

CHEMICAL STUDIES ON DEHYDROCYCLODIPEPTIDES

A thesis presented by

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Abstract

The work contained in this thesis is concerned with the chemistry of dehydro-dioxopiperazines and their derivatives.

The literature on acylimines, related compounds and naturally-occurring dehydropeptides is reviewed.

The oxidation reactions of dioxopiperazines, dihydropyrazinones and dihydropyrazines have been investigated and their limitations exemplified.

The synthesis of a new class of reactive dienones, 5-alkoxy-pyrazin-2(1H)-ones, is described; their potential as biosynthetic precursors of the fungal metabolites, brevianamides A and B, is discussed.

The route to several dehydro-dioxopiperazines, via D.D.Q. oxidation of dihydropyrazinones, is detailed.

A mild method for the addition of sulphur nucleophiles across the double bonds of dehydro-dioxopiperazines is described. The method involves the direct addition of thiols to dehydrocyclodipeptides in the presence of an acid catalyst. Kinetic control affords the α -addition products and this process competes with a slower equilibration leading to the β -adducts. The method is general.

New oxidations of benzylidene dioxopiperazines resulting in the formation of α, β -difunctionalised derivatives are described and a reactive acylimine intermediate is proposed. Such intermediates, dihydropyrazin-2,5-diones, have been isolated and their reactions studied.

Acknowledgements

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The technical assistance and friendliness of Mr. Jack Peppercorn and the staff of the Frankland laboratory are gratefully acknowledged together with the efficient micro-analytical services of Mr. K.I. Jones and his staff.

Many thanks also go to my colleagues, both past and present, in the Heilbron laboratory, especially Tim Wallace, Tom Tyler, Kayhan Göktürk and Dr. Barry Arnold, for providing a continuous source of help and entertainment.

Lastly, my thanks go to my parents and my wife, Mary, whose support and sacrifices over the years have made it all possible.

So, where to now St. Peter
If it's true I'm in your hands
I may not be a Christian
But I've done all one man can
I understand I'm on the road
Where all that was is gone
So, where to now St. Peter
Show me which road I'm on

Bernie Taupin

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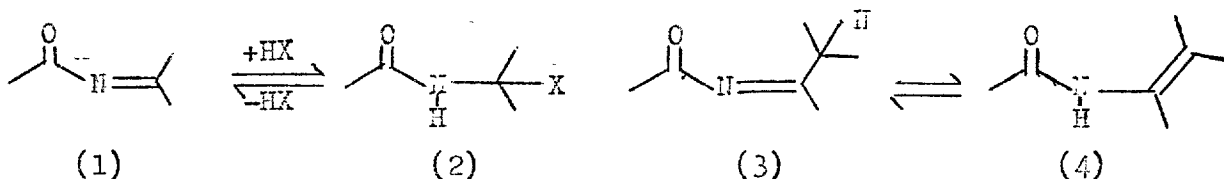
REVIEW

ACYLIMINES AND DEHYDROPEPTIDES

PART 1

A. Acylimines.

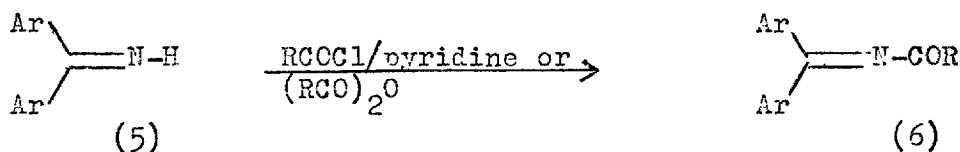
Acylimines possess structure (1). Their chemical behaviour is characterised by their conversion into α -functionalised amides (2) upon the addition of nucleophiles HX. Depending on the nature of X, acylimines can often be



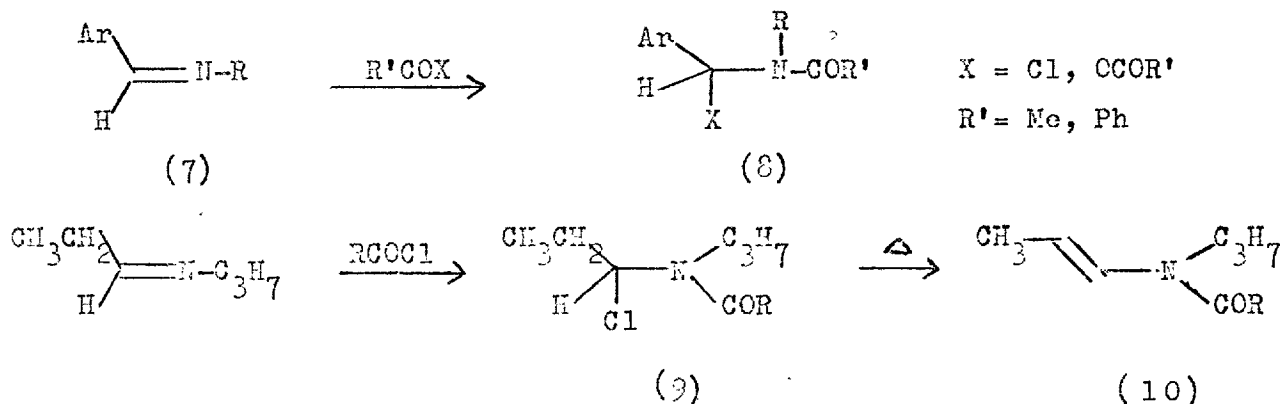
regenerated from these adducts by a thermal or base induced elimination.

Acylimines bearing primary or secondary alkyl substituents (3) can also undergo isomerisation to the enamide structure (4).

(i) Preparation. Aromatic imines (5) can be conveniently acylated¹⁻⁷, by acid chlorides and anhydrides (or ketene⁷) to the corresponding acylimines (6) in good yields. The reactions of N-substituted imines with acid chlorides^{8,9} and anhydrides⁹ have also been investigated. With aromatic aldimines (7), the α -

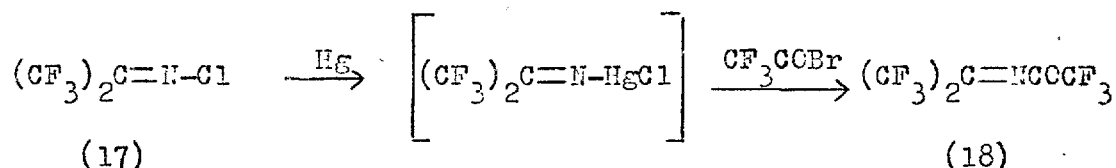


functionalised amides (8) are produced^{8,9}, whilst with alkyl aldimines possessing a β -hydrogen atom, the initial chloro amide e.g. (9), undergoes thermal elimination to produce the isomeric enamide (10)¹⁰. By analogy with

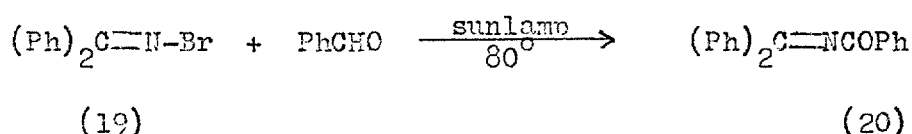


Reformatsky reaction of α -bromoesters with nitrile esters and zinc¹⁵. The synthesis of the hydroxy isoquinoline (16) exemplifies the reaction.

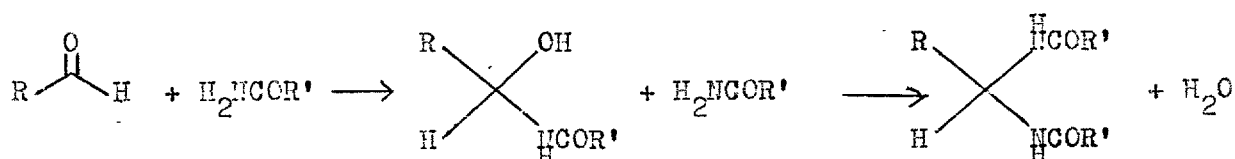
Two preparations involving N-halo ketimines have been published. The first¹⁶ again involves an imine-metal salt. Thus, the N-chloro imine (17) reacts with trifluoroacetyl bromide in the presence of metallic mercury to give the acylimine (18) in 93% yield. A different reaction type is the



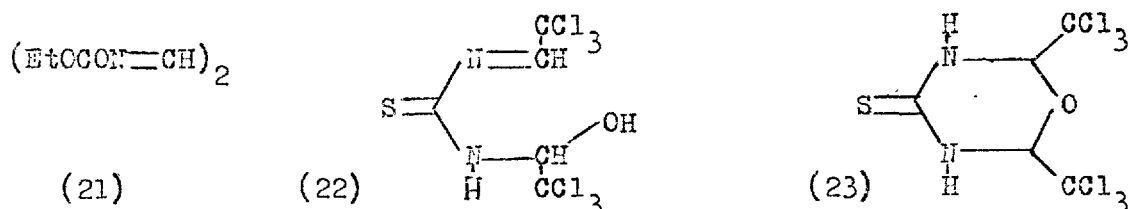
light induced reaction of the N-bromo imine (19) with benzaldehyde¹⁷. The acylimine (20) is produced in 50% yield presumably by a radical mechanism.



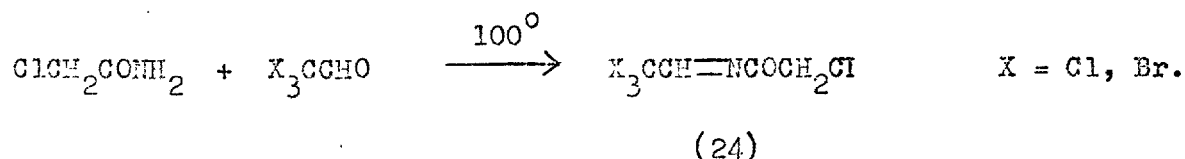
The formation of α -hydroxy amides and 'bis-amides' from aldehydes and primary amides is a well known process¹⁸, but there are several exceptions



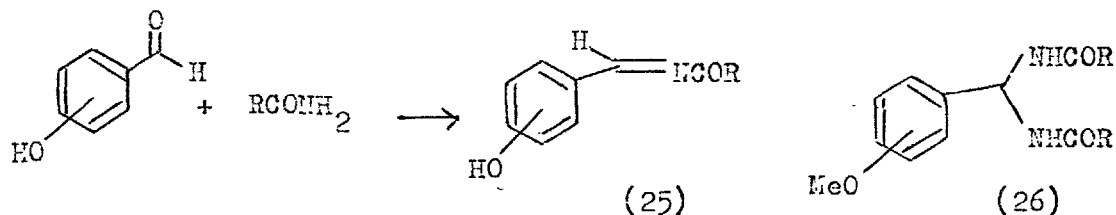
where an acylimine is formed directly by this reaction. Thus, urethane and glyoxal give the bis-acylimine (21)¹⁹ and thiourea and chloral are reported



to give the unusual thioacylimine (22)²⁰. It might be expected that (22) would readily cyclise to (23) but this is not the case. In a similar manner chloral and bromal react with chloroacetamide to give the acylimines (24)²¹.

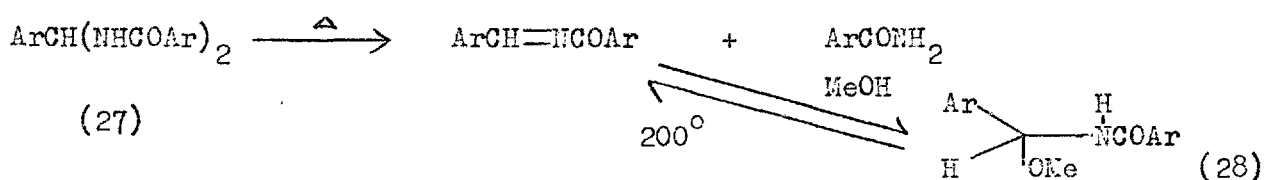


More anomalies are found with aromatic aldehydes^{22,23,24}. *O*-, *m*- and *p*-hydroxy benzaldehydes yield acylimines (25) with propionamide, benzamide and phenylacetamide whereas the corresponding methoxy aldehydes form the bis-amides (26). Similarly, 5-chloro and 3,5-dichlorosalicylaldehyde with

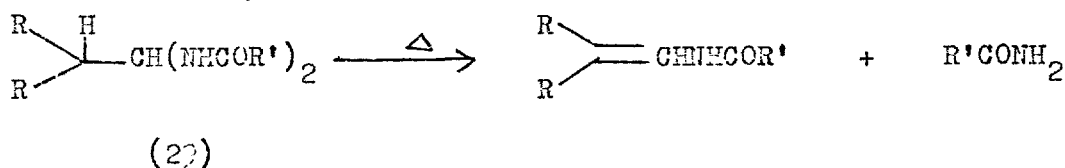


heptanamide and benzamide give only the corresponding acylimines²⁵.

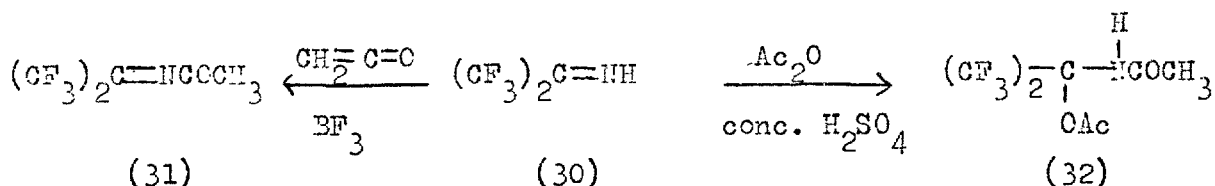
The use of bis-amides as a source of acylimines has been investigated by Ben-Ishai *et al.*^{26,27}. Pyrolysis of arylidene bis-arylamides (27) above their melting points (250° - 280°) results in acylimine formation²⁶. They



are best stored as the methanol adducts (28) and can be regenerated when required by heating at 200° under vacuum. Pyrolysis of alkylidene bis-amides of the type (29), however, results in enamide formation²⁷.

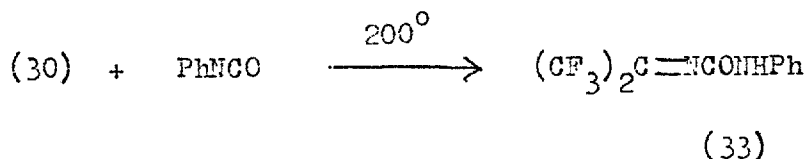


Considerable effort has been directed towards the synthesis of perfluoro acylimines. The hexafluoro imine (30) (readily prepared from hexafluoro acetone^{28,29}) can be acylated with ketene²⁸, but requires the presence of boron trifluoride for reaction to occur. Acylation with acetic anhydride also requires acid catalysis³⁰ but in this case the reaction

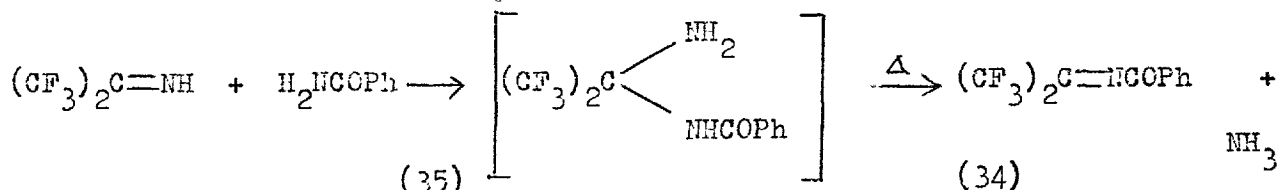


stops at the acetoxamide (32) stage. The acylimine (33) has been prepared

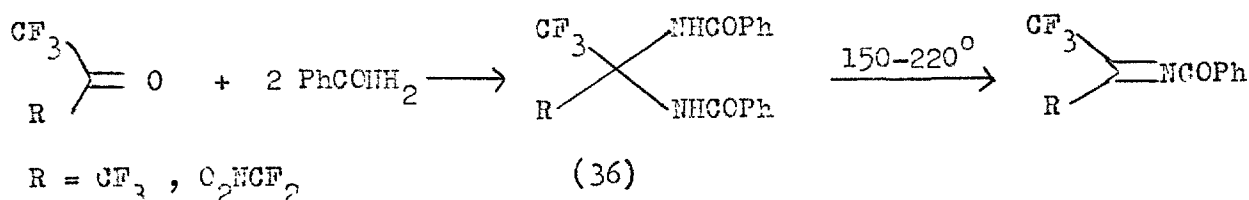
by heating phenyl isocyanate with (30) at 200° for 23 hours³⁰. Similarly,



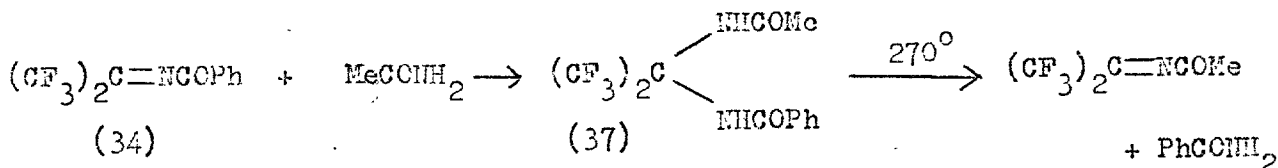
heating benzamide with (30) at 150°-220° affords the benzoylimine (34)³¹. However, this reaction could involve formation of an intermediate α -amino amide (35) rather than nucleophilic attack by the imine on the amide.



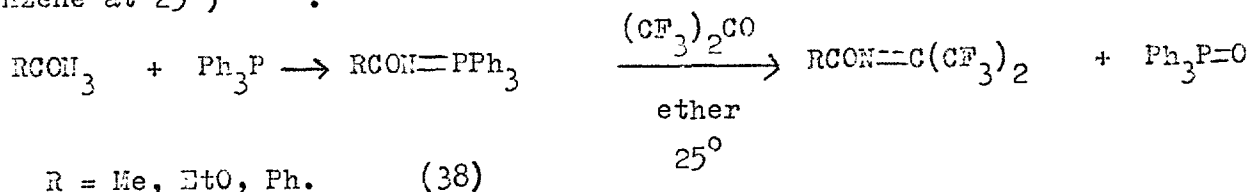
The bis-amides (36), prepared by the reaction of perfluoroketones and an excess of benzamide, yield acylimines on thermolysis^{30,32}. A simple



'acyl-exchange' reaction involves the treatment of the benzoylimine (34) with acetamide; subsequent pyrolysis of the mixed bis-amide (37) gives the acetylimine (31)³².

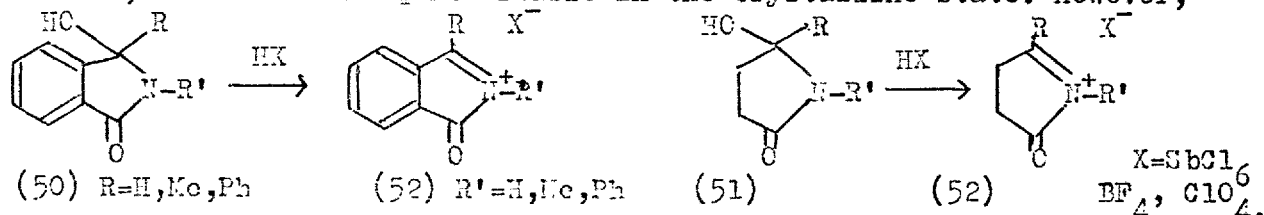


An interesting new development for the preparation of acylimines involves the use of acyl phosphimines (38). These are readily prepared from acyl azides and triphenylphosphine; they react with hexafluoroacetone to give the corresponding acylimines under very mild conditions (ether or benzene at 25°)^{31,32}.

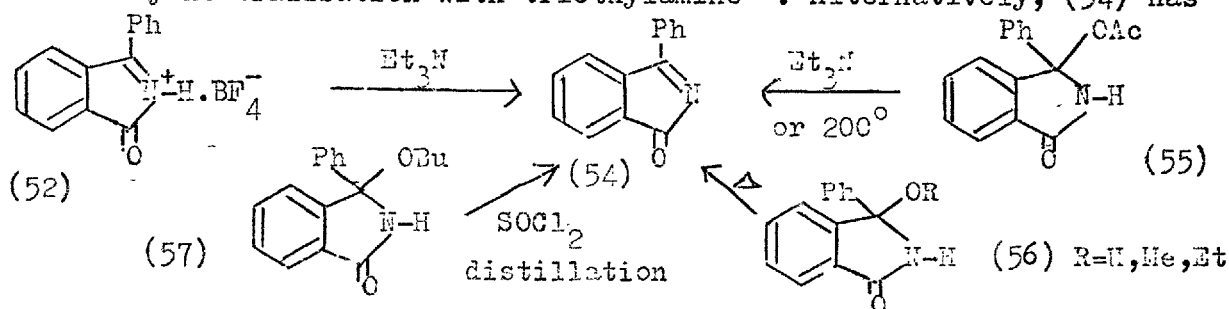


A number of workers have reacted perfluoroketones with amides and carbamates and have isolated the corresponding α -hydroxy amides (39)^{28,33,34}.

enamides, the salts are quite stable in the crystalline state. However,

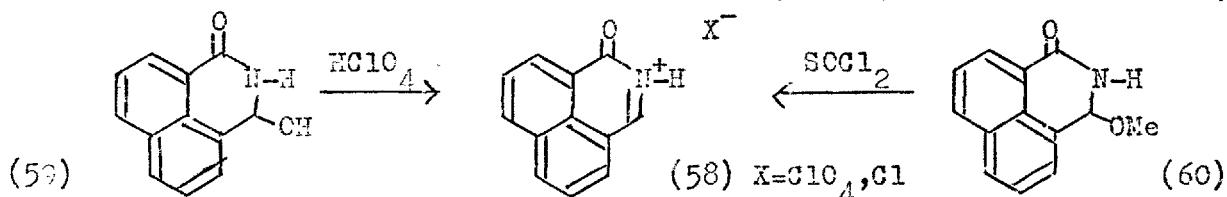


dissolution in D_6 -D.M.S.O. effected the isomerisation, as did attempts to prepare the free acylimines ($R'=H$) by neutralisation with base⁴⁷. Where enamide formation is not possible (52; $R=Ph$, $R'=H$), the acylimine (54) is produced by neutralisation with triethylamine⁴⁷. Alternatively, (54) has

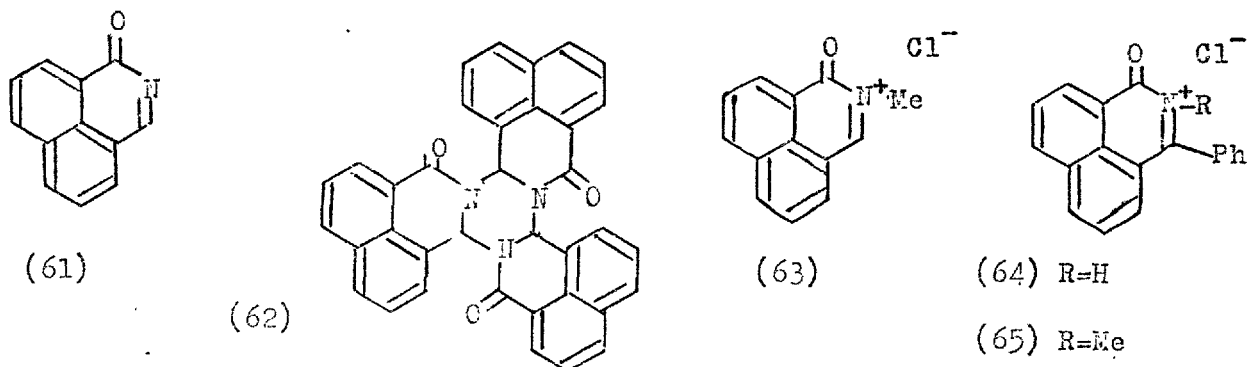


been prepared by both thermolysis and base treatment of the acetate (55)⁴⁷, by thermolysis of the alkoxy derivatives (56)⁴⁸ and by thionyl chloride treatment of the n-butyl ether (57) followed by distillation⁴⁹.

The 2-azaphenalone system has been investigated^{44,50,51}. The perchlorate salt (58; $X=ClO_4$) is formed from the hydroxy amide (59) and perchloric acid⁴⁴. Similarly, the hydrochloride (58; $X=Cl$) is formed from the methoxy amide (60) and thionyl chloride⁵⁰. However, attempts⁵⁰ to liberate the free acylimine (61) either by neutralisation of (58) or by thermolysis of (60)

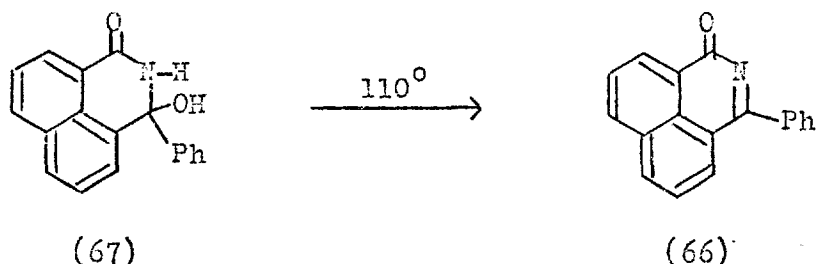


gave a trimer formulated as the s-triazine (62). A series of azaphenalone salts (63), (64) and (65) has been prepared by the thionyl chloride method⁵¹.

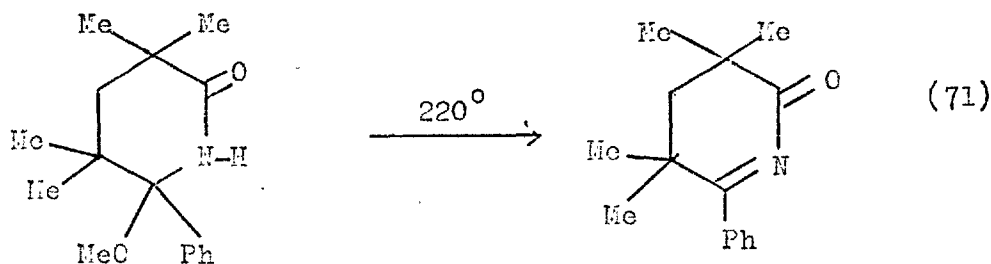
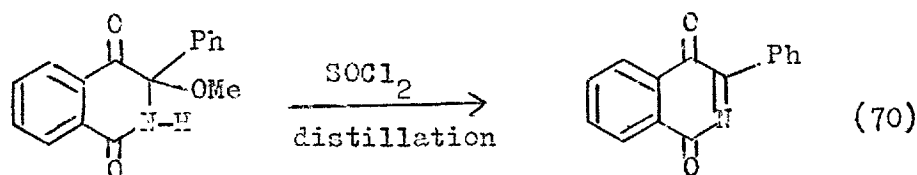
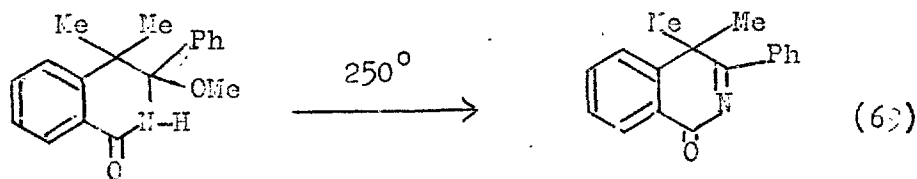
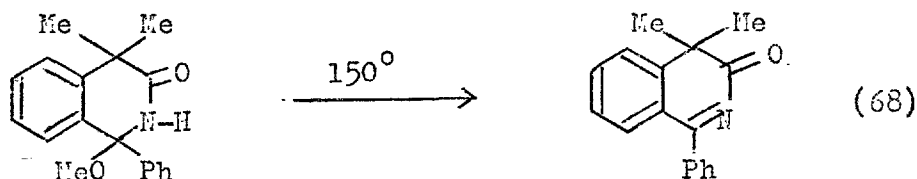


The phenyl substituted salts, (64) and (65), show decreased reactivity toward nucleophiles. This is a consequence of both stabilisation of the imine double bond by conjugation with the extra ring and increased steric hindrance⁵¹.

The acylimine (66) is formed by dehydration of the hydroxy amide (67) in refluxing toluene⁵¹ and polymerisation to the s-triazine does not occur, indicating the decrease in reactivity compared to (61).

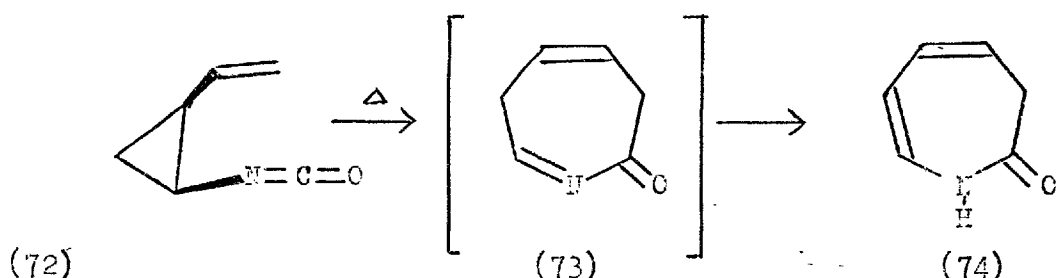


Ben-Ishai has extended the thermolysis reaction to the synthesis of the isoquinolones⁵² (68), (69), (70) and the piperidone⁴⁹ (71). Again, attempts to prepare the corresponding acylimines without the phenyl substituent resulted in s-triazine formation^{49,52}.

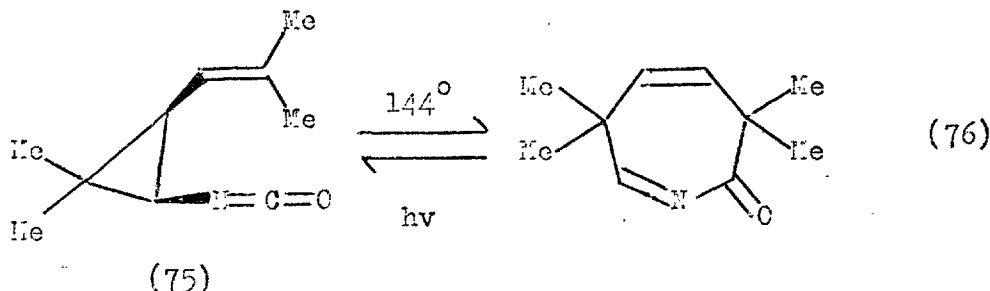


Comparison of the ease of preparation and reactivity towards nucleophiles of these acylimines with the extent of conjugation to the imine function clearly shows the stabilising effect that conjugation has on the system.

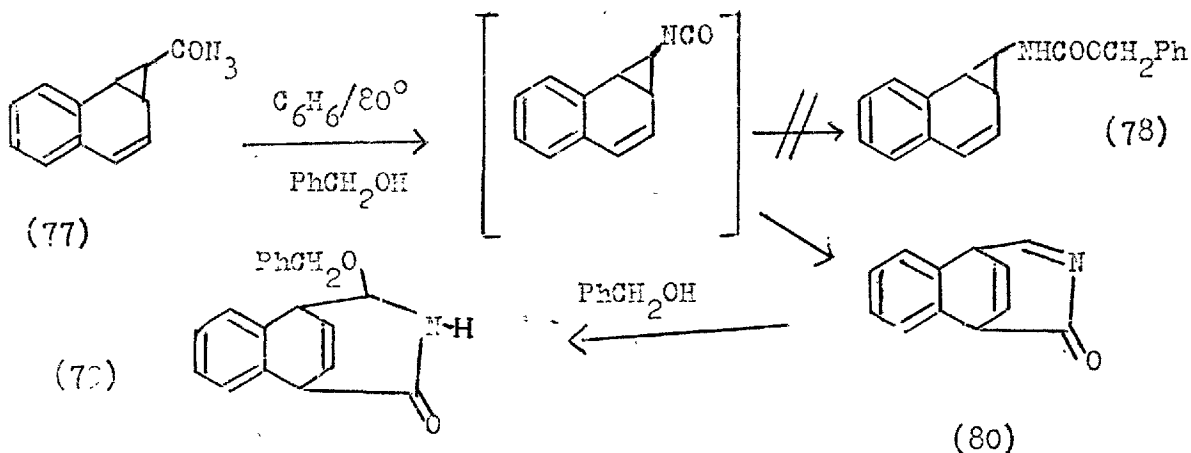
The thermal [3,3] sigmatropic Cope rearrangement of cis-vinylcyclopropane isocyanates should yield acylimines as products. Thus, an acylimine intermediate (73) has been proposed^{53,54} in the thermal rearrangement of (72) to the enamide (74). This has been confirmed⁵⁵ by the use of the tetra-



methyl derivative (75) where enamide formation is not possible; the sole product is the acylimine (76). The reaction is reversed on photolysis.



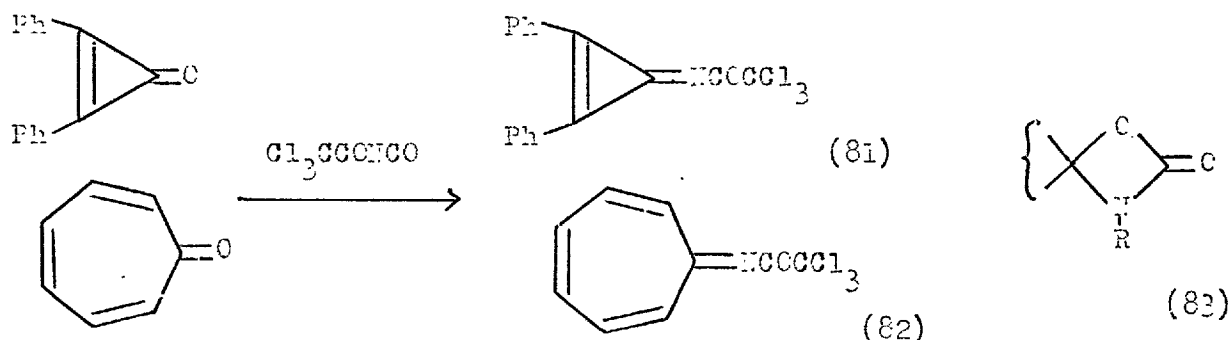
The attempted Curtius reaction of the 1-benzonorcaradiene acylazide (77) carried out in benzene containing benzyl alcohol resulted, not in the expected carbamate (78), but in the α -benzyloxy amide (79)⁵⁶. This reaction



is readily explained by the [3,3] Cope rearrangement of the intermediate

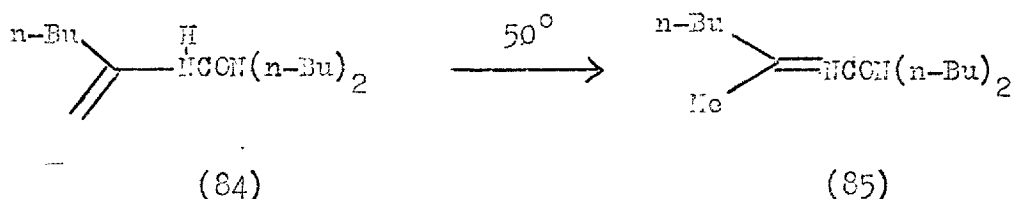
vinyl cyclopropane isocyanate to the acylimine (80) which is then trapped by the benzyl alcohol to give the observed product (79).

The two unusual acylimines (81)⁵⁷ and (82)⁵⁸ have been made by the reaction of trichloroacetyl isocyanate and the corresponding ketones.

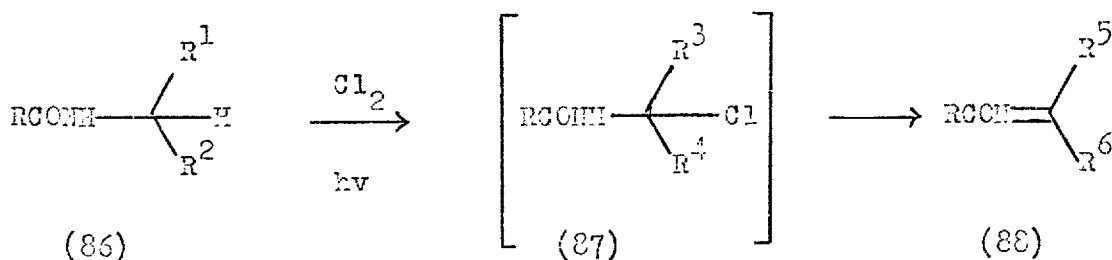


The stepwise or concerted formation of a cyclic intermediate (83) has been postulated⁵⁷.

The thermal isomerisation of N-alkenyl ureas (from N-alkenyl isocyanates and amines) to their acylimines has been investigated by Sato⁵⁹, e.g. (84) → (85).



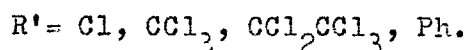
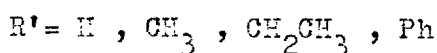
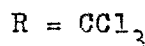
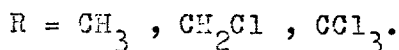
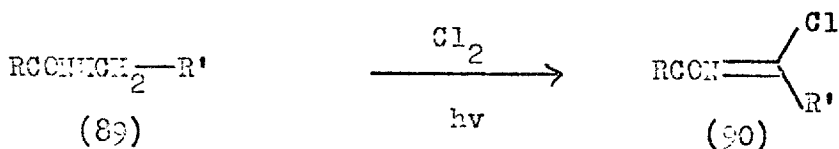
Oxidation of amides can also lead to acylimine formation. For example, photochemical chlorination of the amides (86) gives good yields of the chloro acylimines (88)⁶⁰. The intermediacy of the α-chloro amides (87) is



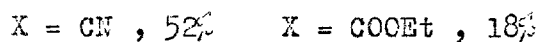
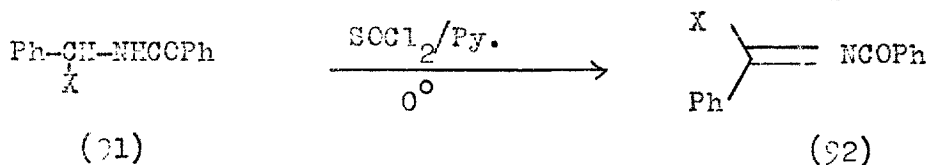
	<u>R</u>	<u>R</u> ¹	<u>R</u> ²
a	CCl ₃	Me	Ph
b	Ph	Me	Me
c	n-Cl.C ₆ H ₅	Me	Me
d	2,5-Cl ₂ .C ₆ H ₄	Me	Me

	<u>R</u> ⁵	<u>R</u> ⁶
a	CCl ₃	Ph
b	CCl ₃	CCl ₃
c	CCl ₃	CCl ₃
d	CCl ₃	CCl ₃

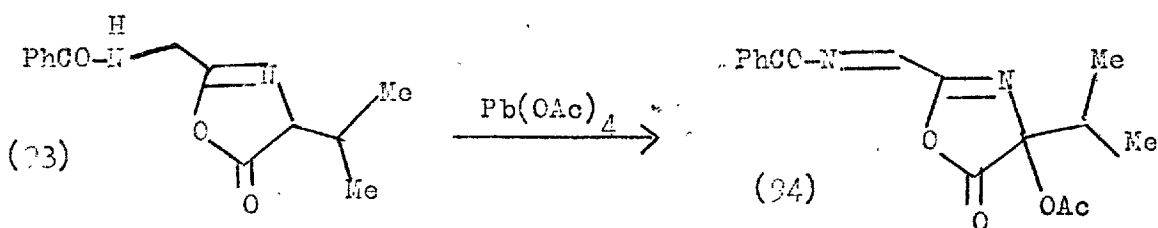
suggested⁶⁰. However, similar chlorination of amides where there is no α -branching in the alkyl chain, e.g. (89), produces the N-acylimide chlorides (90)⁶⁰. Other methods for the preparation of the latter class of



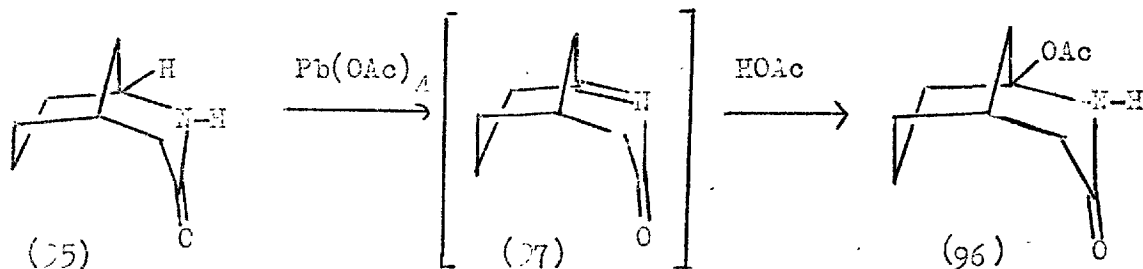
compounds have been described^{60,61}. Related compounds (92) can be prepared, albeit in relatively low yields, by oxidation of some α -functionalised benzamides (91) with thionyl chloride in pyridine at 0°⁶².



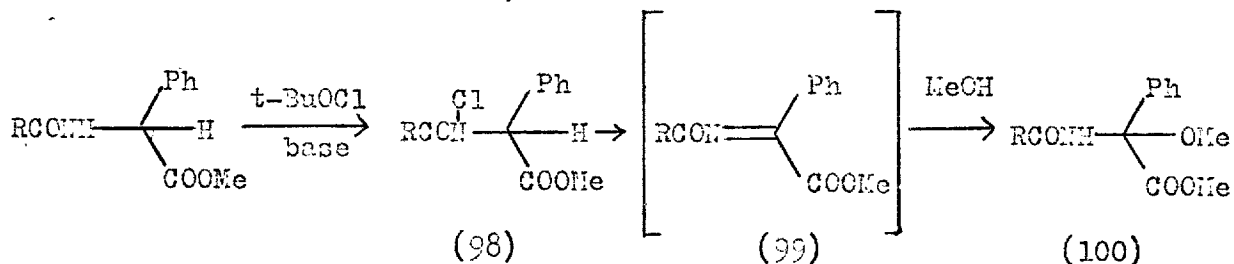
Lead tetraacetate has been utilised for the oxidation of amides. Thus benzoyl glycyl valine oxazolone (93) gives the acylimine (94) on reaction with lead tetraacetate⁶³. Similar oxazolones give products probably



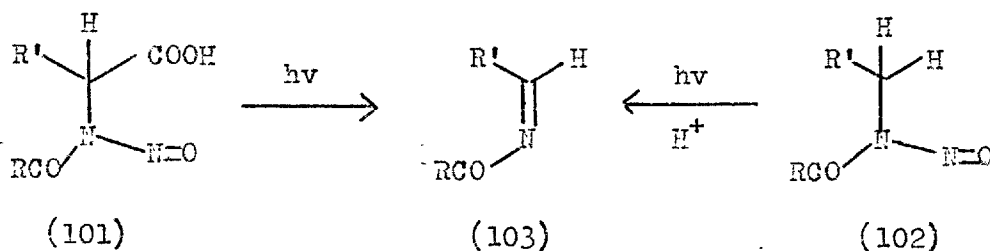
derived from acylimine intermediates on oxidation with lead tetraacetate or mercury (II) acetate. A further example of this type is the oxidation of 3-oxo-2-azabicyclo[3.3.1] non-1-ene (95) to the acetate (96) with lead tetraacetate in benzene⁶⁴. The acylimine (97) is proposed as an intermediate.



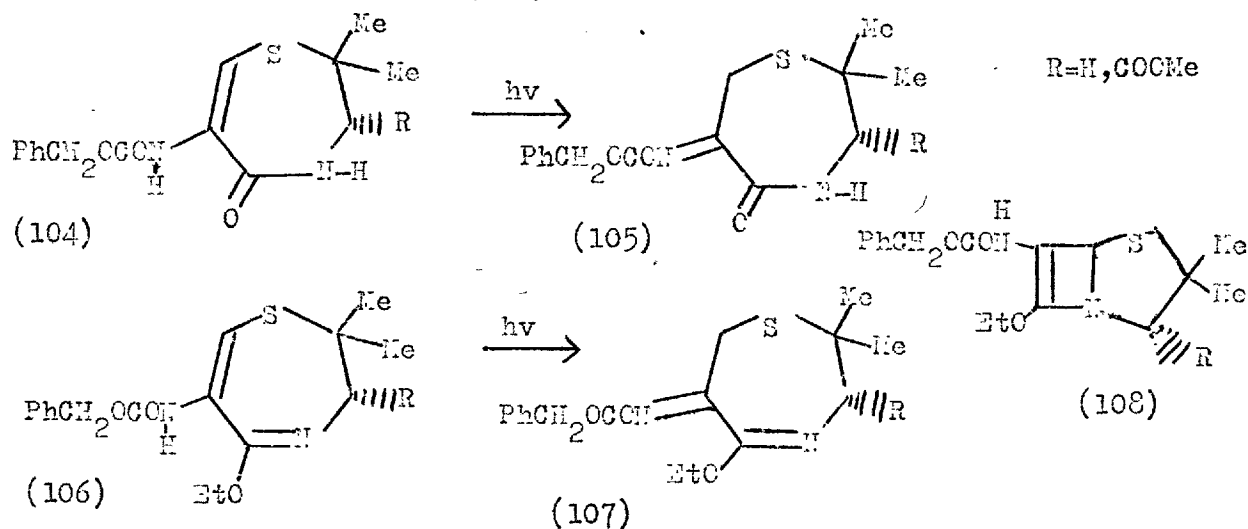
N-chloro amides (98) can be prepared with *t*-butyl hypochlorite⁶⁵; the use of these compounds to generate acylimines by elimination of hydrogen chloride has recently been reported^{66,67}. The acylimines (99) were trapped as their methoxy adducts (100). 6-Methoxy penicillins^{66,67,68} and 7-methoxy cephalosporins⁶⁷ have been prepared in this manner.



The acid catalysed photolyses of N-nitroso- α -amido acids (101) and N-nitroso amides (102) are reported to yield acylimines (103) and products derived therefrom^{69,70}.



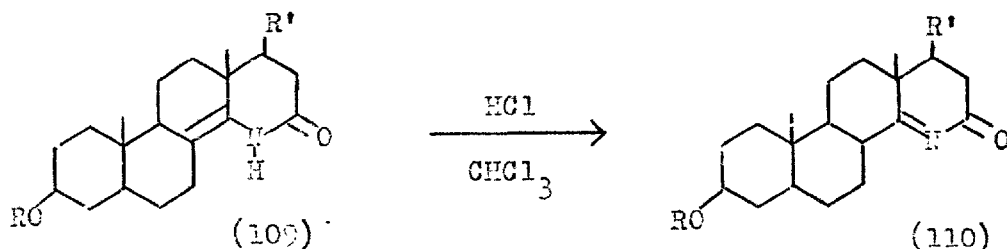
Photolysis of the thiazepines (104) and (106) effects isomerisation to the acylimines (105) and (107)⁷¹. These compounds are unstable towards



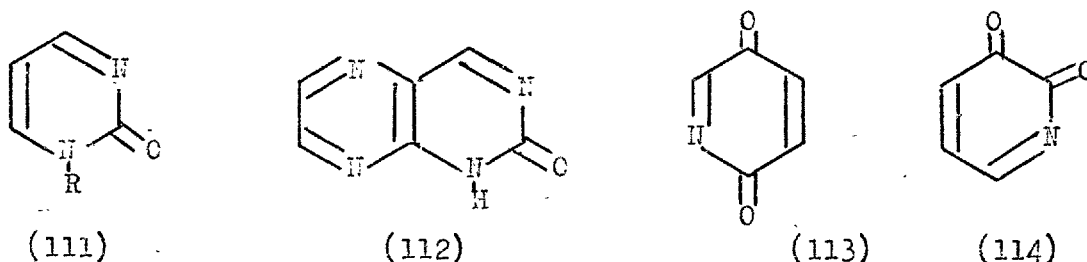
heat, acid and base rapidly reverting to the starting enamides. However, (107) has a half-life of 100 hours in dilute pentane solution (ca. 2×10^{-3} M)

at 25°. The desired cyclisation of (106) to the β -lactam derivative (108) did not occur.

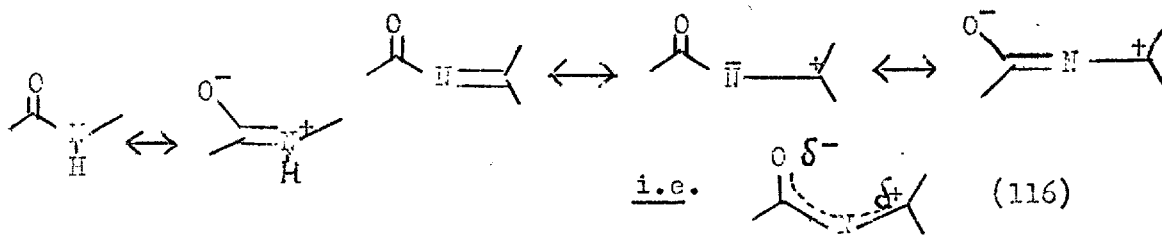
Isomerisation of the enamide azasteroid (109) to the acylimine (110) can be induced simply by treatment with hydrogen chloride in chloroform⁷². In this case the steric strain in (109) is great enough to reverse the normal direction of the isomerisation.



A variety of nitrogen heterocycles resemble acylimines in both their structures and their chemical reactivity. Examples include pyrimidin-2(1H)-ones (111)⁷³, pteridenes (112)⁷⁴ and azaquinones (113) and (114)⁷⁵.

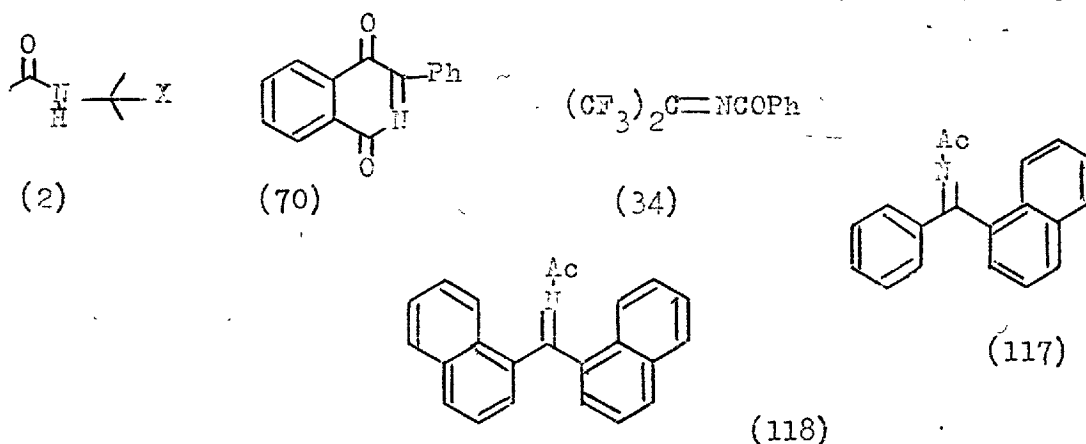


(ii) Reactions. Stabilisation of the amide group by overlap of the nitrogen lone pair with the π -system of the carbonyl group is well established⁷⁶, but with acylimines (1) this kind of overlap is not possible due to the sp^2 nature of the nitrogen atom. However, stabilisation can be re-established if the residual positive charge on the α -carbon atom, derived from the valence bond forms and depicted in (116), can be quenched by an electron donating species and herein lies the electrophilic nature of the acylimine double bond. The facile isomerisation to enamides, where



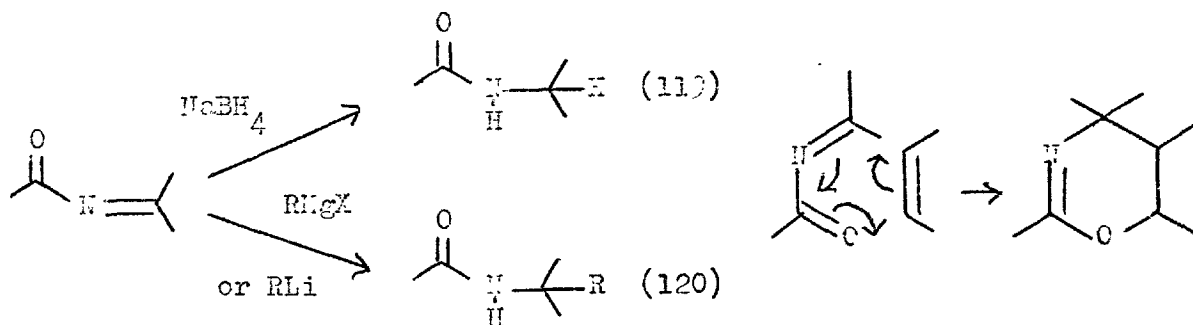
possible, is readily explained by the extra stabilisation gained by overlap of the nitrogen lone pair with the olefinic π -system.

Nearly all acylimines give α -functionalised amides (2) on addition of nucleophiles such as alcohols, thiols, amines, amides, etc.⁷⁷. The reactivity of the acylimine is affected by its immediate environment. Electron-withdrawing substituents such as carbonyl groups e.g.(70), and trihalomethyl groups e.g.(34), enhance reactivity and these acylimines are very reactive indeed^{32,52}. On the other hand, electron-donating substituents stabilise the electrophilic double bond, consequently the

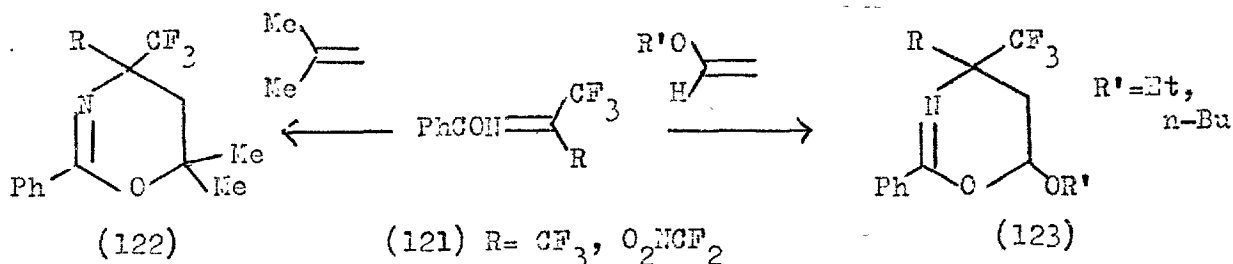


extensively conjugated acylimines (117) and (118) react with nucleophiles with great difficulty, if at all.⁷ A comparison of the reactivity⁵² of the differently conjugated acylimines (68), (69) and (70) has already been remarked upon in the preparation section (page 15). It has also been suggested that addition to the imine is facilitated by an aliphatic rather than aromatic acyl group, which is commensurate with the electron-deficient nature of the system. Steric considerations can affect reactivity, for example, the strain factors in the ring system of the azasteroids (109) and (110) favour the acylimine rather than the enamide⁷².

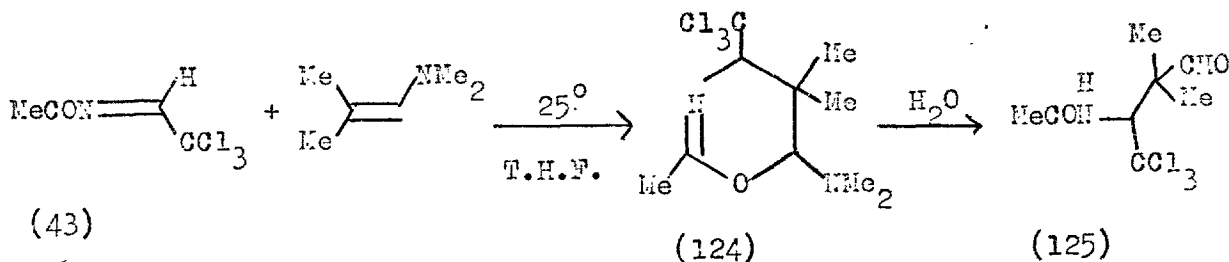
In general, acylimines are reduced with sodium borohydride to the amides (119)^{26,35,49,52,55} and undergo high yield alkylations with Grignard reagents^{11,26,35,37,40} and organo lithium compounds⁷⁸ to give α -substituted amides (120).



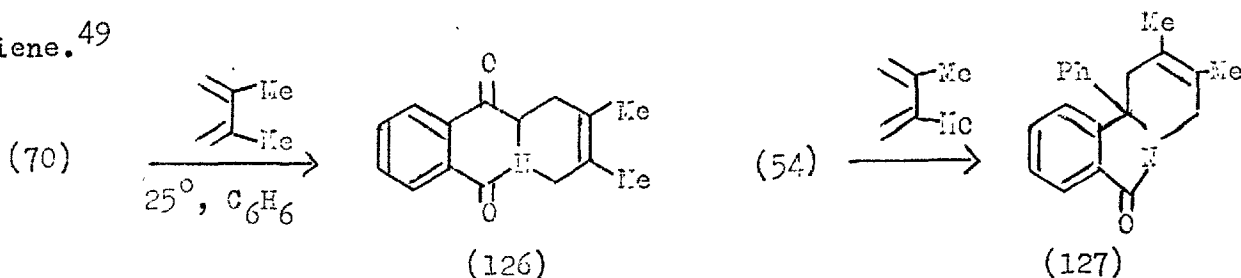
Highly reactive acylimines combine with olefins in what is formally a 1,4-cycloaddition. For example, (121) reacts with isobutylene at high temperatures (100°) to give the oxazine (122)³². With electron-rich olefins reaction occurs smoothly at 25° (121) condensing with enol ethers to give similar products (123)³². Less reactive acylimines do not react



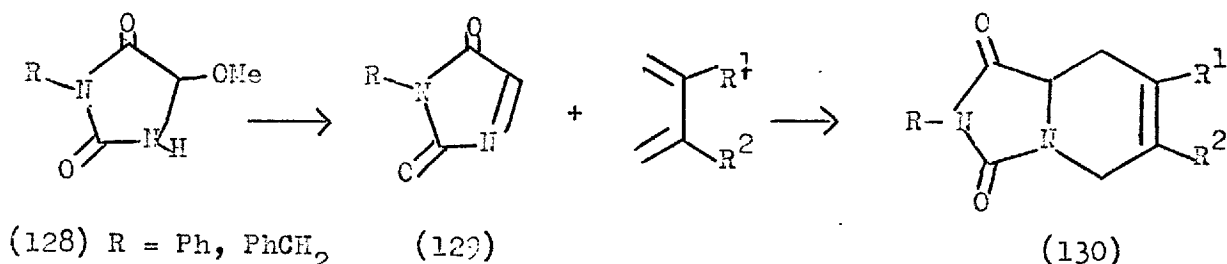
with simple olefins but addition will take place with enamines or enol ethers. The chloral derivative (43) with 1-dimethylamino-2-methylpropene gives an unstable liquid (124) which then reacts exothermically with water to form the aldehyde (125)⁴⁰.



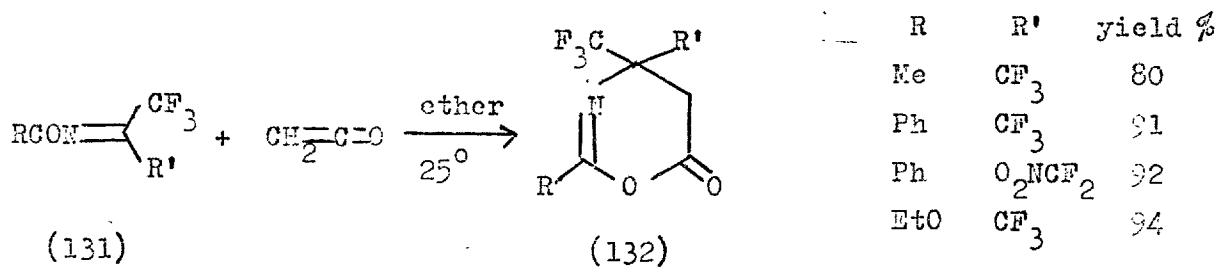
Several acylimines will undergo cyclisation with conjugated dienes. The azaquinone (70) reacts with 2,3-dimethyl butadiene to form the cycloadduct (126)⁵². The oxoisindole (54) gives a similar product with the same diene.⁴⁹



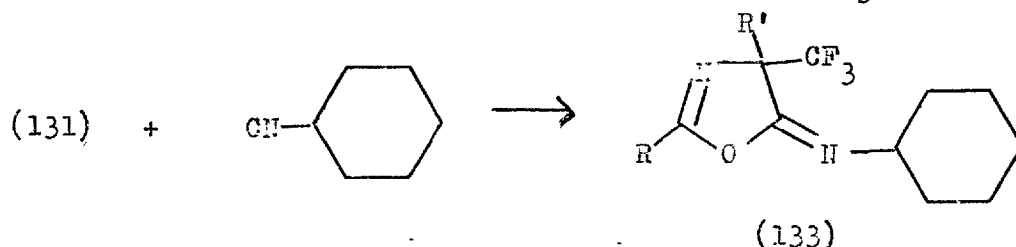
The 5-methoxy hydantoin (128) reacts thermally, presumably via the intermediate acylimine (129), with a variety of 1,3-dienes affording cycloaddition products (130)⁷⁹.



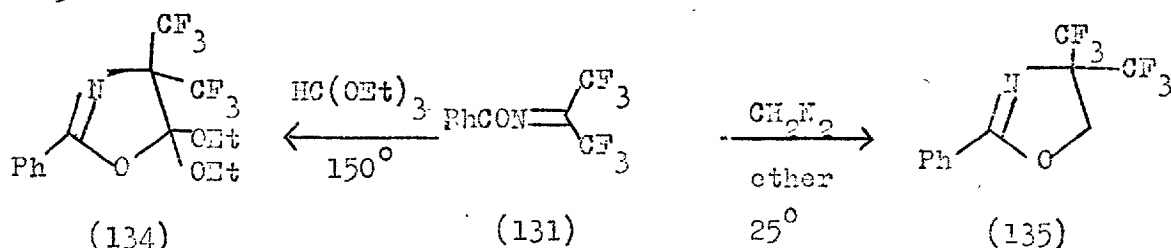
By analogy with the enol ether cycloadditions, perfluoro acylimines (131) with ketene give 1,4-addition products (132) in high yields and under mild conditions.^{30,32,80} Related additions are involved in the



reactions of (131) with isocyanides, ethyl orthoformate and diazomethane. Thus, with cyclohexyl isocyanide, the imino-1,3-oxazolines (133) are formed in good yields (>80%)⁸¹. When (131; R=Ph, R'=CF₃) is heated with

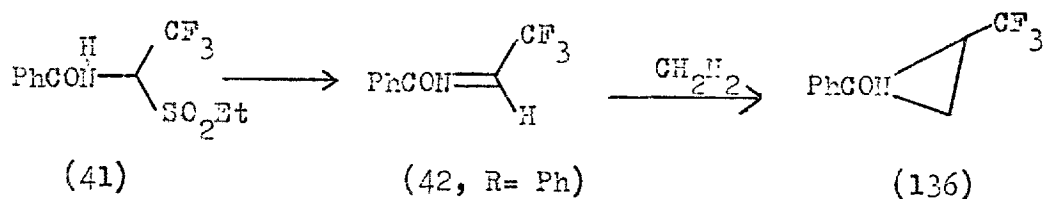


ethyl orthoformate (160°; 15 h.) the 1,3-oxazoline (134) is produced in 92% yield and diazomethane gives the 1,3-oxazoline (135) (91%) in ether at 25°³².

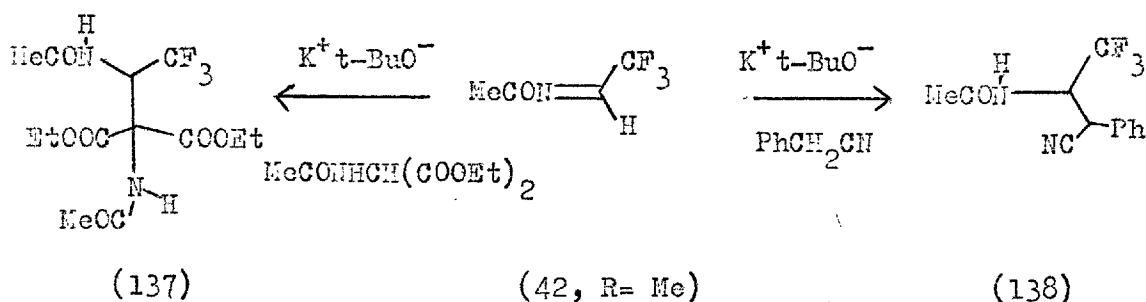


In contrast, the acylimine (42; R=Ph), formed in situ from the sulphone

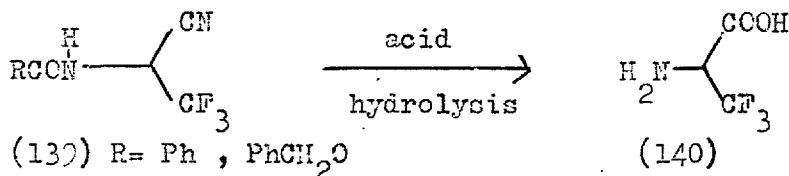
(41; X=SO₂Et), reacts with methanolic diazomethane to give the aziridine (136)³⁶. The structural assignment was based on n.m.r. data.



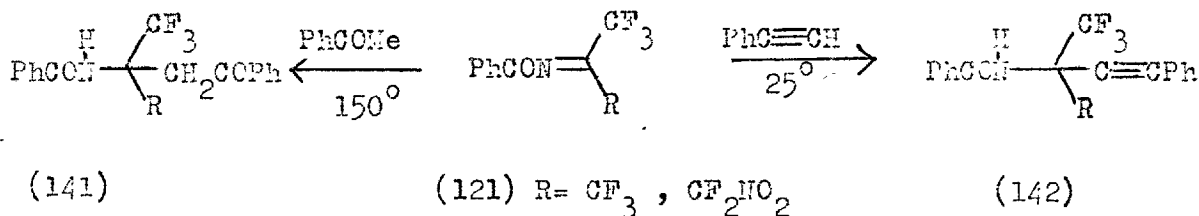
Reactions of active methylene compounds with acylimines are again limited to the perfluoro and azaquinone type of imines. The trifluoro compound (42; R=Me), prepared in situ from (41; X=SO₂Et) with potassium t-butoxide, reacts with acetamido diethyl malonate and benzyl nitrile at 25° to give the corresponding adducts (137) and (138)³⁵.



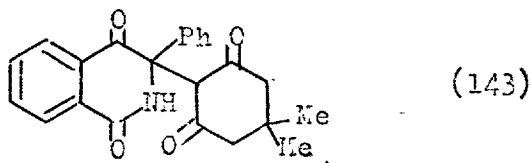
With hydrogen cyanide and (42; R=Ph, PhCH₂O-), prepared in situ from (41; R=Ph, PhCH₂O-, X=Cl) and triethylamine, high yields of the nitrile addition compounds (139) are formed.⁸² This reaction offers a simple high yield route to fluorinated amino acids, e.g. trifluoroalanine (140).



The benzoylimines (121) can either be alkylated by heating with acetophenone for 10 hours at 150° or give high yields of the α-acetylenic amides (142) with phenyl acetylene³².

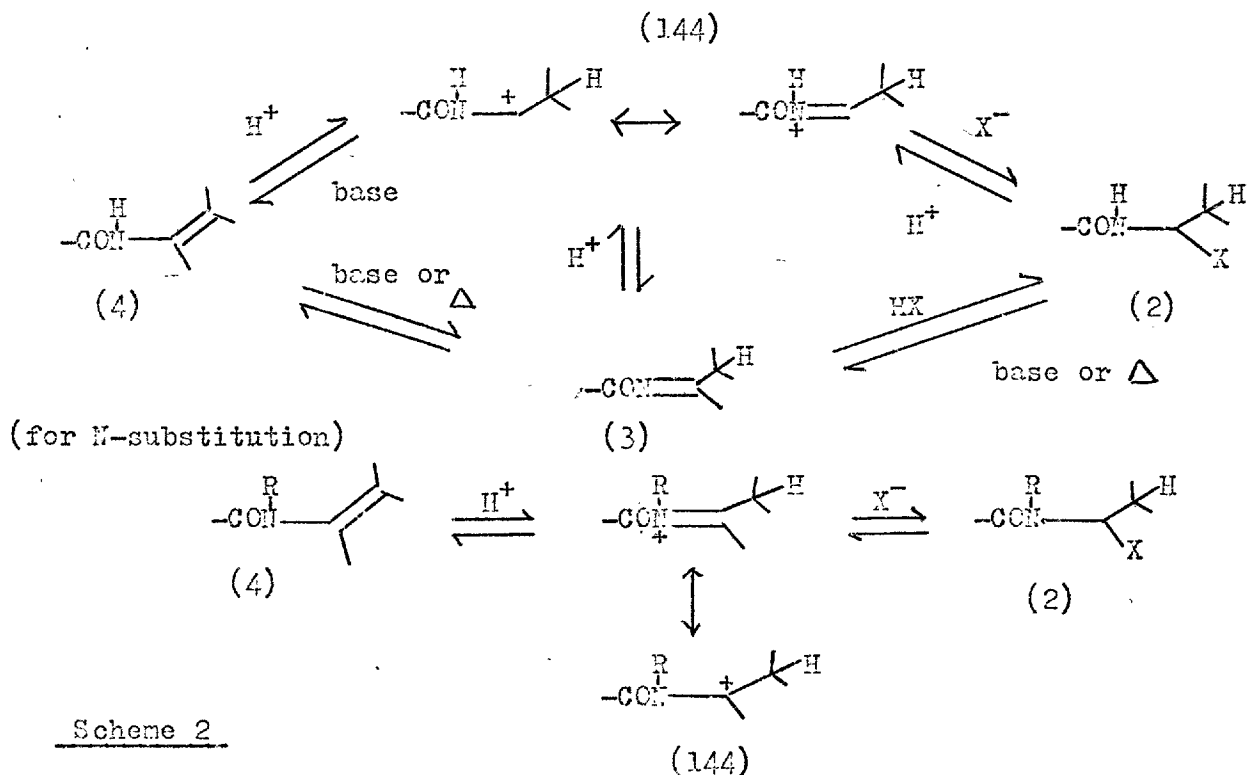


The azaquinone (70) readily forms the adduct (143) on stirring with dimedone in benzene (25°, 20 mins.)⁵².



B. α -Functionalised amides and enamides.

Any discussion on the reactions of acylimines must include the reactions of α -functionalised amides and enamides because the actual reacting species, irrespective of its precursor, is either the acylimine itself (base or thermally induced) or more often the protonated acyliminium cation (144) (acid induced). The equilibria that relate the various species are shown in scheme 2.

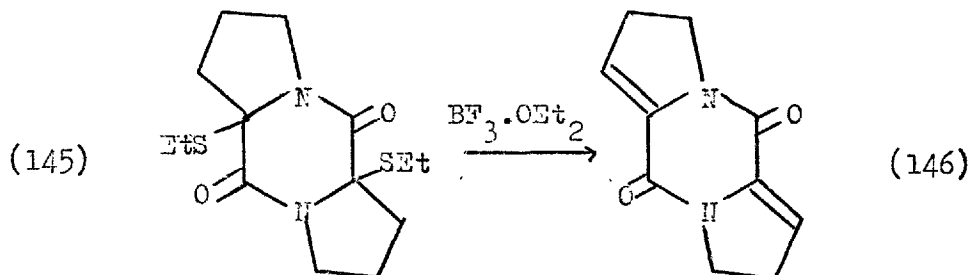


(i) α -Functionalised amides.

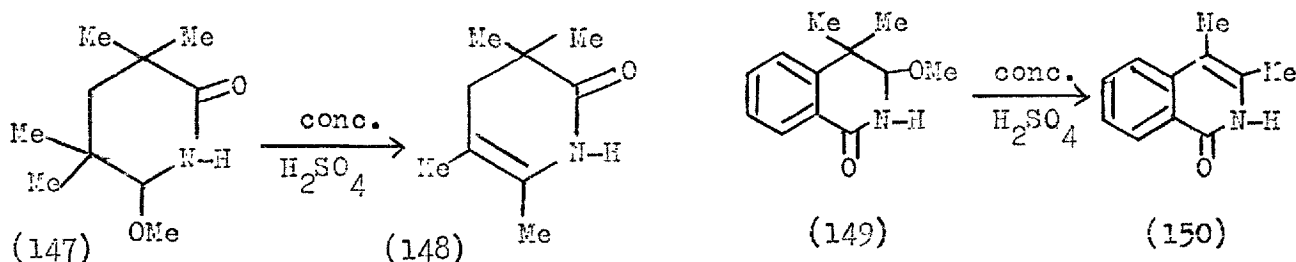
(a) Acid induced reactions- As shown in scheme 2 α -functionalised amides (2) form acyliminium cations (144) upon treatment with acid which are even more electrophilic than the corresponding acylimines (when R=H) due to their

greater electron deficiency.

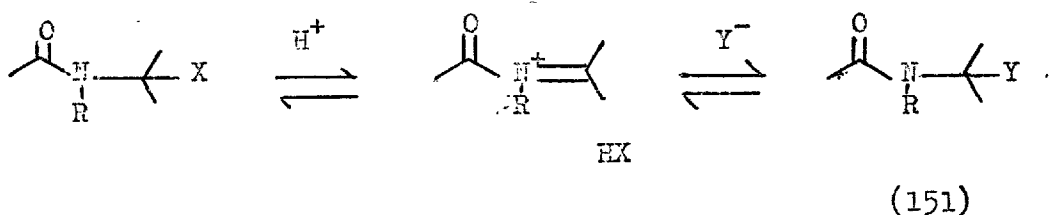
In the absence of an added nucleophile (e.g. acid catalysis by Lewis acids or non-nucleophilic protic acids) elimination to the enamide occurs whenever possible. For example, the dioxopiperazine derivative (145) gives the dehydro compound (146) on treatment with boron trifluoride etherate⁸³. Where enamide



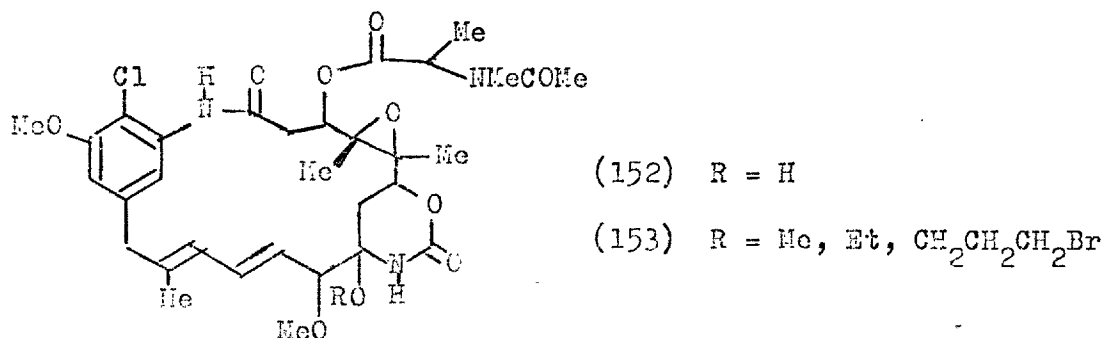
formation by proton transfer is not possible alkyl migrations have been observed. Methyl migration occurs in both (147) and (149) to give (148) and (150) respectively, on reaction with concentrated sulphuric acid^{49,52}.



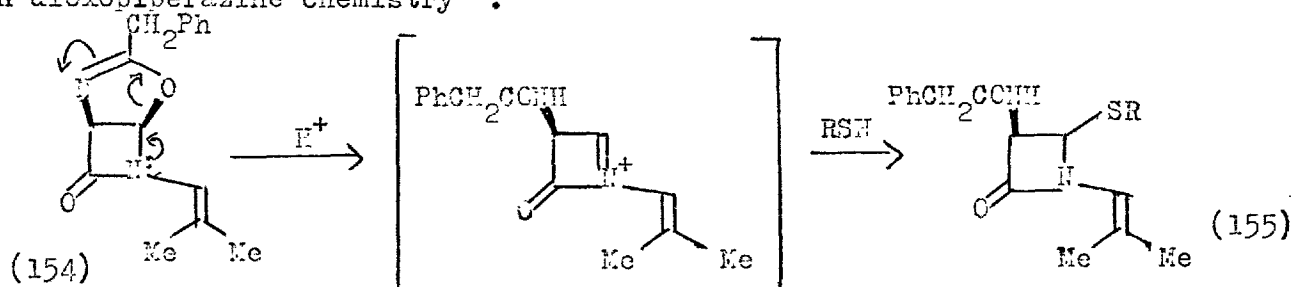
When another nucleophile (Y^-) is present in excess, the equilibria favour the new α -functionalised amide (151) which can then be isolated.



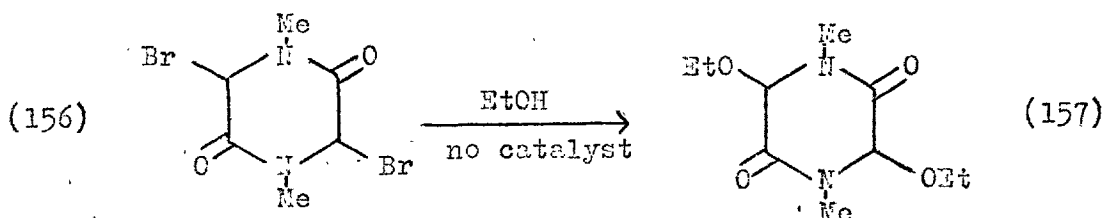
Many derivatives have been prepared in this way and two examples from the current literature are of interest. The first example involves the anti-leukemic ansa-macrolide maytansine (152) which is readily alcoholised to (153) by acid catalysis (e.g. toluene-p-sulphonic acid)⁸⁴.



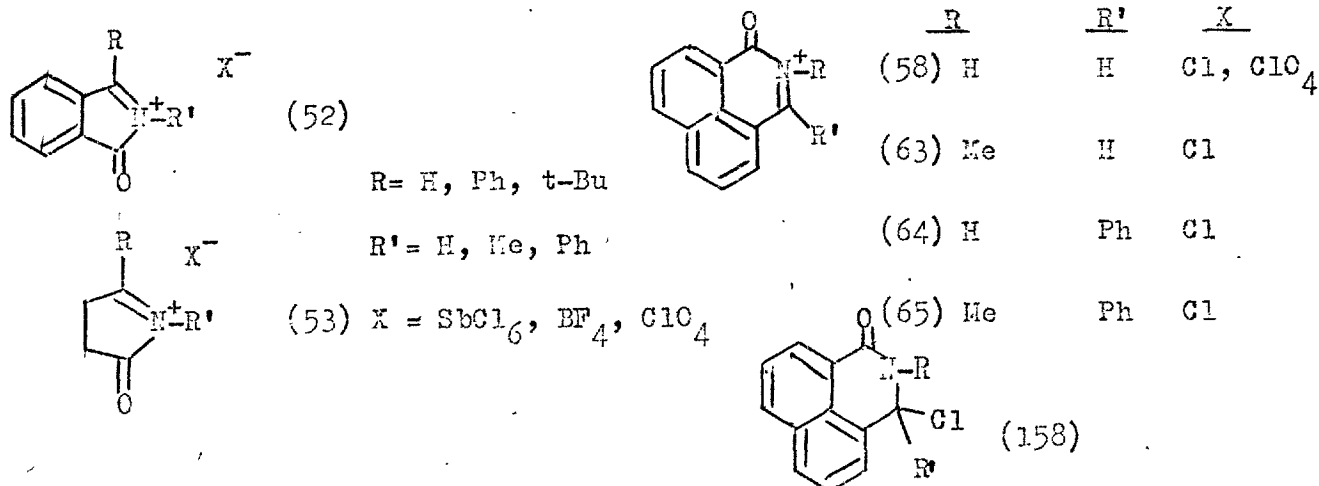
The second example utilises the reaction to prepare several 4-alkylthio azetidin-2-ones (155) from the penicillin derived oxazoline (154)⁸⁵ and has analogies in dioxopiperazine chemistry⁸³.



The nature of the leaving group X is most important and exercises a strong influence on the overall reactivity of the system. The greater the leaving group ability of X, i.e. the more stable is X^- , the more reactive is the α -functionalised amide. Thus, the reactions of many α -halo amides are auto-catalytic in that no external acid catalyst is required, e.g. (156)-(157)⁸⁶.



If anions with no nucleophilic power are used, e.g. BF_4^- , $SbCl_6^-$, ClO_4^- , α -functionalised amides cannot be formed; but, in some cases the corresponding acyliminium salts have been isolated. Compounds (52), (53), and (63)-(65) are examples of this. In the case of the azaphenalones the anion is the chloride ion. The compounds were shown to be true salts and not α -chloro amides of type (158) by their physical properties and infra red spectra^{50,51}. This unusual

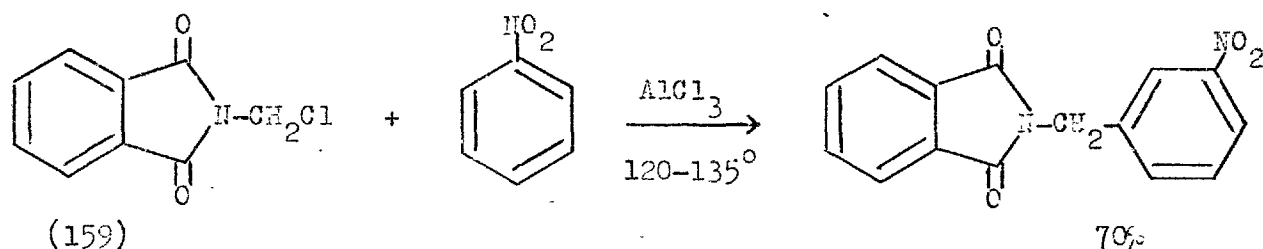


behaviour is another example of the degree of stability conferred on the acyliminium system by conjugation within the molecule.

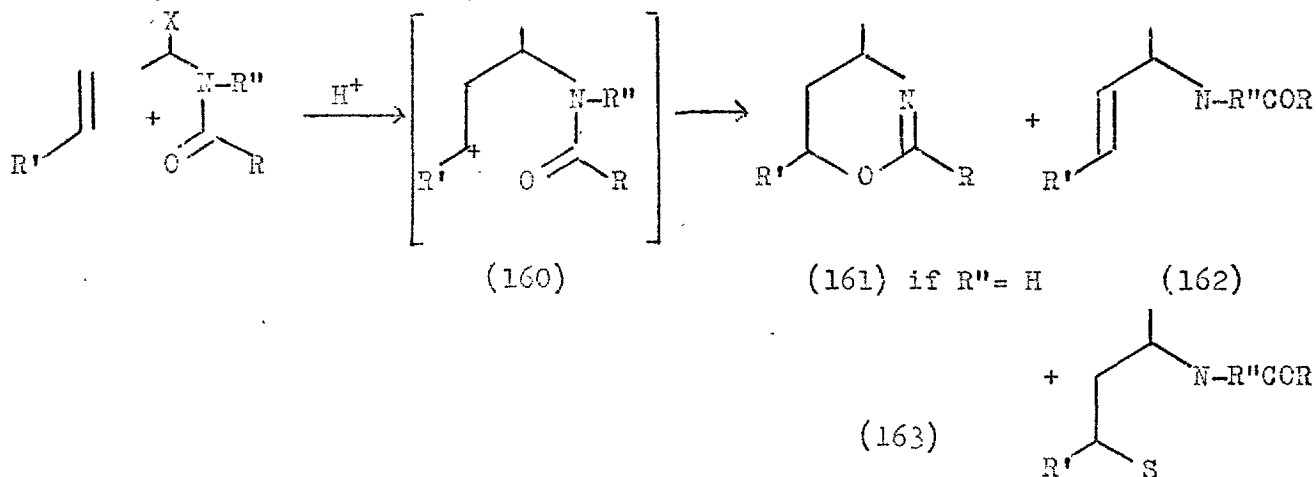
The other reactions of acyliminium cations (*i.e.* α -functionalised amides + acid) parallel those of acylimines but outstrip the latter in both reactivity and degree.

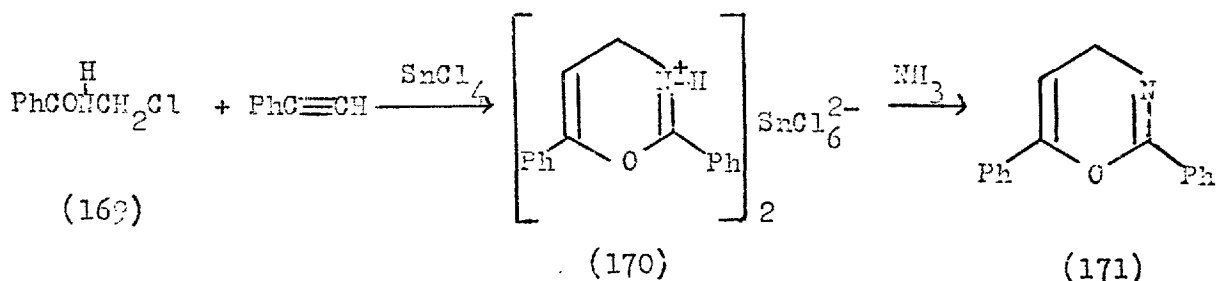
An excellent review¹⁸, quite recently updated⁴⁰, on α -amido alkylations at carbon has been published and this area will not be detailed here. The list of amides used can be represented by $RCON(R')-CH(R'')-X$ where X is $-OH$, $-OR$, $-OCOR$, $-NHCO R$, $-NR_2$, $-NR_3^+$, or halogen. The reviews survey the alkylation of aromatic compounds, monoolefins, conjugated dienes, acetylenes and active methylene compounds. An example of each type is given to illustrate the scope of these reactions.

Aromatic α -amido alkylation, with a variety of acid catalysts, has been known for nearly 80 years¹⁸ and includes the Tscherniac-Einhorn reaction⁸⁷. Even nitrobenzene can be alkylated, for example, by the phthalimide (159)⁸⁸.

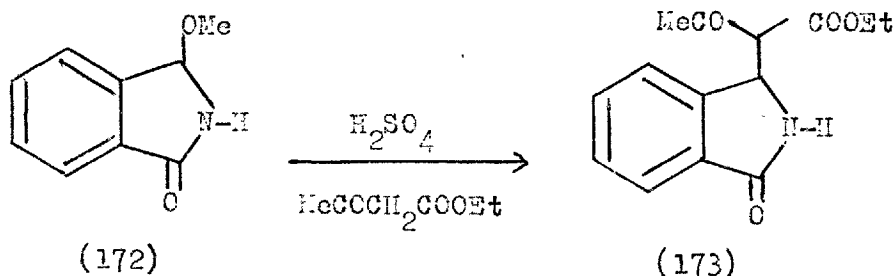


Simple olefins are readily alkylated forming three possible products; (161) is the 1,4-cycloaddition product analogous to the reaction with acylimines (162) is formed by proton loss from the intermediate (160), and (163) can be produced by the reaction of (160) with the solvent. Thus, the benzamido methylol

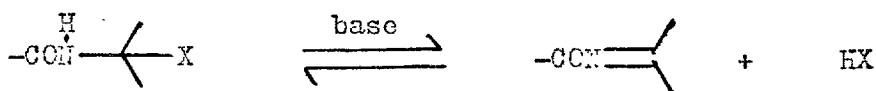




A large number of ketones and activated esters have been reacted with acyliminium salts giving excellent yields of the alkylated amides.⁵¹ Likewise, the methoxy amide (172) is alkylated by ethyl acetoacetate in the presence of conc. sulphuric acid to give (173) in good yield⁴⁹.



(b) Base induced reactions- As shown in scheme 2, base treatment of α -functionalised amides that possess a free N-H group can lead to acylimine formation. Thus, most of the base induced reactions involve acylimines either

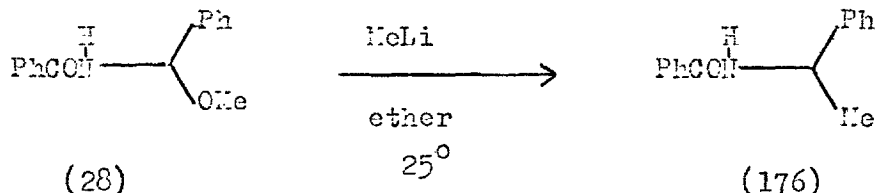


prepared deliberately prior to reaction or as intermediates in the reaction. (All of the former and many of the latter have been already described in previous sections.) The reductions of the acyliminium salt (58)⁵⁰, the methoxy

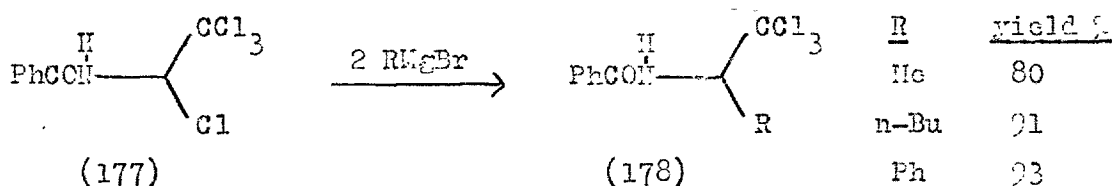


amides (60)⁵¹, (174) and (175)⁵² and the acetoxy amide (96)⁶⁴ with sodium borohydride probably involve initial neutralisation or elimination to the corresponding acylimines which are then reduced as expected. The reactions

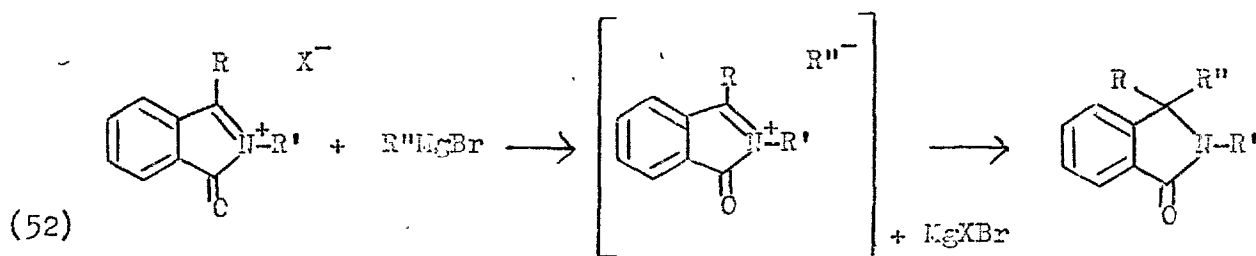
with Grignard reagents follow a similar path. Just as the methoxy amide (60) can be reduced with borohydride it can also be alkylated with Grignard reagents⁵¹. Similarly, alkylation of the methoxy amide (28; R=Ph) with methyl



lithium gives the C-methylated amide (176) and colour changes during the reaction show that the acylimine is an intermediate²⁶. Further proof comes from the yields of the alkylation reactions of the α -chloro amide (177)⁴⁰.



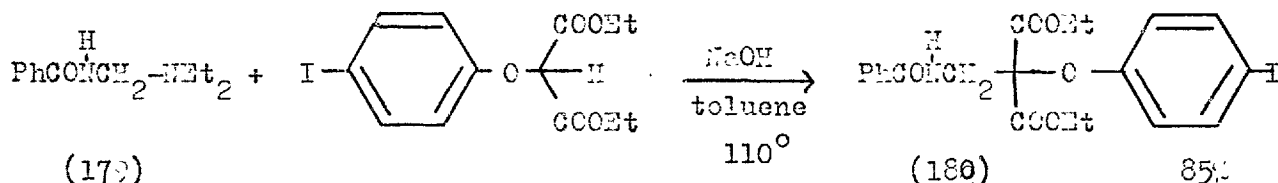
With only one equivalent of the organo-metallic reagent yields of less than 50% were obtained, but with two equivalents the yields of (178) rose to over 80%. Alkylation of the oxoisindolium salts (52) also readily occurs, although if steric crowding becomes too great, e.g. R=R'=R''=Ph, attack occurs at the carbonyl group instead⁹³. In these compounds the stable acylimine cannot be formed due to the N-substituent and alkylation of the free acyliminium cation is enhanced:-



With N-substituted α -functionalised amides, where elimination to the acylimine is not possible, Grignard alkylation requires forcing conditions and long reaction times²⁴; reduction with sodium borohydride also fails²⁶.

Of a similar nature to the Grignard alkylations are the alkylations with active methylene compounds. These reactions have been reviewed⁴⁰ and usually

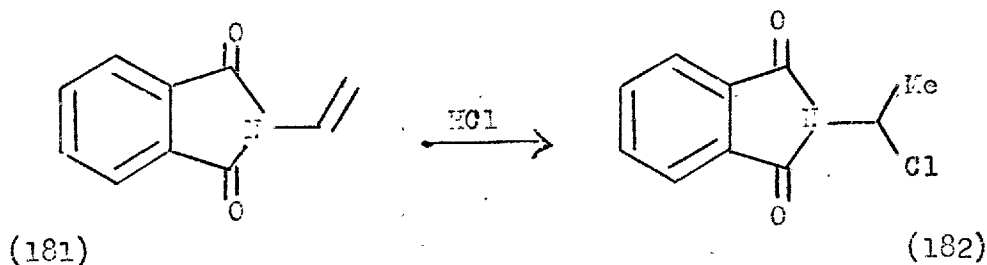
involve the alkali metal salt of the active methylene compound. An example of such a reaction is the high yield alkylation of (179) to (180) with a diethyl malonate derivative. In this case the preferred base is powdered sodium hydroxide⁹⁵.



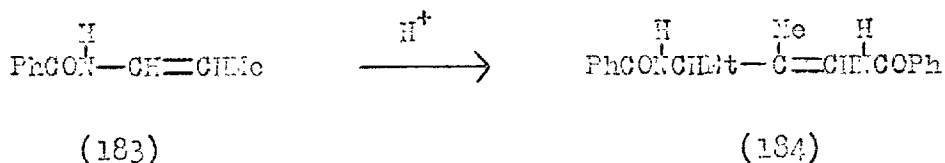
(c) Thermally induced reactions- The thermal eliminations of α -functionalised amides to the corresponding acylimines have been described in the previous sections.

(ii) Enamides.

As indicated in scheme 2 protonation of the enamide double bond can lead to acyliminium ion formation and the whole range of reactions of these intermediates, as described in earlier sections, becomes possible. For example, the addition of dry hydrogen chloride to N-vinyl phthalimide (181) gives a very high yield (93%) of the α -chloro amide (182)⁹⁶. Similarly, various N-vinyl

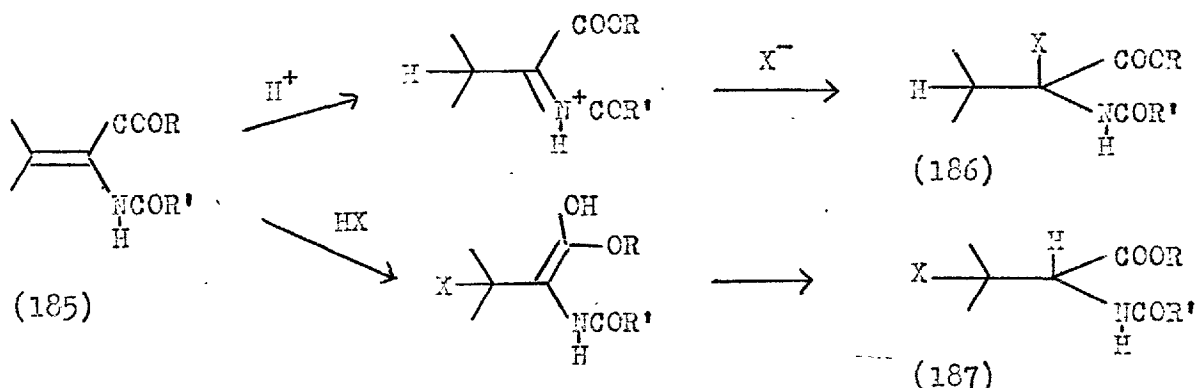


amides and lactams have been α -functionalised by treatment with acid catalysts and nucleophiles^{97,98}. Where no external nucleophile is added the enamide itself can react, as exemplified by the dimerisation of 1-benzoylamino propene



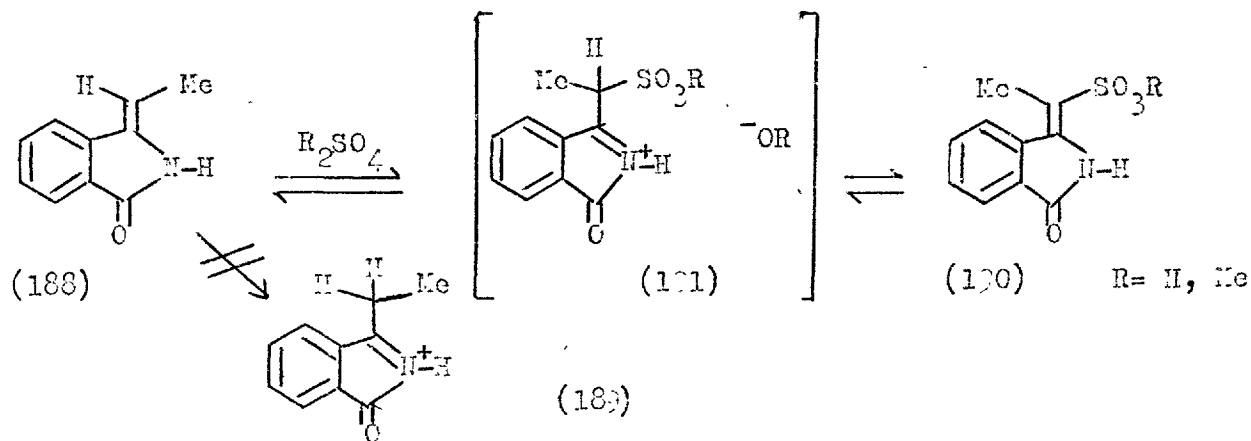
(183) to (184) in the presence of toluene-p-sulphonic acid and the polymerisation of N-vinyl benzamide under similar conditions²⁷.

The addition reactions of dehydro amido acid derivatives (185) have been investigated^{99,100}. In this system, both enamide reactions and 1,4-addition reactions are possible giving products (186) and (187) respectively. The



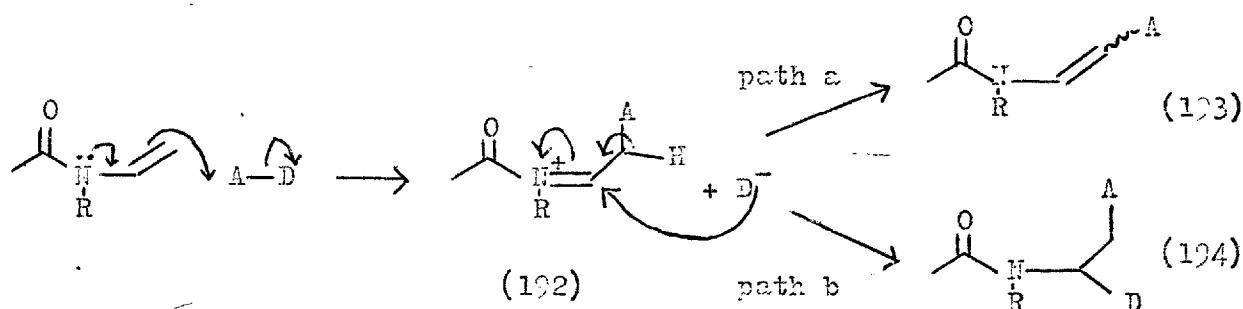
addition of hydrogen bromide to 2-acylamino acrylic acid derivatives over several days affords the thermodynamically controlled 3-bromo derivatives (187; $X=Br$)¹⁰¹. However, following the reaction by n.m.r. spectroscopy shows the initial formation of the kinetically controlled 2-bromo derivatives (186; $X=Br$) which slowly (3 days) revert to the 3-substituted compounds⁹⁹. Although isolation of these reactive α -halo amides is not practical, hydroxy-¹⁰², alkoxy-¹⁰², and mercapto-^{97,103} derivatives are readily prepared by the addition of the corresponding nucleophile. In an exactly analogous manner dehydro-dioxopiperazines have been ring substituted with sulphur nucleophiles in the presence of acid catalysts^{104,105}.

A seemingly exceptional case is the reaction of the oxoisindole (188) with conc. sulphuric acid¹⁰⁶. N.m.r. of the reaction mixture did not show the



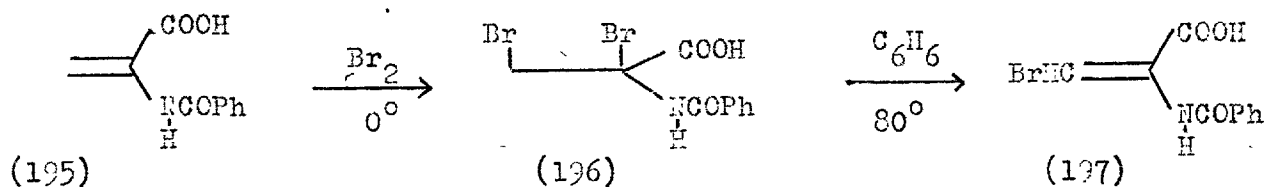
expected acyliminium ion (189) but what is formulated as the sulphonic acid (190; R=H). Although only starting material was isolated on workup, heating (188) with dimethyl sulphate (190⁰, 10 mins.) afforded the sulphonate (190; R=Me) (55%) presumably via the same kind of intermediate (191).

The above reaction and all the acid catalysed reactions are just particular cases of what is a general reaction of enamides. Enamides, like enamines, will seek out any electron deficient centre and attack it with concomitant formation of the acyliminium intermediate (192). Subsequent elimination of a proton

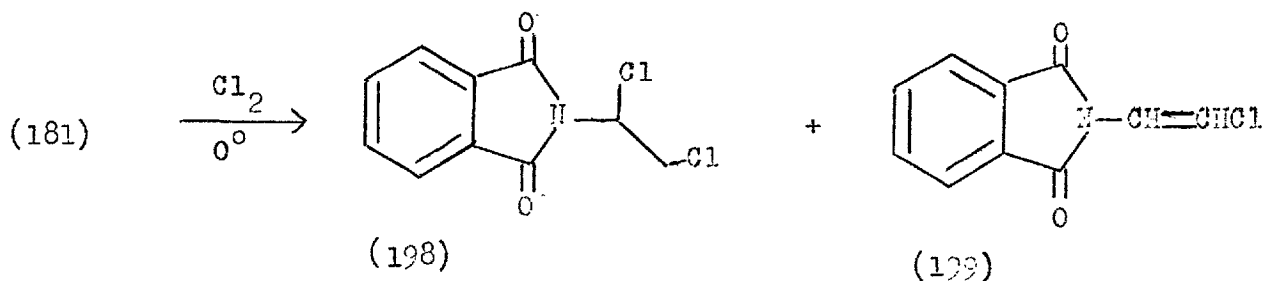


(path a), where possible, gives the substituted enamide (193) whereas attack by the liberated nucleophile D⁻ (path b) gives the α,β -difunctionalised amide (194).

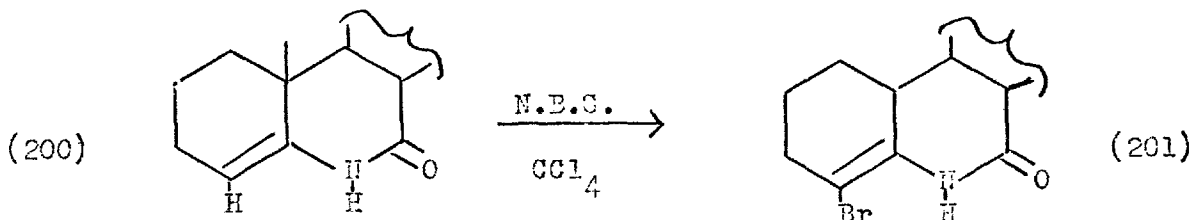
The reactions of enamides with halogens are well known^{100,107-110}. For example, the dehydro-amido acid (195) rapidly forms the dibromide (196) at 0° with bromine. Refluxing in benzene effects elimination to the bromoenamide



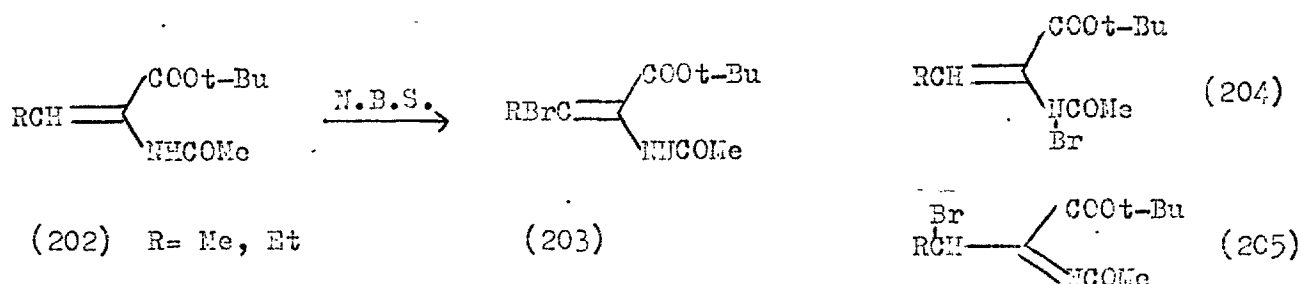
(197)¹⁰⁰. Similarly, N-vinyl phthalimide (181) and chlorine at 0° gives a mixture of the dichloro and monochloro compounds, (198) and (199) respectively¹⁰⁹.



Treatment of 6-aza-4-cholesten-7-one (200) with N-bromosuccinimide affords 85% yield of the bromoenamide (201)¹¹¹. The dehydro-amido esters (202) also

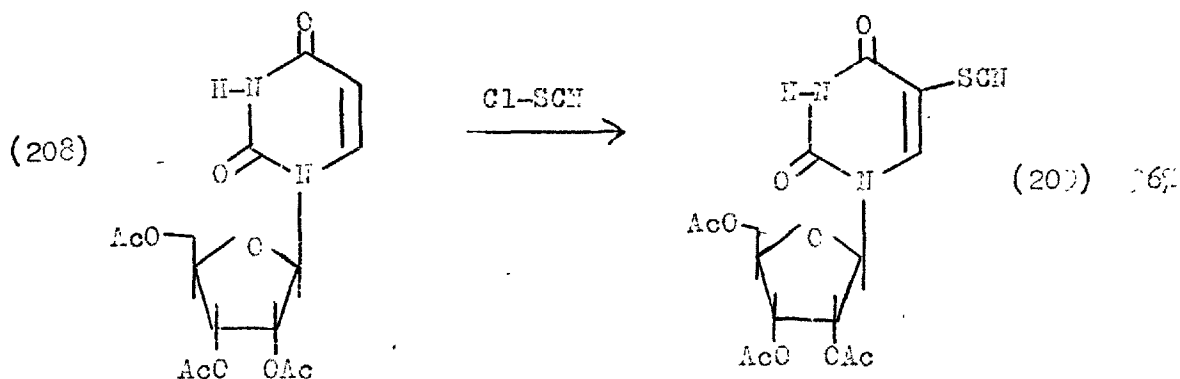
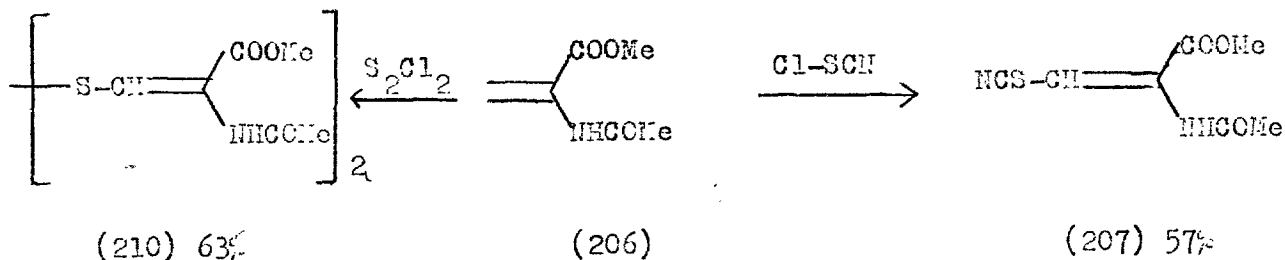


give good yields of the bromoenamides (203) with N-bromosuccinimide in carbon tetrachloride¹¹². The initial product from the reaction is an oil, postulated



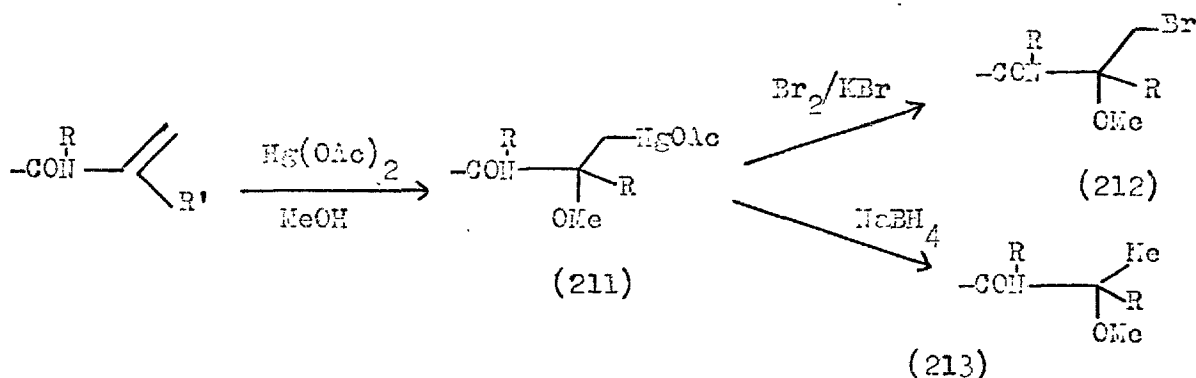
to be the N-bromo amide (204), which on standing rearranges to (203). The only reported evidence for (204) is the infra red spectrum (ν_{max} 1740, 1720, 1680 cm^{-1}) which is far more compatible with expected acylimine (205) than an α,β -unsaturated ester such as (204).

Oxidations of enamides have been reported with thiocyanogen chloride (206) $\rightarrow$ (207)⁹⁹, (208) $\rightarrow$ (209)¹¹³ and with sulphur chloride (206) $\rightarrow$ (210)⁹⁹.



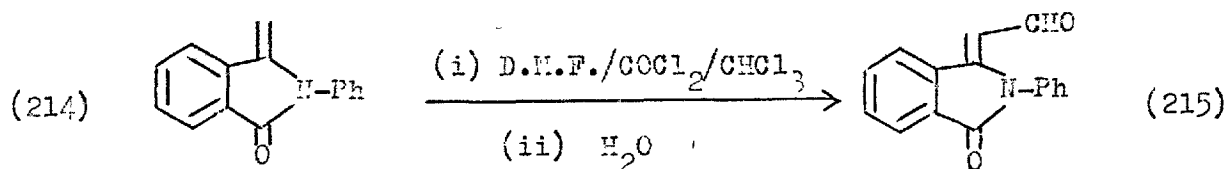
In all three cases dehydrochlorination occurred in situ.

Various enamides e.g. N-vinyl phthalimide¹¹⁴ and 2-acylamino acrylic acids¹¹⁵, can be mercurated in the β -position with mercury (II) acetate. With methanol as solvent the methoxy derivatives (211) are isolated. Subsequent

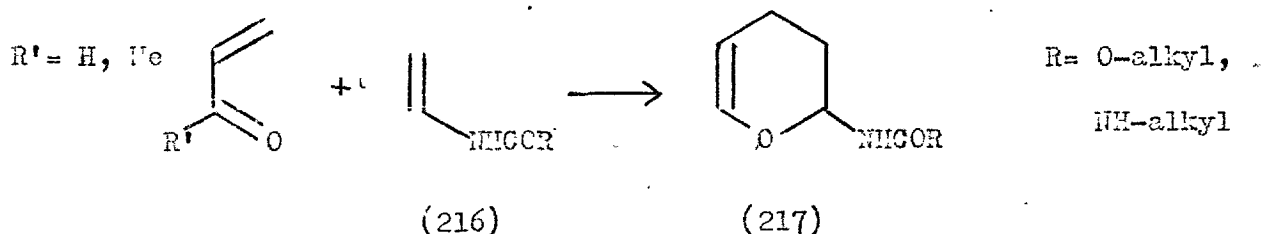


demercuration with potassium bromide/bromine¹¹⁴ affords (212) or with borohydride gives (213)¹¹⁵.

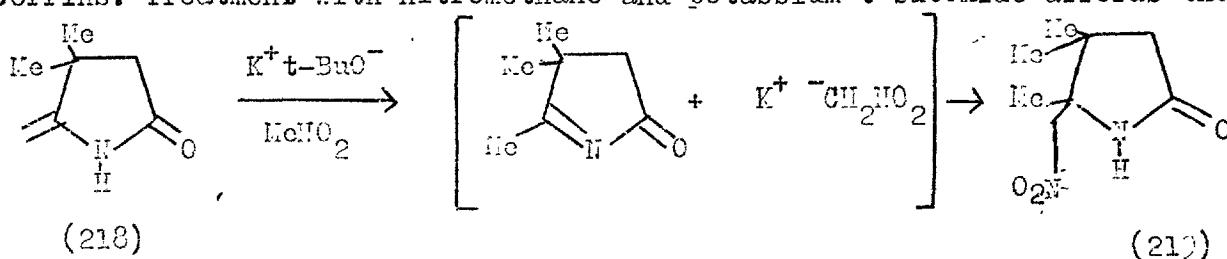
Condensation reactions of enamides have been reported^{116,117}. For example the oxoisindole (214) is readily formylated with dimethylformamide/phosgene to give (215) in 96% yield¹¹⁷. A similar reaction is the addition of α,β -



unsaturated carbonyl compounds to N-vinyl carbamates and ureas (216). Good yields of the dihydropyranyl derivatives (217) are obtained¹¹⁸.



Few base induced reactions of enamides are known. Eschenmoser *et al.*^{119,120} have used the methylene pyrrolidone (218) as a building block in the synthesis of Corrins. Treatment with nitromethane and potassium t-butoxide affords the



alkylated derivative (219).

In absolute ethanol in the presence of sodium ethoxide, N-vinyl benzamide and N-(1-propenyl)benzamides add ethanol in the 1-position to give the ethoxy compounds (220) and in the presence of sodium hydride in boiling benzene both N-(1-propenyl)benzamides and N- β -styryl benzamides undergo cis-trans



isomerisation²⁷. These results must implicate the formation of an acylimine intermediate.

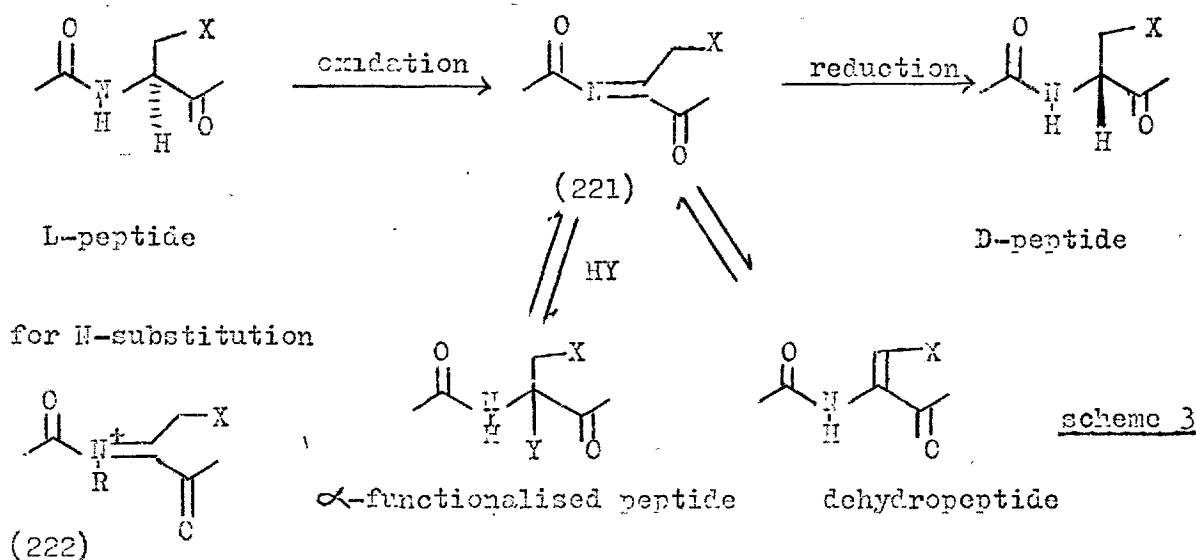
The thermal isomerisations of N-alkenyl carbamates and ureas have already been mentioned⁵⁹.

ACYLIMINES AND DEHYDROPEPTIDES

PART 2

Naturally-occurring dehydropolptides.

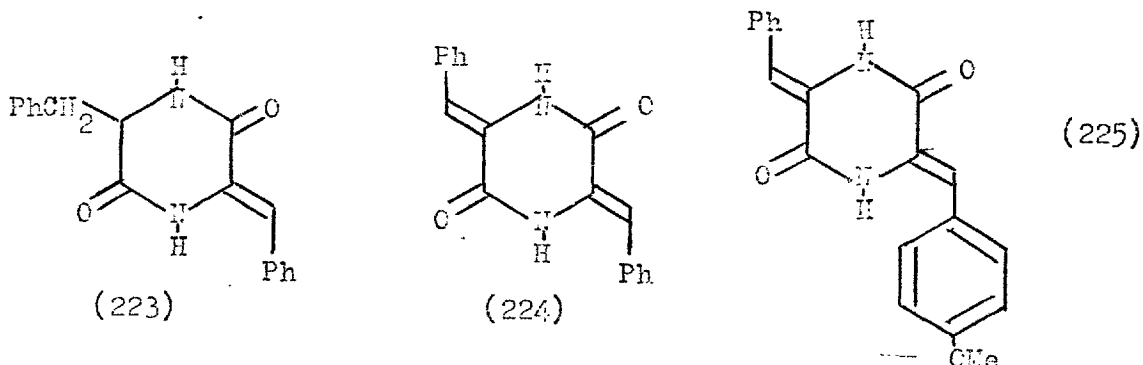
The majority of microbial peptide metabolites are relatively small compounds compared to normal peptide derivatives e.g. proteins¹²¹. Many of these compounds possess some form of biological activity and the majority of them are characterised by their unusually modified amino-acid residues. Many contain 'unnatural' D-amino-acids, dehydroamino acid and α -functionalised amino acid units arranged in rings of varying size and complexity. A proposal made by Bycroft¹²² which explains the formation of D-amino acid and dehydro-amino acid residues is readily extended to include α -functionalised peptides and involves the intermediacy of an acylimine (221) or acyliminium (222) species (scheme 3).



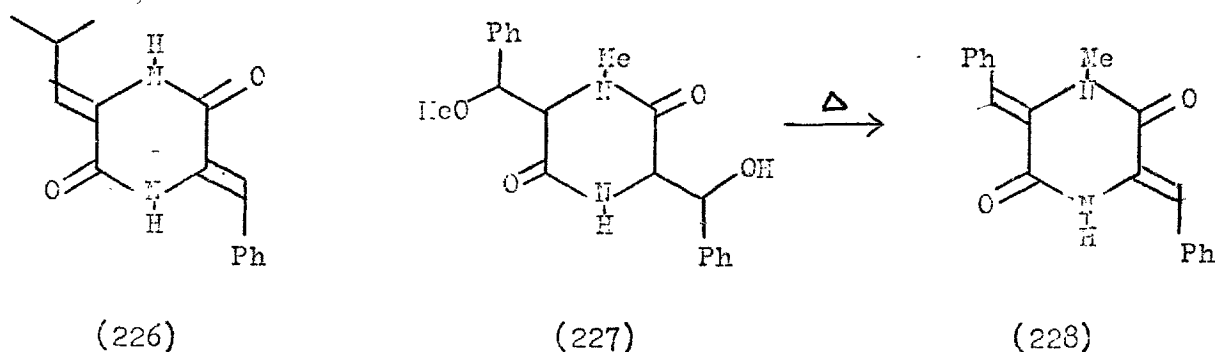
This review will cover naturally-occurring dehydropolptides and α -functionalised peptides but will not include β -functionalised peptides e.g. serine and cysteine residues. The reactivity of acylimines and their related derivatives have been fully detailed in Part 1 and all that remains is to exemplify how nature has utilised this versatile functionality.

2,5-Dioxopiperazines are, without doubt, the most abundant source of

dehydro- and α -functionalised peptides produced in nature. Although the majority of these compounds are derived from simple amino-acids their complexity varies from the sublime to the ridiculous. The benzylidene compounds (223)¹²³, (224)¹²³ and (225)¹²⁴ represent the simplest of the naturally-occurring dehydropeptides. They are readily prepared by condensation of the corresponding



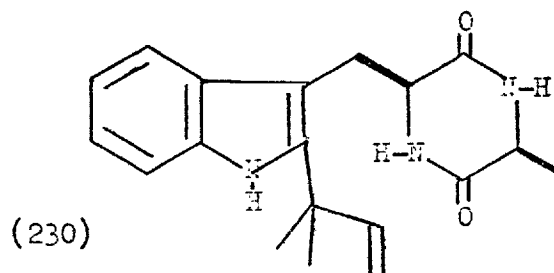
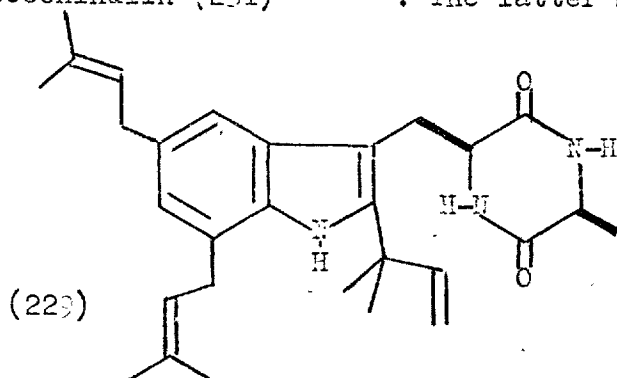
glycine derivative with the aromatic aldehyde, a reaction which has received much attention in the literature^{123,125,126,128}. Albonoursin (226) was isolated from culture preparations of the antibiotics albofungin and nystatin¹²⁷. Its total synthesis has been achieved¹²⁸.



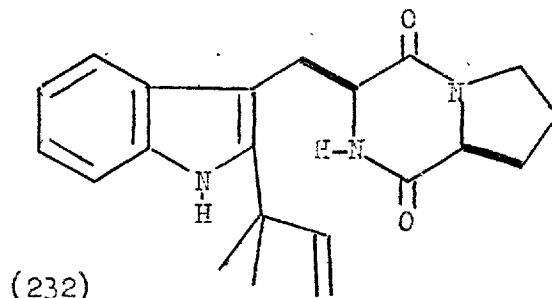
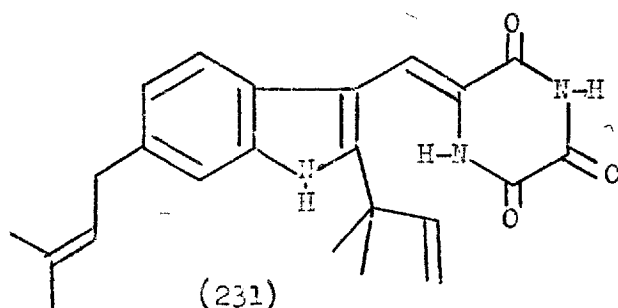
Picrorocellin (227), a lichen product, loses water and methanol on pyrolysis to form the bis-enamide (228), behaviour associated with α -functionalised amides¹²⁹. Its structure was assigned on chemical evidence only and due to doubt about its source the isolation has not been repeated since.

Echinulin (229), although itself not a dehydropeptide, is the first in a series of dioxopiperazines which contain an isoprenylated tryptophan unit¹³⁰. Worthy of note is the inverted C₅-unit at the 2-position of the indole ring which is common to this family of compounds. The structure was proposed

independently by two groups^{131,132} and its total synthesis has also been effected¹³³. Isolated along with echinulin from Aspergillus amstelodami is the simple derivative (230)¹³⁴ and the orange-red dehydrotrioxopiperazine named neoechinulin (231)^{135,136}. The latter metabolite possesses two unusual features.

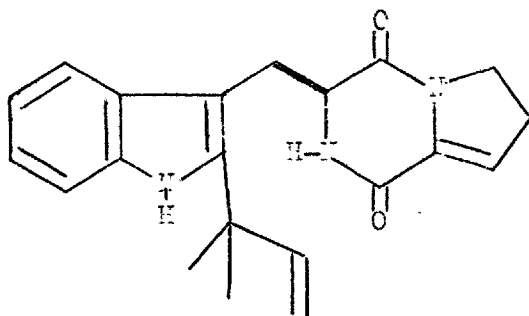


The extra carbonyl group in the dipeptide ring could be formed by an oxidative cleavage of a dehydroalanine residue, a facile in vitro reaction¹³⁷. The position of the second isopentenyl unit is unique in that no other indole alkaloid has an alkyl substituent in the 6-position. It is interesting to

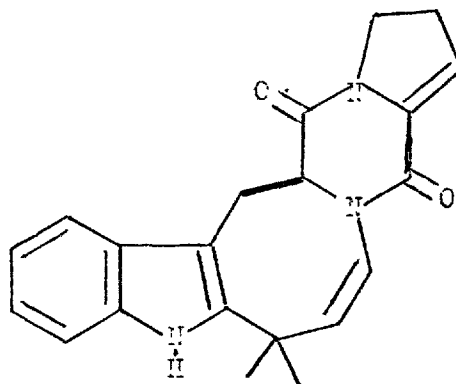


speculate on the biosynthetic relationship between (229), (230) and (231).

A number of metabolites isolated from Aspergillus ustus and Penicillium brevicompactum are derived from L-proline and L-tryptophan. The simpler compounds from A. ustus are (232), analogous to the alanine derivative (230), and the dehydropeptide (233)¹³⁸. Oxidative coupling of the dimethyl allyl group to the free nitrogen of the dioxopiperazine ring is a feature of three other alkaloids, (234), (235) and (236), isolated from A. ustus¹³⁸. In the case of (235) and (236) further oxidation of the indole ring to the corresponding ψ -indoxyl system has also occurred. This oxidation is common both in vivo¹³⁹ and in vitro¹⁴⁰ and is readily reversed in the latter, by treatment with

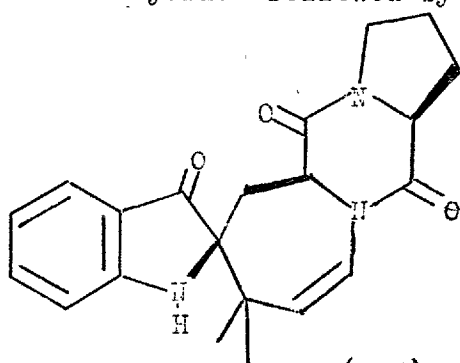


(233)

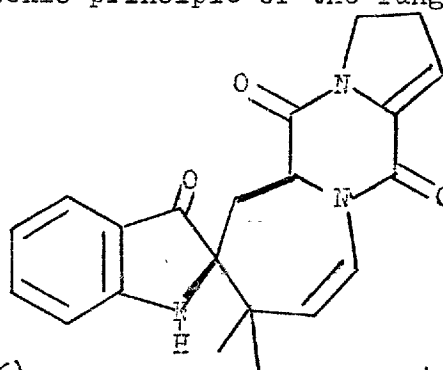


(234)

sodium borohydride followed by acid. The toxic principle of the fungus has



(235)

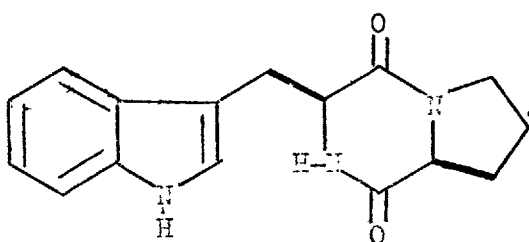


(236)

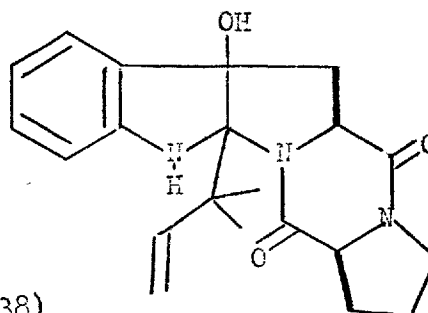
austamide

been identified as austamide (236)¹⁴¹ and its structure has been confirmed by X-ray studies¹⁴².

Derived from the same amino-acids are the brevianamides A to F, isolated from P. brevicompactum^{143,144,145}. Apart from brevianamide F (237) which is cyclo L-prolyl-L-tryptophan¹⁴⁵, the simplest of these metabolites is (238),



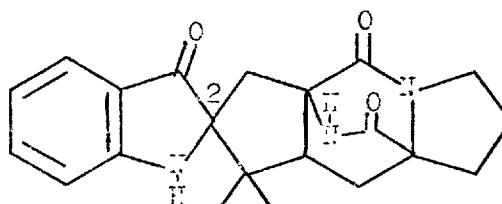
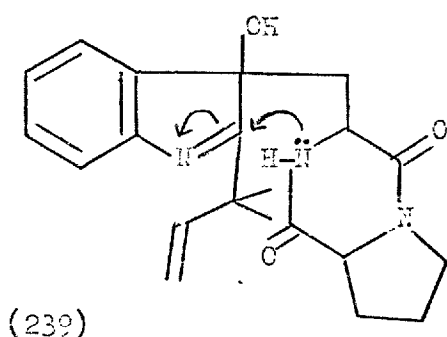
(237)



(238)

brevianamide E^{143,144}. Oxidation of the indole ring has only proceeded to the hydroxyindolenine stage in this compound, the oxidation being followed by an intramolecular addition of the amide N-H group across the imine (239). The other brevianamides are ψ -indoxyl derivatives where the double bond of the dimethyl allyl group is missing. The diastereomeric structures of brevianamides

A (240)¹⁴⁴ and B (241)¹⁴⁵ are based on chemical, spectral and biosynthetic

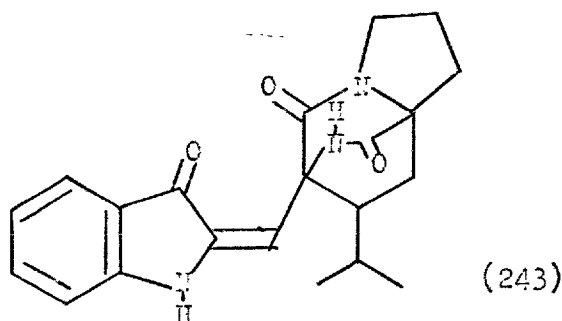
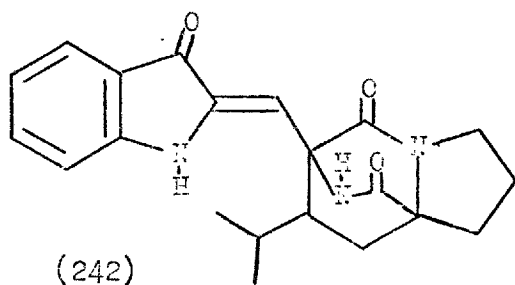


(240) Brevianamide A

(241) Brevianamide B

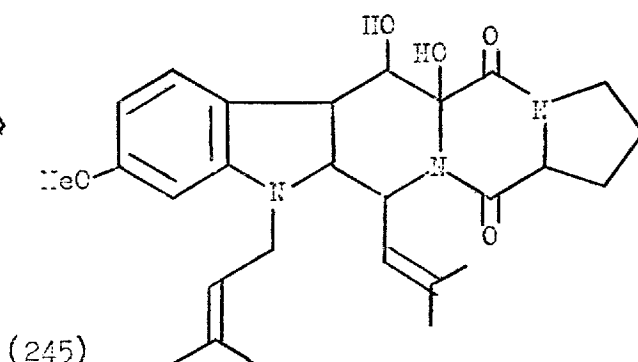
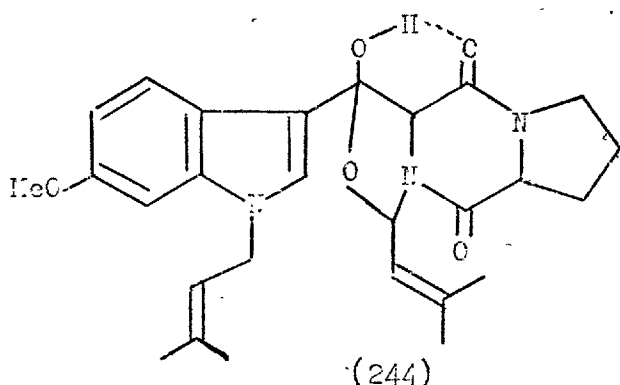
epimers about position 2.

considerations. The formation of the bridged bicyclic system will be examined at length in the discussion (p.79). Brevianamides C (242) and D (243) have been shown to be photochemical artefacts from brevianamides A and B and not



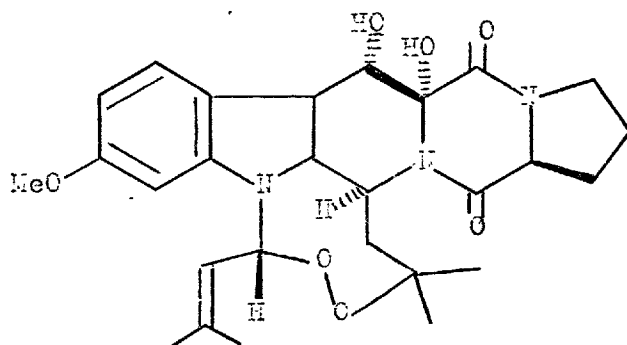
true metabolites of enzymatic origin¹⁴⁵.

Lanosulin, a fungal metabolite isolated from Penicillium lanosum¹⁴⁶, was originally assigned structure (244)¹⁴⁶. However, parallel studies on

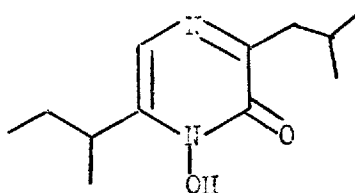


Aspergillus fumigatus¹⁴⁷ have produced two tremorgens (so named because of their tremor inducing effect in test animals), fumitremorgens A and B (245), the latter of which has been shown to be identical with lanosulin¹⁴⁸. The structure of fumitremorgen A is not fully known but the molecular formula, $C_{23}H_{41}N_3O_7$,

suggests the incorporation of a further C_5 -unit. The revised formulation of lanosulin (245) bears a very strong resemblance to the naturally-occurring peroxide verruculogen (246), another tremorgen isolated from Penicillium verruculosum, the structure and relative stereochemistry of which have also been confirmed by an X-ray analysis¹⁴⁹.

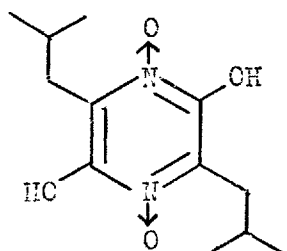


(246)

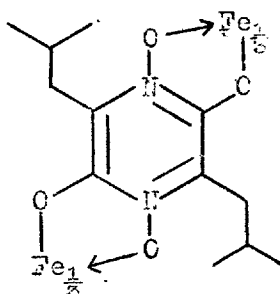


(247)

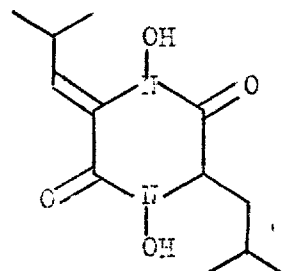
The hydroxamic acid aspergillic acid, isolated from Aspergillus flavus¹⁵⁰, has powerful antibiotic properties. The structure (247) is well established¹⁵¹ and a total synthesis has been achieved¹⁵². Biosynthetic studies on aspergillic acid and related compounds tend to exclude the corresponding dioxopiperazines as biosynthetic precursors¹⁵³. In contrast, cyclo L-leu-L-leu, acts as an efficient precursor¹⁵⁴ to another pyrazine derivative, pulcherriminic acid, via an in vivo aerial oxidation¹⁵⁵. This compound, produced by Candida pulcherrima¹⁵⁶, has the structure (248) on the basis of its n.m.r. spectrum¹⁵⁷. However, the sample originally isolated via the ferric complex, pulcherrimin



(248)



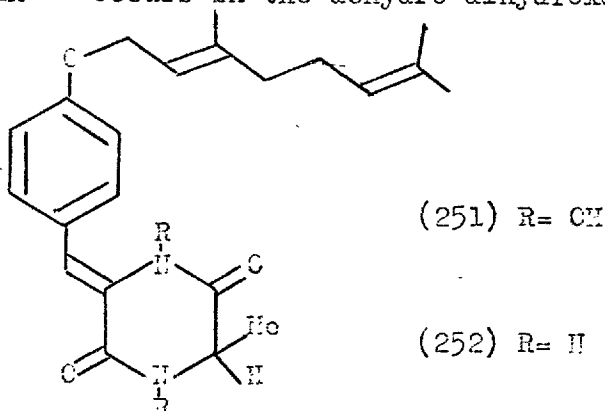
(249)



(250)

(249), exhibited different properties to (248)¹⁵⁸. These differences can be explained by the isomeric dehydro-dihydroxamic acid (250) which may have been formed under the conditions required to free the acid from its metal complex. A total synthesis of pulcherrimin has been claimed¹⁵⁹.

Unlike pulcherrininic acid, mycelianamide isolated from Penicillium griseofulvum Dierckx¹⁶⁰ occurs in the dehydro-dihydroxamic acid form (251)¹⁶¹.



This structure has been confirmed by a total synthesis of racemic deoxy-mycelianamide (252)¹⁶². The chemistry of this unusual metabolite has been reviewed¹⁶³.

A review on pyrazines, many of which may be derived from dehydropeptides, has recently been published¹⁶⁴.

One of the most interesting classes of peptide derivatives is the epipolythiodioxopiperazines which are characterised by their sulphur bridges. A comprehensive list of these metabolites, their structures and their sources is given in tables 1, 2 and 3.

Gliotoxin (253) was first isolated in 1936 from a species of Trichoderma viride¹⁶⁵ and subsequently from a variety of other micro-organisms¹⁶⁶⁻¹⁷⁰.

It is highly antifungal, bacteriostatic and antiviral but its toxicity has made its use prohibitive. Its structure, first proposed on the basis of chemical degradation¹⁷¹, has been confirmed, and its absolute configuration determined, by X-ray analysis¹⁷². Reaction of gliotoxin with quinone oxidants e.g. o-chloranil, affords dehydrogliotoxin (255), also a natural product¹⁷³, in which the cyclohexadienol is converted into the much more stable phenol¹⁷³. Consequently, reactions can now be carried out at the disulphide bridge without interference from the dienol group¹⁷⁴. It is of interest to note that the antiviral and antibacterial activity is absent in related compounds without the strained polysulphide bridge^{171,175}.

In a search for possible biosynthetic precursors of gliotoxin, three

Table 1

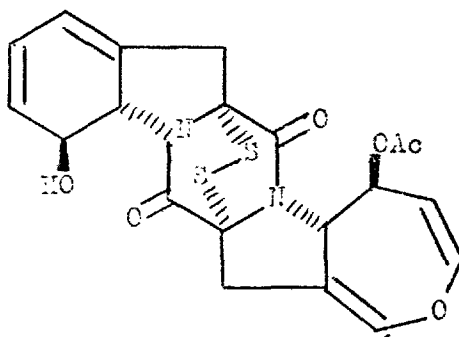
Epipolythiodioxopiperazines belonging to the gliotoxin type.

<u>Name</u>	<u>Structure</u>	<u>Source</u>
Gliotoxin (253)		<u>Trichoderma viride</u> ¹⁶⁵ <u>Gliocladium fimbriatum</u> ¹⁶⁶ <u>Aspergillus fumigatus</u> ¹⁶⁷ <u>Penicillium terlikowski</u> ¹⁶⁸ <u>Aspergillus terreus</u> ¹⁶⁹ <u>Penicillium sp.</u> ¹⁷⁰
Gliotoxin acetate (254)		<u>Penicillium terlikowski</u> ¹⁶⁸
Dehydrogliotoxin (255)		<u>P. terlikowski</u> ¹⁷³
Aranotin R= H (256)		<u>Arachniotus aureus</u> ¹⁷⁷
Acetyl aranotin R= Ac (260) (LL-S88α)		<u>A. aureus</u> ¹⁷⁷ <u>Aspergillus terreus</u> ¹⁶⁹
Bis-dethiodi- (methylthio) acetyl aranotin (261) (LL-S88β)		<u>Arachniotus aureus</u> ¹⁷⁷ <u>Aspergillus terreus</u> ¹⁶⁹

Table 1 contd.

Apoarenotin

(262)



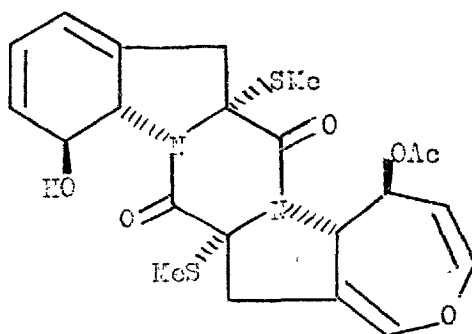
Arachniotus aureus¹⁷⁸

Bis- α -ethiodi

(methylthio)

acetyl apoarenotin

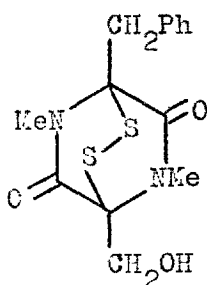
(263)



A. aureus¹⁷⁸

Hyalodendrin

(264)

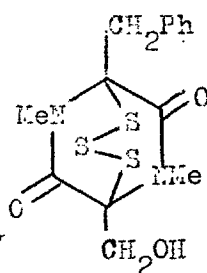


unidentified¹⁸³

Hyalodendron sp.¹⁸⁴

Penicillium turbatum¹⁸⁵

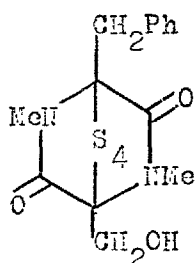
(265)



unidentified¹⁸³

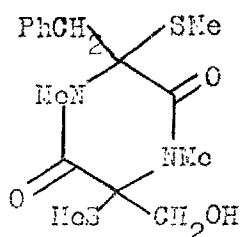
Penicillium turbatum¹⁸⁵

(266)



P. turbatum¹⁸⁵

(267)

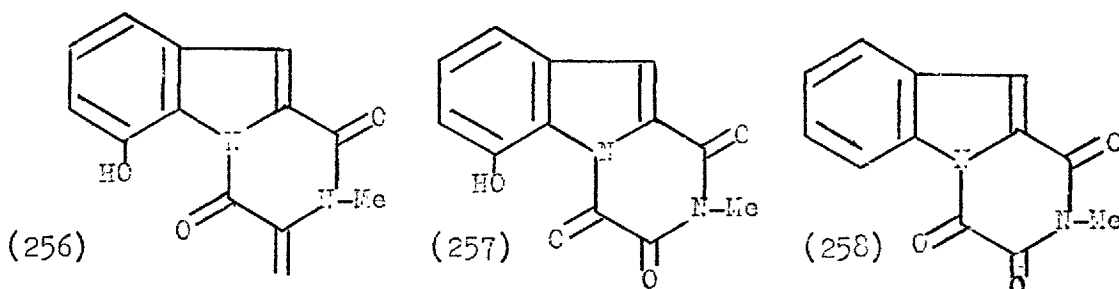


unidentified¹⁸³

Penicillium turbatum¹⁸⁵

dethiodioxopiperazines have been isolated (256), (257) and (258)¹⁷⁶.

Comparison of (257) and (258) with neoechinulin (231) shows the same level



of oxidation present in all three metabolites i.e. the dehydrotrioxopiperazine system.

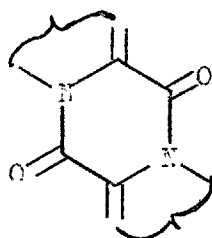
The mould Arachnictus aureus produces five metabolites closely related to gliotoxin. Three of these compounds, aranotin (259), acetyl aranotin (260) and bis-dethiodi(methylthio)acetyl aranotin (261)¹⁷⁷, are characterised by their two dihydrooxepin rings, where as the other metabolites, apoaranotin (262) and bis-dethiodi(methylthio)acetyl apoaranotin (263) contain one seven-membered ring and a cyclohexadienol unit like that found in gliotoxin¹⁷⁸.

The antibiotics LL-S88 α and LL-S88 β isolated from Aspergillus terreus¹⁶⁹, were shown to be identical to acetyl aranotin (260) and the thiomethyl derivative (261) respectively¹⁷⁹. X-ray analyses have confirmed the structures of acetyl aranotin¹⁷⁹, and the thiomethyl derivative (261)¹⁸⁰ and c.d. studies¹⁸¹ identify the configuration at the asymmetric centres in this group of metabolites with that of gliotoxin. Some chemical reactions carried out on the disulphide bridge of acetyl aranotin have been described¹⁸².

Recently, another family of compounds which appears to belong to the gliotoxin type, has been independently isolated by three groups^{183,184,185}. The structures of the disulphide (264), trisulphide (265), tetrasulphide (266) and the bis-dethiodi(methylthio) analogue (267) have been proposed on the basis of spectral data^{183,184,185}, chemical degradation¹⁸⁴ and X-ray studies¹⁸⁵. It should be noted that the c.d. spectrum of the disulphide (264) indicates that the compound isolated from Hyalodendron sp.¹⁸⁴, hyalodendrin, has the opposite configuration to gliotoxin, where as the disulphide isolated from

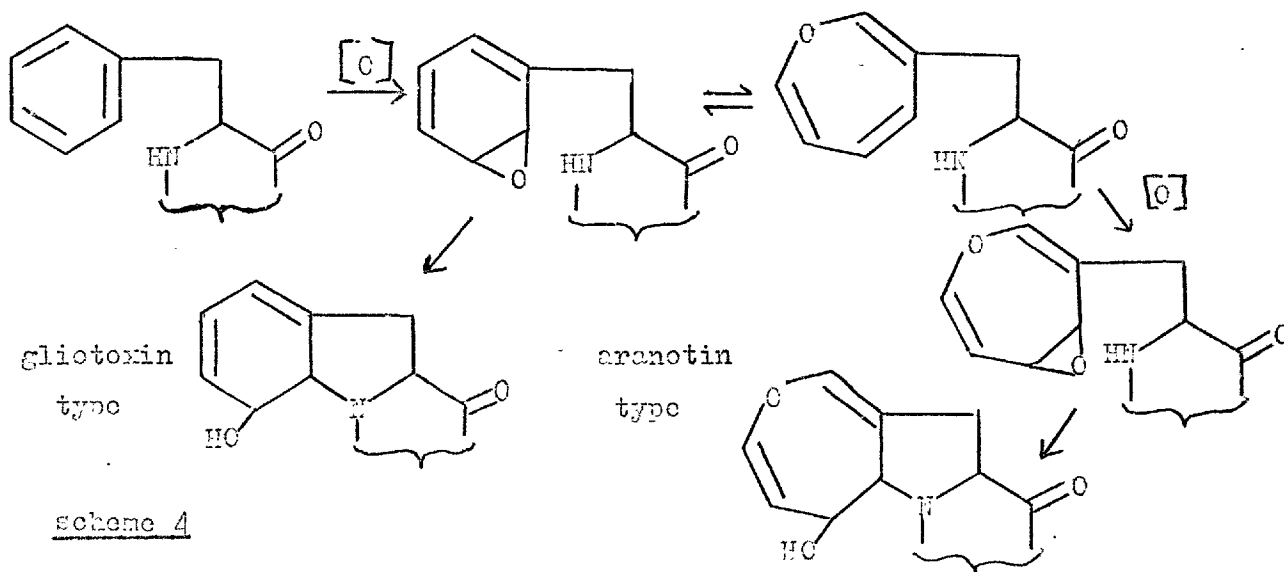
Penicillium turbatum¹⁸⁵ has the same configuration as gliotoxin¹⁸⁶ i.e. the two disulphides are, in fact, enantiomers. The optical rotations as expected have opposite signs and there are small differences in the i.r. and n.m.r. spectra. Whether these two isomers will give rise to different biological activity will be very interesting to observe.

The elucidation of the biosynthesis of this range of compounds has been the subject of considerable effort. Labelling studies have shown that the hydroxy methyl residue in gliotoxin is derived from serine or glycine with N-methylation by methionine^{187,188}. The other part of the ring in both gliotoxin^{189,190} and the aranotins¹⁹¹ is derived from phenylalanine and not as earlier suggested¹⁸⁸ m-tyrosine, tryptophan or acetate¹⁹². D₈-Phenylalanine is incorporated into acetyl aranotin in Aspergillus terreus with retention of all the benzylic deuterons¹⁹¹. Thus, the possibility of a bis-dehydro system (268) acting as a precursor is excluded. A report that dehydrogliotoxin can also act as a precursor has been contradicted¹⁹⁰.

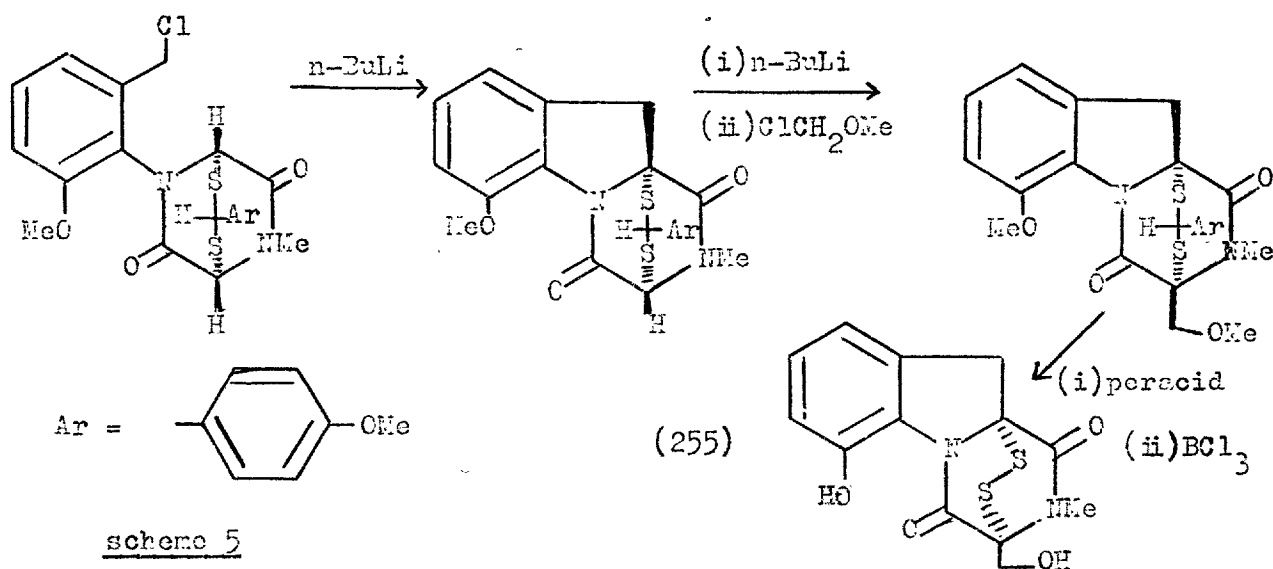


(268)

The most likely proposal¹⁷⁸ for the formation of the cyclohexadienol and the dihydrooxepin systems involves the intermediacy of benzene oxides¹⁹³. This idea is shown in scheme 4.



The synthesis of epipolythiodioxopiperazines¹⁹⁴ has attracted a great deal of attention. The most versatile approach so far involves the protected disulphide developed by Kishi et al.¹⁹⁵. A most elegant synthesis of dehydrogliotoxin (255) has been achieved using this method (scheme 5)¹⁹⁶.



The fungus Pithomyces chartarum first attracted attention because it caused facial eczema in sheep. The toxic metabolite, sporidesmin (269), was isolated and characterised^{197,198} and proved to be the first of seven related compounds, sporidesmins B to G (Table 2) all produced by the same fungus.

The structures of sporidesmin (269) and sporidesmin B (270) were determined by chemical and spectroscopic methods¹⁹⁸. Subsequent confirmation by an X-ray analysis¹⁹⁴ also showed that the configuration of the disulphide bridge is the same as in gliotoxin, as predicted prior to the X-ray by c.d. studies¹⁷². It is interesting to note that the -S-S- dihedral angle in these compounds is not the usual value of ca. 100° but rather 10-20°. The X-ray studies also show that the sulphur atoms are spatially nearer to the carbonyl groups than they are to the nitrogen atoms of the ring. In this context, there is some evidence from n.m.r. that the sulphur bridge in the trisulphide, sporidesmin E (273), can exist in two relatively stable conformations (276) and (277)²⁰⁰. The reduction of (273) to sporidesmin with triphenylphosphine²⁰¹ occurs much faster with one conformer than with the other²⁰⁰.

Table 2

The sporidesmins

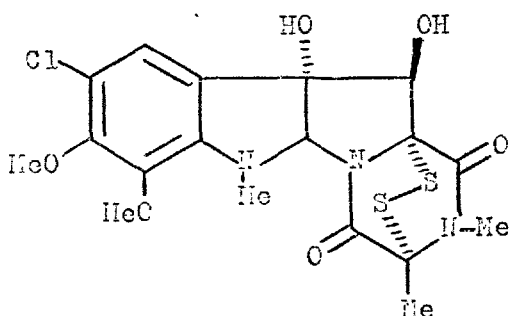
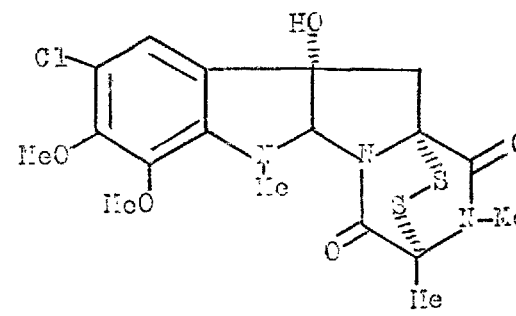
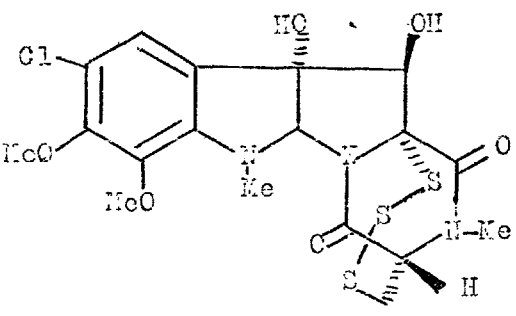
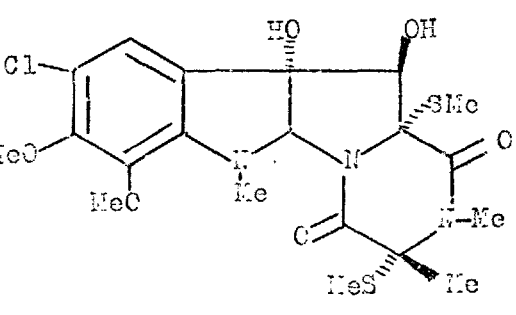
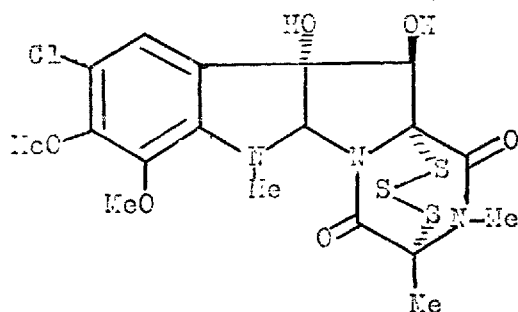
<u>Name</u>	<u>Structure</u>	<u>Source</u>
Sporidesmin (263)		<u>Pithomyces chartarum</u> ¹⁹⁷
Sporidesmin B (270)		<u>P. chartarum</u> ¹⁹⁷
Sporidesmin C (271)		<u>P. chartarum</u> ²⁰⁴
Sporidesmin D (272)		<u>P. chartarum</u> ²⁰⁵

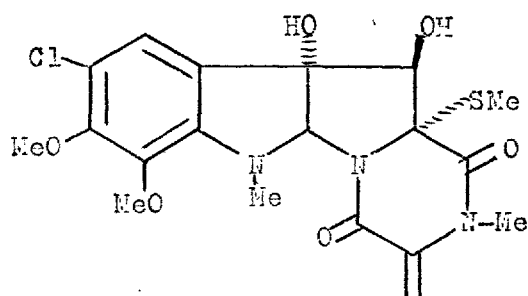
Table 2 contd.

Sporidesmin E
(273)



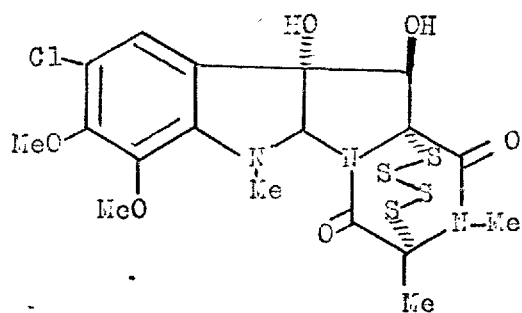
P. chartarum²⁰¹

Sporidesmin F
(274)

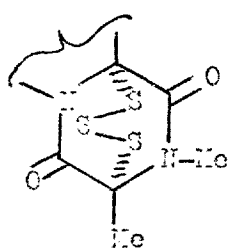


P. chartarum²⁰⁵

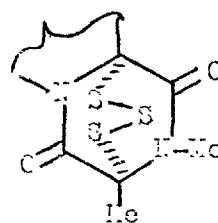
Sporidesmin G
(275)



P. chartarum²⁰²

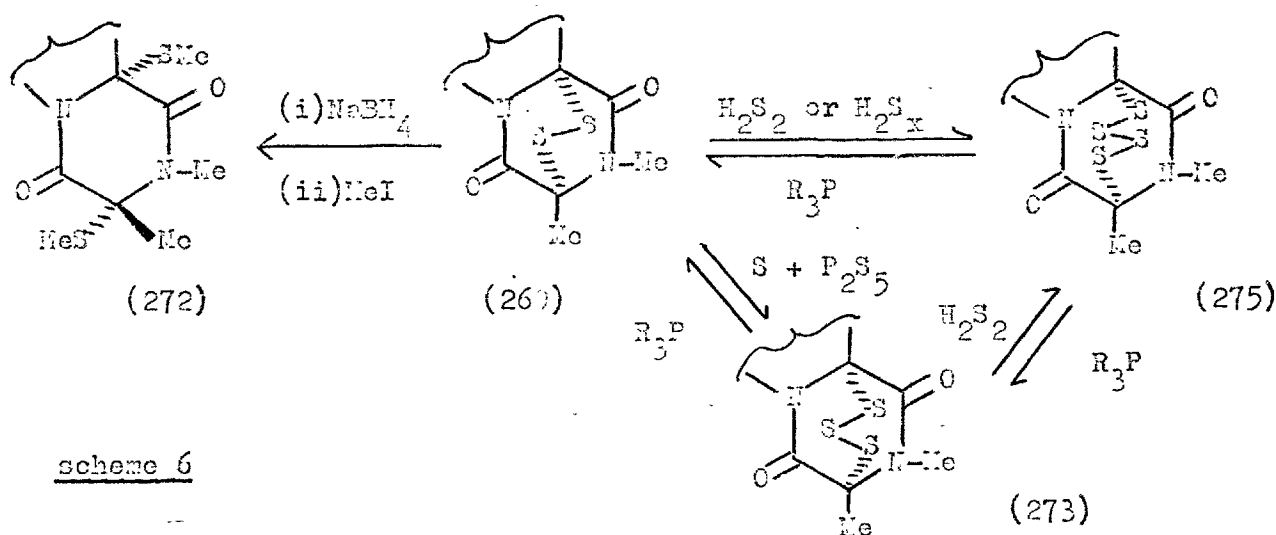


(276)



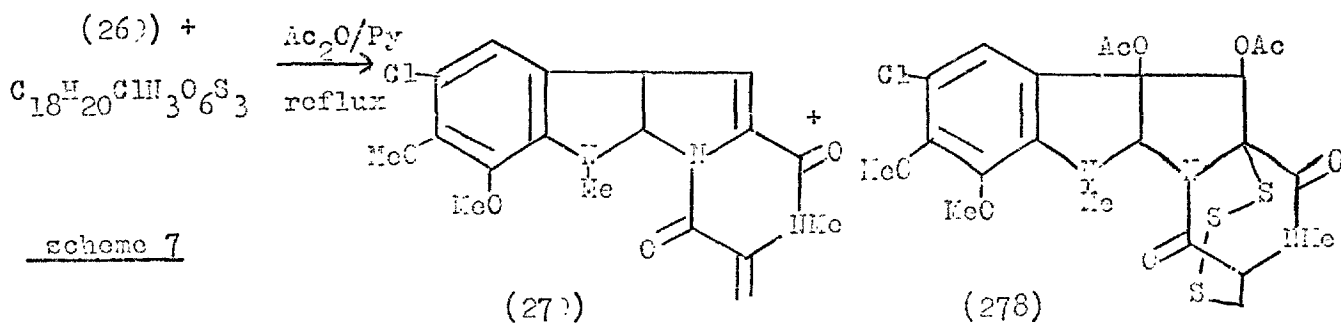
(277)

The structure of the tetrasulphide, sporidesmin G (275), was established by synthesis from sporidesmin²⁰² and subsequently confirmed by an X-ray analysis²⁰³. The chemical interconversions between the sporidesmins are shown in scheme 6.



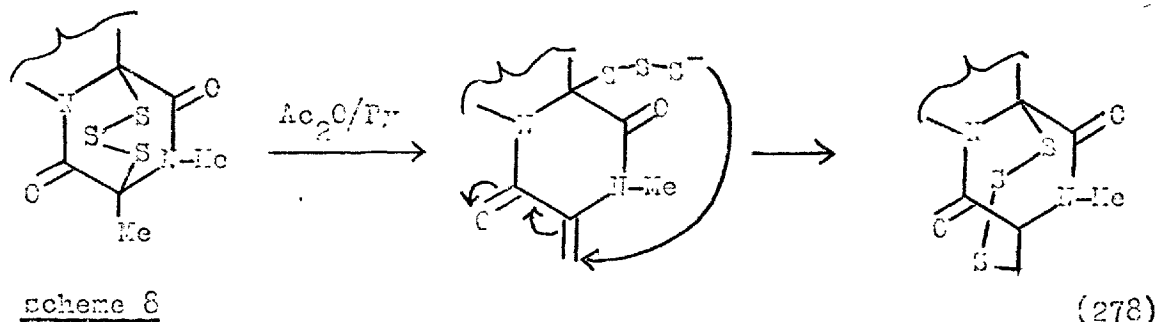
scheme 6

The exceptional structure of sporidesmin C (271) demands careful scrutiny. The only evidence²⁰⁴ for its natural occurrence is provided by two weak peaks at m/e 505, 507 ($C_{18}H_{20}ClN_3O_6S_3$) in the mass spectrum of crude sporidesmin (in addition peaks were present at m/e 537, 539 ($C_{18}H_{20}ClN_3O_6S_4$)). Subsequently, the crude sporidesmin was refluxed with acetic anhydride and pyridine and sporidesmin C diacetate (278) isolated in 0.2% yield from the main product, anhydrodethiosporidesmin (279) (scheme 7). This work was carried out before



scheme 7

the isolation of sporidesmins E²⁰¹ and G²⁰² and it is possible that the minor



components in the mass spectrum in fact, belong to these compounds. The formation of (278) is readily explained (scheme 8) by initial sulphur elimination from the normal epitrithio compound, followed by readdition in the β -position (see p. 32 and refs. 101,104 and 255 for literature precedents).

Very little work has been carried out on the biosynthesis of the sporidesmins but total syntheses of both sporidesmin²⁰⁶ and sporidesmin B²⁰⁷ have been completed in a manner similar to that employed for dehydrogliotoxin. A detailed discussion on the occurrence of the sporidesmins and related compounds has been published²⁰⁸.

Several dimeric metabolites related to the sporidesmins have recently been characterised (Table 3). Chaetocin (280), isolated from Chaetomium minutum, was assigned its structure on the basis of chemical degradation²⁰⁹. Subsequent X-ray analysis confirmed the structure and established the absolute configuration of the molecule²¹⁰. Recently, 11 α ,11 α' -dihydroxy chaetocin (281) has been isolated from Verticillium tenerum²¹¹. Verticillium sp. produces the antibiotics verticillin A (282)²¹² and B (283)²¹³ differing from the chaetocins only in their number of hydroxy groups. It is thought that verticillin C (284) which accompanies A and B is a trisulphide analogue of the latter metabolite²¹³. C.d. studies indicate that all the dimeric alkaloids are antipodal to the gliotoxins, sporidesmins and aranotins, with respect to the configuration of the disulphide bridges, although direct use of c.d. curves in this manner must be treated with caution¹⁸⁶. Whether their lack of antiviral activity is related to this structural feature remains to be seen.

Table 3

Miscellaneous epipolythiodioxopiperazines

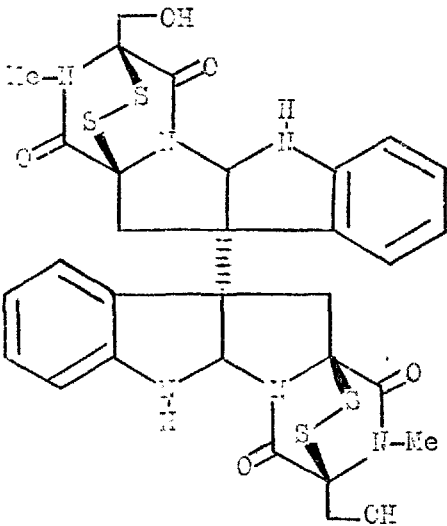
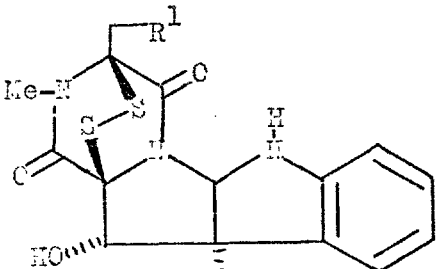
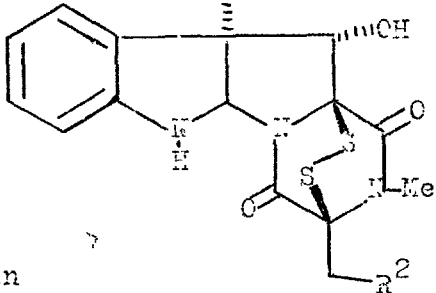
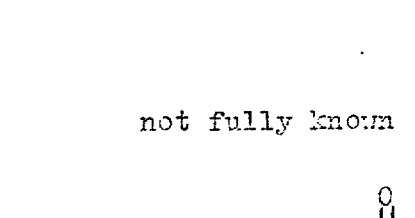
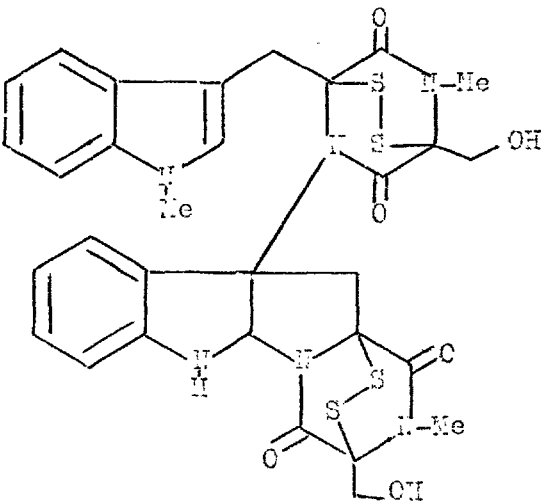
<u>Name</u>	<u>Structure</u>	<u>Source</u>
Chaetocin (280)		<u>Chaetomium minutum</u> ²⁰⁰
Verticillin A (282) $R^1 = R^2 = H$		<u>Verticillium sp.</u> ²¹²
Verticillin B (283) $R^1 = H$ $R^2 = OH$		<u>Verticillium sp.</u> ²¹³
Dihydroxy chaetocin (281) $R^1 = R^2 = OH$		<u>V. tenerum</u> ²¹¹
Verticillin C	not fully known	<u>Verticillium sp.</u> ²¹³
Chetomin (285)		<u>Chaetomium cochliodes</u> ²¹⁴

Table 3 contd.

Oryzanolide²¹⁷ unknown

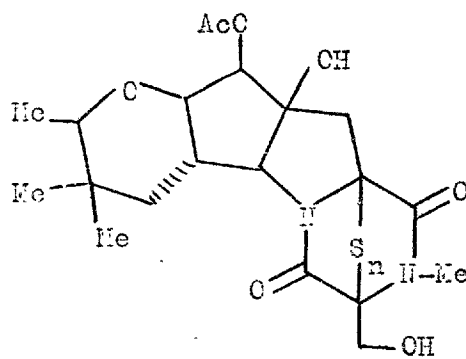
Melinacidins²¹⁸ I, II and III unknown

Sirodesmins²¹⁹

(286) A, n=2

(287) B, n=3

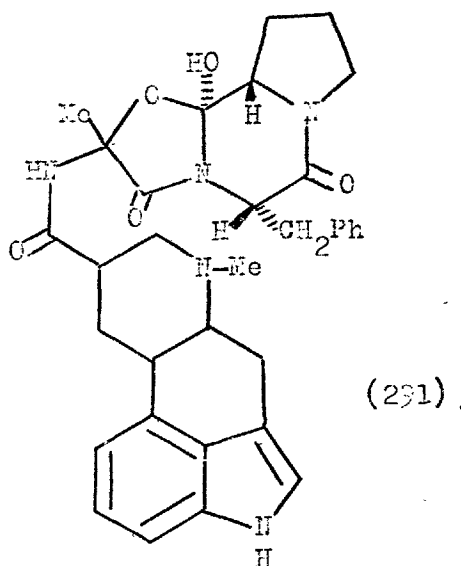
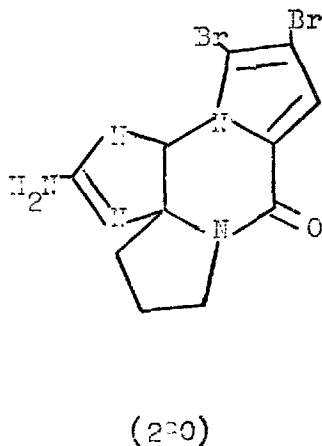
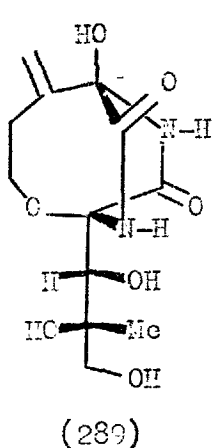
(288) C, n=4



Chetomin was originally isolated in 1944 from a strain of Chaetomium cochliodes²¹⁴. A tentative structure (285) has been proposed for this compound²¹⁵. It should be noted that the c.d. curve is almost identical to that of chaetocin but chetomin has been shown to inhibit specifically viral ribonucleic acid synthesis in a number of cell lines²¹⁶. Other compounds which may be related to the sporidesmins are oryzachloride²¹⁷ and the melinacidins²¹⁸ but their structures are as yet unsolved.

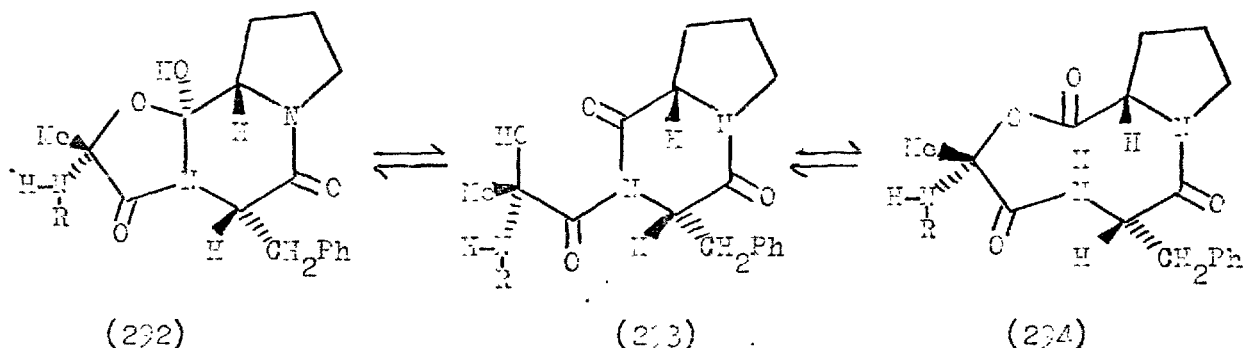
A new group of epipolythiodioxopiperazines has recently been discovered²¹⁹. Named sirodesmin A (286), B (287) and C (288) they are derived²²⁰ from tyrosine rather than phenylalanine but extensive oxidation of the aromatic ring has occurred.

Unlike all previous dioxopiperazines is the antibiotic bicyclomycin (289), isolated from Streptomyces sapporonensis²²¹. Its structure was fully determined by X-ray analysis²²² but its biosynthetic origin is unknown. The unusual dehydropeptide, dibromophakellin (290), has been isolated from the marine sponge, Phakellia flabellata²²³.

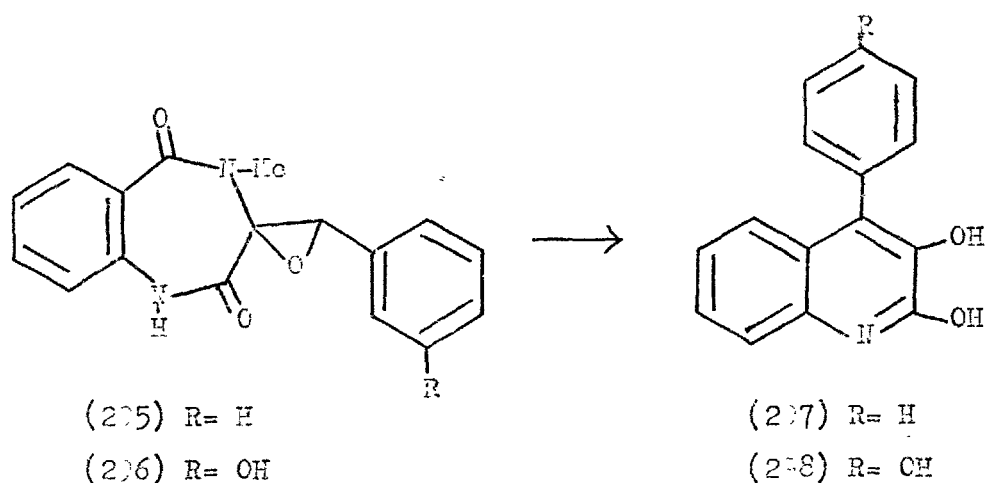


The ergot alkaloid, ergotamine (291)²²⁴ has a side chain tripeptide (292) formally derived from phenylalanine, proline and 2-hydroxy alanine. The conformation of the tripeptide is such that the cyclol form (292) is preferred

over the isomeric dioxopiperazine (293) or the ester (294).

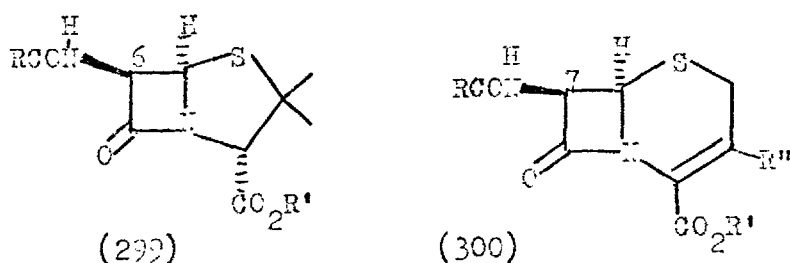


Cyclopenin (295) and cyclophenol (296) are α -epoxy peptides²²⁵, isolated from Penicillium cyclopium and P. viridicatum²²⁶. Both compounds have been



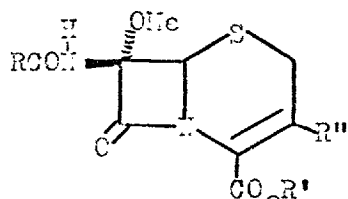
synthesised²²⁷ and are converted either in vivo²²⁸ or in vitro^{226,229} into viridicatin (297) and viridicatinol (298) respectively, also isolated from the same Penicillia^{225,226}.

Both penicillins (299) and cephalosporins (300) are formally derived from bis-dehydro acyl cysteinyl valine precursors and are rightly considered as

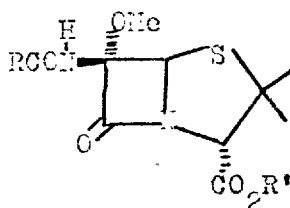


dehydropeptide derivatives. A comprehensive review of their chemistry has been published²³⁰. The discovery^{231,232} of the naturally-occurring 7 α -methoxy cephalosporins (301) has added a new dimension to β -lactam antibiotics due

to their biological activity. These compounds, and the penicillin analogues



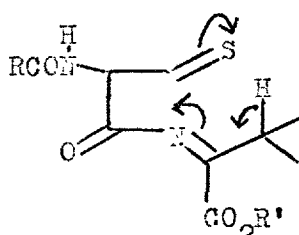
(301)



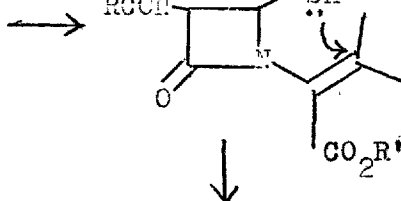
(302)

(302), have been the subject of considerable synthetic effort^{66-68,233}.

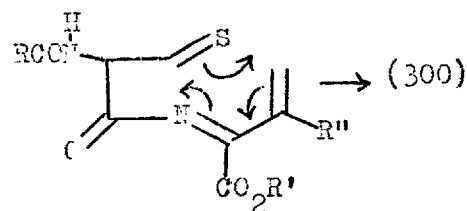
Baldwin²³⁴ has proposed that penicillins and cephalosporins are biosynthesised by the intramolecular cyclisations of acylimine thioaldehydes e.g. (303) and



(303)



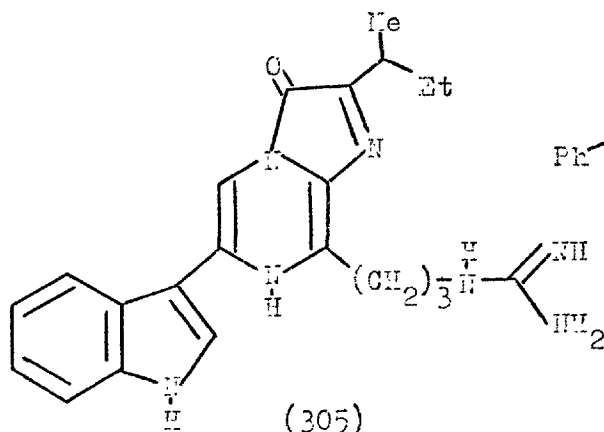
(299)



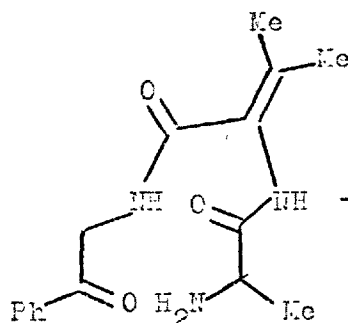
(304)

(304), although this has, so far, only limited in vitro precedent^{235,236}.

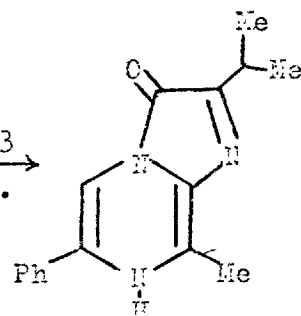
The intermediacy of dehydropeptide derivatives in the biosynthesis of the chemiluminescent luciferin (305), isolated from Cypridina hilgendorfi²³⁷, has been suggested and a model luciferin (306) has been synthesised from such a derivative (307)²³⁸.



(305)



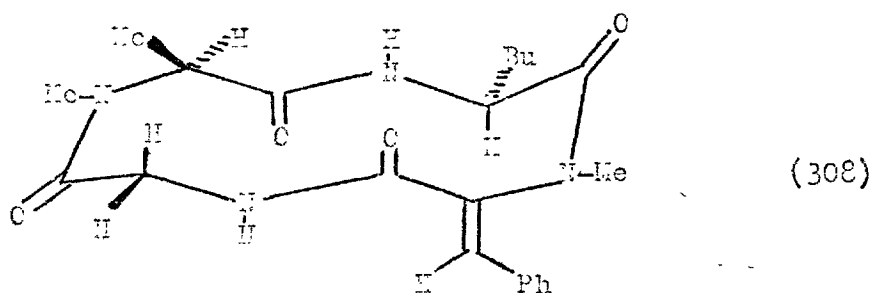
(307)



(306)

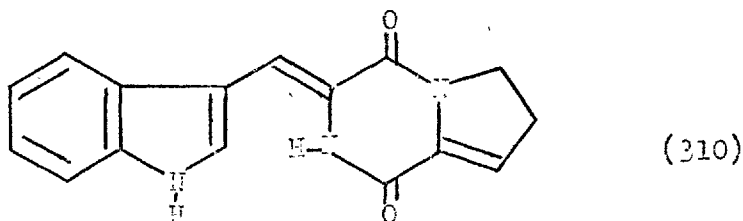
Many larger peptides contain dehydroamino acids and α -functionalised amino acids as structural units. The compounds below, although not rigorously up to date, exemplify the types encountered.

Tentoxin, a phytotoxic metabolite of Alternaria tenuis Nees.²³⁹, is a cyclic tetrapeptide containing an N-methyl dehydrophenylalanine unit. Its

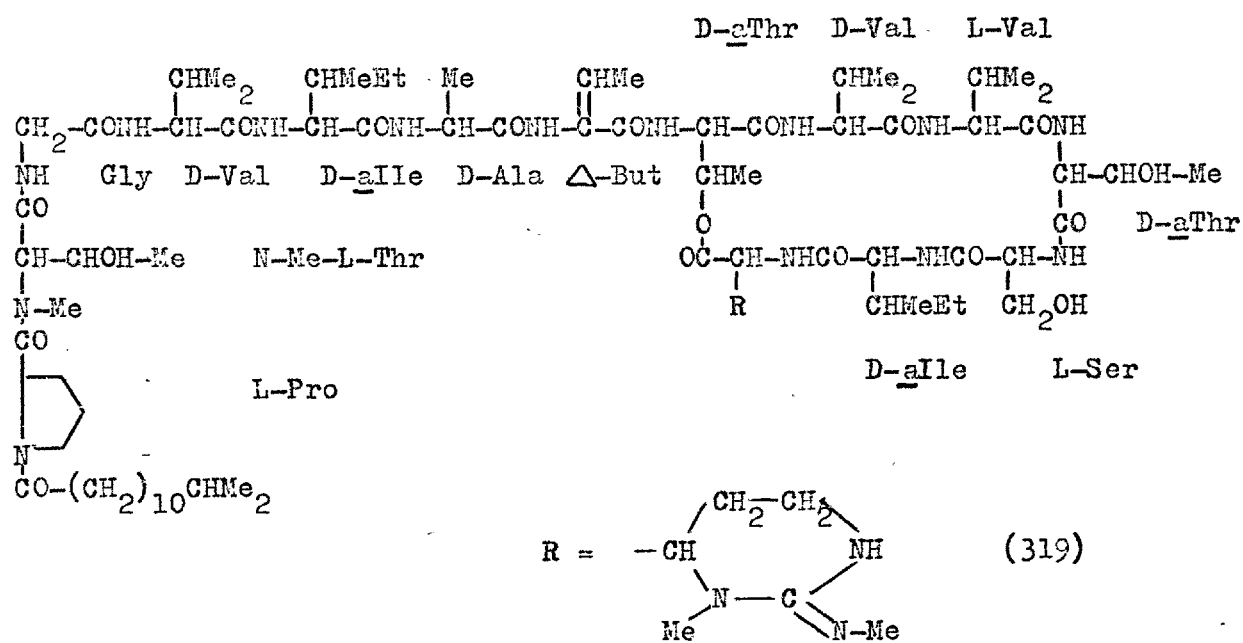


structure (308) has finally been solved by an X-ray analysis of the dihydro-derivative²⁴⁰.

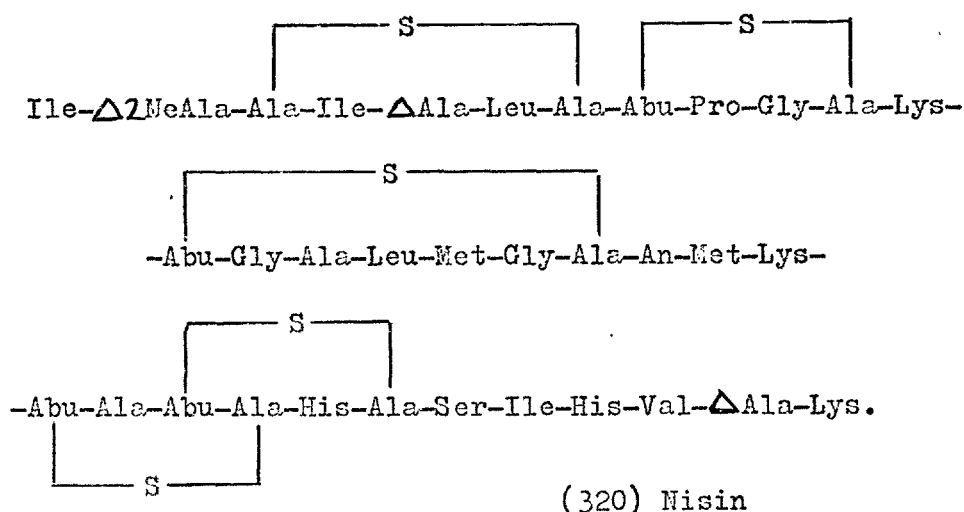
The peptide-lactone antibiotic telomycin, isolated from the culture broth of an unidentified Streptomyces²⁴¹, has the structure (309)²⁴². A dehydrotryptophan unit is responsible for the telomycin chromophore (339 nm.) and the bis-dehydrodioxopiperazine (310) was obtained as an artefact on alkaline hydrolysis.



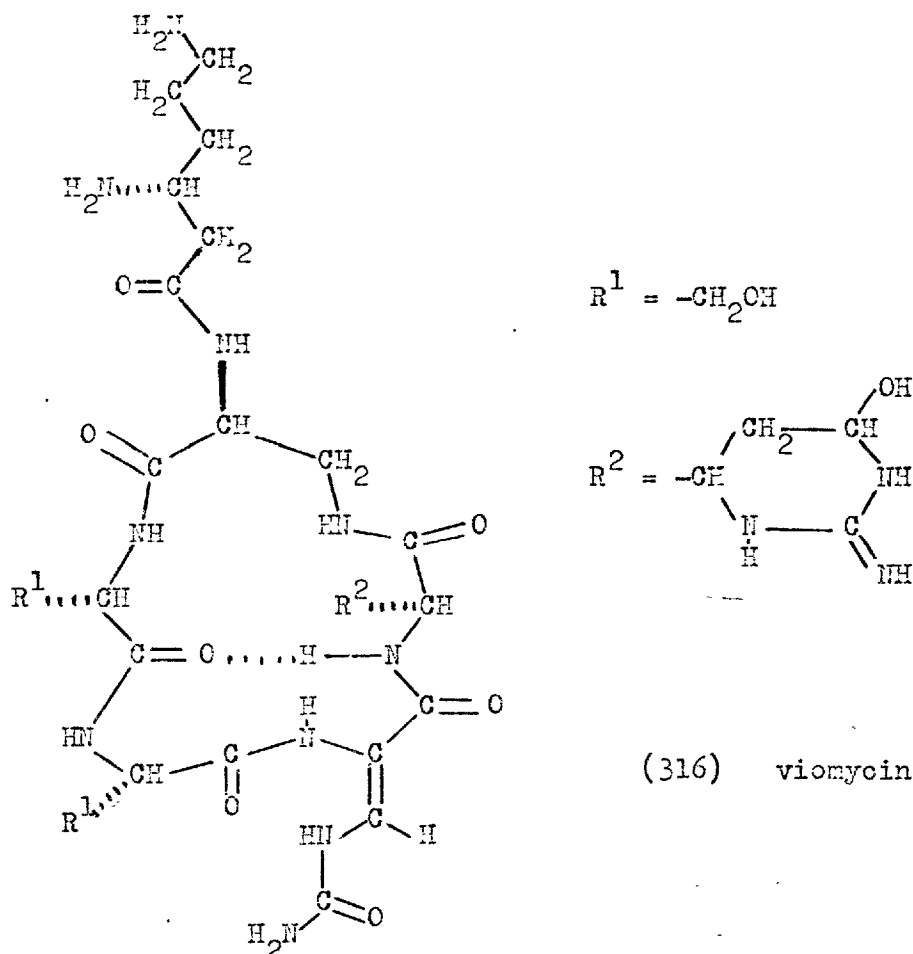
Chemical degradation²⁴³ gives support to the structures (311) and (312) for the antibiotics A-128-OP and A-128-P respectively. Also containing a dehydrotryptophan unit, these compounds differ from telomycin only in the choice of threonine residue used to make up the ring. Mild alkaline hydrolysis



(318) Stendomycin



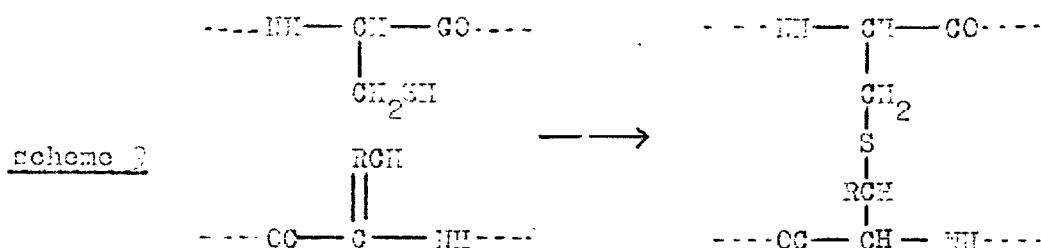
(320) Nisin



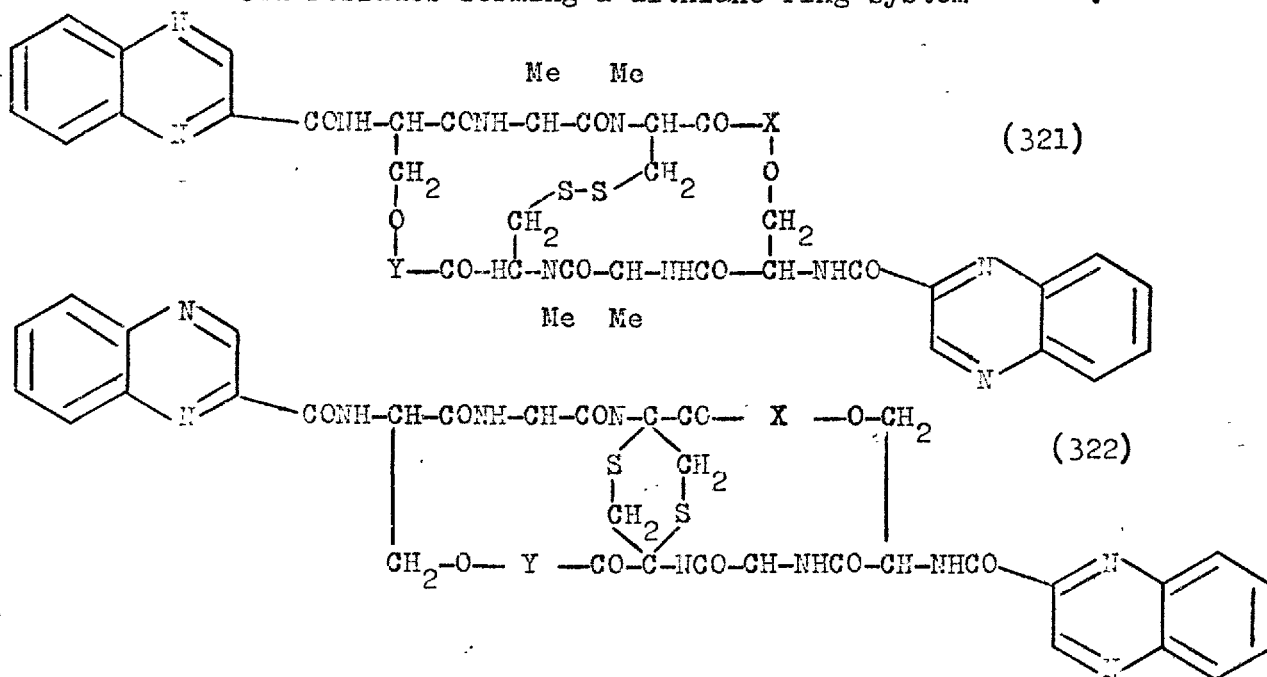
Stendomycin is the name given to a closely related mixture of peptide-lactones which are antifungal antibiotics. To date, the mixture has proved impossible to separate but a structure (318) has been proposed for the dominant member of this family²⁵⁴. Degradative studies have shown that the chromophore in stendomycin is due to the dehydrobutyryne unit (313). Also proposed is an N-methylated guanidine system (319) similar to that found in viomycin.

A structure has been reported for the antibiotic nisin (320)²⁵⁵. This compound contains several lanthionine residues and is the first discovered example of a pentacyclic heterodecetic type peptide containing sulphide bridges. From a biosynthetic point of view, the presence of a number of

dehydropeptide units e.g. dehydroalanine and dehydrobutyrine, is not surprising since it has been shown²⁵⁶ that in addition to serine and threonine, cysteine also takes part in the biosynthesis of lanthionine (scheme 9).



The quinoxaline antibiotics, isolated from various strains related to Streptomyces aureus, are cyclo-octapeptide-lactones linked across the main ring by a sulphur bridge. Two structural types prevail differing only in the nature of the sulphur bridge. The triostins (321) are characterised by a disulphide link formed from two cysteine residues²⁵⁷. Of greater interest are the quinomycins (322) in which the sulphur atoms are bonded to the α -carbons of the amino-acid residues forming a dithiane ring system^{257,258}.



X, Y = N-Me valine, N-Me or N-diMe allo isoleucine

Thiostrepton, first isolated from Streptomyces azureus²⁵⁹, has a complex structure difficult to elucidate by chemical means. However, X-ray analysis

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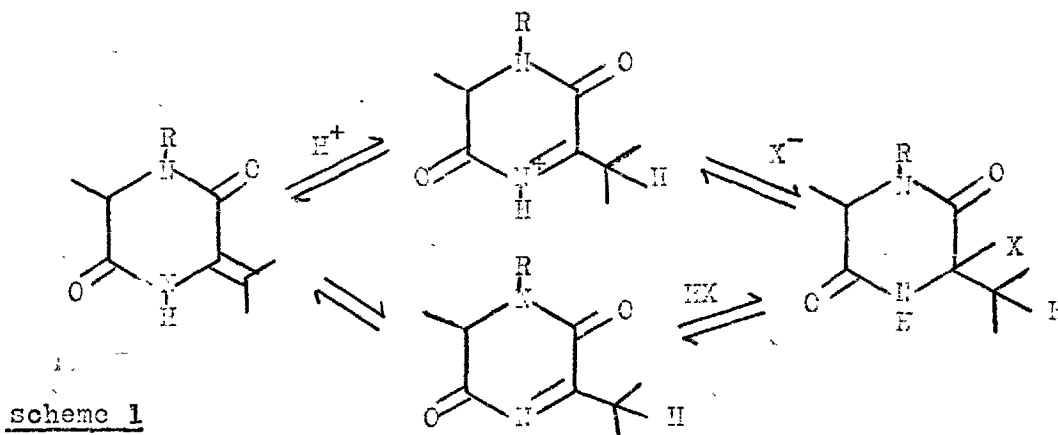
RESULTS AND DISCUSSION

CHEMICAL STUDIES ON DEHYDROCYCLODIPEPTIDES

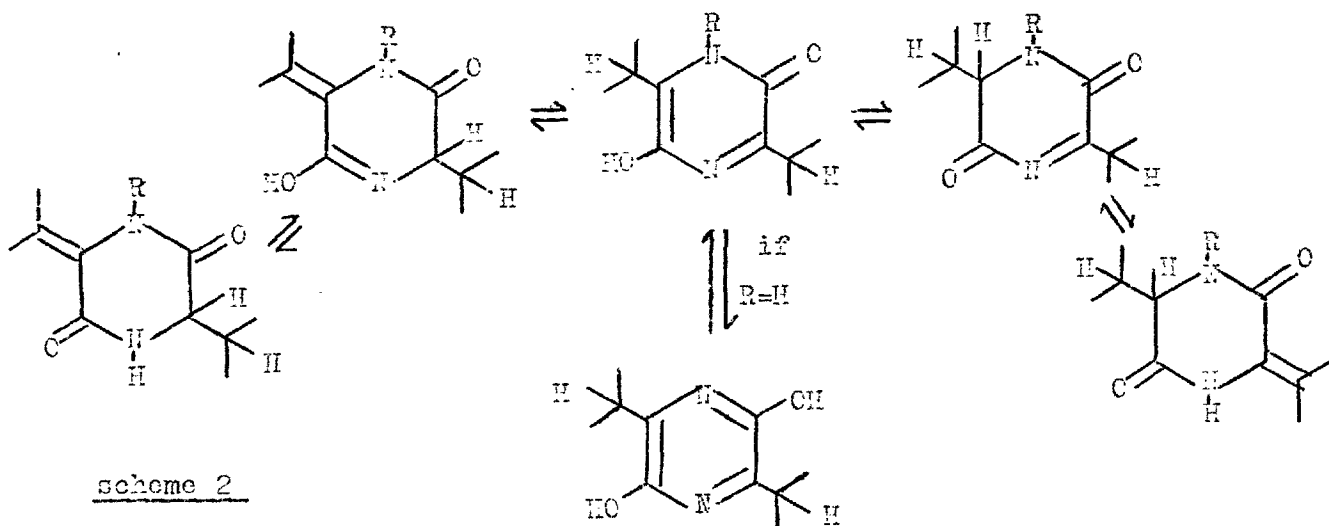
Introduction

The aim of this research project has been to investigate the chemistry of dehydro-2,5-dioxopiperazines in order both to gain insight into their chemical properties and to furnish the chemical knowledge required for the total synthesis of several naturally occurring dioxopiperazines. Furthermore, where possible, the approaches adopted have been aimed at setting in vivo precedents for proposed biosynthetic pathways to these metabolites.

Dehydro-dioxopiperazines are dehydrocyclo dipeptides and therefore should exhibit all the chemical properties associated with enamides as described in Part 1. (scheme 1). In addition, the cyclic nature of these compounds



enables the double bond in one amino acid residue to be transferred to the other amino acid residue via a pyrazine intermediate (scheme 2)

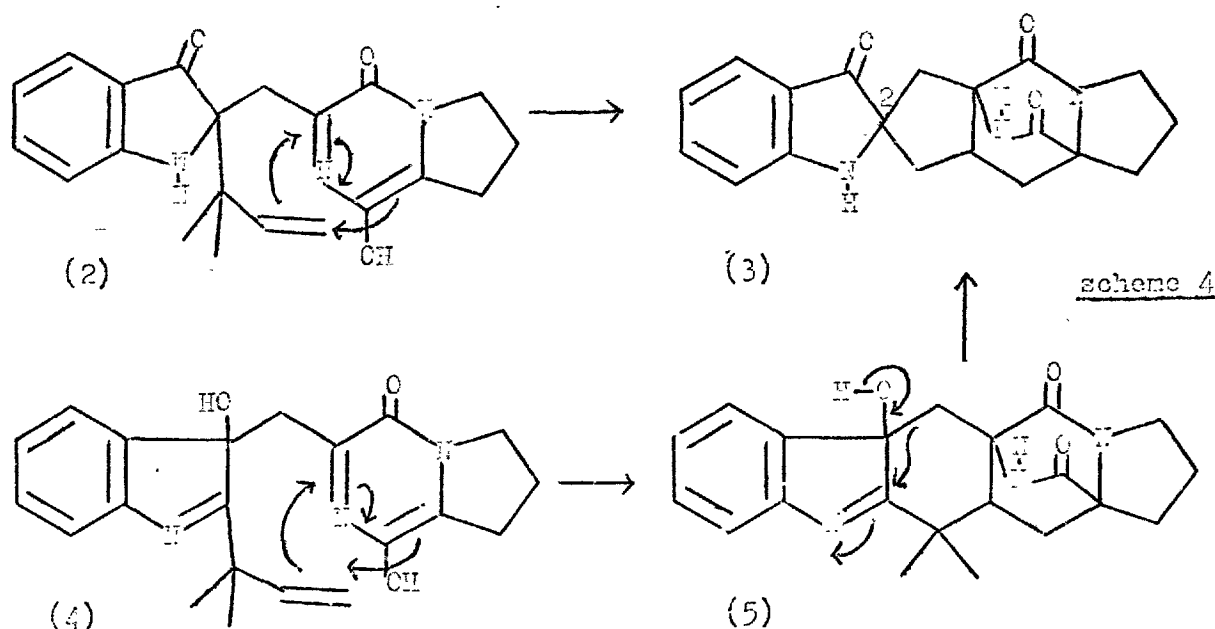


These properties should be borne in mind since the majority of the chemistry described in this thesis is a consequence of them.

A. Brevianamides A and B.

The brevianamides and the austamides (see page 39) isolated from Penicillium brevicompactum¹ and Aspergillus austus², respectively, are closely related fungal metabolites derived from L-tryptophan, L-proline and mevalonate³. Their biosynthesis is not completely understood but a common progenitor (1) has been proposed³, although it has only been isolated from A. austus. Scheme 3 shows some, but not necessarily all of the possible relationships, precursors and intermediates involved in the biosynthesis of these metabolites.

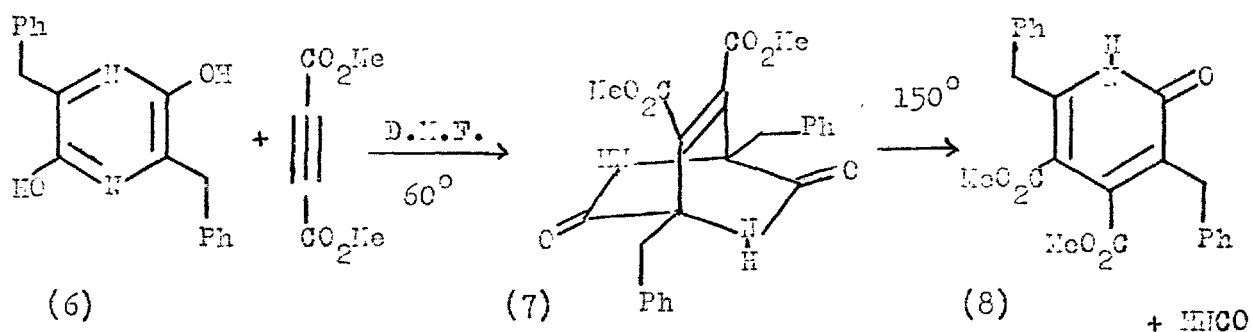
The principle reaction of current interest is the cyclisation of the dimethylallyl group onto the pyrazinone ring which results in formation of brevianamides A and B (3), isomeric about the spiro position 2 (scheme 4).



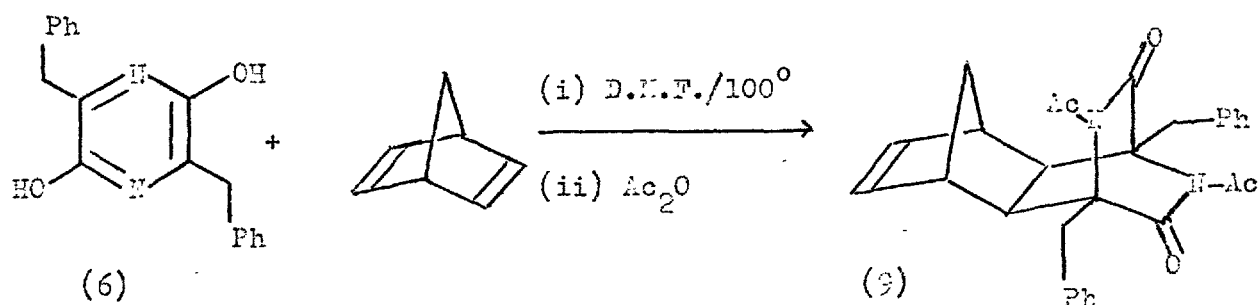
Although radical processes are conceivable an attractive mechanism would involve a Diels-Alder cycloaddition [as shown (2)→(3)] of the olefin with the diene system of the pyrazinone for which there was no literature precedent.

Initially, Porter⁴ showed that dihydroxypyrazines will undergo cycloaddition reaction with electron deficient dienophiles and strained olefins.

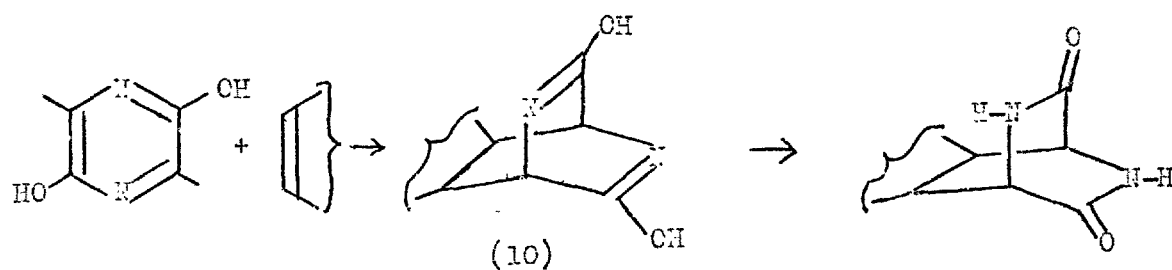
Thus, the dihydroxypyrazine (6) gives the adduct (7) with dimethyl acetylenedicarboxylate (A.D.E.), which readily undergoes a retro Diels-Alder reaction with elimination of cyanic acid and formation of the substituted pyridone (8).



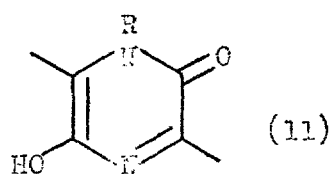
Likewise, (6) reacts with norbornadiene to give, after acetylation, the exo-exo adduct (9). One driving force for these reactions is the stabilisation gained by formation of the amide bonds from the initial adduct (10). However, when



the reaction was attempted with the unactivated olefin cyclopentene, no

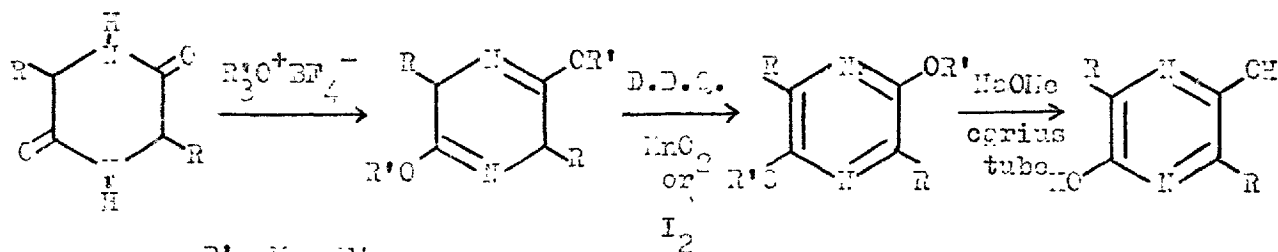


adduct was formed. Consequently, it was thought necessary to prepare a closer analogue to the proposed biosynthetic intermediate in the form of a 1-alkyl-



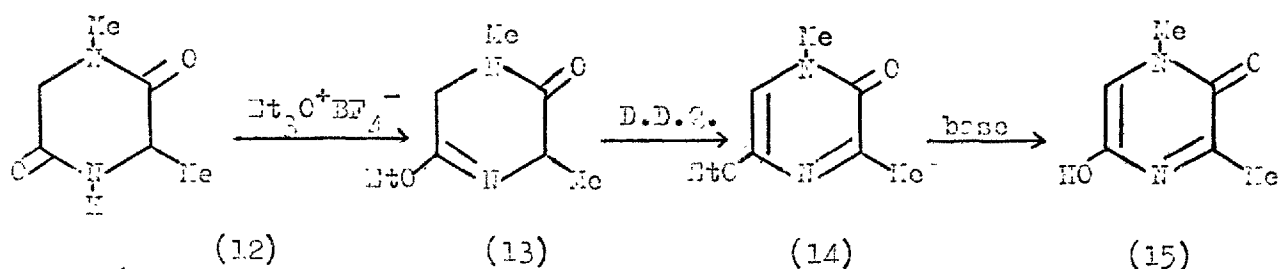
pyrazin-2-one e.g. (11). Dihydroxypyrazines can be prepared ⁵ from dioxipiperazines by alkylation to the bis-imidate, oxidation to the dialkoxypyrazine followed by dealkylation to the desired compounds

(scheme 5). It was hoped that this scheme could be extended to the hydroxy-



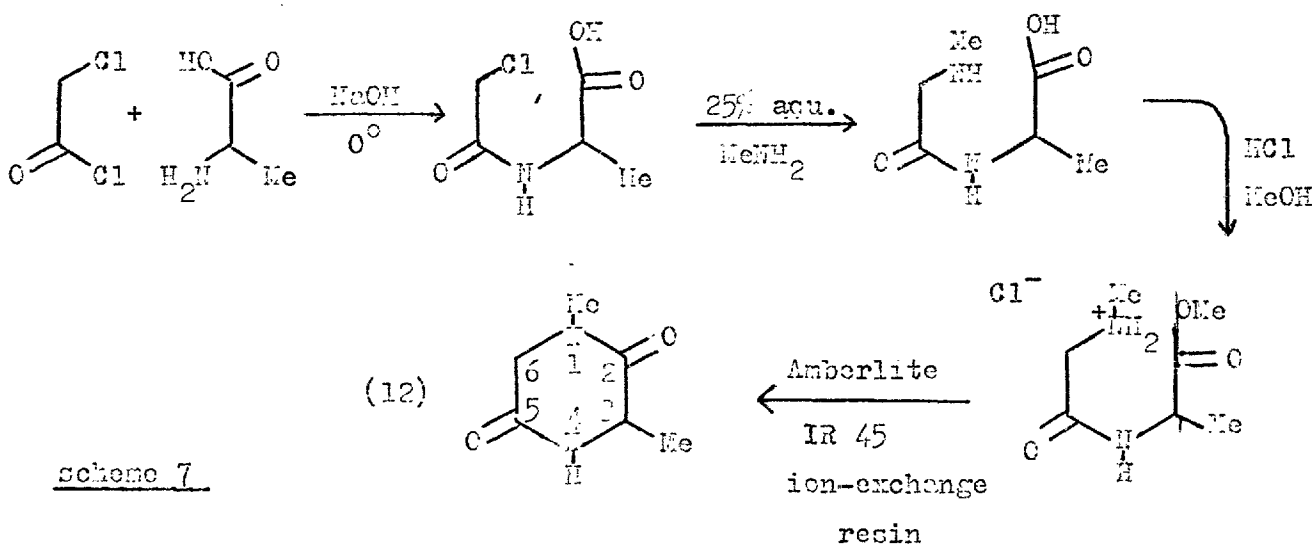
scheme 5

pyrazinone system and an effort to prepare 1,3-dimethyl-5-hydroxypyrazin-2-one (15) was made, although previous attempts to prepare (15) had failed⁶ (scheme



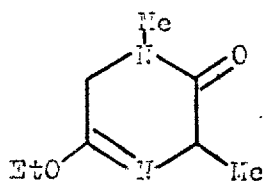
scheme 6

6). The starting material, 1,3-dimethyl-2,5-dioxopiperazine (12) was prepared by reaction of alanine with chloroacetyl chloride in the presence of aqueous sodium hydroxide at 0°, followed by amination with aqueous methylamine. The resultant dipeptide was esterified by the Fischer method and the amino ester hydrochloride cyclised by passage down a basic ion-exchange column⁶. (scheme 7)

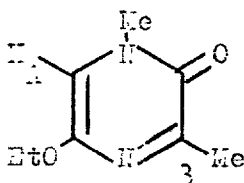


Alkylation of dioxopiperazines with Meerwein salts leads to O- rather than N-alkylation⁵. The dioxopiperazine (12) was reacted with triethyl oxonium fluoborate⁷ in anhydrous dichloromethane for 3 hours. After workup, the oil

slowly crystallised and subsequent high vacuum sublimation (25°, 0.05 Torr) gave large colourless chunks which exhibited the spectral and analytical data expected for the 5-ethoxydihydropyrazinone (13). Oxidation of (13) with activated manganese dioxide or lead dioxide gave only starting material. However, if the imidate was allowed to stand in a benzene solution of dichlorodicyanobenzoquinone (D.D.Q.), at room temperature, reaction did occur.



(13)



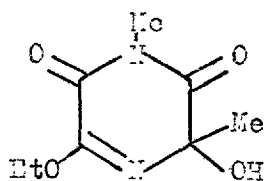
(14)

H ₂	τ 2.40, 1H, q, J 1 Hz.
Me	τ 7.55, 3H, t, J 1 Hz.
N-Me	τ 6.48, 3H, s.
O-Et	τ { 6.63, 3H, t, } J 7 Hz. { 5.80, 2H, q, }

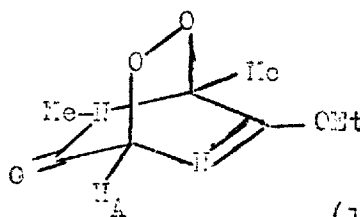
Following the reaction by tlc showed complete disappearance of the starting material with concomitant appearance of a new fluorescent spot. The precipitated quinol was filtered off and the product was isolated by column chromatography on alumina as pale yellow needles (25%). The spectral data was as expected for the ethoxypyrazinone (14). The n.m.r. spectrum showed a rather large long-range coupling (J, 1 Hz.) between the vinylic proton and the methyl group in the 3-position. The conjugated nature of the 1-amide nitrogen resulted in a downfield shift of the N-methyl resonance by 0.5 ppm from its position in the dioxopiperazine (12) and the imidate (13). Its i.r. spectrum showed a sharp vinylic C-H stretch at 3100 cm⁻¹, the amide carbonyl stretch at 1660 cm⁻¹ and the double bond stretching absorptions at 1600 cm⁻¹. The material ran as a single compound in a variety of tlc systems and gave a parent ion at m/e 168 in its mass spectrum, corresponding to the required species, C₈H₁₂N₂O₂. However, satisfactory analytical figures could not be obtained and attempts at purification by recrystallisation or sublimation were abortive. This behaviour is attributed to the considerable reactivity of the compound. For example, slow evaporation of a chloroform solution of the dienone in an open vessel gave not the expected yellow crystals but a colourless oil which partially crystallised after a few hours. Recrystallisation from acetone/

petroleum ether 40-60°(petrol) afforded colourless plates m.p. 83-84°.

Microanalysis and mass spectral data indicated the molecular formula $C_8H_{12}N_2O_4$ which corresponds to the addition of molecular oxygen. The i.r. spectrum showed strong hydroxyl and carbonyl absorptions whilst the n.m.r. spectrum displayed a one proton 'hump' exchangeable with D_2O , as well as a three proton singlet at τ 8.35 consistent with a methyl group on a tetrasubstituted carbon. Thus, the new compound was assigned the hydroxy imide structure (16). The

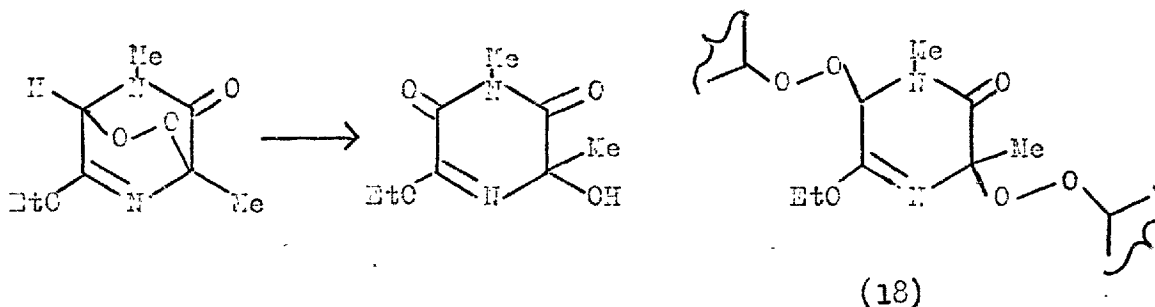


(16)



(17)

reaction was repeated by bubbling air through a solution of the dienone for 4 days, by which time all the starting material had reacted (tlc). The crude reaction mixture gave a positive starch-iodide test and the n.m.r. spectrum of the crude product showed a sharp singlet at τ 6.65 as well as the resonances due to (16). However, crystallisation afforded only the hydroxy imide and the mother liquors no longer gave a positive starch-iodide test. On the basis of these observations, the initial product is probably the endoperoxide (17) which would give a positive starch-iodide test and would also exhibit a singlet methine, H_A , around τ 6.5. Rearrangement to the hydroxy imide as shown, would

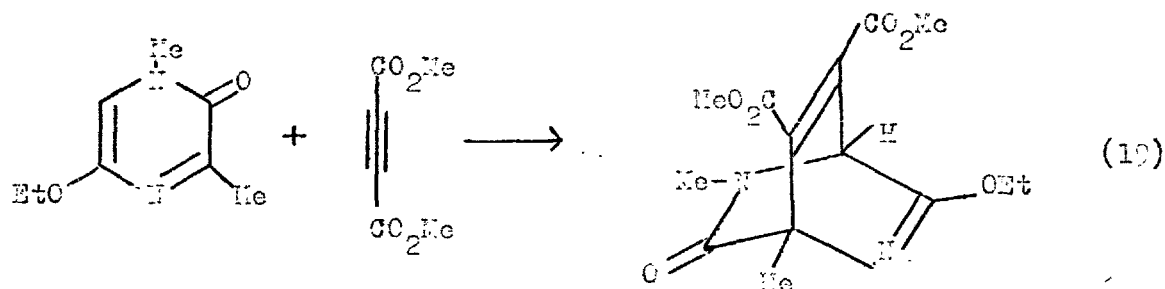


(18)

be expected for a cyclic peroxide of this nature⁸. An alternative polymeric peroxide (18) would also give the starch-iodide test but whether the polymer methines would resonate as a sharp singlet is unlikely. The mechanism of the addition is of interest. That it is not photochemical was confirmed by successfully carrying out the reaction in the dark. Since a direct addition

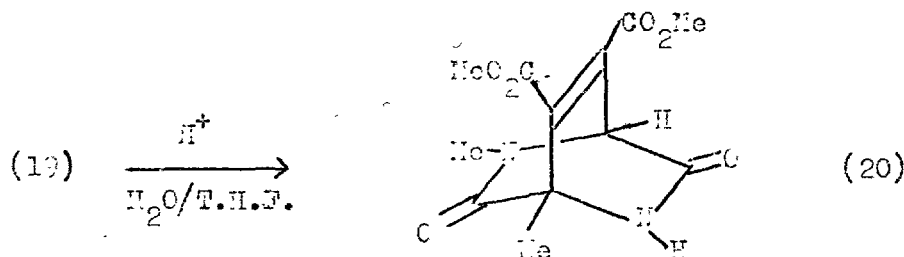
of triplet oxygen to the dienone substrate is improbable, the autoxidation is likely preceded by an electron-transfer process⁹. Due to the reactivity of the dienone towards oxygen, all subsequent preparations were carried out under nitrogen and marginal yield increases were obtained, although the yields in general were greatly variable (15-39%).

The reactivity of the pyrazinone towards dienophiles was investigated. The dienone was reacted with A.D.E. in refluxing dichloromethane under nitrogen. Tlc showed the formation of a new product and the reaction was complete after 20 hours. Workup by preparative layer chromatography (plc) afforded the adduct as a crystalline solid in 60% yield. After recrystallisation the white clusters gave analytical and spectral data consistent with the cycloaddition product (19). Unlike the dihydroxypyrazine adduct (7), this compound showed no tendency



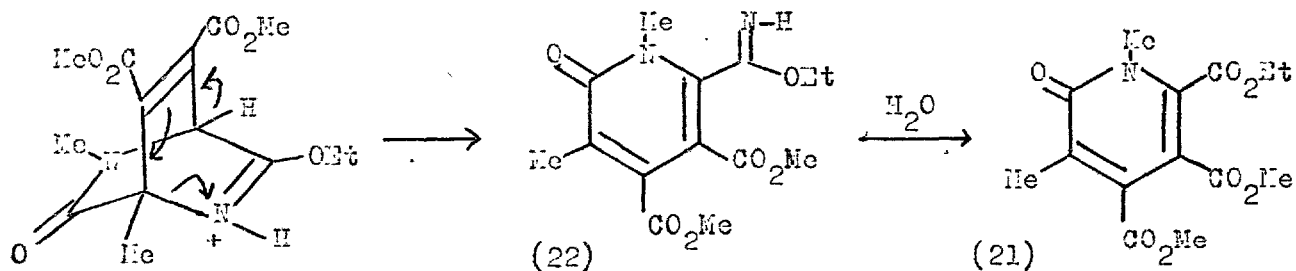
to eliminate the bridging amide group (as methyl isocyanate) even up to 200°. In fact, the mass spectrum indicated that the loss of the ethoxy imine bridge occurred more readily than loss of the amide bridge [$M^+ - 72$ (100%); $M^+ - 57$ (18%)].

The lability of the ethoxy imine towards acid was tested, with a view to isolating the free amide (20). The adduct was dissolved in aqueous tetrahydro-



furan (T.H.F.) containing 2N HCl and after 10 minutes at 25°, tlc showed a one-spot reaction with complete disappearance of the starting material. Workup by neutralisation and extraction gave an oil which crystallised on standing. The

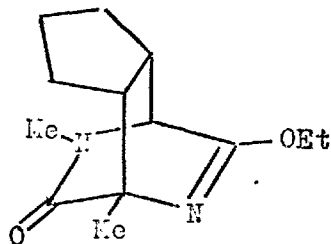
n.m.r. spectrum of this material showed, most interestingly, that the ethoxy group had been retained, thus the bis-amide (20) had not been formed. The i.r. spectrum showed four strong carbonyl absorptions at 1740, 1732, 1725 and 1662 cm^{-1} , i.e. three esters and an amide. The pyridone triester (21) is consistent with these facts and the structural assignment was confirmed by the mass spectral, u.v. and analytical data. Presumably protonation of the imine



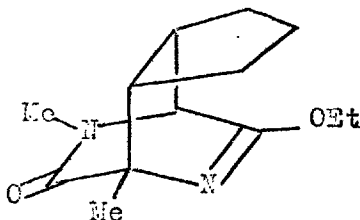
is followed by opening of the bridge to give an intermediate (22), which is immediately hydrolysed to the corresponding ester (21).

As anticipated, the pyrazinone (14) reacted with an isolated double bond. Cyclopentene was chosen as dienophile and solvent although a few drops of ethyl acetate were added to facilitate dissolution of the dienone. The reaction was carried out in a sealed tube, wrapped in foil, in a closed steam bath (100°) for four days. After opening the tube, tlc showed complete consumption of the dienone, a new spot corresponding to the hydroxy imide (16) and a streak near the origin usually associated with product decomposition. However, the presence of a broad multiplet between $\tau 7$ and $\tau 9$ in the n.m.r. spectrum of the crude material indicated incorporation of cyclopentene into the product. Careful analysis of this spectrum showed three major peaks in the N-methyl region (ca. $\tau 7$) one of which, together with other corresponding resonances, was assigned to the hydroxy imide. The remaining resonances were consistent with a 1:1 mixture of the two expected syn- (23) and anti- (24) cyclopentene adducts. (The syn- configuration is arbitrarily taken as that with the cyclopentane ring syn to the N-methyl amide bond). All attempts to resolve the mixture into discrete spots, using different tlc systems, failed; nevertheless the product was applied to plc and afforded 27% of the hydroxy imide (16).

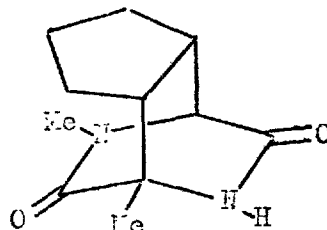
The streak near the origin was eluted from the silica and its n.m.r. spectrum recorded. Surprisingly, it consisted of only two products of which one was present in the original mixture. Tlc now showed two discrete spots and separation was effected by plc. The less polar product, an oil which crystallised on sublimation (25° , 0.1 Torr), showed the presence of ethoxyl, methyl, N-methyl,



(23)

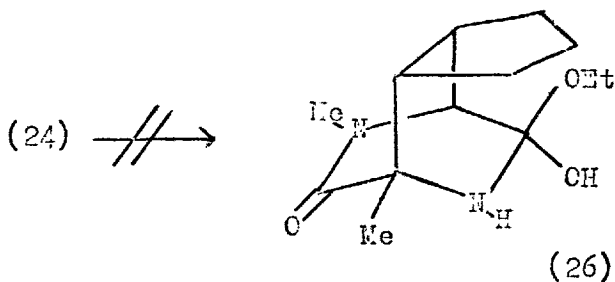


(24)

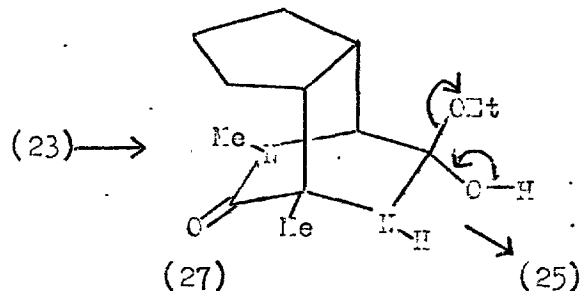


(25)

methine and cyclopentane protons in its n.m.r. spectrum, as well as the expected parent ion at m/e 236 in the mass spectrum. This data is consistent with the primary adduct (24) and a satisfactory analysis was obtained. The more polar product, colourless prisms from acetone, had lost its ethoxy group and the i.r. spectrum showed absorptions typical of a dioxopiperazine N-H group. The mass spectrum gave the parent ion at m/e 208 and the analytical figures also corresponded to the hydrolysed structure (25). The stable primary isomer is assigned as the anti- adduct (24) since in this species the formation of an



(26)



(27)

(25)

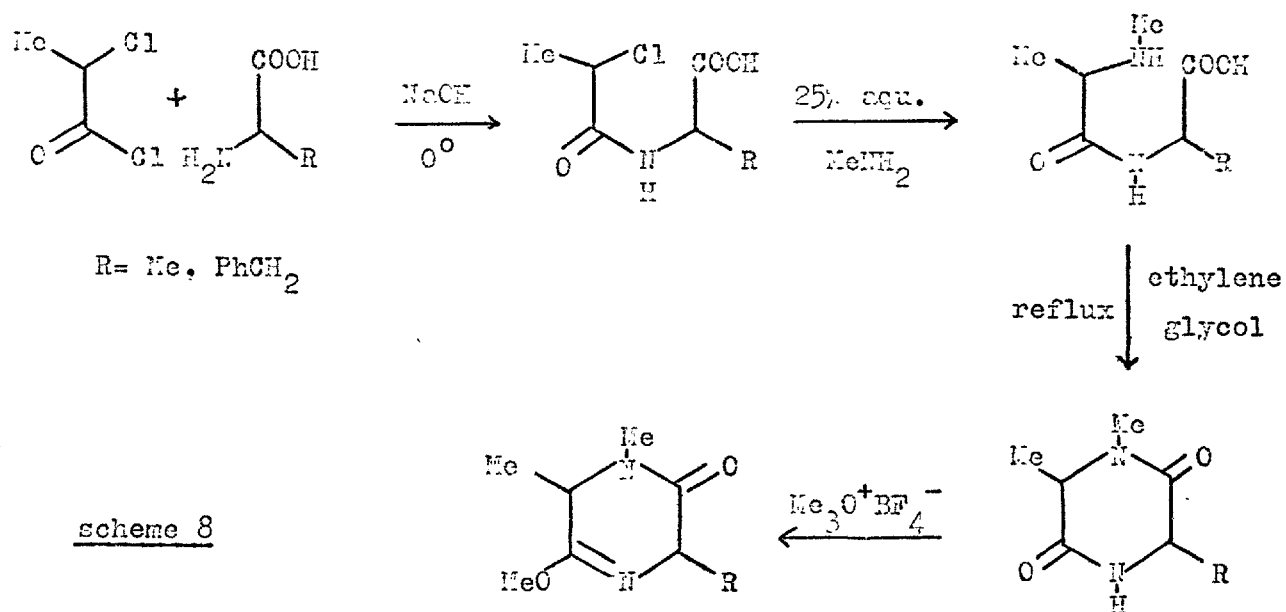
sp^3 -hybridised intermediate (26), required for hydrolysis of the imidate, would be inhibited by steric hindrance from the nearby cyclopentane ring. No such inhibition would be experienced in the formation of the intermediate (27) from the syn- adduct (23) and collapse to the amide (25) could readily occur, as indeed was observed. The selective hydrolysis of the unstable primary adduct on the silica plate was confirmed in a second experiment by stirring

the initial reaction mixture, containing only the primary imidate adducts, with a little silica gel in wet chloroform. Hydrolysis of the syn- adduct was observed whilst the other isomer was stable to these conditions.

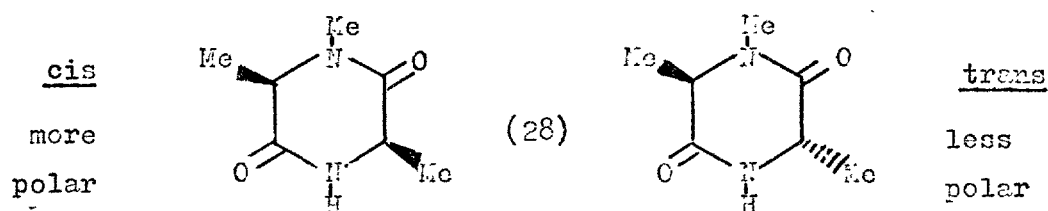
On the basis of the foregoing cycloaddition reactions the biosynthetic scheme proposed for brevianamides A and B appears reasonable, especially when it is considered that a free hydroxypyrazinone, as opposed to the alkoxy derivative described above, would be even more reactive due to the stabilisation gained from formation of the amide group. No attempt to dealkylate the ethoxy pyrazinone (14) was made, but later work (p.157) indicated that the free hydroxy dienones are extremely reactive indeed.

All attempts to increase the yield of the dienone from the oxidation failed. The dienone is stable in solution under anaerobic conditions and does not appear to react with itself in the manner of, for example, cyclopentadiene¹⁰. The precursor imidate (13) does not behave as a dienophile towards the dienone since a mixture of the two compounds remained unchanged after several weeks. However, it was possible that the D.D.Q. was acting as a dienophile giving rise to four possible cycloaddition products. An attempt to prevent this side reaction by adding the oxidant over a period of time had no effect on the yield of the dienone. In addition, since very little starting material remained most of the D.D.Q. must have been reduced and therefore could not participate in cycloaddition reactions. As described earlier carrying out the oxidation and subsequent manipulations under nitrogen did increase the yield to a small extent.

It was decided to prepare some 3,6-disubstituted analogues of the pyrazinone, in order to test the generality of the synthesis and reactivity of the system. The 1,3,6-trimethyl (28; cis and trans) and 1,6-dimethyl-3-benzyl dioxopiperazines (41; cis and trans) were prepared by a modification of the route used for the 1,3-dimethyl compound (12) (scheme 8). It was found essential to prevent the temperature of the initial acylation of the amino acid from rising above 0°, otherwise hydrolysis of the 2-chloropropionyl chloride

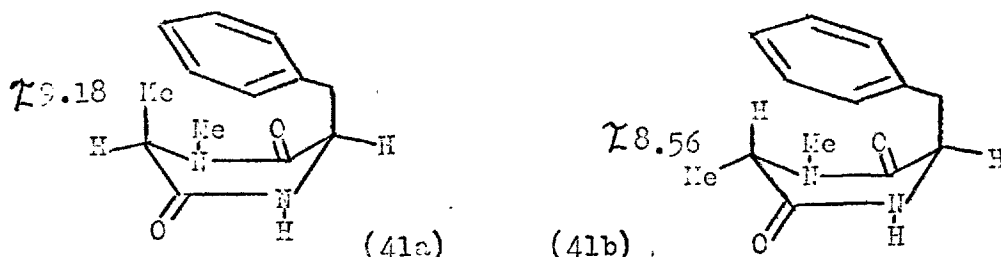


became significant. The amination of the chloro amido acids with aqueous methylamine was straightforward. The resultant dipeptides, N-methyl alanyl alanine and N-methyl alanyl phenylalanine, were cyclised without further purification by thermal dehydration in refluxing ethylene glycol¹¹. The glycol solution was extracted with dichloromethane which was then thoroughly dried with powdered anhydrous calcium chloride by stirring the suspension vigorously until the 'stickyness' disappeared and the solids became finely divided (30 min.) If this drying procedure was omitted or curtailed, contamination with ethylene glycol considerably hindered the eventual isolation of pure product. The crude mixture of isomers readily crystallised from T.H.F. and acetone/petrol affording large prisms of (28) and small prisms of (41), respectively. The isomers ran differently on tlc and a small sample of each pair was separated, recrystallised and satisfactory analyses obtained. For the trimethyl compounds



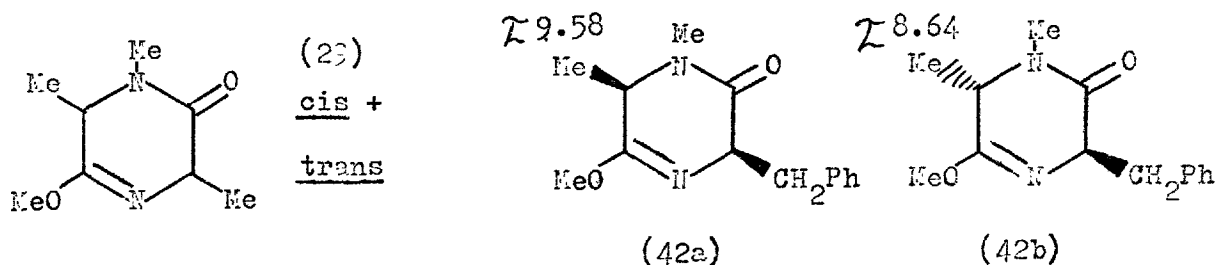
(28) specific assignments for the cis- and trans-isomers could not be determined spectrally and were made by analogy to other cis-trans pairs on the basis

of tlc polarity¹². Thus, the less polar isomer was assigned the trans configuration. The phenylalanine derivatives (41) were readily differentiated by their n.m.r. spectra due to the shielding effects caused by the benzyl substituent¹³. In both isomers the folded conformation is adopted, but only



in the cis-isomer (41a) is the methyl group affected. As expected the trans-isomer was the less polar compound.

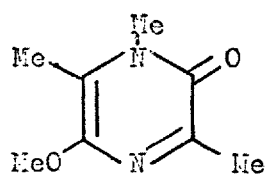
The cis-trans mixtures of the dioxopiperazines were used for the next step. Trimethyl oxonium fluoroborate¹⁴ was preferred to the triethyl salt because of the simplified n.m.r. spectra of the products. The alkylations were carried out in anhydrous dichloromethane at 25° using 1.5 equivalents of the reagent. In the case of the trimethyl derivatives (28) the reaction was over after 4 hours (tlc) and workup afforded a colourless mobile oil in 80% yield. The product was shown by tlc and n.m.r. to consist of two compounds only, corresponding to the cis and trans imidates (29). Methylation of the



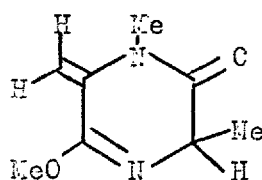
methyl-benzyl derivatives (41) was complete in 3 hours and workup afforded a crystallising oil in 99% yield. Tlc and n.m.r. verified that the product consisted of only two compounds, a 1:1 mixture of the isomeric imidates (42). The benzyl substituent effect, alluded to previously, was even more pronounced in these compounds. A small sample was separated by plc. The more polar cis-compound is a crystalline solid, m.p. 92-94°, where as the less polar trans-isomer remained as an oil. The mixtures of isomers of both (29) and (42) were

used for subsequent oxidations with D.D.Q.

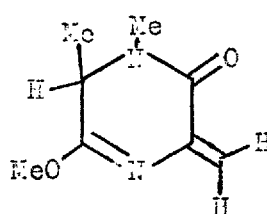
In contrast to the ethoxy imidate (13), the trimethyl isomers (29) remained inert to D.D.Q. at room temperature. However, after 24 hours in refluxing benzene no further change was observed by tlc and only a small amount of starting material could be detected. Tlc also showed two new spots, less polar than starting material, which were readily visualised by u.v. light but were not fluorescent in the manner associated with the pyrazinone system. The precipitated quinol was filtered off and the crude product subjected to n.m.r. study. The vinylic methyl and N-methyl resonances expected for the dienone (30) were absent and the spectrum showed six low-field doublets characteristic of vinylic methylene groups. Therefore, oxidation had occurred, but in the substituent methyl groups and not in the ring. The major product exhibited two one-proton doublets at τ 4.78 and 5.35 and a three-proton doublet, J, 7 Hz. at τ 8.59. Two structures, (31) and (32), could be assigned to this



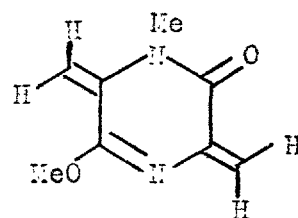
(30)



(31)



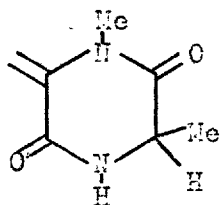
(32)



(33)

compound. The minor product exhibited four one-proton doublets at τ 3.88, 4.41, 4.65 and 5.20, and was assigned the bis-methylene structure (33). The higher field pair of vinylic protons obviously corresponded to the resonances in the major product and on the basis of chemical shifts were considered to belong to the methylene group α to the imidate oxygen rather than α to the carbonyl oxygen. Thus, the major product was the 6-methylene compound (31) as opposed to the 3-methylene compound (32). Column chromatography (alumina, dichloromethane) afforded (31) as a pale yellow oil in 22% yield but no sign of the less polar compound (33) could be found. Although the methylene imidate (31) was distilled (25°, 0.1 Torr) a satisfactory analysis could not be obtained and consequently the compound was fully characterised by hydrolysis to the

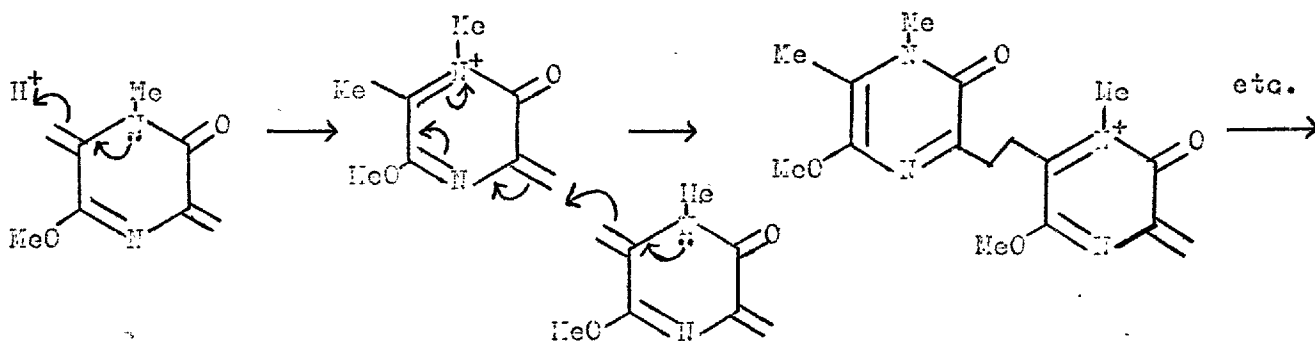
methylene dioxopiperazine (34). This was readily accomplished by stirring with aqueous T.H.F. containing 2N HCl at room temperature for 16 hours.



(34)

Neutralisation, extraction and recrystallisation gave the dioxopiperazine as colourless prisms in 80% yield. The n.m.r. spectrum of this compound showed the vinylic resonances at τ 4.20 and 5.05 confirming the assignment of the methylene group in (31) to the 6-position, since the lower field proton, the one nearest the oxygen function, is the one which experiences the greater shift upon hydrolysis (τ 4.78 to τ 4.20).

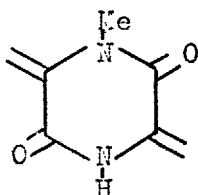
The oxidation was repeated on the same scale (1 mmole) and the n.m.r. spectrum of the crude product showed the same distribution of products as before. Instead of column chromatography, the mixture was subjected to plc (SiO_2 , 1:19 MeOH: CHCl_3). Although the mono-methylene compound could be isolated in this way, the band corresponding to the bis-methylene compound afforded 6%, by weight, of yellow amorphous solid insoluble in the common solvents. This material streaked from the origin on tlc and displayed broad bands in its i.r. spectrum consistent with polymer. At this stage it was felt that the bis-methylene imidate might be susceptible to acid catalysed polymerisation (scheme 9).



scheme 9

Thus, plc on silica or column chromatography using dichloromethane, which often contains small quantities of hydrogen chloride, could initiate polymerisation. Again the oxidation was repeated but on a larger, 7.5 mmole, scale and the crude separated by chromatography on alumina using benzene and acetone:benzene (1:9) as eluents. The first fractions off the column afforded pale yellow

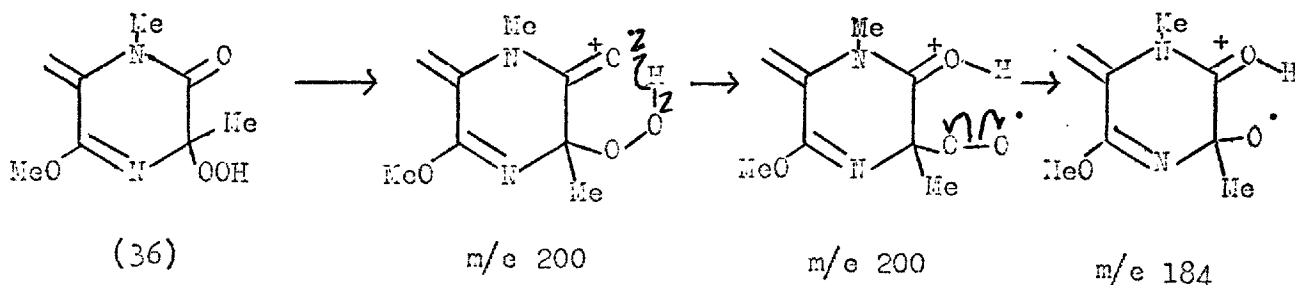
crystals of the bis-methylene compound in 1.5% yield. A conclusive n.m.r. spectrum was obtained but an amorphous precipitate rapidly appeared in the deuteriochloroform solution. An attempt was made, in a subsequent preparation, to hydrolyse the imidate (33) to the bis-methylene dioxopiperazine (35) but



(35)

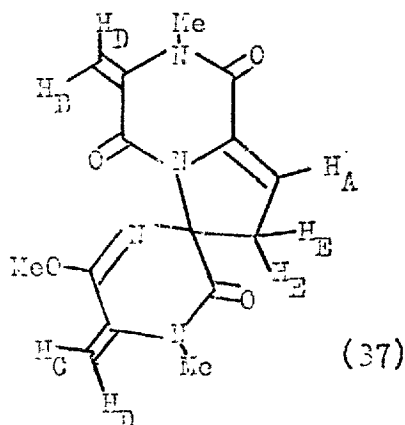
the hydrolysis could not compete with the polymerisation; further characterisation of (33) was abandoned. The second band off the column gave 15% of the methylene imidate (31). The third band off the column was not a discrete compound but a mixture of (31), starting material (29),

a new compound (36) and a fourth compound whose n.m.r. resonances were most consistent with the pyrazinone (30). The mixture was subjected to plc which unfortunately effected decomposition/polymerisation of the pyrazinone. However, the new compound (36) was isolated, in 0.8% yield, as a crystalline solid. The n.m.r. spectrum of the compound was as follows; τ 4.62(1H,d, J 2 Hz.), 5.18 (1H,d, J 2Hz.), 6.12(3H,s), 6.77(3H,s), 6.78(1H,s) and 8.47(3H,s). The methoxy signal at τ 6.12 showed that the imidate function was still intact and consequently the two vinylic protons were assigned to a methylene group in the 6-position. The C-methyl singlet at τ 8.47 indicated that the 3-position was tetrasubstituted and the remaining proton was presumably attached to the fourth substituent at this position. The i.r. spectrum showed a broad hydroxyl absorption at 3300 cm^{-1} , a carbonyl stretch at 1685 cm^{-1} and the double bond absorption at 1615 cm^{-1} ; C-O stretches, also present in the hydroxy imide (16), appeared at 1120 and 1150 cm^{-1} . Although a satisfactory microanalysis was not obtained, the mass spectrum showed a very weak parent ion at m/e 200 and a metastable peak at 169.3 corresponded to a fragmentation from m/e 200 to m/e



184 (scheme 10). Thus, the new compound (36) is the hydroperoxy methylene imidate. The possible significance of this result will be discussed later.

Another unusual compound has been isolated from a D.D.Q. oxidation of the imidate (29). An old sample of (29), containing some of the dioxopiperazine (28) produced by hydrolysis, was used and the products isolated by chromatography. The bis-methylene imidate (33) was isolated in greatly improved yield, 12%, only to polymerise, as before, within minutes of evaporation of the eluent. However, none of the expected methylene imidate (31) was produced but an orange crystalline solid (37) (7%) and the trans-dioxopiperazine (28) (11%) were isolated instead. Recrystallisation of the new compound afforded orange needles which gave a parent ion m/e 316 in the mass spectrum. The molecular formula $C_{15}H_{16}N_4O_4$ was confirmed by the microanalysis results. The n.m.r. spectrum of this dimeric material showed two N-methyl groups but only one methoxy group. The shifts of the two sets of vinylic methylene protons indicated that one methylene group was α to a carbonyl whilst the other was α to the imidate oxygen. The remaining vinylic proton at τ 3.73, a triplet J 3 Hz., was shown by decoupling experiments to be coupled to a two proton double AB quartet centred at τ 6.95. This latter system is similar to that of a 2,3-dehydropyrroline residue. The i.r. spectrum displayed a sharp vinylic C-H stretch at 3100 cm^{-1} as well as carbonyl and double bond absorptions; absorptions due to an N-H group were absent. Consideration of the above data lead to the formulation of structure (37).

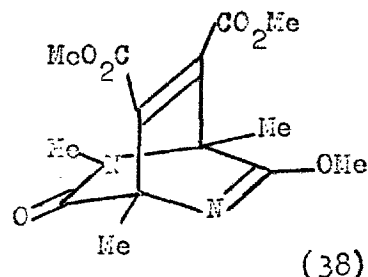
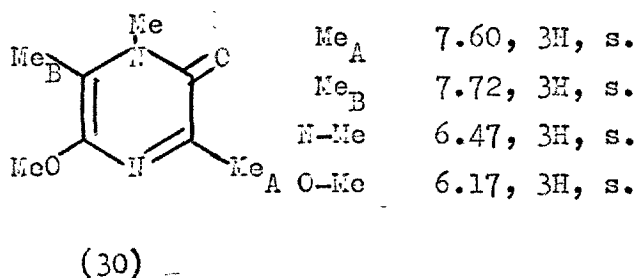


H_A τ 3.73, 1H, t, J 3 Hz.
 H_B τ 4.25, 1H, d, J 2 Hz.
 H_C τ 4.52, 1H, d, J 2 Hz.
 H_D τ 5.08, 2H, d, J 2 Hz. (coincidental)
O-Me τ 6.22, 3H, s.
N-Me τ 6.73, 3H, s, 6.75, 3H, s.
 H_E τ 6.62, 7.22, 2H, dABq, J 3, 18 Hz.

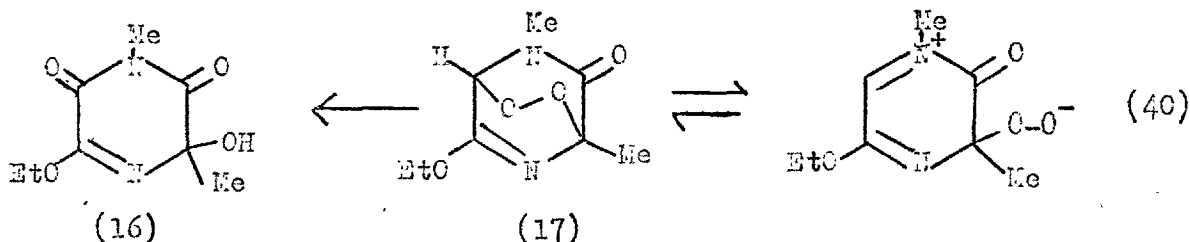
The mechanism for the formation of this compound is not at all clear, but presumably involves radical coupling of a dioxopiperazine and imidate molecule

at an intermediate level of oxidation followed by further dehydrogenation. It should be noted that the dioxopiperazines alone are completely inert to D.D.Q. oxidation. The oxidative coupling of a free amide group in the above manner may have significance for the oxidative cyclisation of the free amide group onto the dimethylallyl unit found in the austamides (see scheme 3).

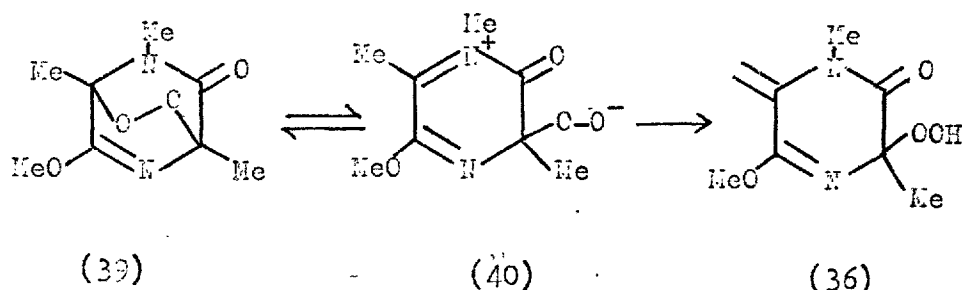
Finally, the D.D.Q. oxidation was repeated on a large scale using freshly prepared material and the products carefully separated by column chromatography. Elution with benzene gave 4% of the bis-methylene imide and 12% of the mono-methylene imide. Further elution with acetone:benzene (1:20) gave 15% of the pyrazinone (30) followed by 28% of the starting material contaminated with dienone. The pyrazinone, a pale yellow oil, exhibited the expected n.m.r. spectrum and was characterised by reaction with A.D.E. in refluxing dichloromethane. After 40 hours the cycloadduct (38) was isolated by plc. The dienone



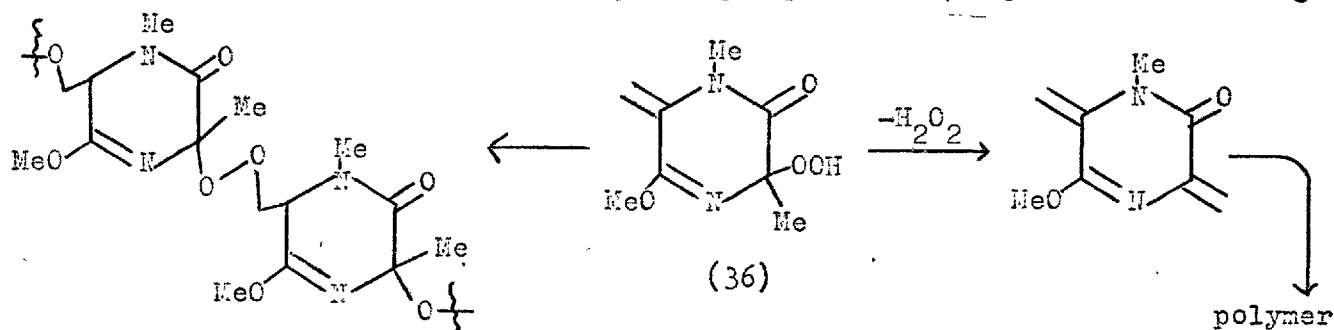
was also found, as expected, to be very reactive towards oxygen. Exposure to air lead to rapid consumption of the compound, but the endoperoxide (39) was not produced. Instead an intractible multicomponent oil was isolated. A plausible explanation is that the initially formed endoperoxide is in equilibrium with the hydroperoxy acyliminium species (40). In the case of the unsubstituted peroxide (17) such an equilibrium would not interfere with the observed rearrangement to the hydroxy imide (16). However, with the substituted



peroxide (39) elimination of a proton from the 6-methyl substituent could give rise to the methylene hydroperoxide (36) a compound already isolated

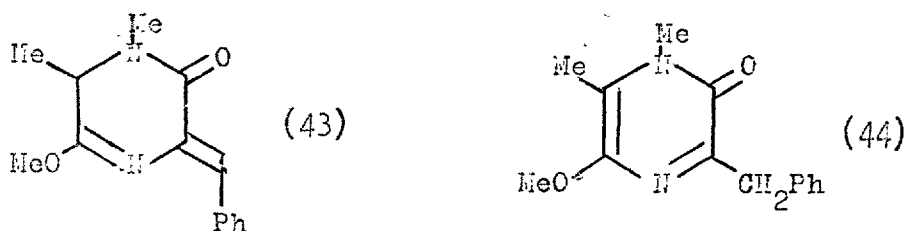


from one preparation of the pyrazinone. Elimination of hydrogen peroxide would give the bis-methylene compound and thence polymer. Alternatively, the hydroperoxide could add to the methylene group in the β -position affording

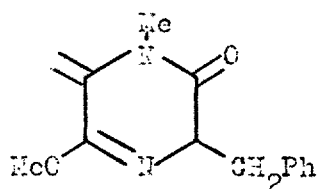


a polymeric peroxide incapable of elimination back to a monomer. Other possibilities include oxidation of the imide function by the hydroperoxide¹⁵ or oxidative cleavage of the methylene functions (see page 157). In reality, probably all of these reactions occur which results in the formation of a multicomponent mixture, as observed.

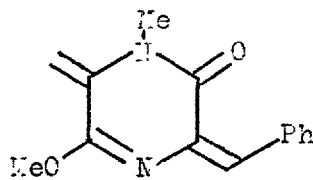
Oxidation of the 3-benzyl-6-methyl imidates (42) with D.D.Q. required 24 hours in refluxing benzene and two products were observed by tlc. The n.m.r. spectrum of the crude material showed no new C-methyl resonances indicating that neither the 6-methyl-3-benzylidene imidate (43) nor the pyrazinone (44) were products. The spectrum did show four vinylic resonances, two major and



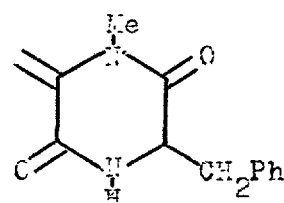
two minor, corresponding to the methylene imidate (45) and the bis-dehydro



(45)



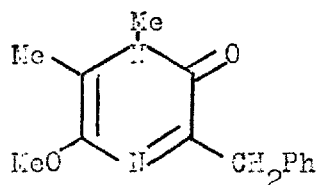
(46)



(47)

imidate-(46). Purification by column chromatography (alumina, dichloromethane) gave (46) as an orange solid in 7.5% yield. Recrystallisation from petrol afforded orange-red needles of analytical purity, m.p. 103-105°. Unlike the bis-methylene imidate (33) this compound showed no tendency to polymerise. The major product (45) was isolated from the column in 36% yield but was contaminated with a deep yellow colouration. Further purification could be effected by either plc or distillation (70°, 1 Torr) but the pale yellow oil refused to crystallise nor could a satisfactory analysis be obtained. Further characterisation was achieved by acid hydrolysis to the dioxopiperazine (47), (aqu. T.H.F., 2N HCl, yield 100%), and after recrystallisation, the colourless prisms gave satisfactory analytical and spectral data. The shielding effect of the phenyl ring is still apparent in the compounds (45) and (47) since the vinylic protons resonate at higher field (ca. 0.3 ppm) than in the other 6-methylene derivatives.

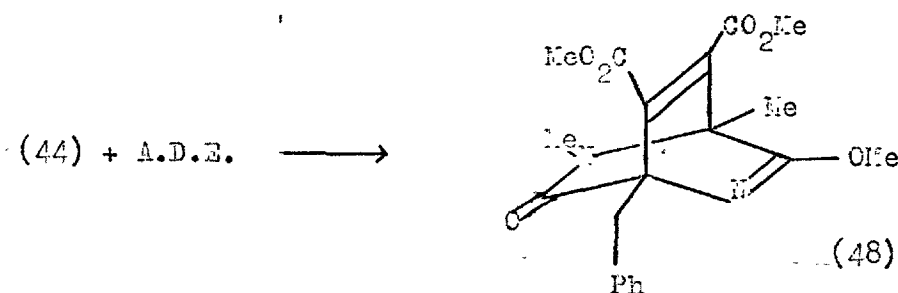
The oxidation was repeated on a 10 mmole scale and the products subjected to column chromatography as before. Besides (45) and (46), some 10-15% of yellow polymeric material was obtained. A further band was eluted from the column composed of (45), trans starting material and a third compound, ca. 30% of the mixture, whose resonances were most consistent with the dienone (44).



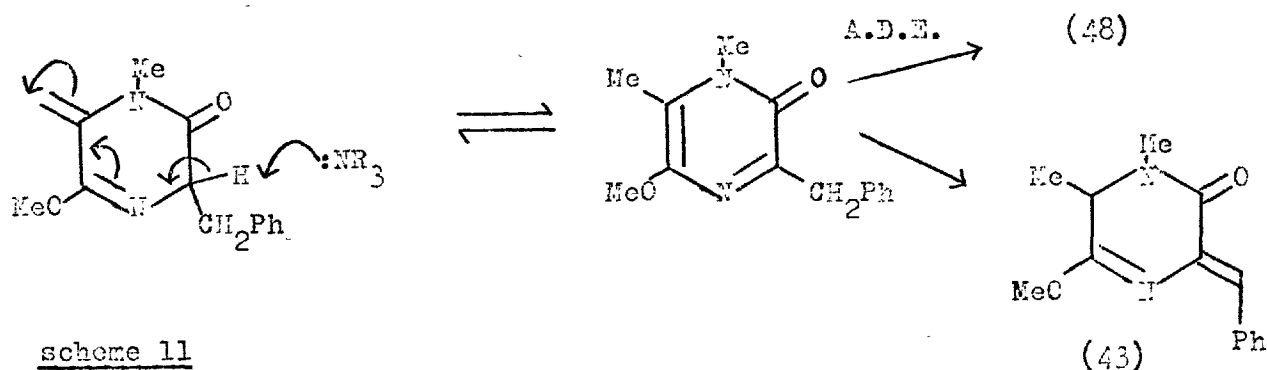
(44)

C-Me τ 7.78, 3H, s.
N-Me τ 6.55, 3H, s.
O-Me τ 6.20, 3H, s.
Ph-CH₂ τ 5.90, 2H, s.
Ph τ 2.73, 5H, s.

The mixture was stored under nitrogen overnight with the intention to separate the dienone by plc. However, during this period the dienone decomposed to polymeric material similar to that previously isolated from the column. Obviously, the dienone is too reactive to be stored for any length of time. In a subsequent oxidation, a sample of the dienone was immediately reacted with A.D.E. in refluxing dichloromethane for 40 hours. The resultant adduct (48) was isolated by plc and fully characterised.



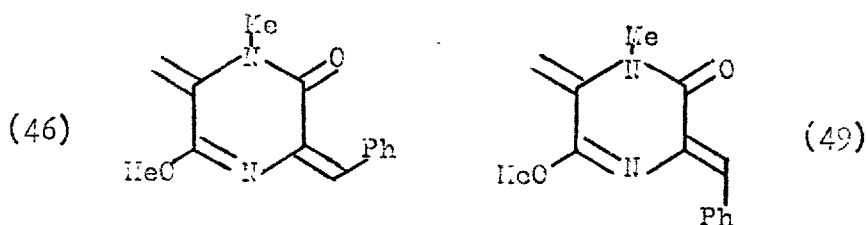
In analogy with the dimethyl pyrazinone (30), reaction of (44) with oxygen gave a complex mixture. Reduction with sodium borohydride failed to simplify the mixture, eliminating the possibility of a polymeric peroxide which would form a monomeric diol on reduction. An attempt was made to prepare the dienone from the methylene imidate (45). Thus, (45) was refluxed in benzene containing diisopropyl ethylamine in the presence of excess A.D.E. (scheme 11).



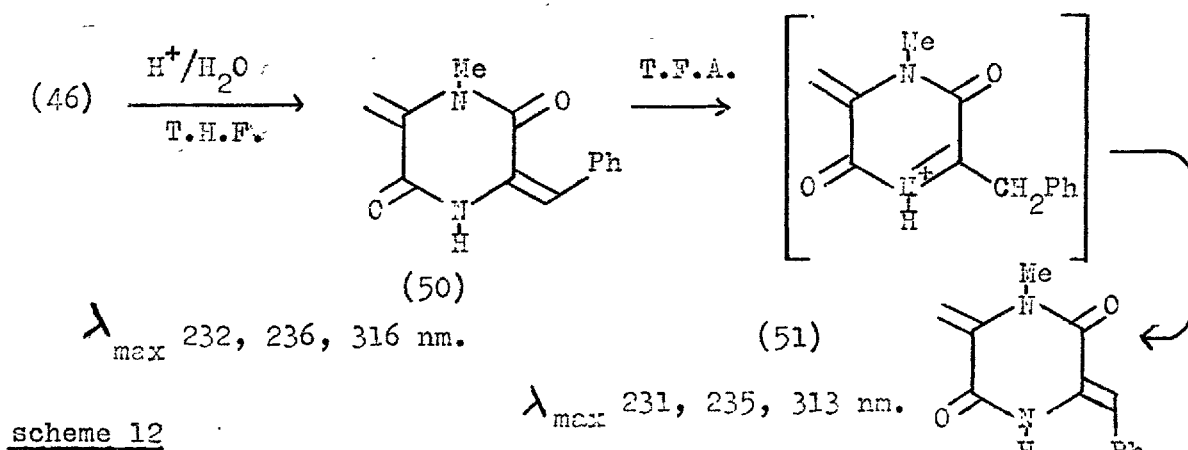
However, after 4 days the mixture was unchanged and neither the isomeric benzylidene imidate (43) nor the dienone-A.D.E. adduct (48) had been formed.

An unusual feature of these oxidations is the configuration of the benzylidene bond in the imidate (46). At first, it was assumed that the molecule possessed the more stable Z-configuration¹⁶, as in (49), but subsequent

experiments showed the contrary to be the case, as in (46). Acid hydrolysis



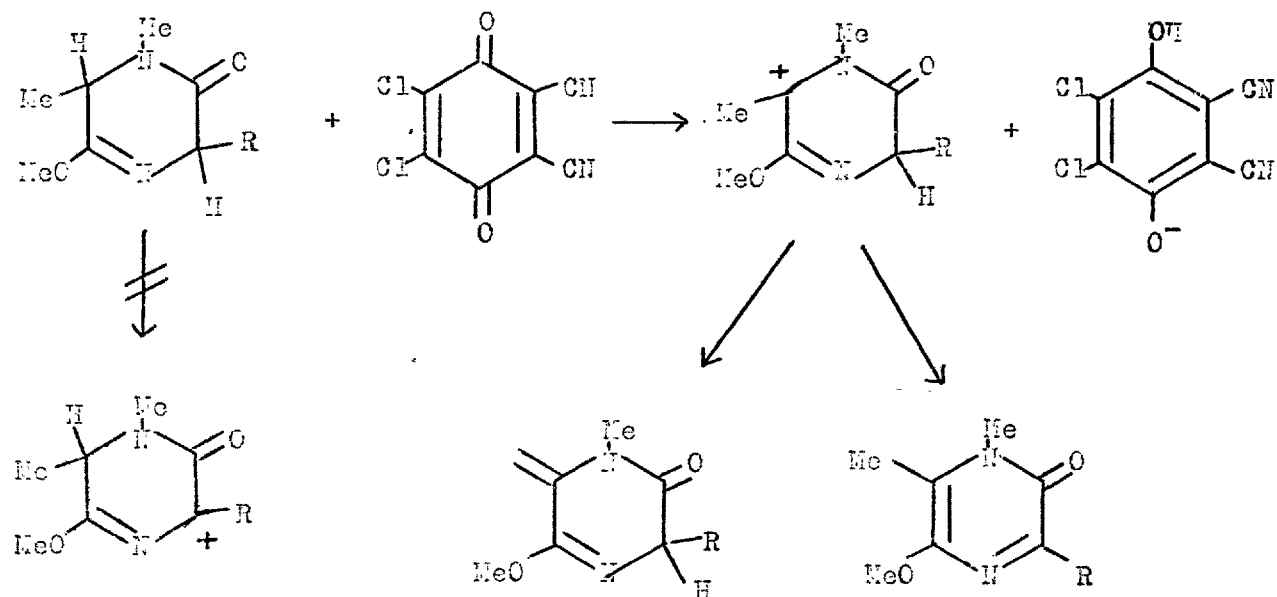
(aqu. T.H.F. 2N HCl) gave, after 5 days, a quantitative yield of the dioxopiperazine (50). Recrystallisation (acetone) afforded yellow prisms, m.p. 145-154°, possessing satisfactory analytical figures. The dioxopiperazine was not very soluble in non-polar solvents, a property usually associated with E-benzylidene dioxopiperazines⁶ (see page 132) and consequently the n.m.r. spectrum could not be recorded in deuteriochloroform. Brief treatment with trifluoroacetic acid (T.F.A.) effected isomerisation to a less polar compound, colourless needles (ethanol) m.p. 115-117°, which gave the same molecular formula on microanalysis. This compound was freely soluble in non-polar solvents and the n.m.r. spectrum was wholly consistent with the Z-benzylidene dioxopiperazine (51). Thus, the initially formed E-compound (50) isomerised in the strong acid to the more stable Z-isomer (51) presumably via an acyliminium intermediate (scheme 12).



The u.v. spectra of the two isomers, although similar, were consistent with the stereochemical formulation since the Z-isomers absorb at slightly shorter wavelength than the corresponding E-isomers¹⁵.

The mechanism of the D.D.Q. oxidation is of great interest. Both isomers were oxidised to the same products, although at slightly differing rates as judged by tlc, so stereochemistry cannot be very important. If a hydride

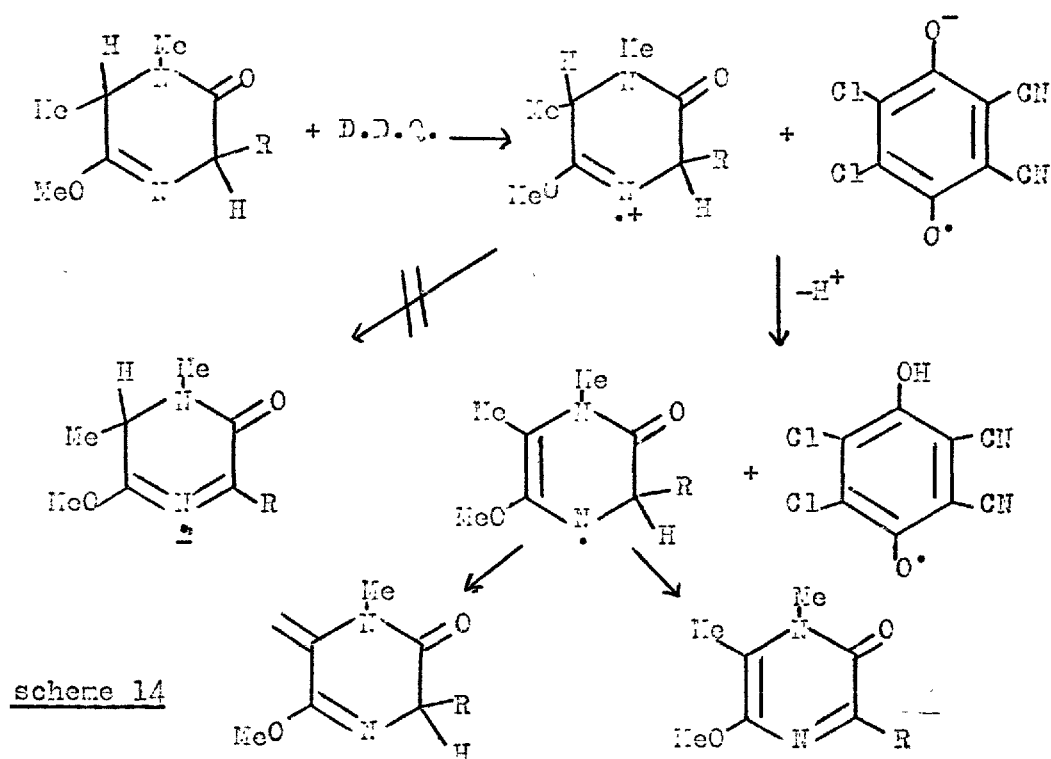
transfer process is considered then the observed products are in accord with the formation of the more stable carbonium ion i.e. in the 6-position. Subsequent loss of a proton from the 3-position gives the pyrazinone structure where as loss of a proton from the 6-substituent, where possible, gives the 6-methylene structure (scheme 13). Where there is no 6-substituent e.g. (13)



scheme 13

the sole product is the pyrazinone, as observed i.e. (13)→(14).

However, if oxygen was bubbled through the oxidation mixture the reaction was completely inhibited; on removal of the gas inlet, the oxidation resumed its normal course. This kind of behaviour is characteristic of a radical mechanism¹⁷, rather than an ionic hydride transfer. A possible mechanism would involve initial electron transfer from the imidate nitrogen to the quinone followed by proton loss from the 6-position. A similar proton loss from the 3-position is not possible as there are no available orbitals on the nitrogen atom. The intermediate radical can then collapse by elimination of a hydrogen radical to give the two observed products, where possible (scheme 14). The formation of the bis-dehydro imidates probably arises by further oxidation of the 6-methylene compounds. This is possible since a sample of the imidate (45) was converted to the methylene benzylidene compound (46) by reaction with D.D.Q. in refluxing benzene. In addition, it was noticed that the highest

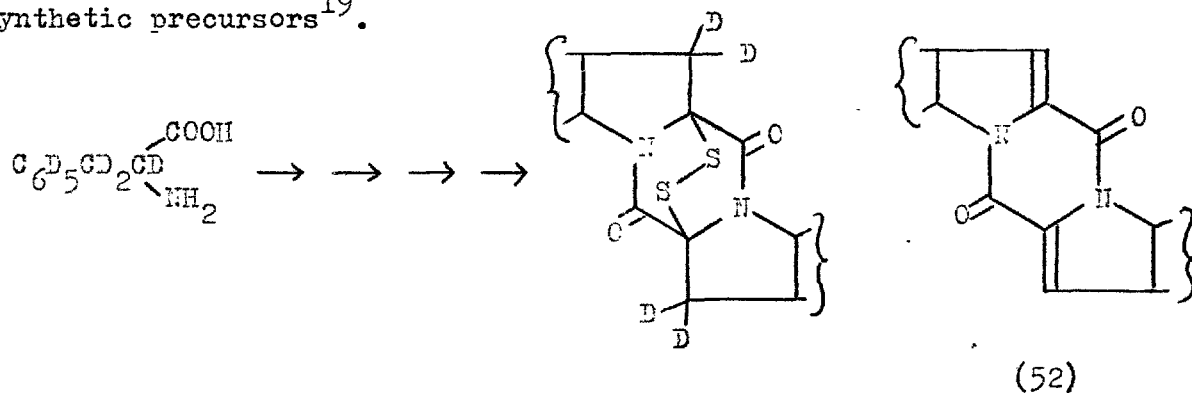


yields of the bis-dehydro compounds coincided with the lowest yields of the mono-dehydro compounds.

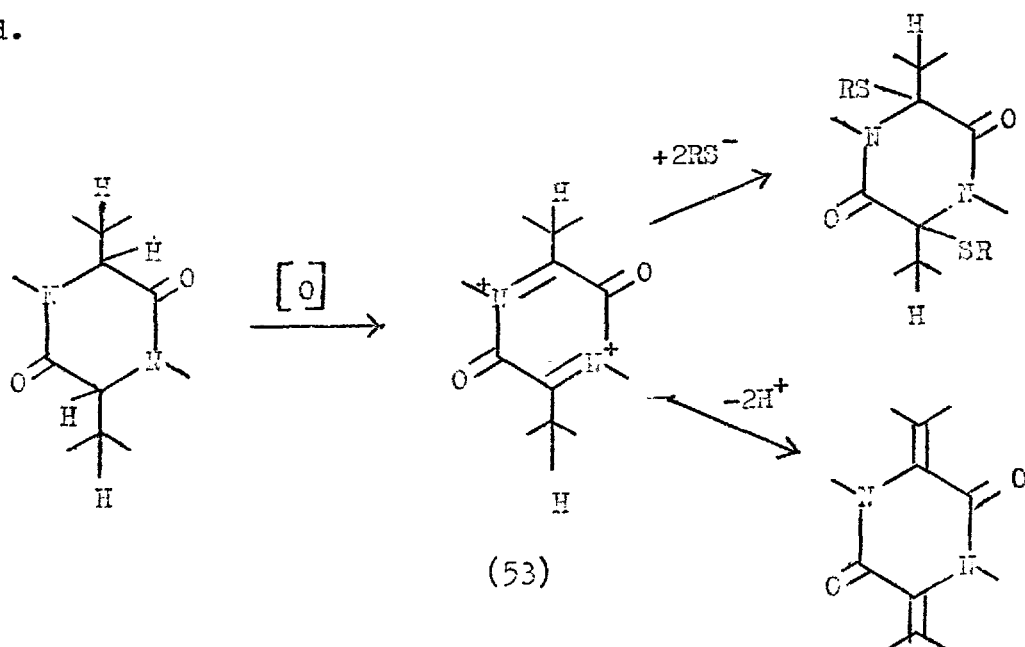
The significance of these oxidations to the biosynthesis of the fungal metabolites may be considerable. It is interesting to note that the in vitro 'unspecific' oxidation produces both the natural features, i.e. the proposed pyrazinone-precursor for the brevianamides and the dehydro-residues found in the austamides.

B. Gliotoxin; the sulphur problem.

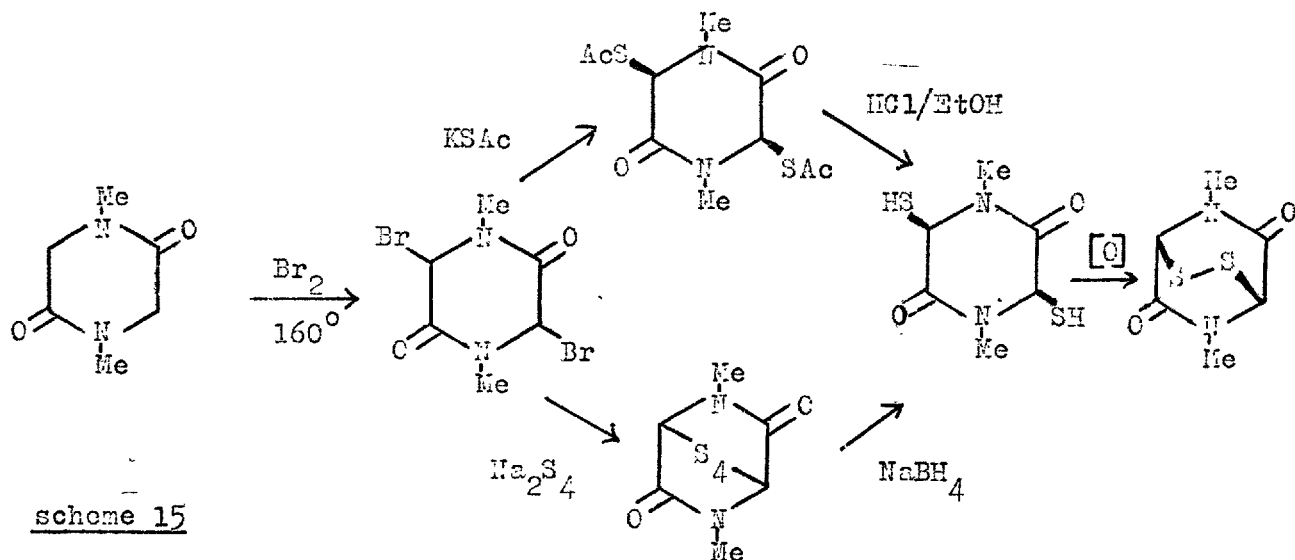
The naturally occurring epipolythiodioxopiperazines (see page 43) present a very interesting problem to the natural product chemist in so far as it is not yet known how and when nature incorporates the sulphur into the dipeptide ring. Feeding experiments¹⁸ with D₈-phenylalanine have shown that a bis-dehydro system, i.e. (52), is not involved in the biosynthesis of acetylaranotin. This observation can probably be extended to include all the other metabolites since a common mechanism for the incorporation of sulphur seems most likely, although bis-dehydro metabolites have been isolated and postulated as biosynthetic precursors¹⁹.



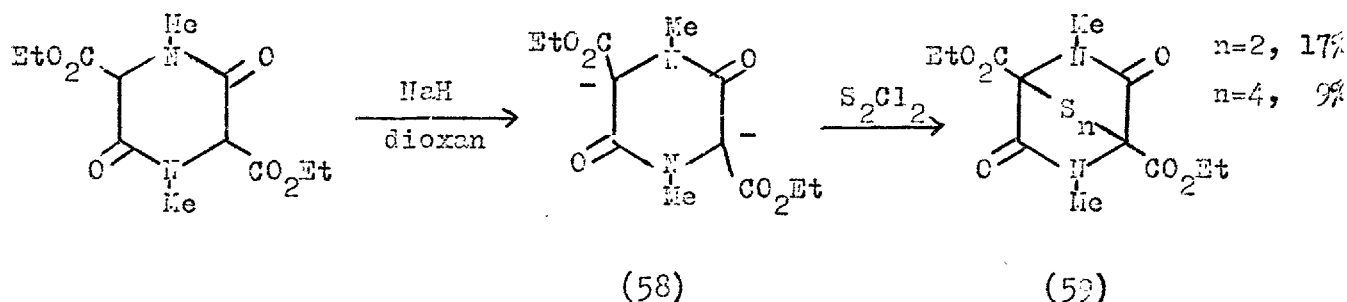
The mechanism may involve oxidation of the dioxopiperazine ring to the corresponding acyliminium intermediate (53) which can then be quenched by a sulphur nucleophile before proton loss converts the intermediate to the dehydro-compound.



Interest in this class of metabolites has stimulated considerable synthetic effort in the area. However, at the outset of this work, in 1971, only two synthetic methods for epidithiodioxopiperazines had been published. Trown²⁰ achieved the first success which involved bromination of sarcosine anhydride to obtain the dibromide (54). Subsequent reaction with potassium thiolacetate, followed by hydrolysis and oxidation gave the disulphide (55) (scheme 15). The N,N'-diphenyl derivative has been prepared by a similar route²¹. Alternatively, the tetrasulphide (56) has been formed²² from the dibromide (54) with sodium tetrasulphide. Reduction with borohydride followed by oxidation gave the disulphide. Unfortunately this method is not general,



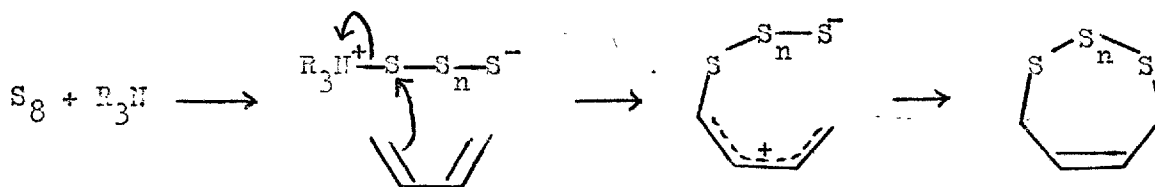
dehydrobromination occurring with 3,6-disubstituted dioxopiperazines. In the second method²³, the activated dioxopiperazine (57) afforded the dianion (58) on treatment with sodium hydride in dioxan. Reaction of (58) with sulphur



chloride gave the disulphide (59) in 17% yield. Neither method offers a general route to the desired disulphides and it was felt that a totally

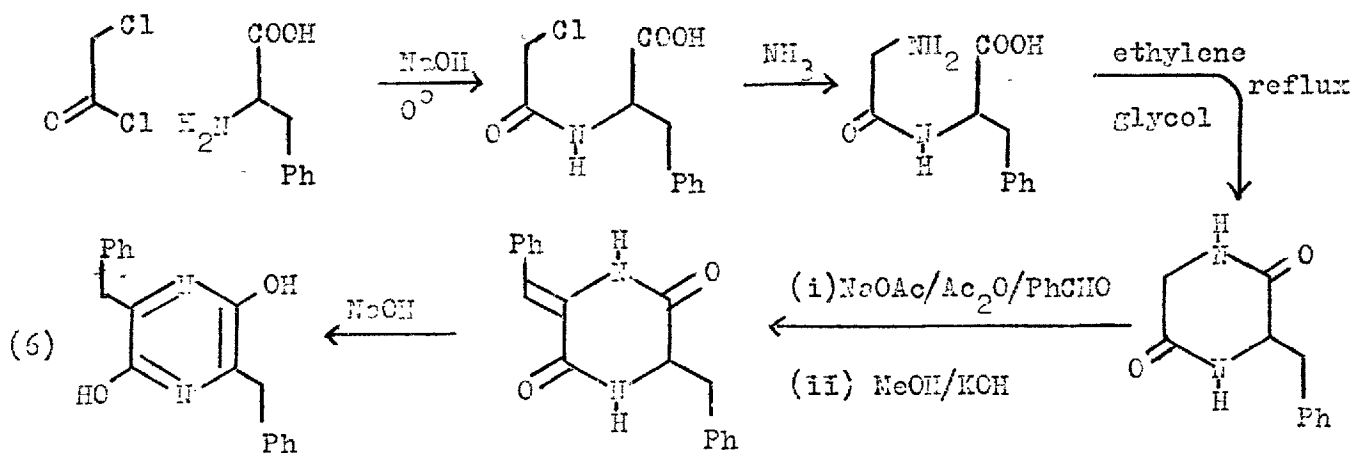
different approach was required.

(i) Reactions with elemental sulphur. The cycloaddition reactions of dihydroxypyrazines and alkoxy-pyrazinones described earlier presented a very attractive route if a suitable sulphur 'dienophile' could be found. The formation of cyclic sulphides from olefins and elemental sulphur, in the presence of tertiary base, has literature precedent²⁴ and this approach was adopted. The mechanism of such a reaction presumably involves rupture of the S_8 -rings to give a dipolar linear species which can then be attacked by the diene (scheme 16).



scheme 16

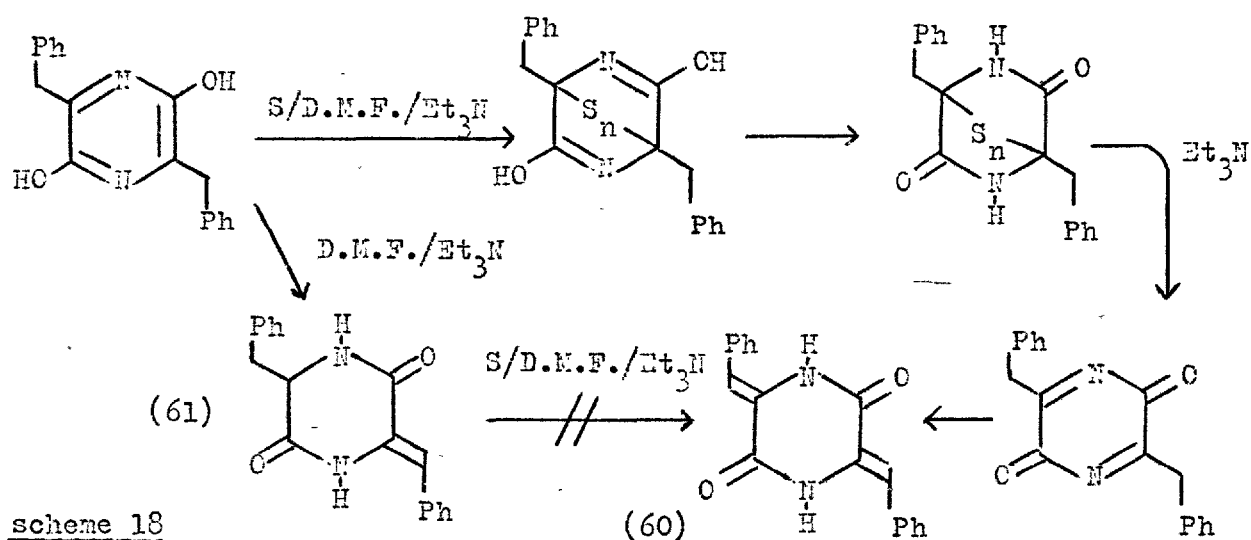
3,6-Dibenzyl-2,5-dihydroxypyrazine (6) was used as the diene and its preparation accomplished by the published route⁴ (scheme 17). The sulphurisation



scheme 17

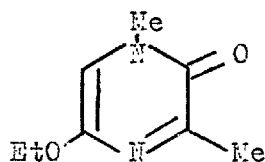
conditions involved heating the pyrazine with sulphur in dimethylformamide (D.M.F.), containing triethylamine, at 60°. After two hours all the solids had dissolved and the mixture had turned from yellow through red and purple to a very dark brown. Heating was discontinued and the solution stirred at 25° overnight whereupon a yellow precipitate appeared. The suspension was cooled in ice and the yellow solids isolated. A sample was recrystallised from D.M.F.

and the yellow needles exhibited m.p. 294-297°. Its i.r. spectrum displayed N-H absorptions and more informatively, a very strong double bond stretching absorption at 1635 cm⁻¹. The n.m.r. spectrum, recorded in T.F.A., showed only a large multiplet around τ 3. The data compares well with that of the known²⁵ bis-benzylidene dioxopiperazine (60) and the assignment was confirmed by the mass and u.v. spectra. Control experiments showed that, without the sulphur,



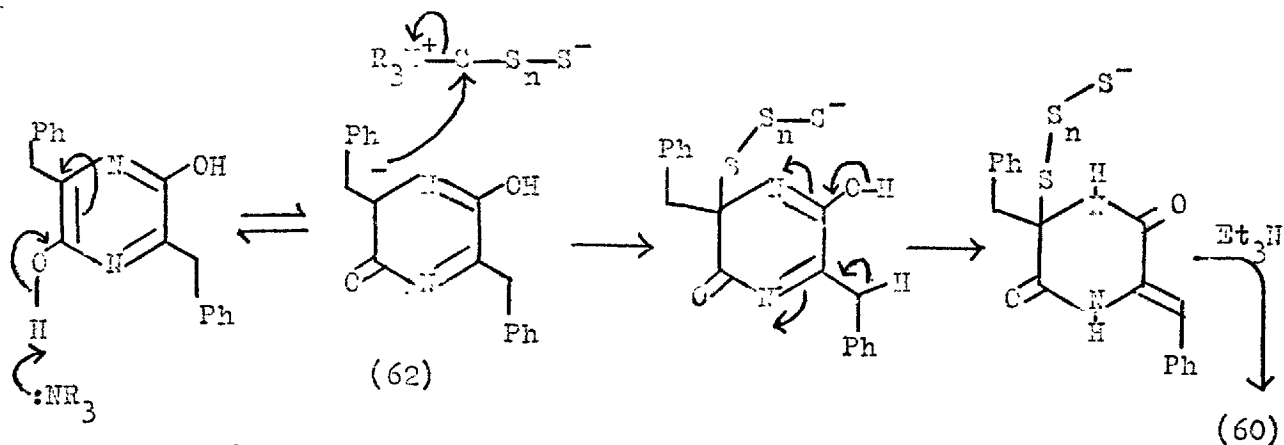
scheme 18

isomerisation to the mono-benzylidene compound (61) was the only reaction. However, the latter derivative was not oxidised in the presence of the sulphur reagent. These findings were encouraging and it seemed possible that an initial sulphur bridged product was undergoing base catalysed elimination to the isolated bis-enamide (scheme 18). It was considered that the elimination could be prevented by blocking the nitrogen atoms with a suitable group. Since this was not possible with the dihydroxypyrazine (dialkoxypyrazines do not undergo cycloadditions⁶) the reactions of the ethoxy pyrazinone (14) were investigated. However, all attempts to persuade this highly reactive dienone

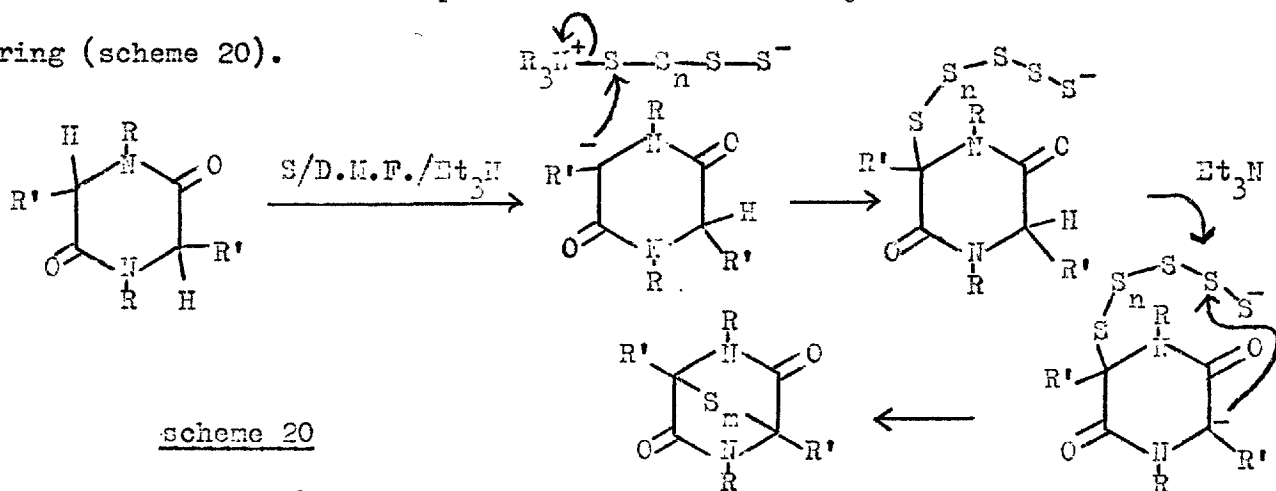


(14)

to react with the sulphur reagent failed. In view of the lack of success with the dienone (14) alternative mechanisms for the formation of (60), not involving thiobridged intermediates, were considered. One such mechanism is shown in scheme 19. Initial attack on the sulphur reagent by the

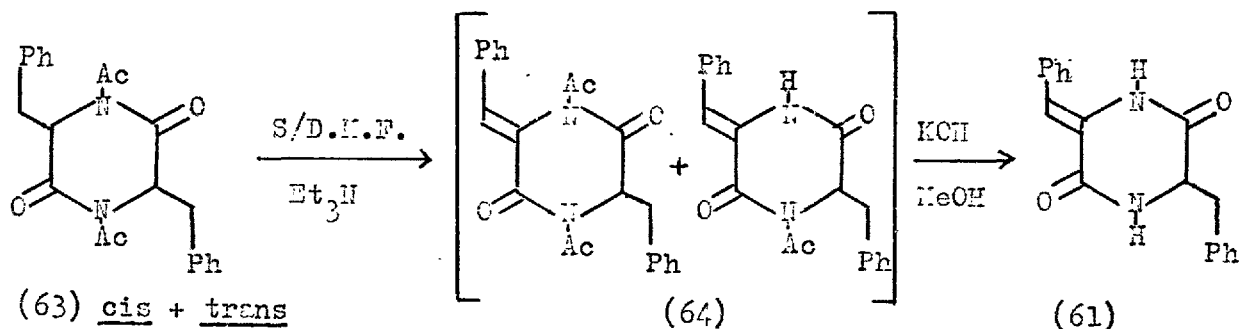


carbanion (62) gives the ring substituted acylimine which might rapidly isomerise to the enamide before attack by the sulphur chain could occur. Subsequent elimination gives the bis-enamide (60). The ethoxy pyrazinone (14) cannot participate by this mechanism and no reaction would be expected as was observed. The carbanion (62) bears some resemblance to the bis-anion (58) and it was felt that, by careful choice of a dioxopiperazine derivative, the direct introduction of sulphur could be obtained by anion formation on the ring (scheme 20).



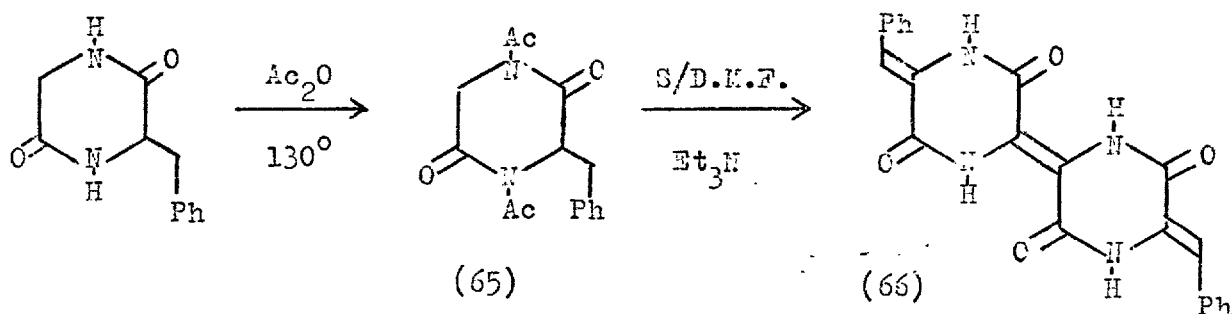
The condensation of aldehydes with glycine derived dioxopiperazines²⁶ (see also scheme 17) is facilitated by the formation of the N,N'-diacetyl derivatives and so it was decided to use these compounds for the sulphur/base reactions. Thus, 3,6-dibenzyl-2,5-dioxopiperazine, prepared by dehydrative dimerisation of phenylalanine in refluxing ethylene glycol (82% yield), was diacetylated with acetic acid/acetic anhydride (reflux, 5 hours, 72%). The diacetyl compound (63) was reacted with sulphur/D.M.F./triethylamine by heating at 60°

for 2 hours followed by stirring at 25° overnight. The brown solution was evaporated and the n.m.r. spectrum of the crude oily product recorded. Comparison with the authentic spectra²⁷ of N-monoacetyl and N,N'-diacetyl benzyl-benzylidene dioxopiperazines (64) showed that the crude product consisted mostly of these compounds. Subsequent hydrolysis of the oil with



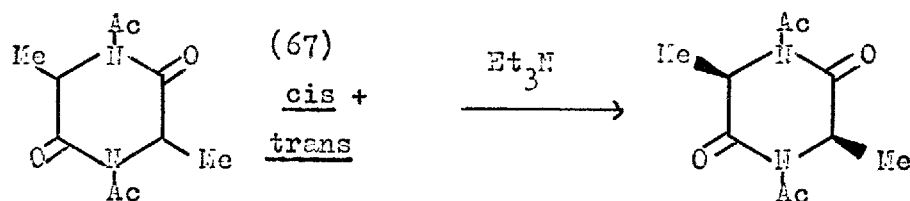
methanolic potassium hydroxide at 25°, afforded 48% of the benzyl-benzylidene compound (61). Other products of the reaction were ring-opened linear dipeptide derivatives and were not of any interest.

When 1,4-diacetyl-3-benzyl-dioxopiperazine (65), prepared from the non-acetylated compound (see scheme 17) with acetic anhydride, was reacted with the sulphur reagent under identical conditions, a bright yellow, highly insoluble compound precipitated out of the reaction mixture in 25% yield. The n.m.r. spectrum of this material, recorded in T.F.A., showed only an aromatic resonance and the compound rapidly crystallised out of the T.F.A. solution in long needles. The i.r. spectrum was very similar to that of the bis-benzylidene compound (60) and the mass spectrum gave a parent ion m/e 400. Analysis of the yellow crystals from the T.F.A. solution gave figures consistent with the formula $\text{C}_{22}\text{H}_{16}\text{N}_4\text{O}_4 \cdot \text{CF}_3\text{COOH}$. On the basis of these facts the dimeric structure (66) was assigned to the new compound. Examination of

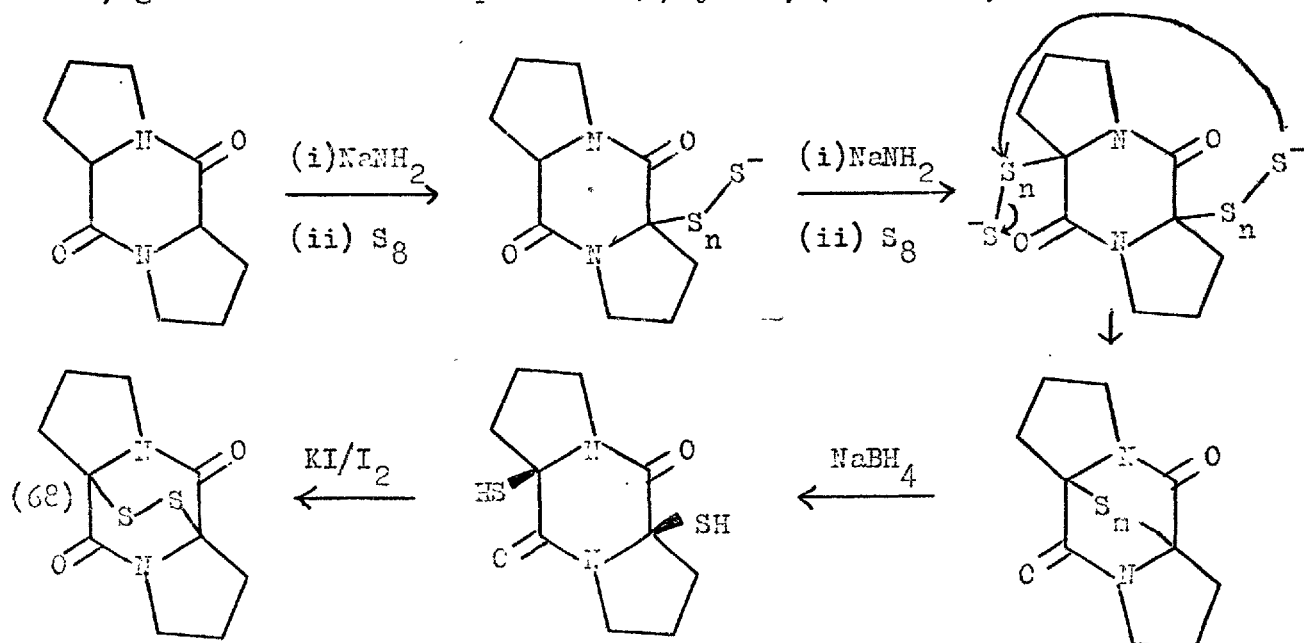


the mother liquors from this reaction showed a multicomponent mixture which was abandoned.

Reaction of the cis-trans-N,N'-diacetyl derivative (67) of alanine anhydride with the sulphur reagent failed to produce any reaction other than epimerisation to the more stable cis-isomer. Such epimerisation could also be effected by treatment with triethylamine alone.



Although these reactions did not succeed with respect to the synthesis of thiobridged compounds, oxidation of the dipeptide ring had certainly occurred in the formation of the dimer (66) and it was felt that the correct choice of reaction conditions, i.e. solvent and base, would lead to the desired incorporation of sulphur. At this point, however, a synthesis of the proline anhydride disulphide (68) was published²⁸. This compound and similar derivatives were prepared by metallation of proline anhydride with sodamide in liquid ammonia. Stepwise treatment with sodamide, sulphur, sodamide and more sulphur afforded the epipolysulphide, which on reduction (borohydride) and oxidation (iodine) gave the crude disulphide in 40% yield, (scheme 21).

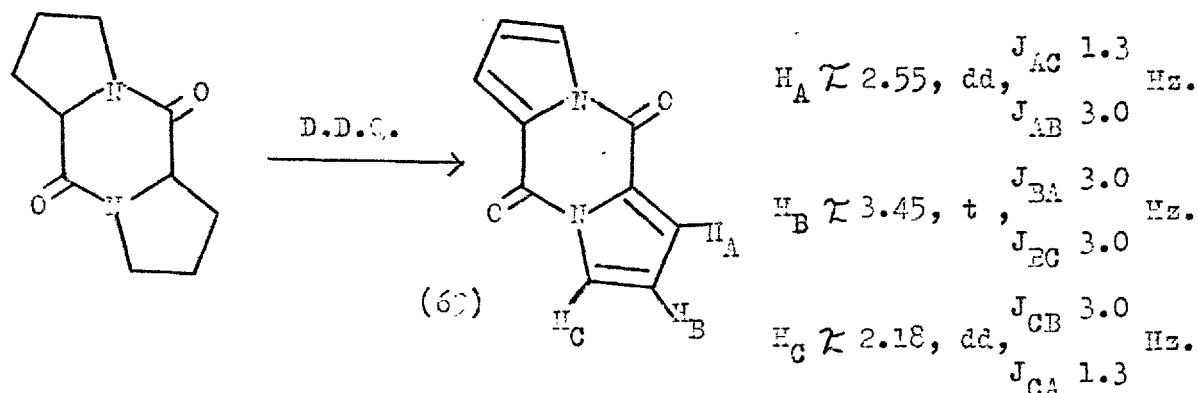


scheme 21

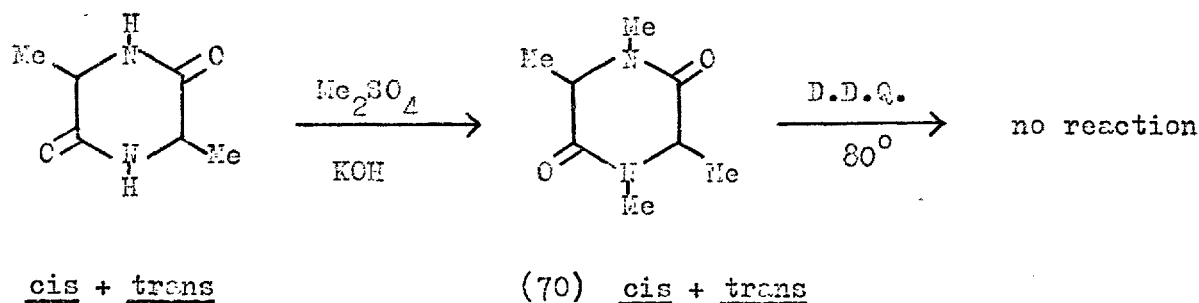
In essence the above synthesis was identical to the first approach of our group. It warranted at this stage, therefore, to approach the synthesis in yet another way.

(ii) Reactions with oxidising agents. The possible intermediacy of an acyliminium species in the biosynthetic incorporation of sulphur into gliotoxin etc., prompted us to carry out several experiments to determine the behaviour of dioxopiperazines and various derivatives towards oxidation. At that point in time there was little literature precedent for such oxidations besides the bromination of N,N'-disubstituted glycine anhydrides^{20,21} and the halogenation of proline anhydride, which leads to dehydrohalogenation and the formation of complex mixtures²⁹. Recently, however, the halogenation of several dioxopiperazines has been reported³⁰.

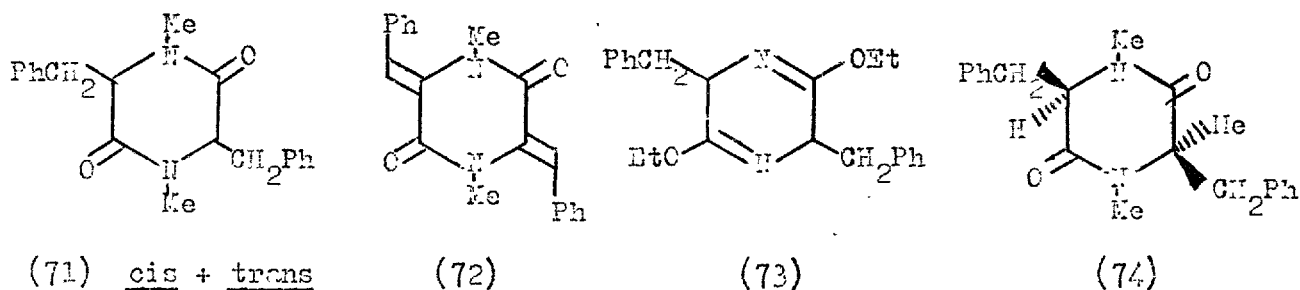
Initially, the reactions of some N,N'-disubstituted dioxopiperazines with D.D.Q. were investigated. Thus, proline anhydride and D.D.Q. in benzene were heated under reflux for 5 days, by which time all the oxidant had been converted to quinol. Tlc of the reaction mixture showed starting material and a new non-polar compound, readily visualised by u.v. light. After removal of the insoluble quinol, the filtrates were evaporated and the residue triturated with the minimum amount of dichloromethane. This removed the starting material and left a quantity, 25% by weight, of highly coloured crystals. Recrystallisation from benzene afforded pale yellow plates m.p. 272°. Its i.r. spectrum showed strong vinylic stretches at 3130 cm⁻¹, a carbonyl stretch at 1705 cm⁻¹ and double bond absorptions at 1560 cm⁻¹. These absorptions are more compatible with an aromatic ketone than a cyclic dipeptide. The n.m.r. spectrum showed nothing but three low-field multiplets. The only possible structure consistent with the spectral data is the cyclic bis-lactam (69) of pyrrole-2-carboxylic acid. This unusual derivative is a known compound, trivial name pyrocoll³¹, first isolated from the pyrolysis of gelatin. Comparison with the published m.p. and u.v. spectrum confirmed the structural assignment.



Considering the quantitative yield obtained when calculated on the amount of oxidant used, such an oxidation was encouraging and extension of the reaction to other dioxopiperazines was rapidly sought. Methylation of cis-trans alanine anhydride, with dimethyl sulphate in aqueous potassium hydroxide, gave the cis-trans-tetramethyl derivative (70), albeit in low yield (40%), but of excellent purity. Although the isomers were not separated the mixture was recrystallised and analysed correctly. (The compounds have recently been

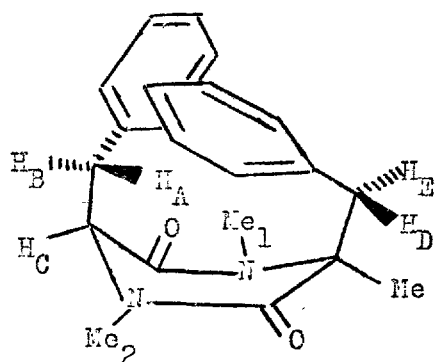


prepared by dehydrative dimerisation of N-methylalanine in refluxing ethylene glycol³⁰). Refluxing (70) with 2 equivalents of D.D.Q. in benzene for one week failed to produce any reaction whatsoever. Similarly, the N,N'-diacetyl derivative (67) was completely inert to D.D.Q. in refluxing benzene. In order to facilitate the oxidation the dibenzyl derivative (71)³² was used as substrate. With this compound dehydrogenation might be expected to occur readily affording



the extensively conjugated dibenzylidene dioxopiperazine (72). The preparation

of (71) merits further discussion. Phenylalanine anhydride was easily methylated with sodium hydride and methyl iodide in D.M.F., a standard reagent for the N-methylation of peptides.³³ Workup after 3 hours reaction time afforded (71) as a cis-trans mixture in 70% yield. Configurational assignments could be made by comparison of the n.m.r. spectrum with those of the known bis-imidates (73)^{5,6} which were found to be very similar. However, if the reaction mixture was left for 20 hours, further alkylation occurred. Crystallisation of the crude product gave needles of the trans-isomer in 38% yield but crystallisation of the residue from the mother liquors did not furnish the cis-isomer. Instead prisms of a new compound were deposited, whose spectral and analytical data were consistent with the trimethyl compound (74). The complicated n.m.r. spectrum can be explained in terms of the preferred conformation shown in (75). Similar C-alkylation reactions have recently been



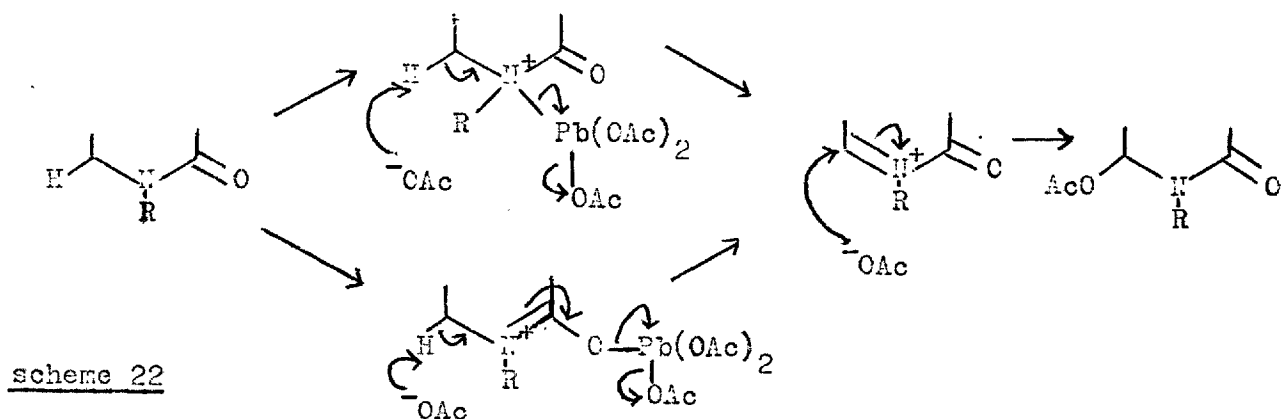
(75)

H_A τ 8.78, dd, J 8, 14.5 Hz.
 H_B τ 7.52, dd, J 4, 14.5 Hz.
 H_C τ 6.05, dd, J 4, 8 Hz.
 H_D τ 7.07 } ABq, J 14 Hz.
 H_E τ 6.86 }
 $N-Me_1$ τ 7.48, s. $N-Me_2$ τ 6.23, s.
 $C-Me$ τ 8.32, s.
 Aromatics τ 2.5-3.1, m.

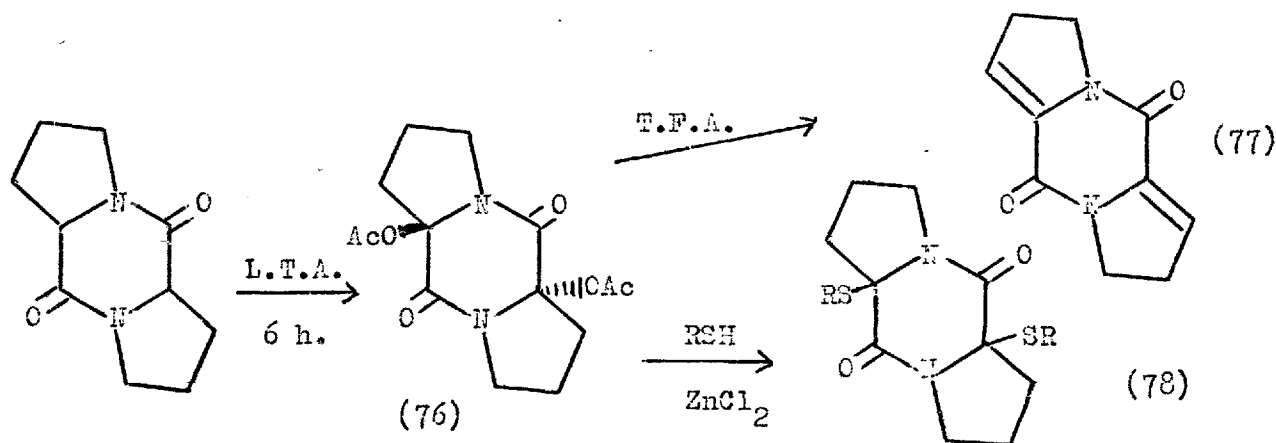
reported.²⁹ Refluxing the dibenzyl compound (71) with D.D.Q. in benzene for 7 days failed to produce any of the bis-benzylidene compound (72) contrary to expectations. Thus, it must be concluded that, in general, oxidation of N,N'-disubstituted dioxopiperazines with D.D.Q. is not a favourable reaction. As described earlier (p. 95) N-monosubstituted dioxopiperazines are inert to D.D.Q. oxidation.

α -Acetoxylation of amides with lead tetraacetate (L.T.A.) has literature precedent³⁴. Attack by the amide on the lead salt can lead to formation of an

acylimine or acyliminium ion which is rapidly quenched by the liberated acetate ion (scheme 22). However, before this reaction could be extended to



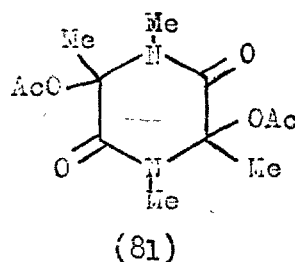
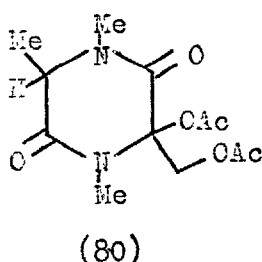
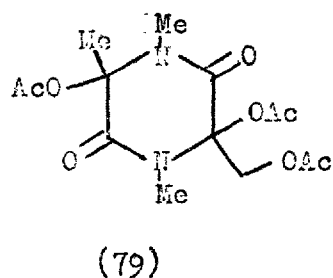
dioxopiperazines, Schmidt *et al.*³⁵ reported that proline anhydride reacts with L.T.A. in benzene at 50-70° to give the diacetate (76) in 32% yield. The diacetate behaves like a normal α -functionalised amide (see Part 1) and



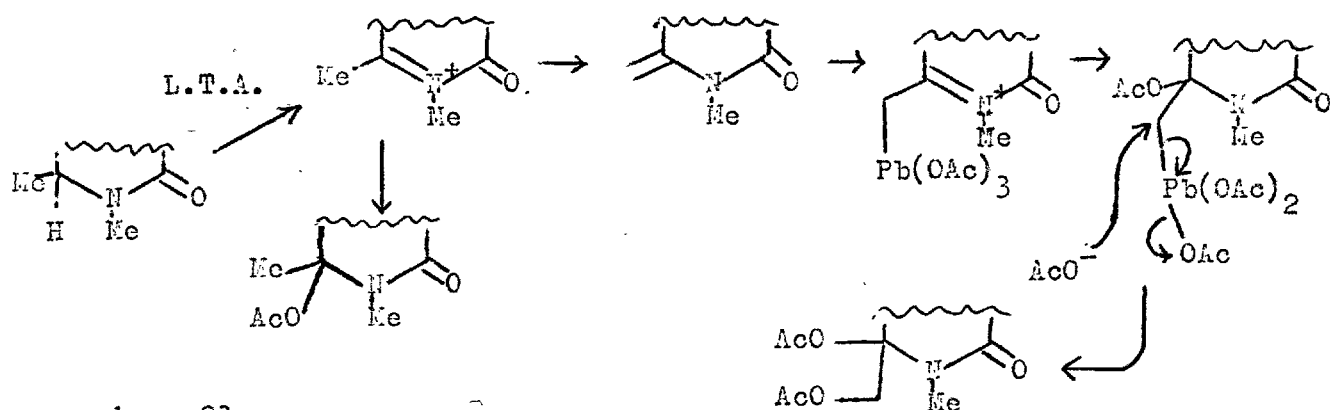
can be readily converted to either the bis-dehydro derivative (77), by acid treatment alone, or to other functionalised derivatives *e.g.* (78), by acid catalysed substitution. In view of the importance of the L.T.A. oxidation, especially as it may well mimic the biosynthetic pathway, it was felt that an investigation of its generality was warranted. The susceptibility of proline anhydride towards oxidation was demonstrated by its reaction with D.D.Q.. Likewise, the ease of oxidation of this dioxopiperazine with L.T.A. had also proved to be somewhat exceptional.

L.T.A. oxidation of the tetramethyl compound (70) required 20 hours in

refluxing benzene before all the oxidant had been consumed. Workup afforded a crystallising oil which, on trituration with ether, gave white crystals (18% yield). However, n.m.r. and tlc showed that this material was a mixture of two compounds which were subsequently separated by plc. The less polar band afforded colourless needles of the triacetate (79) whereas the more polar band constituted the diacetate (80). Both compounds were fully characterised by their spectral and analytical data. The single spot behaviour on tlc and the n.m.r. spectra of these acetates indicated that they were single compounds and not mixtures of isomers. Their formation can be explained in terms of schemes 22 and 23



although the order of acetoxylation is not known. N.m.r. examination of the reaction mother liquors indicated, by the lack of C-methyl singlets around

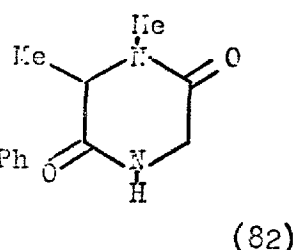
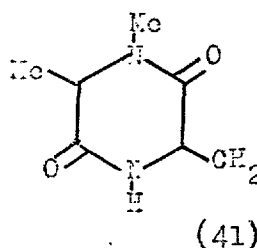
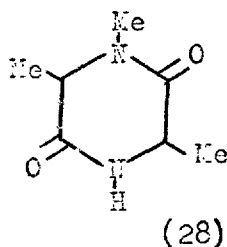
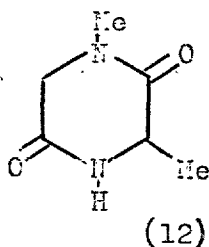


28, that very little (<5%) of the diacetate (81) was present. The facile oxidation of dehydro-dioxopiperazines with L.T.A. will be described later (p136). Attempts to influence the oxidation by changing the solvent (to acetic acid) or by using acid scavengers such as anhydrous calcium carbonate or pyridine resulted in reduced yields or even complete lack of reaction.

Oxidation of 3,6-dibenzyl-1,4-dimethyl-dioxopiperazine (71) with L.T.A. was even less rewarding than in the case of the tetramethyl derivative. Prolonged reaction (4 days) at 80° failed to produce any significant oxidation

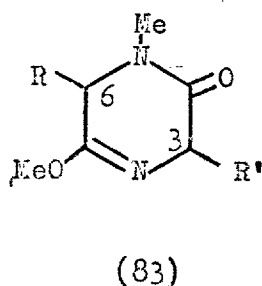
of the substrate.

Several N-monosubstituted dioxopiperazines were treated with L.T.A. in benzene at 80°. In the cases of the 3-methyl derivatives (12) and (28)



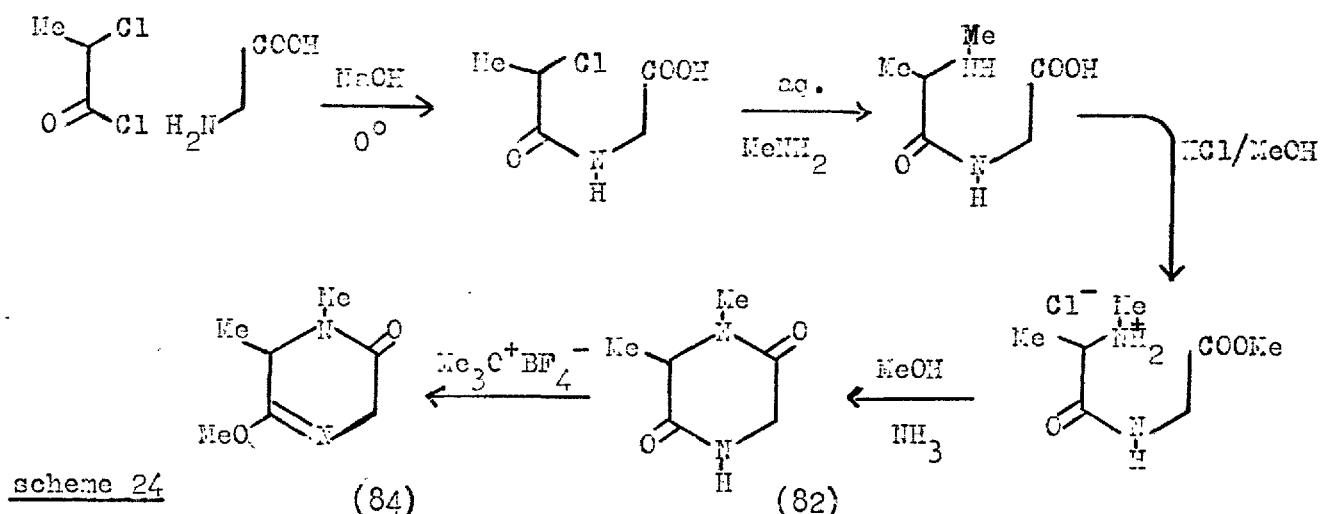
extensive decomposition occurred and no discrete compounds besides starting material could be isolated. In contrast, the 6-methyl derivatives (41) and (82) were unaffected by the oxidant.

The above results indicate that L.T.A. oxidation of dioxopiperazines is not general and each reaction must be taken on its own merits. Nevertheless, it was considered that suitable modification of the dioxopiperazine ring could lead to activation towards oxidation. For example, on the basis of the mechanism in scheme 22, dihydropyrazinones e.g. (83), should readily oxidise in the 6-position because the neighbouring 5-carbonyl group has been converted to

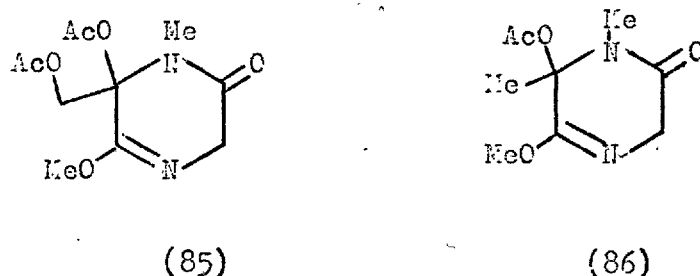


the relatively electron rich imide which would help to stabilise any adjacent, intermediate acyliminium species. Oxidation in the 3-position should be completely inhibited since formation of the acyliminium species is not possible.

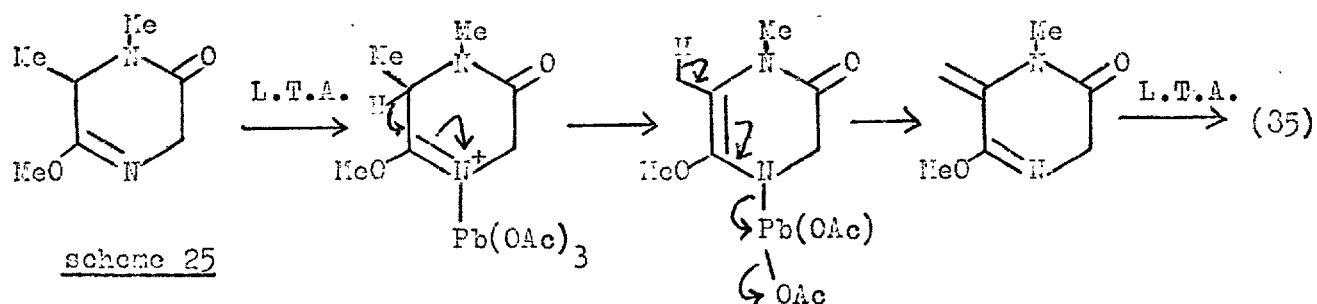
Alternatively, a radical mechanism might also favour oxidation in the 6-position on the grounds used to explain the products in the analogous D.D.Q. oxidations (see p.100). The imide chosen to test these arguments was the 1,6-dimethyl derivative (84). Prepared from glycine and 2-chloropropionyl chloride via the dioxopiperazine (82) (scheme 24), this compound was unusually acid sensitive, readily undergoing hydrolysis to ring opened products. The cyclisation of the dipeptide to the dioxopiperazine (82) was carried out by esterification, followed by base catalysed ring closure rather than by the more usual ethylene glycol dehydration, since the former



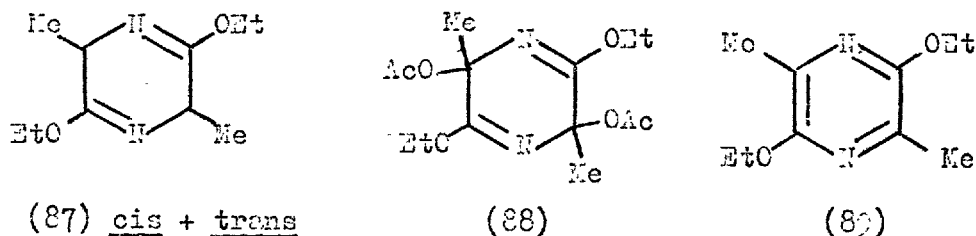
gave a much higher yield (93% compared to 40%). Oxidation of the imide (84) with L.T.A. in benzene occurred smoothly at 80°. Workup afforded the crystalline diacetate (85) as the major product which, after recrystallisation, exhibited the expected spectral and analytical data. Spectral examination of the reaction mixture showed no evidence for the monoacetate (86) nor for acetoxylation in



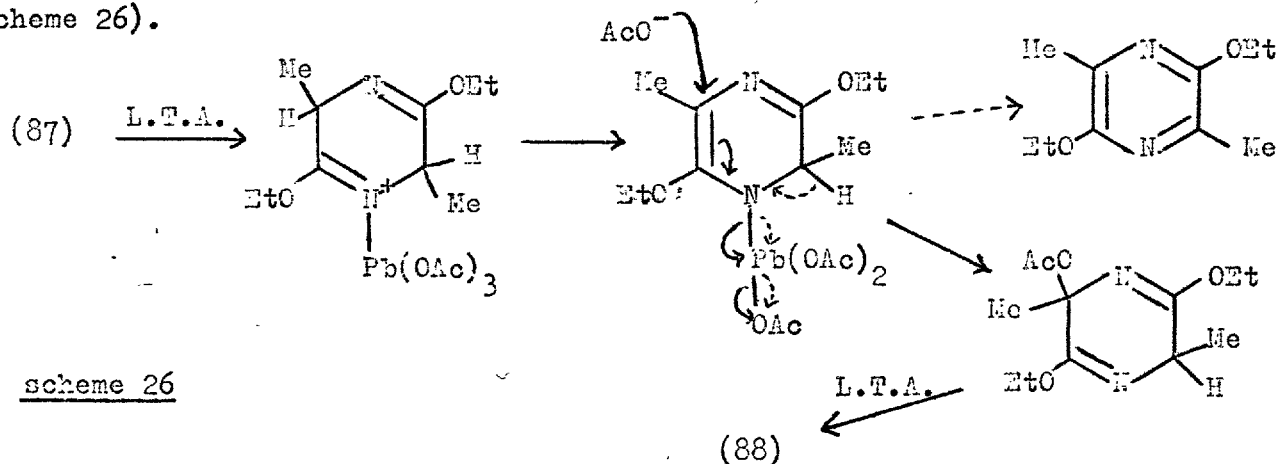
the 3-position. Workup of a subsequent experiment before oxidation was complete gave starting material and the diacetate as the only products. The presence of the amide function is implicit in the mechanisms depicted in schemes 22 and 23, but this need not necessarily be the case. Neglecting radical pathways, an alternative ionic mechanism directly involving the imide function may be operative (scheme 25). Were this the case, L.T.A. oxidation of bis-imides



should be possible. Thus, the bis-ethoxy dihydropyrazine (87), prepared from alanine anhydride and triethyl oxonium fluoroborate in 64% yield⁵, was reacted with L.T.A. in benzene at 80°. Workup after 20 hours gave a crystalline product in excellent yield (ca. 100%). The n.m.r. spectrum of this material indicated that it was a 4:1 mixture of the 3,6-diacetoxy bis-imidate (88) and the known³⁶ diethoxypyrazine (89). One recrystallisation from n-hexane afforded



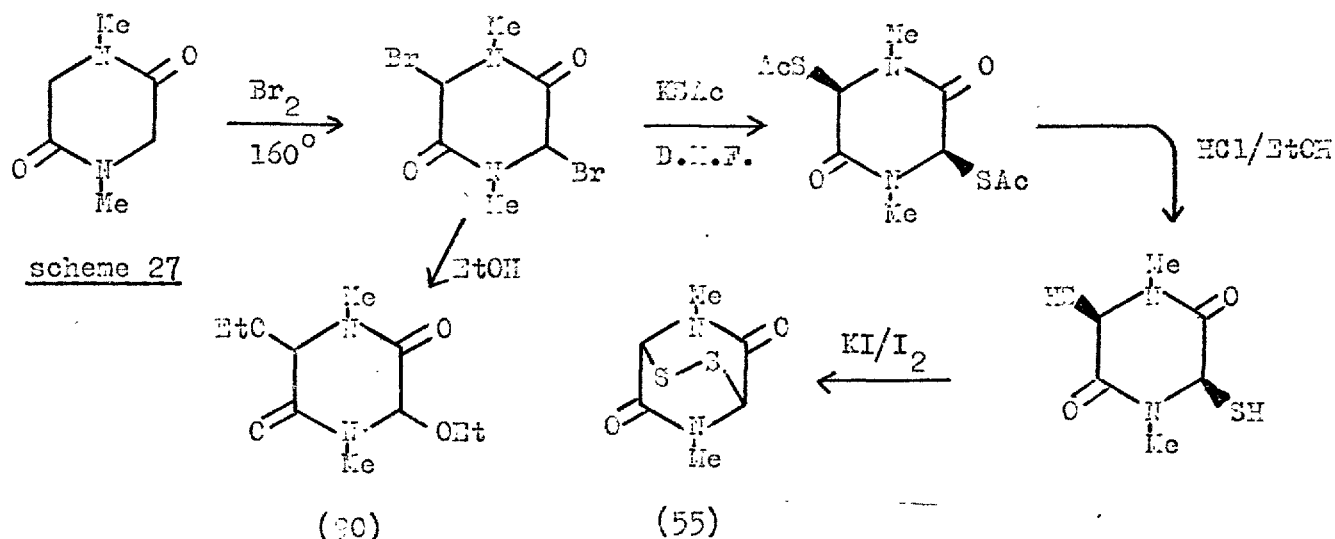
colourless needles of pure bis-imidate, which appeared (tlc, n.m.r.) to be a single compound, although the starting material was a cis-trans mixture. The mechanism in scheme 25 can be easily modified for the bis-imidate oxidation (scheme 26).



scheme 26

(iii) Reactions at the sulphur bridge. Although most of the reactions used in the degradative studies on the natural products have involved destruction of the disulphide bridge, operations can be carried out on this function without its inevitable loss.^{37,38} It was considered that the expertise gained in the preparation and subsequent manipulation of a known epidithiodioxopiperazine might be invaluable to the development of a general route to these compounds.

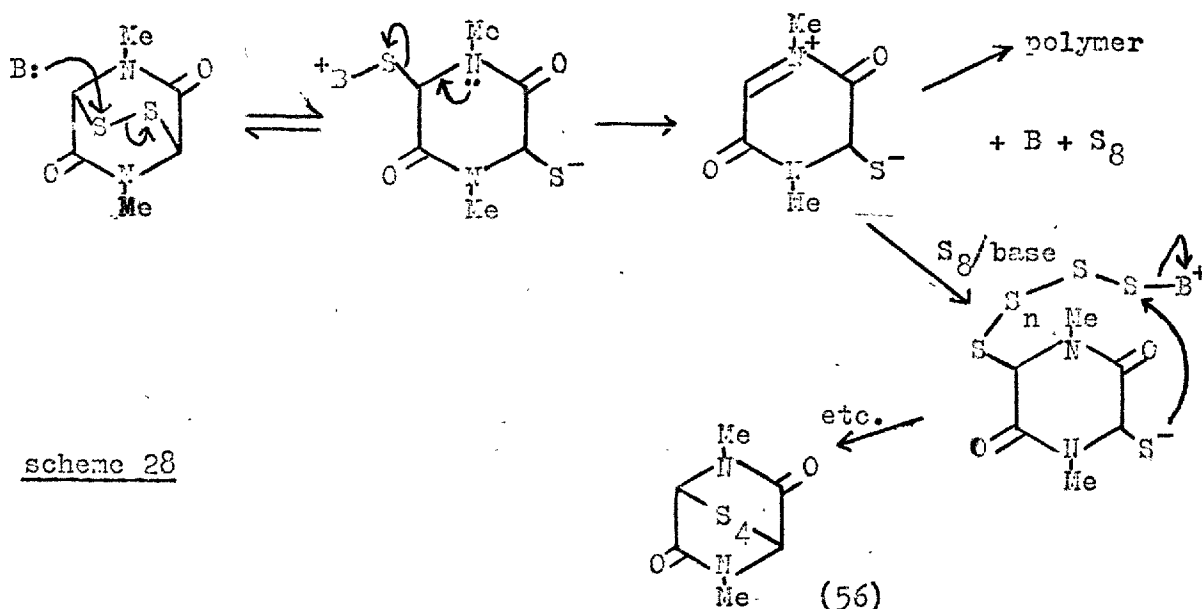
At this point in time the easiest disulphide to prepare was the sarcosine anhydride compound (55) and even then, the published route²⁰ required modification (scheme 27). Sarcosine anhydride, prepared by dimerisation of



sarcosine in refluxing ethylene glycol, was readily dibrominated at 160° in o-dichlorobenzene. However, treatment of this highly reactive dibromide with potassium thiolacetate in ethanol, as reported, gave only 20% of the dithiolacetate. The major product was the diethoxy derivative (90) of unknown configuration, a result of solvolysis. Changing the solvent to D.M.F. eliminated the latter problem but, initially, cis-trans mixtures of the dithiolacetates were obtained. Since only the cis compound can be readily converted into the epidisulphide this reduced the possible yield considerably. However, careful workup of the reaction, avoiding temperatures above 40°, enabled a product to be obtained in 81% yield which was 9:1 in favour of the cis-dithiolacetate. Subsequent hydrolysis with N-ethanolic hydrogen chloride was straightforward and the resultant cis-dithiol was oxidised in 96% yield to the disulphide with iodine in aqueous potassium iodide solution. Conditions of high dilution were required for the latter reaction otherwise considerable amounts of polysulphide were produced.

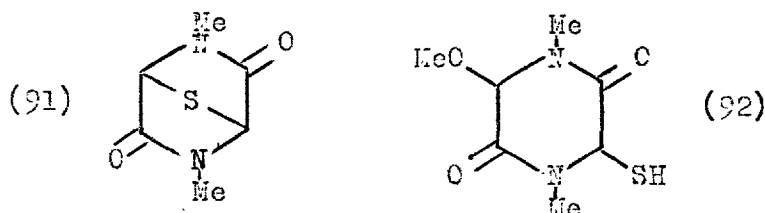
The disulphide, obtained as yellow prisms from acetonitrile, has low solubility in common organic solvents. The compound runs on tlc as a colourless spot which rapidly becomes yellow, on standing, presumably due to loss of

sulphur. Treatment with triethylamine gave a reddish-purple solution of a complex mixture (tlc); but from which a white solid slowly precipitated. This was identified as the known²² tetrasulphide (56) and was also produced, in 80% yield, by reaction of the disulphide with sulphur in pyridine³⁷. With pyridine alone partial polymerisation occurred. These reactions can be explained by initial rupture of the S-S link (scheme 28). The ready formation of the

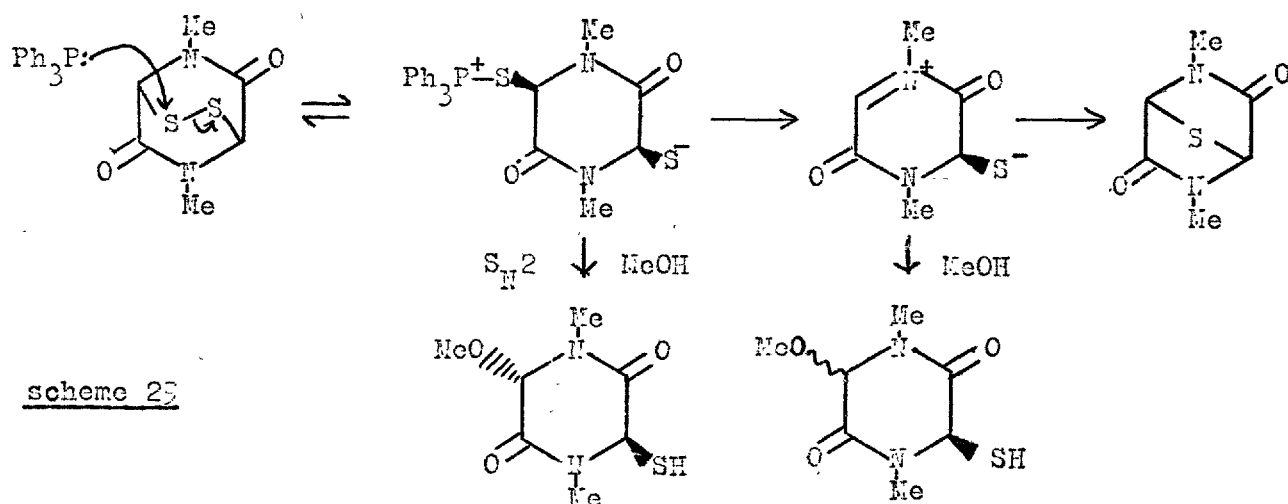


tetrasulphide is a consequence of its stability since it is the simplest epipolythio compound capable of achieving the normal -S-S- dihedral angle of ca. 100°.

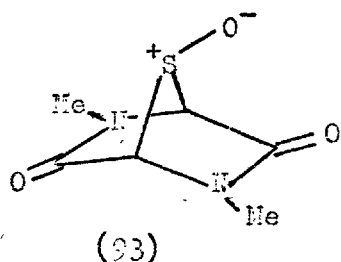
The conversion of dehydrogliotoxin to its monosulphide with thiophiles such as triphenylphosphine has been reported³⁹ and, similarly, the disulphide (55) readily afforded the colourless, crystalline monosulphide (91) with triphenylphosphine or trimethylphosphite in 89% and 98% yields, respectively. This compound, although destroyed by strong base, is remarkably stable to acid.



For example, treatment with methanol in dichloromethane containing boron trifluoride etherate failed to give the methoxy thiol (92) and the monosulphide was recovered unchanged. The methoxy thiols (both isomers) were produced by the reaction of the disulphide with triphenylphosphine in methanol. Presumably, elimination of the triphenylphosphine sulphide occurs before methanol attack and hence both isomers can be formed from the acyliminium ion, as was indeed observed; the alternative S_N2 attack would only give rise to the trans-isomer (scheme 29). The two isomers were unstable to plc but a successful separation



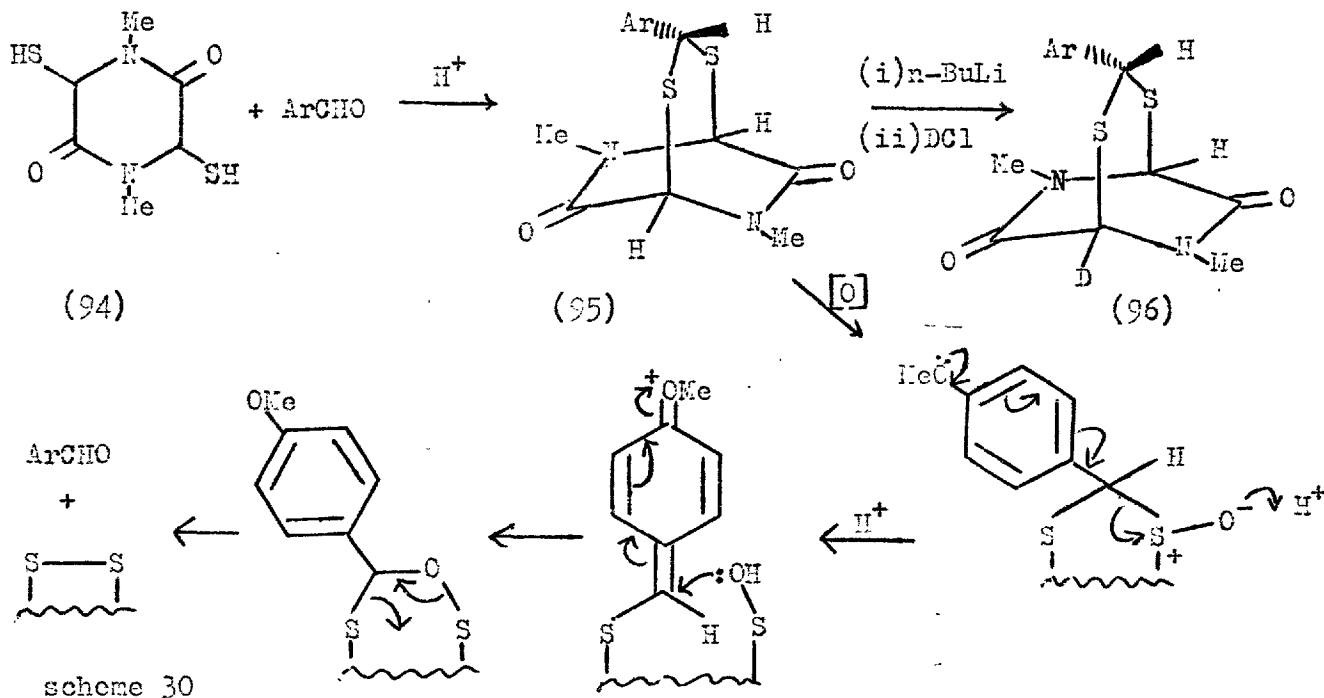
was effected by fractional crystallisation and the compounds were fully characterised by their spectral and analytical data. The monosulphide was converted into the sulphoxide (93) by reaction with one equivalent of *m*-chloro-perbenzoic acid at 0°, and the asymmetry of the molecule was reflected by the different shifts of the N-methyl resonances in the n.m.r. spectrum. Two



equivalents of oxidant produced a complex mixture and did not yield the expected sulphone. Reaction of the disulphide with peracid also produced a complex mixture from which only the tetrasulphide (56) could be isolated.

The recently reported⁴⁰ method for protecting the disulphide bridge deserves mention here. Treatment of the dithiol (94) with anisaldehyde and boron trifluoride etherate gives the dithioacetal (95) which is stable under acidic, basic or reductive conditions. With one equivalent of *n*-butyl lithium

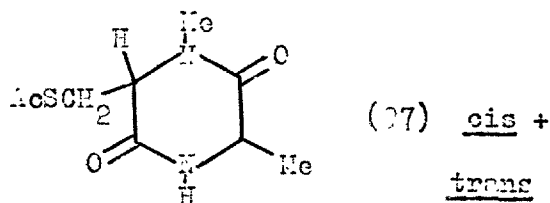
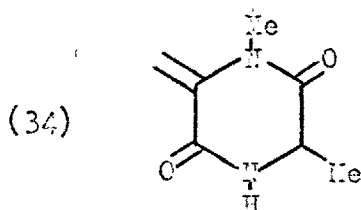
a monocarbanion on the dipeptide ring is formed. However, workup with deuterium chloride gives starting material with only one position specifically deuterated (96). Thus, the chiral nature of the acetal carbon endows selectivity on the anion formation, enabling selective alkylations to be carried out in a stepwise fashion. The disulphide bridge can be reformed by oxidation with *m*-chloroperbenzoic acid followed by acid treatment (scheme 30). The use of this



versatile protecting group has been exemplified in the total syntheses of dehydrogliotoxin⁴¹ and sporidesmins A⁴² and B⁴³.

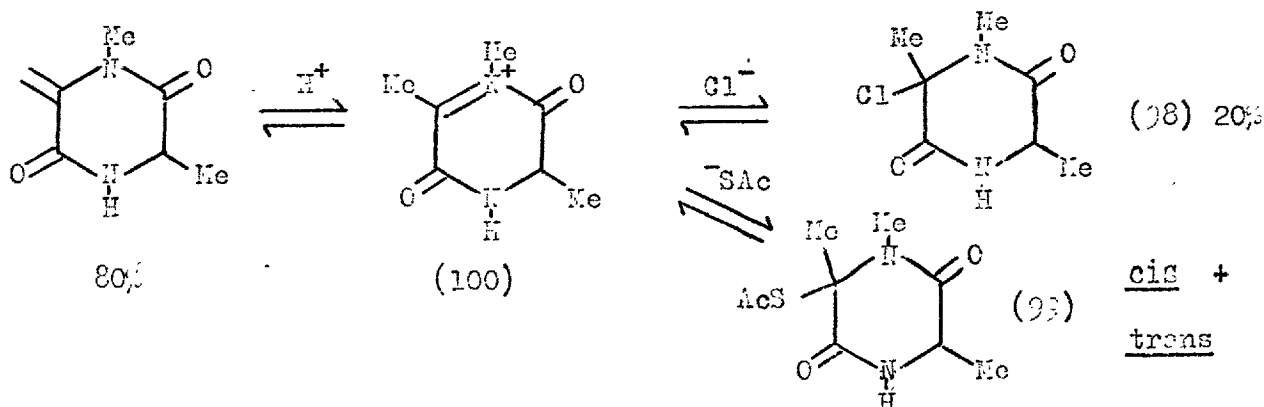
(iv) Reactions with dehydro-dioxopiperazines. As already described feeding experiments have indicated that dehydro derivatives are not biosynthetic precursors to gliotoxin, aranotin, etc., but one of the easiest ways to generate the proposed acyliminium intermediates is by treating such dehydro compounds with acid. This method has many precedents, both in amide chemistry and simple peptide chemistry (see Part 1), but has never been applied to dehydro-dioxopiperazines. Consequently, as a number of dehydro compounds were available to us, we decided to use these derivatives to find a mild and general method for the introduction of sulphur.

The reaction of the methylene dioxopiperazine (34) with thiolacetic acid was carried out in deuteriochloroform and monitored by n.m.r. spectroscopy.



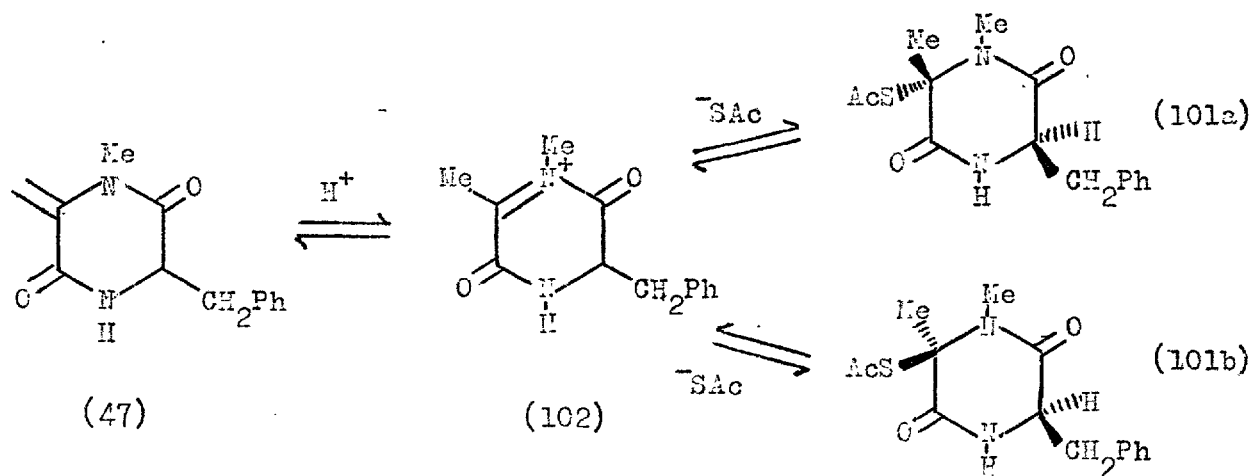
After 4 days at 25° disappearance of the vinylic resonances was complete and the fact that β -addition had occurred was readily ascertained from the new resonances. Besides new N-methyl and acetyl singlets, a multiplet at τ 6.4 ($-\text{S}-\text{CH}_2-\text{CH}$) had appeared and no C-methyl singlets were in evidence. Both possible isomers were formed, as inferred from the two new C-methyl doublets at τ 8.39 and 8.51, due to the methyl substituent in the 3-position, and this was confirmed by two new spots on tlc. The yield of the isomers was quantitative (ca. 1:1) and their separation was effected by plc. Configurational assignments could not be made on the basis of the spectral data but as before it was assumed that the more polar compound, a crystalline solid, was the cis-isomer. The less polar thiolacetate remained an oil despite extensive attempts at crystallisation. β -Addition of this sort is to be expected since thiolacetic acid is not a strong enough acid to promote the acyliminium equilibrium and the β -adduct is the thermodynamically-controlled product (see p.32).

Treatment of (34) with hydrogen chloride in deuteriochloroform effected, after 30 minutes, a 20% conversion to a new compound. The appearance of a new C-methyl doublet at τ 8.39, a C-methyl singlet at τ 7.87 and an N-methyl singlet at τ 6.90 was consistent with the formation of the α -chloro compound (98). After 24 hours and further passage of hydrogen chloride the quantity



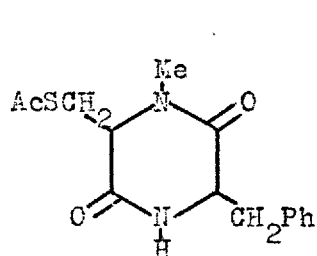
of (98) had still not increased above 20%, i.e. equilibrium had been established. However, on addition of thiolacetic acid to the mixture a rapid reaction occurred and within one hour both the methylene compound and the chloro compound had disappeared. The new resonances were consistent with a mixture of the isomeric α -thiolacetates (99) as sole products. Furthermore, resonances due to β -addition were completely absent. The yield of the thiolacetates was apparently quantitative. Separation of the isomers by plc afforded both compounds as crystalline solids but the isolated yields were considerably lower than expected. The explanation for the low yields, 49% combined, most probably lies in decomposition upon chromatography. Reaction of the methylene dioxopiperazine with thiolacetic acid in the presence of a catalytic amount of hydrogen chloride afforded the same α -thiolacetates confirming that prior formation of the α -chloro compound was not essential⁴⁴. As long as the acyliminium intermediate (100) is produced, α -addition of the nucleophile will occur and the nature of the acid catalyst is not important. Thus, in the recent total syntheses of sporidesmin A⁴² and B⁴³ the addition of hydrogen sulphide to a dehydro-dioxopiperazine was catalysed with boron trifluoride etherate.

An identical reaction occurred with the methylene benzyl dioxopiperazine (47) and thiolacetic acid in the presence of hydrogen chloride. Monitoring the reaction by n.m.r. showed that all the starting material had been consumed

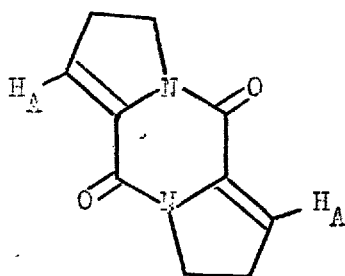


after one hour at 25° giving a 1:1 mixture of the α -thiolacetates (101). The

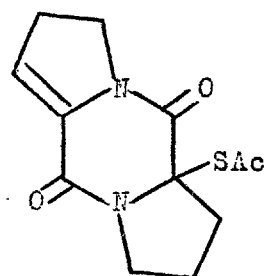
two isomers were readily distinguished by their n.m.r. spectra as before (p. 90) due to the shielding effect of the phenyl ring on the 6-methyl substituent. However, the reaction was not worked up immediately as it was noticed that the ratio of the isomers was slowly changing. After 20 hours, equilibrium had been reached with a 3:1 ratio in favour of the cis-isomer (101a). This observation again emphasises the reversibility of these addition reactions involving acyliminium intermediates e.g. (100) and (102). Workup, by plc, afforded the more polar cis-isomer as colourless needles (49%) and the less polar trans-isomer as prisms (23%). If the reaction mixture was left for several days a complex mixture was formed due to competing β -addition; in fact, a small quantity of material (8 mg.), isolated by plc from such a mixture, showed n.m.r. resonances consistent with the β -thiolacetate (103). However, this compound was not characterised any further.



(103)



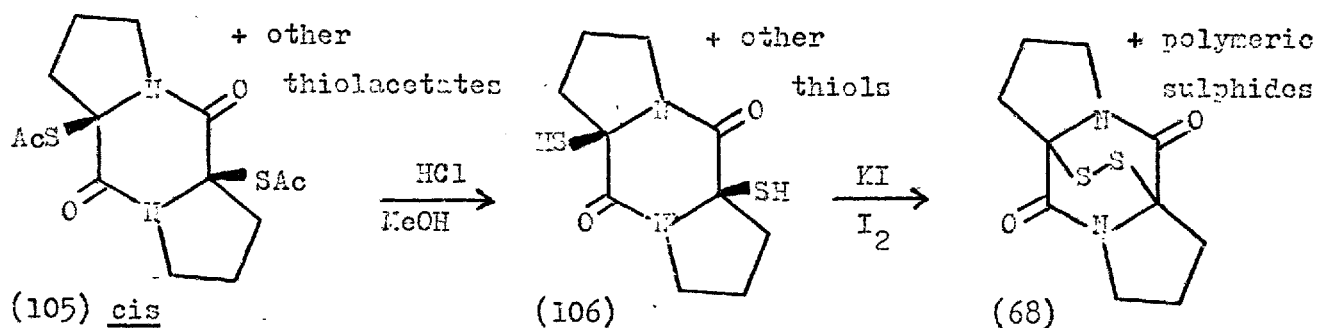
(77)



(104)

To test the generality of these α -addition reactions it was of interest to use bis-dehydro proline anhydride (77) since previous attempts at the α -addition of nucleophiles to this compound had failed⁴⁵. The bis-dehydro compound was reacted with thiolacetic acid in deuteriochloroform containing hydrogen chloride. After 4 hours at 25°, n.m.r. showed that most of the starting material vinylic triplet (H_A) had disappeared and a new vinylic triplet was present. Evaporation of the solution gave a semi-crystalline oil. However, plc of the crude product resulted in extensive decomposition and no single compounds were isolated. The reaction was repeated and after 4 hours some remaining starting material was filtered off. The filtrates were evaporated and the residue dissolved in hot ethyl acetate which on cooling deposited yellow crystals.

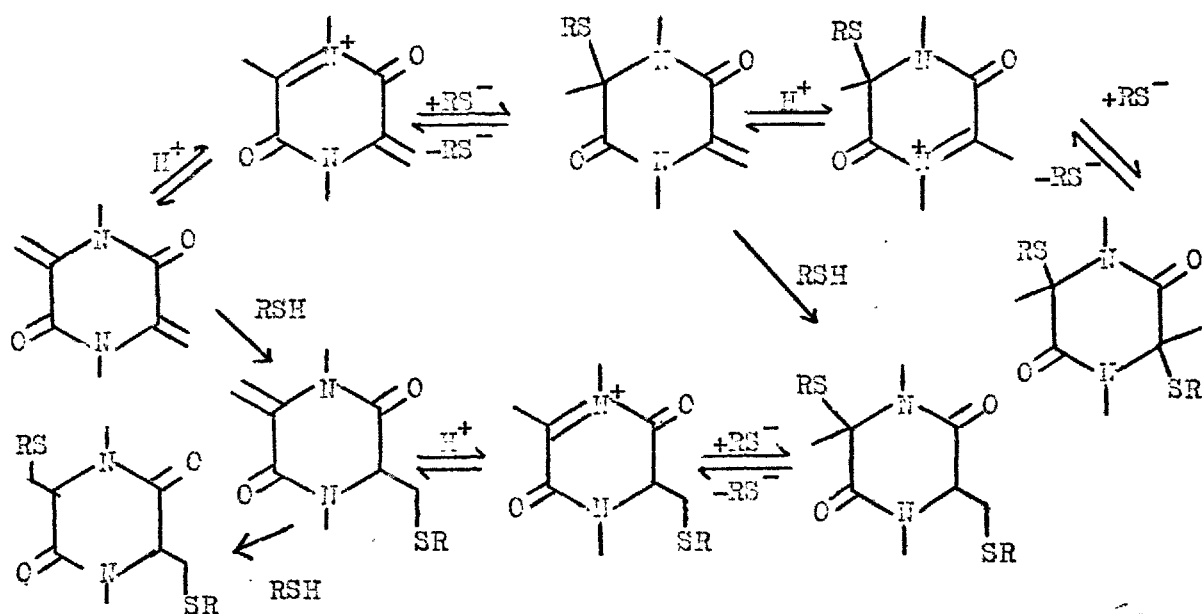
(15%). The n.m.r. spectrum of this material showed that it was basically the same as the crude product from the first reaction. All the spectral data was consistent with the monothiolacetate (104) and, after recrystallisation, the colourless prisms gave confirmatory analytical figures. If the reaction time was increased beyond 4 hours the yield of (104) began to diminish and a very complex mixture was formed. It was considered that with prolonged reaction times, β -addition was competing successfully and that the quantity of bis- α -thiolacetates (105) formed was too small to enable separation from the large number of β -adducts. An attempt was made to hydrolyse the crude mixture after 4 days reaction with a view to oxidising the subsequent thiols. The cis- α -dithiol (106) would give the monomeric cyclic disulphide (68) where as all the other thiols would give polymeric disulphides facilitating separation considerably (scheme 31). The hydrolysis, in refluxing methanolic



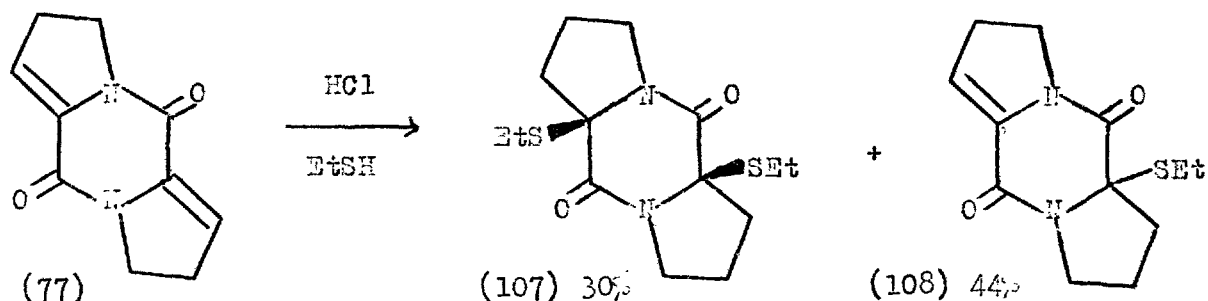
scheme 31

hydrogen chloride, was easily accomplished as was the oxidation with iodine/potassium iodide, but none of the disulphide (68) could be isolated.

Consideration of the problem led to the conclusion that a good nucleophile with poor leaving group properties was required. Rapid formation of the bis- α -adducts would be promoted by a good nucleophile, where as the back reaction through the intermediate acyliminium cations to the dehydro compounds and thence β -addition, would be inhibited by a poor leaving group (scheme 32). The nucleophile chosen was ethanethiol. Tlc of the reaction mixture after 20 hours at 25° showed two new spots, besides a small amount of the bis-dehydro starting material, and the products were separated by plc. The less



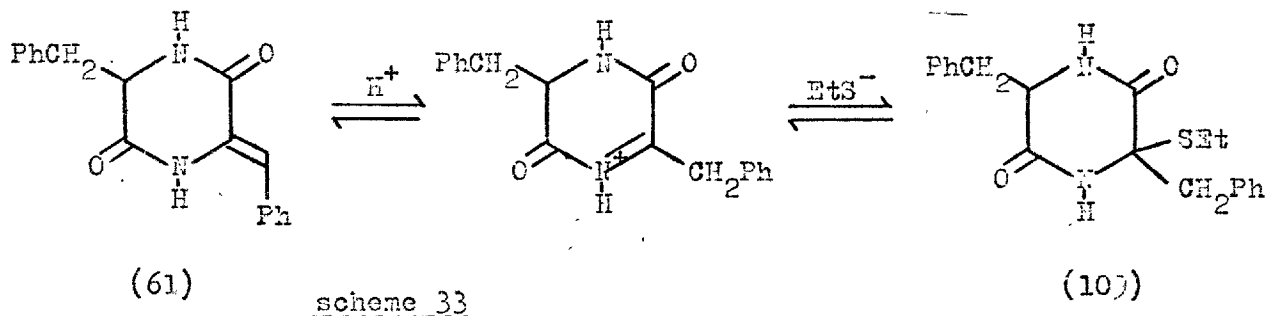
polar band gave a crystalline solid readily identified as the known²⁸ cis-di(ethylthio) adduct (107) (30%) by comparison of its n.m.r. spectrum with the published data. Recrystallisation from n-hexane afforded clusters of prisms m.p. 98-100° (lit.³⁵ m.p. 98-103°). The more polar band gave a non-crystalline foam whose n.m.r. spectrum showed a vinylic triplet as well as a thioethyl group. Its i.r. spectrum was very similar to that of the mono-thiolacetate (104), apart from the acetyl carbonyl stretch at 1690 cm⁻¹ in the latter's spectrum, and consequently the new compound was assigned the mono-ethylthio structure (108). The mass spectrum showed a weak parent ion at



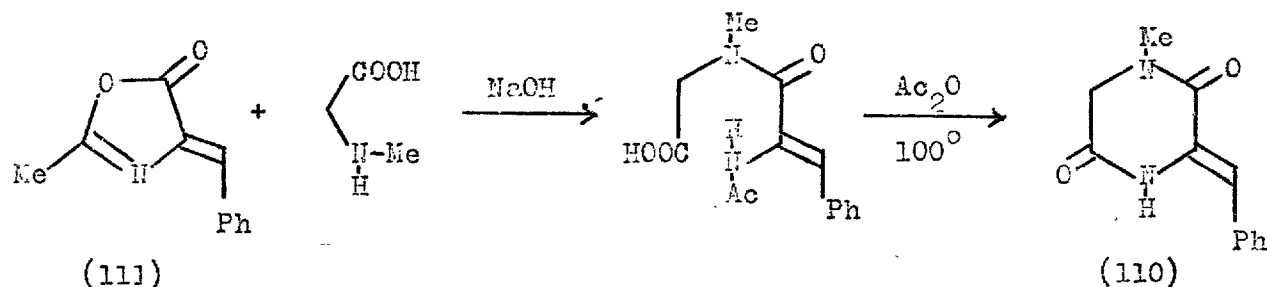
m/e 252 (2%) as expected. All attempts to purify this material further led to its partial decomposition and hence a satisfactory analysis could not be obtained. No sign of the trans-di(ethylthio) derivative could be found, which is in accord with the known²⁸ preference of proline anhydride derivatives for

the cis-configuration.

The generality of the method was further tested with a conjugated dehydro system. For example, the sparingly soluble mono-benzylidene dioxopiperazine (61) was stirred with ethanethiol in D.M.F. saturated with hydrogen chloride, for a week. Filtration of the heterogeneous mixture gave unreacted starting material in 80% yield. Evaporation of the filtrates afforded a yellow oil which, when dissolved in chloroform, rapidly precipitated out the insoluble benzylidene compound with concomitant liberation of ethanethiol. Presumably, the insolubility of (61) in non-polar solvents forces the equilibria (scheme 33) completely over to the left resulting in total

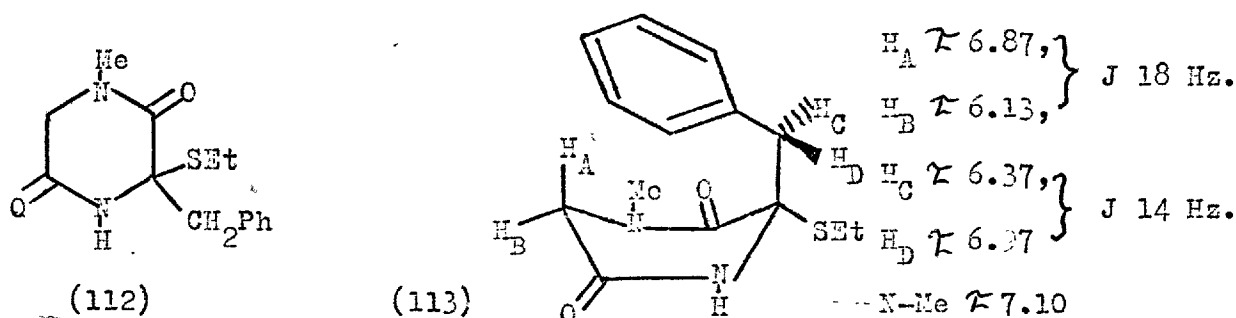


decomposition of the ethylthio adduct (109). To avoid these solubility effects (unsubstituted dioxopiperazines are notoriously insoluble in non-polar solvents) 1-methyl-3-benzylidene dioxopiperazine (110) was used. The compound was prepared from the azlactone (111) by the published route⁴⁶.



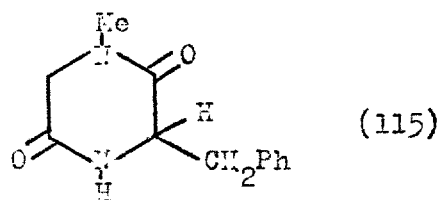
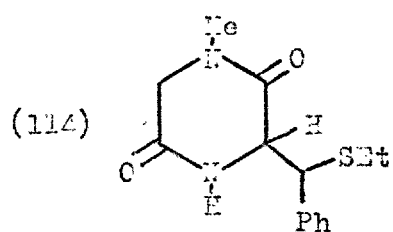
This dioxopiperazine is freely soluble in non-polar solvents and undergoes oxidation reactions typical of enamides (see part C). The compound was treated with ethanethiol in deuteriochloroform saturated with hydrogen chloride. Following the reaction by n.m.r. initially showed little change but, after a

week, a new compound was observed (ca.15%). The solution was left at 25° for a further three weeks by which time the mixture had reached equilibrium at 35% conversion. The new resonances were consistent with the α -ethylthio compound (112). The two protons in the 6-position resonated as an ABq (J 18 Hz) as did the two benzylic protons (J 14 Hz), a consequence of the folded conformation (113) encountered earlier in other benzyl substituted compounds.



β -Addition would lead to resonances between τ 5 and 6 due to the ring proton in position 3 and the remaining benzylic methine, but no such evidence excluded the formation of the β -adduct (114). The mixture was evaporated and subjected to plc. The major band afforded starting material (50%) whilst the ethylthio compound ran slightly higher and was isolated in 25% yield. The compound was a colourless crystalline solid and was fully characterised after recrystallisation. With much weaker nucleophiles e.g. methanol, no addition to (110) was observed, using either T.F.A. or boron trifluoride as catalyst, over a short period of time (1-2 weeks). Thus, the conjugated nature of the dehydro double bond in (110) does inhibit its protonation to the acyliminium cation but with a strong nucleophile and adequate reaction times, addition will occur to an appreciable extent. Where the electrophilic species is more electron deficient than a proton, reaction becomes far more favourable as witnessed by the rapid, high-yield oxidations of (110) (see p.129).

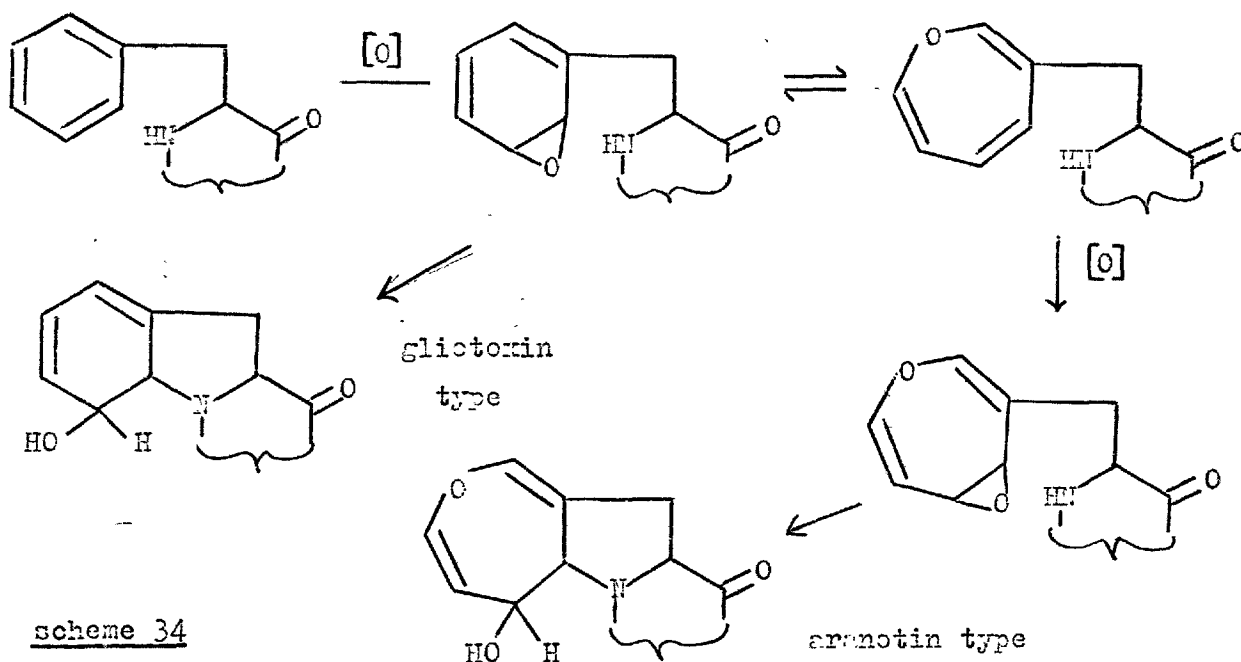
The benzylidene bond in (110) appears to be inert to β -addition since the expected β -ethylthio adduct (114) was not formed with sodium ethanethiolate in D.M.F. Oddly enough, if the sodium thiolate was prepared from ethanethiol and sodium hydride in the presence of the dioxopiperazine, an



efficient reduction of the compound was observed. Workup afforded the known⁴⁷ benzyl dioxopiperazine (115) in 61% yield. Diethyl disulphide was not produced in this reaction and it must be concluded that the reduction was effected by the hydrogen from the thiol-hydride mixture.

C. Gliotoxin; the oxygen problem.

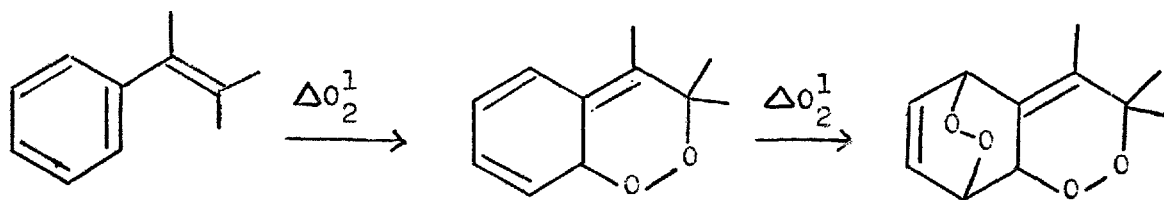
Arene oxides have been postulated as intermediates in the oxidative metabolism of aromatic substrates⁴⁸. In vivo these epoxides undergo⁴⁹ the 'N.I.H.' shift⁵⁰ in rearranging into phenols and, in one instance, an arene oxide has in fact been isolated from a biological system⁵¹. Molloy et al.⁵² proposed that phenylalanine epoxide is involved in the biosynthesis of the cyclohexadienol and dihydrooxepin rings found in gliotoxin and aranotin, respectively, (see p. 47) and feeding experiments^{18,53} support the proposal (scheme 34). To mimic such an oxidation in vitro is very difficult and is



attracting the attention of many chemists at the present time. The following chapter describes our attempts at this process. Although we have so far failed in our objective, the chemistry has proved to be of considerable intrinsic interest.

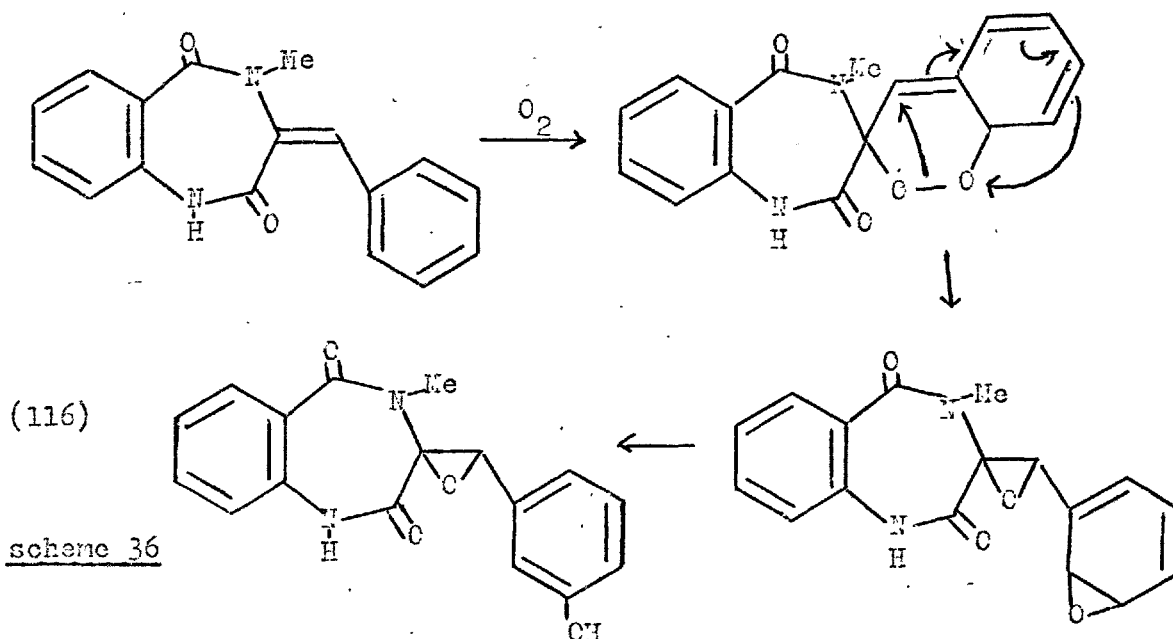
(i) Reactions with singlet oxygen. The common methods for oxidation of aromatic compounds include the use of peracids⁵⁴ and metal salt oxidants⁵⁵, e.g. Pd(OAc)₂, but a large excess of substrate is required to prevent over-oxidation. A reagent for which a large excess of substrate is not necessary

and where overoxidation can be controlled can be found in singlet oxygen. This excited state molecule has been shown to react with styrene derivatives in a 1,4-cycloadditive manner⁵⁶ involving the aromatic ring (scheme 35).



scheme 35

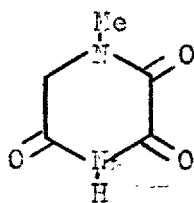
Such a reaction may mimic the behaviour of the dioxygenases. For example, a similar process may be of importance in the biosynthetic incorporation⁵⁷ of molecular oxygen into the fungal metabolite cyclopenol (116), which is formed from phenylalanine and not m-tyrosine or L-Dopa⁵⁸ (scheme 36).



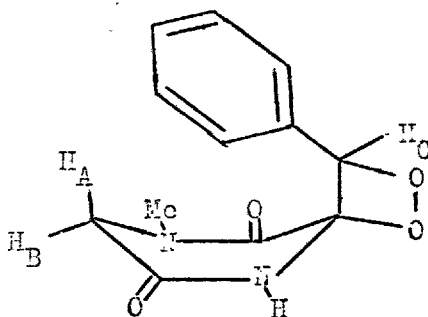
scheme 36

Oxidation of the benzylidene dioxopiperazine (110) with singlet oxygen occurred smoothly at 25°. The reaction was carried out in dichloromethane containing methylene blue (1-2 mg.) as sensitiser. The blue solution was irradiated with a 350 Watt tungsten filament bulb whilst a stream of dry oxygen was passed through the solution. External cooling of the flask with water maintained the temperature at ca.25°. All subsequent singlet oxygen reactions were performed in like manner. Thus, after 60 hours reaction, tlc

showed none of the benzylidene derivative, a non-polar, volatile spot and a polar streak near the origin which gave a weak blue colouration on spraying with the starch-iodide reagent. The reaction mixture itself smelt strongly of benzaldehyde and a crystalline solid had been deposited round the sides of the flask. The colourless crystals were filtered off and their i.r. spectrum recorded. This was dominated by two strong carbonyl absorptions at 1725 and 1690 cm^{-1} and N-H stretches were also present. Insolubility in deuteriochloroform precluded an n.m.r. in this solvent, but in T.F.A. the spectrum showed an N-methyl singlet, a $-\text{CH}_2-$ singlet and a broad N-H singlet; aromatic resonances were completely absent and it was obvious that the molecule no longer possessed the benzyl substituent. The compound proved to be the trioxopiperazine (117), obtained in 76% yield. After evaporation of the reaction mother liquors, the resultant oil was triturated several times with petrol to remove the benzaldehyde (identified by its spectral data). The



(117)



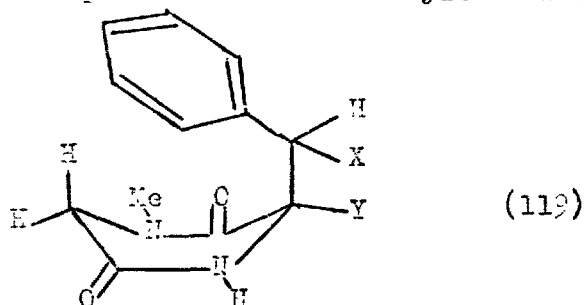
(118)

$\begin{aligned} &H_A \tau 7.48, \\ &H_B \tau 6.44, \end{aligned} \left. \vphantom{\begin{aligned} &H_A \tau 7.48, \\ &H_B \tau 6.44, \end{aligned}} \right\} \text{ABq, } J \text{ 19 Hz.}$

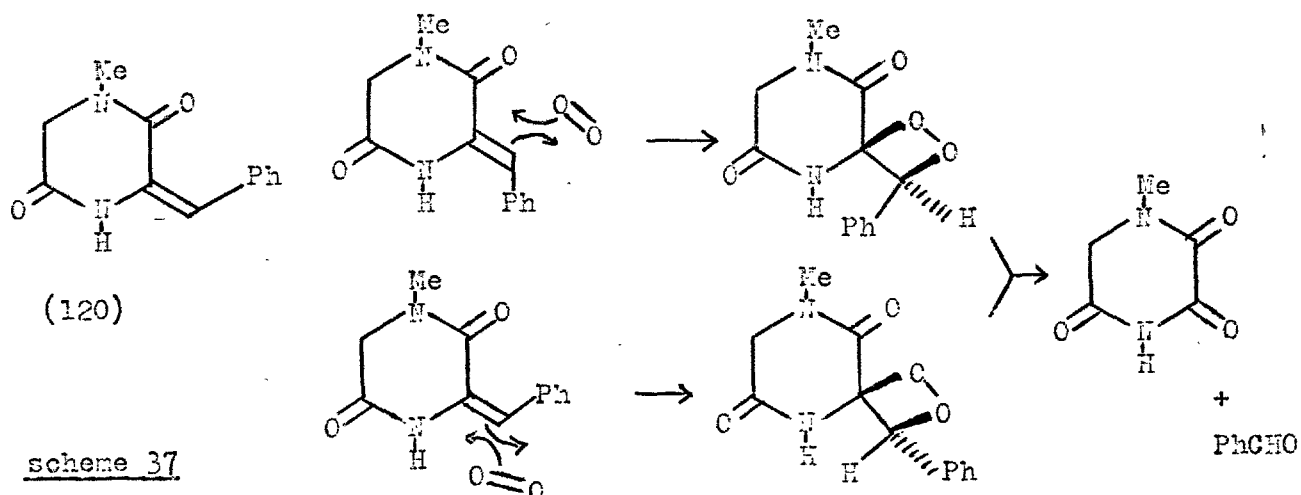
$H_C \tau 4.80, \text{ s.}$
 N-Me $\tau 7.30, \text{ s.}$
 Ph $\tau 2.60, \text{ s.}$
 N-H $\tau 3.00, \text{ bs.}$

residue, which gave a positive starch-iodide test, was examined by n.m.r. spectroscopy. The observed resonances were somewhat broadened due to the ensuing decomposition of the substance which was indicated by the appearance of benzaldehyde resonances and formation of a precipitate of (117) in the deuteriochloroform solution. Nevertheless, resonances consistent with the intermediate dioxetane (118) represented the major peaks in the spectrum. As expected, the two protons in the 6-position resonated as an AB quartet (J 19 Hz), due to the preferred folded conformation cf.(113). The unusually high-field position of the N-methyl resonance at τ 7.30 may also be associated

with the near-by aromatic ring and was later found to be characteristic of the α, β -difunctionalised structural type (119). On standing for several days at 25° decomposition was complete and the total yield of the trioxopiperazine was 100%.

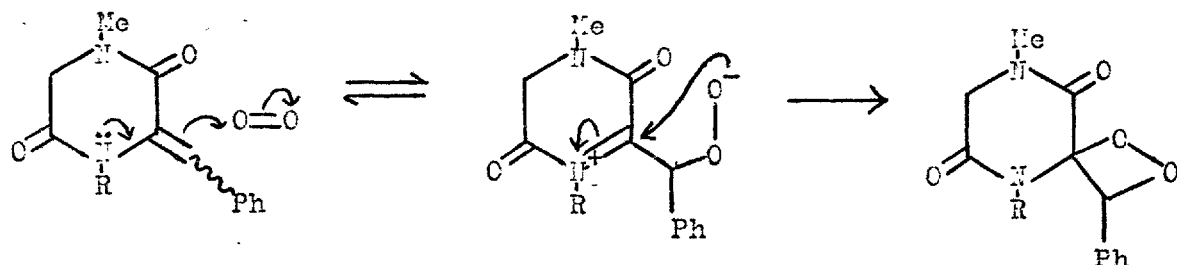


The reactions of olefins with singlet oxygen via a 1,2-cycloaddition are well documented⁵⁹ and the subsequent chemiluminescent cleavage of the dioxetane product may have importance with respect to chemiluminescence in nature⁶⁰. The addition is stereospecific⁵⁹ and therefore it was of interest to react the E-benzylidene dioxopiperazine with singlet oxygen to test if an isomeric dioxetane was produced (scheme 37). The E-isomer was prepared as



follows. Photolysis of the Z-isomer in methanol at 25° under nitrogen in a quartz vessel with a 125 Watt medium pressure mercury lamp gave a photo-equilibrium mixture of the two isomers⁴⁶. Separation by plc afforded the E-isomer as the more polar compound, far less soluble in the common solvents than its Z-isomer. At the same concentration, the E-isomer reacted with singlet oxygen at least twice as fast as the Z-isomer, the reaction being complete within 24 hours. The trioxopiperazine (117) was filtered off, in 74%

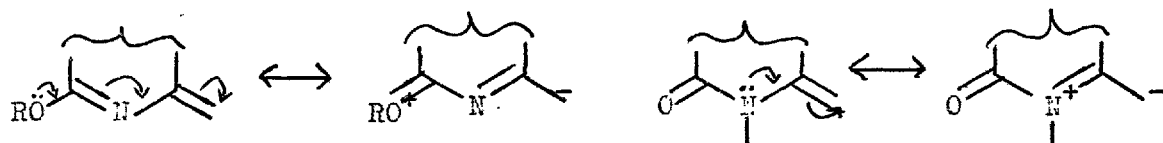
yield, and the filtrates treated as before to remove the benzaldehyde. The n.m.r. spectrum of the intermediate dioxetane was identical to that of (118) leading to the conclusion that the reaction is probably not stereospecific. The susceptibility of enamides to electrophilic attack (see Part 1) prompted us to consider alternative mechanisms. The most likely possibility, involving an acyliminium intermediate, is shown in scheme 38. The peroxy-acyliminium



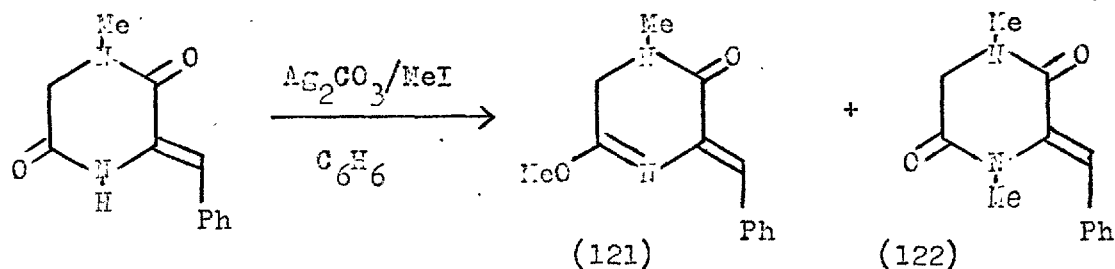
scheme 38

R = Me, H

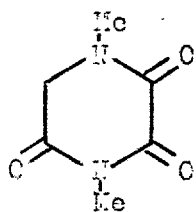
species would be expected to undergo rapid internal addition affording the observed dioxetane. Conversion of the amide into an imide function would prevent the enamide reaction and hence might be expected to alter the course of the oxygenation reaction (donation by the lone pair on the imide oxygen



would involve a greater charge separation compared to the amide system). Consequently, the methoxy imide (121) was prepared by alkylation of the dioxopiperazine with methyl iodide and freshly prepared silver carbonate in benzene⁶¹. The two products of the reaction were readily separated by column chromatography and the less polar imide isolated in 63% yield. The other



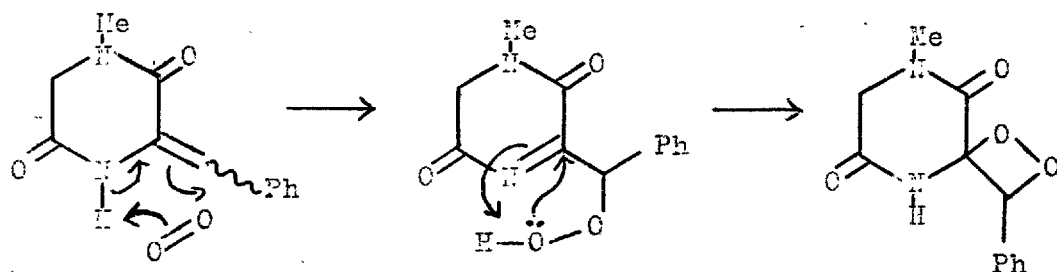
product (37%) was the N,N'-dimethyl derivative (122). Both compounds were highly crystalline and exhibited the expected spectral and analytical data. The imide proved to be completely inert to singlet oxygen and the starting material was recovered unchanged even after seven days reaction. In contrast, the N,N'-dimethyl derivative (122) did react with singlet oxygen (scheme 38). Tlc of the crude product showed the presence of benzaldehyde and this was confirmed by n.m.r., which also showed resonances compatible with the trioxo



(123)

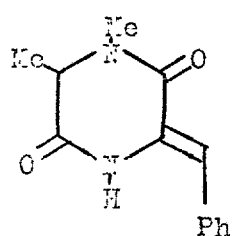
compound (123). Other products were also present and an attempt to purify (123) by plc resulted in its decomposition. The reaction was much slower than that with the unsubstituted dioxopiperazine (110). This may be due either to the steric

influence of the extra methyl group or, that, in the latter case, the possible formation of an acylimine instead of the acyliminium ion is an important rate factor (scheme 39).

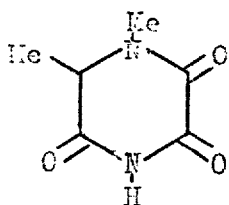


-scheme 39

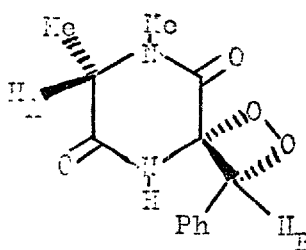
The generality of the oxidation was tested with the 1,6-dimethyl Z-benzylidene derivative (124). The reaction with singlet oxygen required 24 hours for completion and the n.m.r. spectrum of the crude product showed benzaldehyde and resonances consistent with the trioxopiperazine (125) and



(124)



(125)

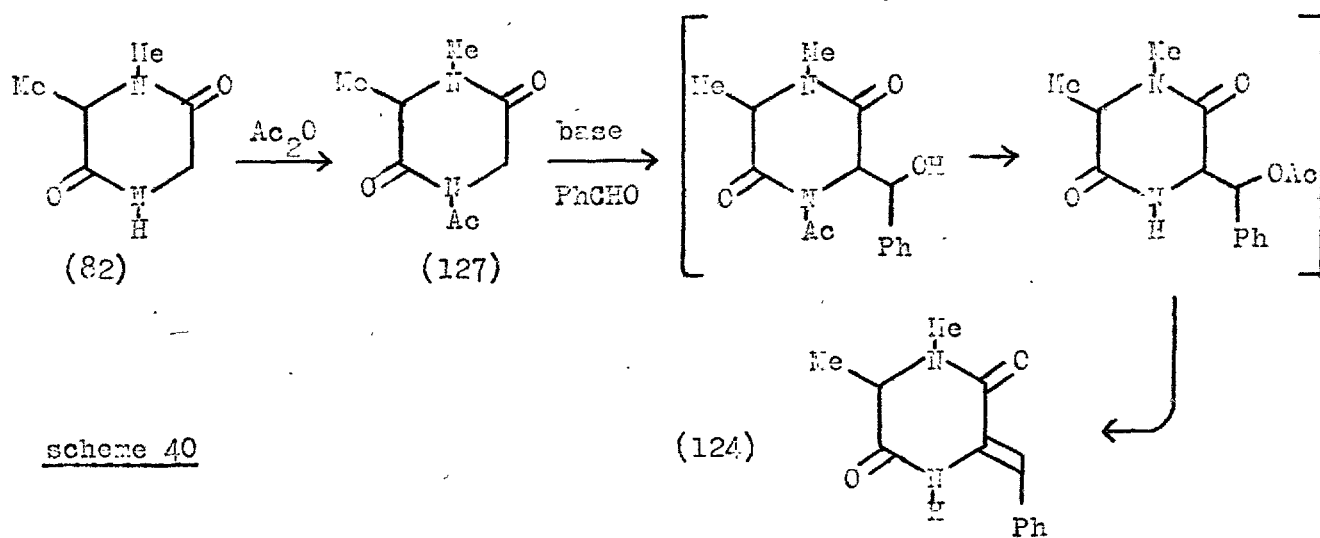


(126)

C-Me τ 8.52, d, J 7 Hz.
 H_A τ 6.83, q, J 7 Hz.
 H_B τ 4.82, s.
 N-Me τ 7.20, s.
 Ph τ 2.73, s.
 H-H τ 3.00, bs.

the dioxetane (126). Again the N-methyl resonance of the dioxetane appeared at higher field than normal, but the shift of the C-methyl group at τ 8.52 indicated that this methyl group was trans to the benzyl group. High field (τ 9.0-10.0) resonances consistent with the cis-isomer were absent. The benzylic methine resonated at ca. τ 4.8 as in the dioxetane (118). The trioxo compound was separated in 68% yield by plc and characterised by its spectral properties (M^+ ; m/e 156) since all attempts to crystallise the oil failed.

The benzylidene dioxopiperazine used in the last reaction was prepared by condensation of the glycine derived dioxopiperazine (82) with benzaldehyde, a reaction which proved to be more difficult than expected. The usual method²⁶ involves heating the dioxopiperazine with the aldehyde and sodium acetate in acetic anhydride at 130° (scheme 40). This reaction produced only the N-acetyl



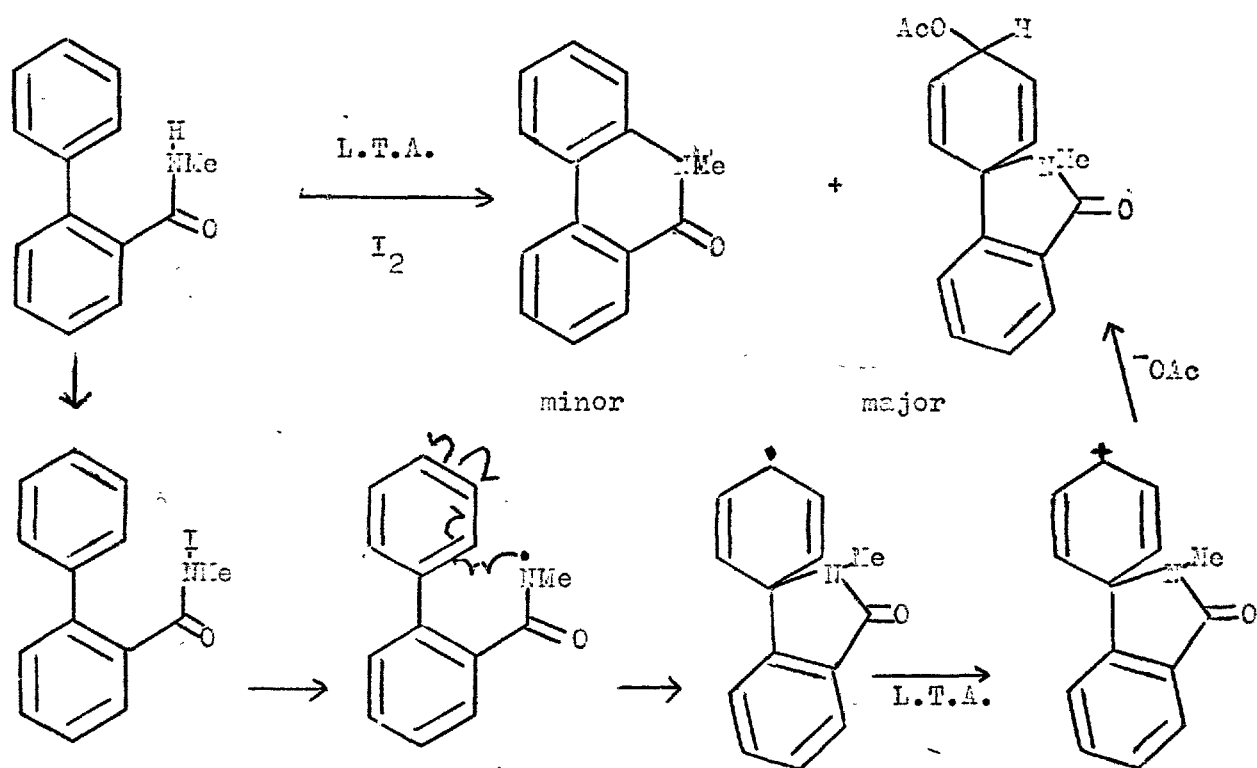
scheme 40

derivative (127). Treatment of (127) with benzaldehyde and triethylamine in D.M.F.⁶² both at 25° and 130° also failed to effect the condensation. A successful preparation was accomplished as follows. A solution of (127) in D.M.F. was cooled to 0° and 0.1 N potassium t-butoxide in t-butanol⁶² was added over 45 minutes. Aqueous workup, followed by column chromatography afforded the benzylidene compound (124) in 40% yield. Recrystallisation gave colourless needles of analytical purity.

No evidence whatsoever has been found in any of these singlet oxygen reactions for 1,4-additions, involving the aromatic ring, and this must mean

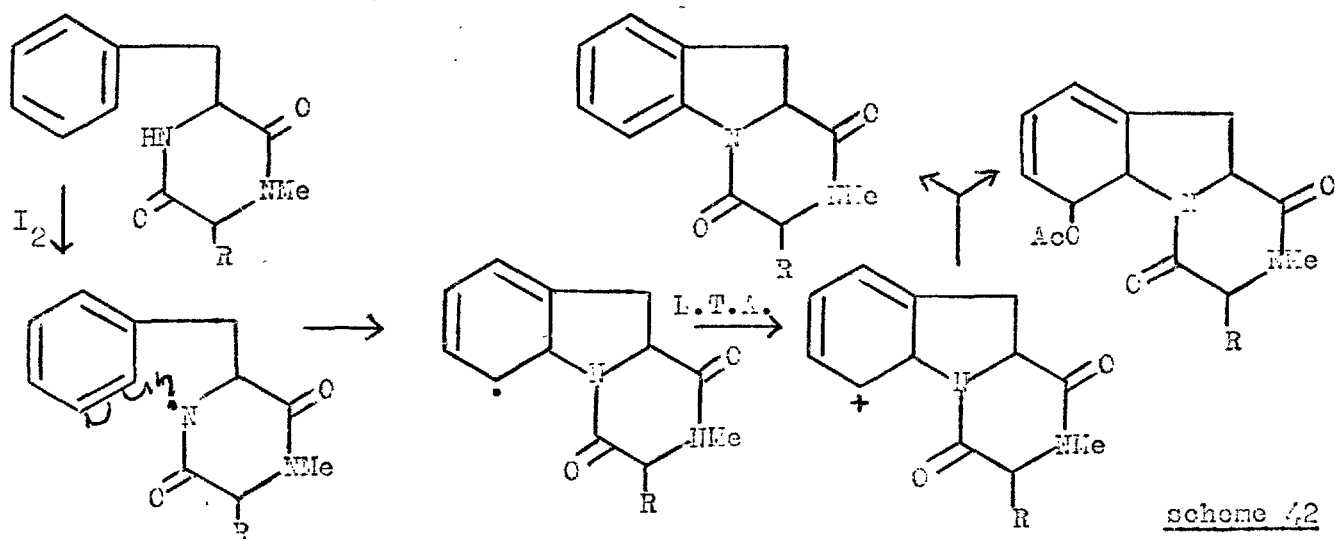
that the 'subtle electronic interactions' quoted by Foote⁵⁶ are absent in our compounds.

(ii) Reactions with lead tetracetate. A recent report⁶³ on the oxidation of an amide with L.T.A./iodine (scheme 41) prompted us to try a similar



scheme 41

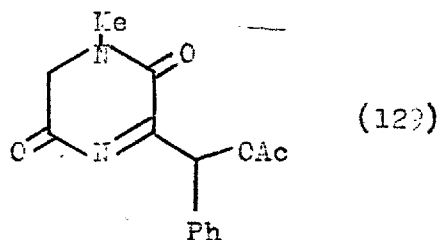
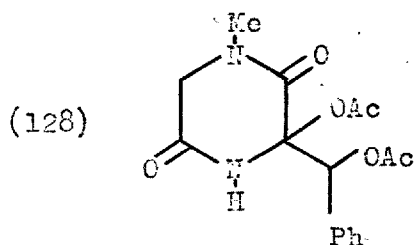
reaction with dioxopiperazine derivatives (scheme 42). Treatment of the benzyl



scheme 42

substituted compound (41) with L.T.A. and iodine in refluxing benzene failed to produce any reaction. However when the benzylidene dioxopiperazine (110)

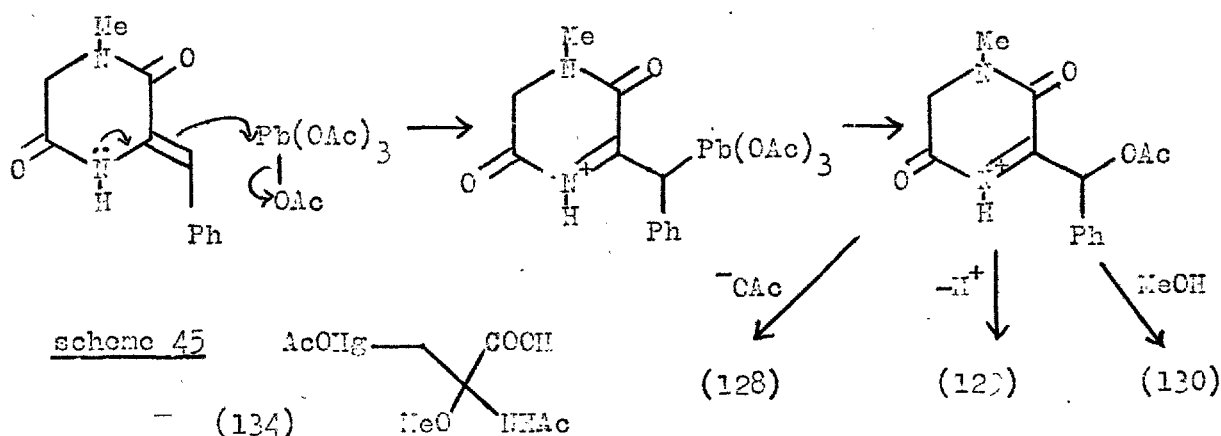
was reacted in like manner, both oxidant and substrate were consumed within one hour and a complex mixture was formed. At 25° a smooth oxidation occurred and workup afforded a foam which gave colourless prisms from ether. The n.m.r. spectrum showed the two protons in the 6-position as an AB quartet (J 19 Hz) and, together with the high field N-methyl resonance at τ 7.38, this indicated a structure of type (119). The presence of two acetate groups (τ 7.83 and 7.88) and the benzylic methine at τ 3.65 suggested the diacetate (128). The 5-proton singlet at τ 2.67 verified that the aromatic ring had remained untouched by the reaction. The crystals gave i.r. and analytical data expected for the diacetate (128). The mass spectrum of the compound did not show a



molecular ion at m/e 334; the highest peak appeared at m/e 274 which corresponds to the unusual acylimine (129). This latter observation is not really surprising considering that the pyrolysis of α -functionalised amides has been used for the preparation of acylimines (see Part 1). The diacetate was not stable to tlc giving a streak which was fluorescent when visualised with u.v. light. This streak was thought to be the acylimine (129) formed by silica (acid) catalysed elimination of acetic acid and an attempt to isolate the compound was made. A sample of the diacetate was run up a preparative plate with MeOH:CHCl₃ (1:19) and the fluorescent band removed. However, the crystalline solid obtained in this way was fully characterised as the methoxy acetate (130). Elution with non-protic solvents e.g. benzene-ethyl acetate, afforded another crystalline solid, characterised as the hydroxy acetate (131). Both compounds showed the AB quartets and high field N-methyl resonances associated with the α, β -difunctionalised structure (119). The compounds could also be prepared, in quantitative yields, by reaction of the diacetate

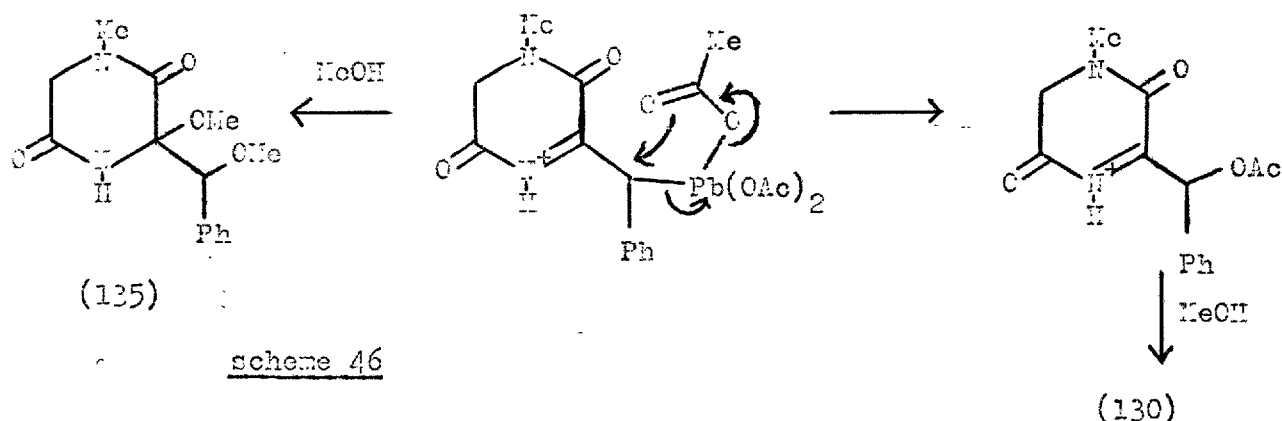
However, with one equivalent of L.T.A. alone, the compound was completely oxidised within one hour at 25°. Examination of the product by n.m.r. showed that the product consisted of 85% diacetate (128) and 15% of another compound whose resonances were compatible with the acylimine (123). Based on these products the isolated yield was ca.100%.

The mechanism of the oxidation was of great interest in view of the formation of α, β -diacetates in the previously described L.T.A. oxidations. The mechanism proposed earlier (p. 113, scheme 23) is an example of the general reaction of enamides towards electrophiles (p. 33) and would lead to the observed products (scheme 45). Other mechanisms have been considered, but



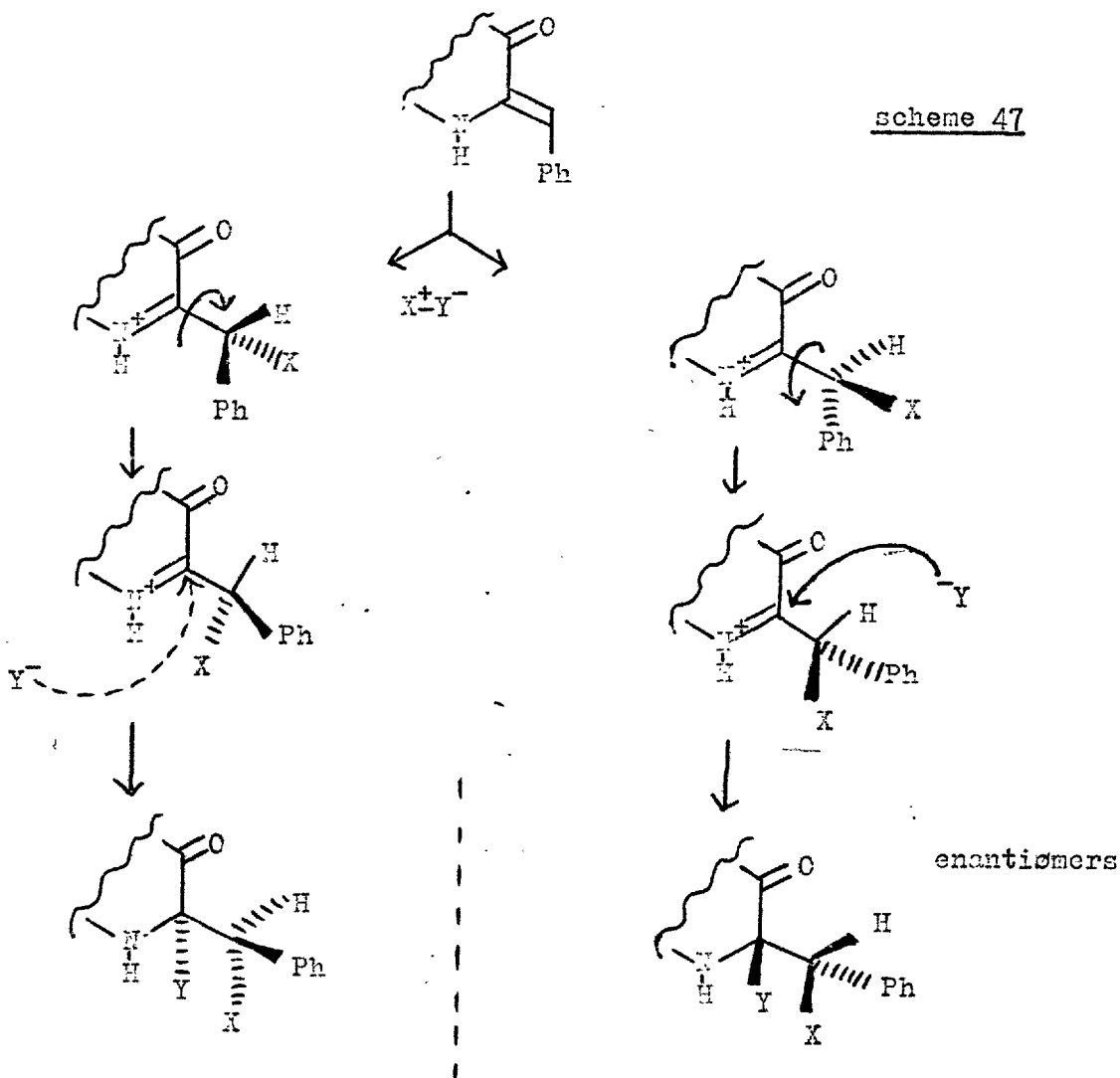
none compare favourably with the proposal described and the isolation⁶³ of a β -organo mercury derivative (134) from a similar oxidation of a dehydro-amido acid with mercury (II) acetate in methanol (see p. 35) lends weight to the enamide type of mechanism shown in scheme 45. Nucleophilic attack is required for formation of both acetate groups and therefore, the inclusion in the reaction of a nucleophile better than acetate should lead to non-acetoxy bearing products. Thus, the oxidation was carried out in benzene containing 5% methanol. Tlc indicated that the reaction was complete within 15 minutes, a remarkable rate enhancement, but the only product formed was the methoxy acetate (130). The reaction was repeated in methanol alone and on addition of the L.T.A. a deep yellow colouration was produced. Within 5 minutes the colour had faded and the reaction was complete. Workup afforded a 1:1 mixture (n.m.r.)

of (130) and a new compound not previously observed. The products were separated by plc and the new compound fully characterised as the crystalline dimethoxy derivative (135). Confirmation of the intermediacy of the acylimine (129) or the ion (132) is given by the facile formation of the α -methoxy derivative in the methanol-benzene solvent. However, the partial formation of the methoxy acetate in methanol alone would indicate that the normal S_N2 displacement of the lead-carbon bond is in competition with a more favourable, intramolecular substitution involving acetate (scheme 46).

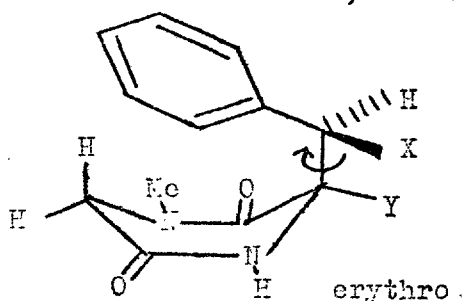


The stereospecificity of this oxidation, the earlier singlet oxygen reactions and the *t*-butyl hypochlorite oxidations described in the next section, is quite pronounced. All the compounds isolated appear to be single isomers and not erythro and threo mixtures (n.m.r. and tlc). Initial attack by the oxidant, X^+-Y^- , at either face of the double bond gives rise to two different relative configurations. Rotation about the benzylic-dipeptide ring bond occurs in the direction required to reduce steric interactions between the two large groups i.e. Ph and X, and the carbonyl oxygen in the 2-position. Subsequent attack by the nucleophile Y^- is governed by product development control and therefore, the attack occurs specifically from the face opposite the phenyl ring in order to afford the preferred folded conformation directly without further bond rotation. Consequently, the products obtained are optical; not configurational isomers and represent the erythro form (sheme 47). The proposal is in accord with the n.m.r. spectra of the derivatives. As already

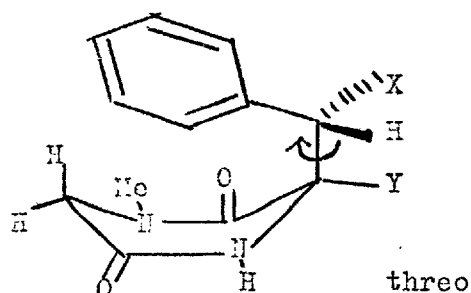
scheme 47



described, the N-methyl resonance in (119) appears at higher field (by ca.0.2-0.4 p.p.m.) than in simple benzyl derivatives e.g. (112) and (115). Such an observation can be explained by shielding of the N-methyl group by the aromatic ring, but obviously requires the ring to be closer to the l-amide group than in the simple examples. This might well be expected, since the rotation required to bring the phenyl ring nearer is in the correct sense for reducing the steric repulsions between the functionalities X and Y. Were the threo configuration adopted, the required rotation would increase the steric repulsions between X and Y, a much more unfavourable situation.



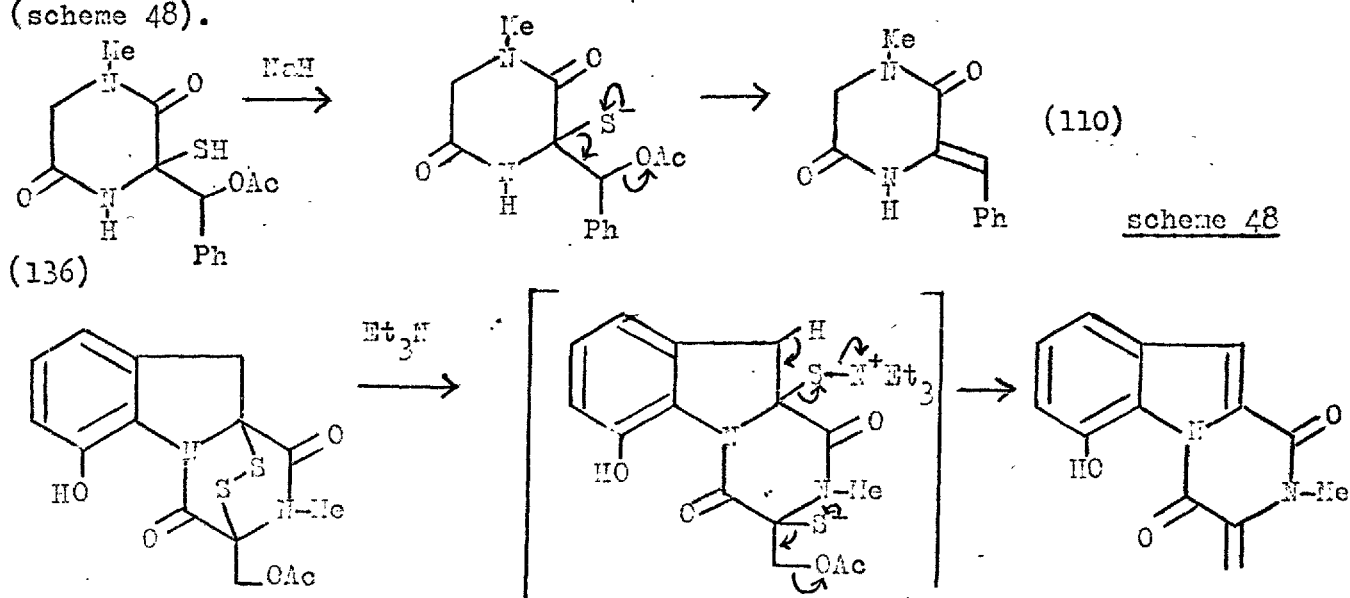
(119)



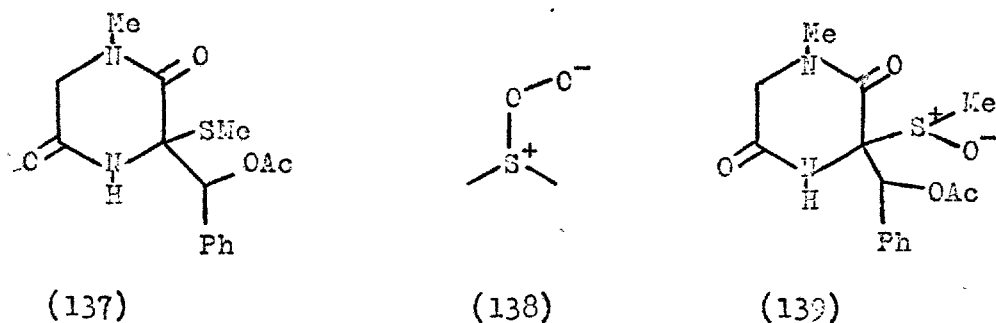
The rate increase observed in the presence of methanol cannot be explained in electronic terms since species of the type $\text{Pb}(\text{OAc})_3\text{OMe}$ and $\text{Pb}(\text{OAc})_2(\text{OMe})_2$ would make the lead atom less electron deficient than in the tetraacetate due to differences in the electron-withdrawing properties of methoxy and acetoxy groups. However, the importance of steric crowding around the 3-position of the dioxopiperazine has already been noted and it may well be that a more favourable overlap of the benzyldiene bond orbitals with the empty orbitals on the lead atom can be achieved when the lead atom is surrounded by methoxy groups rather than the larger acetoxy groups.

In view of the ease of formation of the diacetate (128) and its subsequent substitution, several interesting derivatives were prepared and their reactions investigated. For example, treatment of the diacetate with hydrogen sulphide in dichloromethane containing boron trifluoride etherate afforded a quantitative yield of the crystalline α -thiol (136). Reaction of the thiol with sodium hydride under nitrogen initially produced a bright yellow solution, presumably due to the thiolate anion. However, the colour gradually turned to the deep red characteristic of elemental sulphur in basic solution (p. 104), and tlc confirmed the presence of sulphur in the reaction mixture. Workup, after 20 hours, afforded the benzyldiene compound (110) in 100% yield. This reaction involves an internal reduction and may be of relevance to the desulphurisation of dehydrogliotoxin acetate with triethylamine⁶⁴ commencing with nucleophilic attack by the base on the sensitive disulphide bridge

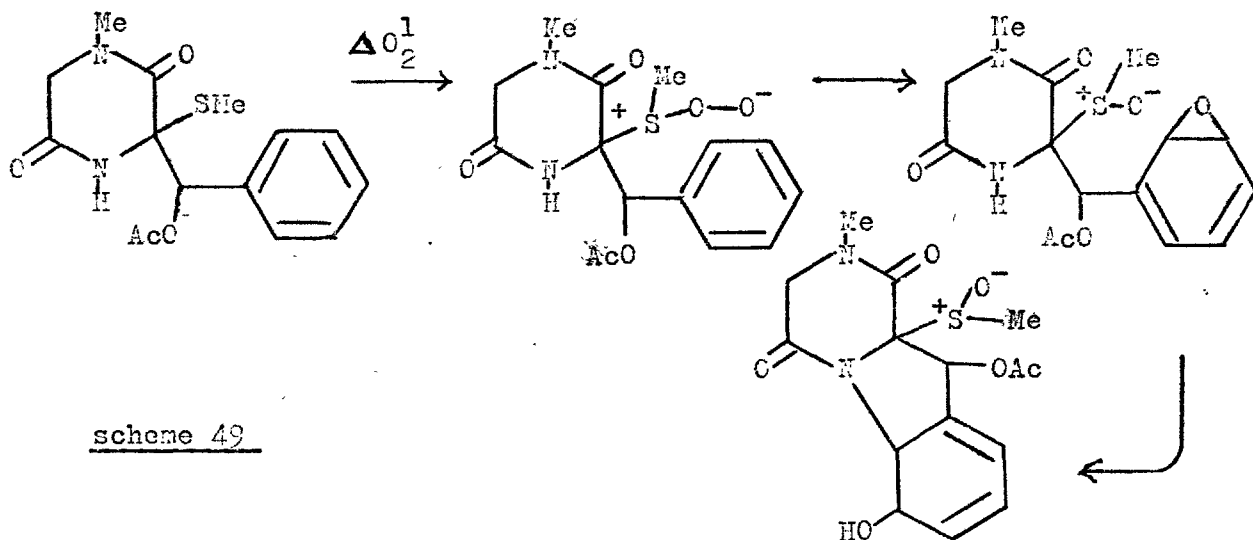
(scheme 48).



In contrast, the thiol group of (136) was readily methylated with methyl iodide and anhydrous potassium carbonate in acetone. Tlc of the mixture showed a 'one-spot' reaction which was complete within 2 hours. Workup gave the crystalline methylthio derivative (137) in quantitative yield. The reaction

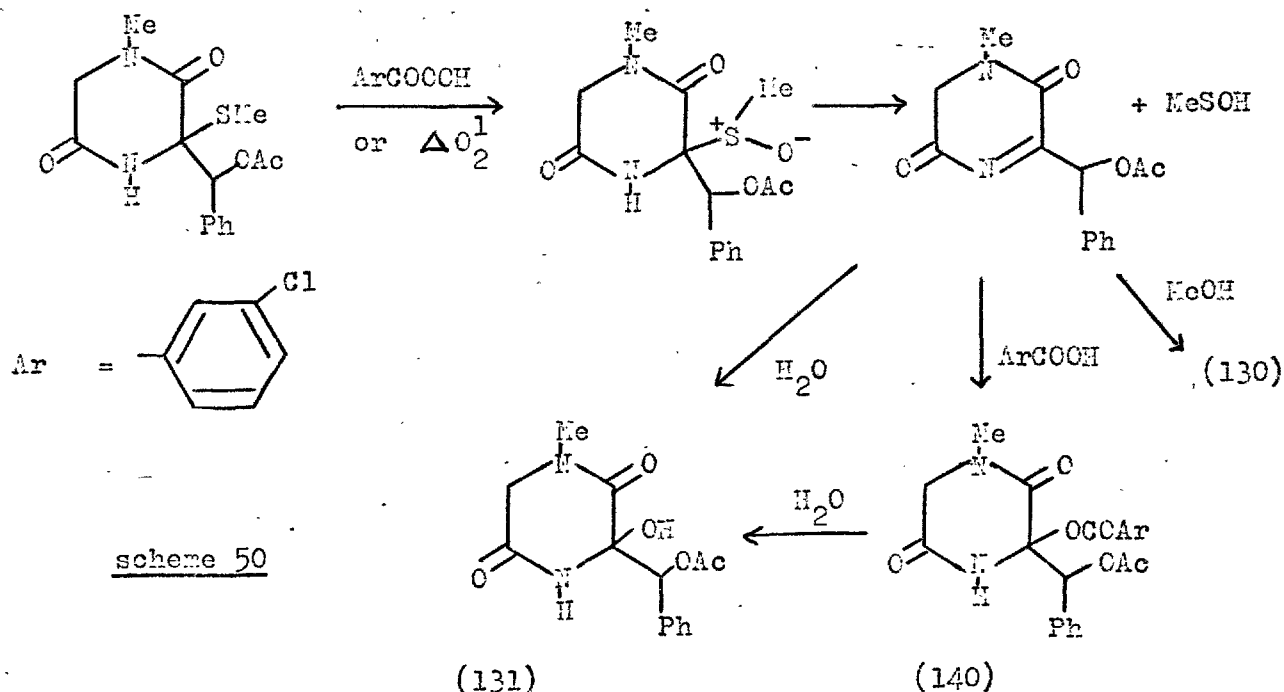


of singlet oxygen with sulphides produces sulfoxides and sulphones via an oxidative intermediate of the type (138)⁶⁵. With such a derivative of the methylthio acetate (137) it was hoped that oxidation of the benzene ring might occur (scheme 49). Before attempting this reaction the susceptibility



of the sulphide to peracid oxidation was tested. The reaction of (137) with *m*-chloroperbenzoic acid at 0° in dichloromethane was virtually spontaneous, but the corresponding sulfoxide (139) was not isolated. The n.m.r. spectrum of the crude product before aqueous bicarbonate workup had removed the *m*-chlorobenzoic acid did show an α,β -difunctionalised dioxopiperazine, but the new S-methyl singlet at τ 7.0 was too low-field for the S-methyl of the sulfoxide group (the sulphide methyl resonated at τ 7.70). After basic

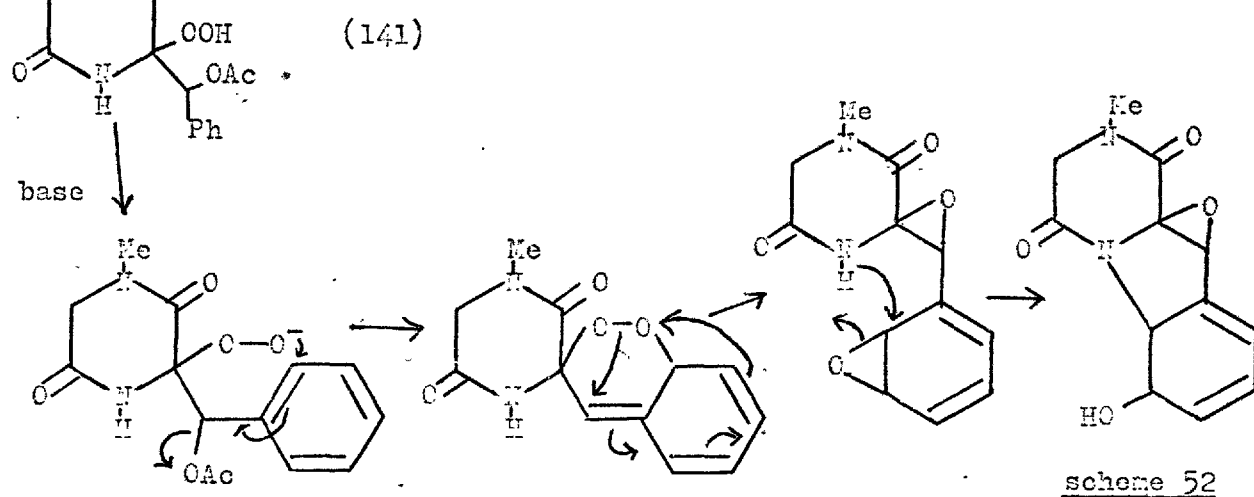
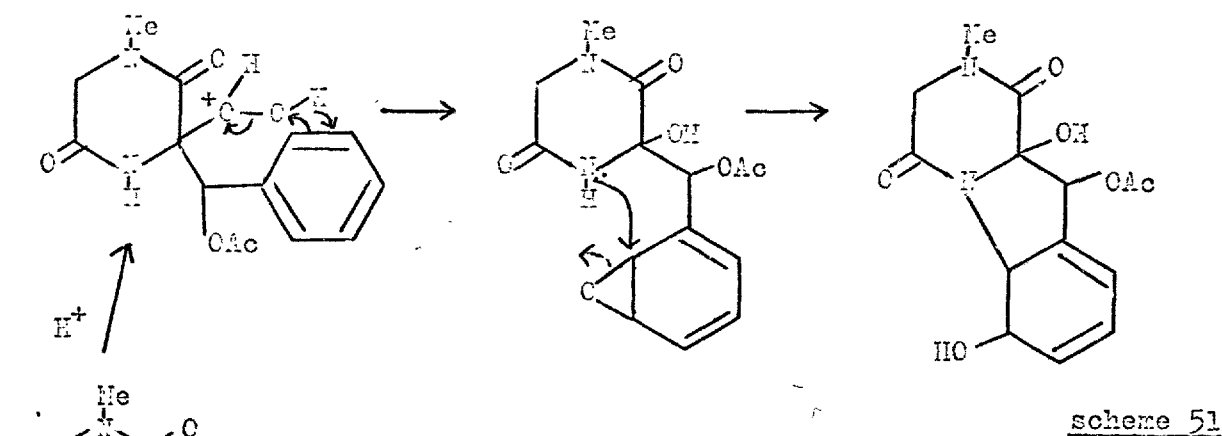
workup the singlet at $\tau 7.0$ had disappeared but aromatic resonances due to a m-chlorobenzoyl unit were still present; the product was a mixture of two compounds, one of which was the hydroxy acetate (131). The remaining resonances were consistent with the m-chlorobenzoate (140) which was even more reactive than the diacetate and all attempts to isolate the compound in a pure state, failed. These observations can be rationalised in terms of the initial sulphoxide (139) undergoing rapid elimination of methyl sulphenic acid ($\tau 7.0$) to the acylimine which is then attacked by the aromatic acid. The resultant diester undergoes partial conversion to the hydroxy ester on aqueous workup (scheme 50). The same results were obtained with singlet oxygen. Although no



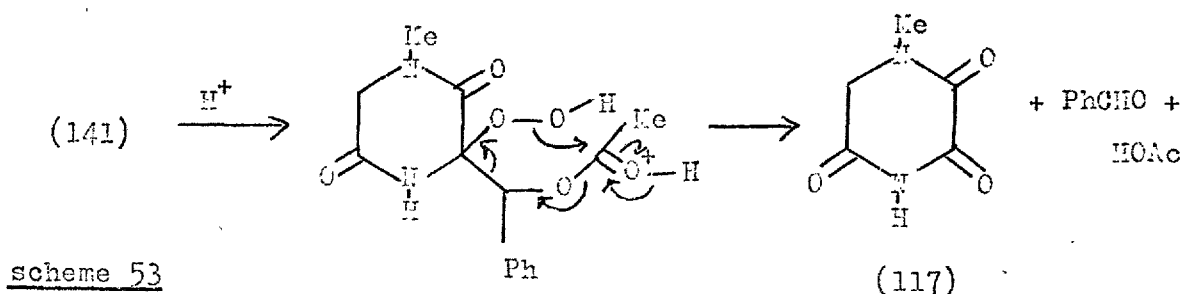
internal nucleophile, besides the sulphenic acid, was present a quantitative yield of the hydroxy acetate was formed, presumably by reaction of the acylimine with water from the surroundings. If solvent containing 5% methanol was used a mixture of the methoxy acetate and the hydroxy acetate was produced (scheme 50). No oxidation of the aromatic ring was observed.

A more favourable oxidation of the aromatic ring was envisaged with the hydroperoxy acetate (141) under either acidic (scheme 51) or basic conditions (scheme 52). The hydroperoxide was prepared from the diacetate with anhydrous hydrogen peroxide⁶⁶ in ether/dichloromethane containing boron trifluoride

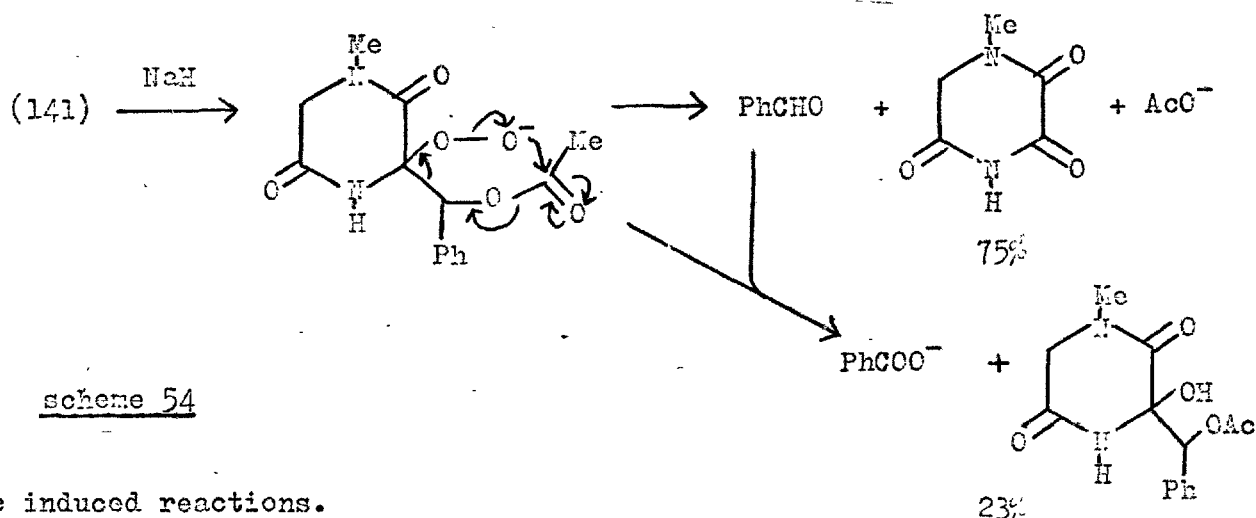
etherate. This unusual derivative was isolated in 95% yield as soft white



needles from dichloromethane. Satisfactory i.r. and analytical data were obtained but the low solubility of the compound precluded an n.m.r. spectrum in deuteriochloroform. The acid catalysed reaction was carried out in T.F.A. solution and followed by n.m.r. spectroscopy. The initial spectrum was fully consistent with the hydroperoxy acetate but the spectrum slowly changed. Oxidation of the aromatic ring was not occurring, however, since the new resonances resembled the trioxopiperazine (117), benzaldehyde and acetic acid. After 24 hours all the hydroperoxide had reacted and evaporation of the solution followed by trituration with ether to remove the aldehyde, afforded a quantitative yield of (117). Such a reaction can be accommodated by the

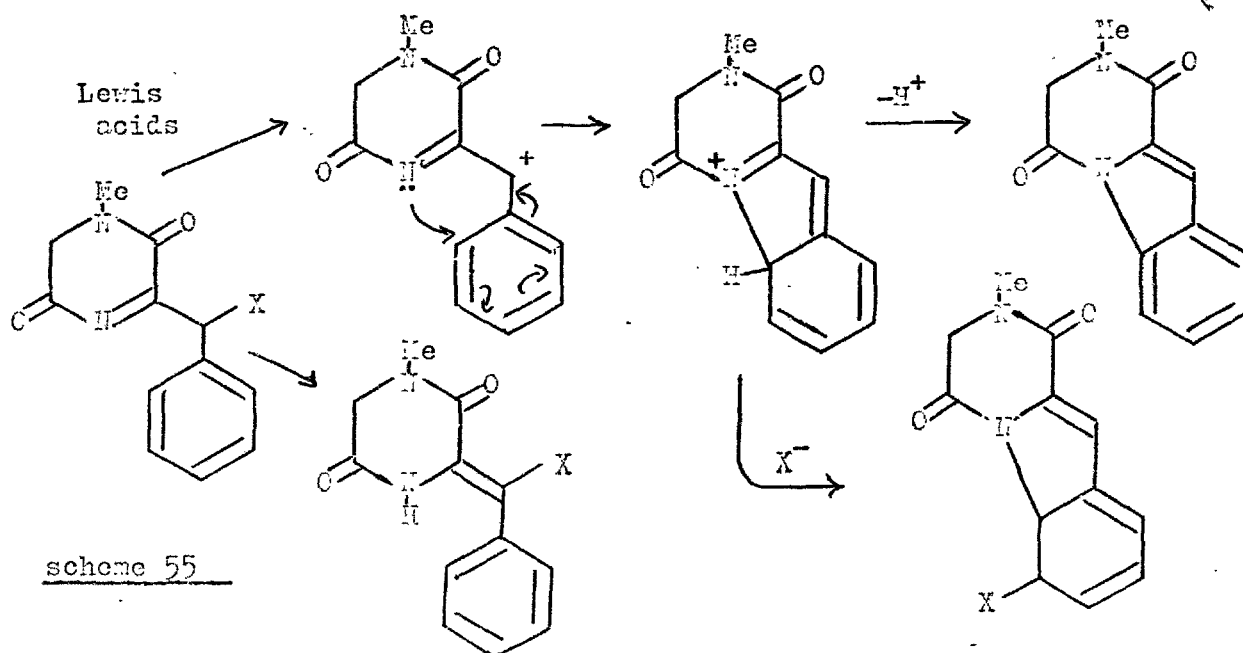


mechanism shown in scheme 53. The reaction under basic conditions was carried out by treatment of the hydroperoxide with sodium hydride in T.H.F. It required 20 hours for the substrate to be consumed (negative starch-iodide test) and then the mixture was neutralised and partitioned between water and dichloromethane. The aqueous phase gave the trioxo derivative (117), which is extremely water-soluble, in 75% yield, whilst the organic phase gave a mixture (n.m.r.) of benzoic acid, benzaldehyde and the hydroxy acetate (131). Trituration with ether removed the benzoyl compounds and afforded a 23% yield of (131). The results can be explained by a slow cleavage reaction which allows oxidation of the aldehyde by remaining hydroperoxide to occur (scheme 54). No oxidation of the aromatic ring was observed in either the acid or

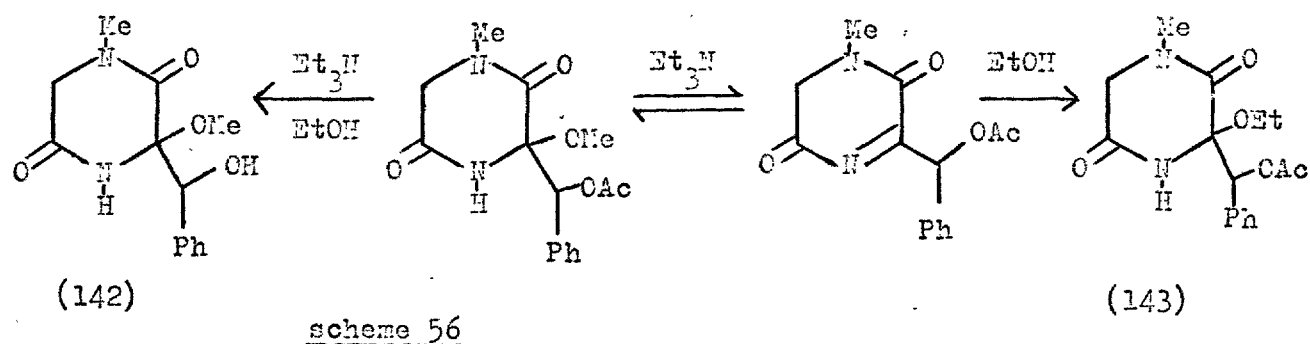


base induced reactions.

An alternative approach to the oxidation of the aromatic ring via an acylimine is shown in scheme 55. The acylimine (129) i.e. X=OAc, is admirably

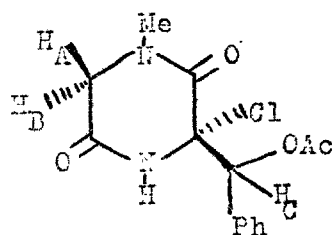


suited to this reaction since, as mentioned previously (p. 138), the alternative isomerisation to the benzylidene compound does not occur with this compound. A method was required for the synthesis of the acylimine as opposed to its acyliminium ion (132), which cannot undergo the desired reaction. An attempt to pyrolyse the methoxy acetate (130) at 200° under high vacuum resulted in its total decomposition. Examination of the residue showed no evidence for the required acylimine. Reaction of the methoxy acetate (130) with triethylamine in dichloromethane did not effect elimination to an observable extent, although a yellow colouration was produced on addition of the base. Whether any elimination was occurring at all was checked as follows. The methoxy acetate was stirred in ethanol containing triethylamine (scheme 56).



Evaporation of the solution after several days afforded a solid residue only partially soluble in dichloromethane. Trituration with this solvent gave a white solid whose i.r. spectrum showed a strong hydroxyl absorption at 3420 cm^{-1} and the acetate carbonyl stretch was no longer present. The n.m.r. spectrum of the compound confirmed the conversion of the acetoxy group into a hydroxy group by the lack of acetyl methyl singlet and the up-field shift of the benzylic methine from $\tau 3.78$ in the methoxy acetate to $\tau 4.77$ in the new compound. The singlet at $\tau 6.63$ confirmed the presence of the methoxy group. Obviously, a base catalysed solvolysis of the acetate had occurred (50% yield) and recrystallisation of the compound afforded prisms of the methoxy alcohol (142) of analytical purity. Examination of the dichloromethane triturate showed only starting material and none of the expected ethoxy acetate (143). For elimination to occur a better leaving group was required and by analogy

with the preparation of simpler acylimines (part 1) the α -chloro derivative (144) was the logical choice. The hydroxy acetate (131) was suspended in deuteriochloroform, an excess of thionyl chloride added, and the reaction followed by n.m.r. Within 15 minutes complete solution was attained and after 90 minutes at 25° all the starting material had reacted giving a mixture of two compounds in the ratio of ca. 8:5. The major compound was assigned the α -chloro structure (144) (later work proved this compound was the chloro-derivative and not the chlorosulphinate). The minor compound was considered to be the acylimine (129) and the resonances were the same as those found in

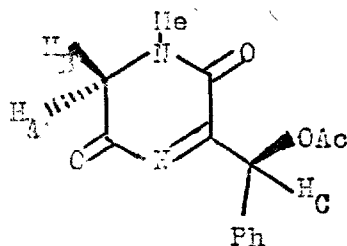


(144)

$$\begin{array}{l} \text{H}_A \tau 5.82, \\ \text{H}_B \tau 6.48, \end{array} \left. \vphantom{\begin{array}{l} \text{H}_A \\ \text{H}_B \end{array}} \right\} \text{ABq, } J \ 19 \text{ Hz.}$$

$$\text{H}_C \tau 3.38, \text{ s.}$$

$$\text{N-Me } \tau 7.12, \text{ s. } \text{Ph } \tau 2.63, \text{ s. } \text{OCOMe } \tau 7.79, \text{ s.}$$



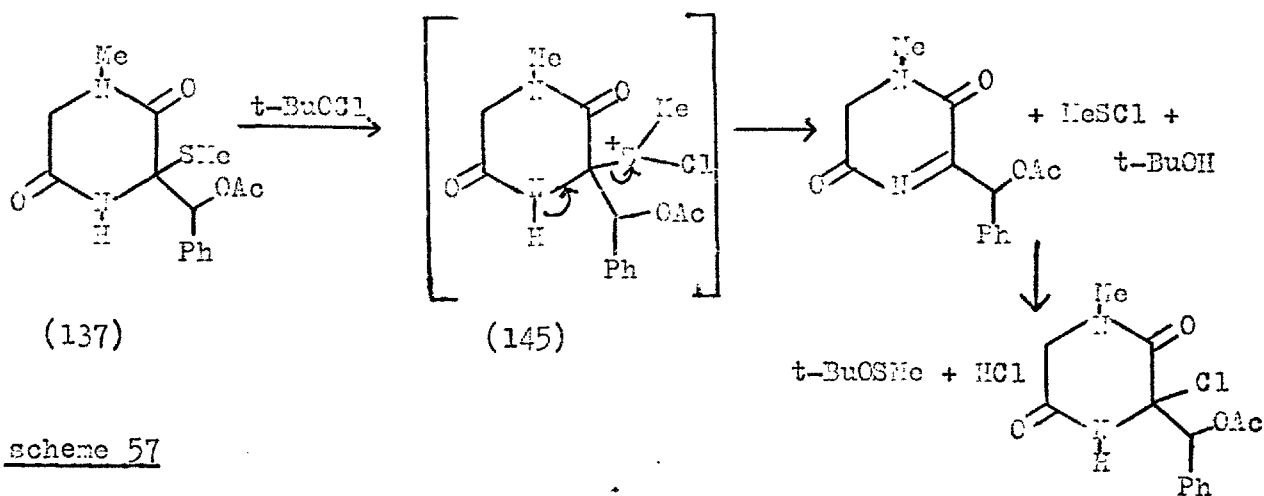
(129)

$$\begin{array}{l} \text{H}_A \tau 5.08, \\ \text{H}_B \tau 5.78, \end{array} \left. \vphantom{\begin{array}{l} \text{H}_A \\ \text{H}_B \end{array}} \right\} \text{ABq, } J \ 18 \text{ Hz.}$$

$$\text{H}_C \tau 3.47, \text{ s.}$$

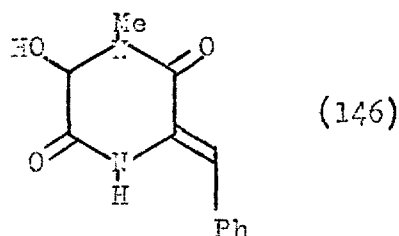
$$\text{N-Me } \tau 6.87, \text{ s. } \text{Ph } \tau 2.45, \text{ s. } \text{OCOMe } \tau 7.90, \text{ s.}$$

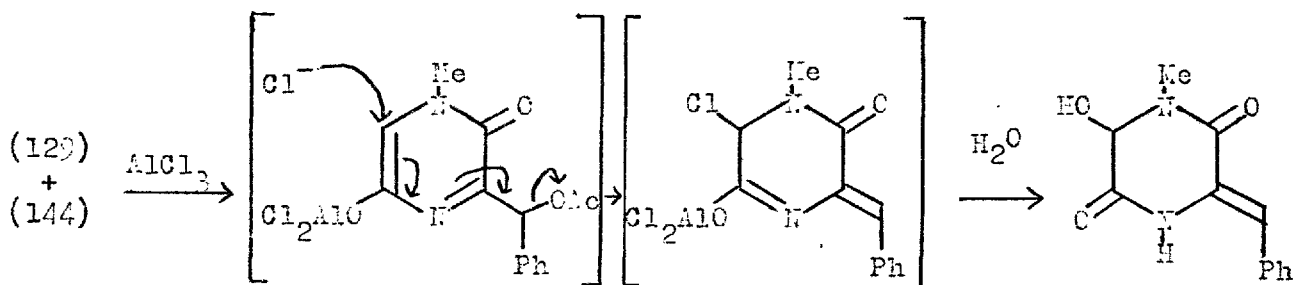
the diacetate preparation reaction mixture (see p. 139). The spectrum of this compound was unusual compared to similar acylimines (see later) in that the N-methyl group was relatively deshielded at τ 6.87 and the two protons in the 6-position appeared as an AB quartet (J 18 Hz.). These effects must be due to the acetate group at the chiral atom. Evaporation of the solvent and the excess thionyl chloride did not unduly change the ratio of the two compounds. A similar equilibrium mixture of the chloro acetate and acylimine was prepared by oxidation of the methylthio derivative (137) with t-butyl hypochlorite, via the sulphonium chloride (145). The t-butanol is too bulky to add to the acylimine itself (see p.153) but reacts with the methyl sulphenyl chloride liberating hydrogen chloride, which can add to the acylimine (scheme 57). The reactions of this two compound mixture were investigated. For example,



scheme 57

treatment with methanol afforded, as expected, a quantitative yield of the methoxy acetate (130). Reaction of the mixture with pyridine, in order to effect complete elimination of the chloro acetate to the acylimine gave, instead, an intractable multicomponent mixture (see p.158). Treatment with boron trifluoride etherate failed to produce any significant reaction; aqueous workup after a week at 25° afforded the hydroxy acetate (131) as the sole product. On the other hand, reaction with aluminium chloride in anhydrous nitromethane did yield a product besides the hydroxy acetate. The new compound isolated by plc in 10% yield, showed O-H and N-H absorptions but no ester carbonyl in its i.r. spectrum; a $\text{C}=\text{C}$ absorption at 1625 cm^{-1} inferred the presence of a benzylidene substituent. This was confirmed in the n.m.r. spectrum by the olefinic proton at τ 2.88 (compared to τ 2.90 in (110)). A broad two-proton singlet at τ 4.80 reduced to a sharp one-proton singlet on shaking with D_2O and combined with the N-methyl group at τ 6.85 this data pointed to a 6-hydroxy substituent. Thus, the new compound was assigned the structure (146). A satisfactory mass spectrum (M^+ ; m/e 232) was obtained from the colourless prisms. The formation of this unusual compound must involve the intermediacy of a pyrazinone species (scheme 58).

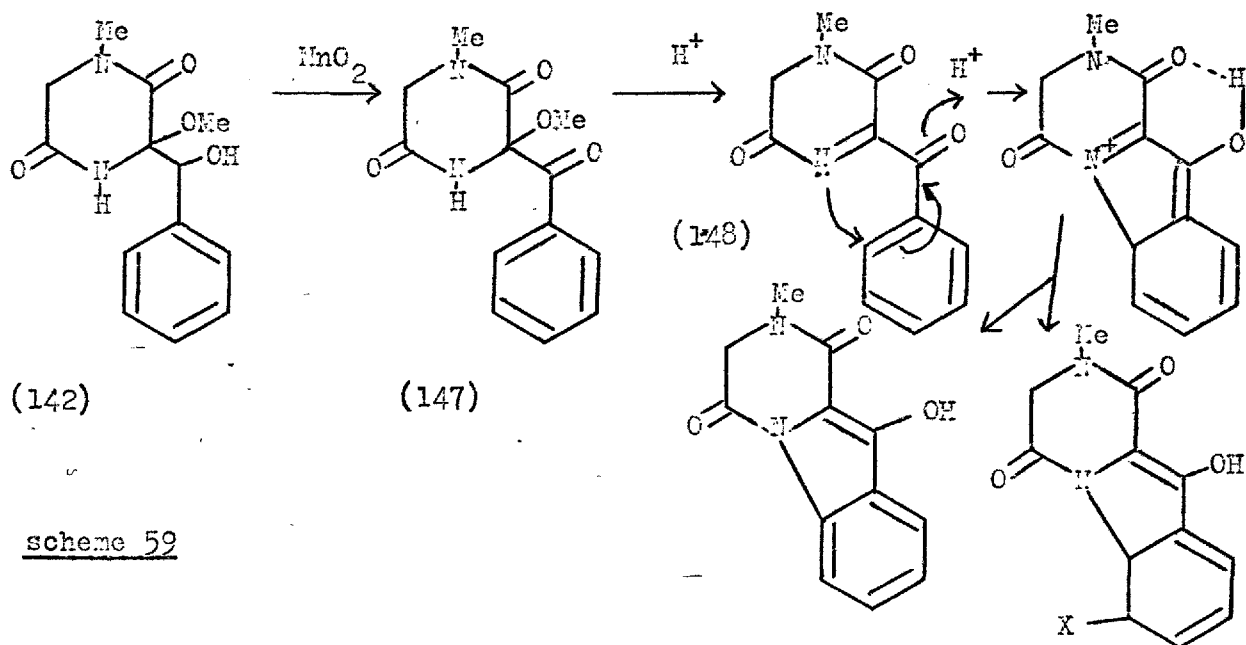




scheme 58

In all of the reactions investigated no products were observed where bonding to the aromatic ring had occurred.

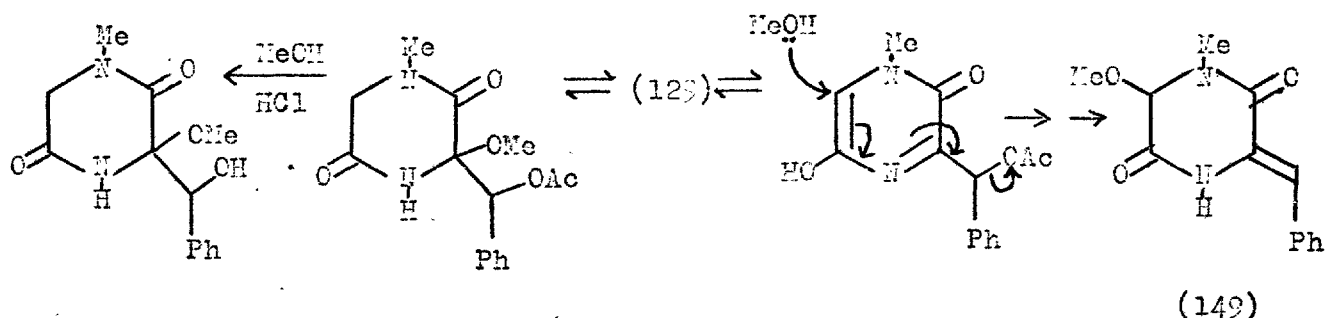
The benzoyl acylimine (148) was also considered as a possible precursor for the desired reaction (scheme 59). The methoxy alcohol (142) was prepared



scheme 59

in 50% yield as described earlier by base catalysed solvolysis of the methoxy acetate (130) with triethylamine in ethanol. The compound could also be prepared in 70% yield by an acid catalysed solvolysis of (130) with 3% HCl in methanol. An interesting side product was formed in the latter reaction. By analogy with the base catalysed solvolysis, the major product was isolated by trituration of the crude product with dichloromethane; however, the triturate did not give unreacted starting material but a new compound which was subsequently purified by plc. Its spectral data closely resembled that of the

6-hydroxy-3-benzylidene dioxopiperazine (146) except for the lack of hydroxyl absorption in the i.r. and the presence of a new methoxy resonance in the n.m.r. spectrum. The mass spectrum (M^+ ; m/e 246) confirmed the 6-methoxy structure (149) for the new compound. Again a pyrazinone intermediate must be involved in the formation of (149) (scheme 60). Oxidation of the alcohol



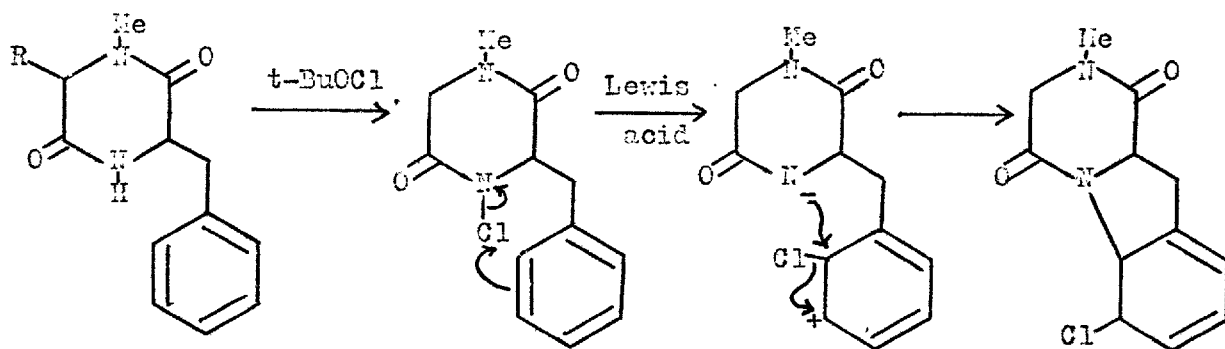
scheme 60

(142) was accomplished with activated manganese dioxide in acetone. This reaction was not very efficient, requiring 2 days to produce a 20% yield of the ketone (147). Longer reaction times did not improve the yield and benzaldehyde was detected in the crude product (n.m.r. and tlc) indicating that oxidative cleavage of the benzyl substituent was also occurring. The n.m.r. spectrum of the ketone was most informative. The N-methyl group resonated at τ 6.97 and the two protons in the 6-position appeared as a singlet at τ 5.90, as opposed to the AB quartet found in all the other α, β -difunctionalised benzyl derivatives. The spectrum gives direct proof for the shielding effects described several times earlier. The sp^2 -benzylic carbon in the ketone prevents the aromatic ring from adopting the preferred folded conformation [cf. (119)], hence the shielding effects are completely absent. It should be noted that asymmetry alone at the 3-position is not always sufficient to render the two protons in the 6-position non-equivalent.

The reaction of the methoxy ketone with boron trifluoride etherate failed to produce any significant change and workup after 5 days at 42° afforded only the starting material. With aluminium chloride reaction did occur but a multicomponent mixture was formed from which no discrete products were

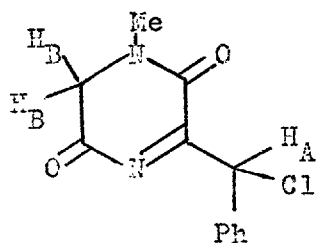
isolated. This approach was abandoned as no evidence for involvement of the aromatic ring was ever found.

(iii) Reactions with t-butyl hypochlorite. Whilst the L.T.A. oxidation and subsequent reactions described in the previous section were being carried out a similar approach using an alternative oxidant was investigated (scheme 61).



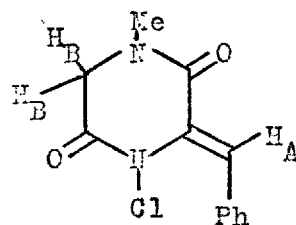
scheme 61

Although N-chlorination of amides with t-butyl hypochlorite is a reportedly⁶⁷ facile process, no reaction occurred with the benzyl-methyl-dioxopiperazine (41) even in the presence of triethylamine. In contrast, the benzylidene compound (110) reacted rapidly with the hypochlorite in dichloromethane at 25° and within one hour all the dioxopiperazine had been consumed. Although tlc indicated a decomposing complex mixture, the n.m.r. spectrum of the yellow, non-crystalline glass obtained by evaporation of the reaction mixture showed a single compound whose resonances were consistent with either the chloro acylimine (150) or the N-chloroamide (151). The chemical shift of the benzylic



(150)

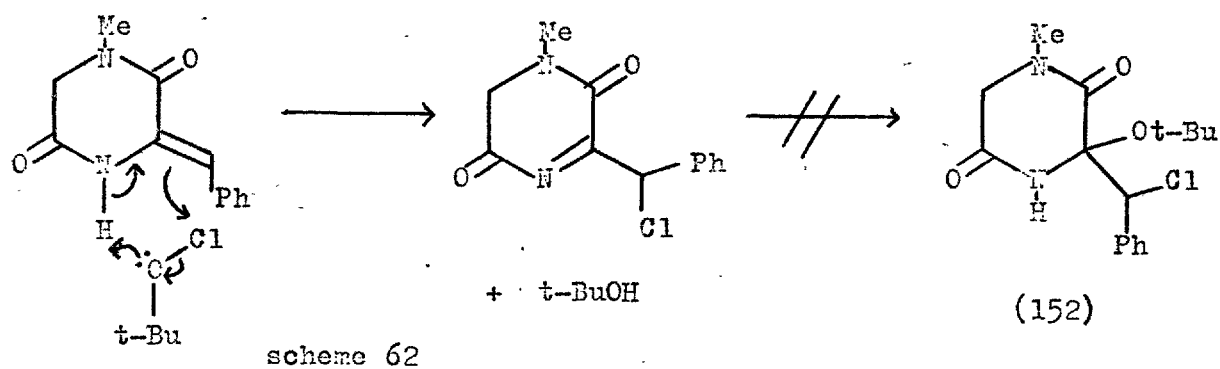
H_B τ 5.70, s.
H_A τ 3.45, s.
N-Me τ 7.00, s.
Ph τ 2.5, m.



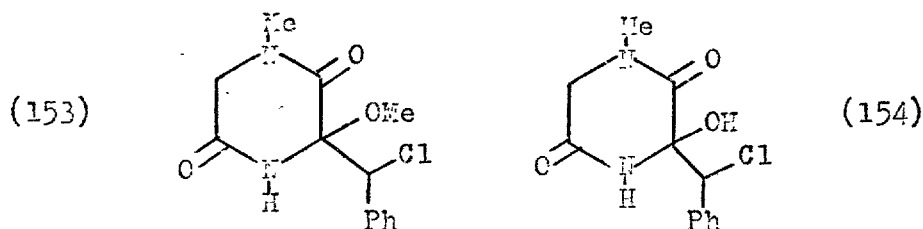
(151)

proton H_A is very similar to that found in the acetoxy acylimine (129) (τ 3.47) but is higher by ca. 0.6 p.p.m. than expected for the benzylidene proton in

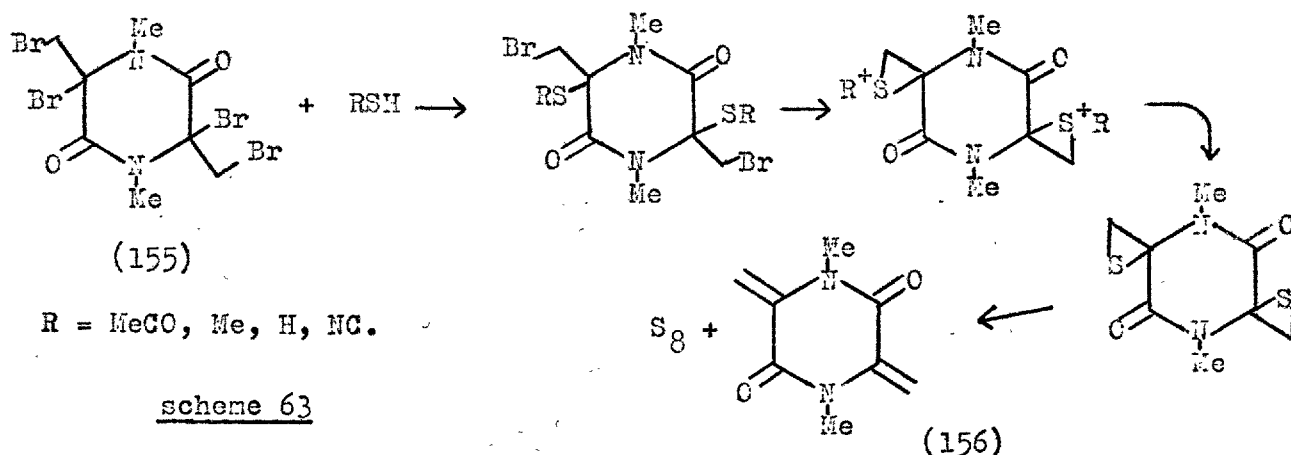
(151) if analogy to other benzylidene compounds is close. The i.r. spectrum showed three bands at 1620 (weak), 1680 (strong) and 1745(weak) cm^{-1} which is more consistent with the acylimine than the chloroamide. Reaction of the yellow material with aqueous sodium sulphite solution failed to produce any of the benzylidene starting material (110) which is evidence against the N-chloroamide (151). On the other hand, the compound did not form the expected cycloaddition adduct with 2,3-dimethylbutadiene in refluxing benzene, probably due to steric inhibition (compare the acetoxy acylimine (129)). An attempt to prepare analytically pure material by using pure (110), purified solvent and freshly distilled oxidant, failed. Despite precautions to keep the material dry, the figures obtained were wholly consistent with the hydroxy chloride (154). The mechanism of the reaction is most probably a further example of the general enamide reaction with electrophiles (see p.33). By analogy with the singlet oxygen oxidation of (110) a cyclic transition state may be of importance (scheme 62). Steric crowding around the 3-position prevents the addition



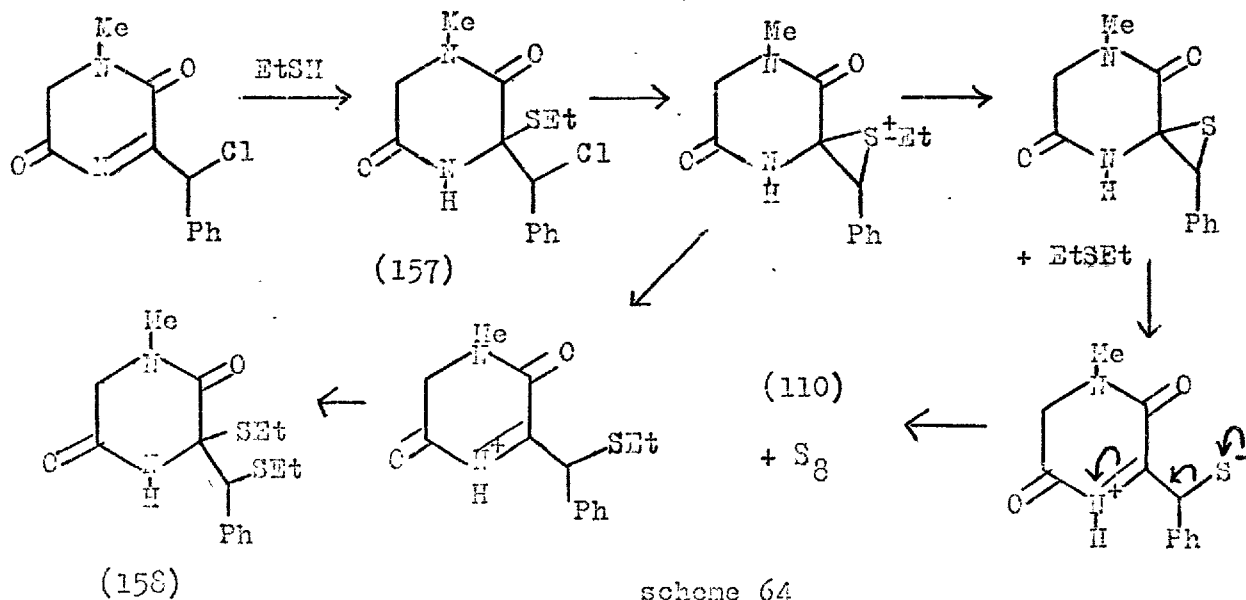
of the bulky t-butanol and so the t-butoxy chloride (152) is not formed. However, when the oxidation was carried out in methanol a quantitative yield of the methoxy chloride (153) was produced. A similar yield of (153) was also



obtained by direct reaction of the acylimine with methanol. This compound, which gave correct analytical figures, was insoluble in chloroform but exhibited the expected resonances, including the high field N-methyl singlet and the AB quartet, in T.F.A. solution. Its mass spectrum, like that of the diacetate (128), showed the highest peak at m/e 251 ($Cl=35$) corresponding to the parent acyliminium ion, rather than the methoxy derivative itself. Reaction of the acylimine with aqueous acetone afforded a quantitative yield of the hydroxy chloride (154). Satisfactory spectral and analytical data were obtained. A recent publication³⁰ reports that reaction of the tetrabromo compound (155) with sulphur nucleophiles led to the formation of the dehydro compound (156) and elemental sulphur via the mechanism shown (scheme 63).

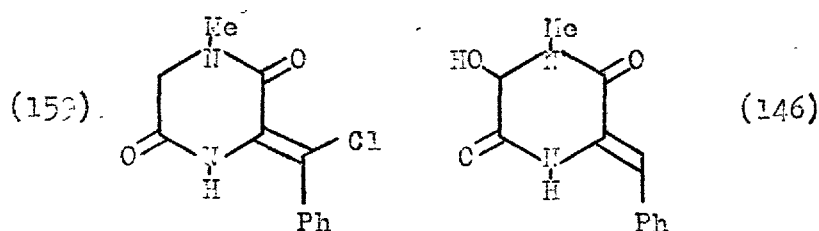


By analogy, reaction of the acylimine with ethanethiol should give either (110) and sulphur, or in view of the lability of sulphonium substituents e.g. (139) and (145), the di(ethylthio) derivative (158) (scheme 64). However, when the



reaction was carried out the ethylthio chloride (157) proved to be a stable crystalline solid and was isolated in 68% yield. The compound was fully characterised by its spectral and analytical data. A second minor product was formed, but its n.m.r. was not that of (110) nor was it consistent with the di(ethylthio) derivative (158). Unfortunately, the compound decomposed upon attempted isolation and was not investigated further.

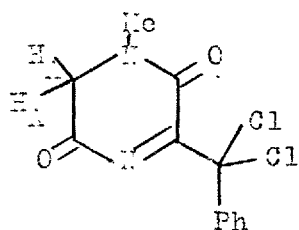
In contrast to the acetoxy acylimine (129), which remained inert to enamide formation, the chloro acylimine (150) slowly rearranged to the chloro-benzylidene compound (159) with Lewis acids. Thus, after 3 days at 25° with boron trifluoride etherate, the dehydro derivative was isolated in greater than 90% yield. The Z-configuration about the benzylidene double bond was assigned by comparison of the n.m.r. spectrum with those of similar compounds. In general, the aromatic protons in the Z-isomer resonate as a broadened singlet, whereas in the E-isomer, interaction between the benzene ring and the 2-carbonyl oxygen causes these protons to appear as a broad multiplet. Attempts to prepare the E-isomer by photolysis, cf. (110) and (120), resulted in slow decomposition of the substrate and none of the required product was formed. With aluminium chloride in nitromethane the chloro acylimine gave quite



a mixture of products. Plc afforded (159) in 20% yield and also the 6-hydroxy benzylidene derivative (146) in 17% yield. As described earlier, the latter compound was also formed by treatment of the acetoxy acylimine (129) with aluminium chloride (see scheme 58). No cyclisation of the amide nitrogen onto the aromatic ring was observed.

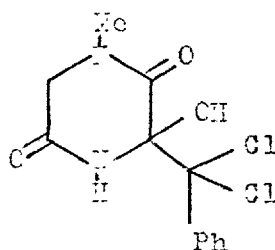
The dichloro acylimine (160) was prepared from the chlorobenzylidene compound (159) with t-butyl hypochlorite in dichloromethane. The oxidation was

somewhat slower than that of (110) requiring 24 hours for completion. The compound was characterised as its crystalline hydroxy adduct (161) which was



(160)

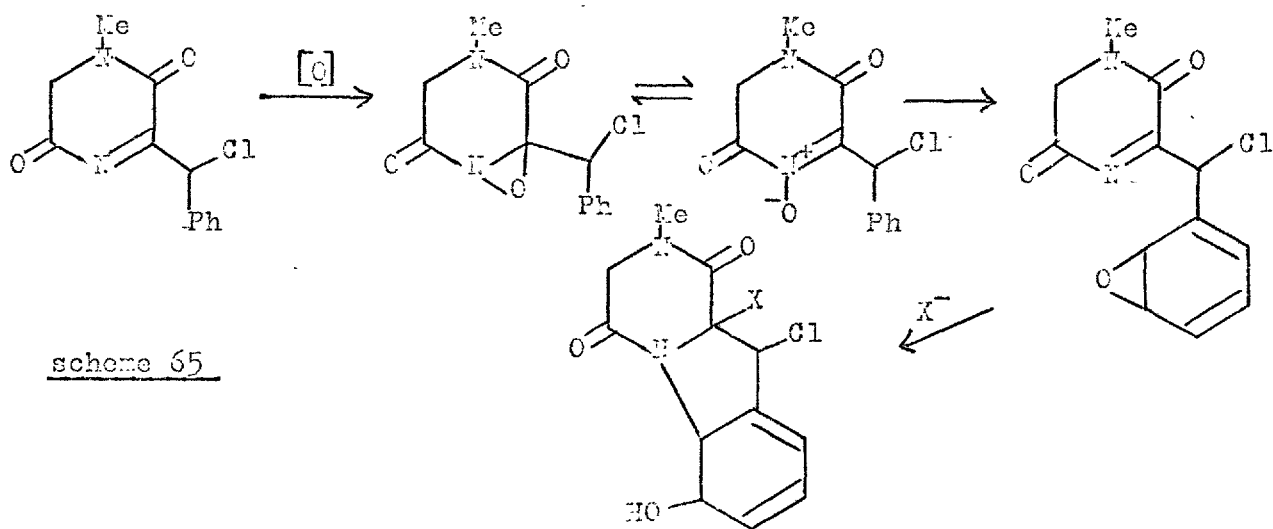
$H_A \tau 5.58, s.$
 $H-Me \tau 7.00, s.$
 $Ph \tau 2.1-2.7, m.$



(161)

formed in 66% yield by treatment of the crude acylimine with aqueous acetone. Reaction of the acylimine with Lewis acids led to the formation of complex mixtures but no involvement of the aromatic ring was observed.

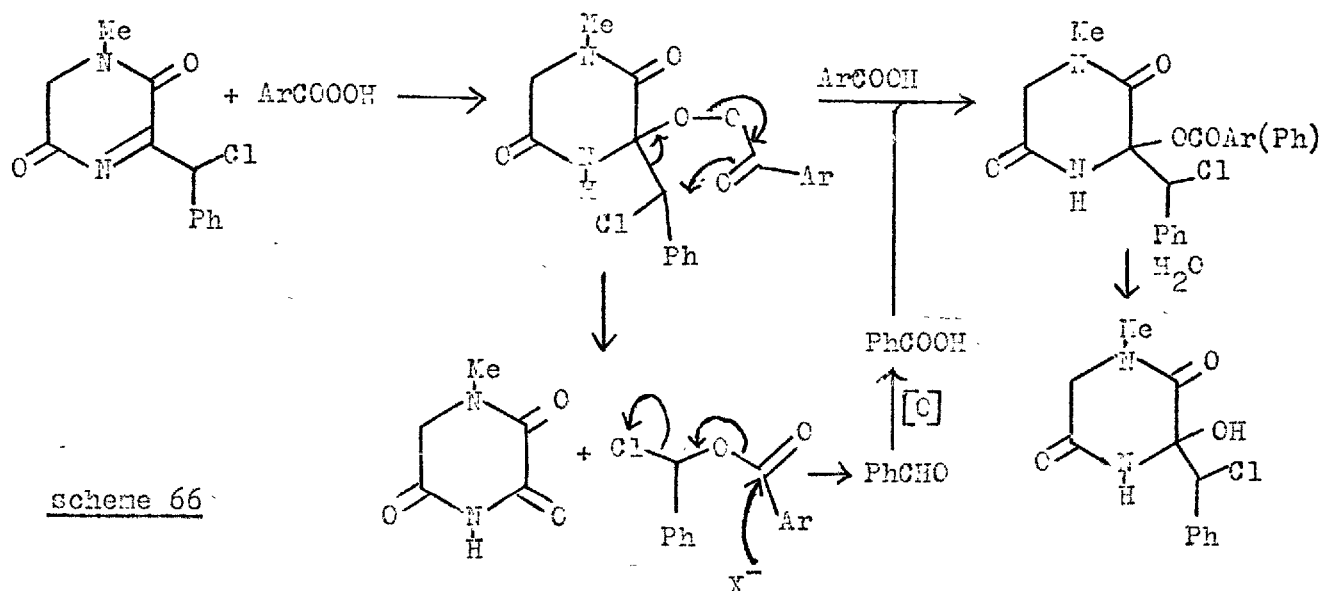
It was considered that the peracid oxidation of the chloro acylimine (150) would give an acyl oxazirane or acylimine N-oxide which, under suitable conditions, could effect oxidation of the aromatic ring (scheme 65). Thus,



scheme 65

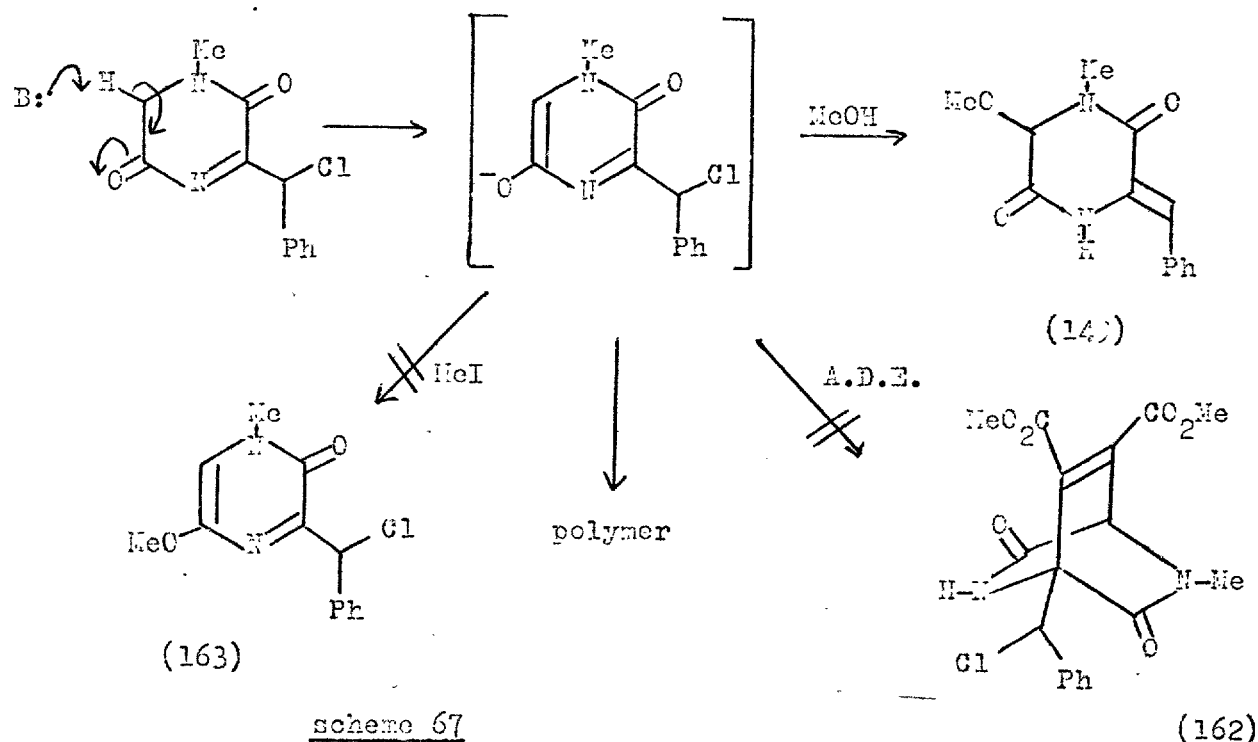
(150) was reacted with m-chloroperbenzoic acid in dichloromethane. The yellow colour was discharged within 15 minutes but the solution still gave a positive starch-iodide test. After 20 hours at 25° the solution was extracted with water which gave the trioxo compound (117) in 33% yield. The organic phase was washed with bicarbonate solution to remove the m-chlorobenzoic acid but the n.m.r. spectrum of the crude product still showed resonances due to such an aromatic unit as well as those of benzaldehyde and benzoic acid. After several days standing open to the atmosphere the product decomposed to the hydroxy

chloride (154) which was separated from the other material (mostly m-chlorobenzoic acid) in 58% yield by trituration with ether. Presumably, nucleophilic attack by the peracid gives a perester which can rearrange to the trioxo compound (117). Oxidation of the liberated benzaldehyde gives the benzoic acids which can displace the perester and themselves be subsequently displaced by water on workup, giving the products observed (scheme 66). No evidence of



oxygenation on nitrogen was forthcoming.

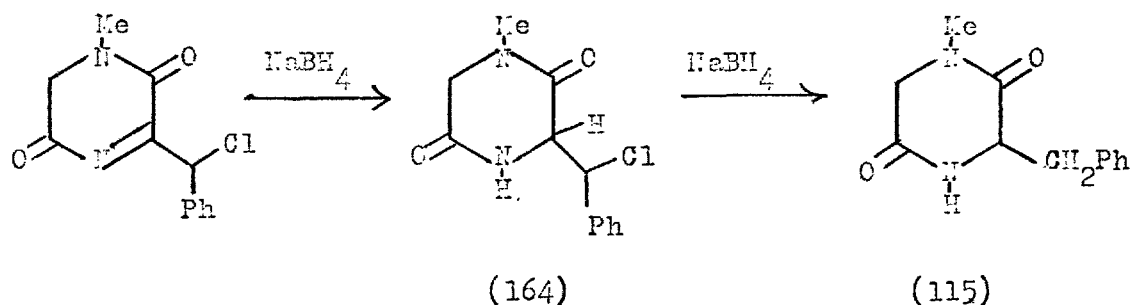
In view of the formation of 6-substituted benzylidene compounds under acidic conditions, it was considered that the same type of pyrazinone intermediate should be formed from the acylimine under basic conditions. Thus, a solution of the acylimine in anhydrous T.H.F. was treated with one equivalent of sodium methoxide. The solution turned from pale yellow to a deep orange colour which did not disappear on workup. The n.m.r. spectrum of the crude product showed one major compound besides broad humps characteristic of polymeric material. The mixture was subjected to plc and the major product, isolated in 38% yield, was shown to be the 6-methoxy derivative (149), previously isolated from the acid catalysed hydrolysis of the methoxy acetate (130). Several attempts to trap the pyrazinone intermediate were made (scheme 67). For example, the acylimine in T.H.F. containing excess A.D.E. was treated



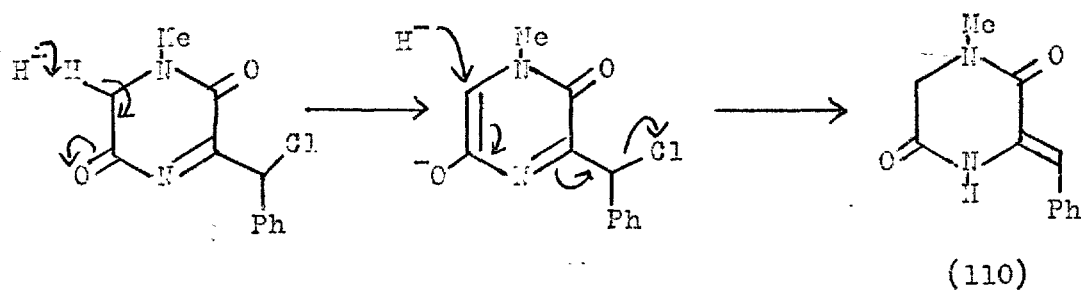
with one equivalent of sodium hydride. A dark orange colour soon developed and tlc showed a very complex mixture. Trituration of the crude product with ether, after aqueous workup, removed the dienophile. The yellow intractible solid thus obtained was polymeric in nature (n.m.r. and i.r.) and was very similar to the polymers produced in the D.D.Q. oxidations of dihydropyrazinones. None of the A.D.E. adduct (162) was observed. A similar result was obtained by stirring the acylimine and the dienophile with one equivalent of triethylamine in dichloromethane. A deep red solution was rapidly formed and workup gave the same yellow polymer. An attempt to stabilise the pyrazinone by formation of the methoxy derivative (163) via reaction of the acylimine and methyl iodide with triethylamine, also produced the polymeric material as the sole product.

The above behaviour explains the formation of a complex mixture when the acetoxy acylimine/chloro acetate was treated with pyridine (p. 149). Another reaction that may also fall into this class is the reduction of the chloro acylimine with sodium borohydride. By analogy with simple acylimines (part 1) the chloro benzyl dioxopiperazine (164) or possibly the fully reduced benzyl derivative (115) would be expected from such a reaction. However, the major

product, isolated by plc in 41% yield, was the benzylidene compound (110).

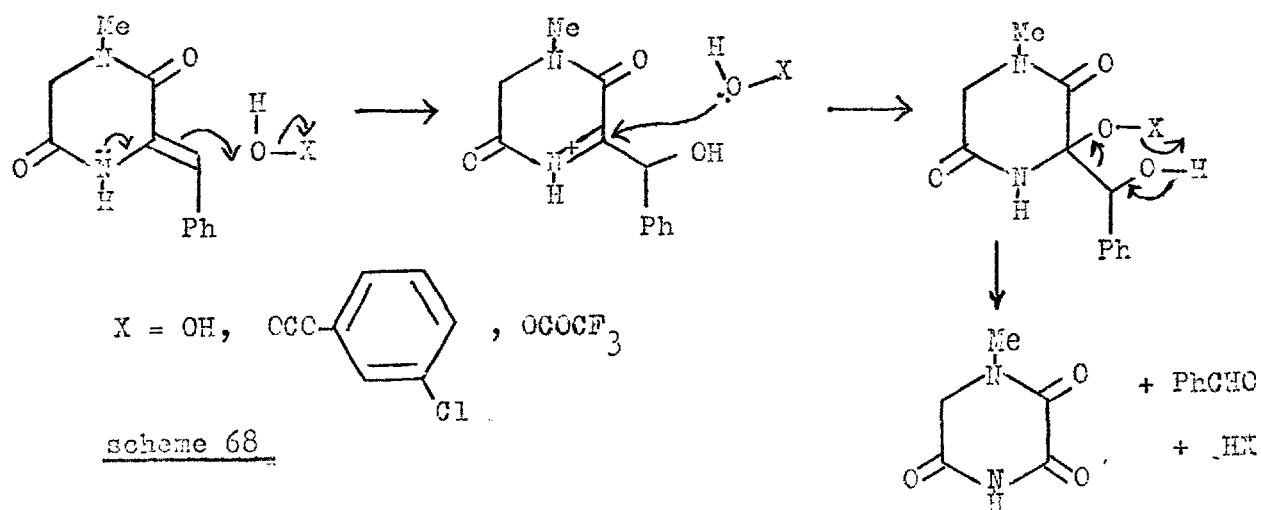


Considering the acidity of the 6-proton in the acylimine, it is possible that the borohydride first acts as a base and then reduces the intermediate pyrazinone to the observed product. A second product decomposed on chromatography but its crude n.m.r. spectrum indicated that reduction of the 5-amide



function had occurred.

(iv) Reactions with other oxidants. The benzylidene dioxopiperazine (110) is only susceptible to oxidation by highly electrophilic species. For example, no reaction occurred with N-bromosuccinimide (N.B.S.) nor with t-butyl hydroperoxide. With peracids and hydrogen peroxide cleavage of the double bond was always the major reaction affording the trioxopiperazine (117) as the main product (scheme 68).



(v) Conclusions. Although oxidation of the aromatic ring has not been achieved the chemistry described in the preceding sections may have biosynthetic significance. For example, the relative ease of conversion of the dehydro-unit into an α, β -difunctionalised residue may be of importance in the biosynthesis of several fungal metabolites e.g. lanosulin, verruculogen and the quinomycins, in which this disubstitution pattern is a structural feature. The acylimines described above are the first examples of a new class of compounds, the dihydrodioxopyrazines. They are also the first stable acylimines derived from simple peptide derivatives although it is probable that the recently described intermediates in the H.B.S. oxidation of acrylamido esters are in fact the corresponding bromo acylimines and not the N-bromoamides as reported⁶⁸.

EXPERIMENTAL

All melting points were determined on a Kofler hot stage and are uncorrected.

Infra-red (i.r.) spectra were recorded on a Perkin-Elmer 157G spectrophotometer for nujol mulls, unless otherwise stated, e.g. film or CHCl_3 solutions.

N.m.r. spectra were recorded, subject to certain stated exceptions, for solutions in deuteriochloroform, with tetramethylsilane as internal reference, using a Varian T60 instrument. The following abbreviations are used in connection with the n.m.r. spectra :

s = singlet	q = quartet
d = doublet	m = multiplet
t = triplet	b- = broadened ———
d- = double ———	

The chemical shifts of AB quartets are quoted as the weighted mid-points of each doublet. Coupling constants are quoted as J values (Hz).

Mass spectra were recorded on either an AEI MS9 or Perkin-Elmer PE270 mass spectrometer.

Where reactions were carried out in deuteriochloroform they were followed by n.m.r. spectroscopy; otherwise thin layer chromatography (tlc) was routinely used for following reactions. Preparative layer chromatography (plc) was carried out on silica plates (Merck Kieselgel GF₂₅₄) of 1mm thickness. Solvent mixtures are described in ratios of volumes.

Ultra-violet (u.v.) spectra were recorded on a Pye-Unicam SP800 spectrophotometer for ethanolic solutions, unless stated to the contrary.

Dichloromethane was dried over phosphorous pentoxide before distillation. D.M.F. was dried over anhydrous barium oxide before distillation. T.H.F. was dried over lithium aluminium hydride before distillation. Boron trifluoride etherate and opichlorohydrin were dried and distilled directly before use in the preparation of trialkyloxonium fluoroborates. Ether and benzene were dried over sodium wire, whilst acetone ('Analar' grade) was stored over 4A sieves.

'Petrol' refers to petroleum ether of boiling range 40-60°.

All organic solvent extracts were dried over anhydrous sodium sulphate before evaporation, unless stated to the contrary.

Singlet oxygen photooxygenations were performed in a 25 ml 3-knecked pear-shaped flask fitted with a gas-inlet tube and calcium chloride drying tubes. A slow stream of oxygen was passed through the dichloromethane solution whilst irradiating with visible light from a 350 Watt tungsten filament bulb. The flask was kept at room temperature by a stream of cold water directed on its outsides. Methylene blue, 1-2 mg, was used as sensitiser.

Thiolacetic acid was distilled directly before use.

Sodium hydride, stored as an oil dispersion, was trituated with dry hexane before use.

1,3-Dimethyl-2,5-dioxopiperazine (12).

Alanine (17.8 g.) was dissolved in N NaOH (200 ml.) and the solution cooled to 0°. Chloroacetyl chloride (20 ml.) and N NaOH (250 ml.) were added dropwise and simultaneously to the stirred solution over 30 minutes. After stirring for a further hour at 0°, N HCl (210 ml.) was added in one portion and the solution evaporated to dryness. The residue was extracted with hot acetone (4x200 ml.) which was filtered, dried and evaporated to yield 26.5 g. 80%, of the chloroamido acid as a white crystalline solid, $v_{\max}$ 3485, 3200-2400, 1730, 1635, 1555 cm^{-1} . This material (16.6 g.) in aqueous methylamine (130 ml. 25%) was stirred at 25° for 2 days. The solution was evaporated, triturated with ethanol, evaporated and the procedure repeated until the dipeptide crystallised. Filtration gave 12.1 g. 75%, of N-sarcosyl alanine. The crude dipeptide (10.1 g.) was suspended in methanol (90 ml.) and the mixture saturated with hydrogen chloride until complete solution was effected. After stirring for 20 hours at 25° the pale yellow solution was evaporated and the oily residue thoroughly degassed on the oil pump. This product, the amino ester hydrochloride, was dissolved in methanol (40 ml.), applied to an ion exchange column (50 g. Amberlite IR 45) and left overnight to effect cyclisation. The column was eluted with methanol and the eluate evaporated to yield pale yellow solids (9.2 g.). Recrystallisation from ethanol afforded colourless needles of the title compound, 6.35 g. 66%, m.p. 135° (lit.⁶⁹ m.p. 135°), $v_{\max}$ 3225, 3090, 1700, 1670, 1470, 1430, 1370, 1330, 1265, 1170 and 840 cm^{-1} , τ 2.30(1H,bs), 5.88(1H,m), 5.98(2H,s), 6.98(3H,s) and 8.49(3H,d, J 7 Hz.).

5-Ethoxy-1,3-dimethyl-3,6-dihydropyrazin-2(1H)-one (13).

1,3-Dimethyl-2,5-dioxopiperazine (2.0 g.) and triethyloxonium fluoro-borate (10.8 g.) in dichloromethane (60 ml.) were stirred at room temperature under nitrogen for 3 h. during which time a white solid appeared. The mixture was poured into cold, saturated NaHCO_3 solution (40 ml.) before separation and extraction of the aqueous phase with more dichloromethane. Evaporation of

the dried organic extracts gave an oil which on sublimation (0.05 Torr, 25°) afforded chunky crystals of the dihydropyrazinone (1.5 g. 64%), m.p. 58-59°, $v_{\max}$ 1710, 1650, 1520, 1465, 1415, 1380, 1275, 1237, 1160, 1053, 900 and 790 cm^{-1} δ 5.6-6.1(5H,m), 7.02(3H,s), 8.56(3H,d, J 7 Hz.), and 8.70(3H,t, J 7 Hz.) (Found: C,56.7; H,8.4; N,16.45. $\text{C}_8\text{H}_{14}\text{N}_2\text{O}_2$ requires C,56.45; H,8.3; N,16.5%).

5-Ethoxy-1,3-dimethylpyrazin-2(1H)-one (14).

5-Ethoxy-1,3-dimethyl-3,6-dihydropyrazin-2(1H)-one (0.40g.) and dichlorodicyanobenzoquinone (D.D.Q. 0.53 g.) in dry benzene (20 ml.) under nitrogen, were allowed to stand at room temperature for 50 minutes. The precipitated quinol was filtered off, the benzene evaporated and the crude product chromatographed through alumina (grade III, 15 g.) with dichloromethane as eluant. Evaporation of the eluate afforded the dienone (14) (0.152 g, 38%) as pale yellow needles, m.p. 61-75°, $v_{\max}$ 3100, 1660, 1600, 1420, 1370, 1340, 1328, 1257, 1190, 1100, 1050, 895, 860, 810 and 758 cm^{-1} , δ 2.40(1H,q, J 1 Hz), 5.89(2H,q, J 7 Hz), 6.48(3H,s), 7.53(3H,d, J 1 Hz) and 8.63(3H,t, J 7 Hz) and M^+ 168.

Reactions of the Pyrazinone (14).

(i) with dimethyl acetylene dicarboxylate (A.D.E.)— The pyrazinone (0.11 g) and the dienophile (0.09 g) in dichloromethane (15 ml) were heated at reflux under nitrogen for 20 h. The products were separated by preparative layer chromatography (plc) (19:1 CHCl_3 -MeOH) to give as the major band a slowly crystallising oil. Recrystallisation from acetone/petrol afforded white clusters of dimethyl 3-ethoxy-1,5-dimethyl-6-oxo-2,5-diazabicyclo[2.2.2]oct-2,7-diene-7,8-dicarboxylate (19) (0.118 g, 59%), m.p. 92-94°, $v_{\max}$ 1740, 1728, 1705, 1637, 1460, 1390, 1370, 1325, 1270, 1200, 1145, 1050, 1010, 815, 780 and 737 cm^{-1} , δ 4.87(1H,s), 5.78(2H,q, J 7 Hz), 6.10(3H,s), 6.15(3H,s), 7.05(3H,s) 8.22(3H,s) and 8.67(3H,t, J 7 Hz), $\lambda_{\max}$ 264.5 nm (ϵ 2400) (Found: C,54.2; H,5.8; N,8.9. $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_6$ requires C,54.2; H,5.9; N,9.0%).

Hydrolysis of the adduct (0.034 g) in 60% aqueous T.H.F. (4 ml) containing 2N HCl (0.5 ml) at room temperature for 10 min, followed by neutralisation with 2N NaOH (0.5 ml) and extraction with dichloromethane, afforded 2-ethyl 3,4-dimethyl 1,6-dihydro-1-methyl-6-oxopyridine-2,3,4-tricarboxylate (21) as colourless needles from acetone/petrol, (0.029 g, 85%), m.p. 68-63°, $\nu_{\max}$ 1740, 1732, 1725, 1662, 1605, 1545, 1443, 1400, 1378, 1265, 1255, 1205, 1178, 1108, 880, 795, 718 and 690 cm^{-1} , τ 5.53(2H,d, J 7 Hz), 6.07(3H,s), 6.20(3H,s), 6.45(3H,s), 7.87(3H,s) and 8.60(3H,t, J 7 Hz), $\lambda_{\max}$ 270 and 306 nm(12,000 and 6500) (Found: C,54.2; H,5.55; N,4.35. $\text{C}_{14}\text{H}_{17}\text{NO}_7$ requires C,54.0; H,5.5; N,4.5%).

(ii) with molecular oxygen — Passing air through a solution of the pyrazinone (0.114 g) in dichloromethane (20 ml) for 4 days, replenishing solvent as required, followed by evaporation, afforded a gummy solid which gave a positive peroxide test (starch-iodide). Its n.m.r. spectrum showed a methine proton signal at τ 6.65. The gummy solid crystallised from acetone/petrol to give colourless plates of 5-ethoxy-3-hydroxy-1,3-dimethylpyrazine-2(3H),6(1H)-dione (16), (0.114 g, 84%), m.p. 83-84°, $\nu_{\max}$ 3340, 1740, 1695, 1670, 1590, 1462, 1410, 1370, 1360, 1335, 1300, 1280, 1200, 1155, 1100, 1080, 1032, 1000, 988, 930, 867, 818, 795 and 760 cm^{-1} , τ 5.61(2H,q, J 7 Hz), 6.55(1H, bs, exchangeable), 6.72(3H,s), 8.35(3H,s) and 8.58(3H,t, J 7 Hz), $\lambda_{\max}$ 218 nm(< 12,000) (Found: C,48.0; H,6.1; N,13.7. $\text{C}_8\text{H}_{12}\text{N}_2\text{O}_4$ requires C,48.0; H,6.0; N,14.0%).

(iii) with cyclopentene — The pyrazinone (0.088 g) was completely dissolved in redistilled cyclopentene (4 ml), by the addition of a few drops of ethyl acetate, in a small carius tube. The tube was sealed, covered in aluminium foil and heated in a steam bath for 4 days. The solution was evaporated and the resultant yellow oil subjected to plc (1:1 EtOAc: C_6H_6) which gave the hydroxy imide (16) (28 mg, 27%) as the only discrete band. The silica from the origin to Rf 0.35 was scraped off and from it a colourless oil (0.061 g) was isolated. After n.m.r. examination the oil was chromatographed (EtOAc)

affording two discrete bands. The less polar of these proved to be 11-ethoxy-1,8-dimethyl-8,10-diazatricyclo[5.2.2.0^{2,6}]undec-10-en-9-one (24) which after crystallisation (petrol) and sublimation(x2, 75⁰, 0.1 Torr) (0.021 g, 17%) exhibited m.p. 36-43⁰, $\nu_{\text{max}}^{\text{film}}$ 3000-2870, 1675, 1640, 1445, 1410, 1375, 1348, 1315, 1260, 1205, 1188, 1070, 1030, 887, 815 and 773 cm⁻¹, τ 5.72(2H,q, J 7 Hz), 6.21(1H,d, J 3 Hz), 7.07(3H,s), 7.28-8.83(8H,m), 8.45(3H,s) and 8.63(3H,s), m/e 236(M⁺, 22%), 179(80), 168(65), 164(94), 150(100), 122(60) and 42(70), (Found: C, 66.3; H, 8.45; N, 11.9. C₁₃H₂₀N₂O₂ requires C, 66.1; H, 8.5; N, 11.85%).

The more polar band gave colourless prisms from acetone (0.018 g, 17%) of 1,8-dimethyl-8,10-diazatricyclo[5.2.2.0^{2,6}]undecane-9,11-dione (25), m.p. 211-212⁰, ν_{max} 3170, 3080, 1700, 1680, 1455, 1395, 1385, 1233, 1068, 762 and 720 cm⁻¹ τ 3.30(1H,bs), 6.25(1H,m), 6.92(3H,s), 7.2-8.8(8H,m) and 8.57(3H,s), m/e 208 (M⁺, 80%), 165(30), 140(100), 137(60), 123(35), 108(80), 93(55), 71(40) and 42(95) (Found C, 63.3; H, 7.6; N, 13.45. C₁₁H₁₆N₂O₂ requires C, 63.4; H, 7.7; N, 13.45%).

N.m.r. examination of the initial reaction mixture showed no peaks due to the hydrolysed adduct (25). However, after treatment of the mixture with a little silica gel in wet chloroform, the hydrolysed adduct was shown to be formed. The ratio of the adducts (24) and (25) was ca. 1:1.

1,3,6-Trimethyl-2,5-dioxopiperazine (28)

Alanine (17.8 g) was dissolved in N NaOH (200 ml) and the solution treated at 0⁰ with 2-chloropropionyl chloride (25 ml) and more N NaOH (250 ml) simultaneously over 30 min. The stirred solution was rigorously maintained below 0⁰ for a further 90 min. and then acidified with N HCl (210 ml). The solution was evaporated to dryness and left on the oil pump overnight to remove traces of water. The sticky white solid was extracted with acetone (3x200 ml), filtered, dried and evaporated to give a white solid, the chloro-amido acid, 25 g, ν_{max} 3350, 3310, 3000-2400, 1710, 1665, 1565. The crude product was stirred with aqueous methylamine (250 ml, 25%) at room temperature

for 5 days. After evaporation of the water, drying was completed by repeated trituration with acetone and evaporation followed by drying on the oil pump. Subsequent trituration with methanol afforded the crude dipeptide (10 g) as white crystals, $\nu_{\max}$ 3375, 3240; 3200-2200, 1670, 1610, 1530 cm^{-1} . The dipeptide was suspended in ethylene glycol and heated at reflux for 16 h. After cooling, the solution was extracted with dichloromethane (3x100 ml) and thoroughly dried with powdered anhydrous calcium chloride by stirring the suspension vigorously until the solids became finely divided (ca. 30 min). Evaporation gave a colourless oil (6.7 g, 79%) which rapidly crystallised. A sample was separated by plc (9:1 CHCl_3 -MeOH). The least polar isomer was assigned as trans-1,3,6-trimethyl-2,5-dioxopiperazine (28), colourless needles from acetone/petrol, m.p. 96-97°, $\nu_{\max}$ 3190, 3050, 1675, 1635, 1365, 1325, 1300, 1250, 1160, 840 and 770 cm^{-1} , τ 2.78(1H,bs), 5.93(1H,q, J 7 Hz), 6.11(1H,q, J 7 Hz), 7.02(3H,s) and 8.51(6H,d, J 7 Hz) (Found: C,53.9; H,7.7; N,18.2 $\text{C}_7\text{H}_{12}\text{N}_2\text{O}_2$ requires C,53.8; H,7.7; N,17.9%).

The more polar material was recrystallised from acetone/petrol to give the cis-isomer, white clusters, m.p. 132°, $\nu_{\max}$ 3170, 3080, 1680, 1660, 1375, 1370, 1320, 1250, 1150, 1090, 1050, 835, 820 and 770 cm^{-1} , τ 2.68(1H,bs), 5.89(1H,dq, J 2,7 Hz), 6.09(1H,q, J 7 Hz), 7.02(3H,s), 8.44(3H,d, J 7 Hz) and 8.49(3H,d, J 7 Hz) (Found: C,53.7; H,7.7; N,18.1%).

The crude mixture of isomers was recrystallised from T.H.F. to give a ca. 1:1 mixture as large prisms.

5-Methoxy-1,3,6-trimethyl-3,6-dihydropyrazin-2(1H)-one (29)

The mixture of cis- and trans-dioxopiperazines (28) (3.6 g) was treated with trimethyloxonium fluoroborate (4.5 g) in dichloromethane (60 ml) at room temperature for 4 h with vigorous stirring. The resulting solution was poured onto cold, saturated NaHCO_3 solution (25 ml) and the organic layer separated. The aqueous phase was re-extracted with dichloromethane (40 ml) and the combined organic phase washed with water (30 ml), dried and evaporated to

yield a colourless mobile oil (2.63 g, 81%). Tlc examination indicated the presence of only two compounds, in similar proportions, corresponding to the cis- and trans- isomers of the title compound. The mixture exhibited $\nu_{\max}$ 1695, 1650, 1490, 1375, 1350, 1315, 1260, 1095, 990, 960 and 755 cm^{-1} , τ 5.58-6.22 (2H,m), 6.27(3H,s), 7.03(3H,s) and 8.40-8.65(6H,m).

Oxidation of the 1,3,6-trimethyl imidate with D.D.Q.

These oxidations offered little consistency with respect to yields and product distribution.

A sample (0.78 g) of the imidate was oxidised with D.D.Q. (1.05 g) in refluxing benzene (100 ml) with stirring for 24 h. The mixture was cooled, precipitated quinol was filtered off and the filtrates evaporated. The residue was chromatographed through alumina (100 g, grade III), eluting with benzene and then with 1:19 acetone-benzene. Three fractions were obtained. The first product was yellow needles (28 mg, 1.5%) of the unstable bismethylenepyrazinone (33), which had τ 3.85(1H,d, J 1.5 Hz), 4.38(1H,d, J 1.5 Hz), 4.62(1H,d, J 2 Hz), 5.20(1H,d, J 2 Hz), 6.08(3H,s) and 6.75(3H,s). This compound rapidly polymerised precluding further characterisation.

The second compound eluted from the column was 5-methoxy-1,3-dimethyl-6-methylene-3,6-dihydropyrazin-2(1H)-one (31) (98 mg, 12%) isolated as a pale yellow oil, $\nu_{\max}^{\text{film}}$ 3000-2840, 1680, 1615, 1355, 1278, 1180, 1110, 1030, 980 and 865 cm^{-1} , τ 4.78(1H,d, J 2 Hz), 5.35(1H,d, J 2 Hz), 5.71(1H,q, J 7 Hz), 6.28(3H,s), 6.88(3H,s) and 8.59(3H,d, J 7 Hz). Hydrolysis of the imidate (31) was accomplished as follows. The imidate (132 mg) was stirred with 60% aqueous T.H.F. (10 ml) containing 2N HCl (1 ml) at room temperature for 16 h. The solution was basified with aqueous NaHCO_3 and extracted with dichloromethane. After drying the organic extract gave 1,3-dimethyl-6-methylene-2,5-dioxopiperazine (34) (134 mg, 80%) as colourless needles from acetone/petrol, m.p. 117-119°, $\nu_{\max}$ 3190, 3040, 1690, 1610, 1455, 1428, 1382, 1374, 1365, 1340, 1295, 1150, 1025, 875, 855 and 755 cm^{-1} , τ 2.43(1H,bs), 4.20(1H,d, J 1.5 Hz), 5.05

(1H,d, J 1.5 Hz), 5.73(1H,dq, J 2,7 Hz), 6.78(3H,s) and 8.44(3H,d, J 7 Hz),

$\lambda_{\max}$ 227 nm(ϵ 14,500) (Found: C,54.7; H,6.6; N,18.0. $C_7H_{10}N_2O_2$ requires C,54.5; H,6.5; N,18.2%).

The final product from the oxidation, eluted from the column as an oil, using the acetone-benzene eluant, was assigned as the pyrazinone (30) (120 mg, 16%) and exhibited λ 6.17(3H,s), 6.47(3H,s) and 7.60 and 7.70(6H,s). This material was acutely unstable to storage even under an inert atmosphere. It was further characterised by refluxing with A.D.E. (200 mg) in dichloromethane (10 ml) for 40 h. Purification by plc gave the cycloaddition adduct (38) as a colourless oil (76 mg), λ 6.17(6H,s), 6.20(3H,s), 7.13(3H,s), 8.10(3H,s), and 8.15(3H,s).

Two other products have been isolated from similar oxidations after extensive chromatography. The crystalline derivative assigned as 5-methoxy-1,3-dimethyl-3-hydroperoxy-6-methylene-3,6-dihydropyrazin-2(1H)-one (36) showed $\nu_{\max}$ 3300, 1685, 1615, 1468, 1380, 1370, 1360, 1280, 1210, 1150, 1120, 1060, 900 and 895 cm^{-1} , λ 4.62(1H,d, J 2 Hz), 5.18(1H,d, J 2 Hz), 6.12(3H,s), 6.77(3H,s), 6.78(1H,s) and 8.47(3H,s), m/e 200(M^+ , 0.1%), 199(1.3), 198(12), 184(9), 183(100), 169.3(M^*), 167(12), 155(11), 141(6) and 139(13). The dimeric material (37) afforded orange needles upon recrystallisation from acetone/petrol, m.p. 175-178°, $\nu_{\max}$ 3100, 1685, 1645, 1605, 1465, 1420, 1405, 1380, 1362, 1352, 1335, 1293, 1280, 1233, 1220, 1200, 1180, 1160, 1108, 1060, 1038, 1002, 990, 974, 955, 895, 880, 830, 802, 773, 755, 740, 730 and 670 cm^{-1} λ 3.73(1H,t, J 3 Hz), 4.25(1H,d, J 2 Hz), 4.52(1H,d, J 2 Hz), 5.08(2H,d, J 2 Hz), 6.22(3H,s), 6.73(3H,s), 6.75(3H,s), 6.62 and 7.22(2H,dABq, J 3,18 Hz), m/e 316 (M^+) (Found: C,56.8; H,5.0; N,17.7. $C_{15}H_{16}N_4O_4$ requires C,57.0; H,5.1; N,17.7%).

1,6-Dimethyl-3-benzyl-2,5-dioxopiperazine (41)

Phenylalanine (33.2 g) was dissolved in N NaOH (200 ml) and the solution treated at 0° with 2-chloropropionyl chloride (25 ml) and more N NaOH (250 ml)

simultaneously over 30 min. The stirred solution was rigorously maintained below 0° for a further 90 min and then acidified with N HCl (210 ml). The solution was evaporated to dryness and left on the oil pump overnight to remove traces of water. The residue was extracted with acetone (3x200 ml), filtered, dried and evaporated to yield an oil which rapidly crystallised. Trituration with ether afforded white crystals of the chloroamido acid (22.4 g 44%) $\nu_{\max}$ 3330, 3200-2400, 1720, 1645, 1555 cm^{-1} . This product was stirred with aqueous methylamine (250 ml, 25%) at room temperature for 5 days. The solution was evaporated and dried thoroughly on the oil pump and the resultant solids triturated with methanol. The crude dipeptide thus obtained (19.4 g, 89%), $\nu_{\max}$ 3300, 3200-2200, 1675, 1630, 1540 cm^{-1} , was suspended in ethylene glycol (60 ml) and heated at reflux for 16 h. After cooling the dark brown solution was extracted with dichloromethane (3x200 ml), dried thoroughly with anhydrous calcium chloride and evaporated to yield 13.1 g, 77% of a pale brown gum which crystallised on prolonged scratching. A sample was separated by plc (19:1 CHCl_3 -MeOH). The least polar isomer was recrystallised (acetone/petrol) to give long colourless needles of trans-1,6-dimethyl-3-benzyl-2,5-dioxopiperazine (41b), m.p. $132-133^{\circ}$, $\nu_{\max}$ 3190, 3060, 1675, 1665, 1400, 1370, 1340, 1310, 1205, 1180, 1078, 1040, 868, 860, 760, 747 and 700 cm^{-1} , τ 2.68 (5H,bs), 3.32(1H,bs), 5.70(1H,m), 6.28-7.20(3H,m), 7.07(3H,s) and 8.56(3H,d, J 7 Hz) (Found: C,67.1; H,6.8; N,12.0. $\text{C}_{13}\text{H}_{16}\text{N}_2\text{O}_2$ requires C,67.2; H,6.9; N, 12.1%).

The more polar compound crystallised from acetone/petrol as colourless needles of the cis-isomer, m.p. $161-162^{\circ}$, $\nu_{\max}$ 3230, 3080, 1680, 1640, 1400, 1340, 1330, 1320, 1300, 1205, 1084, 1070, 1045, 745 and 700 cm^{-1} , τ 2.70(6H, bs), 5.67(1H,m), 6.24(1H,q, J 7 Hz), 6.82(2H,dABq), 7.12(3H,s) and 9.18(3H,d, J 7 Hz) (Found: C,67.05; H,6.8; N,12.0%).

The crude mixture of isomers was recrystallised from acetone/petrol to give a ca. 1:1 mixture as prisms.

3-Benzyl-5-methoxy-1,6-dimethyl-3,6-dihydropyrazin-2(1H)-one (42).

The mixture of cis- and trans-dioxopiperazines (41) (5.8 g) was stirred vigorously with trimethyloxonium fluoroborate (5.9 g) in dichloromethane (100 ml) at room temperature for 3 h. The resulting solution was poured onto cold, saturated NaHCO_3 solution (40 ml) and the organic layer separated. The aqueous phase was re-extracted with dichloromethane (60 ml) and the combined organic phase washed with water (40 ml), dried and evaporated to yield a slightly discoloured oil. This was dissolved in hot petrol and the coloured solid impurity removed by filtration. Evaporation of the petrol gave a crystallising oil (6.1 g, 99%). Tlc examination of the product indicated the presence of two compounds only. A sample was separated by plc (1:1 acetone-n-hexane). The more polar product was identified as cis-3-benzyl-5-methoxy-1,6-dimethyl-3,6-dihydropyrazin-2(1H)-one (42a), isolated as white clusters from petrol, m.p. $92-94^\circ$, ν_{max} 1700, 1630, 1490, 1445, 1310, 1265, 1240, 1090, 1000, 850, 745 and 695 cm^{-1} , τ 2.80(5H,s), 5.52(1H,m), 6.27(3H,s), 6.33-7.10 (3H,m), 7.20(3H,s) and 9.58(3H,d, J 7 Hz) (Found: C,68.4; H,7.4; N,11.6. $\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}_2$ requires C,68.3; H,7.4; N,11.4%).

The less polar material was the corresponding trans-isomer (42b), isolated as an oil, $\nu_{\text{max}}^{\text{film}}$ 3080, 1695, 1645, 1495, 1450, 1430, 1260, 1235, 1090, 745 and 700 cm^{-1} , τ 2.80(5H,s), 5.72(1H,m), 6.30(3H,s), 6.47-6.93(3H,m), 7.17(3H,s) and 8.64(3H,d, J 7 Hz), m/e 246(M^+ ,100%), 231(20), 189(5.5), 174(6.5), 155(44), 127(73) and 91(40).

Oxidation of the 3-benzyl-1,6-dimethyl imidate with D.D.Q.

Again these oxidations offered little consistency with respect to yields and product distribution.

The cis- and trans-imidate mixture (2.0 g) was oxidised with D.D.Q. (1.0 g) in refluxing benzene (100 ml) with stirring for 24 h. After cooling, the precipitated quinol was filtered off, the filtrates evaporated to small bulk and then chromatographed through alumina (150 g, grade III), using benzene as

eluent. The separated products, in order of elution, were as follows.

3,1'-E-3-Benzylidene-5-methoxy-1-methyl-6-methylene-3,6-dihydropyrazin-2(1H)-one (46), (336 mg, 17%), as orange needles from petrol, m.p. 103-105°, $v_{\max}$ 3050, 1673, 1630, 1592, 1492, 1460, 1445, 1430, 1340, 1272, 1175, 1123, 1063, 990, 835, 768, 755, 740 and 690 cm^{-1} , $\lambda_{\max}$ 231, 236, 244, 322, 335 and 348 nm (ϵ 6800, 7900, 7300, 21,000, 25,000, and 23,000), τ 1.85(2H,m), 2.60(4H,m), 4.65(1H,d, J 2 Hz), 5.20(1H,d, J 2 Hz), 5.98(3H,s) and 6.70(3H,s) (Found: C,69.2; H,5.8; N,11.6. $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_2$ requires C,69.4; H,5.8; N,11.6%).

There followed 3-benzyl-5-methoxy-1-methyl-6-methylene-3,6-dihydropyrazin-2(1H)-one (45) (264 mg, 13%), as a yellow oil, $v_{\max}^{\text{film}}$ 3080-2840, 1685, 1609, 1450, 1430, 1350, 1270, 1150, 1090, 985, 852, 747 and 695 cm^{-1} , τ 2.8(5H,s), 5.05(1H,d, J 2 Hz), 5.62(1H,d, J 2 Hz), 5.32(1H,t, J 5 Hz), 6.20(3H,s), 6.77(2H,d, J 5 Hz) and 7.00(3H,s). Hydrolysis of the imidate (45) was accomplished as follows. The imidate (264 mg) was stirred with 60% aqueous T.H.F. (30 ml) containing 2N HCl (5 ml) at room temperature for 4 h. The solution was basified with aqueous NaHCO_3 and extracted with dichloromethane. After drying the organic extract gave 3-benzyl-1-methyl-6-methylene-2,5-dioxopiperazine (47) (251 mg, 100%), as white clusters from acetone/petrol, m.p. 133-137°, $v_{\max}$ 3190, 3060, 1690, 1608, 1425, 1380, 1340, 1155, 875 and 698 cm^{-1} , $\lambda_{\max}$ 233 nm (ϵ 14,000), τ 2.20(1H,bs), 2.80(5H,m), 4.50(1H,d, J 1.5 Hz), 5.38(1H,d, J 1.5 Hz), 5.67(1H,dt, J 2,5 Hz), 6.97(2H,d, J 5 Hz) and 6.98(3H,s). (Found: C,67.7; H,6.1; N,12.2. $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_2$ requires C,67.8; H,6.1; N,12.2%).

3-Benzyl-5-methoxy-1,6-dimethylpyrazin-2(1H)-one (44) (305 mg, 15%), was obtained finally as an oil, contaminated with starting material. It exhibited τ 2.73(5H,s), 5.90(2H,s), 6.20(3H,s), 6.55(3H,s) and 7.78(3H,s). The dienone was characterised by reaction of the freshly obtained material (150 mg) with A.D.E. (200 mg) in dichloromethane (20 ml) at reflux for 40 h. The solvent was removed and the residue purified by plc (1:9 acetone-benzene) to give dimethyl 5,7-diaza-4-benzyl-6-methoxy-1,7-dimethyl-8-oxobicyclo[2.2.2]octa-2,5-diene-2,3-dicarboxylate (48) (150 mg), as colourless prisms from acetone/petrol

m.p. 131-132°, $\nu_{\max}$ 1742, 1725, 1622, 1638, 1620, 1500, 1450, 1435, 1420, 1390, 1368, 1320, 1275, 1230, 1105, 1037, 1010, 970, 790, 742 and 640 cm^{-1} , τ 2.43 (2H,m), 2.73(3H,m), 6.15(3H,s), 6.28(3H,s), 6.30(2H,s), 6.50(3H,s), 7.18(3H,s) and 8.17(3H,s) (Found: C,62.5; H,5.7; N,7.2. $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_6$ requires C,62.2; H,5.7; N,7.25%).

Hydrolysis of the 3-benzylidene-6-methylene imidate (46)

The imidate (52 mg) in 60% aqueous T.H.F. (2 ml) containing 2N HCl (0.5 ml) was stirred at room temperature for 5 days. Workup of the mixture in the usual manner gave 3,1'-E-3-benzylidene-1-methyl-6-methylene-2,5-dioxopiperazine (50) (50 mg, 100%), as yellow prisms from acetone, m.p. 145-154° (decomp.), $\nu_{\max}$ 3290, 3200, 1680, 1625, 1605, 1500, 1450, 1370, 1230, 1185, 1140 and 905 cm^{-1} , $\lambda_{\max}$ 232, 236 and 316 nm(ϵ 8400, 8200 and 23,000) (Found: C,68.25; H,5.5; N,12.0. $\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_2$ requires C,68.4; H,5.3; N,12.3%). The material was too insoluble in deuteriochloroform to record an n.m.r. spectrum. The compound was dissolved in trifluoroacetic acid and then recovered by evaporation to give a colourless substance, which was isomeric to the starting compound (less polar on tlc with 1:19 MeOH- CHCl_3). The new compound was recrystallised from ethanol to give colourless needles of the 3,1'-Z-isomer (51) m.p. 115-117°, $\nu_{\max}$ 3260, 1620, 1625, 1605, 1480, 1430, 1400, 1320, 1255, 1200, 1140 and 905 cm^{-1} , $\lambda_{\max}$ 231, 235, and 313 nm(ϵ 30,000, 13,000 and 13,000), τ 1.80(1H, bs), 2.53(5H,s), 2.83(1H,s), 4.12(1H,d, J 1.5 Hz), 4.97(1H,d, J 1.5 Hz) and 6.65(3H,s) (Found: C,68.3; H,5.3; N,12.2%).

Reaction of 3,6-dibenzyl-2,5-dihydroxypyrazine with sulphur.

3,6-Dibenzyl-2,5-dihydroxypyrazine⁴ (1.0 g) and elemental sulphur (0.22 g) were suspended in dimethyl formamide (D.M.F.) (40 ml). On addition of triethylamine (10 ml) the yellow suspension turned through red and purple to dark brown. The mixture was stirred vigorously, whilst heating at 60° for 2 h and then at room temperature overnight. After cooling in ice, the yellow precipitate

was filtered off, washed well with water and dried in vacuo to yield 3,6-bis-benzylidene-2,5-dioxopiperazine (60) (0.59 g, 60%). Recrystallisation from D.M.F. afforded pale yellow needles which had m.p. 294-297° (lit.²⁵ m.p. 298-300°), $\nu_{\max}$ 3220, 1624, 1635, 1495, 1465, 1410, 1360, 855, 780, 735, 720 and 680 cm^{-1} , χ (T.F.A.) 2.7-3.25(m), $\lambda_{\max}$ 234 and 338 nm (ϵ 8000, and 30,000), m/e 290(M^+ , 100%), 118(87), 117(49), 116(13), 91(27), 90(28) and 89(20).

The reaction was repeated without the sulphur and the product isolated in the same manner was shown to be 3-benzylidene-6-benzyl-2,5-dioxopiperazine²⁵ (61) identical in all respects with an authentic sample of this compound, which was used in the preparation of the dihydroxypyrazine⁴.

cis-3,6-Dibenzyl-2,5-dioxopiperazine.

L-Phenylalanine (10 g) was suspended in ethylene glycol (20 ml) and heated under reflux for 24 h. The mixture was cooled in ice, filtered and the colourless needles washed well with methanol, then ether and dried to yield 7.3 g, 82%. Its properties were identical to those of an authentic sample²⁷.

Cis-trans-1,4-diacetyl-3,6-dibenzyl-2,5-dioxopiperazine (63)

Cis-3,6-dibenzyl-2,5-dioxopiperazine (2 g) was suspended in acetic acid (10 ml) and acetic anhydride (20 ml) and heated at reflux for 5 h. The yellow solution was poured onto ice (100 g) and kept at 0° overnight. The precipitate was filtered and dried. Recrystallisation from ether afforded a mixture of the cis- and trans-isomers of the title compound as white crystals (1.8 g, 72%) which had m.p. 145-151°, $\nu_{\max}$ 1712, 1460, 1380, 1222, 1140, 752, 736 and 694 cm^{-1} , χ 2.60-2.93(10H,m), 4.65(2H,m), 7.20-7.45(4H,m) and 7.48(6H,s).

Reaction of the diacetyl derivative (63) with sulphur.

The 1,4-diacetyl dioxopiperazines (0.57 g) and elemental sulphur (0.09 g) in D.M.F. (4 ml) and triethylamine (1 ml) were stirred at 60° for 2 h and then

at room temperature overnight. The dark brown solution was evaporated to dryness under reduced pressure (oil pump) affording a brown gum. Hydrolysis of this product was accomplished by stirring with N methanolic KOH (25 ml) for 2 h at room temperature. The suspension was acidified with 2N H_2SO_4 , the solids filtered, washed thoroughly with water and methanol and dried to yield 3-benzylidene-6-benzyl-2,5-dioxopiperazine (61) (0.21 g, 48%) identical to an authentic sample.

1,4-Diacetyl-3-benzyl-2,5-dioxopiperazine (65)

3-Benzyl-2,5-dioxopiperazine⁴ (16.4 g) was suspended in acetic anhydride (100 ml) and heated at reflux for 2 h. The cooled, yellow solution was poured onto ice and kept at 0° for 4 h. The crude product was filtered off, treated with decolourising charcoal and recrystallised from aqueous ethanol (1:1) affording white crystals of the title compound (65) (17.7 g, 77%), m.p. 85°, v_{max} 1725, 1465, 1405, 1385, 1372, 1310, 1255, 1230, 1200, 1140, 1050, 760 and 710 cm^{-1} , τ 2.50-3.00(5H,m), 4.52(1H,t, J 5 Hz), 5.46 and 7.46 (2H,ABq, J 19 Hz), 6.73(2H,m), 7.40(3H,s) and 7.42(3H,s).

Reaction of the diacetyl derivative (65) with sulphur.

The 1,4-diacetyl dioxopiperazine (1.73 g) and sulphur (0.38 g) in D.M.F. (14 ml) and triethylamine (4 ml) were stirred at 60° for 4 h. The resultant yellow precipitate was filtered off from the dark brown mixture, washed with methanol and chloroform to yield a bright yellow solid (0.3 g, 25%) which showed m.p. > 320°, v_{max} 3285, 3160, 1660, 1625, 1465, 1390, 985, 760 and 690 cm^{-1} , m/e 400(M^+ , 26%), 399(100), 371(10), 345(M^+), 228(12), 227(10), 118(40), 117(28), 91(24), 90(14) and 89(10), τ (T.F.A.) 2.93-3.21(m), yellow needles readily crystallised out of the n.m.r. solution and their microanalysis was most consistent with the dimeric structure (66) + one mole of T.F.A.:
Found: C, 56.2; H, 3.8; N, 11.3. $C_{24}H_{17}N_4O_6F_3$ requires C, 56.0; H, 3.3; N, 10.9%.

Cis-trans-3,6-dimethyl-2,5-dioxopiperazine.

D,L-Alanine (10 g) was suspended in ethylene glycol (20 ml) and heated under reflux for 24 h. The mixture was cooled in ice, filtered and the colourless plates washed well with methanol, then ether and dried to yield 5.3 g, 68%. Its properties were identical to those of an authentic sample²⁷.

Cis-trans-1,4-diacetyl-3,6-dimethyl-2,5-dioxopiperazine (67).

Cis-trans-3,6-dimethyl-2,5-dioxopiperazine (2.8 g) was suspended in acetic anhydride (15 ml) and heated at reflux for 4 h. The yellow solution was poured onto ice and after 30 min the white crystals were filtered, washed with water and dried to yield a mixture of the cis- and trans-isomers of the title compound (3.5 g, 77%), $\nu_{\max}$ 1720, 1470, 1400, 1380, 1302, 1240, 1150, 1110, 1020, and 968 cm^{-1} , the n.m.r. spectrum clearly showed both isomers and the cis-isomer was assigned τ 4.75(2H,q, J 7 Hz), 7.43(6H,s) and 8.38(6H,d, J 7 Hz). The trans-isomer had τ 5.03(2H,q, J 7 Hz), 7.50(6H,s) and 8.41(6H,d, J 7 Hz).

Reaction of these derivatives with sulphur and triethylamine in D.M.F. gave isomerisation to the more stable cis-isomer as the sole reaction. This epimerisation was also effected by triethylamine alone.

Reaction of proline anhydride with D.D.Q.

Cis-trans-proline anhydride²⁹ (194 mg) and D.D.Q. (227 mg) in benzene (20 ml) were heated at reflux for 5 days. The precipitated quinol was filtered off and the filtrates evaporated. The residue was triturated with dichloromethane (5 ml) and the coloured crystals filtered off (46 mg, 100%). Recrystallisation from benzene gave pale yellow plates of pyrocoll³¹ (69), m.p. 272° (lit.³¹ m.p. 272.5°) $\nu_{\max}$ 3130, 1705, 1560, 1460, 1430, 1415, 1330, 1224, 1155, 1150, 1072, 780, 770, 765 and 740 cm^{-1} , τ 2.18(2H,dd, J 1.3,3.0 Hz), 2.55(2H,dd, J 1.3,3.0 Hz) and 3.45(2H,t, J 3.0 Hz) $\lambda_{\max}^{\text{H}_2\text{O}}$ 235.5, 276.5, 308 and 322 nm(ϵ 26,000, 16,000, 14,000 and 15,000).

Cis-trans-1,3,4,6-tetramethyl-2,5-dioxopiperazine (70)

Cis-trans-3,6-dimethyl-2,5-dioxopiperazine (12 g) and dimethyl sulphate (50 ml) were stirred vigorously in water (500 ml) containing KOH (20 g) for 16 h. The solution was extracted with dichloromethane (3x300 ml), dried and evaporated to yield a white crystalline solid (5.76 g, 40%). The mixture of isomers was recrystallised from acetone/petrol as large prisms, m.p. 116° , $\nu_{\max}$ 1650, 1490, 1455, 1415, 1408, 1370, 1362, 1330, 1305, 1258, 1173, 1093, 1062, 1040, 895, 750, 740 and 710 cm^{-1} , τ 6.07(2H,q, J 7 Hz), 7.02(6H,s), 8.48(6H,d, J 7 Hz) and 6.02(2H,q, J 7 Hz), 7.02(6H,s), 8.44(6H,d, J 7 Hz) (Found: C,56.7; H,8.55; N,16.4. $\text{C}_8\text{H}_{14}\text{N}_2\text{O}_2$ requires C,56.45; H,8.3; N,16.5%).

Methylation of 3,6-dibenzyl-2,5-dioxopiperazine.

Cis-trans-3,6-dibenzyl-2,5-dioxopiperazine (0.88 g) and NaH (0.28 g, 50% oil dispersion) were stirred together in D.M.F. (5 ml) at room temperature for 15 min. The suspension was cooled to 0° and methyl iodide (2 ml) was slowly added whilst vigorous stirring was maintained. The viscous solution was stirred for 3 h at room temperature then poured into water (50 ml), extracted with dichloromethane (2x50 ml), dried and evaporated to yield white crystals of cis-trans-1,4-dimethyl-3,6-dibenzyl-2,5-dioxopiperazine (71) (0.72 g, 75%). The ratio of the isomers was 2:1 in favour of the trans-compound which showed τ 2.75(10H,m), 5.93(2H,dd, J 4.5,6 Hz), 7.07(2H,dd, J 4.5,15 Hz), 7.25(6H,s), 7.76(2H,dd, J 6,15 Hz). The less polar cis-isomer showed τ 2.75(10H,m), 6.60(2H,m), 6.90(4H,m) and 7.15(6H,s).

A similar large scale reaction using 5.9 g of the dioxopiperazine was stirred for 20 h after the addition of the methyl iodide. Workup afforded the crude product (5.0 g) which on recrystallisation from acetone/petrol gave colourless needles of the trans-isomer (71) (2.4 g, 38%) (one spot on tlc), m.p. 161° , $\nu_{\max}$ 1645, 1610, 1500, 1460, 1400, 1355, 1340, 1265, 1255, 1050, 930, 762, 750, 740 and 700 cm^{-1} , n.m.r. as above.

Evaporation of the mother liquors and recrystallisation of the residue

from acetone/petrol gave colourless prisms of cis-3,6-dibenzyl-1,3,4-trimethyl-2,5-dioxopiperazine (74) (1.5 g, 24%), m.p. 163° , $\nu_{\max}$ 1645, 1630, 1605, 1500, 1455, 1395, 1375, 1340, 1290, 1285, 1240, 1140, 1075, 765, 750, 730, 695 and 655 cm^{-1} , χ 2.50-3.12(10H,m), 6.05(1H,dd, J 4,8 Hz), 6.93(3H,s), 6.86 and 7.07(2H,ABq, J 14 Hz), 7.48(3H,s), 7.52(1H,dd, J 4,14.5 Hz), 8.32(3H,s) and 8.78(1H,dd, J 8,14.5 Hz) (Found: C,74.85; H,7.15; N,8.1. $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_2$ requires C,75.0; H,7.2; N,8.3%).

Oxidations with lead tetraacetate (L.T.A.)

(i) With cis-trans-1,3,4,6-tetramethyl-2,5-dioxopiperazine — The dioxopiperazine (0.72 g) and L.T.A. (3.78 g) were stirred together in dry benzene (50 ml) under reflux for 20 h. The cooled mixture was filtered, the lead diacetate washed well with benzene and the filtrates washed with water (25 ml). After drying and evaporation the resultant oily crystals were triturated with ether to give a white solid (0.2 g). This proved to be two compounds (tlc) which were separated by plc (1:19 MeOH- CHCl_3). The less polar band crystallised from carbon tetrachloride as needles of 1,4,6-trimethyl-3-acetoxymethyl-3,6-diacetoxy-2,5-dioxopiperazine (79) (95 mg, 9%) m.p. 163° , $\nu_{\max}$ 1768, 1747, 1690, 1452, 1428, 1390, 1372, 1325, 1285, 1230, 1218, 1130, 1095, 1076, 992 and 776 cm^{-1} , χ 5.10 and 5.50(2H,ABq, J 12 Hz), 6.95(3H,s), 7.02(3H,s), 7.88 (6H,s), 7.90(3H,s) and 8.15(3H,s) (Found: C,48.8; H,5.8; N,8.1. $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_8$ requires C,48.8; H,5.9; N,8.1%).

The more polar band gave 1,4,6-trimethyl-3-acetoxymethyl-3-acetoxy-2,5-dioxopiperazine (80) (80 mg, 9%) as needles from acetone/petrol, m.p. $166-167^{\circ}$, $\nu_{\max}$ 1740, 1680, 1460, 1395, 1375, 1335, 1315, 1262, 1225, 1155, 1059, 1012, 870 and 778 cm^{-1} , χ 5.32 and 5.63(2H,ABq, J 12 Hz), 5.91(1H,q, J 7 Hz), 6.97 (3H,s), 7.10(3H,s), 7.88(3H,s), 7.93(3H,s) and 8.39(3H,d, J 7 Hz) (Found: C,50.55; H,6.4; N,9.8. $\text{C}_{12}\text{H}_{18}\text{N}_2\text{O}_6$ requires C,50.35; H,6.3; N,9.7%).

(ii) With 1,6-dimethyl-5-methoxy-3,6-dihydropyrazin-2(1H)-one (84) (see p.181) The imidate (0.39 g) and L.T.A. (2.66 g) in dry benzene (15 ml) were heated under reflux for 20 h. The lead diacetate was filtered off, washed well with benzene and the filtrates washed with water (25 ml), dried and evaporated to give a colourless, crystallising oil (310 mg). Trituration with ether afforded white crystals of 1-methyl-6-acetoxymethyl-6-acetoxy-5-methoxy-3,6-dihydro-pyrazin-2(1H)-one (85) (134 mg, 25%), m.p. 146-147° (prisms from acetone/petrol), $\nu_{\max}$ 1760, 1745, 1715, 1670, 1470, 1450, 1380, 1280, 1225, 1140, 1090, 1060, 1040, 900, 853, 832 and 750 cm^{-1} , χ 5.52 and 5.60(2H, ABq, J 11 Hz), 5.73(2H,s), 6.28(3H,s), 7.12(3H,s), 7.91(3H,s), and 7.98(3H,s) (Found: C, 48.8; H, 5.8; N, 10.7. $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_6$ requires C, 48.5; H, 5.9; N, 10.3%).

(iii) With 3,6-dimethyl-2,5-diethoxy-3,6-dihydropyrazine⁵ (87)— The bis-imidate (0.2 g) and L.T.A. (5.6 g) in dry benzene (30 ml) were heated under reflux for 20 h. Workup as above gave a crystalline solid (1.22 g) which was shown by n.m.r. to be a 1:4 mixture of the known 3,6-dimethyl-2,5-diethoxy-pyrazine³⁶ (89) and a new diacetate. One recrystallisation from n-hexane gave colourless needles of 3,6-dimethyl-3,6-diacetoxy-2,5-diethoxy-3,6-dihydropyrazine (88) (0.7 g 50%), m.p. 136-137°, $\nu_{\max}$ 1750, 1690, 1460, 1378, 1320, 1265, 1215, 1190, 1090, 1030, 960, 920 and 840 cm^{-1} , χ 5.83(4H,q, J 7 Hz), 7.98(6H,s), 8.33(6H,s) and 8.77(6H,t, J 7 Hz) (Found: C, 53.4; H, 6.9; N, 8.8. $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_6$ requires C, 53.5; H, 7.1; N, 8.9%).

1,6-Dimethyl-2,5-dioxopiperazine (82)

The dipeptide, N-methylalanyl glycine, was prepared from glycine and 2-chloropropionyl chloride followed by amination with methylamine by the procedure described earlier. The dipeptide (33.0 g) was esterified with methanolic hydrogen chloride (250 ml) over 16 h. The free amino ester was liberated from its hydrochloride by treatment with ammonia in dichloromethane. The NH_4Cl was removed, the solvent evaporated and the amino ester cyclised

by stirring overnight in methanolic ammonia. Evaporation of the solvent gave the dioxopiperazine as a white crystalline solid (26.7 g, 93%), m.p. 102° (lit.⁷⁰ m.p. 105°) $\nu_{\max}$ 3200, 3070, 1685, 1660, 1480, 1465, 1408, 1375, 1330, 1305, 1138, 1090, 1037 and 795 cm^{-1} , τ 2.08(1H,bs), 5.98(2H,s), 6.09(1H,q, J 7 Hz), 6.98(3H,s) and 8.48(3H,d, J 7 Hz).

1,6-Dimethyl-5-methoxy-3,6-dihydropyrazin-2(1H)-one (84)

1,6-Dimethyl-2,5-dioxopiperazine (4.26 g) and trimethyloxonium fluoro-borate (6.66 g) were stirred together in dichloromethane (100 ml) at room temperature for 20 h. Workup in the usual manner gave the imidate as a mobile, colourless oil (2.74 g, 59%), $\nu_{\max}^{\text{film}}$ 3000-2820, 1700, 1690, 1650, 1500, 1450, 1405, 1355, 1325, 1275, 1240, 1095, 1075, 1035, 995, 760 and 720 cm^{-1} , τ 5.85(2H,s), 6.09(1H,q, J 7 Hz), 6.28(3H,s), 7.02(3H,s) and 8.58(3H,d, J 7 Hz).

1,4-Dimethyl-2,5-dioxopiperazine

Sarcosine (40 g) was suspended in ethylene glycol (40 ml) and heated under reflux for 20 h. The yellow solution was allowed to cool and extracted with dichloromethane (4x100 ml). The extracts were treated with charcoal, dried over CaCl_2 and evaporated to yield a crystalline solid (13.8 g, 43%). Recrystallisation from T.H.F. afforded colourless prisms of the title compound m.p. 148° (lit.⁷¹ m.p. 148°) $\nu_{\max}$ 1660, 1500, 1450, 1400, 1340, 1250, 1008, 980, 828, 725 and 723 cm^{-1} , τ 6.02(4H,s) and 7.02(6H,s).

3,6-Dibromo-1,4-dimethyl-2,5-dioxopiperazine

1,4-Dimethyl-2,5-dioxopiperazine (6.0 g) was dissolved in o-dichlorobenzene (90 ml) and a solution of bromine (4.65 ml) in o-dichlorobenzene (90 ml) added to the vigorously stirred solution at room temperature over 1 h. The resultant yellow suspension was heated at 170° for 3.5 h, cooled, filtered to remove a small quantity of black material and the filtrates poured into

petrol (1000 ml). After 2 days at 0° the pale yellow crystals of the 3,6-dibromo derivative (10.6 g, 84%) were isolated, $\nu_{\max}$ 1700, 1460, 1393, 1317, 1252, 1010, 730 and 660 cm^{-1} , τ 3.92(2H,s) and 6.88(6H,s).

Reactions of the dibromide with potassium thiolacetate.

(i) Potassium thiolacetate (3.12 g) in ethanol (60 ml) was added dropwise over 90 min to 3,6-dibromo-1,4-dimethyl-2,5-dioxopiperazine (2.73 g) in chloroform (75 ml) at 0°. The mixture was stirred for 45 min at 0°, the KBr filtered off and the filtrates evaporated to give a yellow solid. Trituration with water afforded the crystalline 3,6-dithiolacetate (200 mg). The aqueous triturate was extracted with chloroform (3x50 ml), dried and evaporated to yield a yellow oil (1.68 g, 80%). Crystallisation from ether afforded colourless cubes of 3,6-diethoxy-1,4-dimethyl-2,5-dioxopiperazine (90), m.p. 99°, $\nu_{\max}$ 1675, 1460, 1400, 1354, 1312, 1306, 1250, 1165, 1115, 1070, 1028, 900, 852 and 835 cm^{-1} , τ 5.08(2H,s), 5.92-6.55(4H,m), 6.97(6H,s) and 8.75(6H,t, J 7 Hz) (Found: C, 52.4; H, 7.8; N, 12.4. $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_4$ requires C, 52.2; H, 7.9; N, 12.2%).

(ii) Potassium thiolacetate (10.28 g) in D.M.F. (50 ml) was added dropwise over 45 min to a stirred solution of the dibromide (9.39 g) in dichloromethane (120 ml) at 0°. Stirring was continued at 0° for 30 min and the reaction mixture poured onto ice. The mixture was extracted with dichloromethane (2x100 ml), washed with water (50 ml), dried and evaporated under reduced pressure at a temperature not exceeding 40°. The residue was triturated with ether to give fine white needles of 3,6-di(acetylthio)-1,4-dimethyl-2,5-dioxopiperazine (7.34 g, 81%). This product was shown to be a 9:1 cis:trans mixture (n.m.r.). A sample of the pure cis-isomer, recrystallised from ethanol showed m.p. 204-205° (lit.⁷² m.p. 205°), $\nu_{\max}$ 1690, 1460, 1400, 1296, 1254, 1128, 1021, 950, 800, 759, 692, 640 and 620 cm^{-1} , τ 4.20(2H,s), 7.03(6H,s) and 7.50(6H,s). The trans-isomer had τ 4.33(2H,s), 7.00(6H,s) and 7.57(6H,s).

Cis-3,6-dithiol-1,4-dimethyl-2,5-dioxopiperazine.

The cis-di(acetylthio)-dioxopiperazine (6.0 g) was hydrolysed in refluxing N ethanolic hydrogen chloride (250 ml) for 20 min. The solution was evaporated and the residue triturated with a small volume of methanol to give the title compound as white crystals (3.33 g, 73%), m.p. 110-112° (lit.²⁰ m.p. 111-114°), $\nu_{\max}$ 2560, 1680, 1475, 1408, 1300, 1255, 1200, 1165, 1024, 798, 767, 742, 715 and 630 cm^{-1} , τ 4.94(2H,d, J 7 Hz, collapses to a singlet on exchange), 6.89 (2H,d, J 7 Hz, exchangeable) and 6.90(6H,s).

3,6-Epidithio-1,4-dimethyl-2,5-dioxopiperazine (55)

The cis-dithiol-dioxopiperazine (2.54 g) was dissolved in chloroform (600 ml) in a separatory funnel. Water (150 ml) was added and a solution of iodine (3.13 g) and potassium iodide (9.25 g) in water (60 ml) added dropwise. The oxidation was followed visibly as the colour of the iodine was discharged on vigorous mixing of the two phases. When further addition of iodine produced permanent colouration, a few drops of sodium thiosulphate solution were added to remove the excess iodine. The chloroform phase was separated, dried and evaporated to give the disulphide (2.42 g, 96%). Recrystallisation from acetonitrile afforded pale yellow prisms, m.p. 184-185° (lit.⁷² m.p. 185°), $\nu_{\max}$ 1690, 1460, 1443, 1390, 1295, 1260, 1238, 1225, 1150, 1000, 848, 788, 664 and 615 cm^{-1} , τ 4.77(2H,s) and 6.88(6H,s).

Reactions of 3,6-epidithio-1,4-dimethyl-2,5-dioxopiperazine.

(i) With triethylamine — The disulphide (51 mg) was suspended in chloroform (1 ml) and triethylamine (25 mg) added. The suspension became very dark after a few minutes and a white solid was deposited. This was filtered off to yield finely crystalline 3,6-epitetraethio-1,4-dimethyl-2,5-dioxopiperazine (56) (18 mg, 55%), m.p. 194-205° (lit.⁷² m.p. 195-205°), $\nu_{\max}$ 1675, 1660, 1463, 1440, 1408, 1305, 1255, 1208, 780, 744 and 690 cm^{-1} , τ (T.F.A.) 4.37(2H,s) and 6.75(6H,s), m/e 268(M^+ , 8%), 236(1.1), 204(3.2), 172(39) and 140(100).

(ii) With pyridine and sulphur — the disulphide (51 mg) and sulphur (32 mg) were stirred together in dry pyridine (5 ml) at room temperature for 3 h. The precipitate was filtered off and washed with dichloromethane to yield the tetrasulphide (50 mg, 80%) identical in all respects to the material isolated in (i).

(iii) With triphenylphosphine in T.H.F. — the disulphide (1.02 g) and triphenylphosphine (1.31 g) were suspended in anhydrous T.H.F. (50 ml) and stirred, under nitrogen, at room temperature for 20 h. The solution was evaporated and the residue chromatographed through silica. The first band, triphenylphosphine sulphide, was eluted from the column with benzene and the second band, the desulphurised product, eluted with dichloromethane. The solid (0.77 g, 80%) was recrystallised from acetone/petrol to give colourless needles of 3,6-epimonothio-1,4-dimethyl-2,5-dioxopiperazine (91), m.p. 124-130° (decomp.), $\nu_{\max}$ 1706, 1455, 1432, 1378, 1240, 1203, 1086, 972, 848, 810, 780, 752 and 669 cm^{-1} , τ 5.27(2H,s) and 7.02(6H,s), m/e 172(M^+ , 0.7%), 140(4.4) and 115(100), (Found: C, 41.6; H, 4.7; N, 16.5; S, 18.5. $\text{C}_8\text{H}_8\text{N}_2\text{O}_2\text{S}$ requires C, 41.85; H, 4.7; N, 16.3; S, 18.6%).

(iv) With trimethylphosphite — the disulphide (102 mg) was suspended in dry T.H.F. (10 ml) under nitrogen and a solution of trimethylphosphite (62 mg) in dry T.H.F. (10 ml) added to the stirred suspension. A colourless solution was rapidly attained and reaction was complete within 5 min. The solution was evaporated and the residue triturated with petrol, to remove the trimethylthiophosphate, yielding the monosulphide (91) (84 mg, 98%) identical to the material isolated in (iii).

(v) With triphenylphosphine in methanol — the disulphide (102 mg) and triphenylphosphine (131 mg) were stirred together, under nitrogen, in dry methanol (10 ml). During 30 min the pale yellow colour was discharged and a

white precipitate, of triphenylphosphine sulphide, was deposited. After filtration the solution was evaporated and the residue triturated with ice-cold methanol (5 ml), filtered and the filtrates evaporated to yield a crystalline solid (97 mg, 95%). This was shown to be a cis-trans mixture of 3-methoxy-6-thiol-1,4-dimethyl-2,5-dioxopiperazine (92). The major isomer crystallised out of acetone/petrol in white clusters, m.p. 103-106°, $\nu_{\max}$ 2485, 1680, 1440, 1435, 1410, 1305, 1240, 1164, 1092, 1024, 1015, 906, 877, 767 and 630 cm^{-1} , χ 4.87(1H,d, J 6 Hz, collapses to a singlet on exchange), 5.03(1H,s), 6.55(3H,s), 6.88(3H,s), 6.93(3H,s) and 7.05(1H,d, J 6 Hz, exchangeable), m/e 204 (M^+), (Found: C,41.4; H,5.9; N,13.6; S,15.65. $C_7H_{12}N_2O_3S$ requires C,41.2; H,5.9; N,13.7; S,15.7%).

The mother liquors were evaporated and, by repeated recrystallisation, the other isomer was obtained as colourless prisms from acetone/petrol, m.p. 120-121°, $\nu_{\max}$ 2550, 1675, 1450, 1407, 1303, 1259, 1164, 1065, 1024, 955, 877, 775 and 745 cm^{-1} , χ 5.22(1H,d, J 6 Hz), 5.33(1H,s), 6.40(3H,s), 6.87(1H,d, J 6 Hz), 6.88(3H,s) and 6.93(3H,s), m/e 204 (M^+), (Found: C,41.0; H,6.1; N,13.8%).

3,6-Episulphoxide-1,4-dimethyl-2,5-dioxopiperazine (93)

3,6-Epimonothio-1,4-dimethyl-2,5-dioxopiperazine (86 mg) and m-chloroperbenzoic acid (90 mg) in dichloromethane (20 ml) were kept at 0° for 20 h. The solution was washed with dilute Na_2CO_3 solution, dried and evaporated to yield crude (93) (47 mg, 50%). Recrystallisation from dichloromethane/ether gave soft white needles of 3,6-episulphoxide-1,4-dimethyl-2,5-dioxopiperazine, m.p. 128-129°, $\nu_{\max}$ 3050, 1702, 1455, 1440, 1380, 1218, 1120, 1077, 977, 753 and 655 cm^{-1} , χ 5.37(2H,bs), 6.80(3H,s) and 6.91(3H,s), m/e 188 (M^+ , 9.5%), 172(1.2), and 40(100) (Found: C,38.25; H,4.3; N,14.8; S,16.9. $C_6H_8N_2O_3S$ requires C,38.3; H,4.3; N,14.9; S,17.0%).

Reaction of 1,3-dimethyl-6-methylene-2,5-dioxopiperazine (34) with thiolacetic acid.

(i) Absence of acid catalyst. The dioxopiperazine (50 mg) and thiolacetic acid (30 mg) dissolved in deuteriochloroform (1.0 ml) in a stoppered n.m.r. tube were kept at room temperature for 4 days, after which no starting material remained (n.m.r.). The two products were separated by plc (1:19 MeOH-CHCl₃) to afford cis- and trans-6-acetylthiomethyl-1,3-dimethyl-2,5-dioxopiperazine (97). The more polar cis-isomer crystallised from acetone/petrol as prisms (29 mg, 38%), m.p. 163-165°, $\nu_{\max}$ 3310, 1620, 1640, 1470, 1460, 1420, 1330, 1320, 1300, 1165, 1135, 960, 765, 695 and 640 cm⁻¹, τ 2.40(1H,bs), 5.80(2H,m), 6.42(2H,d, J 4 Hz), 6.98(3H,s), 7.63(3H,s) and 8.39(3H,d, J 7 Hz) (Found: C,47.1; H,6.2; N,12.15; S,14.2. C₉H₁₄N₂O₃S requires C,46.9; H,6.1; N,12.2; S,13.9%).

The less polar trans-isomer was isolated as an oil (24 mg, 32%), τ 2.67(1H,bs), 5.80(2H,m), 6.43(2H,m), 6.98(3H,s), 7.63(3H,s) and 8.51(3H,d, J 7 Hz).

(ii) With acid catalyst. The dioxopiperazine (60 mg) and thiolacetic acid (100 mg) were added to deuteriochloroform (1.0 ml) to which had been added several drops of solvent saturated with anhydrous hydrogen chloride. After 3 h at room temperature the reaction was complete (n.m.r.) and the solvent was evaporated. The residue was separated by plc (1:19 MeOH-CHCl₃) to give cis- and trans-6-acetylthio-1,3,6-trimethyl-2,5-dioxopiperazine (99). The more polar cis-isomer afforded prisms from acetone/petrol (17 mg, 12%), m.p. 140-143°, $\nu_{\max}$ 3200, 3080, 1685, 1655, 1500, 1460, 1432, 1384, 1370, 1330, 1298, 1270, 1178, 1120, 1100, 1070, 990, 954, 842, 790, 755 and 630 cm⁻¹, τ 3.55(1H,bs), 5.73(1H,q, J 6 Hz), 6.98(3H,s), 7.72(3H,s), 8.18(3H,s) and 8.35(3H,d, J 6 Hz) (Found: C,47.1; H,6.2; N,12.3. C₉H₁₄N₂O₃S requires C,46.9; H,6.1; N,12.2; S, 13.9%).

The less polar trans-isomer gave prisms from ethyl acetate/petrol (28 mg, 30%), m.p. 114-115°, $\nu_{\max}$ 3200, 3080, 1685, 1665, 1455, 1420, 1390, 1375, 1330, 1320, 1265, 1170, 1160, 1125, 1100, 845 and 640 cm⁻¹, τ 3.45(1H,bs), 5.41(1H, q, J 7 Hz), 6.98(3H,s), 7.72(3H,s), 8.12(3H,s) and 8.48(3H,d, J 7 Hz) (Found: C,46.8; H,6.25; N,12.1; S,13.9%).

Reaction of 3-benzyl-1-methyl-6-methylene-2,5-dioxopiperazine (47) with thiolacetic acid.

The dioxopiperazine (58 mg) and thiolacetic acid (100 mg) in deuteriochloroform (1.0 ml) containing dry hydrogen chloride were left at 25° for 1 h, after which time no starting material remained (n.m.r.). After a further 20 h the ratio of the two isomeric products had changed from ca. 1:1 to 3:1 and at this point tended to remain constant. The products were separated by plc (1:19 MeOH-CHCl₃) to give, as the more polar band, cis-6-acetylthio-3-benzyl-1,6-dimethyl-2,5-dioxopiperazine (101a) (37 mg, 49%), colourless needles from acetone/petrol, m.p. 181-182°, $\nu_{\max}$ 3190, 3060, 1695, 1685, 1665, 1500, 1460, 1390, 1370, 1270, 1245, 1215, 1120, 1095, 960, 868, 850, 792, 760, 720, 700 and 630 cm⁻¹, τ 2.67(5H,s), 2.83(1H,bs), 5.28(1H,dt, J 2,5 Hz), 6.78(2H,d, J 5 Hz), 7.10(3H,s), 7.75(3H,s) and 8.70(3H,s) (Found: C,58.6; H,5.7; N,9.1; S,10.3. C₁₅H₁₈N₂O₃S requires C,58.8; H,5.9; N,9.1; S,10.5%).

The less polar band afforded the trans-isomer (101b) (18 mg, 23%), colourless prisms from ethyl acetate/petrol, m.p. 124-126°, $\nu_{\max}$ 3190, 3060, 1695, 1685, 1655, 1500, 1450, 1380, 1270, 1120, 1095, 955, 742, 700 and 630 cm⁻¹, τ 2.60(5H,s), 3.17(1H,bs), 5.67(1H,m), 6.30(2H,m), 6.92(3H,s), 7.67(3H,s) and 8.20(3H,s), m/e 306(H⁺,16%), 305(82), 263(100), 231(58), 203(40) and 139(38).

Addition reactions of 2,3,7,8-tetrahydrodipyrrolo[1,2-a:1',2'-d]pyrazine-5,10-dione (77)

(i) With thiolacetic acid. Bis-dehydropyrroline anhydride⁴⁵ (190 mg) and thiolacetic acid (200 mg), in deuteriochloroform (2 ml) containing hydrogen chloride, were stirred at room temperature for 4 h. Unchanged starting material was filtered off, the filtrates evaporated and the residue recrystallised from ethyl acetate to give the major reaction product, 5a-acetylthio-2,3,5a,6,7,8-hexahydrodipyrrolo[1,2-a:1',2'-d]pyrazin-5,10-dione (104) (29 mg, 15%), as colourless prisms, m.p. 138-141° (decomp.), $\nu_{\max}$ 1690, 1670, 1655, 1460, 1430, 1375, 1358, 1338, 1200, 1115, 1090, 1010, 958, 915, 725 and 630 cm⁻¹,

τ 3.72(1H,t, J 3 Hz), 5.93(2H,t, J 3 Hz), 5.68-6.55(2H,m), 7.18(2H,dt, J 3,9 Hz), 7.46-8.04(4H,m) and 7.73(3H,s) (Found: C,54.4; H,5.5; N,10.4; S,11.7. $C_{12}H_{14}N_2O_3S$ requires C,54.1; H,5.3; N,10.5; S,12.0%).

(ii) With ethanethiol. The dioxopiperazine (95 mg) and ethanethiol (0.1 ml) in dichloromethane (25 ml) containing dry hydrogen chloride were left at room temperature for 20 h. After evaporation of the solvent the residue was separated by plc (1:19 MeOH-CHCl₃). The less polar band was identified as the known cis-5a,10a-diethylthio-derivative (107) (39 mg, 30%). Recrystallisation from hexane afforded clusters of prisms, m.p. 98-100° (lit.³⁵ m.p. 98-103°), $\nu_{\max}$ 1660, 1460, 1445, 1400, 1378, 1340, 1195, 1168, 1147, 1093, 1055, 1018, 979, 940, 900, 890, 775, 625 and 670 cm⁻¹, τ 6.11-6.52(4H,m), 7.23(4H,q, J 7 Hz), 7.43-8.18(8H,m) and 8.77(6H,t, J 7 Hz), m/e 314(M⁺).

The more polar band gave the monoethylthio-adduct (108) (47 mg, 44%) as an unstable, non-crystalline foam, $\nu_{\max}^{\text{film}}$ 3000-2860, 1670, 1642, 1420, 1370, 1205, 1010, 915, 735 and 675 cm⁻¹, τ 3.77(1H,t, J 3 Hz), 5.67-6.40(4H,m), 7.17(2H,dt, J 3,9 Hz), 7.41(2H,q, J 7 Hz), 7.50-8.20(4H,m) and 8.80(3H,t, J 7 Hz), m/e 252(M⁺, 2%) and 191(100).

Reaction of 3,1'-Z-3-benzylidene-1-methyl-2,5-dioxopiperazine (110) with ethanethiol.

The benzylidene dioxopiperazine⁴⁶ (54 mg) and ethanethiol (0.1 ml) in deuteriochloroform (1.0 ml) containing dry hydrogen chloride were left at room temperature for 4 weeks. After this time the slow addition reaction had attained equilibrium at 35% conversion. The solvent was evaporated and the residue separated by plc (1:2 acetone-hexane). The more polar band gave starting material (25 mg, 50%) whilst the less polar band afforded 3-ethylthio-3-benzyl-1-methyl-2,5-dioxopiperazine (112) (17 mg, 25%), as colourless needles from acetone/hexane, m.p. 149-152°, $\nu_{\max}$ 3190, 3085, 1690, 1675, 1640, 1455, 1440, 1420, 1380, 1325, 1275, 1230, 1120, 1050, 968, 898, 765 and 715 cm⁻¹

2.68(5H,s), 3.20(1H,bs), 6.13 and 6.87(2H,ABq, J 18 Hz), 6.37 and 6.97(2H, ABq, J 14 Hz), 7.10(3H,s), 7.28(2H,q, J 7 Hz) and 8.75(3H,t, J 7 Hz)

(Found: S, 11.5. $C_{14}H_{18}N_2O_2S$ requires S, 11.6%).

Reaction of the benzylidene dioxopiperazine (110) with sodium ethanethiolate.

The dioxopiperazine (216 mg) and sodium hydride (40 mg) in dry D.M.F. (2 ml) were treated with ethanethiol (1 ml). After the evolution of gas had subsided the solution was heated at 60° for 3 days. The dark yellow solution was taken up in dichloromethane (40 ml) and washed with 2N HCl (20 ml). The aqueous phase was back-extracted with dichloromethane (40 ml), the combined extracts washed with water (20 ml), dried and evaporated to yield a yellow oil. The n.m.r. spectrum of this product showed <5% of diethyl disulphide. Drying on the oil pump overnight removed residual D.M.F. whereupon the oil crystallised. The crystals were triturated with ether and filtered off (135 mg, 61%). The filtrates were evaporated to give starting material (40 mg, 20%). After further purification by plc (1:19 MeOH-CHCl₃) the product was recrystallised from ethyl acetate to yield soft white needles of 3-benzyl-1-methyl-2,5-dioxopiperazine (115), m.p. 180° (lit.⁴⁷ m.p. 180-181°), ν_{max} 3270, 1650, 1460, 1450, 1430, 1410, 1375, 1332, 1320, 1200, 1182, 1102, 1025, 870, 750 and 705 cm⁻¹, τ 2.42(1H,bs), 2.70(5H,m), 5.67(1H,m), 6.45 and 7.18(2H,ABq, J 18 Hz), 6.85 (2H,m) and 7.18(3H,s).

If the ethanethiolate was prepared prior to the addition of dioxopiperazine no reaction occurred and after 7 days at 60° a quantitative yield of starting material was isolated.

Reaction of the benzylidene dioxopiperazine (110) with singlet oxygen.

The dioxopiperazine (0.43 g) in dichloromethane (15 ml) containing methylene blue (2 mg) as sensitiser was photooxygenated at room temperature

for 60 h, after which time no starting material remained (tlc). The precipitate was filtered off, washed well with dichloromethane and dried to yield 1-methyl-2,3,5-trioxopiperazine (117) (0.21 g, 76%), colourless prisms from acetone, m.p. 201-202°, $\nu_{\max}$ 3170, 3030, 1725, 1690, 1505, 1450, 1410, 1380, 1340, 1295, 1225, 995, 840, 815, 730 and 675 cm^{-1} , ζ (T.F.A.) 2.97(1H,bs), 5.80(2H,s) and 7.18(3H,s) (Found: C,42.1; H,4.4; N,19.9. $\text{C}_5\text{H}_6\text{N}_2\text{O}_3$ requires C,42.3; H,4.3; N,19.7%).

The filtrates were evaporated and the resultant oil triturated with petrol (5x10 ml). The triturate was evaporated to give benzaldehyde (spectral data) whilst the residue, which gave a positive starch-iodide test, was shown to consist of the intermediate dioxetane (118), ζ 2.60(5H,s), 3.00(1H,bs), 4.80 (1H,s), 6.44 and 7.48(2H,ABq, J 19 Hz) and 7.30(3H,s). This product was slowly decomposing into the trioxo compound and benzaldehyde.

Reaction of 3,1'-E-3-benzylidene-1-methyl-2,5-dioxopiperazine (120) with singlet oxygen.

The E-benzylidene dioxopiperazine was prepared from its Z-isomer (110) by photoequilibration followed by plc^{46} . The dioxopiperazine (96 mg) in dichloromethane (10 ml) containing methylene blue (1 mg) was photooxygenated at room temperature for 24 h. Workup as above gave the crystalline trioxopiperazine (117) (46 mg, 74%) and a residue containing benzaldehyde and the decomposing dioxetane (118).

Methylation of 3,1'-Z-benzylidene-1-methyl-2,5-dioxopiperazine (110).

The dioxopiperazine (432 mg) in benzene (40 ml) was stirred with freshly prepared silver carbonate (700 mg) and methyl iodide (5 ml) for 7 days in a flask protected from the light. The mixture was filtered through celite which was washed well with benzene and the filtrates evaporated. The crystalline residue was chromatographed through silica (70 g) eluting with 1:4 acetone-hexane. The first band was 3,1'-Z-benzylidene-1-methyl-5-methoxy-3,6-dihydro-

pyrazin-2(1H)-one (121) (193 mg, 63%), colourless plates from hexane, m.p. 149°
 $\nu_{\max}$ 1665, 1610, 1595, 1500, 1440, 1400, 1378, 1275, 1250, 1210, 1015, 930,
 840, 775, 770, 720, 703, 697 and 665 cm^{-1} , τ 1.95(2H,m), 2.63(3H,m), 2.68(1H,
 s), 5.90(2H,s), 6.08(3H,s) and 6.97(3H,s), $\lambda_{\max}$ 220, 224, 231, 295, 307 and
 320 nm(ϵ 11,500, 13,500, 12,000, 27,000, 32,500, and 23,000) (Found: C,67.65;
 H,6.3; N,12.1. $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_2$ requires C,67.8; H,6.1; N,12.2%).

The second band was 3,1'-Z-benzylidene-1,4-dimethyl-2,5-dioxopiperazine
 (122) (113 mg, 37%), colourless prisms from hexane, m.p. 122° , $\nu_{\max}$ 1685,
 1665, 1625, 1450, 1425, 1370, 1315, 1295, 1240, 1225, 1210, 988, 948, 770, 750
 and 705 cm^{-1} , τ 2.60(5H,s), 2.67(1H,s), 5.87(2H,s), 6.90(3H,s) and 7.10(3H,s),
 $\lambda_{\max}$ 224 and 289 nm(ϵ 21,000 and 29,000) (Found: C,68.0; H,5.95; N,12.4%).

1,6-Dimethyl-4-acetyl-2,5-dioxopiperazine (127)

1,6-Dimethyl-2,5-dioxopiperazine (82) (5.2 g) and fused sodium acetate
 (3.8 g) in acetic anhydride (15 ml) were heated at 100° for 90 min. The cooled
 mixture was poured into water (100 ml) and stirred until homogeneous. The
 solution was extracted with dichloromethane (2x100 ml) and the extracts treated
 with water (50 ml) and solid sodium carbonate until all the acetic acid was
 neutralised. (If neutralisation was attempted before extraction into the
 organic solvent the product was completely hydrolysed to ring opened compounds).
 Drying and evaporation gave crystalline solid (6.39 g, 95%). Recrystallisation
 from ether afforded colourless prisms of 1,6-dimethyl-4-acetyl-2,5-dioxopip-
erazine (127), m.p. $78-79^{\circ}$, $\nu_{\max}$ 1720, 1700, 1665, 1505, 1465, 1442, 1400,
 1385, 1365, 1333, 1270, 1230, 1090, 1065, 1045, 990, 810, 740 and 690 cm^{-1} ,
 τ 5.17 and 5.06(2H,ABq, J 18 Hz), 5.93(1H,q, J 7 Hz), 6.98(3H,s), 7.41(3H,s)
 and 8.44(3H,d, J 7 Hz) (Found: C,52.0; H,6.45; N,15.1. $\text{C}_8\text{H}_{12}\text{N}_2\text{O}_3$ requires
 C,52.2; H,6.6; N,15.2%).

3,1'-Z-Benzylidene-1,6-dimethyl-2,5-dioxopiperazine (124)

The N-acetyl dioxopiperazine (127) (0.92 g) was dissolved in dry D.M.F.

(10 ml) and an atmosphere of dry nitrogen established. After the addition of benzaldehyde (1.1 ml) the stirred solution was cooled to 0° and potassium t-butoxide (0.56 g) in t-butanol (10 ml) added dropwise over 45 min. The pale yellow solution was stirred at room temperature for a further 6 h, then neutralised with acetic acid (0.5 ml) and poured into water (100 ml). The solution was washed with petrol (50 ml) and extracted with ethyl acetate (2x 100 ml) which was then washed with dilute sodium bisulphite solution (2x50 ml), saturated NaHCO₃ solution (50 ml) and water (50 ml). After drying and evaporation, the residue was chromatographed through silica eluting with benzene to give 3,1'-Z-benzylidene-1,6-dimethyl-2,5-dioxopiperazine (124) (0.45 g, 40%), colourless needles from ethyl acetate/petrol, m.p. 106°, $\nu_{\max}$ 3170, 3080, 1690, 1668, 1630, 1500, 1460, 1450, 1400, 1372, 1365, 1265, 1030, 880, 765 and 692 cm⁻¹, τ 1.95(1H,bs), 2.60(5H,s), 2.95(1H,s), 5.94(1H,q, J 7 Hz), 6.92(3H,s) and 8.42(3H,d, J 7 Hz), $\lambda_{\max}$ 224 and 296.5 nm(ϵ 16,000 and 24,000) (Found: C,68.05; H,6.1; N,12.4. C₁₃H₁₄N₂O₂ requires C,67.8; H,6.1; N,12.2%).

Reaction of the benzylidene dioxopiperazine (124) with singlet oxygen.

The dioxopiperazine (100 mg) in dichloromethane (10 ml) containing methylene blue(1 mg) was photooxygenated at room temperature for 24 h. The solution was evaporated and the residue triturated with petrol (5x10 ml) to remove the benzaldehyde. The n.m.r. spectrum of the crude product showed the trioxopiperazine (125) and the intermediate dioxetane (126), τ 2.73(5H,s), 3.00(1H,bs), 4.82(1H,s), 6.83(1H,q, J 7 Hz), 7.20(3H,s) and 8.52(3H,d, J 7 Hz). Purification by plc (1:19 MeOH-CHCl₃) gave 1,6-dimethyl-2,3,5-trioxopiperazine (125) (43 mg, 68%) as an oil, $\nu_{\max}^{\text{film}}$ 3195, 3095, 3000-2820, 1720, 1680, 1450, 1410, 1330, 1290, 1230, 1095, 912, 780 and 735 cm⁻¹, τ 2.68(1H,s), 5.71(1H, q, J 7 Hz), 6.90(3H,s) and 8.31(3H,d, J 7 Hz), m/e 156 (M⁺).

Reaction of 3,1'-Z-3-benzylidene-1-methyl-2,5-dioxopiperazine (110) with lead tetraacetate.

(i) The dioxopiperazine (1.08 g) and L.T.A. (2.22 g) in dry benzene (80 ml) were stirred vigorously at room temperature for 1 h, after which time no starting material remained (tlc). The lead diacetate was filtered off, washed well with benzene and the filtrates evaporated. The residue was taken up in dichloromethane (100 ml) washed with water (30 ml), dried and evaporated to yield a non-crystalline foam (1.59 g, 100%). Crystallisation from ether gave colourless prisms of 3-acetoxy-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (128), m.p. 140-144°(decomp.), $\nu_{\max}$ 3190, 3080, 1755, 1690, 1460, 1425, 1380, 1335, 1230, 1198, 1090, 1045, 1015, 970, 945, 755 and 710 cm^{-1} , τ 2.38(1H,bs), 2.67(5H,s), 3.65(1H,s), 6.10 and 6.77(2H,ABq, J 19 Hz), 7.38(3H,s), 7.83(3H,s) and 7.88(3H,s) (Found: C,57.4; H,5.5; N,8.2. $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_6$ requires C,57.5; H,5.4; N,8.4%).

(ii) The dioxopiperazine (0.22 g) and L.T.A. (0.56 g) in 1:19 methanol-benzene (20 ml) were stirred at room temperature for 15 min. A thick solution was rapidly formed and after this time the reaction was complete (tlc). The solution was decanted from the sticky lead diacetate which was washed well with dichloromethane and the combined liquors were evaporated to dryness. The residue was dissolved in dichloromethane (50 ml), washed with water (20 ml), saturated NaHCO_3 solution (20 ml), dried and evaporated to yield a crystalline solid (0.28 g, 92%). Recrystallisation from acetone/petrol afforded colourless prisms of 3-methoxy-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (130), m.p. 198-202°(decomp.), $\nu_{\max}$ 3180, 3060, 1745, 1680, 1455, 1430, 1420, 1405, 1370, 1320, 1230, 1190, 1100, 1045, 1000, 940, 850, 820, 795, 750 and 705 cm^{-1} , τ 2.43(1H,bs), 2.68(5H,s), 3.78(1H,s), 6.38 and 7.37(2H,ABq, J 18 Hz), 6.68 (3H,s), 7.32(3H,s) and 7.83(3H,s) (Found: C,58.7; H,5.8; N,9.05. $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_5$ requires C,58.8; H,5.9; N,9.15%).

(iii) The dioxopiperazine (108 mg) was dissolved in methanol (5 ml) and L.T.A. (225 mg) added. The solution immediately became deep yellow but the colour faded over 5 min by which time the reaction was complete (tlc). The solution was poured into dichloromethane (40 ml), washed with water (15 ml), dried and evaporated to yield a yellow foam (160 mg). The n.m.r. spectrum of this product showed it to be a 1:1 mixture of the methoxy acetate (130) and a new compound. The products were separated by plc (1:19 MeOH-CHCl₃) and the band corresponding to the new compound, slightly less polar than (130), gave 3-methoxy-3-methoxybenzyl-1-methyl-2,5-dioxopiperazine (135) (42 mg, 30%), prisms from acetone/petrol, m.p. 200-202°(decomp.), $\nu_{\max}$ 3230, 3100, 1670, 1460, 1440, 1410, 1320, 1275, 1185, 1100, 990, 848, 750, 712 and 645 cm⁻¹, τ 2.58(5H,s), 3.32(1H,bs), 5.23(1H,s), 6.40 and 7.37(2H,ABq, J 18 Hz), 6.57 (3H,s), 6.65(3H,s) and 7.37(3H,s) (Found: C,60.4; H,6.4; N,10.0. C₁₄H₁₈N₂O₄ requires C,60.4; H,6.5; N,10.1%).

Acid catalysed reactions of 3-acetoxy-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (128).

(i) The diacetate (0.33 g) was stirred in dichloromethane (20 ml) containing boron trifluoride etherate (0.5 ml) and methanol (3 ml) at room temperature for 30 min. The solution was washed with water (10 ml), dried and evaporated to yield a crystalline solid (0.30 g, 100%). Recrystallisation from acetone/petrol gave colourless prisms of the methoxy acetate (130) described above.

(ii) The diacetate (0.33 g) was stirred vigorously in dichloromethane (20 ml) containing boron trifluoride etherate (0.5 ml) and water (5 ml) at room temperature for 1 h. The organic phase was separated, dried and evaporated to yield a crystalline solid (0.29 g, 100%). Recrystallisation from acetone/petrol afforded colourless prisms of 3-hydroxy-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (131), m.p. 158-161°(decomp.), $\nu_{\max}$ 3190, 3090, 1745, 1665, 1460, 1375, 1325, 1230, 1125, 1060, 1045, 825, 795, 750 and 710 cm⁻¹,

τ 2.57(5H,s), 3.25(1H,bs), 3.80(1H,s), 5.58(1H,bs), 6.44 and 7.31(2H,ABq, J 19 Hz), 7.22(3H,s) and 7.77(3H,s) (Found: C,57.8; H,5.75; N,9.7. $C_{14}H_{16}N_2O_5$ requires C,57.5; H,5.5; N,9.6%).

(iii) The diacetate (1.67 g) was stirred in dichloromethane (40 ml) containing boron trifluoride etherate (0.3 ml) whilst a slow stream of hydrogen sulphide was passed through the solution for 1 h. The gas-saturated solvent was evaporated and the residue redissolved in dichloromethane (50 ml), washed with water (20 ml), dried and evaporated to yield a foam (1.54 g, 100%). Crystallisation was induced by trituration with ether and recrystallisation from acetone/petrol afforded white rosettes of 3-thiol-3-acetoxymethyl-1-methyl-2,5-dioxopiperazine (136), m.p. 170-173°, v_{max} 3195, 3080, 2510, 1745, 1680, 1460, 1415, 1375, 1320, 1238, 1050, 940, 810, 720, 710 and 625 cm^{-1} , τ 2.38 (1H,bs, exchangeable), 2.60(5H,s), 3.63(1H,s), 6.23 and 7.05(2H,ABq, J 18 Hz), 6.62(1H,s, exchangeable), 7.25(3H,s) and 7.77(3H,s) (Found: C,54.7; H,5.5; N,8.9. $C_{14}H_{16}N_2O_4S$ requires C,54.5; H,5.2; N,9.1%).

(iv) The diacetate (167 mg) was dissolved in 1:1 ether-dichloromethane (15 ml) containing boron trifluoride etherate (0.1 ml). Anhydrous ethereal hydrogen peroxide (5 ml, ca. 10%) was added and the solution stirred at room temperature for 1 h. The solvents were evaporated, the residue taken up in dichloromethane (20 ml) and washed with water (10 ml). The aqueous phase was extracted with more dichloromethane (20 ml), the combined extracts dried and evaporated to small volume whereupon a mass of soft, white needles crystallised out of the solution. Filtration afforded 3-hydroperoxy-3-acetoxymethyl-1-methyl-2,5-dioxopiperazine (141) (146 mg, 95%), m.p. 135-137°, v_{max} 3190, 3110, 1760, 1675, 1460; 1378, 1342, 1220, 1082, 1050, 1035 and 950 cm^{-1} , τ (T.F.A.) 2.70 (5H,s), 3.73(1H,s), 6.10 and 7.00(2H,ABq, J 20 Hz), 7.30(3H,s) and 7.80(3H,s) (Found: C,54.55; H,5.2; N,9.0. $C_{14}H_{16}N_2O_6$ requires C,54.5; H,5.2; N,9.1%). The compound gave a very strongly positive starch-iodide test as expected.

Desulphurisation of 3-thiol-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (136)

The dioxopiperazine (154 mg) in dry T.H.F. (5 ml) was stirred with sodium hydride (20 mg) under nitrogen. The initial release of gas gave rise to a yellow solution which gradually, over 2 h, turned deep red. After stirring for a further 10 h the solution was poured into dichloromethane (20 ml) and washed with water (not acid) which removed most of the colour. Drying and evaporation afforded a crystalline solid (110 mg, 100%) identified as the 3-benzylidene dioxopiperazine (110), by comparison with an authentic sample.

Methylation of 3-thiol-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (136)

The dioxopiperazine (1.54 g) in Analar acetone (50 ml) was stirred with anhydrous potassium carbonate (2.50 g) and methyl iodide (25 ml). After 2 h no starting material remained (tlc) and the solids were filtered off, washed well with acetone and the filtrates evaporated. The residue was digested with dichloromethane (70 ml), washed with water (30 ml), dried and evaporated to yield a foam (1.61 g, 100%). Crystallisation was induced by trituration with ether and recrystallisation from acetone/petrol afforded colourless prisms of 3-methylthio-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (137), m.p. 176-178°, $v_{\max}$ 3190, 3100, 1755, 1690, 1666, 1495, 1455, 1405, 1372, 1313, 1225, 1172, 1047, 930, 810, 770, 711 and 625 cm^{-1} χ 1.98(1H,bs), 2.57(5H,s), 3.55(1H,s), 6.13 and 6.92(2H,ABq, J 18 Hz), 7.23(3H,s), 7.70(3H,s) and 7.78(3H,s) (Found: C,55.9; H,5.8; N,8.7; S,10.0. $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_4\text{S}$ requires C,55.9; H,5.6; N, 8.7; S,9.95%).

Oxidations of 3-methylthio-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (137)

(i) The dioxopiperazine (161 mg) was dissolved in dichloromethane (6 ml) and m-chloroperbenzoic acid (123 mg, 70%) was added in portions. After all the oxidant had been added no starting material remained (tlc). The solution was poured into more dichloromethane (15 ml), washed with saturated NaHCO_3 solution (10 ml), dried and evaporated and the residue crystallised from ether

to give the hydroxy acetate (131) (61 mg, 40%). Plc (1:19 MeOH-CHCl₃) of the crystallisation mother liquors gave (131) and the methoxy acetate (130) as the only products.

(ii) The dioxopiperazine (80 mg) in dichloromethane (10 ml) containing methylene blue (1 mg) was photooxygenated for 18 h, after which time the starting material had been consumed. The solvent was evaporated and the residue triturated with ether to give the hydroxy acetate (131) (74 mg, 100%).

The reaction was repeated in solvent containing 5% methanol. N.m.r. examination of the product showed a ca. 4:1 mixture of the methoxy acetate (130) and the hydroxy acetate (131).

Reactions of 3-hydroperoxy-3-acetoxylbenzyl-1-methyl-2,5-dioxopiperazine (141).

(i) With acid. The dioxopiperazine (23 mg) was dissolved in T.F.A. (0.7 ml) in a stoppered n.m.r. tube and the reaction followed by n.m.r. After 4 h resonances due to starting material (43%) and the trioxopiperazine (117) (57%) as well as benzaldehyde and acetic acid could be discerned. The solution was left at room temperature for 20 h and the solvent evaporated. Trituration with ether to remove the benzaldehyde gave the crystalline 1-methyl-2,3,5-trioxopiperazine (117) (10.6 mg, 100%).

(ii) With base. The dioxopiperazine (77 mg) in dry T.H.F. (2 ml) was treated with sodium hydride (6 mg). The suspension was stirred at room temperature for 20 h after which time it gave a negative starch-iodide test. The mixture was poured into dichloromethane (20 ml), neutralised with N HCl (0.25 ml) in water (5 ml) and the aqueous phase back-extracted with more dichloromethane (10 ml). The combined extracts were dried, evaporated and the residue triturated with ether, to remove the benzoyl compounds, affording the hydroxy acetate (131) (17 mg, 23%) as colourless prisms. The aqueous phase was evaporated to dryness and the residue triturated with hot acetone. Filtration

removed the inorganic material and the acetone was evaporated to give the trioxopiperazine (117) (27 mg, 75%) as white crystals.

Preparation and reactions of 3-chloro-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine and 3-acetoxybenzyl-1-methyl-5,6-dihydropyrazin-2,5-dione mixture.

3-Hydroxy-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (26 mg) was suspended in deuteriochloroform (1 ml) in an n.m.r. tube and thionyl chloride (0.2 ml) added. Complete solution was attained after 15 min and within 90 min all the starting alcohol had reacted. Evaporation of the solvent and excess reagent gave a mixture (8:5) of the 3-chloro-dioxopiperazine (144), τ 1.70 (1H,bs), 2.63(5H,s), 3.38(1H,s), 5.89 and 6.48(2H,ABq, J 19 Hz), 7.12(3H,s) and 7.79(3H,s) and the 3-acetoxybenzyl-pyrazindione (129), τ 2.45(5H,s), 3.47 (1H,s), 5.78 and 5.98(2H,ABq, J 18 Hz), 6.87(3H,s) and 7.90(3H,s) as an oil.

A similar mixture of (144) and (129) was also prepared by reaction of 3-methylthio-3-acetoxybenzyl-1-methyl-2,5-dioxopiperazine (137) (100 mg) in dichloromethane (5 ml) with t-butyl hypochlorite (0.2 ml) at room temperature for 30 min.

Reactions of the mixture were investigated as follows.

- (i) Treatment with methanol for 10 min at room temperature gave, after evaporation, a quantitative yield of the methoxy acetate (130).
- (ii) Treatment with pyridine at room temperature rapidly produced a deeply coloured solution which on evaporation gave an intractible yellow foam.
- (iii) The mixture (from 146 mg of hydroxy acetate) in dichloromethane (10 ml) containing boron trifluoride etherate (0.1 ml) was stirred at room temperature for 1 week. Aqueous workup gave the hydroxy acetate (90 mg, 63%) as the sole product.
- (iv) The mixture (from 146 mg of hydroxy acetate) in dry nitromethane (8 ml) was treated with anhydrous aluminium chloride (100 mg) and the solution stirred at room temperature for 5 days. The solution was poured into dichloromethane (20 ml), washed with water (10 ml), dried and evaporated to give a multi-

component oil (77 mg). The mixture was separated by plc (1:19 MeOH-CHCl₃). The major component was the hydroxy acetate (131) (23 mg, 16%). Only one other single compound was isolated, as colourless prisms from ether, 3,1'-Z-3-benzylidene-6-hydroxy-1-methyl-2,5-dioxopiperazine (146) (12 mg, 10%), m.p. 148-149°, $\nu_{\max}$ 3225, 1710, 1680, 1625, 1452, 1395, 1360, 1295, 1262, 1212, 1030, 855, 765 and 692 cm⁻¹, τ 1.90(1H,bs), 2.62(5H,s), 2.88(1H,s), 4.78(2H,bs, collapses to a sharp singlet, 1H, on exchange) and 6.85(3H,s), m/e 232(M⁺, 100%), 216(6), 215(4), 187(14), 143(8), 119(22), 118(14), 117(41), 116(8), 91(8), 90(14) and 89(11).

Solvolysis of 3-methoxy-3-acetoxymethyl-1-methyl-2,5-dioxopiperazine (130).

(i) Base induced. The methoxy acetate (100 mg) was stirred in ethanol (4 ml) containing triethylamine (0.25 ml) for 2 days at room temperature. By this time the initial yellow colouration had faded. The solution was evaporated to dryness and the crystalline residue triturated with dichloromethane (5 ml). The solids were filtered off and dried to yield 3-methoxy-3-hydroxymethyl-1-methyl-2,5-dioxopiperazine (142) (39 mg, 45%), as colourless prisms from acetone, m.p. 215-218°(decomp.), $\nu_{\max}$ 3420, 3200, 3070, 1690, 1660, 1455, 1435, 1415, 1380, 1335, 1328, 1270, 1205, 1185, 1110, 1095, 1075, 1060, 990, 838, 787, 742, 710 and 642 cm⁻¹, τ 2.78(5H,s), 3.40(1H,bs), 4.67(1H,s), 4.77(1H,s), 6.45 and 7.48(2H,ABq, J 19 Hz), 6.63(3H,s) and 7.27(3H,s) (Found: C, 59.2; H, 5.85; N, 10.6. C₁₃H₁₆N₂O₄ requires C, 59.1; H, 6.1; N, 10.6%).

The triturate afforded crystalline starting material (50 mg, 50%).

(ii) Acid induced. The methoxy acetate (306 mg) was stirred with 3% hydrogen chloride in methanol (15 ml) for 2 days at room temperature. The solution was evaporated and the residue triturated with dichloromethane (2x5 ml). Filtration afforded the methoxy alcohol (142) (202 mg, 70%).

The triturate was evaporated and the residue separated by plc (1:19 MeOH-CHCl₃). The most intense band gave 3,1'-Z-3-benzylidene-1-methyl-2,5-dioxopiperazine (149) (15 mg, 6%), as an oil, $\nu_{\max}^{\text{film}}$ 3220, 3000-2840, 1710, 1680,

1450, 1405, 1350, 1220, 1255, 1200, 1020, 858, 765 and 690 cm^{-1} , τ 2.03(1H, bs), 2.57(5H,s), 2.85(1H,s), 5.25(1H,s), 6.47(3H,s) and 6.83(3H,s), m/e 246 (M^+ ,100%), 215(8), 188(11), 187(78), 160(11), 149(23), 119(9), 118(25), 117(13), 91(9), 90(32) and 89(25).

3-Methoxy-3-benzoyl-1-methyl-2,5-dioxopiperazine (147)

3-Methoxy-3-hydroxybenzyl-1-methyl-2,5-dioxopiperazine (230 mg) was stirred with activated manganese dioxide (5 g) in acetone (30 ml) at room temperature for 2 days. The mixture was filtered through celite, washed well with acetone and the filtrates evaporated to dryness. The residue was triturated with hot ether (3x10 ml), unreacted starting material filtered off and the triturate evaporated to give 3-methoxy-3-benzoyl-1-methyl-2,5-dioxopiperazine (147) (46 mg, 20%), colourless plates from ether, m.p. $160-162^\circ$, $v_{\max}$ 3190, 3070, 1695, 1600, 1580, 1465, 1450, 1420, 1380, 1320, 1250, 1240, 1205, 1175, 1165, 1150, 1105, 1052, 1005, 1000, 885, 792, 745, 695 and 652 cm^{-1} τ 1.65(2H,m), 2.50(3H,m), 2.85(1H,bs), 5.90(2H,s), 6.53(3H,s) and 6.97(3H,s) (Found: C,59.5; H,5.4; N,10.6. $C_{13}H_{14}N_2O_4$ requires C,59.5; H,5.4; N,10.7%).

Reaction of 3,1'-Z-3-benzylidene-1-methyl-2,5-dioxopiperazine (110) with t-butyl hypochlorite.

The dioxopiperazine (0.54 g) in dichloromethane (25 ml) was treated with t-butyl hypochlorite (2 ml) and allowed to stand at room temperature for 1 h. After this time all the starting material had reacted (tlc) and the yellow solution was evaporated. Thorough drying on the oil pump, to remove residual t-butanol, afforded 3-chlorobenzyl-1-methyl-5,6-dihydropyrazin-2,5-dione (150), (0.63 g, 100%), as a yellow, non-crystalline glass, $v_{\max}$ ($CHCl_3$) 1745, 1680, 1620, 1455, 1410, 1320, 1260, 1155 and 695 cm^{-1} , τ 2.5(5H,m), 3.45(1H,s), 5.70(2H,s) and 7.00(3H,s), $\lambda_{\max}^{THF}$ 232, 250(sh), 274 and 378 nm(qualitative).

Reactions of the 3-chlorobenzyl-acylimine (150) with nucleophiles.

(i) The acylimine (125 mg) was treated with methanol (2 ml) at room temperature for 15 min. The yellow colour was quickly discharged and evaporation of the solvent gave a white crystalline solid (140 mg, 100%). Recrystallisation from methanol gave clusters of 3-methoxy-3-chlorobenzyl-1-methyl-2,5-dioxopiperazine (153), m.p. 199-202°(decomp.) $\nu_{\max}$ 3190, 3080, 1680, 1445, 1415, 1380, 1324, 1275, 1205, 1180, 1100, 1065, 1000, 985, 854, 780, 735, 710, 668 and 635 cm^{-1} , χ (T.F.A.) 1.90(1H,bs), 2.78(5H,s), 4.70(1H,s), 6.36 and 7.54(2H,ABq, J 19 Hz), 6.78(3H,s) and 7.45(3H,s) (Found: C,55.3; H,5.4; N,10.0; Cl, 12.7. $\text{C}_{13}\text{H}_{15}\text{N}_2\text{O}_3\text{Cl}$ requires C,55.2; H,5.35; N,9.9; Cl,12.5%).

The same derivative was also prepared by oxidation of the benzylidene dioxopiperazine (110) with t-butyl hypochlorite in methanol for 1 h at room temperature. The yield was quantitative.

(ii) The acylimine (125 mg) was treated with acetone (2 ml) containing water (0.1 ml). The yellow colour was immediately discharged and a white precipitate formed. The solvent was evaporated and the product dried on the pump (132 mg, 100%). Recrystallisation from acetone afforded colourless prisms of 3-hydroxy-3-chlorobenzyl-1-methyl-2,5-dioxopiperazine (154), m.p. 207-208°(decomp.), $\nu_{\max}$ 3330, 3240, 3080, 1690, 1670, 1452, 1415, 1380, 1330, 1270, 1238, 1192, 1125, 1100, 1040, 935, 865, 830, 808, 772, 705 and 672 cm^{-1} , χ (T.F.A.) 2.78 (5H,s), 4.73(1H,s), 6.53 and 7.48(2H,ABq, J 19 Hz), and 7.38(3H,s) (Found: C,53.5; H,4.9; N,10.4; Cl,13.2. $\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_3\text{Cl}$ requires C,53.6; H,4.9; N,10.4; Cl,13.2%).

(iii) The acylimine (250 mg) was treated with ethanethiol (1 ml) in dichloromethane (5 ml). After 20 h. at room temperature the solution was evaporated and the resultant oil triturated with ether to induce crystallisation.

Filtration gave 3-ethylthio-3-chlorobenzyl-1-methyl-2,5-dioxopiperazine (157) (210 mg, 68%), colourless chunks from ether, m.p. 140-142°, $\nu_{\max}$ 3180, 3060,

1688, 1665, 1460, 1430, 1408, 1380, 1324, 1276, 1038, 833, 795, 772, 706, 658 and 625 cm^{-1} , τ 2.57(5H,s), 2.90(1H,bs), 4.37(1H,s), 6.29 and 7.20(2H,ABq, J 18 Hz), 7.23(2H,q, J 7 Hz), 7.32(3H,s) and 8.72(3H,t, J 7 Hz) (Found: C,53.8; H,5.3; N,8.95; S,10.1. $\text{C}_{14}\text{H}_{17}\text{N}_2\text{O}_2\text{ClS}$ requires C,53.75; H,5.5; N,9.0; S,10.25%).

N.m.r. examination of the ether triturate excluded the presence of the benzylidene dioxopiperazine (110), but did show another product besides the ethylthio chloride (157). However, decomposition occurred upon attempted purification by plc.

Reactions of the 3-chlorobenzyl-acylimine (150) with Lewis acids.

(i) The acylimine (1.25 g) in dichloromethane (30 ml) containing boron trifluoride etherate (0.5 ml) was allowed to stand at room temperature for 3 days. The solution was washed with water (15 ml), dried and evaporated to yield a pale yellow crystalline solid (1.25 g, 100%). Recrystallisation from acetone/petrol afforded white clusters of 3,1'-Z-3(1'-chloro)benzylidene-1-methyl-2,5-dioxopiperazine (159), m.p. $179-180^\circ$, ν_{max} 3220, 1705, 1675, 1610, 1598, 1465, 1435, 1385, 1323, 1307, 1225, 962, 885, 830, 765, 740 and 695 cm^{-1} , τ 1.80(1H,bs), 2.60(5H,s), 5.83(2H,s) and 7.05(3H,s) λ_{max} 223.5 and 259 nm (ϵ 29,000 and 28,000) (Found: C,57.5; H,4.6; N,11.4; Cl,14.1. $\text{C}_{12}\text{H}_{11}\text{N}_2\text{O}_2\text{Cl}$ requires C,57.5; H,4.4; N,11.2; Cl,14.1%).

(ii) The acylimine (125 mg) in dry nitromethane (3 ml) was treated with anhydrous aluminium chloride (40 mg) and the solution stirred at room temperature for 3 days. The thick solution was diluted with dichloromethane (20 ml) washed with saturated NaHCO_3 solution (10 ml), water(10 ml), dried and evaporated to give a yellow oily solid (94 mg). The products were separated by plc (1:19 MeOH- CHCl_3 , two elutions) to yield the chlorobenzylidene dioxopiperazine (159) (24 mg, 20%), the 6-hydroxy-3-benzylidene dioxopiperazine (146) (21 mg, 17%) and the hydroxy chlorobenzyl dioxopiperazine (154) (16 mg, 12%) in order of increasing polarity.

Oxidation of 3,1'-Z-3(1'-chloro)benzylidene-1-methyl-2,5-dioxopiperazine (159) with t-butyl hypochlorite.

The chlorobenzylidene compound (125 mg) in dichloromethane (5 ml) was stirred with t-butyl hypochlorite (0.5 ml) at room temperature for 20 h by which time all the starting material had reacted (tlc). The solution was evaporated to dryness to give a yellow foam of crude 3-dichlorobenzyl-1-methyl-5,6-dihydropyrazin-2,5-dione (160), λ 2.1-2.7(5H,m), 5.58(2H,s) and 7.00(3H,s). This dichlorobenzyl-acylimine was characterised by reaction with acetone (3 ml) containing water (1 ml). The decolourised solution was evaporated to dryness, after 20 min, and the residue triturated with ether-dichloromethane (10:1) to give 3-hydroxy-3-dichlorobenzyl-1-methyl-2,5-dioxopiperazine (161) (98 mg, 66%), colourless prisms from acetone, m.p. 210-213°(decomp.), $\nu_{\max}$ 3270, 1670, 1455, 1382, 1330, 1260, 1190, 1143, 1124, 829, 804, 750, 705, and 670 cm^{-1} ,

λ 2.17(2H,m), 2.50(3H,m), 3.30(1H,bs), 4.95(1H,bs), 6.38 and 7.03(2H,ABq, J 18 Hz) and 7.08(3H,s) (Found: C,47.5; H,4.1; N,9.1; Cl,23.5. $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_3\text{Cl}_2$ requires C,47.55; H,4.0; N,9.2; Cl,23.4%).

Peracid oxidation of the 3-chlorobenzyl-acylimine (150).

The acylimine (125 mg) in dichloromethane (7 ml) was treated dropwise with a solution of m-chloroperbenzoic acid (123 mg, 70%) in dichloromethane (5 ml) at room temperature. The yellow colour disappeared over 10 min, but the solution still gave a positive starch-iodide test so stirring was continued overnight. The solution was diluted with dichloromethane (20 ml) and washed with water. The aqueous phase, upon evaporation, afforded 1-methyl-2,3,5-trioxopiperazine (117) (24 mg, 33%). The organic phase was washed with saturated NaHCO_3 solution (10 ml), dried and evaporated to yield an oil (135 mg). This product was allowed to stand open to the atmosphere for 4 days and was then triturated with ether to remove benzoyl compounds. There remained the hydroxy chlorobenzyl dioxopiperazine (154) (77 mg, 58%).

Reaction of the 3-chlorobenzyl-acylimine (150) with sodium methoxide.

The acylimine (250 mg) in dry T.H.F. (10 ml) was stirred with sodium methoxide (54 mg) at room temperature. The solution rapidly became a deep orange colour and after 2 h was poured into water (20 ml) containing 2N HCl (0.5 ml), extracted with dichloromethane (2x30 ml), dried and evaporated to yield an orange oil. The mixture was separated by plc (1:19 MeOH-CHCl₃) to give 3,1'-Z-3-benzylidene-6-methoxy-1-methyl-2,5-dioxopiperazine (149) (93 mg, 38%) as the only isolable product.

Reaction of the 3-chlorobenzyl-acylimine (150) with sodium borohydride.

The acylimine (250 mg) in dry D.M.F. (2 ml) was reacted with sodium borohydride (11 mg). The mixture became warm and most of the colour was discharged. Stirring was continued for 24 h and the mixture poured into dichloromethane (25 ml) and washed with 2N HCl (10 ml). The aqueous phase was back-extracted with dichloromethane (20 ml) and the combined extracts dried and evaporated to yield a yellow oil. Overnight drying on the oil pump removed residual D.M.F. and the two major products were separated by plc (1:19 MeOH-CHCl₃). The less polar band afforded crystals of the 3,1'-Z-3-benzylidene-1-methyl-2,5-dioxopiperazine (110) (88 mg, 41%). The more polar band gave an oil (60 mg) whose n.m.r. spectrum could only be explained by a mixture of at least two compounds. Further purification (plc) led to more decomposition and the attempt was abandoned.

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