38)[71].

The reactions of imidazoles with singlet oxygen have been studied in some detail. The parent compound is transformed into 4,5-diethoxy-2-oxo-imidazole (39)[72]. In other cases, the reaction pathway is dependent on the substitution pattern of the ring (Scheme 22). β-Elimination of a proton to form an α-alkoxy or α-hydroxy ketone is frequently the preferred mechanism[73].

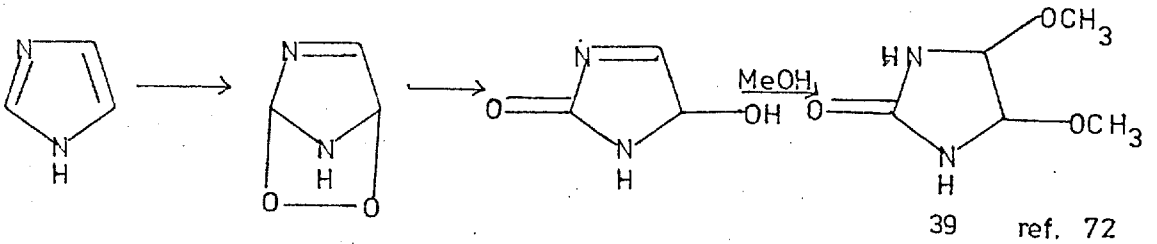
Isotopic studies on oxazoles with labelled molecular oxygen provided evidence for the intermediacy of an endoperoxide rather than for a dioxetane (Scheme 23)[76]. Photooxygenation of oxazoles has also supplied a new route to the synthesis of nitriles. An oxazole fused to a saturated macrocycle is cleaved to an alkyl chain carboxylic acid with a cyano function at the opposite terminus (40)[77].

Irradiation of triphenylisoxazole results in its conversion to the oxazole which subsequently reacts with singlet oxygen to form benzonitrile, benzoic anhydride, and triphenacyl amine (Scheme 24)[78].

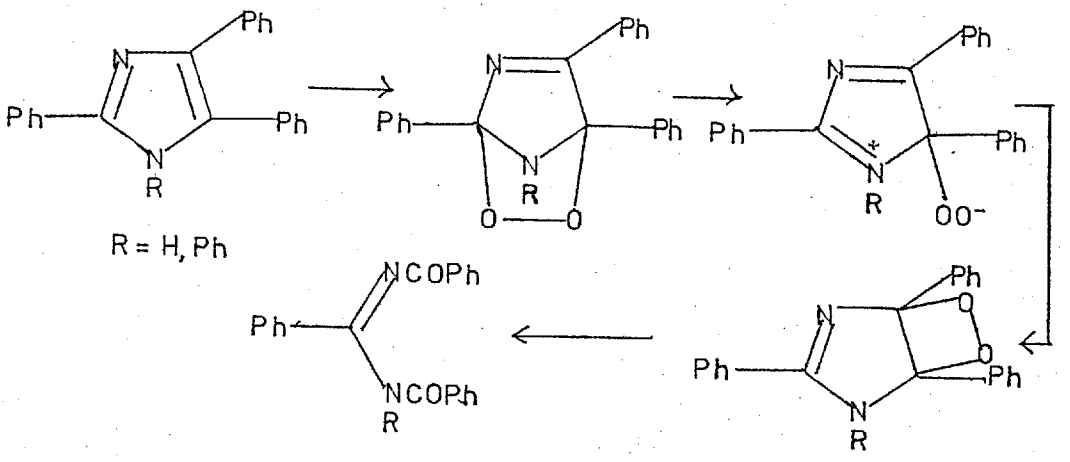
Thiazoles are also oxidatively cleaved by singlet oxygen, the pathway depending on the solvent employed. In methanol, 1,2-dioxo-1,2-diphenylethane and benzamide are formed, whereas reaction in chloroform gives N,N-diphenacylthiobenzamide[79].

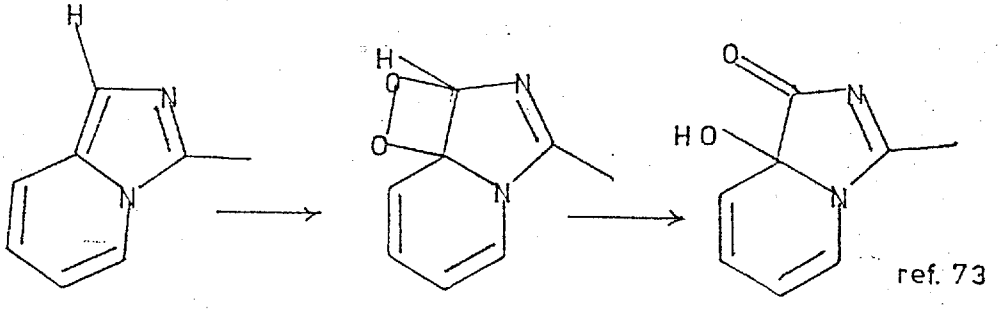
Oxygen heterocycles (e.g. 41 and 42) undergo conversion to the corresponding aldehydes (43 and 44)[80].

Reactions of several five membered aromatic heterocycles

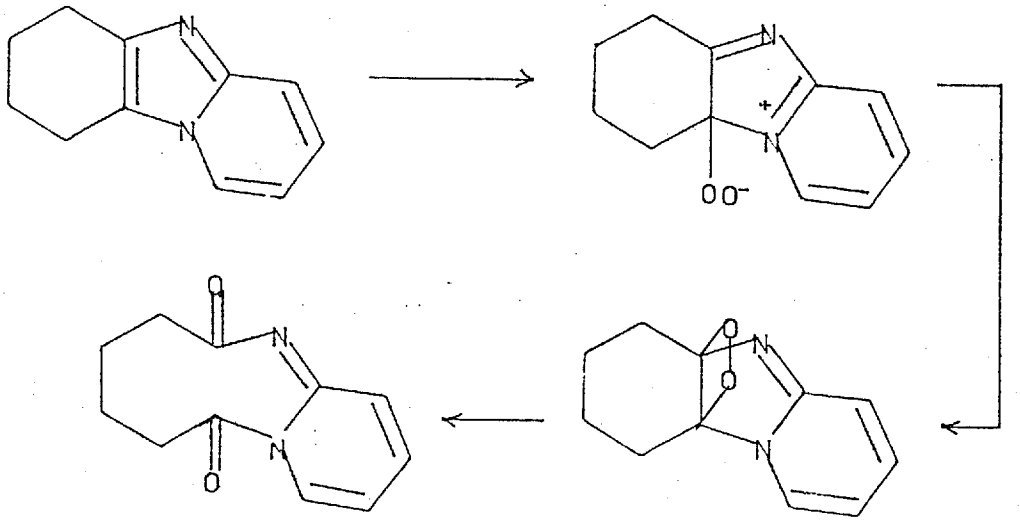


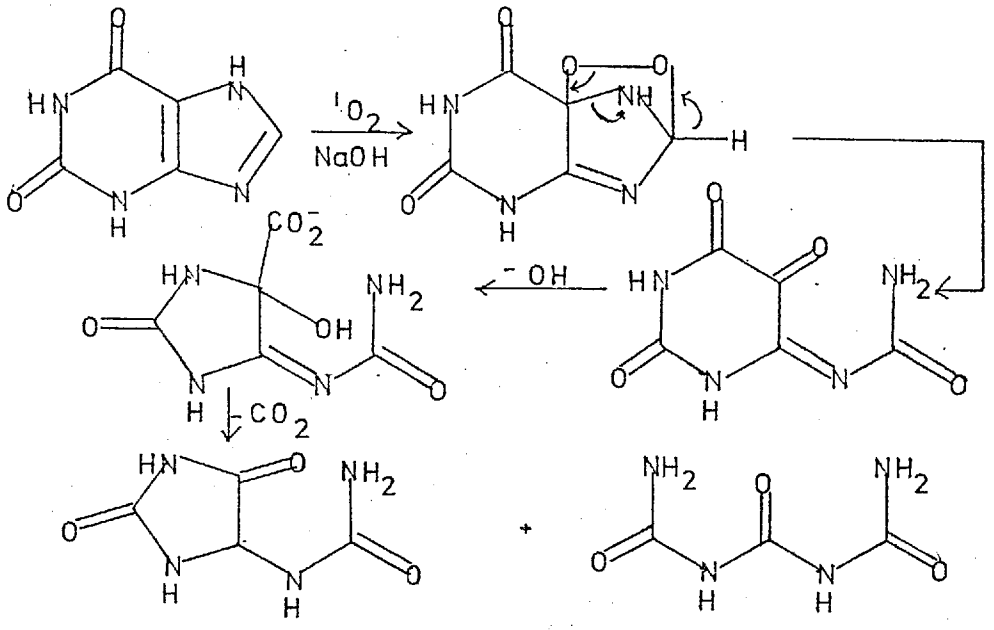
39 ref. 72



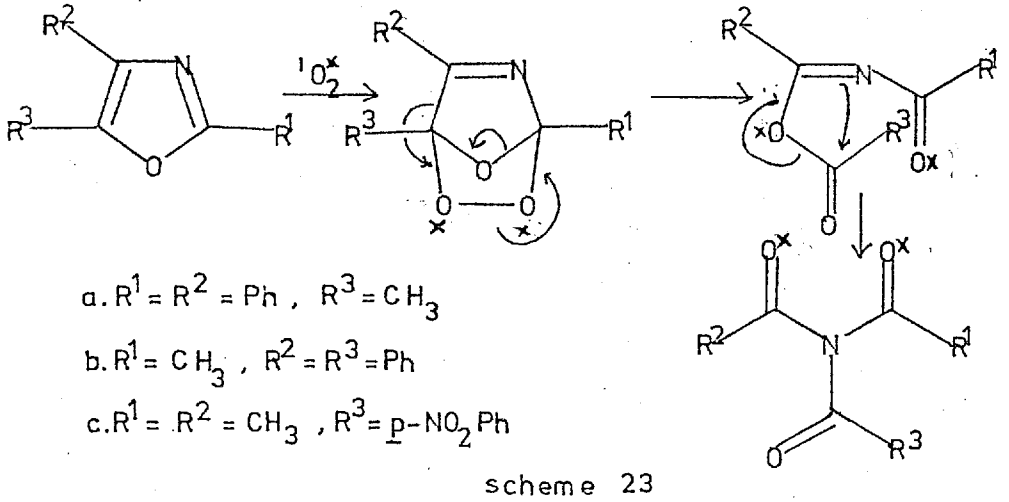


ref. 73





scheme 22 ref. 74,75

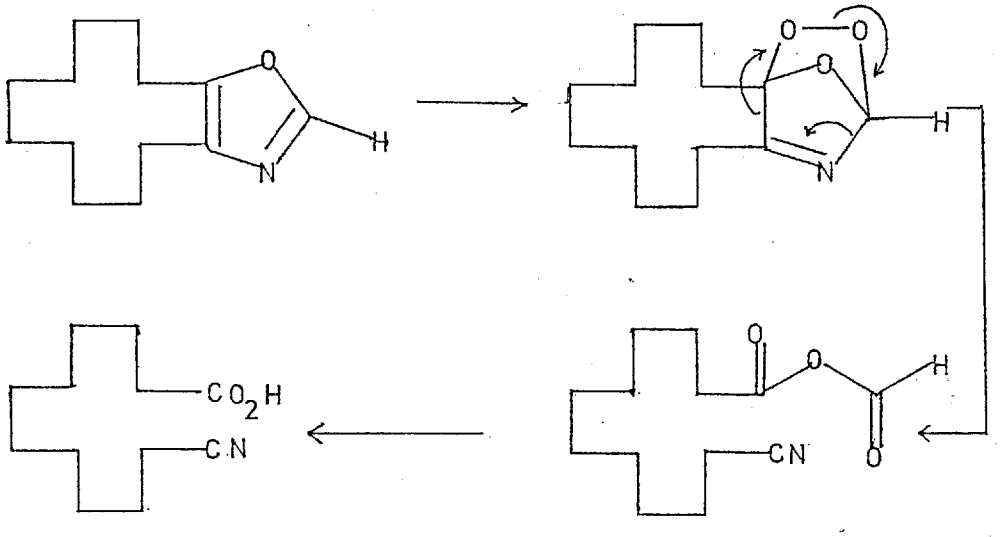


a. $R^1 = R^2 = Ph$, $R^3 = CH_3$

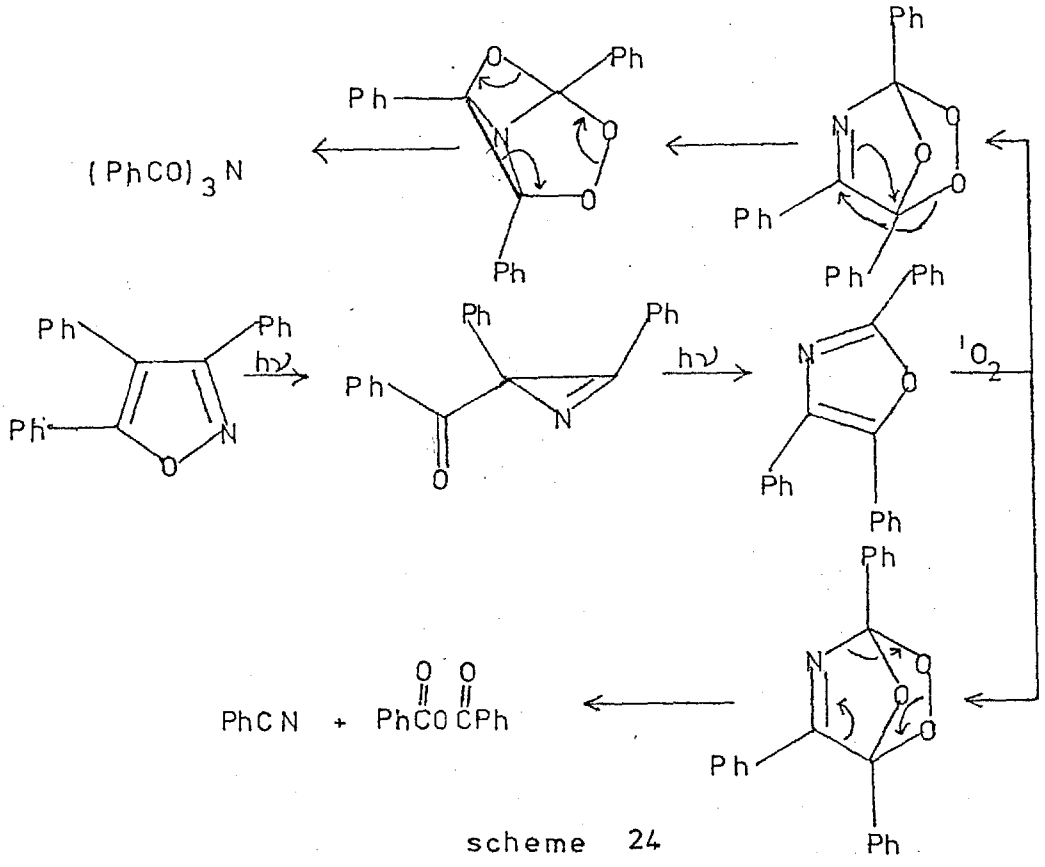
b. $R^1 = CH_3$, $R^2 = R^3 = Ph$

c. $R^1 = R^2 = CH_3$, $R^3 = \underline{p}\text{-}NO_2Ph$

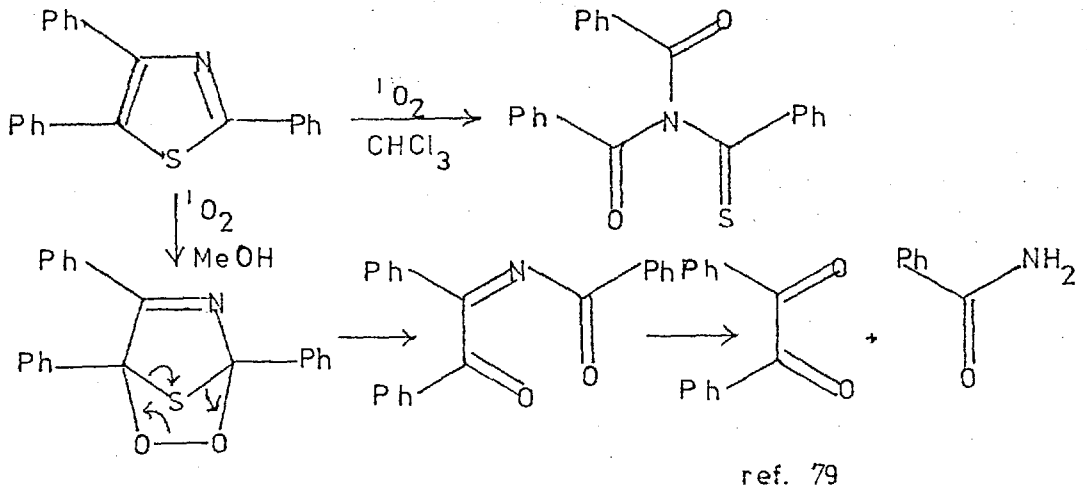
scheme 23



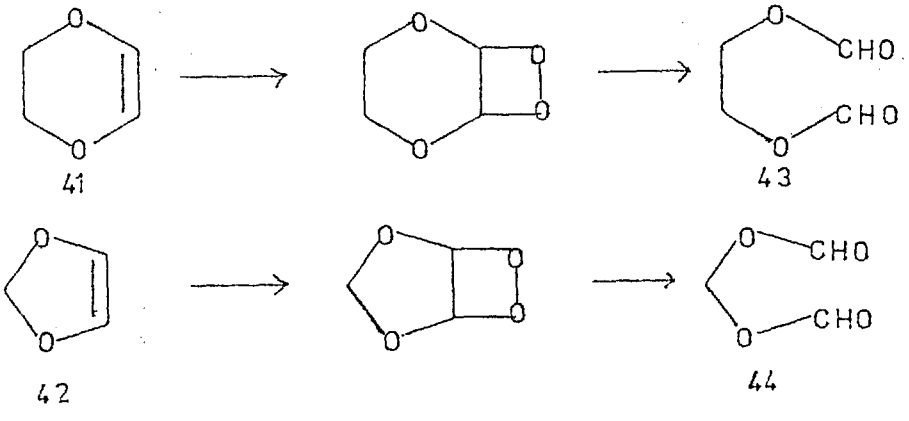
40



scheme 24



ref. 79



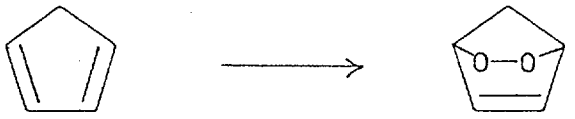
41 43

42 44

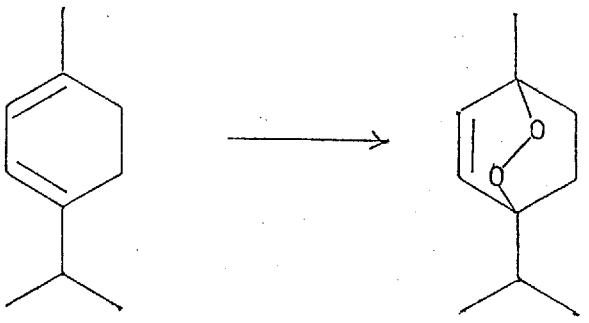
with singlet oxygen have been summarised[73].

Although endoperoxides have frequently been postulated as intermediates in the reaction of heterocycles with singlet oxygen, they have not been isolated. Several unsaturated hydrocarbons, however, yield the endoperoxide as the major product. For example, cyclopentadiene, 1-isopropyl-4-methyl-1,3-cyclohexadiene, and napthalene are oxygenated to the derived peroxides (45, 46, 47)[81]. It has been found recently that some steroidal dienes of the type (48) give the 'ene' reaction with singlet oxygen instead of the cycloaddition reaction[82].

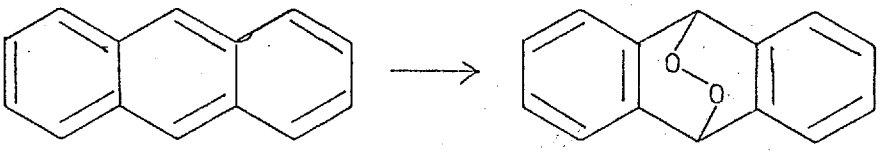
Reports of additions of singlet oxygen to six membered heterocycles are rare. Working on the hypothesis that singlet oxygen reacts with heterocycles to form endoperoxides, as demonstrated for carbocycles, it was thought that addition of singlet oxygen to a pyrazine and reductive workup would result in isolation of the 2,5-dihydroxydihydropyrazine. As mentioned in the preceding chapter, 2,5-dioxopiperazines are readily converted to their aromatic counterparts, the dialkoxy pyrazines, by O-alkylation and subsequent DDQ oxidation. The first pyrazine tried as a substrate, 3,6-dibenzyl-2,5-diethoxypyrazine, was quantitatively transformed into the endoperoxide (49) on treatment with singlet oxygen. The endoperoxide was isolated in spite of standard reductive procedures during workup. The peroxide was identified largely on the basis of the ^{1}H n.m.r. spectrum, whose simplicity indicated the presence of a symmetrical moiety. The most informative signal in the spectrum was that due to the methylene protons which had suffered an upfield shift of 1 ppm. No hydroxyl absorption



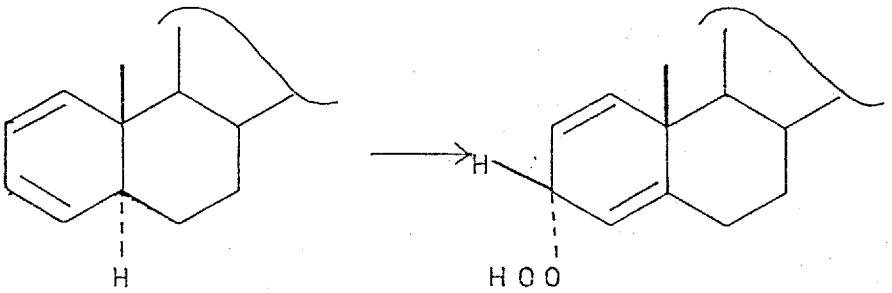
45



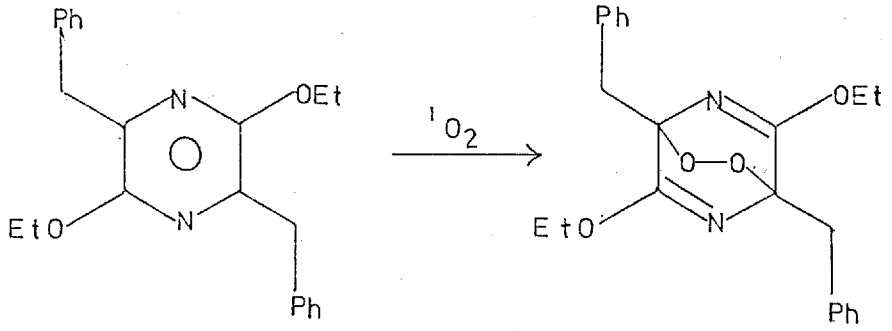
46



47



48



49

was present in the i.r. spectrum. Because endoperoxides have no characteristic absorption in the u.v. spectrum, and no strong diagnostic bands in the i.r. spectrum, and frequently eliminate molecular oxygen to regenerate the parent compound in the mass spectrum, identification of peroxides is dependent on chemical tests (e.g. starch/iodide), process of elimination on the basis of other spectral data, and the shifts, especially of α-protons, in the n.m.r. spectrum. Compound (49) proved to be quite stable, as shown by its resistance to reduction by aqueous potassium iodide solution during work-up, and exhibited no inclination to detonation, one of the more spectacular characteristics of some peroxides. Control experiments irradiating the pyrazine in a dichloromethane solution containing methylene blue but without an oxygen stream, and irradiating the pyrazine in solution without the sensitiser both gave three products (tlc), the major one of which was the peroxide. The oxygenated, photosensitised reaction was significantly more efficient, supporting the assumption that singlet oxygen is the reactive species. The formation of peroxide in the absence of a sensitiser or a stream of oxygen may be explained by considering that the highly conjugated dibenzyl pyrazine is acting as its own sensitiser to utilise molecular oxygen dissolved in solution.

After this initial success, a search was begun for other heterocyclic substrates that would react similarly. 3-Methyl-isoquinolinium methiodide (50) adds olefins across the 1,4-positions,[83] and it seemed likely that it would also serve as a receptor for singlet oxygen. However, no reaction was observed, and, after

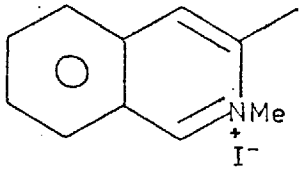
alkaline work-up, only hydrolysis products (51) of the starting material were isolated.

2,6-Dimethyl-4-pyrimidone (52) circumvents one of the problems encountered with the isoquinolinium salt, specifically its low solubility in organic solvents. Photooxygenation of the pyrimidone for up to seven hours produced no reaction, whereas longer reaction times led to the formation of an intractable mixture of highly coloured compounds.

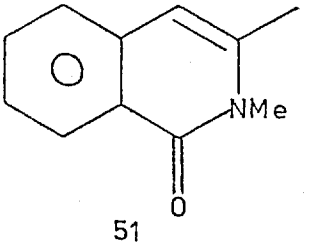
A similar heterocycle, 4,6-dihydroxy-2-methylpyrimidine (53) was next tested. Although this dihydroxypyrimidine is again insoluble in organic solvents, O-alkylation with ethyl Meerwein salt in situ (alkoxy and dialkoxy pyrimidines are notoriously volatile) afforded a freely soluble derivative (54). Addition of singlet oxygen proceeded smoothly to give the expected endoperoxide (55), an unstable crystalline solid.

Though the alkylation of the dihydroxypyrimidine was performed on the hypothesis that the insolubility of the substance prevented its oxygenation, the alkylation also generated a fully aromatic system with no possibility of tautomerism to a non-aromatic isomer. It appeared that the aromaticity, rather than the solubility, of the substrate was the determining factor. O-alkylation of 2,6-dimethyl-4-pyrimidinone and subsequent photooxygenation gave the endoperoxide (57) as an oil which decomposed rapidly on isolation.

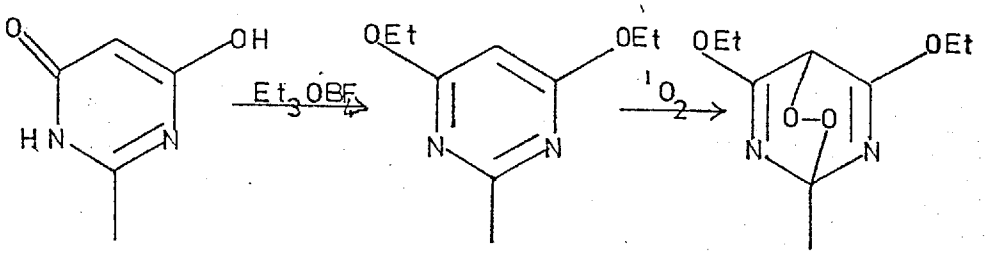
A third pyrimidine, 5-butyl-4,6-dihydroxy-2-methyl pyrimidine (58), has no C-5 proton and might, therefore, react with singlet oxygen without the prior necessity of O-alkylation



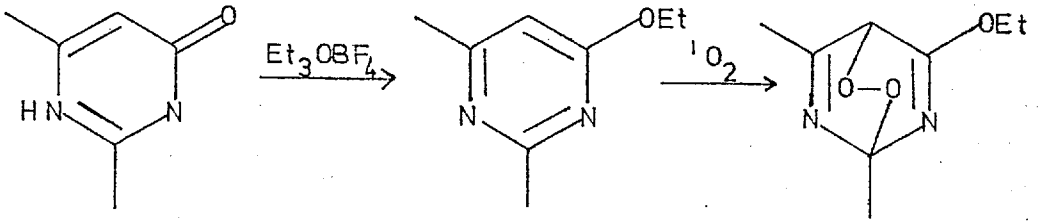
50



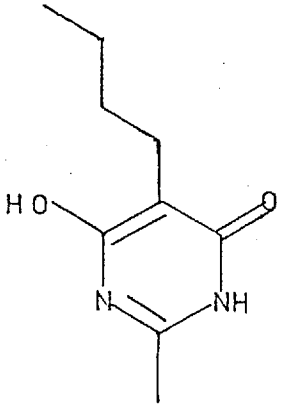
51



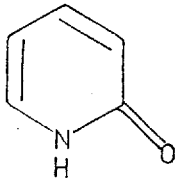
53 54 55



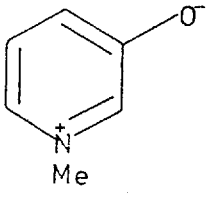
52 56 57



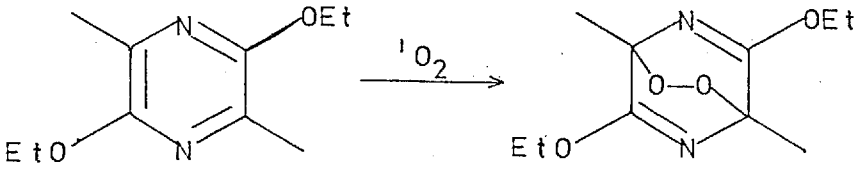
58



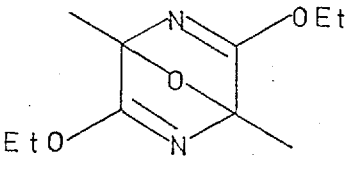
59



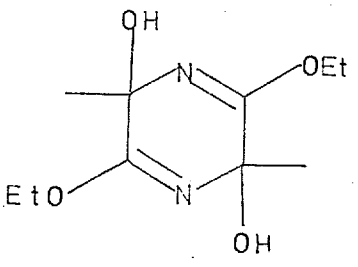
60



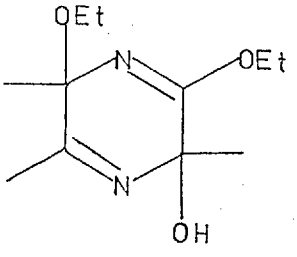
61



73



74



75

to give a more stable peroxide than those obtained from the other two pyrimidines. No reaction was observed on photooxygenation. After O-alkylation, the pyrimidine was consumed and peroxidic products formed under oxidation conditions, but no evidence for the endoperoxide was seen. It appears then, that the absence of a ring proton is not a prerequisite for peroxide formation, although it seems reasonable that the presence of such a proton would admit of facile degradation.

It was attempted to add singlet oxygen across two other unrelated heterocycles. Photooxygenation of α-pyridone (59) appeared to give a reaction (tlc, positive starch-iodide test), but only starting material could be recovered from the reaction solution. Efforts to trap the peroxide by oxidation in methanol or by reduction with triphenylphosphine produced complex mixtures of highly coloured compounds. Treatment of 3-hydroxypyridinium methiodide betaine (60) with singlet oxygen showed no evidence of reaction, although the betaine is reported to undergo cycloadditions with N-phenylmaleimide, acrylonitrile, methyl acrylate, and methylmethacrylate[84].

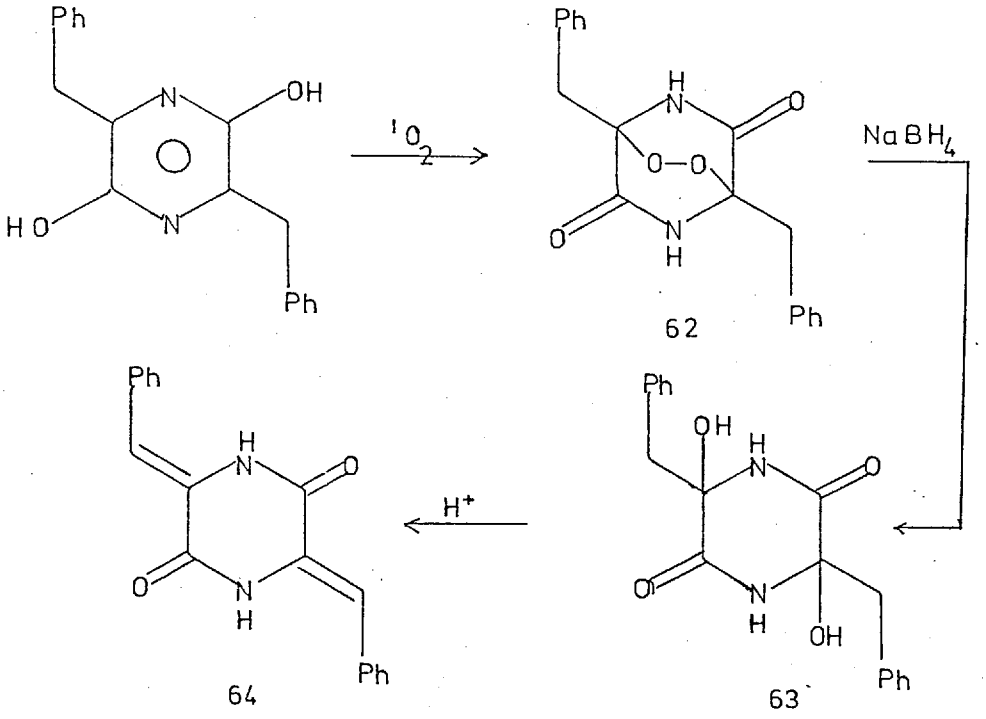
A return to the use of pyrazine substrates was more rewarding. The sole product of the oxygenation of 3,6-dimethyl-2,5-diethoxypyrazine was the corresponding peroxide (61), isolated as a stable, crystalline solid. Control experiments without a sensitiser, and with a sensitiser in the dark demonstrated the photolytic and sensitised nature of this reaction.

Singlet oxygen also added readily to 3,6-dibenzyl-2,5-dihydroxypyrazine to produce the peroxide (62). The advantage of

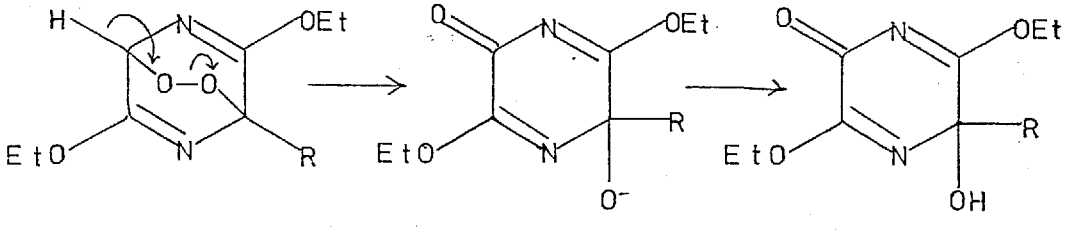
dihydroxypyrazines is that their photooxygenation leads directly to the dioxopiperazine, thus avoiding the need to hydrolyse imino ethers to amides. Reduction of the endoperoxide to the diol (63) and dehydration by p-toluenesulphonic acid in refluxing methanolic benzene provided 3,6-dibenzylidenyl-2,5-dioxopiperazine (64), identical by i.r. and mass spectral comparisons to an authentic sample (Scheme 25)[24,85,86].

Although photooxygenation of 2,5-diethoxypyrazine and 2,5-diethoxy-3-hydroxypropylpyrazine (22) resulted in the consumption of starting material, no discrete products could be isolated, and the organic material recovered appeared to be polymeric. The presence of ring protons is evidently responsible for the instability of the endoperoxide. A mechanism such as that illustrated in Scheme 26 might be expected to contribute to the decomposition of the peroxide.

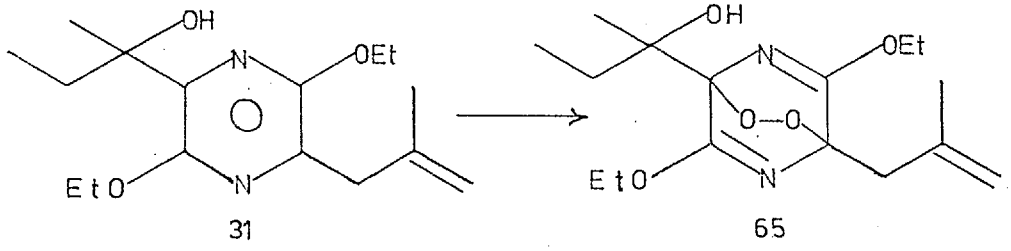
More successful was the oxygenation of the unsymmetrically disubstituted pyrazine (30). There are several possible reaction products from this substrate. In addition to conversion of the pyrazine to the endoperoxide, the exocyclic double bond is susceptible to attack by singlet oxygen in the 'ene' reaction. In this case, there are two isomers that could be formed. If the double bond reacted more rapidly than the ring, it would be expected that the double bond would move into conjugation with the aromatic system. Any of the compounds (65-69) would, therefore, be reasonable products from the photooxygenation. In the event, the only product isolated was the endoperoxide (65), indicating that the ring is more reactive to singlet oxygen. The shifts of

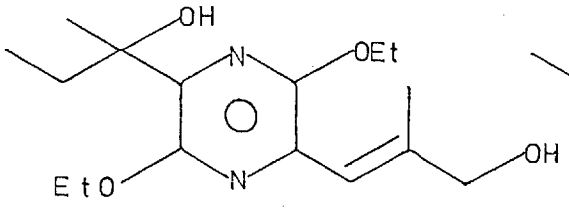


scheme 25

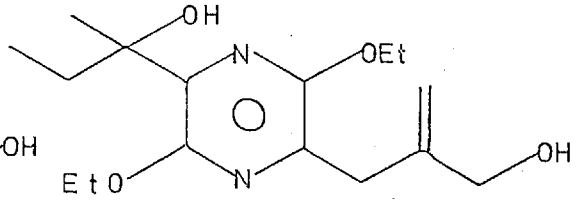


scheme 26

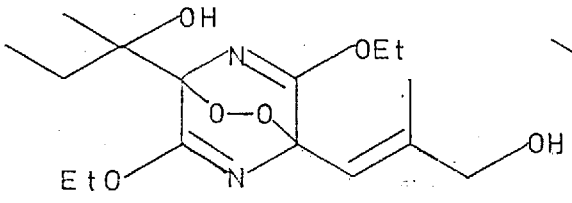



OH
N
OEt
EtO
N
OH

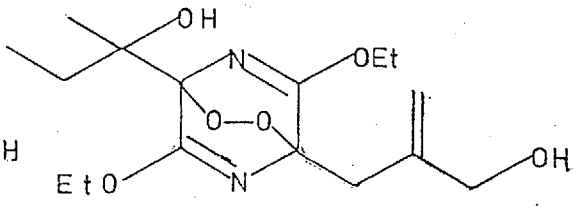
66


OH
N
OEt
EtO
N
OH

67


OH
N
OEt
O—O
EtO
N
OH

68


OH
N
OEt
O—O
EtO
N
OH

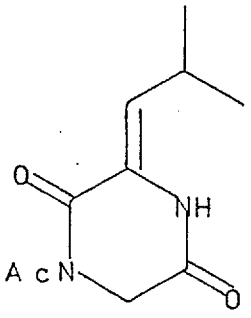
69

signals in the n.m.r. spectrum to positions more nearly those for a dihydropyrazine than for a pyrazine and the persistance of signals arising from the terminal methylene protons and the vinylic methyl group, as well as the occurrence of an absorption at 890 cm^{-1} in the i.r. spectrum, led to this assignment.

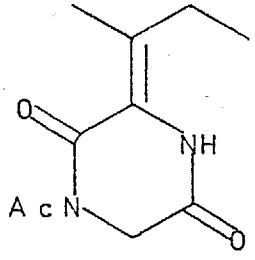
An earlier effort to add singlet oxygen by the 'ene' mechanism to 1-acetyl-3-isobutylidenyl-2,5-dioxopiperazine (70) had been unsuccessful, and starting material was recovered, thus eliminating the possibility that singlet oxygen addition to 1-acetyl-3-(1-methylpropylidenyl)-2,5-dioxopiperazine (71) would generate the desired 1-acetyl-3-(1-ethylethylenyl)-3-hydroxy-2,5-dioxopiperazine (72).

In summary it appears that the cycloaddition of singlet oxygen to aromatic N-heterocycles is a general reaction, provided that the substrate is fully aromatic and cannot tautomerise, for example, from hydroxy imidates to amides. Ring protons tend to assist the decomposition of the endoperoxides formed.

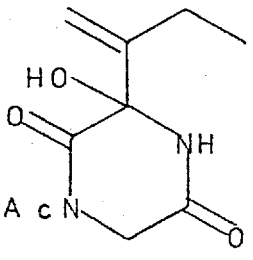
The peroxides presented an attractive entry into the epidithiodioxopiperazine systems (Scheme 27) found in gliotoxin, sporidesmin, the aranotins, and related fungal metabolites. Reduction to the diol and hydrolysis of the dihydropyrazine to the dioxopiperazine, followed by sulphur exchange[87] and oxidation[88] would generate this system. A similar route to mercaptodioxopiperazines has since been published[89]. However, the peroxides proved to be surprisingly resistant to reduction. Standard procedures, such as washing with aqueous solutions of potassium iodide or sodium thiosulphate, were ineffective. The peroxide of 2,5-diethoxy-3,6-



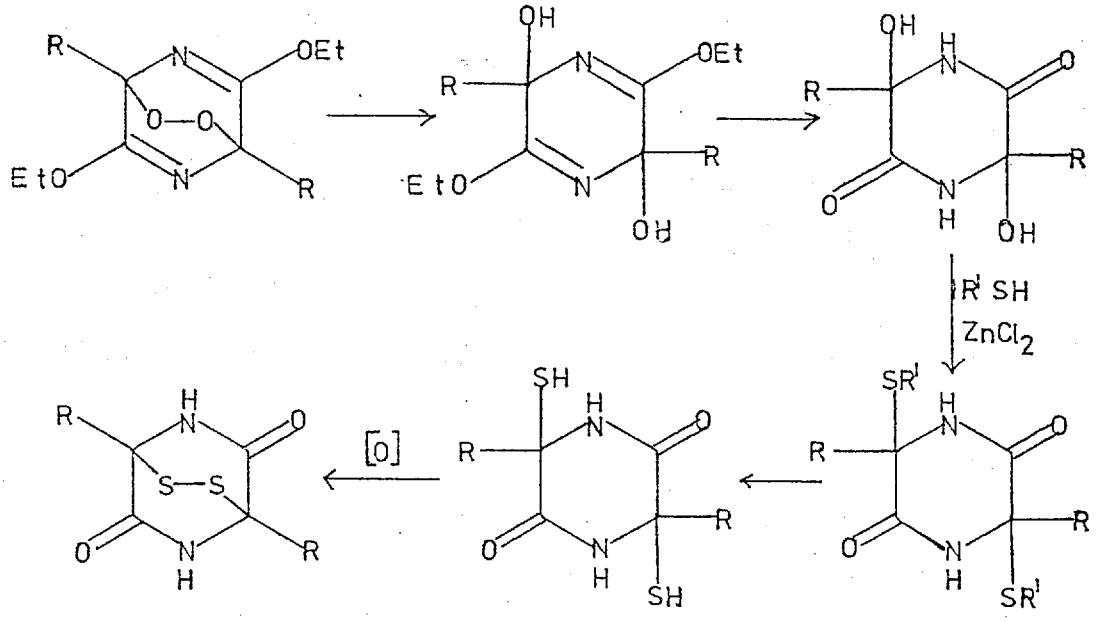
70



71



72



scheme 27

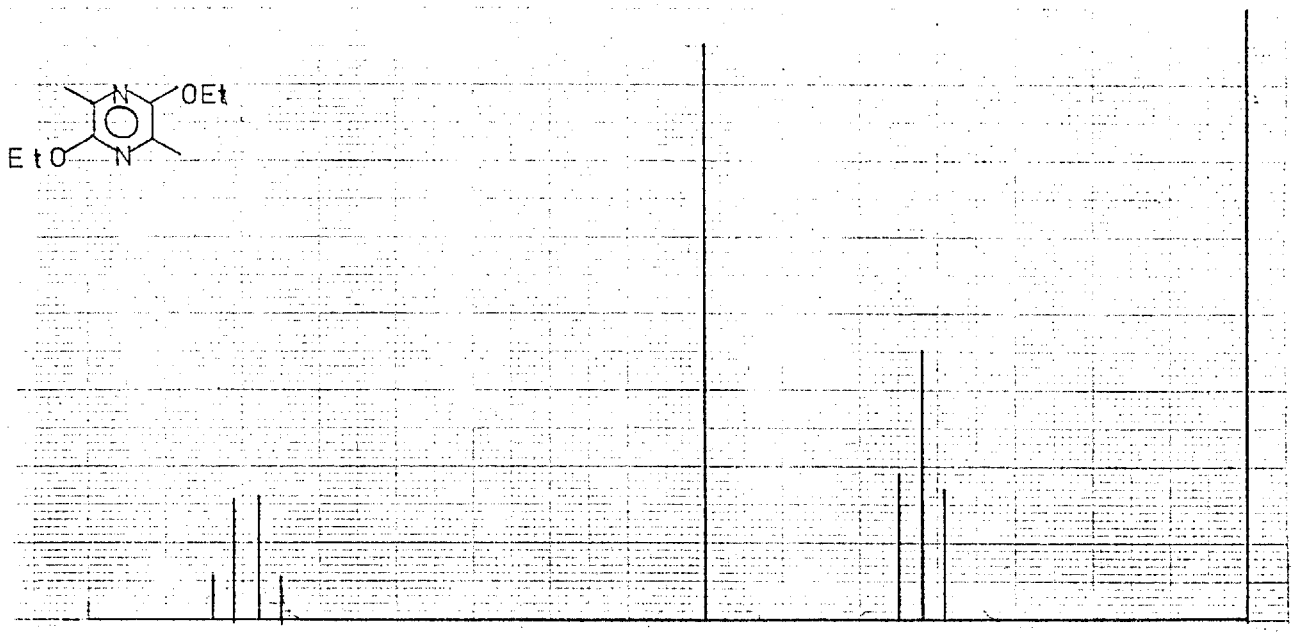
dimethylpyrazine (61) could be reduced by stirring in ethanol at room temperature with potassium iodide or sodium borohydride, but the complex pattern in the high field region of the n.m.r. spectrum suggested that the reduction product had undergone partial exchange with the solvent to give a 2,3,5-triethoxy compound. The product appeared to be unstable under the conditions used, and little material was recovered. Stirring in aqueous acetone with potassium iodide left the peroxide intact. Treatment of the peroxide in aqueous THF with sodium borohydride reduced the peroxide, but again the product appeared to be something other than the expected dihydroxydihydropyrazine. Two methyl signals in the n.m.r. spectrum and complex splitting patterns in the ethoxy signals strongly indicated an unsymmetrical product, which indication was supported by two absorptions in the imine region of the i.r. spectrum. The highest molecular weight fragment of any significance occurred at 215 mass units in the mass spectrum, although it does not seem likely that an odd molecular weight signal would arise from the molecular ion. It may be that the product consists of a cis, trans mixture of the dihydroxydihydropyrazines. Mechanistic and geometrical considerations dictate that the endoperoxide be cis-fused. A mixture of isomeric diols would arise either by exchange of the initial reduction product with the solvent, or by nucleophilic attack of hydroxyl ion on the peroxide. Loss of fifteen mass units in the mass spectrum could be explained by radical formation at an alcoholic oxygen atom, followed by loss of a methyl group to generate a protonated keto compound. Alternatively,

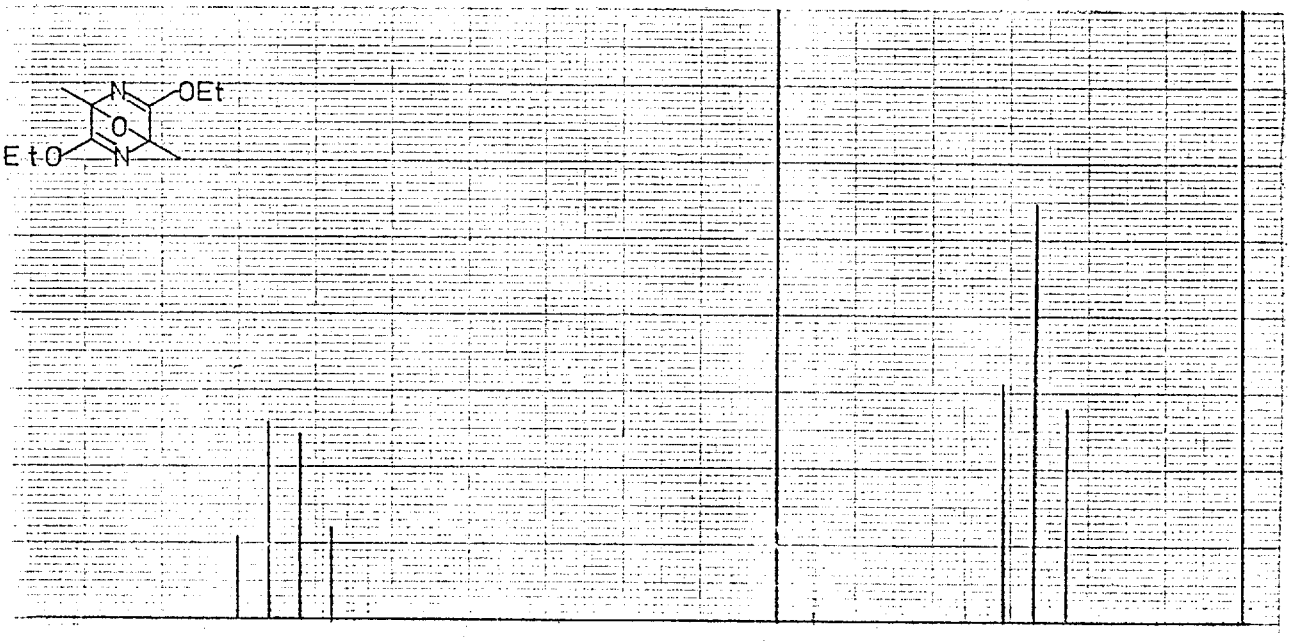
the product may be an unsymmetrically substituted pyrazine. As yet, no structure considered has been unequivocally consistent with all the observed data.

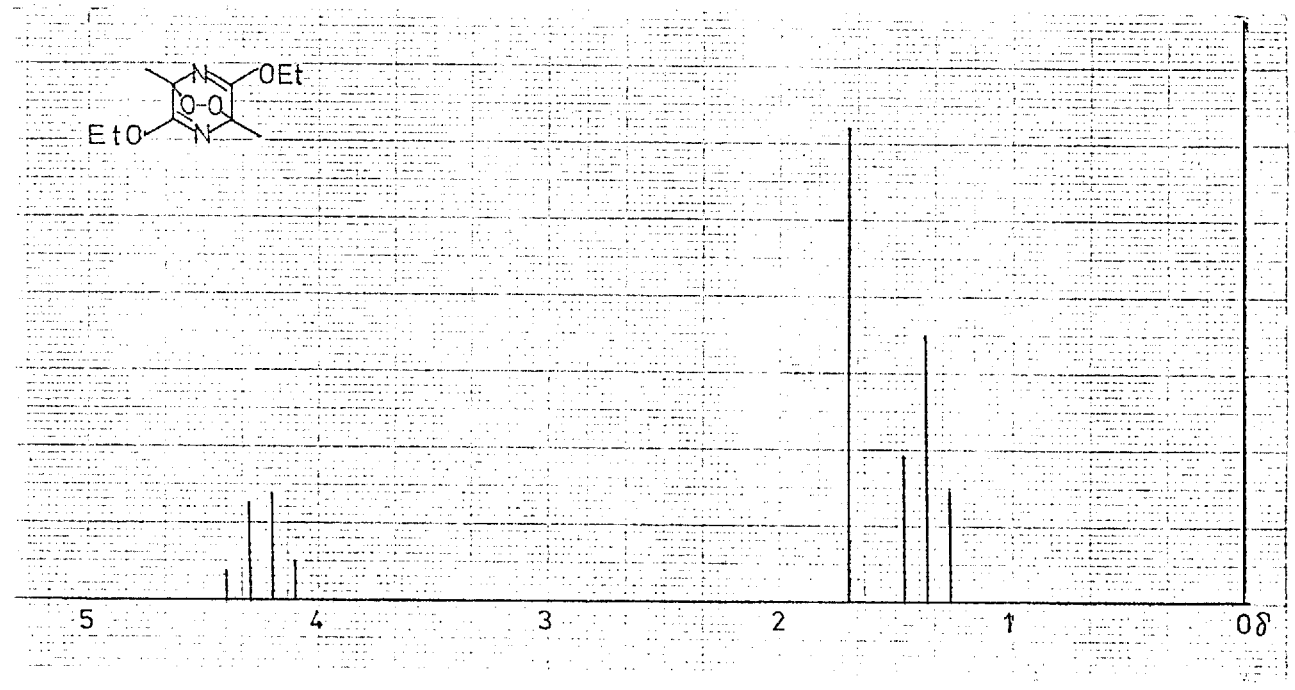
Reaction of the peroxide with triphenylphosphine appeared encouraging in the first instance. The methyl signal in the n.m.r. spectrum had shifted to a position intermediate to the corresponding signals in the pyrazine and the peroxide, as might be expected for an oxide structure. However, attempts to repeat the initial result were unsatisfactory. Subsequent reductions with triphenylphosphine gave irreproducible and inconsistent results. An analogous reaction has been used in the conversion of epidithiodioxopiperazines to the monosulphides [88,90,91].

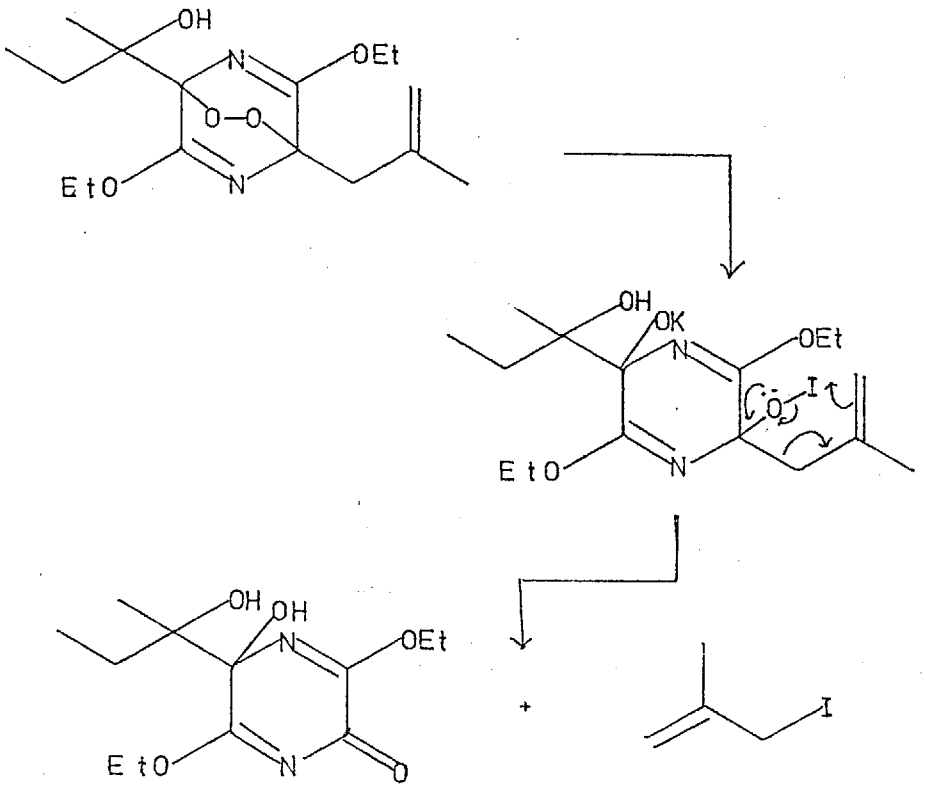
The unsymmetrical peroxide (65) was also resistant to aqueous potassium iodide or thiosulphate solutions. Prolonged stirring of the peroxide in aqueous acetone with potassium iodide resulted in decomposition with loss of the methylallyl side chain. Loss of the side chain may arise from elimination of methylallyl iodide *via* a cyclic mechanism (Scheme 28). In addition, the iodide ion may be sufficiently nucleophilic to cleave the ethoxy groups of the imino ethers. Treatment of the same peroxide with sodium borohydride again resulted in decomposition. The only soluble organic material recovered was a minute quantity of starting material. It appears that the reduction of this peroxide poses special problems.

That 2,5-dioxopiperazines are themselves susceptible to oxidation at the 3,6-positions has recently been demonstrated


OEt
EtO
N
N


OEt
EtO
N
O
N


OEt
EtO
N
O-O
N
5
4
3
2
1
0δ



scheme 28

by the isolation of 3-hydroperoxy-2,5-dioxopiperazines from samples of dioxopiperazines that were unprotected from light and air. The hydroperoxydioxopiperazines are autoxidation products, but can be generated photochemically on exposure to molecular oxygen[89].

The reaction of singlet oxygen, therefore, with pyrazines provides an efficient means for the oxygenation of the 3,6-positions of the heterocyclic ring. The chemistry of the resultant peroxides is somewhat baffling, at this stage.

V. Summary: Bicyclomycin synthesis to date

A practical route to the total synthesis of the antibiotic bicyclomycin is now becoming clear. It has been determined that, in principle, the carbon skeleton can be assembled by means of alkylations and condensations on dihydropyrazines. The dihydropyrazines are conveniently oxidised to the aromatic pyrazines by reaction with DDQ. Oxygenation of the pyrazine ring at the 3,6-positions is effected in high yield by addition of singlet oxygen. Some problems have been encountered in the hydrolysis of dihydropyrazines to dioxopiperazines, but use of dihydroxypyrazines, possibly derived by boron tribromide cleavage of the dialkoxypyrazines, should circumvent this problem. Using this route, it should be possible to create analogues of bicyclomycin containing the appropriate side chains and oxidation level. Cyclisation to form the eight-membered cyclic ether has yet to be demonstrated, and

GENERAL TECHNIQUES

All melting points were taken on a Reichert hot stage apparatus and are uncorrected. Infra-red (i.r.) spectra were recorded on a Perkin-Elmer 157b spectrometer as films, except where specified as nujol mulls or solutions. Ultraviolet (u.v.) spectra were recorded on a Pye-Unicam SP800 spectrometer on solutions in ethanol. Nuclear magnetic resonance (n.m.r.) spectra were taken of solutions in deuterochloroform with tetramethylsilane as an internal standard, except where specified to the contrary, on Varian T60 and Jeol MH100 instruments. The following abbreviations are used in describing spectra: s(singlet), d(doublet), t(triplet), q(quartet), m(multiplet), and bs(broad singlet). Mass spectra were performed on an AEIMS 9 spectrometer, and accurate mass measurements were taken by the Physico-Chemical Measurements Unit, Harwell, Berks.

Reactions were followed by thin layer chromatography (t.l.c.) on silica plates (Merck Kieselgel GF_{254}). Preparative layer chromatography (p.l.c.) was performed on 1 mm silica plates. Solvent systems are given as ratios of volumes.

Dichloromethane was dried by refluxing over and distilling from phosphorous pentoxide, and was stored on molecular sieves (4A) in the dark. Benzene was distilled from lithium aluminium hydride (LAH) and stored over molecular sieves (4A). Tetrahydrofuran (THF) was distilled from LAH immediately before use. N,N-Dimethylformamide (DMF) was distilled under reduced pressure from barium oxide and stored over molecular sieves (4A). Acetone was dried by prolonged standing on molecular sieves.

Organic extracts of reaction mixtures were dried over sodium sulphate or magnesium sulphate.

The ion exchange resins used were Amberlite 120(H^+) and 120(OH^-) for acidic and basic catalysis, respectively.

"Pet. eth." refers to the petroleum ether fraction boiling between 60^o and 80^o.

1,4-Dipropoxybutan-2-one (7). -

Mercury (II) p-toluenesulphonamide (12.5g., 0.026 mole)[92,93,94,95] and 1,4-but-2-yne diol (25.01g., 0.28 mole) were heated in refluxing n-propanol (380 ml) for 24 h. The mixture was cooled, filtered, and concentrated to ca. 60 ml. by distillation at atmospheric pressure. Distillation of the residue afforded 1,4-dipropoxybutan-2-one as a colourless liquid (21%), b.p. 61.9° (2 mm Hg), δ 4.6 (2H, s), 3.5 (4H, m), 2.6 (4H, q), 1.6 (4H, m), 1.0 (6H, m), ν_{max} 3040-2900, 1730, 1470, 1330, 1230, 1080, 770, 680 cm^{-1} (Found: C, 63.92; H, 10.47. $C_{10}H_{20}O_3$ requires C, 63.60; H, 10.79%).

3,6-Di-(1-hydroxybenzyl)-1,4-dimethylpiperazine-2,5-dione (12). -

To a cold, stirred solution of 1,4-dimethylpiperazine-2,5-dione (0.28g., 2 mmole) and benzaldehyde (0.94 ml; 8 mmole) in dry DMF (4 ml) was added potassium t-butoxide (3.3 ml; 0.6M solution in t-butanol; 2 mmole). The slurry was allowed to warm to room temperature, and stirring was continued for 5½ h. The mixture was acidified with glacial acetic acid (0.5 ml.), poured into water (20 ml.), and extracted with ethyl acetate (3 x 20 ml). The organic layer was dried and concentrated to yield a yellow oil which crystallised on standing. Purification by p.l.c. ($CHCl_3$/MeOH, 10:1) and recrystallisation from chloroform / pet. eth. afforded 3,6-di-(1-hydroxybenzyl)-1,4-dimethyl-2,5-dioxopiperazine, apparently one isomer of undetermined configuration, as fine white needles (69%), m.p. 229.9°, δ (DMSO-d_6) 2.35 (6H, s), 4.2 (2H, d, J=3Hz), 5.4 (2H, t; collapses to doublet on D_2O exchange, J=3Hz), 6.5 (2H, d, J=4Hz,

exch. D_2O), 7.5 (10H, m), ν_{max} (nujol) 3340, 1690, 1640, 1060, 755 cm^{-1} λ_{max} 225, 242 nm (ε 5130, 3240). (Found: C, 67.72; H, 6.16; N, 7.79. $C_{20}H_{22}N_2O_4$ requires C, 67.78; H, 6.26; N, 7.91%).

Attempted dehydration of 3,6-di-(1-hydroxybenzyl)-1,4-dimethyl-2,5-dioxopiperazine. -

(a) With acetic anhydride

1. The bis alcohol (0.05 g) was suspended in acetic anhydride (10 ml.) and heated with stirring at 100-110° for 2½h. At the end of this time, water (50 ml) was added and stirring was continued until homogeneity was obtained. The solution was extracted with dichloromethane (2 x 50 ml). The organic layers were combined, treated with sodium carbonate, filtered, dried and concentrated. No dehydration product was observed (n.m.r. spectroscopy).

2. The alcohol (0.03 g) was refluxed overnight in acetic anhydride. The solution was worked up as above. A mixture of acetylated products and starting material was obtained (n.m.r. spectroscopy).

(b) With phosphorous oxychloride

To a solution of the bis alcohol (0.04 g., 1 mmole) in dry pyridine (10 ml) was added with stirring and cooling phosphorous oxychloride (0.05 ml). Stirring was continued overnight, after which time the solution was poured into ice (12 g) and extracted with dichloromethane (5 x 10 ml). The dichloromethane fractions

were collected, washed with 2 x 20 ml portions of saturated aqueous sodium chloride solution, dried, and concentrated. The resultant oil was taken up into chloroform, washed with dilute, aqueous hydrogen chloride solution until neutral, dried and concentrated. Only decomposition products were detected (n.m.r. spectroscopy).

3,6-Di-(1-acetoxybenzyl)-1,4-dimethyl-2,5-dioxopiperazine (13). -

The 3,6-di-(1-hydroxybenzyl)-1,4-dimethyldioxopiperazine (0.12 g., 0.5 mmole) was dissolved in dry pyridine (2 ml). Acetic anhydride (1 ml) was added, and the mixture stirred at room temperature for 16 h. under a drying tube. The solution was poured into dichloromethane and washed 3 x with 2N HCl, 3 x with saturated aqueous sodium hydrogen carbonate solution and once with water. The organic layer was separated, dried, and concentrated. Recrystallisation from pet. eth. afforded the title compound as colourless rhombic crystals (50%), m.p. 215° (subl.), δ 2.2(6H, s), 2.8 (6H, s), 4.3 (2H, d, J=4Hz), 6.36 (2H, d, J=4Hz), 7.36 (10H, s), ν_{max} 2940, 1745, 1670, 1450, 1400, 1370, 1225, 1020, 920, 770, 760, 730, 700 cm^{-1}., M^+ 332(14), 290(10), 230(28), 183(86), 141(100), 113(14), 107(18). (Found: C, 55.28; H, 4.98; N, 5.19. $C_{24}H_{26}N_2O_6$. $4\frac{1}{2}$ H_2O requires C, 55.49; H, 6.74; N, 5.37%. Found: M^+ 438.1781. $C_{24}H_{26}N_2O_6$ requires 438.1790).

3,6-Dibenzylidenyl-1,4-dimethyl-2,5-dioxopiperazine (14). -

The diacetate (13) (0.08 g) was heated in a sealed tube at 160° for 19 h. The resultant dark brown gum was taken up in dichloromethane, washed with saturated aqueous sodium hydrogen

carbonate solution, and water. The organic layer was separated, dried, and concentrated to give a brown solid identical by ^1H n.m.r. spectroscopy and t.l.c. behaviour to an authentic sample.

3-(2-Methylpropylideny1)-2,5-dioxopiperazine (16). -

To a cold (0^o), stirred solution of 1,4-diacetyl-2,5-dioxopiperazine (0.4 g.,2 mmole) and freshly distilled methacrolein (0.7 ml; 8 mmole) with a trace of di-t-butylmethylphenol in dry DMF (4 ml) under nitrogen was added potassium t-butoxide (0.23 g., 2 mmole). The mixture was allowed to warm to room temperature, and stirring was continued for $6\frac{1}{4}$ h. The mixture was acidified with acetic acid (0.5 ml), poured into water (10 ml), and extracted with ethyl acetate. The organic layers were dried and concentrated. The residue was chromatographed (Al_2O_3, grade III; 40 g), eluting consecutively with pet. eth., chloroform, and ethyl acetate. The chloroform fractions gave, on concentration, a solid identified as the title condensation compound, which was recrystallised from chloroform (36%), m.p. 149.5^o, δ 1.96 (3H, s), 4.09 (2H, s), 5.1 (2H, d, J=9Hz), 6.4 (1H, s), 6.8 (1H, bs, exch. D_2O), 8.0 (1H, bs, exch. D_2O), ν_{max} 340, 1685, 1630 cm^{-1}, λ_{max} 280 nm (ε 5370), M^+ 166(100), 165(50), 164(38), 153(54), 139(62), 124(44), 108(46), 100(66), 83(56), 68(64). (Found: M^+166.0738. $C_8H_{16}N_2O_2$ requires 166.0742).

1-Acetyl-4-hydroxymethyl-3-propylideny1-2,5-dioxopiperazine (17). -

The 1-acetyl-3-propylidenyldioxopiperazine (0.2 g) was heated with paraformaldehyde (0.2 g) overnight in glacial acetic acid. The orange solution was poured into water and extracted with dichloromethane. The organic layers were dried and concentrated.

Residual acetic acid was removed under vacuum, and the remaining oil was purified twice by p.l.c. ($CHCl_3$/5% MeOH) to give the title compound. δ 1.12 (3H, t, J=4Hz), 2.12 (3H, s), 2.08-2.3 (2H, q, J=4Hz), 4.28 (2H, s), 5.52 (2H, s), 6.28 (1H, t, J=3Hz), 8.76 (1H, bs), ν_{max} 3480, 3200, 3080, 2960, 1740, 1680, 1640, 1420, 1360, 1320, 1220, 1190, 1020, 950, 810, 840 cm^{-1}.

3-Oxopropyl-1-tetrahydropyranyl ether -

Acetaldehyde (10 ml) was refluxed with tartaric acid (0.03 g) under a column packed with glass helices. When the refluxing mixture reached the top of the column, a 36% solution of formaldehyde in water (4 g) containing 3M NaOH (0.14 ml) was added dropwise through the column head. Refluxing was continued for 20 min. after addition was complete. The mixture was cooled, and distilled *in vacuo*. The small quantity of a colourless liquid that collected in the condenser (b.p. 36-40°, 1-2 mm Hg; lit.[32] 38°, 2 mm Hg) was washed out with diethyl ether. Dihydropyran (0.5 ml) and concentrated hydrochloric acid (2 drops) were added, and the solution shaken and allowed to stand overnight at room temperature. Removal of the solvent left the 3-oxopropyl-1-tetrahydropyranyl ether as approximately 25% of a pale liquid which decomposed rapidly on standing at room temperature. δ 1.6 (10H, m), 3.7 (2H, m), 5.0 (1H, bs), 9.66 (1H, s), ν_{max} 3410, 2960-2820, 1720, 1685, 1440, 1380, 1350, 1320, 1280, 1260, 1200, 1160-950, 900, 800, 830-805, 780, 750 cm^{-1}.

3-Hydroxypropyl-1-tetrahydropyranyl ether. -

To propane-1,3-diol (20 ml, 0.3 mole) was added dihydropyran (29 ml, 0.3 mole) and concentrated hydrochloric acid (3 drops). The solution was shaken and allowed to stand at room temperature overnight. After neutralisation with sodium hydroxide (2 pellets), the solution was fractionally distilled to yield the mono-tetrahydropyranyl ether as a colourless liquid, b.p. 73-76° (2 mm Hg), δ 1.4-1.8 (8H, m), 3.0-4.0 (7H, m; 1 exch. D_2O), 4.6 (1H, bs), ν_{max} 3520-3300, 2930, 2860, 1650, 1440, 1350, 1200, 1140, 1120, 1070, 1030, 900, 860, 810 cm^{-1}.

Attempted oxidation of 3-hydroxypropyl-1-tetrahydropyranyl ether with chromium trioxide-pyridine complex. -

Anhydrous chromium trioxide (2.00 g., 0.02 mole) was added to a stirred solution of dry pyridine (3.2 ml, 0.04 mole) in dry dichloromethane (60 ml). The slurry was stirred under a drying tube at room temperature for 15 minutes. The alcohol (0.56 g, 0.033 mole) was added in one portion as a solution in dry dichloromethane (10 ml). Stirring was continued for a further 15 minutes. The solvent was decanted from the precipitated tar. The residue was washed with diethyl ether. The combined organic layers were washed with 5% aqueous sodium hydroxide solution (4 x 50 ml), 2N hydrogen chloride solution (50 ml), saturated sodium hydrogen carbonate solution (50 ml), saturated aqueous sodium chloride solution (50 ml), dried, and concentrated. The minute quantity of residue obtained gave a positive 2,4-dinitrophenylhydrazine test and showed aldehydic signals in its n.m.r. spectrum, but

decomposed on attempted purification by p.l.c.

1,4-Dimethyl-3,3,6-triallyl-2,5-dioxopiperazine (18). -

Into a flanged flask fitted with a soda lime drying tube, a mechanical stirrer, a nitrogen inlet, a serum cap, and a dry ice/acetone condenser was distilled several hundred mls of liquid ammonia. Hydrated ferric nitrate (0.10 g), followed by sodium (0.11 g) was introduced. To this stirred suspension was added more sodium (0.20 g). After stirring for 45 minutes, 1,4-dimethyldioxopiperazine (1.00 g, 7 mmole) was added. Stirring was continued for 20 minutes before addition of allyl bromide (0.6 ml, 7 mmole). The suspension was stirred for 1 h. Dry dichloromethane (80-90 ml) was added, and the ammonia was allowed to evaporate. Water (120 ml) was added to the residue. The mixture was then extracted with dichloromethane (3 x 50 ml), dried and the solvent removed. P.l.c. ($CHCl_3$) afforded the trialkylated compound (18), (3%), m.p. 74.7-75.2°, δ 2.0-2.8 (6H, m), 2.95 (3H, s), 3.0 (3H, s), 4.0 (3H, t, J=4Hz), 5.9-6.8 (9H, m), ν_{max} 3090, 2950, 1650, 1450, 1400, 1350, 1340, 1250, 1080, 1000, 930, 680 cm^{-1}. (Found: C, 68.60; H, 8.33; N, 10.62. $C_{15}H_{22}N_2O_2$ requires C, 68.67; H, 8.44; N, 10.68%).

Attempted alkylation of 1,4-diacetyl-2,5-dioxopiperazine. -

To a stirred suspension of sodium hydride (0.025 g, 1 mmole) in dry DMF (5 ml) was added a solution of the dioxopiperazine (0.2 g, 1 mmole) in dry DMF (5 ml). After stirring for one hour, 3-chloropropyl-1-tetrahydropyranyl ether (1 mmole) in dry DMF (1 ml) was

added, and the resultant mixture heated at 85-100° with stirring for 2h. The mixture was cooled, neutralised with acetic acid (0.25 ml), and poured into water. No compounds soluble in organic solvents were obtained.

Attempted alkylation of 1,4-dimethyl-2,5-dioxopiperazine. -

To a solution of sarcosine anhydride (0.28 g, 2 mmole) in dry DMF (4 ml) was added with cooling and stirring a solution of 0.6M potassium t-butoxide in t-butanol (4 ml, 2 mmole). The solution was allowed to stir for 40 min. before addition of allyl bromide (0.15 ml, 2 mmole). Stirring was continued at room temperature for 6 h. The mixture was then acidified with glacial acetic acid (0.5 ml) and poured into water. The aqueous solution was extracted with 3 x 20 ml portions of dichloromethane. The organic layers were collected, washed with 2 x 20 ml portions of water, dried, and the solvent removed to give a small quantity of the alkylation product as a yellow oil (6%), δ 2.7 (2H, m), 3.0 (6H, s), 4.0 (3H, t), 5.0-6.0 (3H, m). Attempted purification by p.l.c. ($CHCl_3$) resulted in loss of material.

Attempted alkylation of 1,6-dimethyl-2,5-dioxopiperazine. -

To a solution of 1,6-dimethyl-2,5-dioxopiperazine (0.10 g, 0.7 mmole) in dry THF at -25° was added dropwise with stirring a solution of n-butyl lithium in hexane (0.75 ml of 0.87M solution, 1.4 mmole). After stirring for 40 min, 3-bromopropyl-1-tetrahydropyranyl ether (0.15 g., 0.7 mmole) was added. The slurry was allowed to warm to room temperature, and stirring was continued

overnight, after which time, t.l.c. showed no reaction. Workup by pouring the mixture into cold, dilute acetic acid, extraction with ethyl acetate, and column chromatography (Al_2O_3, grade III) of the residue from the organic extracts, afforded unreacted alkylating agent.

Attempted alkylation of 1-methyl-2,5-dioxopiperazine. -

As above, the dioxopiperazine (0.10 g, 0.78 mmole) was suspended in dry THF and treated with n-butyl lithium (1.56 mmole) and 3-bromopropyl-1-tetrahydropyranyl ether (0.78 mmole). Workup yielded the unreacted alkylating agent and a decomposition product thereof.

Attempted preparation of 1,4-diformyl-2,5-dioxopiperazine. -

(a) In formic acid

Glycine anhydride (1.0 g, 8.7 mmole) was heated in refluxing 90% formic acid (25 ml) for 20 h. The solvent was removed under reduced pressure to leave a deep yellow oil. δ (D_2O) 3.7 (4H, s), 8.2 (2H, s), ν_{max} 3420-3220, 3190-3000, 2760, 2450, 1710-1610, 1600-1440, 1360, 1260, 985, 920-890 cm^{-1}, M^+ 142, 133, 144, 104, 103, 86, 75, 59, 58, 46, 45; thus the highest ion corresponded to a mono-formylated product. This was not characterised further.

(b) In formic-acetic anhydride

Glycine anhydride (1.0 g, 8.7 mmole) was dissolved in 90% formic acid (25 ml), and the solution was heated to 50^{o}. Acetic anhydride was added at a rate such that the temperature did

not rise above 60°. The solution was allowed to cool and then stirred overnight at room temperature. The solution was diluted with water (30 ml), and the solvents removed. No evidence for reaction was observed, the product resembling (t.l.c. and physical properties) starting material.

1,4-Di(trifluoro)acetyl-2,5-dioxopiperazine. -

To cold glycine anhydride (1 g, 8.7 mmole) was added trifluoroacetic anhydride (TFAA) (3 ml). The suspension was heated at reflux for 1 h, during which time the dioxopiperazine went into solution. After cooling to room temperature, TFA and excess TFAA were removed under vacuum to leave a viscous yellow oil which crystallised on trituration with organic solvents or on scratching, m.p. 120° (dec.), δ (TFA) 4.38 (4H, s), ν_{max} (nujol) 3200-3140, 1760-1640, 1440, 1340, 1210-1160, 1105, 1005, 925, 840, 810, 750 cm^{-1}, M^+ 306(7), 209(8), 181(8), 134(6), 133(5), 126(28), 109(4), 97(5), 96(7), 78(9), 69(100). (Found: M^+ 306.0072. $C_8H_4N_2O_4F_6$ requires 306.0075).

Hydrolysis of 1,4-di(trifluoro)acetyl-2,5-dioxopiperazine. -

(a) With methanolic potassium hydroxide

The trifluoroacetyl dioxopiperazine (0.25 g) was stirred at room temperature in 25 ml of 1M methanolic potassium hydroxide for $2\frac{3}{4}$ hr. The slurry was neutralised with 1M sulphuric acid (25 ml). The precipitate was filtered and washed with ethanol, and the combined alcoholic layers were concentrated to a semi-

solid residue, not consisting of the desired dioxopiperazine (i.r. spectroscopy).

(b) With ion exchange resin

The trifluoroacetyl dioxopiperazine (0.1 g) was suspended in ethanol (10 ml) and stirred with basic ion exchange resin (0.1 g) for 3-4 h at room temperature. The slurry was filtered, and the solids washed with water to dissolve glycine anhydride. The combined filtrates were concentrated to dryness, giving an off-white solid identical to an authentic sample of glycine anhydride (i.r. spectroscopy).

Alkylation of 2,5-dioxopiperazine. -

(a) With dimethylsulphate

To a slurry to glycine anhydride (0.5 g, 4.3 mmole) in dry dichloromethane was added dimethyl sulphate (1.4 ml, 8.6 mmole). The reaction was stirred at room temperature under a drying tube. After stirring overnight, no reaction had occurred (t.l.c.). The volume of the mixture was increased, and the slurry was then refluxed overnight. As no reaction had occurred after this time, 50% aqueous potassium carbonate (1.5 ml) was added, and the mixture stirred at room temperature. No reaction was observed (t.l.c.; no change in physical properties).

(b) With methylfluorosulphonate

To a suspension of glycine anhydride (0.5 g, 4.5 mmole) in dry dichloromethane under a drying tube was added methylfluoro-sulphonate (1.25 g, 9 mmole). The mixture was stirred at room

temperature for 24 h. Unchanged starting materials were recovered.

(c) With trimethyloxonium fluoroborate

1. In dichloromethane

To a suspension of glycine anhydride (2.04 g, 18 mmole) in dry dichloromethane under nitrogen was added, with stirring, a slurry of trimethyloxonium fluoroborate (12.3 g, several fold excess) in dry dichloromethane. The resulting slurry was stirred at room temperature for 3 days. The mixture was washed with saturated aqueous sodium hydrogen carbonate solution (20 ml) and water (3 x 15 ml). The organic layer was dried and concentrated to give impurities from the Meerwein salt. Unchanged dioxopiperazine was recovered from the aqueous phase.

2. In nitromethane

To a solution of trimethyloxonium fluoroborate (16 g, 36 mmole) in dry nitromethane was added glycine anhydride (1.0 g, 9 mmole). The mixture was stirred at room temperature under dry nitrogen. After 3 h, the solids had dissolved and a precipitate began to collect. After stirring overnight, the mixture was worked up as above. Concentration of the aqueous layer afforded 2,5-dimethoxy-3,6-dihydropyrazine (19a) as an intractable, inhomogeneous residue with organic salts, $\delta(D_2O)$ 4.0 (6H, s), 4.08 (4H, s).

Alkylation of 3,6-dimethyl-2,5-dioxopiperazine. -

To a solution of trimethyloxonium fluoroborate (2.0 g, 14 mmole) in dry nitromethane under nitrogen was added alanine

anhydride (0.5 g, 3.5 mmole). The reaction was stirred at room temperature for 2 days, a second portion of trimethyloxonium fluoroborate (2.0 g, 14 mmole) was added, and stirring was continued for a further 3 days. After workup as above, concentration of the aqueous layer afforded 2,5-dimethyl-3,6-dimethoxy-2,5-dihydropyrazine (19b) contaminated with inorganic salts, $\delta(D_2O)$ 1.59 (6H, d, J=4Hz), 3.86 (6H, s), 4.22 (4H, q, J=4Hz).

General procedure for alkylations and condensations on 2,5-diethoxydihydropyrazines.[51,52] -

To a cooled (-78°), stirred solution of dry diisopropylamide (1.1 eq.) in dry THF under dry nitrogen was added n-butyl lithium (1.1 eq). The solution was stirred 30 min. The imino ether (1 eq.) was then added as a solution in dry THF and stirring was continued for a further 90 min. The alkylating agent, aldehyde, or ketone (1 eq.) was then injected, and stirring was continued at -78° for up to 3 h, at which time the mixture was allowed to warm to room temperature to stir 4-21 h. The reaction mixture was next poured into diethyl ether. The organic layer was washed with saturated aqueous sodium chloride solution, dried and concentrated.

2-Allyl-3,6-diethoxy-2,5-dihydropyrazine (20a). -

3,6-Diethoxydihydropyrazine (0.2 g, 1.2 mmole) was metalated with lithium diisopropylamide and alkylated with allyl bromide (0.14 ml, 1.2 mmole). After stirring overnight, t.l.c. showed incomplete reaction. After workup and purification by

p.l.c. ($CHCl_3$), the title compound was obtained as a yellow oil, (52%), δ 1.24 (6H, t, J=3 Hz), 2.48 (2H, t, J=3 Hz), 4.0(7H, m), 4.9-5.84 (3H, m), ν_{max} 2970, 2920, 1690, 1435, 1365, 1340, 1240, 1135, 1090, 1030, 910 cm^{-1}, M^+ 228(25), 227(33), 210(8), 169(100), 153(25), 141(58), 113(58), 85(100), 57(42), 55(33). (Found: M^+ 228.1468. $C_{11}H_{18}N_2O_2.H_2O$ requires 228.1474).

3,6-Diethoxy-2,5-dihydro-2-methallylpyrazine (20b). -

Using the standard procedure, the diethoxydihydropyrazine (0.5 g, 3 mmole) was alkylated with methallyl bromide[96]. After workup and chromatography (Al_2O_3, grade III; 20 g) eluting with pet. eth., the product was obtained as a nearly colourless oil (70%), δ 1.24 (6H, t, J=3 Hz), 1.72 (3H, s), 2.38 (2H, d, J=2 Hz), 3.88-4.08 (7H, m), 4.68 (2H, d, J=4 Hz), ν_{max} 3000-2800, 1690, 1440, 1365, 1340, 1240, 1140, 1090, 1030, 890 cm^{-1}, M^+ 224(42), 222(7), 170(9), 169(100, 141(45), 113(38), 85(45), 56(10), 55(9). (Found: M^+ 224.1524. $C_{12}H_{20}N_2O_2$ requires 224.1525).

3,6-Diethoxy-2,5-dihydro-2-(3-tetrahydropyranyloxypropyl)pyrazine (20c). -

The diethoxydihydropyrazine (0.2 g, 1.2 mmole) was alkylated with 3-bromopropyl-1-tetrahydropyranyl ether (0.3 g, 1.2 mmole). The alkylated pyrazine was isolated as an oil after column chromatography (Al_2O_3, grade 3; eluted $CHCl_3$) (67%), δ 1.2(6H, t, J=3 Hz), 1.4-2.1 (10H, m), 3.3-3.8 (4H, m), 3.9-4.2 (7H, m), 4.5(1H, bs), ν_{max} 2980-2860, 1690, 1480-1440, 1370, 1345, 1240, 1135, 1120, 1030, 900, 815, 755 cm^{-1}, M^+ 312(7), 229(9), 228(77), 227(100), 211(31), 119(27), 183(45), 181(20), 169(47), 155(10), 154(11), 153(39), 85(84), 84(13),

71(12), 70(13), 67(12), 55(27). (Found: M^+ 312.2053. $C_{16}H_{28}N_2O_4$ requires 312.2050).

2,5-Diethoxy-3-(3-tetrahydropyranyloxypropyl)pyrazine (21). -

The dihydropyrazine (1 g, 3.2 mmole) was heated at reflux in dry benzene with dichlorodicyanobenzoquinone (DDQ; 0.73 g, 3.2 mmole) overnight. The mixture was cooled, filtered, concentrated, and chromotographed (Al_2O_3, grade III; 70 g, eluting with $CHCl_3$) to give the title pyrazine as an oil. Further purification was effected by distillation in vacuo (83%), b.p. 103° (2 mm Hg), δ 1.3(6H, t, J=3 Hz), 1.4-1.6 (6H, m), 2.06 (2H, t, J=2 Hz), 3.3-3.9(6H, m), 4.2 (4H, q, J=3 Hz), 4.5 (1H, bs), 7.6 (1H, s), ν_{max} 2940, 2860, 1630, 1440, 1340, 1260, 1200, 1130, 1115, 1070, 1030, 980 cm^{-1}, λ_{max} 345, 245 (ε 3125, 8750), M^+ 310(9), 226(81), 195(57), 182(100), 167(33), 153(48), 149(29), 126(33), 97(29), 85(90), 68(29), 57(43). (Found: M^+ 310.1896. $C_{16}H_{26}N_2O_4$ requires 310.1892).

2,5-Diethoxy-3-(3-hydroxypropyl)pyrazine (22). -

The (tetrahydropyranyloxypropyl)pyrazine (0.46 g) was hydrolysed by stirring with acidic ion exchange resin (0.26 g) for 3 h at room temperature in ethanol. The resultant slurry was filtered, concentrated, and chromatographed (Al_2O_3, grade III; 10 g) eluting successively with pet. eth. and chloroform. Concentration of the chloroform extract afforded the pyrazine (0.08 g) as an orange oil (21%), δ 1.38 (6H, t, J=3 Hz), 2.0 (2H, m), 2.84 (2H, t, J=2 Hz), 3.68 (2H, t, J=2 Hz), 4.28 (4H, m), 7.56 (1H, s), ν_{max} 3500-3200, 2990-2860, 1580, 1540, 1440, 1380, 1330, 1310, 1270,

1170, 1150, 1130, 1040, 920, 880 cm^{-1}, M^+ 226(44), 195(46), 182(100), 167(29), 154(24), 153(27), 139(22), 126(24), 125(24), 97(12), 68(12), 57(17). (Found: M^+ 226.1314. $C_{11}H_{18}N_2O_3$ requires 226.1317).

3,6-Diethoxy-2,5-dihydro-2-(1-hydroxybenzyl)pyrazine (23a). -

Diethoxydihydropyrazine (0.2 g, 1.2 mmole) was condensed with benzaldehyde (0.15 ml, 1.2 mmole). The aldol intermediate was obtained as white needles after workup and recrystallisation from pet. eth. (51%), m.p. 121-122°, δ 1.26 (6H, t, J=3Hz), 2.88-4.56 (8H, m), 5.12 (1H, bs, sharpens on D_2O exch.), 7.15 (5H, m), ν_{max} 3420, 1685, 1370, 1250, 1130, 1090, 1080, 1060, 1030, 725, 695 cm^{-1}, λ_{max} 208 nm (ε 8585). (Found: C, 65.61; H, 7.46; N, 10.11. $C_{15}H_{20}N_2O_3$ requires C, 65.21; H, 7.24; N, 10.14%).

3,6-Diethoxy-2,5-dihydro-2-(1-hydroxy-2-methylallyl)pyrazine (23b). -

Using the procedure detailed above, the diethoxydihydropyrazine (0.2 g, 1.2 mmole) was condensed with freshly distilled methacrolein (0.1 ml; 1.2 mmole). The residue, after workup, was chromatographed (Al_2O_3, grade III; 20 g) eluting with $CHCl_3$ subsequent p.l.c. ($CHCl_3$/2% MeOH) afforded one major condensation product as a deep yellow oil (45%), δ 1.28 (6H, t, J=3 Hz), 1.84 (3H, s), 2.40 (1H, bs, exch. D_2O), 4.2 (8H, m), 4.9 (2H, d, J = 2 Hz), ν_{max} 3600-3100, 2970, 2930, 1690, 1445, 1370, 1340, 1250, 1140, 1090, 1030, 940, 900, 890, 755 cm^{-1}. M^+ 240(3), 170(40), 169(35), 142(100), 141(40), 114(60), 113(54), 85(43), 70(54), 57(19). (Found: M^+ 170.1047. $C_{12}H_{20}N_2O_3$-C_4H_6O requires 170.1055).

3,6-Diethoxy-2,5-dihydro-2-(1-hydroxy-1-methylallyl)pyrazine (23c). -

Following the standard procedure, methyl vinyl ketone (0.05 ml; slight excess) was condensed with diethoxydihydropyrazine (0.1 g, 0.6 mmole), and the crude product was purified by p.l.c. ($CHCl_3$/2% MeOH) to give the title compound as a yellow oil (47%), δ 1.28 (6H, t), 1.4 (3H, s), 4.08 (7H, m), 5.5 (3H, m), ν_{max} 3450, 2970, 1685, 1440, 1370, 1340, 1240, 1135, 1085, 1030, 920, 755 cm^{-1}, M^+ 240(0.3), 224(2), 170(28), 169(7), 141(9), 113(8), 87(9), 85(67), 83(100), 48(9). (Found: M^+ 240.1473. $C_{12}H_{20}N_2O_3$ requires 240.1474).

Oxidation of 3,6-diethoxy-2,5-dihydro-2-(1-hydroxybenzyl)-pyrazine. -

The dihydropyrazine (0.1 g, 0.4 mmole) and DDQ (0.18 g, 0.8 mmole) were heated in refluxing dry benzene for 4½ h. The mixture was cooled overnight, filtered, concentrated, and the DDQ residues removed by p.l.c.($CHCl_3$/1% MeOH). Further purification by p.l.c. separated two products. The less polar compound was 2,5-diethoxy-3-benzoylpyrazine (25), obtained as an oil, δ 1.3 (6H, m), 4.24 (4H, m), 7.1-7.8 (6H, m), ν_{max} 3060-2840, 1675, 1597, 1580, 1460, 1440, 1380, 1345, 1300, 1165, 1155, 1130, 1090, 1040, 910, 815, 760, 715, 700 cm^{-1}, λ_{max} 207, 220, 253, 329 (ε 12000, 11000, 5857, 5230), M^+ 212(19), 243(12), 215(6), 149(12), 122(114), 105(100), 77(45). (Found: M^+ 272.1160. $C_{15}H_{16}N_2O_3$ requires 272.1161).

The more polar product was identified as the desired alcohol (24), also as an oil, δ 1.24-1.52 (6H, m), 4.2-4.6 (5H, m), 5.8 (1H, d, collapses to singlet on D_2O exch), 7.16-7.28 (5H, m), 7.56 (1H, s),

ν_{max} 3600-3300, 3100-2800, 1440, 1210, 1050, 920, 755, 665 cm^{-1}, M^+ 274(69), 246(51), 217(14), 169(100), 141(33), 105(31), 83(20), 77(27). (Found: M^+ 274.1818. $C_{15}H_{18}N_2O_3$ requires 274.1317).

3,6-Diethoxy-2,5-dihydro-2,5-di(1-hydroxybenzyl)pyrazine (26).-

The usual procedure was followed to condense the mono-addition product of the diethoxydihydropyrazine with benzaldehyde (0.08 g, 0.3 mmole) with a second molecule of benzaldehyde. The initial deep yellow colour of the solution of the mono-condensation product with lithium diisopropylamide (2 eq) was discharged on addition of benzaldehyde (0.06 ml, 0.3 mmole). Workup as usual afforded, after chromatography (Al_2O_3, grade III) eluting with pet. eth., an isomeric mixture of the condensation products as an oil which slowly crystallised on standing (52%), δ 1.28 (6H, t, J=4Hz), 3.60-5.16 (6H, m), 7.00-7.32 (10H, m), ν_{max} 3350, 2980-2880, 2240, 1685, 1440, 1370, 1340, 1250, 1140, 1080, 1030, 960, 730, 700 cm^{-1}, M^+-$PhCH_2OH$ 274(5), 258(5), 245(2), 170(100), 142(39), 141(37), 114(26), 113(32), 106(47), 105(58), 85(21), 77(58). (Found: M^+ 274.1318. $C_{15}H_{18}N_2O_3$ requires 274.1317).

3,6-Diethoxy-2,5-dihydro-2-(1-hydroxy-1-methylethyl)pyrazine (27). -

Following the standard procedure, the diethoxydihydropyrazine (0.5 g, 2.9 mmole) was condensed with acetone (0.2 ml, 2.9 mmole) using lithium diisopropylamide (3.2 mmole) as base. After elution through a column of Al_2O_3 (grade III, 10 g) with 40-60° petroleum ether, the (hydroxymethylethyl)pyrazine was obtained as a pale yellow oil (51%), δ 1.16 (12H, t and s), 3.44 (1H, exch. D_2O), 4.04-4.3 (7H, m), ν_{max} 3440, 2970, 2930, 2900,

1685, 1460, 1370, 1335, 1240, 1140, 1080, 1030, 910, 820, 790, 760 cm^{-1}, M^+ 228(3), 213(6), 170(100), 169(50), 142(28), 141(44), 113(39), 87(17). (Found: M^+ 228.1468. $C_{11}H_{20}N_2O_3$ requires 228.1474).

3,6-Diethoxy-2-(1-hydroxy-1-methylethyl)pyrazine (28). -

The dihydropyrazine (27) (0.42 g, 1.9 mmole) and DDQ (0.43 g, 1.9 mmole) were heated in refluxing dry benzene for 1h. The mixture was cooled, filtered, concentrated, and the residue eluted through Al_2O_3 (grade III; 10 g) with 40-60° petroleum ether. The pyrazine was obtained as an orange oil (52%), δ 2.16 (6H, t, J=4 Hz), 1.46 (6H, s), 3.60 (1H, bs), 4.2 (4H, m), 7.6 (1H, s), ν_{max} 3450, 2970, 1450, 1370, 1335, 1260, 1160, 1095, 1040, 920, 880, 780, 750 cm^{-1}, M^+ 226(31), 211(100), 183(62), 155(46), 137(23), 93(15), 72(15), 59(23), 57(23). (Found: M^+ 226.1316. $C_{11}H_{18}N_2O_3$ requires 226.1317).

2,5-Diethoxy-3-isopropenylpyrazine (29). -

The pyrazine (28) (0.089 g) was heated in refluxing dry benzene for 1 h with a trace of toluene-p-sulphonic acid and molecular sieves. The mixture was cooled, filtered, and concentrated. Purification by p.l.c. ($CHCl_3$/1% MeOH) afforded the dehydration product as the major component, δ 1.46 (6H, t, J=4 Hz), 2.2 (3H, s), 4.2-4.4 (4H, q, J=4 Hz), 5.9 (2H, d, J=15 Hz), 7.76 (1H, s), ν_{max} 2980, 1430, 1380, 1340, 1310, 1190, 1110, 1040, 1030, 920 cm^{-1}, M^+ 208(100), 194(19), 182(25), 181(13), 166(25), 154(31), 151(25), 150(25), 125(25), 108(19), 92(63), 73(25), 69(31), 57(69). (Found:

M^+ 208.1214. $C_{11}H_{16}N_2O_2$ requires 208.1212).

Attempted alkylation of 2,5-diethoxy-3-isopropenylpyrazine (28). -

Using the standard procedure for alkylations, a solution of the substituted pyrazine (28) (0.064 g, 0.3 mmole) in dry THF was added to a cold, stirred solution of lithium diisopropylamide (0.4 mmole) in dry THF. An intense red colouration was produced. After stirring for 90 min, redistilled chloromethylmethyl ether (0.03 ml, 0.3 mmole) was added. Stirring was continued overnight. Workup afforded unchanged starting material as the only component isolated from the organic layer (n.m.r. spectrum).

3,6-Diethoxy-2,5-dihydro-2-(1-hydroxy-1-methylpropyl)-5-methylallyl-pyrazine (30). -

Using the normal procedure, the methylallyldihydropyrazine (20b) (0.20 g, 0.9 mmole) was reacted with ethylmethyl ketone (0.1 ml, 0.9 mmole). After stirring for $3\frac{3}{4}$ h, the solution was worked up as usual. The fluid, yellow oil so obtained was chromatographed (Al_2O_3, grade III; 20 g), eluting successively with pet. eth. and $CHCl_3$ to give the title compound (0.17 g) as a mixture of isomers (61%), δ 0.8-1.4 (14H, m), 1.68 (3H, s), 2.45 (2H, m), 3.9-4.4 (7H, m), 4.7 (2H, d, J=17 Hz), ν_{max} 3560, 3480, 2980, 2920, 1690, 1440, 1370, 1240, 1140, 1090, 1030, 890 cm^{-1}, M^+ 296(2), 267(8), 224(30), 195(8), 169(100), 141(88), 113(32), 85(34), 73(22), 55(44). (Found: 296.2102. $C_{16}H_{28}N_2O_3$ requires 296.2100).

2,5-Diethoxy-3-methylallyl-6-(1-hydroxy-1-methylpropyl)pyrazine (31). -

The starting material (30) (0.12 g, 0.4 mmole) and DDQ (0.10 g, 0.4 mmole) were heated in refluxing dry benzene for 1½ h. The mixture was cooled, filtered and concentrated. After purification by p.l.c. ($CHCl_3$), the product (31) was isolated as an orange oil contaminated with the diethoxymethylallylpyrazine, δ 0.7 (3H, t, J=3 Hz), 1.36 (6H, t, J=4 Hz), 1.52 (3H, s), 1.76 (3H, s), 1.96 (2H, q, J=3 Hz), 3.44 (2H, s), 4.36 (4H, m), 4,76 (2H, d, J=6 Hz), 4.90 (1H, exch. D_2O), ν_{max} 3460, 2960, 1650, 1420, 1380, 1340, 1190, 1090, 1040, 890 cm^{-1}, M^+ 294(5), 266(32), 265(100), 237(29), 222(35), 221(31), 207(27), 193(19), 165(21), 82(18), 55(20). (Found: M^+ 294.1939, $C_{16}H_{26}N_2O_3$ requires 294.1943).

2,5-Diethoxy-3-methylallylpyrazine. -

The corresponding dihydropyrazine (20b) was heated in refluxing dry benzene with DDQ (0.19 g) for 1½ h. After workup as above and chromatography on Al_2O_3 (grade III), eluting with pet. eth., the title pyrazine was obtained as an orange oil, δ 1.40 (6H, t, J=3 Hz), 1.80 (3H, s), 3.46 (2H, s), 4.3 (4H, dq), 4.76 (2H, d, J=4 Hz), 7.58 (1H, s), ν_{max} 2970, 2920, 1440, 1380, 1350, 1310, 1280, 1260, 1215, 1155, 1130, 1090, 1040, 890, 740 cm^{-1}, M^+ 222(100), 221(85), 207(77), 193(46), 179(38), 165(62), 151(30), 82(38), 55(46). (Found: M^+ 222.1362. $C_{12}H_{18}N_2O_2$ requires 222.1368).

3,6-Dibenzyl-2-ethoxy-5-hydroxypyrazine (32). -

The 3,6-dibenzyl-2,5-diethoxypyrazine[46] (0.2 g, 0.6 mmole) in dry dichloromethane was added dropwise and with stirring over a

period of 40 min to a cold (-78°), stirred solution of boron tribromide (0.1 ml, 1.2 mmole) in dry dichloromethane. The resultant slurry was allowed to warm gradually to room temperature, and stirring was continued overnight. The orange slurry was stirred with water for 20 min. The organic layer was separated, dried and concentrated. Chromatography (Al_2O_3, grade III; 60 g) eluting consecutively with 40-60° petroleum ether and 40-60° petroleum ether/chloroform afforded the title compound (20 mg) as pale yellow clusters of fine needles after recrystallisation from pet. eth. (10%), m.p. 155-157°, δ 1.36 (3H, t, J=4 Hz), 3.96 (2H, s), 4.04 (2H, s), 4.28 (4H, q, J=4 Hz), 7.12-7.4 (10H, m), ν_{max} 3700-3000, 1630, 1440, 1420, 1330, 1260, 1170, 1120, 1040, 900 cm^{-1}, M^+ 320(100), 305(11), 292(26), 291(31), 263(8), 214(10), 187(8), 146(11), 119(35), 118(34), 105(18), 91(90), 85(18), 82(29), 77(16). (Found: M^+ 320.1519. $C_{20}H_{20}N_2O_2$ requires 320.1525).

Treatment of 2,5-diethoxy-3-methallylpyrazine with boron tribromide. - The pyrazine (0.1 g, 0.45 mmole) in dry dichloromethane was added dropwise and with stirring to a cold (-78°) solution of boron tribromide (0.1 ml, 0.9 mmole) in dry dichloromethane. The resultant yellow slurry was allowed to warm gradually to room temperature, during which time the precipitate dissolved. After stirring overnight, the solution was washed with two portions of saturated aqueous sodium chloride solution, dried, and concentrated to give a complex mixture of pyrazinoid compounds (t.l.c. and n.m.r. spectrum) as a solid residue.

3,6-Dihydro-5-ethoxy-2-oxo-1(1H)-pyrazine (33). -

3,6-Diethoxy-2,5-dihydropyrazine (0.10 g, 0.6 mmole) was added as a solution in dry dichloromethane dropwise and with stirring to a cold (-78°) solution of boron tribromide (0.11 ml, 1.2 mmole) in dry dichloromethane. A white precipitate formed during the addition. The mixture was allowed to warm gradually to room temperature and was stirred overnight. Extraction with saturated aqueous sodium chloride solution and concentration of the aqueous layer afforded the mono-dealkylated compound as a white solid (50%), m.p. 168-171° (subl.), δ (D_2O) 1.32 (3H, t, J = 3 Hz), 3.96 (4H, s), 4.32 (2H, q, J=3 Hz), ν_{max} 3400-3200, 1740, 1610, 1230, 1090, 1040, 1000, 900, 850, 800 cm^{-1}.

Treatment of the diethoxydihydropyrazine with 4 equivalents of boron tribromide, under the same conditions, again resulted mainly in mono-dealkylation.

Singlet oxygen oxygenations.

All singlet oxygen additions were performed in dichloromethane (10 ml) with a dye sensitiser. This sensitiser was most frequently methylene blue (1-2 mg), but polymer-bound rose bengal and eosin[97,98] were also used. Oxygen was bubbled through the solution during overnight irradiation with a 350 watt tungsten lamp. The solution was water cooled. Polymer-bound sensitisers were removed by filtration; methylene blue was removed chromatographically. The peroxides were purified by p.l.c.

3,6-Dibenzyl-2,5-diethoxy-3,6-epidioxy-3,6-dihydropyrazine (49). -

A solution of 3,6-dibenzyl-2,5-diethoxypyrazine (0.06 g) and methylene blue (1 mg) was photooxygenated. After 19 h, t.l.c. showed complete conversion of starting material. The mixture was purified by p.l.c. ($CHCl_3$) to give the title peroxide in virtually quantitative yield as a colourless oil, δ 1.34 (6H, t, J=6 Hz), 3.36 (4H, s), 4.24 (4H, q, J=6 Hz), 7.34 (10H, m), ν_{max} 3140-2820, 1745, 1630, 1500, 1375, 1325, 1225, 1080, 1040, 1020, 865, 800, 750, 695 cm^{-1}. (Found: C, 69.29; H, 6.23; N, 7.18, $C_{22}H_{24}N_2O_4$ requires C, 69.47; 6.32; N, 7.39%).

Control experiments

(a) Dibenzyldiethoxypyrazine (20 mg) in dichloromethane and under oxygen was irradiated for 19 h with external cooling. Traces of the peroxide were observed (t.l.c., n.m.r. spectroscopy).

(b) The pyrazine (20 mg) in dichloromethane with methylene blue was irradiated for 19 h. Traces of the peroxide were observed (t.l.c., n.m.r. spectroscopy).

Photooxygenation of 3-methylisoquinolinium methiodide (50). -

3-Methylisoquinolinium methiodide (0.05 g) was photo-oxidised as above. A dichloromethane solution of the residue was washed with aqueous 10% sodium hydroxide solution (3 x 20 ml). The organic layer was dried, concentrated, and purified by p.l.c. ($CHCl_3$/MeOH, 10:1) to yield the isoquinolone (51).[99] For identification, the isoquinolone was prepared by shaking the methiodide salt (0.1 g) with aqueous 10% sodium hydroxide

solution, extracting with dichloromethane, and purifying by p.l.c., δ 2.4 (3H, s), 3.6 (3H, s), 6.4 (1H, s), 7.2-8.4 (4H, m).

Attempted photooxygenation of 2,6-dimethylpyrimidone (52). -

The pyrimidone (0.03 g) was photooxidised. After irradiation, the solution was washed with 10% aqueous potassium iodide solution, dried, and the solvent removed. T.l.c. ($CHCl_3$) showed a complex mixture of highly coloured products.

4,6-Diethoxy-2-methylpyrimidine (54). -

4,6-Dihydroxy-2-methylpyrimidine (53) (0.1 g, 0.8 mmole) was suspended in dry dichloromethane and a solution of triethyloxonium fluoroborate (1.0 g) in dry dichloromethane was added. The resultant slurry was heated at reflux until the solids dissolved ($4\frac{1}{2}$ h). The solution was cooled, washed successively with saturated aqueous sodium hydrogen carbonate solution, dried and concentrated. Purification by p.l.c. ($CHCl_3$/1% MeOH) afforded the alkylation product (20 mg) as an oil. The small quantity of material isolated is a reflection of the volatility of the dialkoxy pyrimidine (12%), δ (CCl_4) 1.0 (6H, t, J=3 Hz), 2.08 (3H, s), 3.94 (4H, q, J=3 Hz), 5.30 (1H, s), ν_{max} 2980, 2920, 2900, 2860, 1600-1530, 1380, 1350, 1340, 1300, 1240, 1170, 1070, 1020, 990 cm^{-1}, M^+ 182(6), 177(35), 154(11), 139(23), 121(29), 119(100), 117(97), 84(45), 72(29), 68(23), 49(45).

4,6-Diethoxy-2,5-epidioxy-2-methyl-2,5-dihydropyrimidine (55). -

The dihydroxypyrimidine (53) (0.11 g) was heated at

reflux with triethyloxonium fluoroborate (1.0 g) overnight in dry dichloromethane under nitrogen. The solution was cooled, filtered, and photooxidised for 8 h. After this time, the solution was poured into saturated aqueous sodium hydrogen carbonate solution, washed with water, dried, and purified by p.l.c. ($CHCl_3$) to afford the endoperoxide (55), in high yield as an unstable solid melting below room temperature, δ 1.33 (6H, t, J=6 Hz), 2.50 (3H, s), 4.33 (4H, q, J=6 Hz), 5.8 (1H, s), ν_{max} 2940, 1585, 1420, 1385, 1347, 1250, 1155, 837 cm^{-1}.

2,6-Dimethyl-4-ethoxypyrimidine (56). -

2,6-Dimethyl-4-hydroxypyrimidine (52) (0.07 g, 0.5 mmole) was dissolved in dry dichloromethane, and a solution of triethyloxonium fluoroborate (2 g) was added. The resultant solution was heated at reflux for 2 h. After this time, the solution was cooled, washed with saturated aqueous sodium hydrogen carbonate solution and water, dried, and concentrated. Purification by p.l.c. ($CHCl_3$/ 1% MeOH) afforded the title compound as an oil in low yield. Considerable material was lost during workup due to evaporation (12%),δ1.0 (3H, t, J=3 Hz), 1.96 (3H, s), 2.08 (3H, s), 3.96 (2H, q, J=3 Hz), 5.80 (1H, s), ν_{max} 2980, 2920, 2860, 1730, 1590, 1440, 1400, 1380, 1365, 1340, 1170, 1070, 1030, 960, 840 cm^{-1}, M^+ 152(9), 137(20), 124(17), 121(30), 118(86), 117(100), 86(29), 84(51), 82(17), 67(17), 49(37).

2,6-Dimethyl-2,5-epidioxy-4-ethoxy-2,5-dihydropyrimidine (57). -

To a solution of the pyrimidine (52) (0.1 g) in dry

dichloromethane was added a solution of triethyloxonium fluoroborate (2 g) in dry dichloromethane. The solution was stirred at room temperature under nitrogen for 2 h and then irradiated overnight in the presence of oxygen and methylene blue. The photooxidation mixture was poured into saturated aqueous sodium hydrogen carbonate solution and shaken gently until vigorous gas evolution ceased. The organic layer was separated, washed with water, dried, and the solvent removed. The residue was purified by p.l.c. ($CHCl_3$/1% MeOH) to give the endoperoxide (57) as an unstable oil, δ 1.4(3H, t, J=6 Hz), 2.4 (3H, s), 2.6 (3H, s), 4.43 (2H, q, J=6 Hz), 6.55 (1H, s), ν_{max} 2950, 1605, 1355, 1187, 1090, 750 cm^{-1}.

Photooxygenation of 5-butyl-4,6-dihydroxy-2-methylpyrimidine (58). -

A suspension of the butylpyrimidine (0.042 g) was treated with singlet oxygen. After overnight irradiation, no evidence of addition was observed (physical properties, t.l.c.). A sample of the pyrimidine (0.044 g) was then heated at reflux in dry dichloromethane with ethyl Meerwein salt (0.41 g) under nitrogen. Workup with saturated aqueous sodium hydrogen carbonate solution and purification by p.l.c. ($CHCl_3$/1% MeOH) as before gave three peroxidic products, which were not characterised further.

Attempted photooxygenation of α-pyridone (59). -

α-Pyridone (0.10 g) was subjected to the singlet oxygen addition conditions overnight. After workup and purification by p.l.c., unchanged starting material was recovered (n.m.r.

spectroscopy).

Attempted photooxygenation of 3-hydroxy-1-methylpyridinium betaine (60). -

3-Hydroxy-1-methylpyridine methiodide (0.05 g) was stirred overnight with basic ion exchange resin (0.20 g) in dry acetonitrile at room temperature. After filtration, the pale violet solution was irradiated overnight in the presence of oxygen and methylene blue. No reaction was detectable by t.l.c. (MeOH).

2,5-Diethoxy-3,6-dimethyl-3,6-epidioxy-3,6-dihydropyrazine (61). -

The pyrazine was prepared by the published route[46]. Subjection to the singlet oxygen conditions, as above, led to the quantitative formation of the endoperoxide, purified by p.l.c. ($CHCl_3$), m.p. 81°(dec.), δ 1.33 (6H, t, J=7 Hz), 3.63 (6H, s), 4.23 (4H, q, J=7 Hz), ν_{max} 1625, 1320, 1275, 1140, 1012, 905, 730, 647 cm^{-1}, M^+ 228(3), 196(100), 181(43), 168(31), 140(45), 112(20), 111(21), 86(30), 70(25), 71(33). (Found: C, 52.48; H, 7.13; N, 12.30. $C_{10}H_{16}N_2O_4$ requires C, 52.63; H, 7.02; N, 12.28%).

Control Experiments

(a) A sample of diethoxydimethylpyrazine was irradiated in dichloromethane for 19 h under a stream of oxygen in the absence of a sensitiser. No peroxide was detected (t.l.c., starch-iodide test).

(b) A sample of the pyrazine was stirred in the dark in dichloromethane under a slow stream of oxygen in the presence of methylene

blue. Traces of peroxide were observed (t.l.c.).

3,6-Dibenzyl-3,6-epidioxy-2,5-dioxopiperazine (62). -

The dibenzyldihydroxypyrazine[100] (0.1 g) was suspended in a mixture of dimethylsulphoxide and dichloromethane (1:10). The suspension was photooxygenated for 45 h. The mixture was filtered and concentrated to give the 3,6-peroxide (62) as an insoluble yellow solid. δ 2.9 (4H, s), 7.3 (10H, m), ν_{max} 3600-2650, 1690, 1640, 1370, 1290, 1135, 1020, 1000, 760, 700 cm^{-1}, λ_{max} 313, 220 (ε 3.1 x 10^3, 7.7 x 10^3), M^+ 324(0.6), 306(2.6), 290(4.2), 105(44), 94(39), 91(36), 84(100), 79(76), 77(32), 66(100), 46(69).

3,6-Dibenzyl-3,6-dihydroxy-2,5-dioxopiperazine (63). -

The dibenzylperoxide (62) (0.03 g) was stirred with an excess of sodium borohydride in ethanol at room temperature for 1 h. The solution was neutralised with 10% hydrogen chloride solution, filtered, and concentrated to leave the dihydroxydioxopiperazine (63) as a pale yellow, high melting solid. Attempted purification by p.l.c. (MeOH) resulted in decomposition, δ 1.03 (4H, s), 7.3 (10H, m), ν_{max} 3700-3000, 1650, 1400, 1185 cm^{-1}, λ_{max} 287, 210 (ε 2.52 x 10^3, 5.14 x 10^2), $M^+ - H_2O_2$ 292(29), 290 (34), 276(18), 201(34), 153(18), 118(34), 117(28), 103(13), 91(100).

3,6-Dibenzylideny1-2,5-dioxopiperazine (64). -

The dihydroxydioxopiperazine (63) was refluxed in methanolic benzene with toluene-p-sulphonic acid and molecular

sieves. After refluxing overnight, the benzylidenyldioxopiperazine was isolated, and was identical to an authentic sample[24,85,86] (i.r. and m.s. spectroscopy), m.p. 298° (dec.).

Attempted oxygenation of 2,5-diethoxypyrazine. -

The pyrazine was prepared by the literature method[46]. The pyrazine (0.09 g, 0.5 mmole) was dissolved in dichloromethane and irradiated under a slow stream of oxygen in the presence of polymer-bound rose bengal (0.08 g). After overnight irradiation and standard workup, no starting material remained, but a mixture of products had been produced. This mixture was not examined further.

Attempted oxygenation of 2,5-diethoxy-3-(3-hydroxypropyl)pyrazine (22). -

The hydroxypropylpyrazine (22) was dissolved in dichloromethane and irradiated under a slow stream of oxygen in the presence of polymer-bound rose bengal (0.08 g) overnight. The mixture was filtered, and the filtrate washed with saturated aqueous potassium iodide solution until evolution of yellow colouration ceased and the starch-iodide test was negative. After drying, concentrating, and purifying by p.l.c. ($CHCl_3$/ 1% MeOH), no starting material remained, but a mixture of products was formed which was not investigated further.

3,6-Diethoxy-2,5-epidioxy-2-methallyl-5-(1-hydroxy-1-methylpropyl)-2,5-dihydropyrazine (65). -

The pyrazine (31) (0.058 g) was photooxidised with polymer-bound rose bengal (0.08 g) in dichloromethane under a slow stream of oxygen for $6\frac{1}{2}$ h; all starting material had, by then, been consumed. The mixture was filtered to remove the polymer-bound sensitiser, filtered again through silica to remove dye residues leached into solution, and concentrated to give the peroxide as a yellow oil, δ 1.0 (3H, t, J=3 Hz), 1.28-1.44 (9H, m), 1.92 (3H, s), 1.72-2.04 (2H, q, J=3 Hz), 2.8 (2H, d, J=2 Hz), 4.14-4.28 (4H, m), 4.96 (2H, s), ν_{max} 3540, 2980, 2920, 1720, 1600, 1440, 1380, 1240, 1020, 890 cm^{-1}., M^+-O_2 294(5), 265(100), 237(20), 222(43), 221(38), 207(29), 193(20), 165(20), 128(14), 110(12), 84(20), 83(25), 56(54). (Found: M^+ 294.1939. $C_{16}H_{26}N_2O_3$ requires 294.1943).

Attempted photooxidation of 1-acetyl-3-isobutylidenyl-2,5-dioxo-piperazine (70). -

The isobutylidenyl derivative (70) was prepared by the published route[26,27]. The derivative (0.03 g) was treated overnight with singlet oxygen. After reduction with aqueous 10% potassium iodide solution and purification by p.l.c. ($CHCl_3$/MeOH, 10:1), starting material was recovered (n.m.r. spectroscopy).

Reaction of the peroxide (61) with triphenylphosphine. -

To a solution of 2,5-diethoxy-3,6-dimethyl-3,6-epidioxy-3,6-dihydropyrazine (0.035 g, 0.15 mmole) in dry benzene was added a solution of triphenylphosphine (0.80 g, 3.0 mmole) in dry benzene. After stirring overnight at room temperature, the reaction product (73) was isolated by p.l.c. ($CHCl_3$). Attempts to

reproduce this experiment were of limited success, the product ether (73) evidently being very unstable, δ 1.7 (6H, t, J=7 Hz), 2.06 (6H, s), 4.07 (4H, q, J=7 Hz).

Reduction of 2,5-diethoxy-3,6-dimethyl-3,6-epidioxy-3,6-dihydropyrazine (61). -

(a) With potassium iodide

The peroxide (0.09 g) was dissolved in a saturated ethanolic potassium iodide solution, and the resulting solution was stirred at room temperature for 30 min. The solution was then neutralised with aqueous hydrochloric acid, filtered, and concentrated. The residue was dissolved in chloroform, washed with water, dried, and concentrated. After p.l.c. ($CHCl_3$) a crystalline solid was obtained, formulated originally as the diol (74) but later as the triethoxy compound (75), m.p. 76-77°, δ 1.2-1.96 (15H, m), 2.0 (1H, bs,exch. D_2O), 3.4 (6H, q, 5=6 Hz), ν_{max} 3000-2900, 1625, 1440, 1365, 1320, 1270, 1210, 1145, 1100, 895, 860, 805, 745 cm^{-1}.

(b) With sodium borohydride in ethanol

To a solution of the peroxide (0.07 g, 0.5 mmole) in ethanol was added sodium borohydride (0.01 g). The mixture was stirred at room temperature for 1 h, by which time starting material had been consumed (t.l.c., $CHCl_3$). The mixture was neutralised with 10% aqueous hydrochloric acid, filtered, and concentrated. The residue was dissolved in chloroform, washed with water, dried, and concentrated to give the reduction product in good yield.

(c) With sodium borohydride in aqueous THF

The diethoxydimethylpyrazine peroxide (0.10 g) was stirred in aqueous, peroxide-free THF for $5\frac{1}{2}$ h with excess sodium borohydride at room temperature. The solution was neutralised with 2N aqueous hydrochloric acid, filtered, and concentrated to give the reduction product, formulated as a cis, trans mixture of the diols (74) as a pale yellow oil, δ 1.30 (6H, t, J=4 Hz), 1.56 (3H, s), 1.62 (3H, s), 2.16 (2H, bs, exch. D_2O), 4.08-4.32 (4H, m), ν_{max} 3390, 3320, 2980, 1740, 1670, 1440, 1360, 1340, 1270, 1200, 1140, 1100, 1020, 960, 900, 850, 780, 750, 710 cm^{-1}, M^+ 215(41), 174(47), 142(100), 128(21), 114(26), 112(32), 87(21), 61(38).

Reduction of 2,5-diethoxy-3,6-epidioxy-3-(1-hydroxy-1-methylpropyl)-6-methallyl-3,6-dihydropyrazine (65). -

(a) With potassium iodide.

The peroxide (0.08 g) was dissolved in aqueous acetone and stirred with potassium iodide for 30 h (shorter reaction times left the starting material largely unchanged). The solution was poured into ethyl acetate, and washed with water, 2 x with aqueous sodium thiosulphate solution, and again with water. The organic layer was separated, dried, and concentrated. Only decomposition products were observed in the residual oil.

(b) With sodium borohydride.

The peroxide (0.09 g) was dissolved in freshly distilled THF. Water and excess sodium borohydride were added, and the

mixture was stirred at room temperature for 1 h (monitored by starch-iodide tests and t.l.c.). The mixture was neutralised with 2N hydrochloric acid, filtered, and poured into dichloromethane. The organic layer was separated, dried, and concentrated to yield a minute quantity of starting material (n.m.r. evidence).

REFERENCES

1. T. Miyoshi, N. Miyairi, H. Aoki, M. Kohsaka, H. Sakai, and H. Imanaka, J. Antibiotics, 1972, 25, 569.

2. S. Miyamura, N. Ogasawara, H. Otsaka, S. Niwayama, N. Tanaka, T. Take, T. Uchigama, and H. Ochiai, J. Antibiotics, 1973, 26, 479.

3. H. Saka, T. Miyoshi, N. Miyairi, H. Aoki, M. Kosaka, and H. Imanaka, Japan Kokai, 74 94, 898 (Cl. 36(2) D914) 9 Sept. 1974. Appl. 73, 11, 404, 27 Jan. 1973.

4. Y. Tokumi, S. Koda, T. Miyoshi, and Y. Morimoto, Bull. Chem. Soc. Jap., 1974, 47, 18.

5. T. Kamiya and S. Maeno, Belg. 815, 530 (Cl. C12d), 25 Nov. 1974. Appl. 815, 530, 24 May 1974.

6. _____, Ger. Offen. 2, 421, 427 (Cl. CO7D), 13 Nov. 1975, Appl. p. 24 21, 427.5, 03 May 1974.

7. _____, S. African 74 02, 795 (Cl. CO7D), 26 Mar. 1975. Appl. 74 2795, 02 May 1974.

8. T. Kamiya, S. Maeno, M. Hashimoto, and Y. Mine, J. Antibiotics, 1972, 25, 576.

9. _____, Ger. Offen. 2, 344, 927 (Cl. CO7d), 14 Mar. 1974, Japan. Appl. 72, 90, 675, 8 Sept. 1972.

10. J. V. Scudi, J. Amer. Chem. Soc., 1937, 59, 1403.

11. P. M. Pojer and I. D. Rae, Aust. J. Chem., 1972, 25, 1737.

12. A. Wohl and L. H. Lips, Ber., 1907, 40, 2312.

13. G. DuPont, Ann. Chim. (Paris), 1913, 30, 535.

14. F. Leonard, A. Wajngurt and A. Horn, J. Org. Chem., 1956, 21, 1402.

15. H. A. Bruson, F. W. Grant and E. Bobko, J. Amer. Chem. Soc., 1958, 80, 3635.

16. A. W. Johnson, U.S. 2, 468, 881, 3 May 1949; Brit. 597, 119, 19 Jan. 1948.

17. W. Reppe, et al., Annalen, 1955, 596, 38.

18. _____, ibid., 112.

19. H. Wynberg, J. Amer. Chem. Soc., 1958, 80, 364.

20. H. Wynberg and A. Bantjes, Org. Syn. Coll. Vol. 4, 1963, 534.

21. E.G.E. Hawkins, J. Chem. Soc., 1959, 248.

22. R.J. Oullette and R.J. Bertsch, J. Org. Chem., 1976, 41, 2782.

23. K. Ogura, M. Yamashita, S. Furukawa, M. Suzuki, and G. Tsuchihashi, Tetrahedron Letters, 1975, 2767.

24. T. Sasaki, Ber., 1921, 54B, 163.

25. K. W. Blake and P. G. Sammes, J. Chem. Soc. (C), 1970, 980.

26. C. Gallina and A. Liberatori, Tetrahedron Letters, 1973, 1135.

27. _____ , Tetrahedron, 1974, 30, 667.

28. B. W. Dominy and R. G. Lawton, J. Org. Chem., 1969, 34, 2013.

29. A.E.A. Porter and P.G. Sammes, J. Chem. Soc. (C), 1970, 2530.

30. S. Rajappa and B.G. Advani, J.C.S. Perkin I, 1975, 349.

31. Courtesy of Dr. P. J. Machin.

32. Y. Ogata, A. Kawasaki and K. Yokai, J. Chem. Soc. (B), 1967, 1013.

33. J.T. Hays, G.F. Hager, H.M. Engleman, and H.M. Spurlin, J. Amer. Chem. Soc., 1951, 73, 5369.

34. R.H. Hall and D.S. Stern, J. Chem. Soc., 1950, 490.

35. J.J. Tufariello and J.J. Tegeler, Tetrahedron Letters, 1976, 4037.

36. A.I. Meyers, S. Schwartzman, G.C. Olson and H.-C. Cheung, Tetrahedron Letters, 1976, 2417.

37. E. Abderhalden and W. Stix, Z. Physiol. Chem., 1924, 132, 238.

38. D.H.R. Barton, P.G. Sammes, M.V. Taylor and E. Wierstuck, J. Chem. Soc. (C), 1970, 1977.

39. H. Meerwein in Houben-Weyl, "Methoden der Organischen Chemie," 4th ed., vol. 6., no. 3, D. Müller, ed., Georg Thieme Verlag, Stuttgart, 1965, p. 329.

40. Toyo Rayon Co., Ltd., Fr. 1, 409, 957 (Cl. C08 g), Sept. 3, 1965, Appl. Aug. 6, 1964.

41. A.N. Nesmeyanov, L.G. Makarova and T.P. Tolstaya, Tetrahedron, 1957, 1, 145.

42. R. Kreher, Ang. Chem., Int. Ed. Engl., 1973, 12, 1022.

43. T.J. Curphey, Org. Syn., 1971, 51, 142.

44. F. Johnson, L.G. Duquette, W.L. Parker and W.A. Nasutavicus, J. Org. Chem., 1974, 39, 1434.

45. T. Kato, A. Takada and T. Ueda, Chem. Pharm. Bull., 1974, 22, 984.

46. K.W. Blake, A.E.A. Porter, and P.G. Sammes, J.C.S. Perkin I, 1972, 2494.

47. A.I. Meyers, A. Nabeya, H.W. Adiches, and J.R. Politzer, J. Amer. Chem. Soc., 1969, 91, 763.

48. A.I. Meyers, H.W. Adiches, J.R. Politzer and W.N. Beverung, ibid, 765.

49. A.I. Meyers, et al., ibid, 764.

50. H.W. Adiches, J.R. Politzer and A.I. Meyers, ibid., 2155.

51. A.I. Meyers, et al., J. Org. Chem., 1973, 38, 36.

52. B. M. Trost and R.A. Kunz, J. Amer. Chem. Soc., 1975, 97, 7152.

53. K.W. Blake, Ph.D. Thesis, University of London, 1970.

54. E.A. Braude, Quart. Rev., 1950, 4, 404.

55. E.I. Klimova and Y.A. Arbuzov, Dokl. Akad. Nauk. SSSR, 1970, 190, 102; Chem. Abs. 72:111143e.

56. Y. Sugimura, K. Iino, Y. Iwano, T. Saito, and T. Hiraoka, Tetrahedron Letters, 1976, 1307.

57. T.B. Windholz and D.B.R. Johnson, Tetrahedron Letters, 1967, 2555.

58. D. St. C. Black and A.M. Wade, J.C.S. Chem. Commun., 1970, 871.

59. A.E.A. Porter, personal communication.

60. G.B. Bachman and R.M. Schisla, J. Org. Chem., 1957, 22, 858.

61. G.B. Bachman and R.M. Schisla, ibid., 1302.

62. V.K. Smith, Jr., and S. Kushner, U.S. 2, 677, 686, May 4, 1954.

63. J.D. Behun and R. Levine, J. Amer. Chem. Soc., 1959, 81, 5157.

64. M.R. Kamal and R. Levine, J. Org. Chem., 1964, 29, 191.

65. T. Caronna, G.P. Gardini and F. Minisci, J.C.S. Chem. Commun., 1969, 201.

66. P. deMayo and S.T. Reid, Chem. Ind. (London), 1962, 1576.

67. H.H. Wasserman and A. Liberles, J. Amer. Chem. Soc., 1960, 82, 2086.

68. J. Martel, Compt. Rend., 1957, 244, 626.

69. G.O. Schenck, Annalen, 1953, 584, 156.

70. T. Saito, I. Moritani, and M. Matsuyama, Tetrahedron Letters, 1969, 5113.

71. H.H. Wasserman and W. Strehlow, Tetrahedron Letters, 1970, 795.

72. H.H. Wasserman, K. Stiller and M.B. Floyd, Tetrahedron Letters, 1968, 3277.

73. H.H. Wasserman, Ann. N.Y. Acad. Sci., 1970, 171, 108.

74. T. Matsuura and I. Saito, J.C.S. Chem. Commun., 1967, 693.

75. _____, Tetrahedron, 1968, 24, 6609.

76. H.H. Wasserman, F.J. Vinick, and Y.C. Chang, J. Amer. Chem. Soc., 1972, 94, 7180.

77. H.H. Wasserman and E. Druckrey, J. Amer. Chem. Soc., 1968, 90, 2440.

78. H.W. Kurtz and H. Schechter, J.C.S. Chem. Commun., 1966, 689.

79. T. Matsuura and I. Saito, Bull. Chem. Soc., Jap., 1969, 42, 2973.

80. A.P. Schaap, Tetrahedron Letters, 1971, 1757.

81. D. R. Kearns, Chem. Rev., 1971, 71, 419.

82. J.W. Huffmann, J. Org. Chem., 1976, 41, 3847.

83. C.K. Bradsher and F.H. Day, Tetrahedron Letters, 1971, 409.

84. A.R. Katritzky and Y. Takeuchi, J. Chem. Soc. C, 1971, 874.

85. S. Khoklov and G.B. Lokshin, Tetrahedron Letters, 1963, 1881.

86. R. Brown, C. Kelly, and S. E. Wibberley, J. Org. Chem., 1965, 30, 277.

87. H. Schmidt, et al., Chem. Ber., 1973, 106, 396.

88. P.J. Machin, Ph.D. Thesis, London University, 1974.

89. U. Schmidt and J. Häusler, Angew. Chem. Int. Ed. Eng., 1976, 15, 497.

90. S. Safe and A. Taylor, J. Chem. Soc. C., 1971, 1189.

91. T. Sato and T. Hino, Tetrahedron, 1976, 32, 507.

92. M.W. Goldberg, R. Aeschbach, and E. Hardegger, Helv. Chim. Acta, 1943, 26, 680.

93. L. Fieser and M. Fieser, Reagents for Organic Synthesis, John Wiley, (1967), New York, p. 660.

94. J.W. Williams, W.T. Rainy, Jr., and R.S. Leopold, J. Amer. Chem. Soc., 1942, 64, 1738.

95. V. Dessaignes, Annalen, 1852, 82, 231.

96. P.L. Nichols, Jr., A.N. Wrigley, and E. Yanovsky, J. Amer. Chem. Soc., 1946, 68, 2020.

97. A.P. Schaap, A.L. Thayer, E.C. Blossey and D.C. Neckers, J. Amer. Chem. Soc., 1975, 97, 3741.

98. A.P. Schaap, A.L. Thayer, E.C. Blossey and D.C. Neckers, J. Amer. Chem. Soc., 1973, 95, 5820.

99. R.M. Acheson, An Introduction to the Chemistry of Heterocyclic Compounds, 2nd ed., John Wiley (1967), London, p. 270-274.

100. P.J. Machin, A.E.A. Porter and P.G. Sammes, J.C.S. Perkin I, 1973, 404.