SYNTHETIC APPROACHES TO TRYPTOPHAN BASED DIPEPTIDE ALKALOIDS

A thesis presented by

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Abstract

The occurrence and biosynthesis of alkaloids containing isolated isoprenyl units are reviewed.

Attempts directed towards a synthesis of naturally occurring 2-(1',1'-dimethyl)allyltryptophan derived dioxopiperazines by rearrangement of N-(3',3'-dimethyl)allyltryptophan derivatives are described.

The novel, reversible, intramolecular trapping of indolenines produced by protonation of an indole is reported and the synthetic utility of the resulting indolines is investigated.

An example of a highly unusual direct substitution of an indole in positions 2 and 7 is provided.

An unprecedented oxidation of a 1, 2, 3-trialkylindole at the 1-alkyl group is described and the reactions of the product are reported at length.

Carbon-13 nuclear magnetic resonance spectra of a number of cyclo-prolyltryptophyl compounds are listed.

Acknowledgements

I thank Professor Peter G. Sammes for his help and encouragement, and for the privilege of working in his department. I also thank Professor Sir Derek H.R. Barton, F.R.S., for the privilege of working in his department and the Science Research Council for financial support.

I am grateful to the technical staff of the chemistry departments of Imperial College and the City University without whom this work would not have been possible, and I acknowledge the friendship and stimulation provided by my colleagues in the research laboratories of these institutions.

Finally, my warmest thanks go to my parents for their support during my years as a student.

Contents

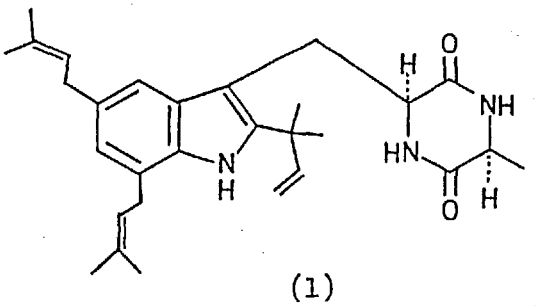
REVIEW

Introduction

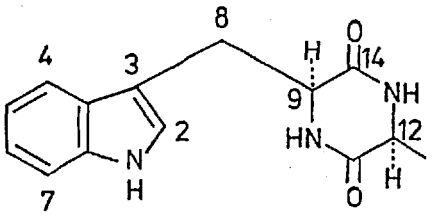
The occurrence and biosynthesis of alkaloids containing hemiterpenoid derived units are reviewed with the exception of the ergot alkaloids and related natural products derived from 4-(3',3'-dimethyl)-allyltryptophan and the prenylated phenols possessing a nitrogen containing side chain (for example some of the pyrrolizidine alkaloids[252]). The biosynthesis of ergot alkaloids and related compounds has recently been reviewed.[59]

Isoprenylated indole alkaloids and related compounds containing a tryptophan derived dioxopiperazine ring

In 1943 Italian workers reported[1] the isolation of the mould metabolite echinulin, (1), from Aspergillus echinulatus and subsequently this natural product has been isolated from four other micro-organisms.[2,3,4] In the last 6 years approximately 30 related metabolites have been obtained from mould growths and these compounds all resemble echinulin in that they possess a tryptophan moiety with a 1',1'-dimethylallyl group in position 2 of the indole nucleus. This highly unusual coupling of an isoprene unit in an inverted configuration is almost unique amongst indole alkaloids.



(1)



(2)

(2a), opposite stereochemistry about position 9

Following the isolation of echinulin there was a stay of almost 20 years before modern methods of instrumental analysis allowed determination of the structure. After early work,[5,6] the structure was eventually shown to be (1) by a combination of spectral and chemical means,[7] the substitution pattern in the benzene ring being confirmed by degradation to known aniline derivatives. The absolute configurations of the two asymmetric centres were determined by comparison of the optical rotatory dispersion (o.r.d.) curve of echinulin with those of the four possible isomers of cyclo-alanyltryptophyl.[8] Echinulin was found to have the same characteristics as cyclo-L-alanyl-L-tryptophyl, (2), suggesting the S-configuration at both chiral centres. This was confirmed by hydrolysis and oxidation of echinulin which gave L-alanine and L-aspartic acid.[9]

The structure of echinulin suggests that in the organism it is derived from the amino-acids L-tryptophan and L-alanine along with three isoprene units. A number of feeding experiments using Aspergillus amstelodami, which is a good source of echinulin,[2] have confirmed that this is so; thus tryptophan labelled with tritium in the methylene group was incorporated into echinulin as, incidentally, were [2-^{13}C]glycine and [1-^{13}C]glycine.[10] Carbon-13 n.m.r. spectroscopy was used to demonstrate that [2-^{13}C]glycine and [1-^{13}C]glycine were specifically incorporated into echinulin in positions 9 and 14 respectively confirming that tryptophan is biosynthetically derived from glycine.[11] This result had previously been reported by Birch[12] although he did not determine the site of incorporation into echinulin when [1-^{14}C]glycine was fed to Aspergillus amstelodami. Birch also found that D,L-tryptophan labelled with ^{14}C in the side chain methylene position was incorporated into echinulin;[13] the site of the label was not rigorously determined but hydrolysis of the isolated product gave alanine containing no label

confirming that it still resided in the tryptophan half of the molecule. Evidence that tryptophan is incorporated intact into echinulin has come from the work of two groups; Italian workers have found that tryptophan doubly labelled with tritium and ^{14}C at the methylene position is converted into echinulin with equal incorporation of both labels.[14] Also tryptophan labelled at any one of positions 2, 1', 2' or 3' was incorporated equally irrespective of the site of the label.[15] Degradation of echinulin derived from [1'-^{14}C]tryptophan revealed the incorporated label in the expected position proving that no randomization had taken place. It was also found[15] that L-tryptophan was incorporated more efficiently than D-tryptophan supporting the configuration assigned to position 8 of echinulin.

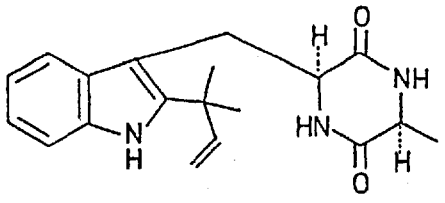
Incorporation of alanine into echinulin has been demonstrated by Birch[12] who fed D,L-[1-^{14}C]alanine to *A. amstelodami* and succeeded in isolating radioactive echinulin.

The dimethylallyl groups of echinulin are very probably mevalonate derived; feeding either [2-^{14}C]mevalonic acid or [2-^{14}C]acetate resulted in incorporation of label into the tryptophan half of the echinulin molecule.[12] The point along the biosynthetic pathway at which the C_5 units are introduced into echinulin has also been examined. In theory, isoprenylation of tryptophan could occur either before or after coupling with alanine and before or after formation of the dioxopiperazine ring. It has been found that *cyclo*-L-alanyl-L-tryptophyl, (2), labelled at position 8 is efficiently incorporated into echinulin in *A. amstelodami* suggesting that the dioxopiperazine (2) can be formed from tryptophan and alanine prior to attachment of the C_5 units.[16] The degree of incorporation into echinulin of label from *cyclo*-L-alanyl-L-tryptophyl was found to be much greater than into tryptophan isolated from the microorganism and the incorporation was not lessened by addition of tryptophan

along with the labelled dioxopiperazine. These facts strongly suggest that cyclo-L-alanyl-L-tryptophyl, (2), is incorporated intact and is further along the biosynthetic pathway than tryptophan. They also imply that isoprenylation occurs after the formation of the dioxopiperazine ring but do not rule out the possibility that isoprenylation can occur before this event as an alternative route. The absolute configuration assigned to echinulin was also supported in this work by the observation that cyclo-L-alanyl-L-tryptophyl, (2), was a more efficient precursor of echinulin than the L,D isomer.[16]

Formation of cyclo-L-alanyl-L-tryptophyl, (2), prior to isoprenylation has been confirmed by isolation of the enzyme which carries out part of the process. A cell free extract of A. amstelodami has been prepared[17] which will convert dioxopiperazine (2) into cyclo-L-alanyl-L-(2-(1',1'-dimethyl)allyl)tryptophyl, (4), in the presence of dimethylallylpyrophosphate. Furthermore no formation of the allylindole (4) is observed when no dimethylallylpyrophosphate is present or when, instead of (2), tryptophan, tryptophylalanine or alanyltryptophan are presented as substrates. In a further experiment[18] doubly labelled cyclo-L-alanyl-L-(2-(1',1'-dimethyl)allyl)tryptophyl, (4), was prepared from the dioxopiperazine (2) using the isolated enzyme and was fed to A. amstelodami

(4)



with the result that doubly labelled echinulin was formed. This suggests that compound (4) is incorporated intact and is a natural biosynthetic intermediate. Compound (4) has not yet been isolated from A. amstelodami but has been found in two other closely related micro-organisms, which

strongly supports its status as a biosynthetic intermediate rather than as an unnatural biosynthetic precursor.

On the basis of these results it appears that the biosynthetic route to echinulin in A. amstelodami involves coupling of L-tryptophan and L-alanine to give cyclo-L-alanyl-L-tryptophyl, (2), which is isoprenylated first to give cyclo-L-alanyl-L-(2-(1',1'-dimethyl)allyl)-tryptophyl, (4), and then echinulin. Although it has been shown that the allylindole (4) is a direct precursor of echinulin, it is also possible that isoprenylation of the benzene ring of the indole nucleus can occur before position 2; the isolation of a number of metabolites possessing isoprene units in position 2 only and the failure to detect analogues prenylated only in the benzene ring suggests that this is not a major pathway.

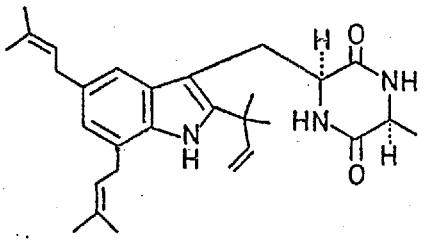
Eight other cyclo-dipeptides apparently derived from tryptophan and alanine and bearing an inverted isoprene unit at position 2 have been isolated from A. amstelodami. These compounds differ from echinulin and each other in the degree and position of substitution in the benzene ring and the oxidation pattern of the dioxopiperazine ring. They are listed in table 1. It is of interest to note that although the proportion of echinulin to all the other 2-isoprenylated cyclo-alanyltryptophyl derivatives in A. amstelodami is in the ratio 40:1, none of them carry the same substitution pattern in the benzene ring.[21]

The neoechinulins A-D have been shown to be derived from cyclo-L-alanyl-L-tryptophyl, (2). Feeding the cyclo-dipeptide (2) labelled in both the tryptophyl and alanyl halves of the molecule to A. amstelodami[24] resulted in spectacular incorporations into the neoechinulins; for neoechinulin A, 88% and for neoechinulins B, C and D, 43, 43 and 34% respectively. No change in the ratio of the double labels was observed demonstrating that the dipeptide was incorporated intact. The relative

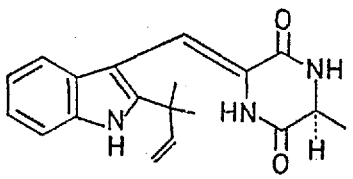
Table 1

Cyclo-L-alanyl-L-(2-(1',1'-dimethyl)allyl)tryptophyl derivatives isolated from Aspergillus amstelodami

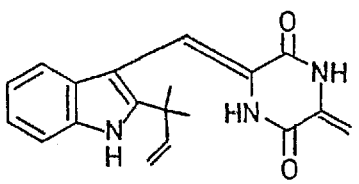
echinulin[2]
(1)



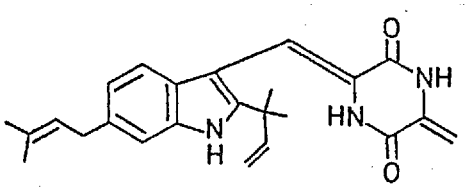
neoechinulin A[20]
(5)



neoechinulin B[20]
(6)



neoechinulin C[10]
(cryptoechinulin A)[20]
(7)



neoechinulin D[22]
(8)

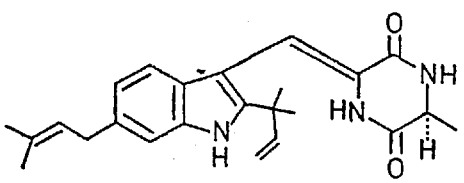
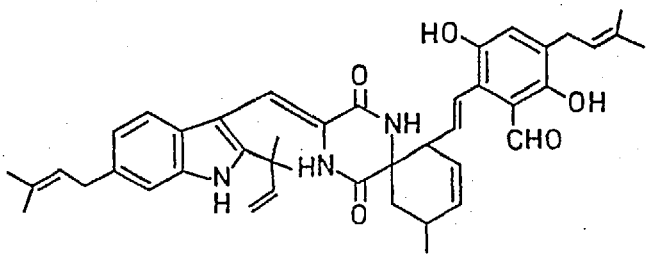
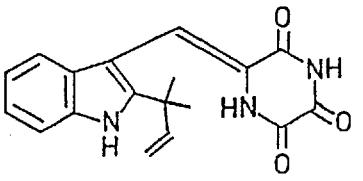


Table 1 (Continued)

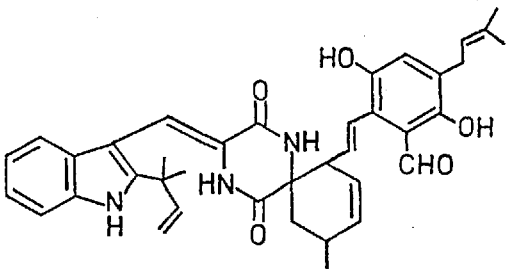
cryptoechinulin B[23]
(9)



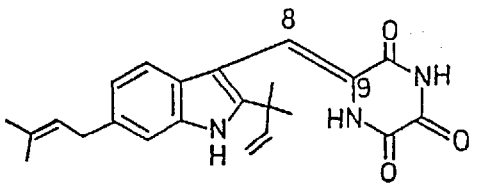
cryptoechinulin C[21]
(10)



cryptoechinulin D[23]
(11)



neoechinulin[19]
(12)

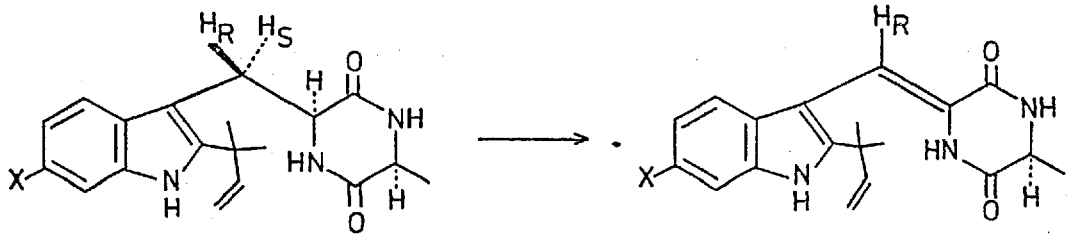


The stereochemistry around the 8,9-bond of these compounds is tentatively assigned as shown from ^{1}H and ^{13}C n.m.r.[14,24] The stereochemistry around the spiro junction in cryptoechinulins B and D is not known.

magnitude of incorporation figures in metabolites isolated from a plant or micro-organism generally reflects their distance along the biosynthetic pathway;[234] applying this rule here, cyclo-L-alanyl-L-tryptophyl, (2), is a more direct precursor of neoechinulin A than of neoechinulins B, C or D. Unfortunately the relative magnitudes of the incorporations of label into the last 3 compounds do not allow any conclusions to be drawn as to the order of the biosynthetic steps - i.e. the chronology of isoprene insertion and dioxopiperazine ring oxidation.

It has also been found[24] that cyclo-L-alanyl-D-tryptophyl (2a) is not a precursor of the neoechinulins in A. amstelodami. This is not surprising since all the neoechinulins are derived from the dipeptide (2) or (2a) by dehydrogenation of the 8,9-bond and inversion of configuration at position 9 would be expected to interfere with the enzymic dehydrogenation mechanism. As expected, neoechinulin obtained from tryptophan labelled non-stereospecifically in the side chain methylene group with a tritium atom and also with ^{14}C in the same molecule lost half of the tritium label relative to the ^{14}C internal standard. Similarly, neoechinulin C isolated from A. amstelodami retained all the tritium when (3'R)[3'-^{3}H,3'-^{14}C]-L-tryptophan was administered to the mould but lost all of the tritium label when the corresponding (3'S) isomer was fed, indicating that the pro-S hydrogen atom is lost.[14] This implies

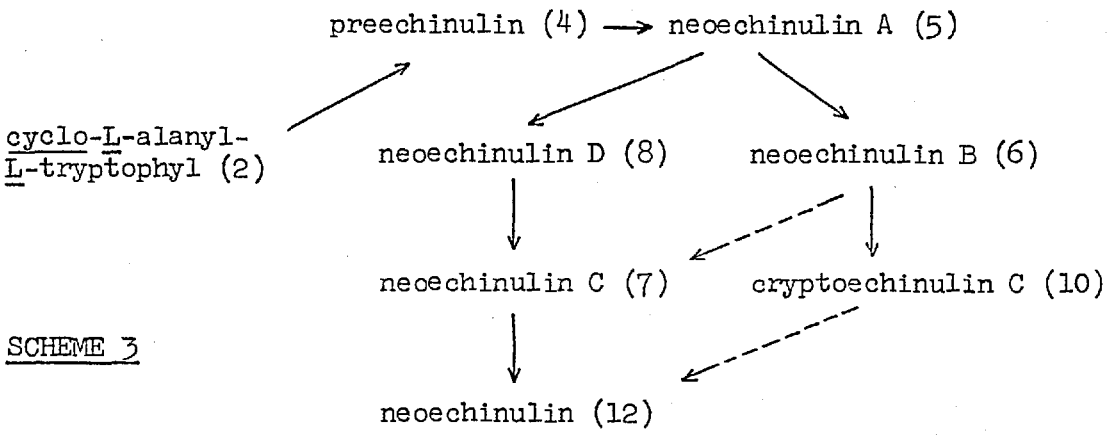
SCHEME 2



(13) (14)

that if the double bond of neoechinulin C possesses the Z-configuration as proposed,[14] then dehydrogenation proceeds by a cis elimination (scheme 2). In the scheme, X represents either a hydrogen atom or a dimethylallyl group depending upon whether isoprenylation of the benzene ring occurs before or after dehydrogenation of the dioxopiperazine ring.

Inspection of the functionality of the natural products from A. amstelodami suggests that as with echinulin the first step in their biosynthesis following dioxopiperazine ring formation is 2-isoprenylation succeeded by dehydrogenation of the 8,9-bond. Subsequently isoprenylation of position 6 or dehydrogenation of the alanyl side chain can apparently occur in any order. The progression neoechinulin A → neoechinulin B → cryptoechinulin C and the analogous series neoechinulin D → neoechinulin C (i.e. cryptoechinulin A) → neoechinulin suggests that these compounds are intermediates along similar biosynthetic pathways where the alanyl side chain is oxidatively

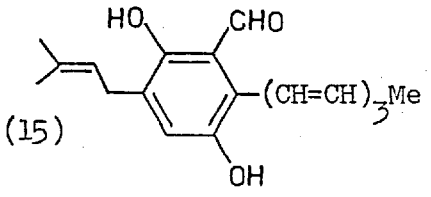


SCHEME 3

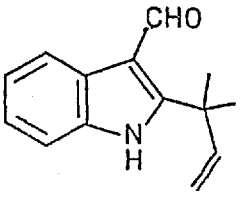
degraded. These possible metabolic pathways for A. amstelodami are shown in scheme 3. It is feasible in this tentative scheme that routes also exist between neoechinulin B and C and between cryptoechinulin C and neoechinulin as shown by the broken lines.

The two related compounds[23] cryptoechinulin B, (9), and cryptoechinulin D, (11), are of interest in that they appear to be derived

from a Diels-Alder reaction between neoechinulins B and C and the natural product auroglaucin, (15), which has previously been isolated from an Aspergillus species closely related to A. amstelodami.[25] The chemical viability of this route has been demonstrated in vitro by the formation of cryptoechinulin D from the thermal cycloaddition of neoechinulin C and auroglaucin.[23]



(15)



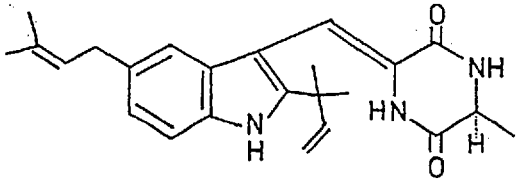
(19)

Some of the dioxopiperazines shown in table 1 have also been isolated from sources other than A. amstelodami. These compounds are summarized in table 2 along with 3 related cyclo-alanyltryptophyl derivatives, isoechinulins A, B and C, which have been found in A. ruber. These alkaloids bear a close resemblance to neoechinulins C and D, the main difference being that the dimethylallyl group in the benzene ring is at position 5 in the isoechinulins and position 6 in the neoechinulins. In isoechinulin C this dimethylallyl group has undergone oxidation in preference to the dehydro-alanyl moiety whereas according to scheme 3 the isomeric compound, neoechinulin C, undergoes preferential oxidation in the reverse manner leading to neoechinulin. This may suggest either that a different oxidative pathway is available in A. ruber or that in A. amstelodami neoechinulin is formed by isoprenylation of cryptoechinulin C rather than neoechinulin C. Another possibility is that the oxidising system in A. ruber is less selective; thus 2-(1',1'-dimethyl)allylindole-3-carboxaldehyde, (19), has been isolated from A. ruber, and could possibly arise from oxidative cleavage of neoechinulin A present in the same organism.

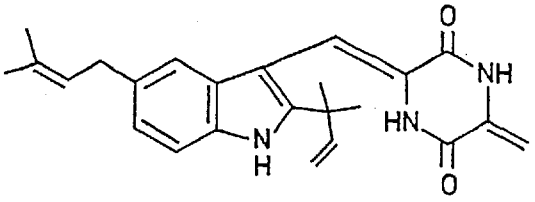
Table 2

Cyclo-L-alanyl-L-(2-(1',1'-dimethyl)allyl)tryptophyl derivatives isolated from Aspergillus species other than A. amstelodami

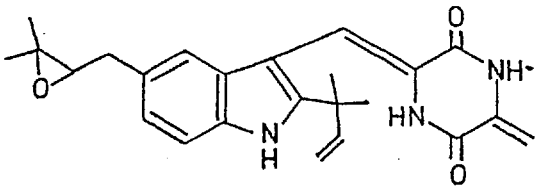
Name	No.	Source	Reference
echinulin	(1)	A. echinulatus	1
		A. repens	3
		A. ruber	3
		A. chevalieri	4
preechinulin	(4)	A. chevalieri	4,26
neoechinulin A	(5)	A. glaucus	27
		A. ruber	28
neoechinulin D	(8)	A. ruber	28
isoechinulin A	(16)	A. ruber	29
isoechinulin B	(17)	A. ruber	29
isoechinulin C	(18)	A. ruber	29



isoechinulin A



isoechinulin B

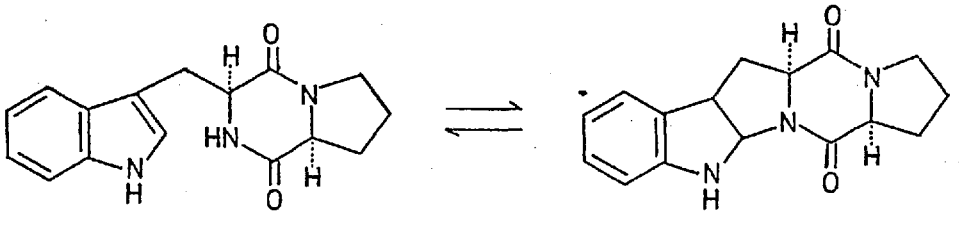


isoechinulin C

Biosynthetic studies into the mode of introduction of the dimethylallyl groups into the benzo-ring of the compounds listed in tables 1 and 2 have been the subject of only one publication.[30] The authors found that introduction of a dimethylallyl group could only be achieved *in vitro* after the indole ring had been hydroxylated and this led them to search for evidence of an N.I.H. shift during the formation of echinulin and neoechinulin from tryptophan in *A. amstelodami*. Accordingly [3'-^{14}C, 5-^{3}H,7-^{3}H]-L-tryptophan was administered to the mould and echinulin and neoechinulin were isolated. It was found that the echinulin contained no tritium label and that the neoechinulin had retained all the tritium label relative to the internal ^{14}C standard. In a second experiment, feeding [3'-^{14}C,4-^{3}H,6-^{3}H]-L-tryptophan gave echinulin which had retained all its tritium and neoechinulin which had lost half its tritium activity. These experiments demonstrate conclusively that the dimethylallyl group is inserted directly without loss of the flanking protons and rules out the intermediacy of hydroxy-indole intermediates derived from an N.I.H. shift mechanism.

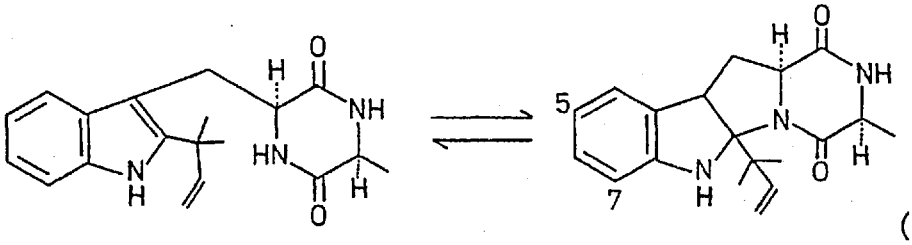
It is possible that isoprenylation in the benzene ring to give echinulin, or the isoechinulins, may occur *via* an indoline intermediate; indolines are known to be reactive towards electrophiles in positions 5 and 7 and their intermediacy would therefore explain the observed substitution pattern. It has been found in work described in this thesis that cyclo-L-prolyl-L-tryptophyl derivatives can undergo reversible acid

SCHEME 4



(38)

SCHEME 4 (continued)



(4) (35)

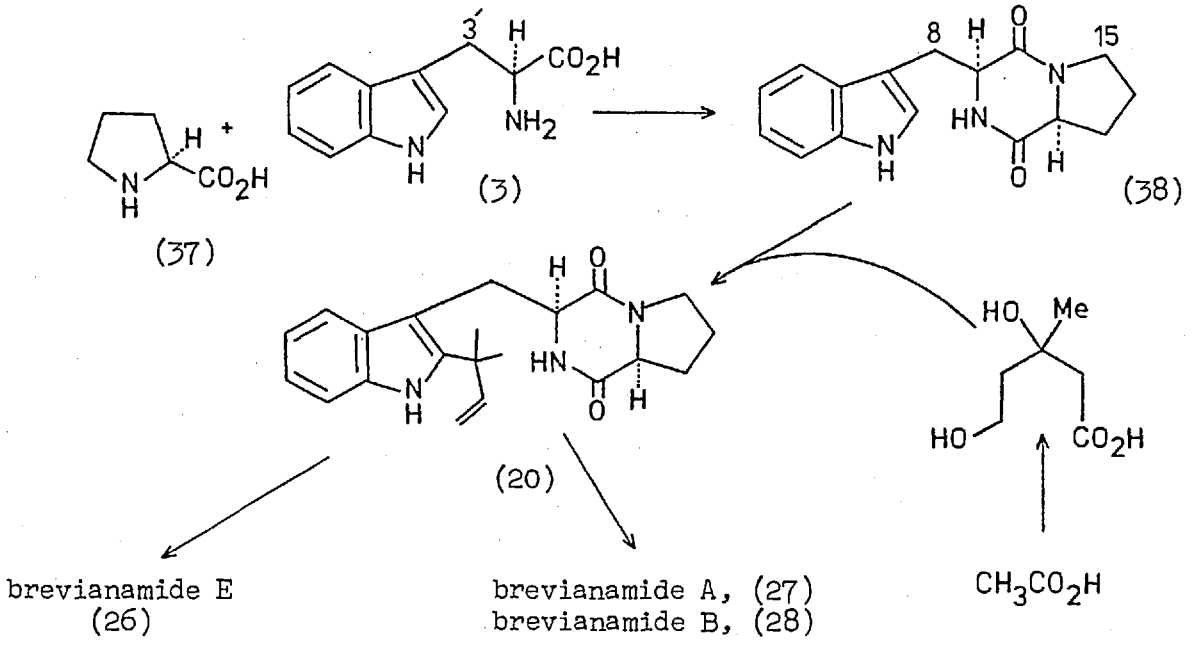
acid catalysed cyclization to give stable indolines (scheme 4) and it is feasible that 2-isopentenyl-cyclo-L-alanyl-L-tryptophyl derivatives such as compound (4) could do the same to give intermediates of the type (35), which could then react with dimethylallylpyrophosphate at positions 5 and/or 7 readily. This scheme does not, however, explain the formation of the neoechinulins and cryptoechinulins which apparently arise from electrophilic substitution of the dimethylallyl group at the non-activated position 6.

In the laboratory electrophilic substitution into the benzene ring of indole is not a selective process and positions 4, 5, 6 and 7 are all found to be of very similar reactivity.[42] In vivo no problem appears to exist; thus selective hydroxylation of tryptophan in position 5 is an important process[43] and ergot alkaloids are derived from 4-(3',3'-dimethyl)allyltryptophan.[44] The latter compound has been shown to be produced by direct prenylation of position 4 by 3,3-dimethylallylpyrophosphate in the presence of an enzyme isolated from a Claviceps species.[45] Floss and co-workers have investigated this reaction[46] and proposed that the enzyme prenylates position 4 of tryptophan by inhibiting attack at other equally reactive or more reactive positions. It was suggested that the enzyme might fit around the lower part of the benzene and pyrrole rings of the indole nucleus leaving only position 4 open to electrophilic attack. In order to test this theory Floss synthesized a series of tryptophan derivatives with a methyl group in each of the benzene ring positions; it was predicted that tryptophan with a

substituent in position 5, 6 or 7 would not be able to bond to the enzyme and so would not be selectively prenylated. In the event it was found that 5, 6 or 7-methyltryptophan were all selectively prenylated at position 4 although less readily than free tryptophan. However the ease of substitution apparently increased as the substituent was moved further from position 4 which is the opposite effect to that expected. Floss concludes that the enzyme in fact controls the reaction by binding to the two reactants (i.e. dimethylallylpyrophosphate and tryptophan) in a spacial arrangement such that reaction at position 4 is optimized. This conclusion can equally well be applied to explain the varying patterns of prenylation observed in the benzene rings of the metabolites shown in tables 1 and 2.

A group of 9 fungal metabolites have been isolated which appear to be derived from cyclo-L-prolyl-L-tryptophyl and which resemble the echinulins in possessing an inverted isopentenyl unit in position 2 of the indole nucleus. These are the so called austamides[31,32] and brevianamides[33,34] and they are listed in table 3. The brevianamides were isolated from Penicillium brevicompactum by Birch et al. who also determined their structures by spectral means[33,34] and carried out some biosynthetic studies. It was found that[3'-^{14}C]-D,L-tryptophan, (3), [2-^{14}C] mevalonic acid, (36), [2-^{14}C]acetate and [U-^{14}C]-L-proline, (37), were all incorporated into brevianamide A although the incorporations were unusually low for a mould. In a later paper Birch was able to report[48] improved incorporations of the same labelled compounds and further found that cyclo-L-prolyl-[8-^{14}C]-L-tryptophyl, (38), was incorporated into brevianamide A. The cyclo-dipeptide (38) was confirmed as a natural biosynthetic intermediate by its isolation from the P. brevicompactum[34] and was given the trivial name brevianamide F. It was also demonstrated that brevianamide F was incorporated intact and not as the

SCHEME 5



individual amino-acids following hydrolysis *in vivo* by feeding the doubly labelled dipeptide *cyclo*-[15-^{3}H]-*L*-prolyl-[8-^{14}C]-*L*-tryptophyl which resulted in the isolation of doubly labelled brevianamide A with no change in isotope ratio.

These labelling observations led Birch to propose that the major biosynthetic pathway to brevianamide A is that shown in scheme 5. The intermediate (20), named desoxybrevianamide E and analogous to pre-echinulin, (4), has not been isolated from *P. brevicompactum*, although a search has been made,[48] but it has been found[31] in *Aspergillus ustus* which is a source of other 2-isopentenyl-*cyclo*-prolyltryptophyl compounds, *viz*. the austamides (table 3). Birch has also demonstrated[33] *in vitro* the relationship between desoxybrevianamide E, (20), and brevianamide E, (26); mild reduction of the latter gave (20) which could be readily autoxidized back to the starting material.

It has been proposed[49,50] that brevianamides A and B are formed from desoxybrevianamide E *via* an intramolecular cycloaddition between the 2-isopentenyl group and an oxidized dioxopiperazine ring, as shown in scheme 6. The stereochemistry of oxidation of the indole determines

Table 3

Cyclo-L-prolyl-L-(2-(1',1'-dimethyl)allyl)tryptophyl derivatives

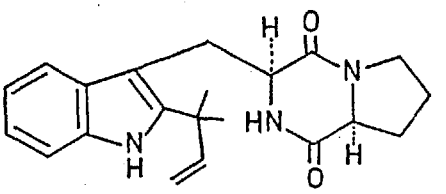
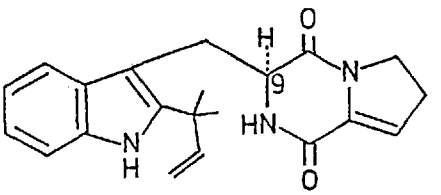
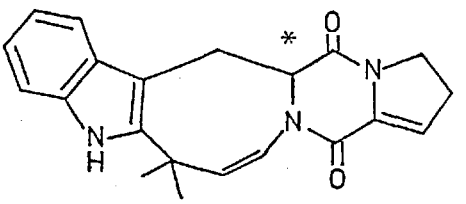
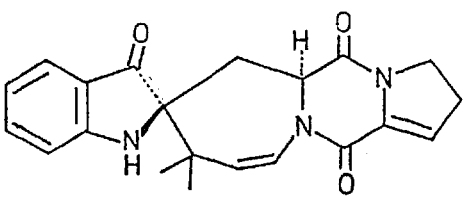
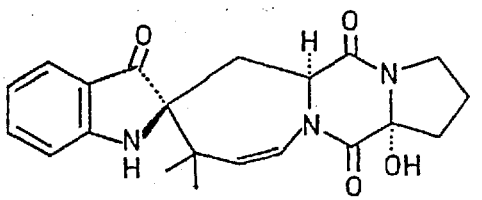
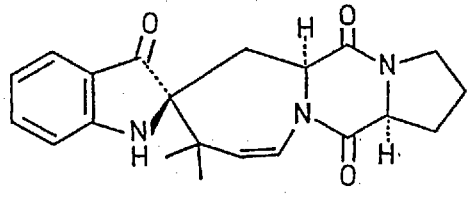
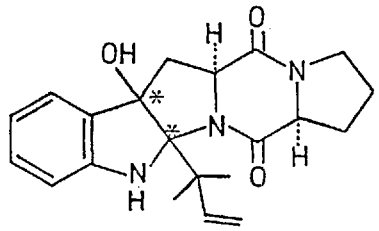
	(20)	desoxy-brevianamide E	Aspergillus ustus[31]
	(21)	-	A. ustus[31]
	(22)	-	A. ustus[31]
	(23)	austamide	A. ustus[31]
	(24)	-	A. ustus[32]

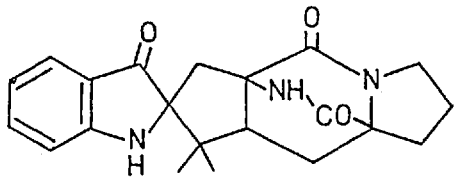
Table 3 (continued)



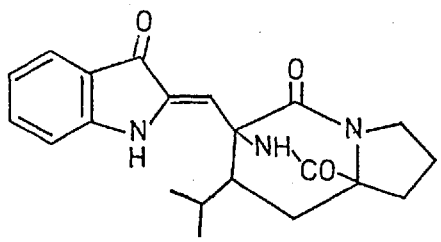
(25) - *A. ustus*[31]



(26) brevianamide E — *Penicillium brevicompactum*[33]



(27)** brevianamide A, brevianamide B — *P. brevicompactum*[33,34]



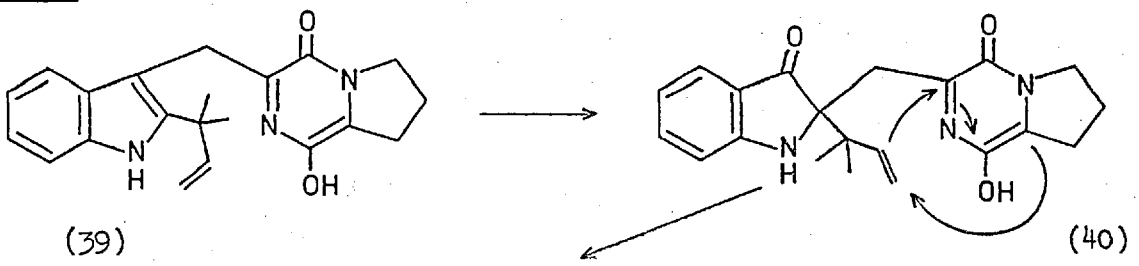
(28)*** brevianamide C, brevianamide D — *P. brevicompactum*[34]

* stereochemistry at these centres unknown

** brevianamide A and brevianamide B possess opposite configurations about the spiro junction

*** brevianamide C and brevianamide D possess opposite geometries about the double bond

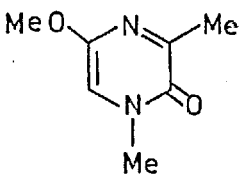
SCHEME 6



(39) (40)

brevianamide A/B

the configuration of the spiro junction in the product and hence the formation of either brevianamide A or B. The viability of this cycloaddition, which is unusual in that it involves an inverse demand Diels-Alder reaction with an unactivated double bond adding across an electron deficient diene system, has been demonstrated by model studies.[49,50] For example a Diels-Alder reaction readily occurred between cyclopentene and the diene system of the methoxy pyrazinone (41) to give a mixture of the two possible regioisomers. The formation of brevianamide A or B by this cycloaddition requires an oxidation of the indole nucleus and



(41)

of the dixopiperazine ring. These transformations are evidently possible in vivo as is clear from inspection of the closely related compounds isolated from A. ustus (table 3); thus the dioxopiperazine (21) is at the correct oxidation level for a cycloaddition to occur, although initial double bond isomerization is required, and the indole systems of the natural products (23), (24) and (25) have undergone an oxidative rearrangement in the required fashion.

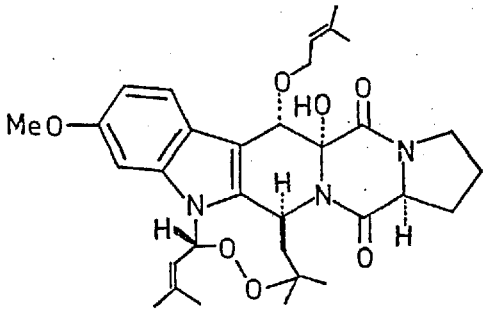
The group of compounds (20)-(25), named the austamides after the major component, were isolated from A. ustus by Steyn[31,32] who determined their structures from spectral evidence. The stereochemistry of the

asymmetric centres of desoxybrevianamide E, (20), were determined by comparison of the ^{1}H n.m.r. spectrum and optical rotatory dispersion curve with those of cyclo-L-prolyl-L-tryptophyl and cyclo-L-prolyl-D-tryptophyl and also by hydrolysis which gave L-proline. The absolute stereochemistry of position 9 of compound (21) was determined by reduction which gave a single diastereoisomer identical with the reduction product of desoxybrevianamide E. The absolute stereochemistry of the asymmetric centres of the most abundant alkaloid, austamide, was determined by ^{1}H n.m.r. and chemical evidence and confirmed by X-ray crystallographic analysis of the 5-bromo-tetrahydro derivative. Like compound (21), hydrogenation of austamide gave a single diastereoisomer. This compound was identical with the reduction product of compound (25) thus relating its stereochemistry to that of austamide. The stereochemistry of compound (24) was assigned by spectral comparison with the other compounds in table 3.

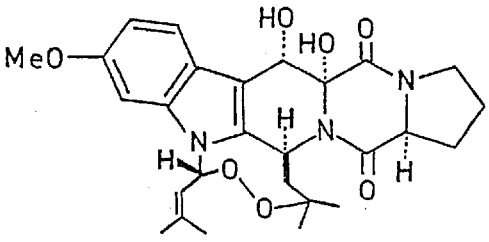
No biosynthetic studies have been carried out on the austamides. However consideration of their close structural similarity to the brevianamides strongly suggests that they are derived from L-tryptophan and L-proline via cyclo-L-prolyl-L-tryptophyl, (38), which is prenylated to give desoxybrevianamide E, (20). Subsequent oxidation of both the indole and dioxopiperazine rings and oxidative cyclization of the allylic side chain then leads to the observed natural products. The order in which the oxidation steps occur appears to be random judging by the isolated products and perhaps takes place indiscriminately until all 3 centres have been oxidized. This is in accordance with the fact that austamide, (23), is the most highly oxidized member of this group of natural products and is also the major component suggesting that it is the end product of the metabolism.

Table 4

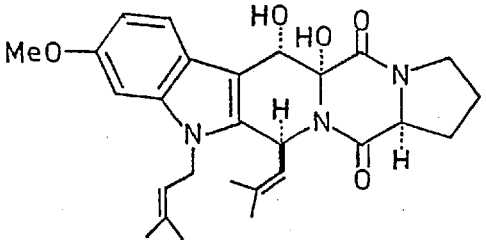
Cyclo-L-prolyl-L-tryptophyl derivatives



(29) fumitremorgen A — Aspergillus fumigatus[35]



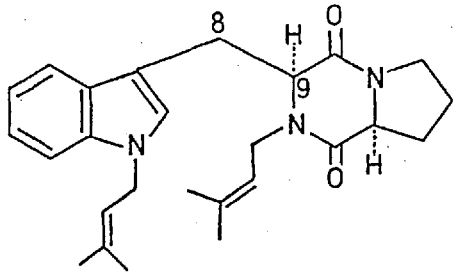
(30) verruculogen — Penicillium verruculosum[36]



(31) fumitremorgen B (lanosulin) — A. fumigatus[35,37] P. lanosum

Three other mould metabolites have been isolated which appear to be derived from cyclo-L-prolyl-L-tryptophyl and which contain isoprene units. These compounds are named fumitremorgen A, (29),[35] fumitremorgen B, (31),[35,37] and verruculogen, (30);[36] fumitremorgen B is also known as lanosulin. The structures of these alkaloids have all been determined by X-ray crystallographic analysis and they are shown in table 4. No biosynthetic studies have been reported for these compounds, which are evidently closely related, but by comparison with the brevianamides it is reasonable to suggest that they are derived from L-proline and L-tryptophan via cyclo-L-prolyl-L-tryptophyl, (38), which is then

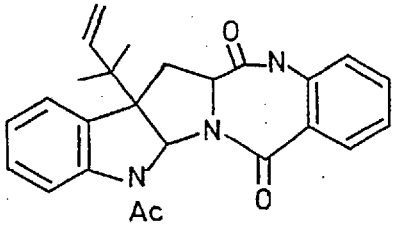
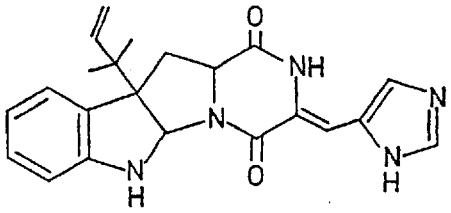
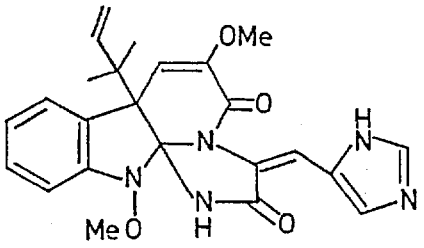
(42)



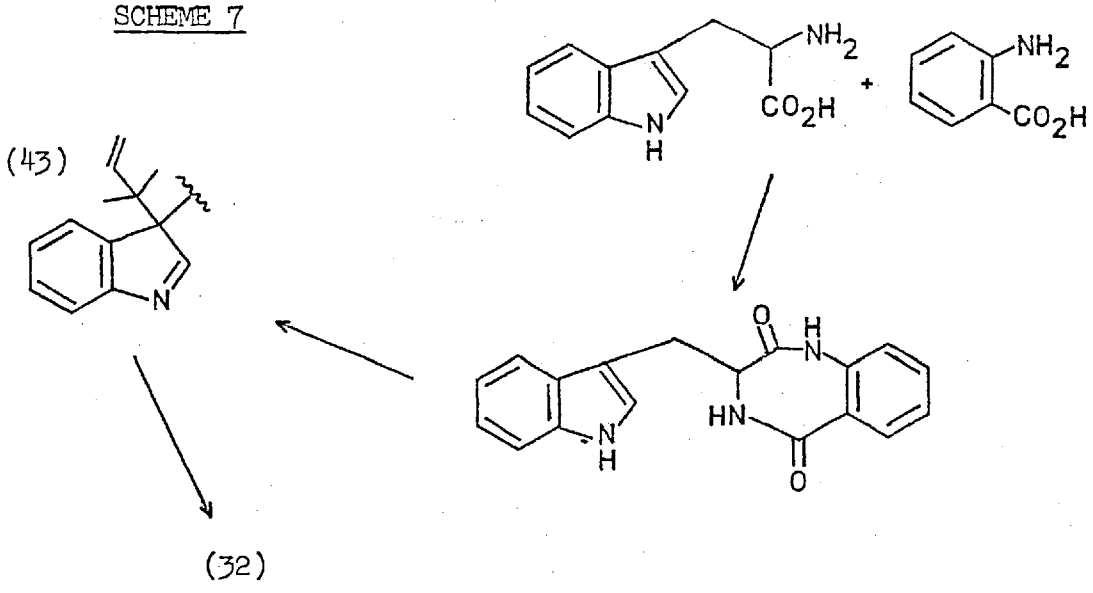
prenylated to give cyclo-L-prolyl-L-(N,N'-di(3',3'-dimethyl)allyl)-tryptophyl, (42). Dehydrogenation of the 8,9-bond as in the echinulins followed by cis hydroxylation along with further oxidation of the molecule in the benzene ring and the allylic side chains can then produce the observed natural products. The presence of a methoxyl group in position 6 is worthy of note; hydroxylation of the indole nucleus normally occurs at position 5 in vivo and also position 6 is the site of isoprenylation in the neoechinulins and cryptoechinulins. The presence of dimethylallyl groups on the dioxopiperazine and indole nitrogen atoms in these compounds is of relevance to the problem of the mechanism of introduction of the inverted 2-isopentenyl unit in the echinulins, austamides and brevianamides which is discussed below.

Another three isoprenylated alkaloids apparently derived from tryptophan have recently been isolated and they are shown in table 5. These compounds differ from the echinulins, austamides and brevianamides in that the inverted isopentenyl group is present at position 3 rather than position 2. The simplest of the three, compound (32), was isolated from an unidentified Aspergillus species[38] and in the organism is probably derived from condensation of tryptophan, anthranilic acid and dimethylallylpyrophosphate as shown in scheme 7. The validity of this scheme and its implications for the biosynthesis of 2-isopentenyl-tryptophan derivatives is discussed below. The structure was assigned on the basis of spectral data only and the relative stereochemistry of the chiral centres was not determined.

Table 5

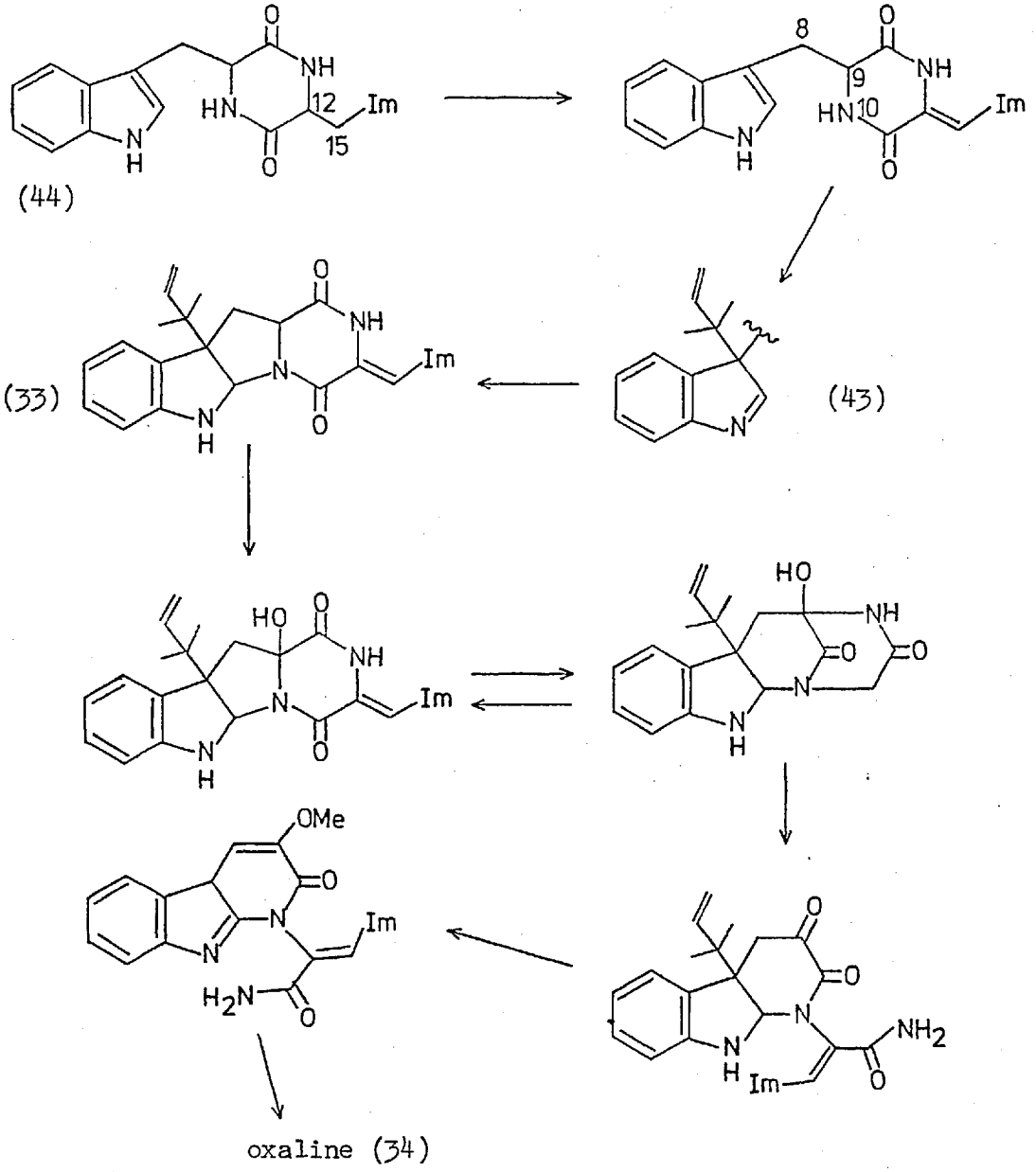
	(32)		unident. Aspergillus[38]
	(33)	roquefortine	P. roqueforti[39,40]
	(34)	oxaline	P. oxalicum[41]

SCHEME 7



Roquefortine, (33), was simultaneously isolated by two groups[39,40] from the same mould, Penicillium roqueforti, and the structure was assigned on the basis of chemical degradation and spectral data.[39] Biosynthetically, the structure is consistent with formation from tryptophan and histidine via cyclo-histidinyltryptophyl, (44), which can undergo dehydrogenation of the 12,15-bond and condense with dimethylallylpyrophosphate in an analogous fashion to compound (32), as shown in scheme 8.

SCHEME 8



The structure of the third natural product in table 5, oxaline, was determined by X-ray crystallographic analysis. Like roquefortine, this compound can also be regarded as being derived from tryptophan and histidine *via* *cyclo*-histidinyltryptophyl, (44), followed by oxidation of position 12 and prenylation of position 3. At this point the paths diverge and instead of attack upon the imine group of structure (43) in scheme 8 by the tryptophyl nitrogen atom of the dioxopiperazine ring to give roquefortine, the histidinyl nitrogen atom quenches the imine followed by fragmentation of the dioxopiperazine ring due to oxidative cleavage of the 9,10-bond. Subsequent oxidation and cyclization can then lead to oxaline. A route to oxaline *via* elaboration of roquefortine is shown in scheme 8; the chronology of the various oxidation and prenylation steps is purely speculative and roquefortine need not necessarily be an intermediate.

The isopentenyl unit present in position 2 of the austamides, brevianamides and echinulins possesses an extremely unusual inverted configuration. Normally an isopentenyl group is incorporated as a 3',3'-dimethylallyl unit, as in the benzo-ring of the echinulins and in the aromatic rings of many naturally occurring phenols.[54] This orientation arises from the fact that *in vivo* the isopentenyl unit is generally derived directly from dimethylallylpyrophosphate which acts as an electrophile with pyrophosphate as a leaving group. Steric hindrance by the methyl groups results in the predominance of the S_N2 mechanism over S_N2' when a nucleophile such as an aromatic system attacks the dimethylallylpyrophosphate, with the consequence that 3',3'-dimethylallyl groups are commonly observed in natural products and 1',1'-dimethylallyl groups are rare.

It has been suggested that the inverted configuration of the isopentenyl unit in the austamides, brevianamides and echinulins arises *via*:

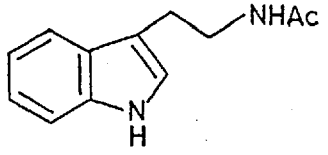
(i) initial substitution at the indole nitrogen atom in the normal manner to give an N-(3',3'-dimethyl)allyl derivative, followed by rearrangement with inversion into position 2;[13]

(ii) initial substitution at the dioxopiperazine nitrogen atom in the normal manner followed by rearrangement into position 2;[48]

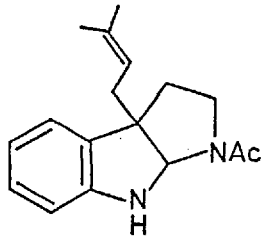
(iii) direct allylation at position 3 by either an S_N2 or S_N2' mechanism followed by an electrocyclic or Wagner-Meerwein rearrangement;[51]

(iv) direct allylation at position 2 by an S_N2' mechanism.[52]

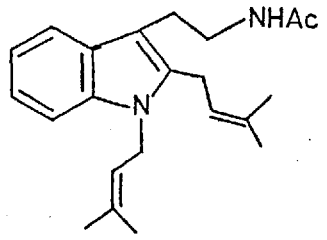
The feasibility of direct allylation at position 2 to give 2-(1',1'-dimethyl)allylindoles has been investigated *in vitro*.[52] Treatment of skatole with dimethylallyl bromide under "biological conditions" - *i.e.* in sodium acetate buffered aqueous acetic acid - gave 2-(3',3'-dimethyl)allylskatole, whilst indole itself gave 3-(3',3'-dimethyl)allylindole. N-Acetyltryptamine, (45), gave a mixture of the



(45)

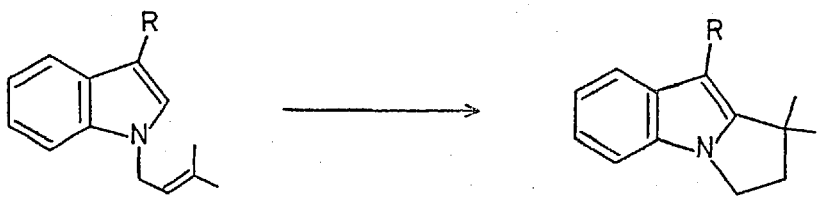


(46)



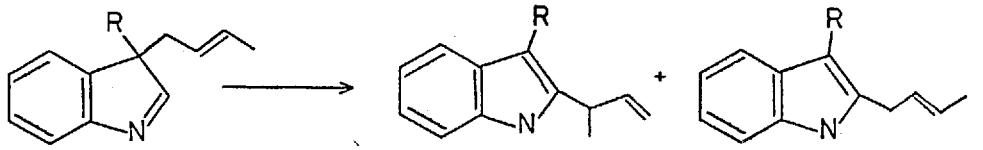
(47)

3-(3',3'-dimethyl)allylpyrrolo-indoline (46) and 1,2-di(3',3'-dimethyl)-allyl-N-acetyltryptamine, (47). These reactions are all consistent with the well authenticated mode of reaction between indoles and electrophiles, *viz.* initial substitution at position 3 followed by Wagner-Meerwein arrangement to position 2 if position 3 is already occupied.[42,53] As might be expected from this mechanism, no products derived from S_N2' attack by position 3 of the indoles upon dimethylallyl bromide were observed suggesting that this may not occur *in vivo* either. However



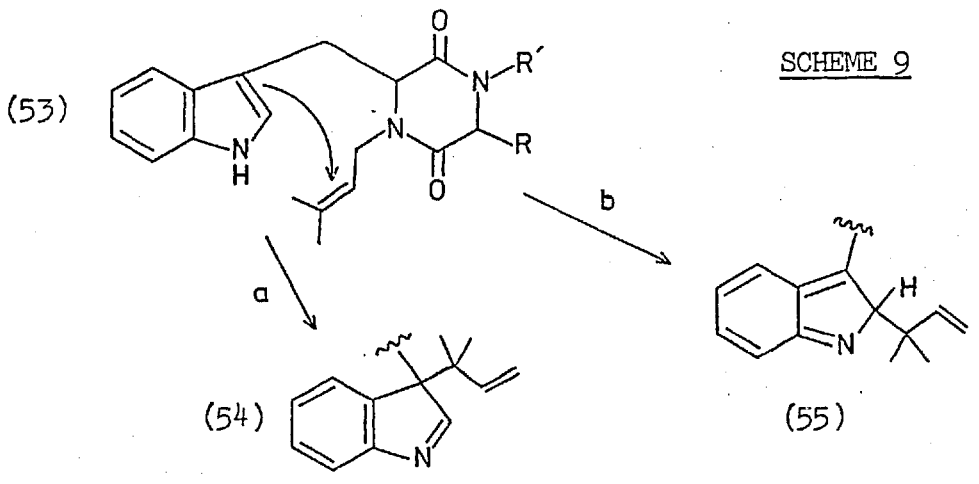
(48) (49)

evidence is supplied in the results section of this thesis that direct 2-substitution of an indole can occur although the reaction is much slower than the rate of substitution at position 3. Thus when the indole of part structure (48) was treated with acid catalysts one of the major products was the pyrrolo-indole (49). Under these conditions the only reasonable mechanism for this reaction is direct Friedel-Crafts alkylation of position 2. This implies that, in vivo, if the enzyme regulating isoprenylation at position 2 can hold the indole substrate and dimethylallylpyrophosphate in the correct spacial configuration, then direct S_N2' attack by the 2-position of the indole upon the allyl derivative can occur to give a 2(1',1'-dimethyl)allylindole.



(50) (52) (51)

Formation of 2(1'-methyl)allyl-3-alkylindoles by initial substitution at position 3 followed by rearrangement into position 2 has been investigated by Jackson's group.[51] It was found that 3-alkyl-3-alkenylindolenines such as (50) rearrange under acidic conditions to give the 3-alkyl-2-alkenylindole (51) via a Wagner-Meerwein mechanism and did not form any 2-(1'-methyl)allyl-3-alkylindole (52). Similarly heating the indolenine (50) led to an N-allyl-3-alkylindole and no substitution in position 2.[60] These results suggest that in vitro insertion of a 2-isopentenyl group does not involve initial prenylation at position 3 of the indole unless an S_N2' mechanism is involved.



The proposal that the inverted 2-isopentenyl group may arise from S_N2' displacement of a 3',3'-dimethylallyl unit on the dioxopiperazine nitrogen atom by position 2 of the indole comes from Birch[48] and is illustrated in scheme 9b. Extending Birch's ideas this intramolecular substitution could go into either position 2 or 3 of the indole nucleus; in the former case proton loss would give the 2-allylindole directly whilst in the latter (c.f. scheme 9a) the indolenine (54) can either undergo Wagner-Meerwein rearrangement to give the 2-allylindole or the tryptophyl nitrogen atom of the dioxopiperazine can quench the imine function to give the 3-(1',1'-dimethyl)allylindoline system seen in roquefortine and oxaline. The presence of a dimethylallyl group on a dioxopiperazine nitrogen atom in the fumitremorgens and verruculogen (table 4) supports these proposals.

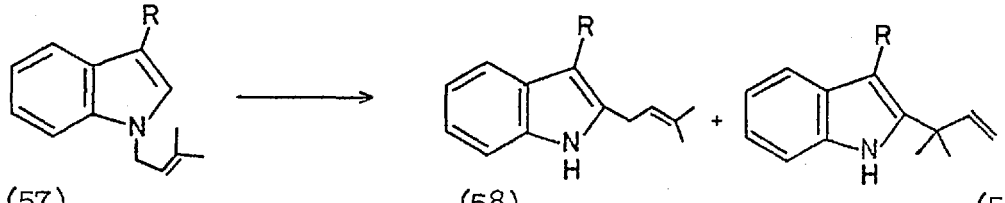
The transfer mechanism described above is really only an elaboration of the previous suggestion that the prenylating enzyme acts by holding the substrate and dimethylallylpyrophosphate in a suitable spacial arrangement. If such an intramolecular transfer mechanism is involved in the formation of 2-isopentenyl alkaloids then the presence of an enzyme would still be required because in its absence an indole nucleus could displace a dimethylallyl group from a dioxopiperazine ring by an S_N2 mechanism more easily than by an S_N2' mechanism.

Not long after it was first shown that echinulin possessed an inverted isoprenyl unit in position 2, Birch pointed out[12] that it might arise by rearrangement of an N-(3',3'-dimethyl)allylindole. This idea has subsequently received support from the isolation of the fumitremorgens and verruculogen (table 4) which all have a dimethylallyl group attached to the indole nitrogen atom. A number of publications have also appeared reporting that, in vitro, N-allylindoles rearrange to 2 or 3-allylindoles under thermal[57] or photolytic[59] conditions and in the presence of acid catalysts.[55,56,58]

Under extreme thermal conditions it was found that N-crotylindole rearranges to 3-(1'-methyl)allylindole only and this rearranges further to 2-(1'-methyl)allylindole at slightly higher temperature.[57] The stereochemistry of the products suggests that the rearrangements are concerted despite the extremely high temperatures required (ca 470°C) which might otherwise be expected to indicate a fragmentation-recombination mechanism.

Photolysis of N-allylskatole was found to produce 3-allyl-3-methylindolenine[59] but no work was performed to determine whether or not this rearrangement proceeds with inversion of the allyl group.

The acid catalysed rearrangement of the 3-alkyl-N-(3',3'-dimethyl)-allylindole (57) has been found to give mixtures of 3-alkyl-2-(3',3'-dimethyl)allylindole (58) and 3-alkyl-2-(1',1'-dimethyl)allylindole (59).[55,56] The relative amounts of the two products were found to be



(57) (58) (59)

dependent upon the size of the 3-substituent; when this was large the proportion of isomer (59) fell. This is consistent with a rearrangement

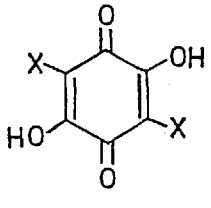
mechanism proceeding *via* a 3-alkyl-3-alkenylindolenine, a conclusion which is supported by work described in the results section of this thesis and by a report that N-crotylindole rearranges under acid catalysis to 3-(1'-methyl)allylindole.[58]

The conclusions to be drawn from these *in vitro* studies are that the inverted 2-isopentenyl group present in the austamides, brevianamides and echinulins and the inverted 3-isopentenyl group present in the compounds listed in table 5 can be formed *in vivo* either by intramolecular transfer of a 3',3'-dimethylallyl group from the indole or dioxopiperazine nitrogen atoms or by direct, enzyme regulated S_N2' attack of the indole upon dimethylallylpyrophosphate. In all 3 of these mechanisms initial substitution in the indole ring could occur at either position 2 or 3, the latter subsequently undergoing a Wagner-Meerwein shift if necessary.

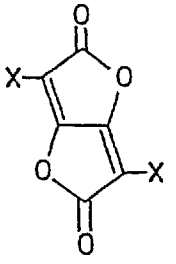
Two experiments are required to distinguish between these 3 mechanisms. In the first, administration to *A. amstelodami* of *cyclo*-L-alanyl-L-tryptophyl possessing a labelled dimethylallyl group on the indole nitrogen and measurement of the degree of incorporation into echinulin would reveal if rearrangement from the indole nitrogen atom occurs. In the second experiment, feeding *cyclo*-L-alanyl-L-tryptophyl containing a labelled dimethylallyl group on the dioxopiperazine nitrogen atom and measurement of the amount of incorporation into echinulin would similarly reveal if rearrangement from the dioxopiperazine nitrogen atom occurs. If no incorporation was obtained in either experiment then this would indicate that direct prenylation of the indole nucleus occurs.

Isoprenylated indole alkaloids not containing a dioxopiperazine ring

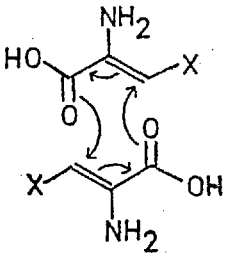
Apart from the natural products of the ergot fungus, only two other simple prenylated indoles have been isolated from a mould. These are cochliodinol, (60), and cochliodinone, (61), which are highly coloured compounds obtained from Chaetomium globosum and C. cochliodes. The structures of these metabolites were assigned from chemical degradation and spectral data and feeding experiments with labelled precursors indicated that they were both derived from tryptophan and mevalonate. Cochliodinol is most probably formed by dimerization of two dehydrotryptophan units as shown in representation (62), whilst cochliodinone is an oxidation product of cochliodinol. The latter reaction has precedent in the known oxidative rearrangement of 2,5-dihydroxybenzoquinones to give dilactones[62] and was confirmed by the formation of cochliodinone when cochliodinol was treated with acetic anhydride and dimethylsulphoxide.[61] The stage at which the isoprene units are inserted is not



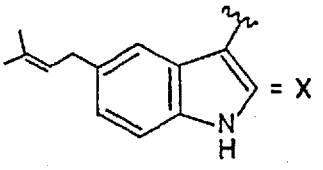
(60)



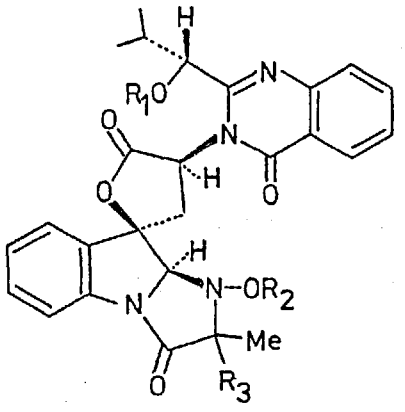
(61)



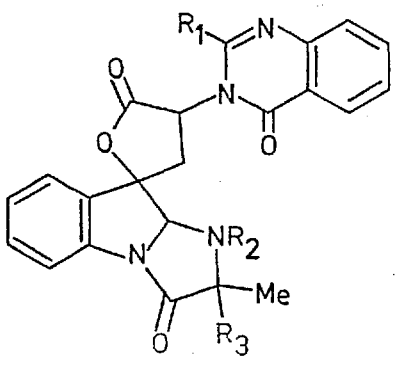
(62)



known; it is of interest, however, to note that they are in the same position as in the isoechinulins (table 2). Apart from echinulin itself, all of the 11 echinulins which have so far been isolated and which possess isoprenyl groups in the benzo-ring, have also undergone dehydrogenation of the 8,9-bond bringing the tryptophan unit to the same oxidation level as cochliodinol. Conversely not all of the echinulins which contain a dehydrotryptophan unit have isoprenyl groups in the benzo-ring, perhaps suggesting that dehydrogenation normally precedes isoprenylation.



Name	No.	R_1	R_2	R_3	Source	Ref.
tryptoquivaline	(71)	Ac	H	Me	A. clavatus A. fumigatus	74 75
isotryptoquivaline (fumitremorgen C)	(72)	H	Ac	Me	A. fumigatus	75
norisotryptoquivaline	(73)	H	Ac	H	A. fumigatus	75



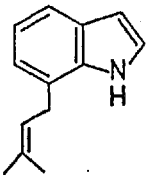
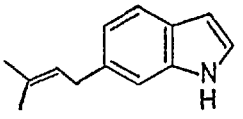
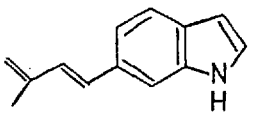
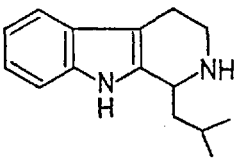
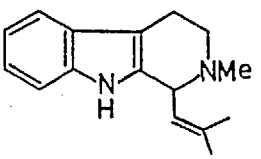
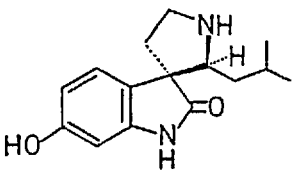
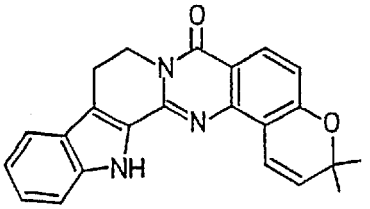
Name	No.	R_1	R_2	R_3	Source	Ref.
tryptoquivalone	(74)		OH	H	A. clavatus	74
fumitremorgen E	(75)	H	OH	H	A. fumigatus	75
fumitremorgen F	(76)	H	H	H	A. fumigatus	75
fumitremorgen G	(77)	H	OH	Me	A. fumigatus	75
fumitremorgen H	(78)	H	OH	H	A. fumigatus	75

A group of complex fungal metabolites have been isolated from Aspergillus fumigatus[75] and A. clavetus.[74] The former fungus is also a source of fumitremorgens A and B mentioned in table 4. These alkaloids have been given the names tryptoquivalines or fumitremorgens C-H (structures (71)-(78)) and their structures were determined from chemical and spectral evidence. The structure of one of these compounds - tryptoquivaline, (71) - has been confirmed by X-ray crystallographic analysis. Inspection of the ring structure of the tryptoquivalines suggests that they are derived from tryptophan and anthranilic acid; in addition some of them contain a C_5 unit which is possibly derived from dimethylallylpyrophosphate but may also come from degradation of leucine (c.f. dolichotheline and the isoquinoline alkaloids described below). A novel feature common to all of the tryptoquivalines is the C_3N or C_4N group fused on to what was the indole nucleus. This has no biosynthetic precedent but is perhaps amino acid derived.

Table 6 lists the indole derived alkaloids containing isoprenyl groups which have been isolated from plants. The two simple indoles (63) and (64) were both isolated from a pair of liverwort species and were used to confirm the taxonomic relationship between the two plants.[63] The positions of substitution were assigned from their ^1H n.m.r. spectra by comparison with the spectra of methylindoles. No investigations into their biosynthesis have been reported but free indole has frequently been isolated from plants[68] and is thought to be derived from tryptophan or possibly tryptophan precursors such as anthranilic acid derivatives.

The isoprenylindole (65) was isolated from the seeds of a Nigerian plant[64] and its structure, including the double bond geometry, has been confirmed by synthesis.[69] It was suggested[64] that the compound is formed in the plant by direct substitution of indole by dimethylallylpyrophosphate or by elaboration of the benzene ring before formation of the indole

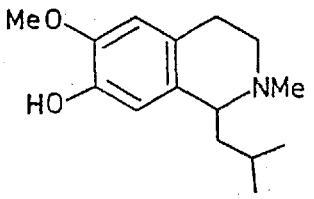
Table 6

Structure	No.	Source	Ref.
	(63)	Riccardia sinuata R. incurvata	63
	(64)	R. sinuata R. incurvata	63
	(65)	Monodora tenuifolia	64
	(66)	Eleagnus commutata	65
	(67)	Borreria verticillata	66
	(68)	E. commutata	67
	(70)	Euxylophora paraensis	73

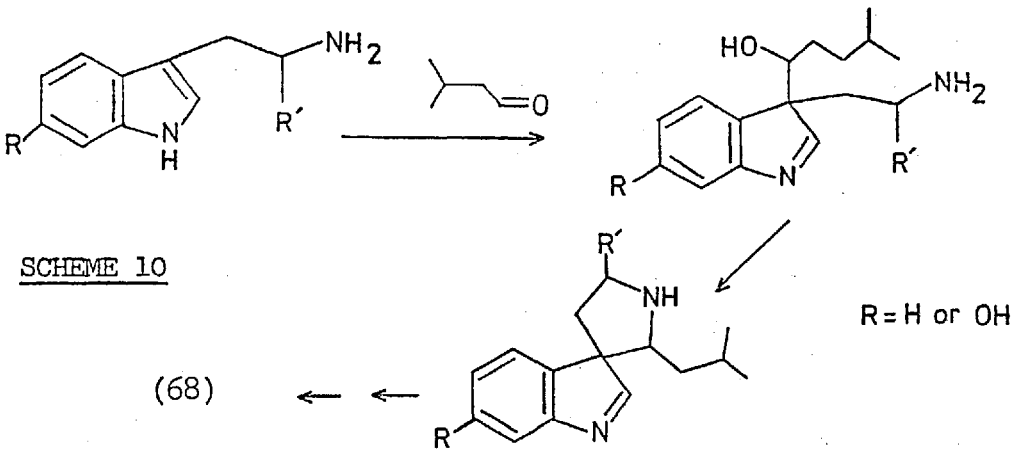
nucleus. It is also possible, in view of the higher oxidation level compared with compound (64), that 3-methyl-but-4-enal (senecialdehyde) or a derivative is the more direct precursor.

The alkaloids (66) and (67) are evidently closely related, despite their isolation from different plants.[65,66] A reasonable biosynthetic route to these compounds would be condensation of tryptophan with a suitable C_5 unit along with decarboxylation and, in the case of (67), methionine methylation. This pathway is suggested by analogy with lophocerine, (69), a cactus alkaloid[70] which has been shown to be derived from tyrosine.[71] The origin of the C_5 unit in lophocerine has been shown

(69)



to be both mevalonate and leucine[72] and it was suggested that both of these are incorporated <u>via</u> 3-methylbutanal. Compound (66) was found to be optically inactive; this may suggest that condensation with a C_5 unit does not occur until after decarboxylation.



SCHEME 10

(68)

The alkaloid (68) was isolated from the bark of the tree <u>Eleagnus commutata</u>, the same source as compound (66), and the structure was determined by X-ray crystallographic analysis.[66] No biosynthetic studies

have been reported but the structure suggests that it is derived from tryptophan *via* decarboxylation, hydroxylation and condensation with a C_5 unit as shown in scheme 10. The C_5 unit could well be identical with that incorporated into compound (66) - *viz.* a 3-methylbutanal derivative.

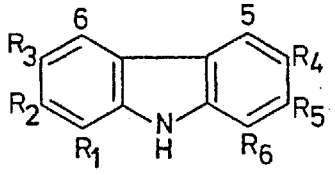
The final alkaloid in table 6, compound (70), was isolated from *Euxylophora paraensis* which is a species belonging to the Rutaceae family. Many species of Rutaceae contain alkaloids possessing the same basic hexacyclic structure as compound (70) which is clearly derived from tryptophan, anthranilic acid and formate.[256] Similarly many species of Rutaceae contain prenylated alkaloids - for example the carbazole, quinoline and furoquinoline alkaloids reviewed below - and so it is not too surprising to find compound (70) in a Rutaceae species also. Compound (70) does, however, possess the unique structural feature of a hydroxylated, prenylated anthranilic acid moiety.

Carbazole alkaloids from Rutaceae species

The family Rutaceae has yielded a number of carbazole alkaloids which contain dimethylallyl groups or which appear to be derived from prenylated precursors. These compounds are listed in tables 7, 8 and 9. Most of the structures were deduced from spectral data and were confirmed by synthesis in many cases. The occurrence of these natural products was reviewed in 1970[107] and again in 1973[256] and since that time new members of the group have been reported, increasing their number by a quarter.

The most notable property of these metabolites is that all 28 of them possess a methyl group (or an oxidation product thereof) in either position 4 or 7 of the carbazole nucleus and this suggested the first theories about their biosynthesis. Chakraborty and co-workers proposed

Table 7



No.	R_1	R_2	R_3	R_4	R_5	R_6	Name	Source	Ref.
(79)	H	H	H	Me	H	H	-	Clausena heptaphylla	76
(80)	H	H	MeO	Me	H	H	glycozoline	Glycosmis pentaphylla	77
(81)	H	H	H	CHO	H	MeO	murrayanine	C. heptaphylla	76,78
(82)	H	H	H	CHO	HO	X	heptaphylline	C. heptaphylla	79
(83)	H	X	H	CHO	HO	H	clausanitine	C. anisata	80
(84)	H	H	H	CO_2H	H	MeO	mukoeic acid	Murraya koenigii	81
(85)	H	H	MeO	Me	MeO	H	glycozolidine	G. pentaphylla	82
(86)	H	H	H	CHO	X	MeO	indizoline	C. indica	83
(87)	HO	H	H	CHO	HO	X	heptazoline	C. heptaphylla	84
(88)	H	H	MeO	CHO	HO	X	6-methoxy-heptaphylline	C. indica	85

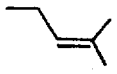
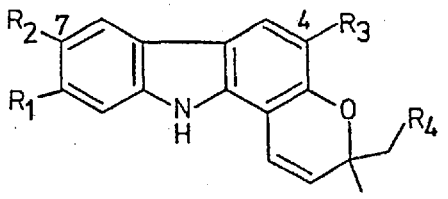
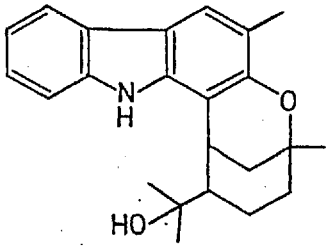
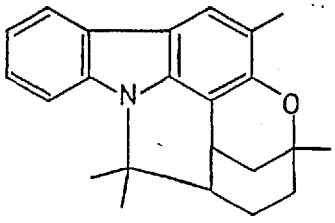
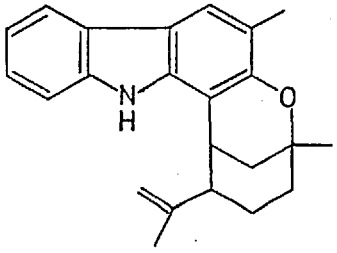
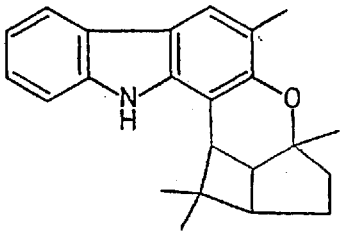
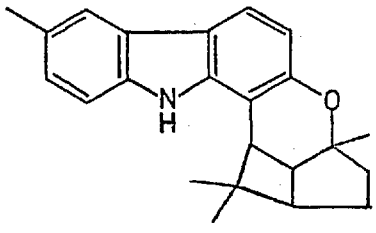
X = 

Table 8



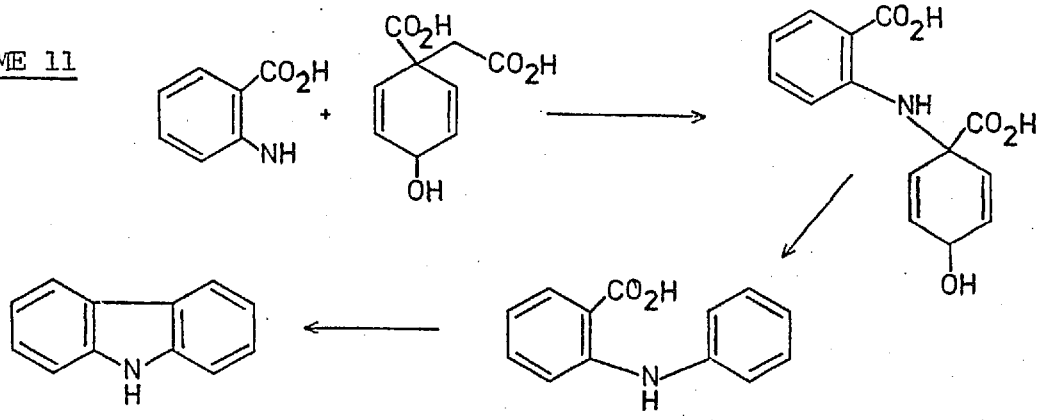
No.	R_1	R_2	R_3	R_4	Name	Source	Ref.
(90)	H	H	Me	H	girinimbine	Murraya koenigii	87
(91)	H	H	CHO	H	murrayacine	M. koenigii, Clausena heptaphylla	88,89
(92)	H	HO	Me	H	koenine	M. koenigii C. heptaphylla	90,91
(93)	H	MeO	Me	H	koenimbine	M. koenigii	92
(94)	H	Me	MeO	H	heptazolidine	C. heptaphylla	93
(95)	HO	MeO	Me	H	koenigine	M. koenigii	91
(96)	MeO	MeO	Me	H	koenimbidine (koenigicine, koenidine)	M. koenigii	94
(97)	H	H	Me		mahanimbine	M. koenigii	95
(98)	H	Me	H		isomahanimbine (mahanimbicine)	M. koenigii	96,97
(99)	H	H	CHO		murrayacinine	M. koenigii	98
(100)	H	HO	Me		mahanine	M. koenigii	91
(101)	H	H	Me		mahanimbinine	M. koenigii	99

Table 9

No.	Structure	Name	Ref.
(103)		murrayazolinine	103
(104)		mahanimbidine (murrayazoline, curryangine)	104 105
(102)		murrayazolidine (cyclomahanimbine) (curryanin)	100 101 102
(105)		bicyclomahanimbine	106 96
(106)		bicyclomahanimbicine	100 106

All these alkaloids were isolated from Murraya koenigii

SCHEME 11



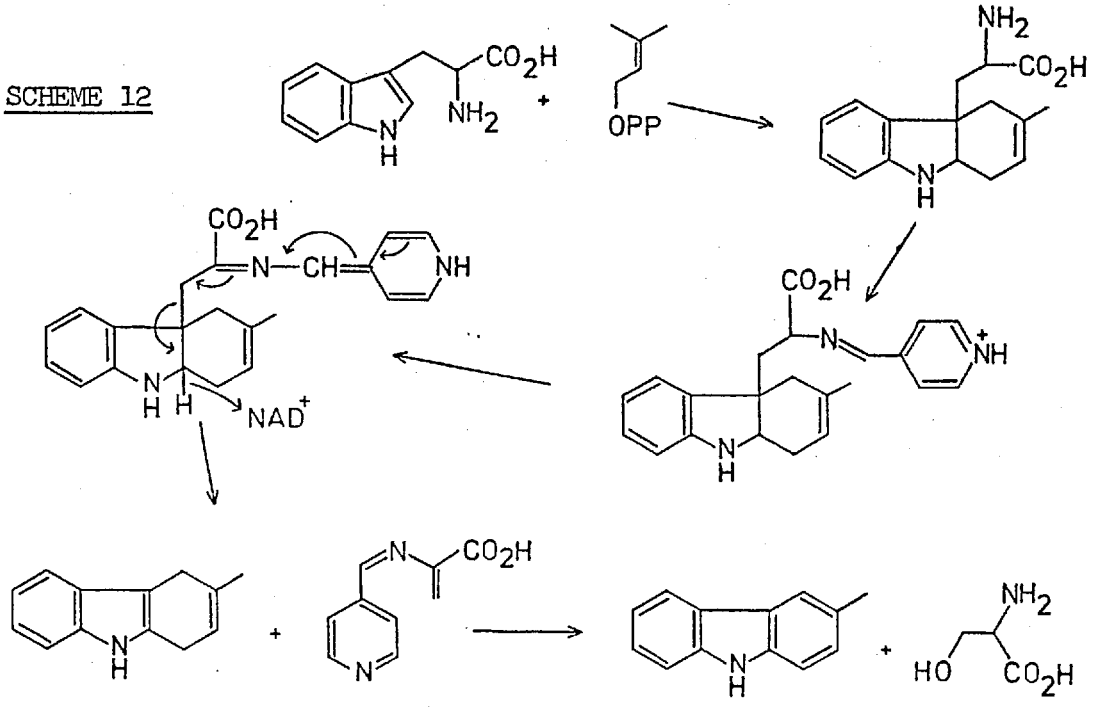
that anthranilic acid could be a precursor and that condensation with prephenic acid would eventually lead to carbazole as shown in scheme 11.[77,88,108] The extra methyl group, it was thought, could be incorporated from methionine or formaldehyde prior to, or after carbazole formation. In support it was noted that the methyl group is always found in positions 4 or 7 which are the most reactive sites in carbazole towards electrophilic substitution. This theory suffers from the weakness that it does not explain why every single one of the carbazole alkaloids is apparently methylated prior to further elaboration and overlooks the fact that carbazole itself has never been isolated from any of the species of Rutaceae named in tables 7, 8 and 9.

Kapil has proposed the hypothesis that the 4 or 7-methylcarbazoles are formed from condensation of indole or an indole derivative with a suitable C_5 unit.[94,109] This suggestion has been extended by Narasimhan who has published[110] a possible biosynthetic route shown in scheme 12. It is claimed in its support that loss of serine from tryptophan in the presence of pyridoxal coenzyme is well known.

A third proposal is that a 3-dimethylallylquinoline derivative may, following ring contraction and further elaboration, be the precursor of 4 and 7-methylcarbazoles.[111] This is a reasonable suggestion in so far as anthranilic acid is known to be converted to 3-dimethylallylquinolines

in some Rutaceae species (for a discussion of this, see the following section) but the subsequent reactions are without precedent.

SCHEME 12

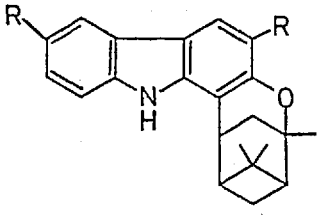


Brief details of only one investigation into the biosynthesis of these methylcarbazole alkaloids have been published.[107] It was found that ^{14}C-methionine fed to Murraya koenigii resulted in incorporation of label into the methoxy groups of koenigicine, (96), but not into the methyl group in position 4. Also [2-^{14}C] and [2-^{3}H] mevalonic acid gave radioactive koenimbine, (93), koenigicine, (96), and mahanimbine, (97), but the position of the label was not determined. These results tend to rule out Chakraborty's proposals but do not distinguish between the other theories.

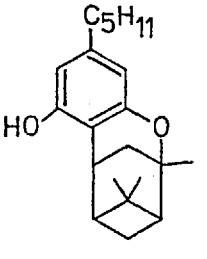
Inspection of the natural products in table 7 suggests the biosynthetic fate of a 4-methylcarbazole molecule in vivo; the most common reaction appears to be ring hydroxylation, mainly in position 3, along with degradation of the methyl group. Prenylation of position 2 is also an important process and leads to the natural products shown in table 8,

which arise from oxidative cyclization of the allylic side chain. The compounds (90)-(96) are derived from various apparently indiscriminate ring hydroxylations whilst compounds (97)-(101) result either from a second prenylation reaction or more probably were formed by reaction of the parent carbazole with geraniol. It is interesting to note that some of the compounds in table 8 exhibit the ring methyl group in position 7. If Kapils mechanism is correct[94,109] then this implies that in the biosynthesis of compounds (94) and (98) condensation occurs between a tryptophan derivative and a C_5 unit rather than with a C_{10} or a C_{15} unit, as could be the case with compounds (90) and (97) respectively.

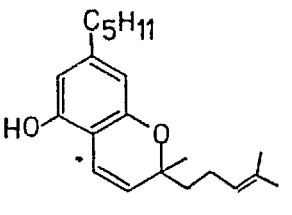
The interesting structures shown in table 9 may be derived from acid catalysed cyclization of mahanimbine, (97), to give murrayazolidine, (102). It is then chemically viable for hydration to murrayazolinine, (103), and cyclization to mahanimbidine, (104), to occur. Bicyclomahanimbine, (106), can be formed from mahanimbicine, (98), in a similar manner. The conversion of murrayazolinine to mahanimbidine has been performed <u>in vitro</u> using phosphorous oxychloride, and the reverse process has been carried out by treatment of mahanimbidine with aqueous acid.[103] Bicyclomahanimbine, (105), and bicyclomahanimbicine, (106), are evidently derived from an intramolecular [2+2] cycloaddition of mahanimbine, (97), and mahanimbicine, (98), respectively. Originally bicyclomahanimbine and bicyclomahanimbicine were assigned the structure (107) by analogy with cannabicyclol, (108), the product of cyclization of geraniol-



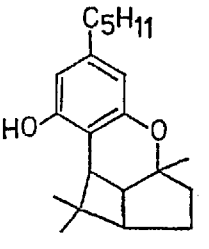
(107)



(108)



(109)



(110)

substituted olivetol, (109). However an X-ray crystallographic analysis of cannabicyclol indicated its true structure to be (110) and hence the structures of bicyclomahanimbine and bicyclomahanimbicine were reassigned as those shown in table 9.[106]

Some doubt exists as to whether cyclomahanimbine, bicyclomahanimbicine and bicyclomahanimbine are true natural products rather than artefacts. Thus it has been found that if a chloroform solution of mahanimbine is shaken in the presence of HCl a quantitative yield of cyclomahanimbine is obtained.[107] Similarly if a benzene solution of mahanimbine is shaken with silica for 48 hours, bicyclomahanimbine is formed.[100]

Quinoline and furoquinoline alkaloids of Rutaceae

The Rutaceae family has yielded a large group of heterocyclic natural products which possess a furan ring fused on to an aromatic system such as a quinoline or coumarin and biosynthetic studies have shown this ring to be isoprene derived. The occurrence, structure determination and synthesis of the furoquinolines was reviewed in 1966[179] and their occurrence and distribution amongst species of Rutaceae were reviewed in 1973.[256] In 1966 some 36 members of the group had been isolated and identified and at the time of writing this number has increased to 94. These compounds are not found outside the Rutaceae but are widely distributed amongst the species within the family; for example in 1973 the furoquinoline alkaloid skimmianine had been isolated from 83 different species out of a total of 182 which had been screened up to that time.[256]

In this section the occurrence and biosynthesis of the furoquinolines and prenylated quinolines found in the Rutaceae family are reviewed.

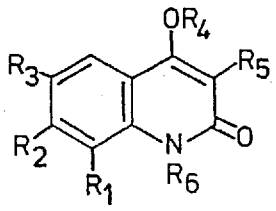
Due to lack of space and the proximity of the last review,[256] the distribution of these natural products amongst the species will not be reviewed here; instead each compound will be referenced to its first recorded isolation and identification only.

The earliest furoquinoline alkaloids isolated possessed the general structure shown at the top of table 11 and more recently a number of other general structural types have been reported; these are shown at the heads of tables 10, 12, 13 and 14. Tables 15 and 16 list related natural products whose structures are more novel. Taken together, tables 10-16 list all the prenylated quinoline and furoquinoline alkaloids of Rutaceae known up to 1976.

Up until 1966 the biosynthetic origin of furoquinoline alkaloids had been the subject of much speculation. To a large extent this was misdirected effort because at that time most of the known members of the group possessed the furoquinoline structure shown at the head of table 11 and consequently the origin of the furan ring was not obvious. It was early considered that by analogy with the acridone alkaloids (q.v.) the basic ring system of quinoline alkaloids was derived from anthranilic acid[180] although Robinson suggested that it could also be derived from tryptophan[181] by oxidation along the lines indicated in scheme 13. Anthranilic acid was envisaged as condensing with acetate to give 4-hydroxy-2-quinolone which could then be elaborated to give the observed natural products.

Experiments with labelled precursors have shown that both anthranilic acid and tryptophan are incorporated into furoquinoline alkaloids, although anthranilic appears to be the more efficient precursor. Japanese workers found that ring tritiated anthranilic acid was incorporated into skimmianine, (149), in Skimmia japonica and subsequent degradation showed that the label resided in the benzene ring as predicted.[182] When tryptophan

Table 10



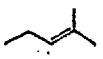
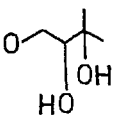
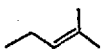
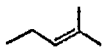
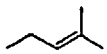
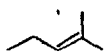
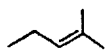
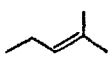
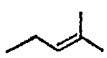
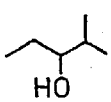
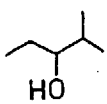
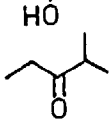
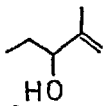
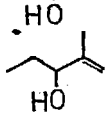
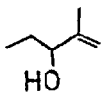
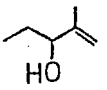
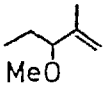
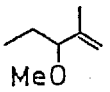
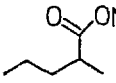
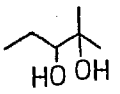
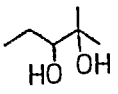
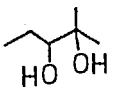
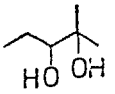
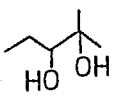
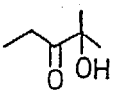
No.	R_1	R_2	R_3	R_4	R_5	R_6	Name	Ref.
111	H	H	H		H	Me	ravenine	112
112		H	H	Me	H	Me	foliosidine	113
113	H	H	H			H	-	114
114	H	H	H	Me		Me	-	115
115	H	H	H	Me		H	atanine	116
116	O - CH_2 - O		H	Me		H	-	117
117	OMe	OMe	H	Me		H	preskimmianine	118
118	OMe	O - CH_2 - O		Me		Me	ptelecortine	119
119	H	H	H	H		Me	ravenoline	112
120	OMe	H	H	Me		Me	lunacridine	120
121	O - CH_2 - O		H	Me		Me	lunidine	121
122	O - CH_2 - O		H	Me		Me	lunidonine	121
123	OMe	H	OMe	Me		Me	ptelefoline	122
124	H	O - CH_2 - O		Me		Me	ptelefolidine	123
125	H	OMe	OMe	Me		Me	isoptelefoline	123

Table 10 (continued)

No.	R_1	R_2	R_3	R_4	R_5	R_6	Name	Ref.
126	O - CH_2 - O		OMe	Me		Me	ptelefructine	123
127	O - CH_2 - O		H	Me		Me	ptelefolidine Me ether	123
128	OMe	H	OMe	Me		Me	ptelefoline Me ether	119
129	H	H	OMe	-CMe_2-CH=CH-		H	haplamine	124
130	H	OMe	OMe	-CMe_2-CH=CH-		Me	oricine	125
131	H	H	H	-CH(CMe_2OH)-CH_2-		Me	araliopsine	126
132	OMe	H	H	-CH(CMe_2OH)-CH_2-		Me	pseudo-balfourodine	126
133	O - CH_2 - O		H	Me		Me	pteleoline	119
134	H	H	H	Me		Me	eduline	127
135	OMe	H	H	Me		Me	hydroxy-lunacridine*	128
136	OMe	H	H	Me		Me	balfourolone*	129
137	O - CH_2 - O		H	Me		Me	hydroxy-lunidine	130
138	O - CH_2 - O		H	Me		H	nororixine	131
139	H	H	H	Me		Me	hydroxy-lunidonine	132

* enantiomers

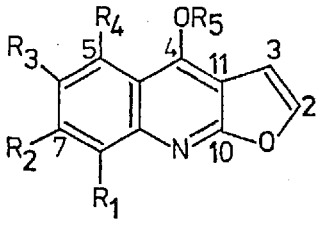


Table 11

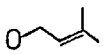
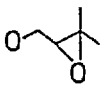
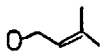
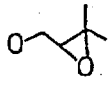
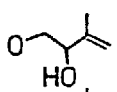
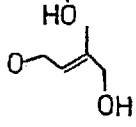
No.	R_1	R_2	R_3	R_4	R_5	Name	Ref.
139	H	H	H	H	Me	dictamnine	133
140	HO	H	H	H	Me	robustine	134
141	H	OH	H	H	Me	confusameline	135
142	OMe	H	H	H	Me	gamma-fagarine	136
143	H	OMe	H	H	Me	evolitrine	137
144	H	H	OMe	H	Me	pteleine	138
145		H	H	H	Me	haplophydine	139
146	H		H	H	Me	-	140
147	OMe	OH	H	H	Me	haplopine	141
148	H	OH	OMe	H	Me	heliparvifoline	142
149	OMe	OMe	H	H	Me	skimmianine	143
150	O - CH_2 -	O	H	H	Me	kokusagine	144
151	OMe	H	OMe	H	Me	maculosidine	145
152	H	OMe	OMe	H	Me	kokusaginine	144
153	H	O - CH_2 -	O	H	Me	maculine	145
154	OMe		H	H	Me	-	146
155	OMe		H	H	Me	-	140
156	OMe		H	H	Me	evoxine	146
157	OMe		H	H	Me	evodine	146

Table 11 (continued)

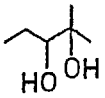
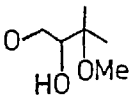
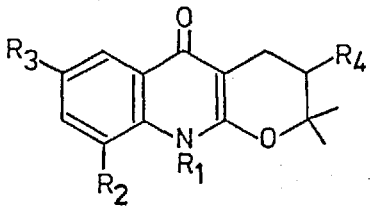
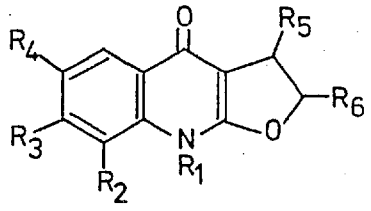
No.	R_1	R_2	R_3	R_4	R_5	Name	Ref.
158	OMe		H	H	Me	haplatine	147
159	H	OMe	O-CH(CMe_2OH)CH_2-		Me	choisyine	148
159a	H	OMe	O-CMe_2—CH=CH_2-		Me	acronidine	178,148
160	OMe	OMe	OMe	H	Me	halfordinine	149
161	OMe	O - CH_2-	O	H	Me	flindersiamnine	150
162	H	OMe	OMe	OMe	Me	acronycidine	151
163	H	O - CH_2-	O	H		maculosine	152
164	OMe		H	H	Me	methylevoxine	153
165	O-CMe_2-CH_2CH_2-		H	H	Me	foliminine	154

Table 12



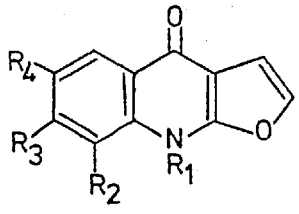
No.	R_1	R_2	R_3	R_4	Name	Ref.
166	H	H	H	H	haplofoline	155
167		H	H	H	haplobucharine	156
168	Me	H	H	OH	ribalinine	126
169	Me	H	OH	OH	ribalinidine	157
170	Me	OMe	H	OH	isobalfourodine	129
171	H	O - CH_2-	O	OH	ptelefoine	132

Table 13



No.	R_1	R_2	R_3	R_4	R_5	R_6	Name	Ref.
172	Me	OMe	H	H	H	CMe_2OH	balfourodine	129
173	Me	H	H	H	H	CMe_2OH	isoplatydesmine	158
174	Me	OMe	H	H	H	CMe_2OH	hydroxylunacrine	130
175	Me	H	H	OH	H	CMe_2OH	ribaline	157
176	Me	O - CH_2 - O		H	H	CMe_2OH	hydroxylunine	130
177	Me	H	H	H	H	$C(Me)=CH_2$	ptelefolidone	132
178	Me	OMe	H	OMe	H	$C(Me)=CH_2$	-	159
179	Me	OMe	H	H	H	$CHMe_2$	lunacrine	120
180	Me	O - CH_2 - O		H	H	$CHMe_2$	lunine	160
181	Me	H	H	H	Me_2	Me	ifflaiamine	161
182	Me	H	H	H	Me	Me_2	spectabiline	162

Table 14



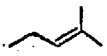
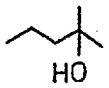
No.	R_1	R_2	R_3	R_4	Name	Ref.
183	Me	H	H	H	isodictamnine	164
184		H	OMe	H	acrophylline	163
185		H	OMe	H	acrophyllidine	163
186	Me	OMe	H	OMe	isomaculosidine	165

Table 15

Quinolinium salts of Rutaceae

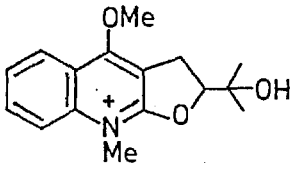
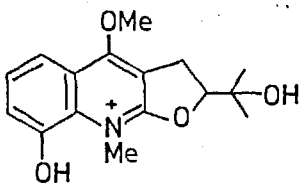
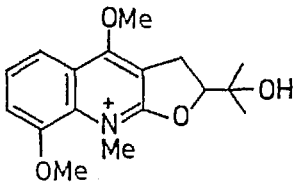
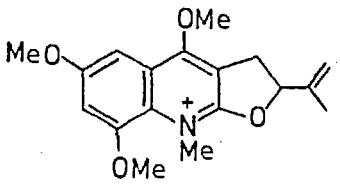
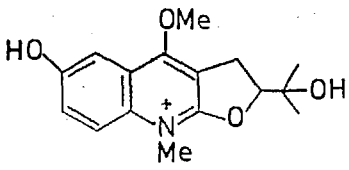
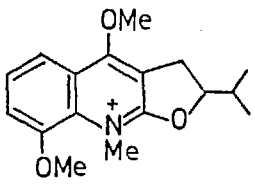
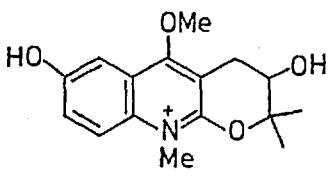
No.	Structure	Name	Ref.
187		N-Me platydesminium salt	166
188		pteleatinium salt	167
189		O-Me balfourodinium salt	168
190		O-Me ptelefolonium salt	169
191		N-Me ribalinium salt	170
192		lunasine	171
193		rutalinium salt	170

Table 16

Miscellaneous prenylated quinoline alkaloids of Rutaceae

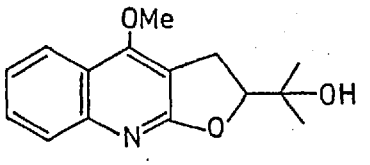
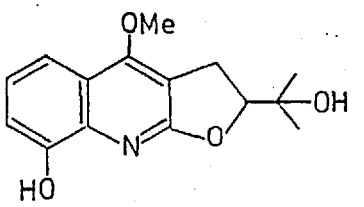
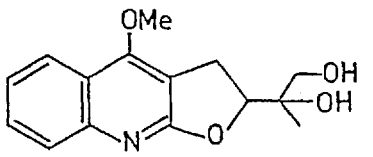
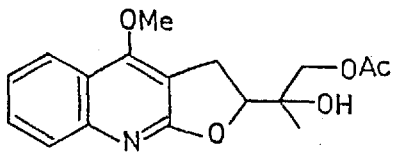
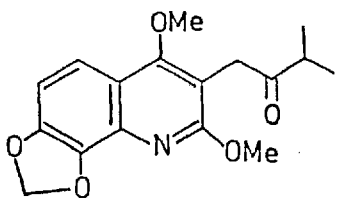
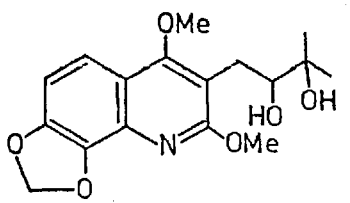
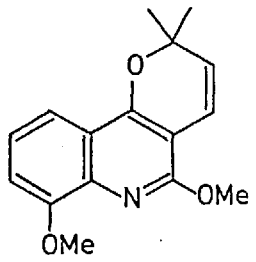
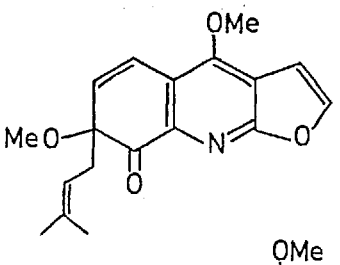
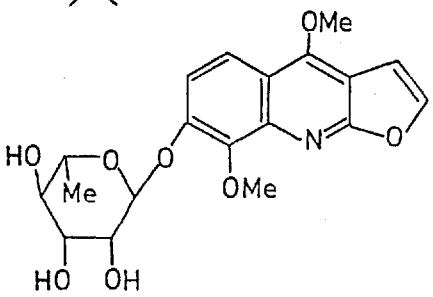
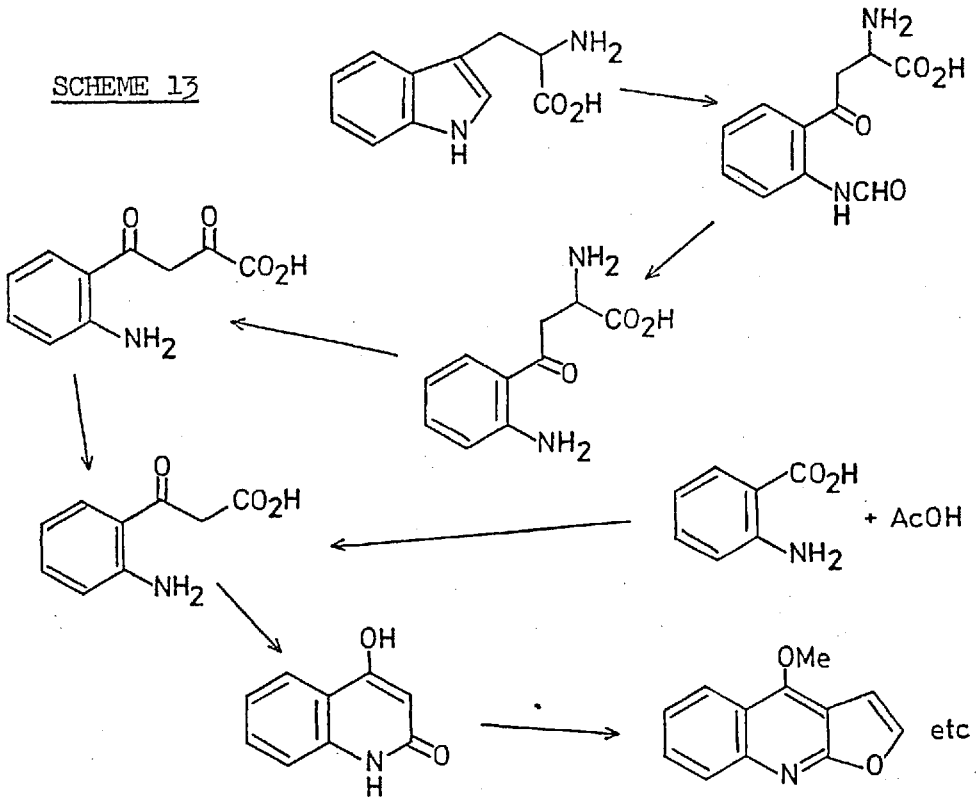
No.	Structure	Name	Ref.
(194)		platydesmin	138 177
(195)		-	167
(196)		dubinidine	172
(197)		dubinine	172
(198)		orixinone	173
(199)		orixine	144

Table 16 (continued)

(200)		flindersine	174
(201)		perfamine	175
(202)		glycoperine	176

SCHEME 13



labelled with ^{14}C in the methylene group of the side chain was fed to the plant it was incorporated into skimmianine to a much smaller extent and degradation indicated that the heavy isotope was located at position 11*. The incorporation of tryptophan labelled in this position rules out the possibility that it is first degraded to anthranilic acid but rather supports the intermediacy of the anthranoyl acetate intermediate shown in scheme 13.

Canadian workers have also found that carboxyl labelled anthranilic acid is efficiently incorporated into dictamnine, (139), in Dictamnus albus[183] but were unable to detect any incorporation of tryptophan.

These experiments indicate that both anthranilic acid and, to a much lesser extent, tryptophan are precursors of furoquinolines when added to the plant nutrient; it does not necessarily show that these acids are the real biosynthetic precursors or intermediates since they have not been isolated from the plants in these experiments. Their status as intermediates here can probably be assumed since they are such simple building blocks and almost bound to be present in the plant if looked for; however proof of this could only be obtained by their isolation or by trapping experiments. In the biosynthetic experiments described below it may be assumed that such trapping experiments have been carried out or that the labelled compound being administered has previously been isolated. In some cases it may be that the isolation has been made from a different, closely related species. This extrapolation is justified by the large range of structural types of furoquinolines which have been isolated from all species of the Rutaceae and the common occurrence of many natural products (e.g. skimmianine) in different species. This range suggests that the biosynthetic pathways are very similar in all the Rutaceae and that the different levels of the

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* the numbering system used is shown at the head of table 11

reservoirs of each natural product in different species merely reflect the varying metabolic balances. In other words, it is reasonably safe to conclude that a proven intermediate in one species is also an intermediate in a closely related species if it is shown to be incorporated into metabolites further along the biosynthetic pathway in that species.

Several hypotheses have been advanced to account for the origin of the furan ring of furanoquinolines and these include suggestions that the four carbon atoms are derived from succinic acid[184] or α-oxoglutaric acid.[185] Price[186] and Birch[187] have both suggested that the furan ring is derived from a prenyl unit by analogy with the biosynthesis of coumarins which are also isolated from Rutaceae. Robinson proposed that contraction of one of the aromatic rings of the acridones could yield furoquinolines or alternatively that they could arise from condensation of a suitable fragment with 4-hydroxy-2-quinolone.[181] Robinson's former idea is attractive in so far as acridones have been isolated from furoquinoline-yielding plants whilst the latter suggestion, in conjunction with Price and Birch's proposals, are greatly supported by the isolation of the 3-prenylated-4-alkoxy-2-quinolines shown in table 10 and their cyclization products listed in tables 12, 13, 15 and 16. Thus loss of a C_3 unit from the 2-isopropanol substituted dihydrofuroquinolines (172)-(176) (see table 13) would yield furoquinolines of the type shown in table 11. Labelling studies have shown these hypotheses to be largely correct and the metabolic relationships between most of the quinoline alkaloids containing a prenyl derivative, which are suggested by these findings, are shown in scheme 16 (p.59). The labelling studies which point towards this scheme will now be considered.

Administration of [1-^{14}C]-acetate and [2-^{14}C]-acetate to Skimmia japonica[182] and Dictamnus albus[183] results in incorporation of label into positions 10 and 11 respectively of both dictamnine, (139), and

skimmianine, (149), whilst α-oxoglutarate was not incorporated.[183] No label from acetate found its way into positions 2 or 3 of the furoquinoline nuclei, a result obtained elsewbere when both labelled acetate and mevalonate were fed to S. japonica.[188] Subsequently Argentinian workers have reported[189] that [4-^{14}C] and [5-^{14}C] mevalonic acid and [1-^{14}C] dimethylallylalcohol are all incorporated into skimmianine in Fagara coco. Degradations revealed that the radioactive isotopes were located at positions 2, 3 and 3 respectively. They also found that [1-^{14}C] dimethylacrylic acid and labelled acetate were not good precursors for positions 2 and 3 of skimmianine although acetate was incorporated into the 10 and 11 positions as before.

These results are consistent with condensation of anthranilic acid with acetate to give a 4-hydroxy-2-quinolone followed by prenylation with dimethylallylpyrophosphate to give a 3-(3',3'-dimethyl)allyl-4-hydroxy-2-quinolone. This can then be converted to the furoquinoline nucleus as shown in scheme 13.

The incorporation of mevalonate and dimethylallylalcohol but not acetate or dimethylacrylic acid into positions 2 and 3 of skimmianine is a little unusual because the latter two are known to be common precursors of the former elsewhere. It has been suggested[189] in explanation that at the sites of alkaloid biosynthesis the rate of conversion of acetate into mevalonate is slow compared with its rate of incorporation into 4-hydroxy-2-quinolone. The failure of some workers to observe mevalonate incorporation[188] into positions 2 and 3 presumably reflects the inability of fed mevalonate to reach the site of synthesis of 3-prenylquinolones in the plants investigated.

4-Hydroxy-2-quinolone, (203), has been confirmed as a possible precursor by feeding a labelled sample to Ruta graveolens.[190] Good incorporation of the intact quinolone into skimmianine, (149), with no

randomization of label was observed. The degree of incorporation was greater than that of anthranilic acid and acetate supporting its later role in the biosynthetic pathway as a more direct precursor of skimmianine.[191] Irish workers have repeated the experiment[193] with doubly labelled 4-hydroxy-2-quinolone and 4-methoxy-2-quinolone and these compounds were incorporated intact into dictamnine, (139), skimmianine, (149), and N-methylplatydesmine, (187). Feeding 4-hydroxy-2-quinolone to cell suspensions of Ruta graveolens was also reported[194] to give incorporation into dictamnine, gamma-fagarine, (142), skimmianine, kokusaginine, (152), and eduline, (194).

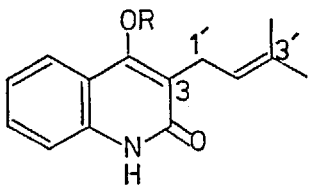
The demonstration that 4-hydroxy-2-quinolone and mevalonic acid are good precursors of furoquinolines and the identification of products bearing dihydrofuran rings with an isopropanol residue in position 2 (e.g. compounds (172)-(176), table 13) suggests that the next step in the biosynthetic conversion of 4-hydroxy-2-quinoline to furoquinolines is prenylation at position 3. This idea is supported by the number of 2-quinolones isolated from Rutaceae possessing a 3,3-dimethylallyl group or an oxidized derivative at position 3 (e.g. compounds (113)-(139), table 10).

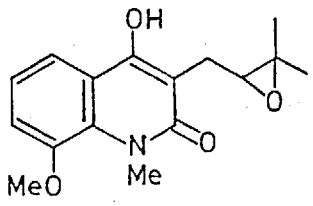
Experiments with labelled 4-hydroxy-3-(3',3'-dimethyl)allyl-2-quinolone, (204) and its 4-methoxy derivative, (205), have shown that these compounds are also incorporated into dictamnine, (139), gamma-fagarine, (142), skimmianine, (149), kokusaginine, (152), and eduline, (194),[192,193,194] when administered to Ruta graveolens and Skimmia japonica. Furthermore, both compounds were incorporated rather more efficiently than the non-prenylated quinolone implying that they are even more direct precursors.

Inspection of the structures of the compounds listed in tables 13 and 15 suggests that one of the major metabolic fates of the prenyl quinolones

(204) and (205) is epoxidation of the prenyl group and cyclization to give dihydroquinolines or dihydroquinolones followed by loss of a C_3 unit to give the furoquinolines shown in table 11. This sequence has received support from labelling studies; administration of doubly labelled prenyl quinolones (204) and (205) and of labelled platydesmine, (194), to Skimmia japonica revealed that whilst the former two compounds were incorporated intact into dictamnine, (139), to much the same extent, platydesmin was incorporated four times more efficiently.[193]

(204), R=H
(205), R=Me



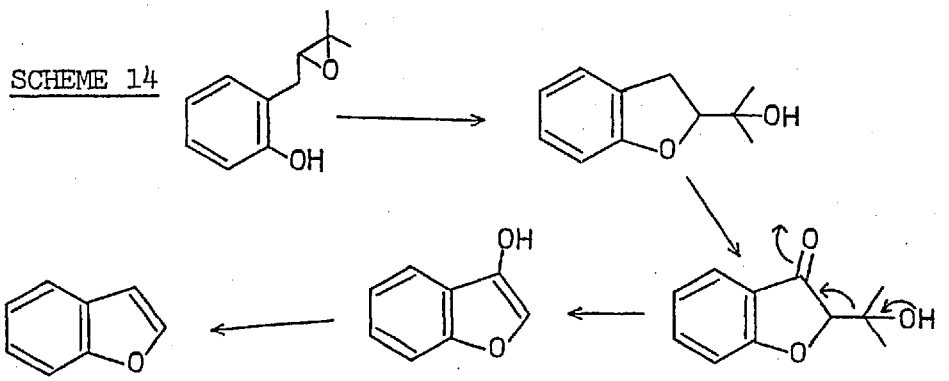


(206)

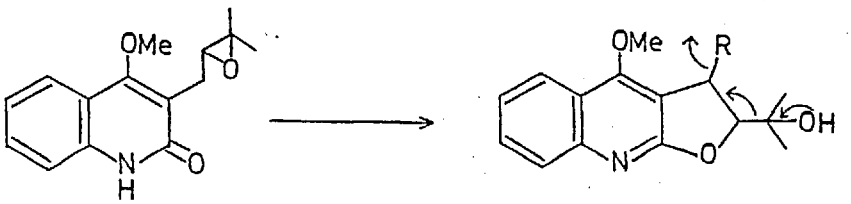
Chemical evidence for the cyclization of an epoxidized 3-prenylquinolone and for the loss of a C_3 unit by fragmentation of platydesmin to dictamnine has been reported. Thus treatment of optically active balfourodine, (172), with base yields on intermediate epoxide, (206), which could be trapped with methyl iodide or converted into optically active pseudobalfourodine, (132).[195] The optical purity of the products imply that the epoxide (206) is produced in vivo in a stereospecific manner presumably through the mediation of an epoxidase. This reaction is also observed in the substituents of the benzene ring of a number of Rutaceae derived quinolines: for example the 2-quinolone (112) bears a hydroxylated dimethylallyl group in position 8, which could be epoxide derived, and furoquinolines (146) and (155) possess epoxidised dimethylallyl groups at position 7.

Loss of an isopropyl fragment is illustrated by the reaction of platydesmin, (194), with lead tetraacetate and iodine in the presence of calcium carbonate. Illumination of this mixture with visible light gave a 34% yield of dictamnine, (139), presumably via a radical cleavage mechanism.[196]

SCHEME 14



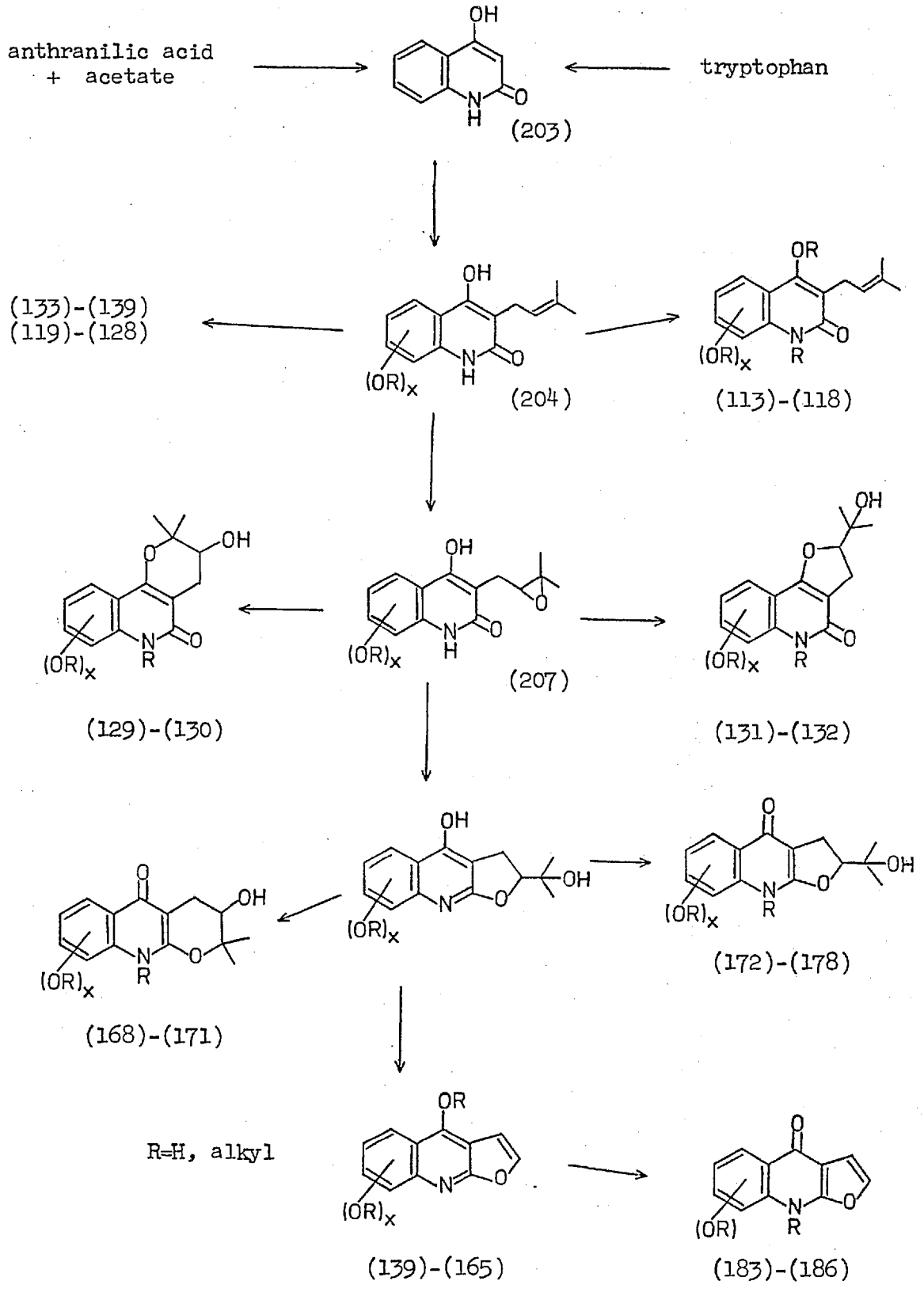
SCHEME 15



Birch has suggested[187] that in the biosynthesis of benzofurans, cyclization of an oxidized prenyl group of a 2-prenylated phenol is followed by benzylic oxidation and elimination of a C_3 unit as illustrated in scheme 14. It has been shown[197] that this mechanism does not apply to the biosynthesis of furoquinolines; feeding 4-methoxy-3-[2'-$^{3}H_2$,2'-^{14}C](3',3'-dimethy)allyl-2-quinolone, (205), to Choisya ternata gave skimmianine, (149), from which only half the tritium label had been lost. Consequently a 3-hydroxyfuroquinoline analogous to Birch's 3-hydroxybenzofuran cannot be an intermediate and an alternative mechanism such as shown in scheme 15 is suggested. In the scheme R represents direct loss of hydride or loss of a leaving group such as hydroxide previously incorporated by oxidation.

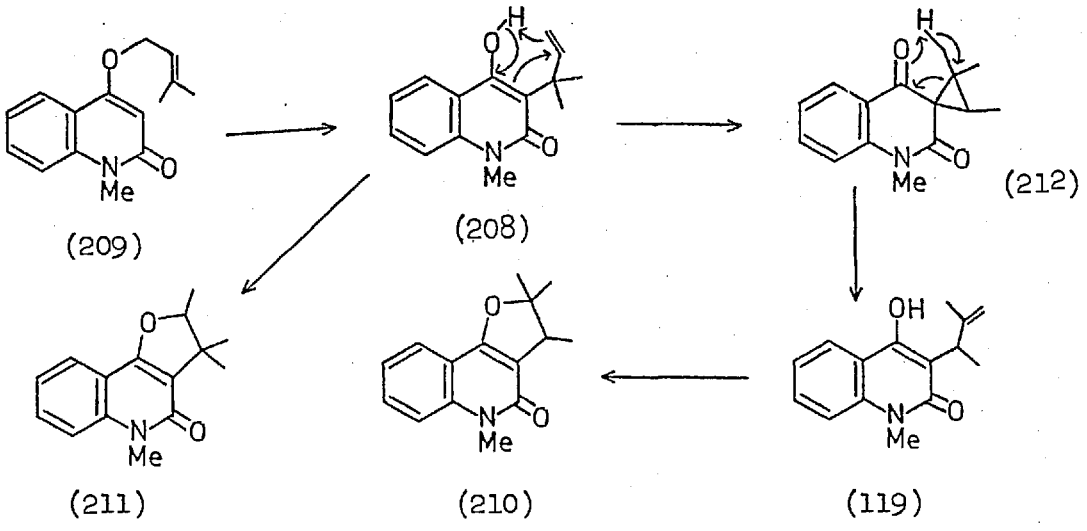
Apart from the timing of ring hydroxylation and O and N methylation, the investigations reported above account for the biosynthetic pathways to most of the alkaloids shown in tables 10-16. These pathways are summarized in scheme 16. Formation of the 2,2-dimethyldihydropyranoquinolines (166) and (167) and of the 2-isopropyldihydrofuroquinolines (179), (180) and (192) can be envisaged as arising either by cyclization

SCHEME 16


anthranilic acid
+ acetate
tryptophan
(203)
(133)-(139)
(119)-(128)
(204)
(113)-(118)
(129)-(130)
(207)
(131)-(132)
(168)-(171)
(172)-(178)
R=H, alkyl
(139)-(165)
(183)-(186)

of the epoxide (207) followed by reduction of the alcohol function or by acid catalysed cyclization of the 3-prenylquinolone (204).

SCHEME 17



The formation of ifflaiamine, (181), spectabiline, (182), and ravenoline, (119), has been explained by the intermediacy of 3-(1',1'-dimethyl)allyl-4-hydroxy-2-quinolone, (208). As was noted in the section on 2-(1',1'-dimethyl)allyltryptophyl compounds, this unusual inverted configuration of the dimethylallyl group has been regarded as arising either by direct S_N2' substitution at the tertiary centre of dimethylallyl-pyrophosphate[54] or by a Claisen rearrangement mechanism.[198] The latter pathway appears to be followed in the formation of ravenoline because when labelled 4-(3',3'-dimethyl)allyloxy-N-methyl-2-quinolone, (209), is fed to Ravenia spectabilis good incorporation into optically active ravenoline was observed.[162] The reaction was found to be reproduceable in vitro; heating the allylether (209) at 130° gave a mixture of racemic ravenoline and its cyclization products (210) and racemic spectabiline, (182).[200] Also present was the cyclization product (211) which is clearly derived from the proposed intermediate 2-(1',1'-dimethyl)-4-hydroxy-2-quinolone, (208). When the reaction was carried out in the

presence of acetic anhydride and N-methylpiperidine it was found possible to trap out (208) as its O-acetate so confirming its intermediacy.[199] The mechanism of formation of ravenoline, (119), from (208) is said to be that of the "abnormal" Claisen rearrangement, analogous to a vinyl cyclopropane rearrangement, via the intermediate (212)[200] (scheme 17). The optical activity of isolated ravenoline confirms that it is not an artefact and also suggests that the rearrangement (208)→(212) is enzymatically controlled.

The O-methyl groups of skimmianine, (149), have been shown to be both methionine[183] and formate[189] derived by feeding ^{14}C-methyl methionine and ^{14}C-sodium formate to Fagara coco and Skimmia japonica. It is presumed that the N-methyl groups of other prenylated quinolines are similarly derived. The point at which methylation of nitrogen or oxygen functions of furoquinolines occurs has not been determined. Inspection of the quinolines in table 10 suggests that both O and N-methylation can occur at least as early as this stage in the biosynthetic sequence, before rearrangement of the allyl group. The presence of an N-methyl group in a 3-prenyl-2-quinolone does not appear to prevent its conversion into desmethylfuroquinolines in later states of biosynthesis since N-methyl and N-desmethyl-3-(3',3'-dimethyl)allyl-4-methoxy-2-quinolone are efficiently incorporated into dictamnine, (139), by S. japonica to an equal extent.[197] This implies that the enzyme system is capable of demethylating N-methylquinolones easily. The mechanism of demethylation has not been investigated but it does not appear to involve quinolinium salts of the type shown in table 15; for example N-methylplatydesminium salt is incorporated only slightly into dictamnine in S. japonica.[193] However this low incorporation may only reflect solubility factors preventing efficient transfer of the salt to the relevant enzyme site.

It is interesting to note that N-methylanthranilic acid has been isolated from Acronychia baueri,[219] a furoquinoline containing species, and has been found to be a precursor of N-methyl-9-acridones in Evodia xanthoxyloides,[219] which is also a furoquinoline containing plant. As will be seen in the following section 9-acridones are formed in Rutaceous species via 4-hydroxy-2-quinolones in identical fashion to furoquinolines. These observations suggest that N-methylation can occur as early as the anthranilic acid stage in the biosynthesis.

In contrast to the "reversibility" of N-methylation in furoquinolines, it has been shown that O-demethylation cannot occur. Double labelling of 3-(3',3'-dimethyl)allyl-4-methoxy-2-quinolone upon the methoxy group and in the methylene group of the allylic side chain and the administration of this compound to S. japonica has shown that the methoxyl group is retained in skimmianine, (149),[197] whilst feeding labelled 2,4-dimethoxy quinoline resulted in no incorporation of the quinoline nucleus.[197]

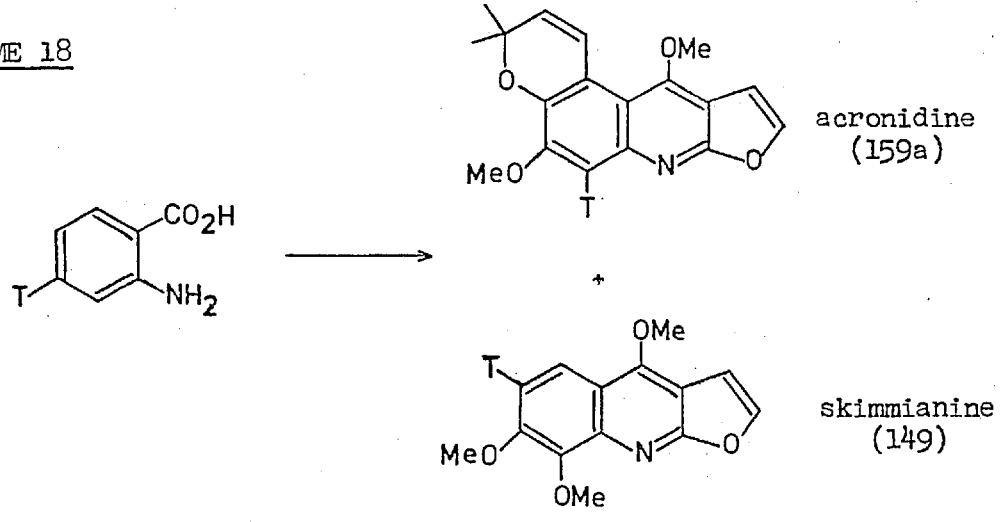
The point at which O-methylation occurs does not appear to be critical; 4-hydroxy- and 4-methoxy-2-quinolone, 4-methoxy- and 4-hydroxy-3-(3',3'-dimethyl)allyl-2-quinolone are all incorporated into S. japonica[193] with very little difference in degree of incorporation between the methylated and non-methylated compounds. The proven irreversibility of O-methylation however suggest that once methylation of the 4-hydroxy group has occurred then furoquinoline synthesis is stimulated at the expense of the route to the N-methyl-4-quinolone compounds shown in tables 12, 13 and 14, which becomes blocked.

Aromatic hydroxylation of furoquinolines appears to be able to occur at any stage during the biosynthetic sequence. Thus the large number of ring hydroxylated 3-prenylated quinolines which have been isolated (see table 10) suggests that hydroxylation can occur at least

as early as this point whilst the incorporation of dictamnine into gamma-fagarine,[194] (142), skimmianine,[201] (149), choisyine,[201] (159) and evoxine,[201] (156), demonstrates that hydroxylation can also occur at a late stage. No labelling studies with hydroxylated anthranilic acid or hydroxylated quinolones have been reported and so the earliest point at which hydroxylation occurs is not known.

The mechanism of ring hydroxylation has been the subject of only one paper.[202] The occurrence of a large number of quinolones (see table 10) and quinolines (table 11) bearing oxygen functions in both positions 7 and 8 led to speculation that they might be inserted in a one step process by a dioxidase enzyme rather than the more commonly encountered process involving arene oxide intermediates. The latter mechanism would be expected to exhibit an N.I.H. shift and accordingly Australian workers administered [3-^{1}H] anthranilic acid to Acronychia baueri in an attempt to detect such a shift.[202] Their results are illustrated in scheme 18. The major furoquinoline alkaloids, viz. skimmianine and acronidine, were isolated and were found to have retained between 10 and 20% of the tritium originally present thus demonstrating that an N.I.H. shift had occurred and supporting a two step mechanism of hydroxylation.

SCHEME 18



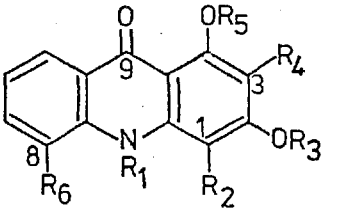
Acridone alkaloids

The acridone alkaloids constitute a group of natural products derived from 9-acridone by aromatic hydroxylation. Like the furoquinoline alkaloids described above they are found in the Rutaceae family and frequently in the same species. A number of the acridone alkaloids bear isoprenoid side chains and these compounds are reviewed here; those without such side chains have been reviewed previously.[216] Some 21 isoprenylated acridone alkaloids are known and they are listed in tables 17-20. It will be seen from the tables that these compounds are all N-methyl-2,4-dihydroxy-9-acridones and that the majority of them possess a derivatized dimethylallyl group in position 2. The isolation of the acridones from the same sources as furoquinoline alkaloids suggests that they might be subject to the same type of metabolic processes and this is supported by the structures of the acridones which have been isolated. Thus some of the acridones have undergone ring hydroxylation (in position 8) and in most the dimethylallyl side chain has undergone oxidative cyclization. Similarly both O and N methyl groups are common.

Several hypotheses have been proposed to explain the biogenesis of these compounds. One of the first was that o-aminobenzaldehyde, or its biochemical equivalent, might condense with phloroglucinol to give 2,4-dihydroxyacridine which can then be oxidised further to the known alkaloids.[217] Robinson has suggested[181] that anthranilic acid may condense with acetate derived 3,5-diketohexanoic acid to give 2,4-dihydroxy-9-acridone or alternatively that tryptophan can be metabolised to the same compound (scheme 19) in similar fashion to his proposal for furoquinoline biosynthesis (scheme 13).

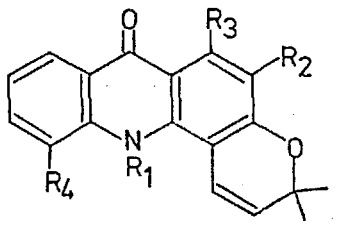
German investigators administered non-specifically ring tritiated anthranilic acid to Glycosmis arborea and isolated the non-isoprenoid acridone alkaloid arborinine, (234).[218] Nitric acid degradation yielded

Table 17



No.	R_1	R_2	R_3	R_4	R_5	R_6	Name	Source	Ref.
213	Me	H		OMe	H	H	evoprenine	Evodia elata	203
214	H		H		H	OH	atalaphylline	Atalantia monophylla	204
215	Me		H		H	OH	N-Me-atalaphylline	Atalantia monophylla	204

Table 18



No.	R_1	R_2	R_3	R_4	Name	Source	Ref.
216	Me	H	OMe	H	acronycine	Acronychia baueri, Bauerella baueri	205 206
217	Me	H	OH	H	noracronycine	Glycosmis pentaphylla	207
218	H	H	OMe	H	des-10-Me-acronycine	G. pentaphylla	207
219	H	H	OH	H	des-10-nor-acronycine	G. pentaphylla	207
220	H	H	OH	OH	atalaphyllidine	Atalantia monophylla	208
221	Me	H	OH	OH	-	A. ecylanica	209
222	Me		OH	OH	-	A. ecylanica	209

Table 19. Acridone alkaloids from Ruta graveolens

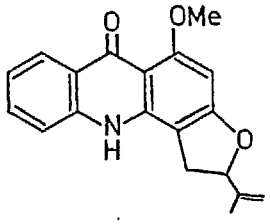
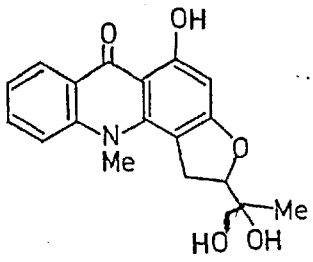
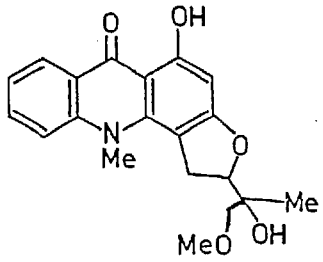
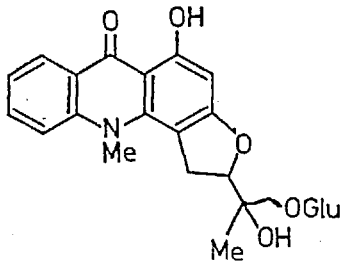
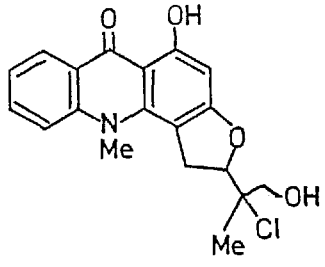
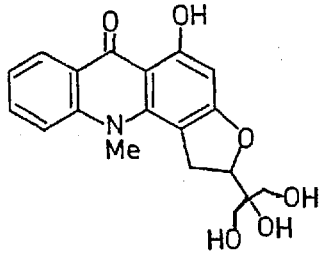
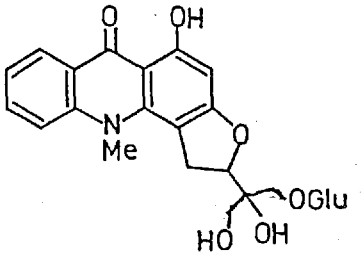
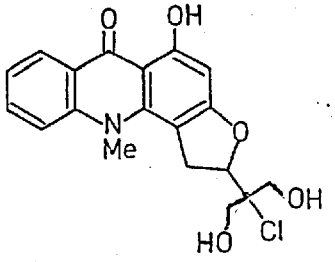
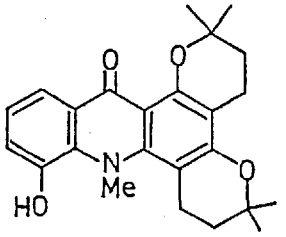
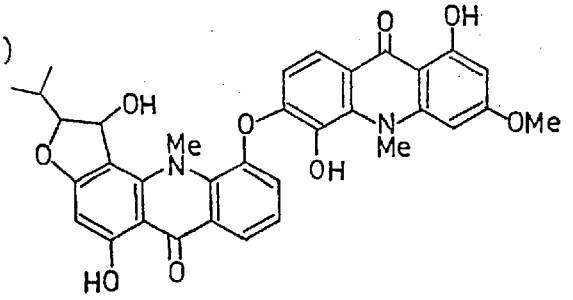
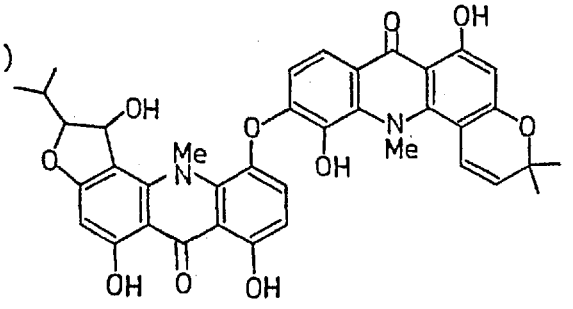
No.	Structure	Name	Ref.
(223)		rutacridone	210 211
(224)		gravacridondiol	212
(225)		gravacridondiol monomethyl ether	212
(226)		-	213
(227)		gravacridonchlorine	212
(228)		gravacridontriol	213

Table 19 (continued)

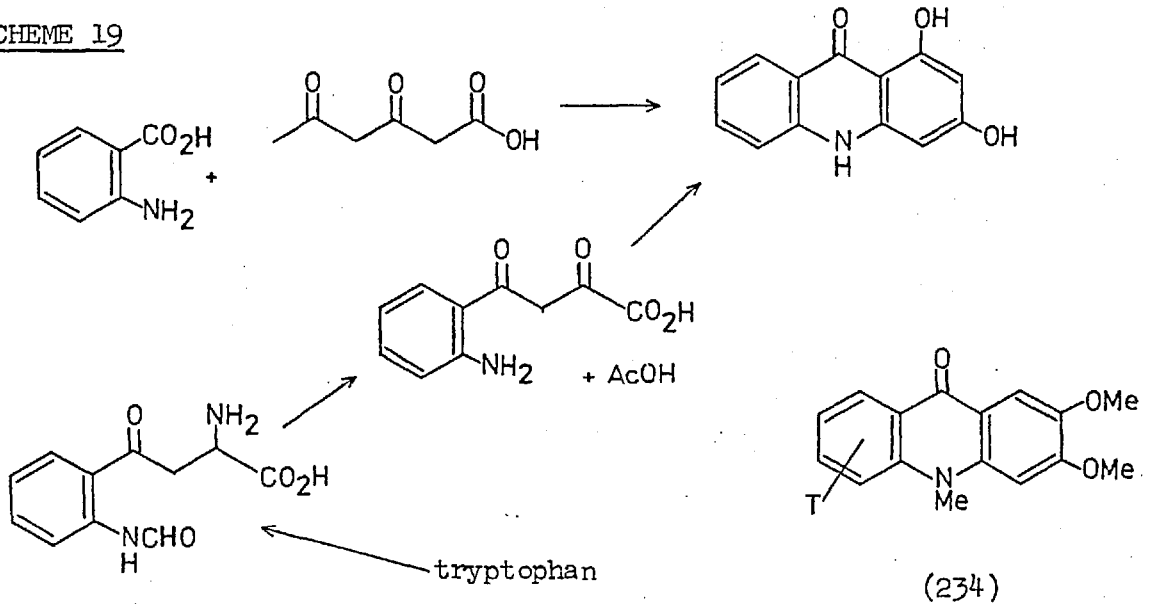
(229)		-	213
(230		gravacridonolchlorine	212

Glu = glucose

Table 20

No.	Structure	Source	Ref.
(231)		Atalantia monophylla	214
(232)		A. ceylonica	215 209
(233)		A. ceylonica	215 209

SCHEME 19



(234)

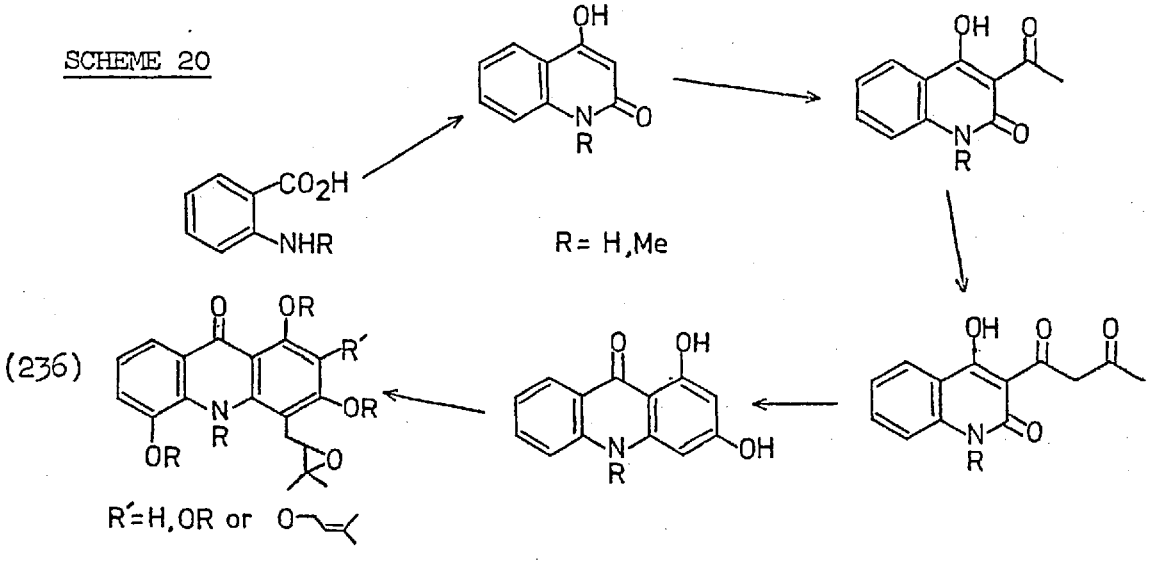
N-methyl-4-quinolone-3-carboxylic acid which contained all of the label in ring A indicating that it is derived from anthranilic acid. This result has been confirmed elsewhere[219] where it was found that ring tritiated anthranilic acid was incorporated into the A ring of several acridone alkaloids in Acronychia baueri. It was also found that tryptophan labelled in the side chain methylene group was not incorporated in A. baueri possibly ruling out Robinson's second proposed mechanism. In further experiments 4-hydroxy-2-quinolone and its N-methyl derivative were incorporated, the latter more efficiently suggesting that it is a more direct precursor. It is interesting to note that both of these compounds were found to be precursors of the furoquinoline alkaloids (see previous section) in related species of Rutaceae perhaps implying a common path for acridone and furoquinoline biosynthesis.

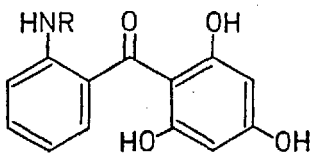
In further experiments designed to determine at what stage N-methylation occurred[219] ring labelled N-methylanthranilic acid was fed to Evodia xanthoxyloides. The label was detected in the A ring of the isolated acridone alkaloids confirming N-methylanthranilic acid as a possible precursor. Its status as a true intermediate rather than as an acceptable artificial precursor was demonstrated by the isolation from Acronychia baueri of labelled N-methylanthranilic acid following

the feeding of ring labelled anthranilic acid.[219] The presence of enzymes capable of demethylating N-methyl-3-(3',3'-dimethyl)allyl-2-quinolone in Skimmia japonica[197] means however that the incorporation of methyl-labelled N-methylanthranilic acid into acridone alkaloids would have to be proved before it can be stated categorically that N-methylation occurs before incorporation of anthranilic acid into acridone alkaloids. It is also not known if N-methylation is required before incorporation into a quinolone system can take place; if this were the case then it might explain the evolution of N-demethylating enzymes in Rutaceae species so as to allow the formation of the furo-quinolines.

Most of the proposed biosynthetic pathways to acridone alkaloids require that the C ring is acetate derived and administration of labelled acetate to A. baueri has indeed yielded radioactive acridone alkaloids.[219] Unfortunately no degradations were carried out to demonstrate the position of the label. In the light of the incorporation of both anthranilic acid and 4-hydroxy-2-quinolone it is probable that a major route leading to acridone alkaloids is via the condensation of a 4-hydroxy-2-quinolone (possibly already N-methylated and hydroxylated in the benzene ring) with two equivalents of acetate (scheme 20).

SCHEME 20



(235) 

As an alternative to the route shown in scheme 20, it has been suggested[220] that 9-acridones could arise by way of a pathway analogous to the biosynthesis of xanthones, viz. from 2-aminobenzophenone precursors. Such a route has been shown to be viable in vitro; thus intramolecular condensation of 2'-methylamino-2,4,6-trihydroxybenzophenone, (235), in the presence of sodium hydride in hot dimethylsulphoxide gave 2,4-dihydroxy-N-methyl-9-acridone in reasonable yield.[221] In vivo the intermediate benzophenone, (235), is envisaged as arising from condensation of anthranilic acid and acetate.[220] It is evident that further labelling studies are required to distinguish between these various routes.

Inspection of tables 17-20 indicates that all of the prenylated acridone alkaloids possess the basic 2,4-dimethoxy-9-acridone skeleton strongly implying that this is a common intermediate in their biosynthesis. If this is so then subsequent elaboration to give the observed alkaloids must involve aromatic hydroxylation at position 8 (and for evoprenine, (213), in position 3) and the incorporation of a dimethylallyl group, commonly at position 1 but also at position 3 or on oxygen. The intermediate epoxide (236) is a likely precursor for most of the alkaloids listed in tables 18 and 19 which can then arise by intramolecular opening of the epoxide; good precedent exists for this amongst the furoquinoline alkaloids (q.v.). As in the case of furoquinoline alkaloids lunasine, (171), lunine, (180), lunacrine, (179), haplobucharine, (167), and haplofoline, (166), the acridone alkaloid N-methyl-

bicycloatalaphylline, (231), may be regarded as the product of acid catalysed cyclization of N-methylatalaphylline, (215), or as the product of cyclization of the di-epoxide of (215) followed by reduction.

The two bis-acridones (232) and (233) are noteworthy in that the dimethylallyl group appears to have isomerized, with the double bond moving into conjugation with the aromatic ring prior to epoxidation and cyclization.

Guanidine alkaloids

The small group of alkaloids which appear to be derived from guanidine and one or more isoprenyl units are shown in table 21. They are isolated from leguminous plants, which is consistent with their highly nitrogenous nature.

The simplest members of the group, compounds (237)-(241), are isoprenylated guanidines. Biosynthetic studies with ^{14}C-labelled arginine have shown that the guanidyl carbon atom is incorporated into both galegine,[235] (237), and hydroxylgalegine,[236] (238), in Galega officinalis. It was also found that the guanidyl carbon atom of guanidinoacetic acid was incorporated into galegine.[237] In a later repetition of this experiment[238] guanidinoacetic acid was also incorporated into arginine and further investigations showed that a trans-amidinase enzyme was present in G. officinalis. Consequently guanidinoacetic acid or arginine may not necessarily be natural precursors of galegine and the immediate precursor may be an amidine-enzyme complex.

In experiments aimed at determining the origin of the dimethylallyl group in these compounds, labelled leucine, valine, hydroxyleucine, and dimethylallylamine were all administered to G. officinalis but were not incorporated into galegine or hydroxygalegine.[236] Mevalonic acid

Table 21 Guanidine alkaloids

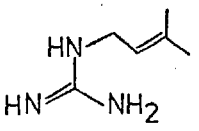
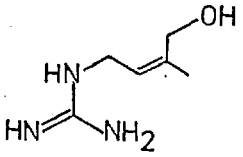
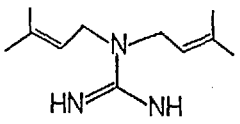
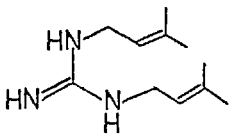
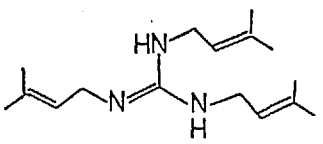
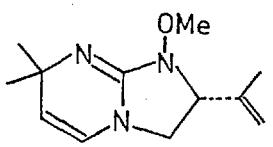
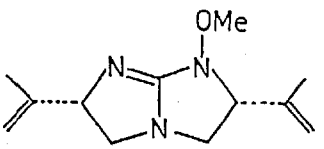
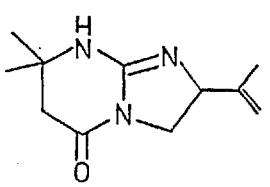
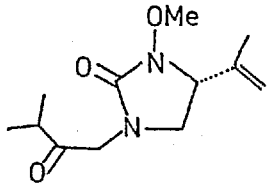
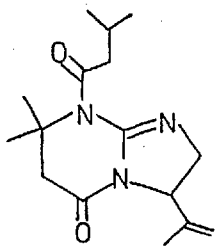
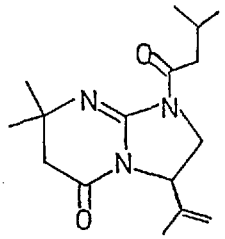
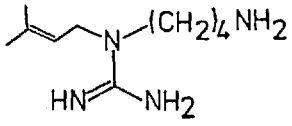
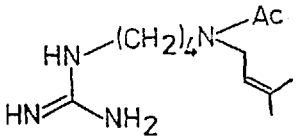
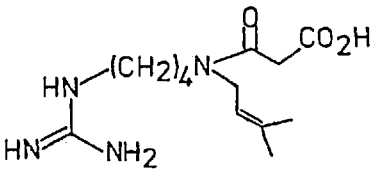
No.	Structure	Name	Source	Ref.
(237)		galegine	Galega officinalis	222
(238)		hydroxy galegine	G. officinalis	223
(239)		pterogynine	Pterogyne nitens	224
(240)		pterogynidine	P. nitens	228
(241)		-	Alchornea javanensis	225
(242)		alchorneine**	A. floribunda A. hirtella	226
(243)		isoalchorneine	A. floribunda	227
(244)		alchornine**	A. javanensis	

Table 21 (Continued)

No.	Structure	Name	Source	Ref.
(245)		alchomeinone	A. floribunda	227
(246)				
		alchorinidine*	A. javanensis	225
(247)				
(248)		spherophysine	Smirnovia turkestana Sphaerophysa salsula G. officinalis	230 229 231
(249)		smirnovine	Smirnovia turkestana G. officinalis	230 231
(250)		smirnovinine	Smirnovia turkestana G. officinalis	230 231

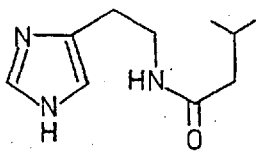
* (246) and (247) are alternative structures for alchorinidine

** structures determined by X-ray crystallography

was found to be a poor precursor whilst pyruvate was efficiently incorporated.[239] This result is surprising since pyruvate must enter the fatty acid cycle and be converted to acetyl coenzyme A and then to mevalonic acid before it can be incorporated into a dimethylallyl group.[11] It may be that in this instance the relative efficiencies of mevalonate and pyruvate incorporation do not reflect their directness as precursors of galegine but rather some other factor such as their rates of transportation to the sites of alkaloid biosynthesis.[234]

No biosynthetic studies on the compounds (242)-(247) have been reported. However the structures of alchorneine, (242), and isoalchorneine, (243), are consistent with their formation by oxidative cyclization of pterogynine, (239), followed by dehydration and N-oxidation at some point. Alchornine, (244), alchorneinone, (245), and alchornidine, (246) or (247), may also arise from oxidative cyclization of at least one N-dimethylallyl group but it is also possible that one of the C_5 units could be more directly derived from leucine, valine or isovaleric acid. A similar isovaleryl moiety has been identified in the cactus alkaloid dolichotheline, (251), (q.v.) and labelling studies have shown that the major precursor of the C_5 unit in this case is leucine.[233]

(251)

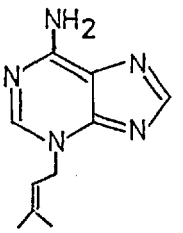


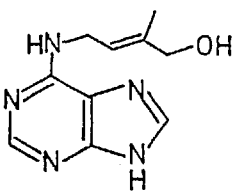
The remaining three alkaloids in table 21, namely spherophysine, smirnovine and smirnovinine, have been known for many years. In Sphaerophysa salsula it has been demonstrated that the guanidyl carbon atom of arginine is incorporated into spherophysine as is [2-^{14}C]-ornithine.[239,240,241] No experiments have been reported which show if the tetramethylene chain of arginine is incorporated or if arginine or

ornithine are incorporated intact. As with galegine, both pyruvate and mevalonate were incorporated into spherophysine, the latter less efficiently.[239]

Miscellaneous isoprenylated alkaloids

A number of isoprenylated aminopurines have been isolated from plants as a result of their activity as plant growth hormones. The first of these was triacanthine, (252), isolated from Gleditsia triacanthos[242] and later Holarrhensa mitis.[243] Zeatin, (253), is a 6-aminopurine bearing a hydroxylated dimethylallyl group which has been isolated from Zea mays.[244] Its structure has been confirmed by synthesis[245] as has that of the non-natural cis isomer.[246] Zeatin is a very potent plant hormone and this aspect of its properties has been the subject of many papers; however no reports have appeared relating

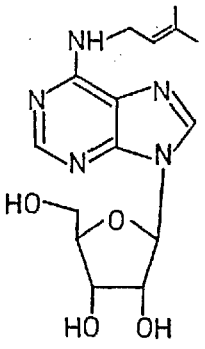
(252) 

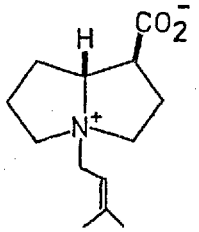
 (253)

to its biosynthesis. This is presumably due to its extremely low concentration in the plant which would make labelling studies difficult. The dihydro derivative of Zeatin has also been isolated, this time from Lupinus luteus,[247] and its absolute configuration has been determined.[248]

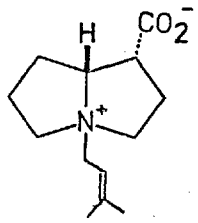
Isoprenylated 6-aminopurines have also been isolated from micro-organisms where they exist in relatively high concentrations. Thus N^6-(3',3'-dimethyl)allyladenosine, (255), has been isolated from yeast R.N.A. following enzymatic hydrolysis of the macromolecule[249] and an enzyme has been concentrated from cell free extracts of Escherichia coli which catalyses the synthesis of compound (255) in transfer R.N.A.[250]

(255)





(256)

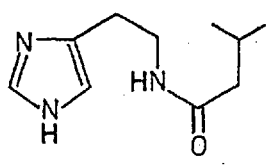


(257)

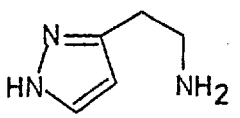
A pair of isoprenylated pyrrolizidine alkaloids, anodendrine, (256), and alloanodendrine, (257), have been isolated from Anodendron affine and their structures have been confirmed by synthesis.[251] Pyrrolizidine alkaloids have frequently been found bearing side chains containing an isoprenylated phenol[252] and so the isolation of compounds (256) and (257) is not too surprising.

Cactus alkaloids

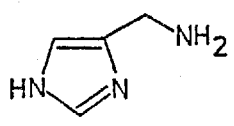
A number of alkaloids have been isolated from cactii which contain a C_5 unit resembling a 3',3'-dimethylallyl group. These are dolichotheline,[253] (251), lophocerine,[254] (69) and pilocerine,[257] (258). Dolichotheline was isolated from Dolichothele sphaerica and its biosynthesis has been independently investigated by two groups. Histidine and histamine were both found to be precursors of the imidazole portion of the molecule[254,255] with the latter being more efficiently incorporated.[254]



(251)



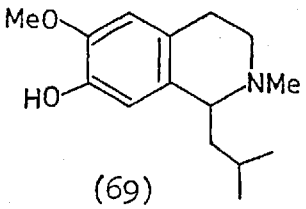
(259)



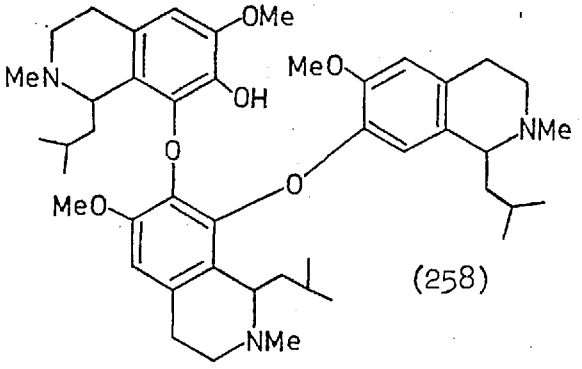
(260)

The C_5 side chain was found to be derived from leucine[254,255] valine,[254] isovaleric acid[254,255] and mevalonate;[255] however of these leucine was

incorporated much more efficiently than mevalonate suggesting that the major biosynthetic pathway involves the former, presumably *via* transamination and decarboxylation. *Dolichothele sphaerica* was also found capable of accepting a number of unnatural precursors and converting them to dolichotheline analogues.[233] Thus cinnamic acid, isocaproic acid and compounds (259) and (260) were all incorporated into dolichotheline-like amide derivatives.

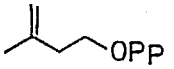


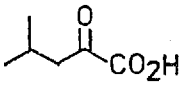
(69)

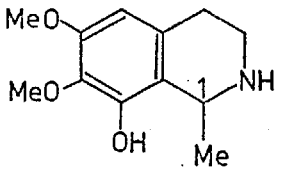


(258)

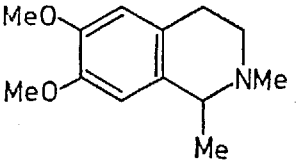
Lophocerine, (69), and pilocereine, (258), are isoquinoline alkaloids isolated from the cactii *Lophocereus schotti*[254,257] and *Pachycereus marginatus*[260] and their biosynthetic relationship has been demonstrated by feeding of doubly labelled lophocerine which was efficiently incorporated intact into pilocereine.[258] Such phenolic coupling of isoquinolines in Nature is well documented.[259] The origin of the isoquinoline nucleus was found to be tyrosine[71] and the C_5 unit incorporated label from both leucine and mevalonate, the latter more efficiently.[71] Also incorporated were[72] isopentenyl-pyrophosphate, (261), 3-methylbutanol and 3-methylbutanal but not 3-methylbutanoic acid. On the basis of these results it was suggested that leucine is not incorporated *via* mevalonate since according to accepted biogenetic theory this would involve initial conversion to 3-methylbutanoic acid.[72]

(261) 

(262) 



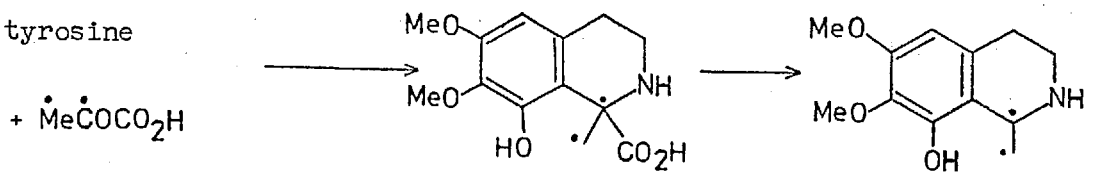
(263)



(264)

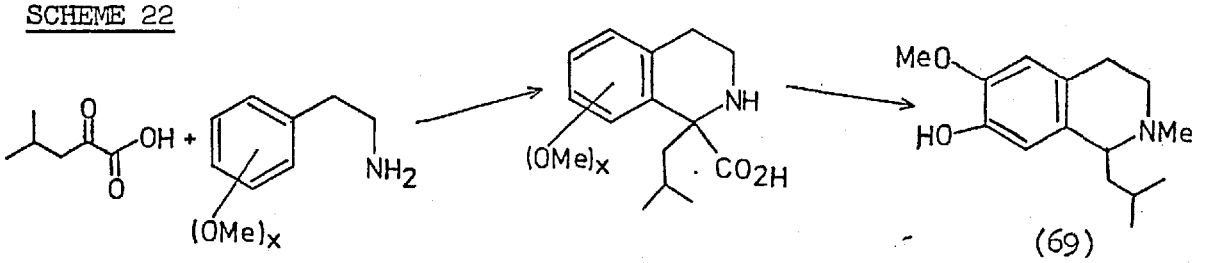
In the biosynthesis of dolichotheline it was proposed[254] that leucine is incorporated via the keto-acid (262). That this is also the intermediate in the biosynthesis of lophocerine from leucine is implied by the finding that naturally occurring 1-methylisoquinolines (e.g. anhalonidine, (263)) are derived from tyrosine and pyruvate but not acetate. Thus labelled acetate was only randomly incorporated into compound (264)[262] and in anhalonidine the 1-methyl group was preferentially derived from the methyl group of pyruvate.[263] This route was confirmed by the isolation from the same source of compound (265) and its efficient incorporation into anhalonidine[261] (scheme 21). By analogy, the biosynthetic pathway to lophocerine from leucine and tyrosine may be that shown in scheme 22.

SCHEME 21



(265) (263)

SCHEME 22



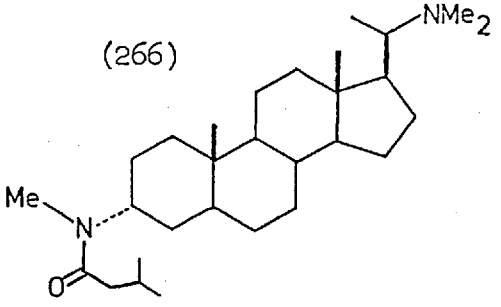
(69)

Steroidal alkaloids

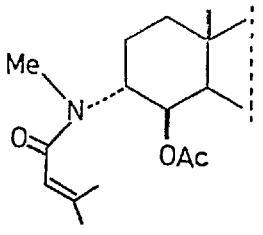
A large number of steroid alkaloids have been found in species of the Buxaceae family and from one species - Pachystrandra terminalis - a group of 6 steroid alkaloids have been isolated which bear an unusual C_5 unit. These are compounds (266)-(271) shown in table 22. Each of these natural products possess a dimethylacrylic acid (senecioic acid) moiety which in some cases has apparently undergone intramolecular cyclization with an N-methyl group to give a 7-membered lactone in compound (269) and a beta-lactam in compounds (270) and (271). No biosynthetic studies of these compounds have been reported.

Table 22

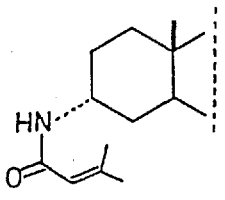
(266)



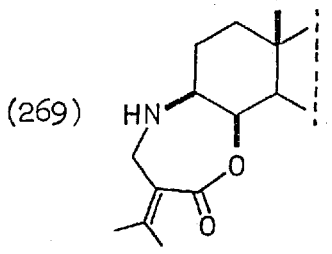
(267)



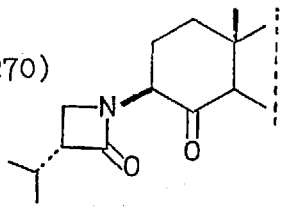
(268)



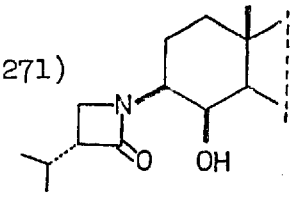
(269)



(270)



(271)



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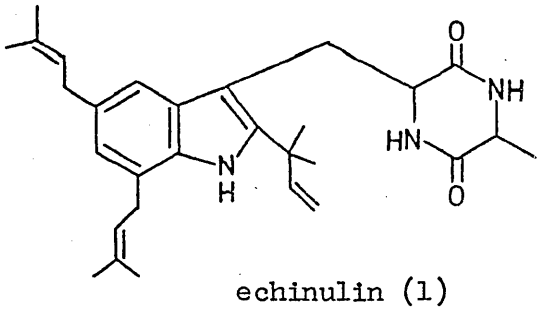
RESULTS AND DISCUSSION



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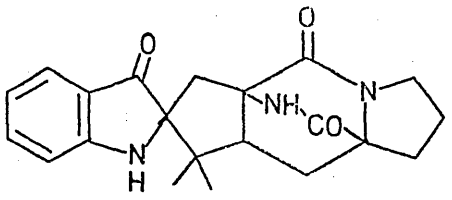

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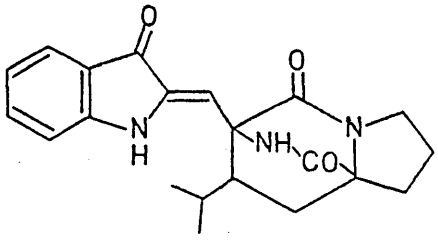

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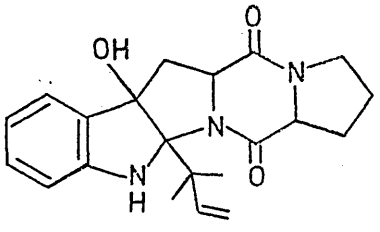

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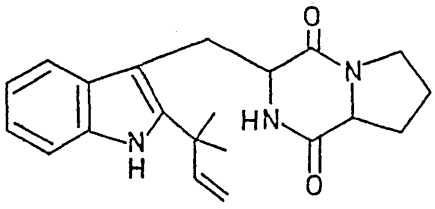

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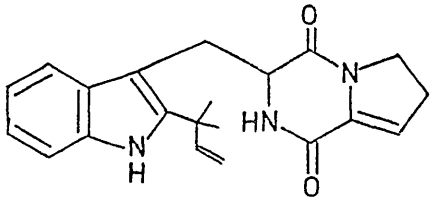

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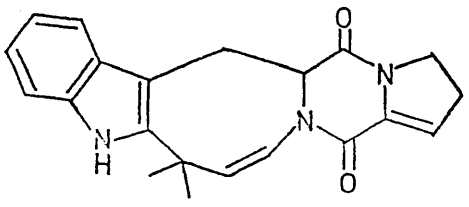

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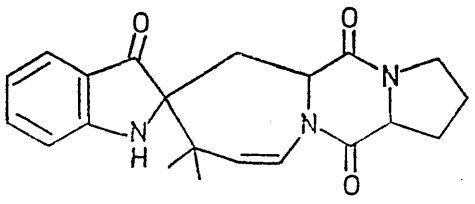

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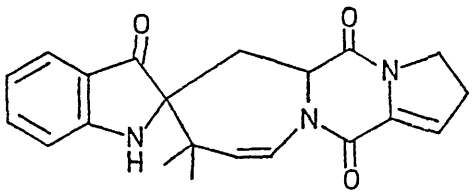


austamide (9)

* brevianamide B possesses the opposite configuration at the spiro junction

** brevianamide D possesses the opposite geometry about the double bond

Introduction

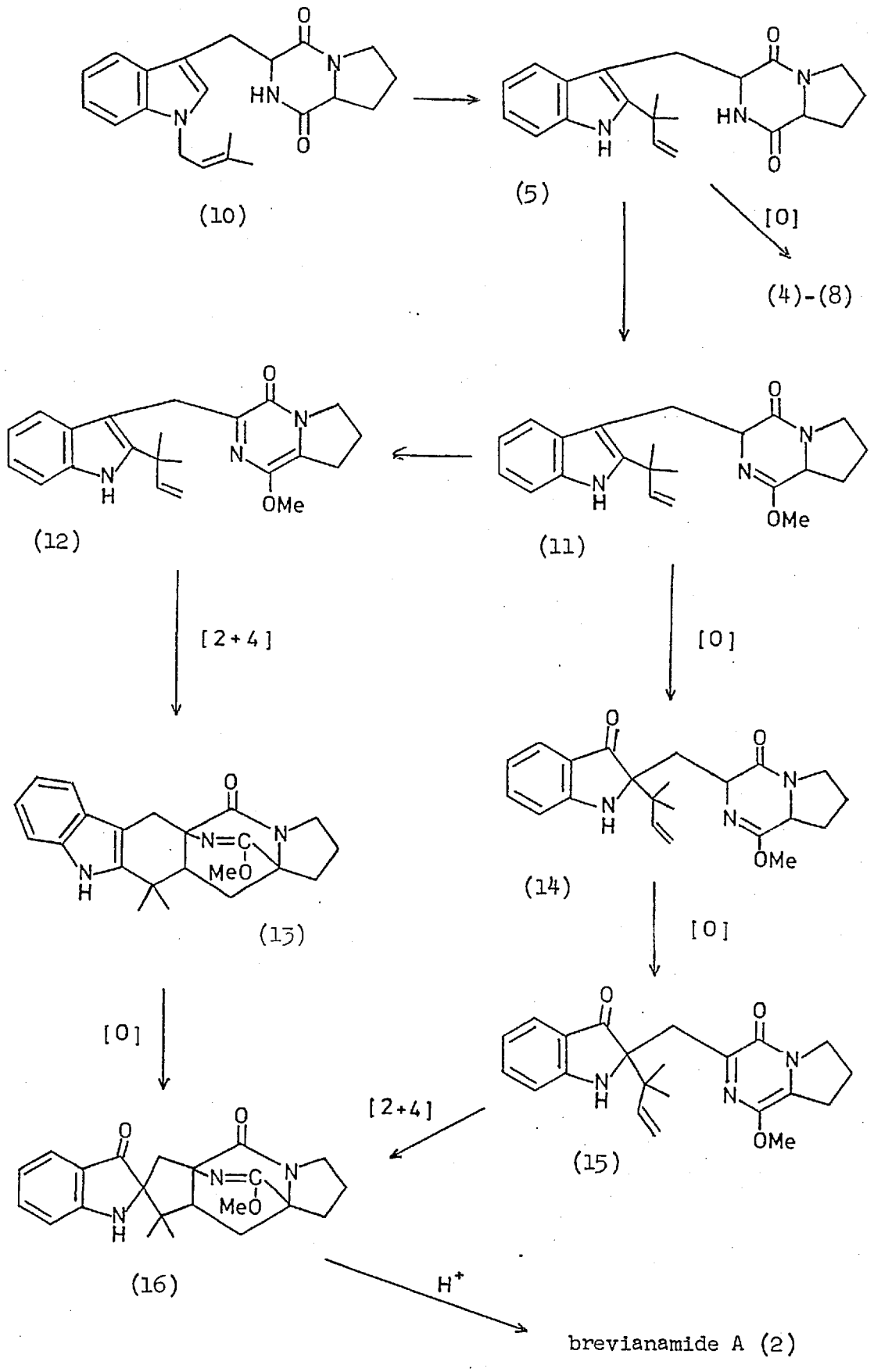
At the time of writing (summer 1976) some 30 natural products containing a dioxopiperazine ring and incorporating a 2-(1',1'-dimethyl)allyltryptophan unit are known. The occurrence of these compounds and what is known or postulated about their biosynthesis is reviewed in the preceding section. When the work about to be described in this thesis was commenced in 1973 only a handful of these natural products had been reported, *viz.* echinulin[1] (1), the brevianamides[2] (2), (3), (4) and the austamides[3] (5)-(9), of which only echinulin had been synthesized.[4] The aim of the present work was to attempt a synthesis of the brevianamides and hence the austamides by a possibly biomimetic route illustrated in scheme 1.

The initial step in this route involves rearrangement of *cyclo*-L-prolyl-L-(N^a-(3',3'-dimethyl)allyl)tryptophyl, (10), into desoxybrevianamide E, (5), itself a natural product[3] whose facile interconversion with brevianamide E, (4), has been exemplified.[2,3] The rearrangement of N-(variously substituted)allylindoles to give 2-substituted products also has plentiful precedent.[42-45].

It has been suggested that conversion of desoxybrevianamide E into brevianamides A and B may proceed *in vivo* by way of a [2 + 4] cycloaddition of the 2-isopentenyl unit across the dioxopiperazine ring following its oxidation to a pyrazinone[5] and model studies have shown this to be viable.[5b] Two plausible laboratory routes using this sequence are shown in scheme 1; the two pathways differ only in the order of the transformations.

The work described in the following pages was directed towards the achievement of the very first step of this scheme, the rearrangement of the allylindole (10), and subsequent elaboration of the products.

SCHEME 1

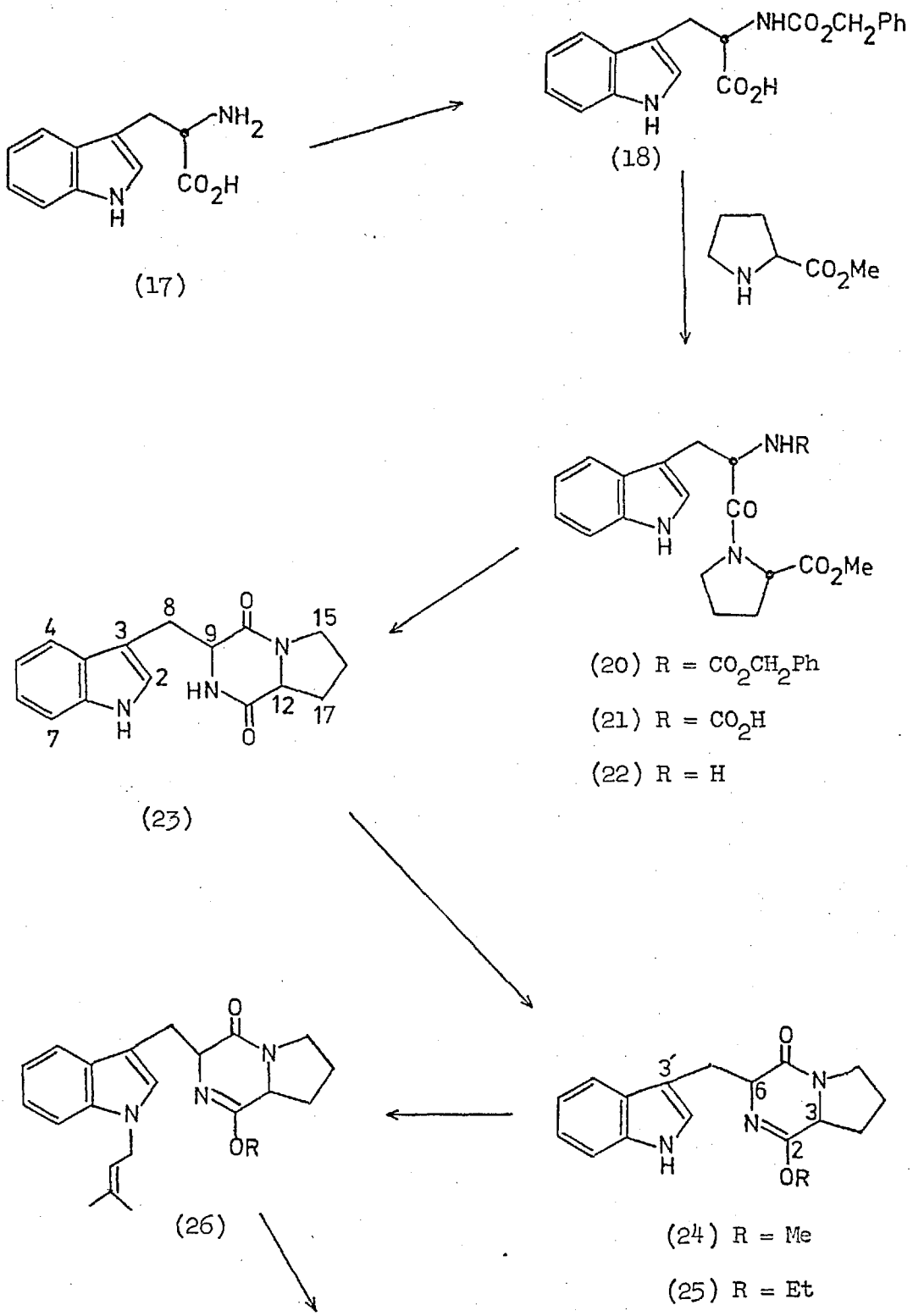


Synthesis of cyclo-L-prolyl-L-[N^a-(3',3'-dimethyl)allyl]tryptophyl, (10)

The route originally selected for the preparation of the allyl-indole (10) is shown in scheme 2. N-Carbobenzoxy-L-tryptophan, (18), was prepared in high yield and purity from L-tryptophan and carbobenzoxy chloride by a published procedure[7] and was condensed with L-proline methyl ester in the presence of dicyclohexylcarbodiimide (D.C.C.) in dry dichloromethane. Removal of precipitated dicyclohexylurea and evaporation of solvent gave N-carbobenzoxy-L-tryptophyl-L-proline methyl ester, (20), contaminated with a small amount of dicyclohexylurea. Apart from the latter impurity the dipeptide was assessed to be sufficiently pure by thin layer chromatography (t.l.c.) to be used directly in the next stage, viz. hydrogenation over palladized charcoal containing a trace of acetic acid. The hydrogenolysis of the benzyl group gave the unstable carbamic acid (21) which was detected by t.l.c. on silica but not isolated. Decomposition of the carbamic acid by mild base gave cyclo-L-prolyl-L-tryptophyl, (23), presumably via the amine (22) which was not observed. The dioxopiperazine (23) was obtained as a gum which solidified to a white powder following trituration with ether. Crystallization from acetone gave a sample with melting point and optical rotation in good agreement with the literature values.[2] The dioxopiperazine (23) had previously been prepared in a similar manner by condensation of carbobenzoxy-L-tryptophan and L-proline benzyl ester[2] and recently a report has appeared describing the preparation of (23) by the same method used here.[75]

It was mentioned above that the dipeptide (20) was contaminated with dicyclohexylurea and that this impurity was allowed to be carried through the synthesis into the product, dioxopiperazine (23). Unfortunately the similarity between the solubility and chromato-

SCHEME 2

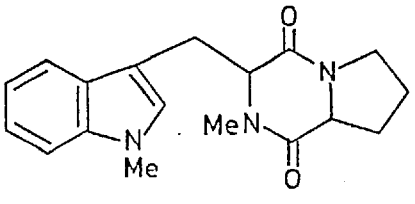

NH2
CO2H
N
H
(17)
NHCO2CH2Ph
CO2H
(18)
CO2Me
NHR
CO
(20) R = CO2CH2Ph
(21) R = CO2H
(22) R = H
(23)
(24) R = Me
(25) R = Et
OR
(26)
(10)

graphic properties of the simple dioxopiperazines and dicyclohexylurea combined with the relative unreactivity of the latter resulted in the survival and gradual accumulation of the urea in subsequent reaction products further along the synthetic route to and beyond the allylic dioxopiperazine (10). This was so even after chromatographic purification and crystallization at each stage. Consequently an alternative synthetic pathway to the dioxopiperazine (23) was considered; treatment of N-carbobenzoxy-L-tryptophan, (18), with phosphorous pentachloride in ether gave N-carbobenzoxy-L-tryptophyl chloride in accordance with a published route.[8] Condensation of this acid chloride with L-proline methyl ester in the presence of triethylamine gave the desired dipeptide (20) which was converted to the dioxopiperazine as before. Whilst this sequence worked well on a small scale problems were experienced in larger reactions (i.e. more than 10g of tryptophan) where the crude dioxopiperazine (23) was obtained in purities of only 50% or thereabouts. This level of purity was unacceptable, partly because of the loss of expensive starting material it represented and also because of difficulties encountered in purifying the mixture. Cyclo-tryptophyl-prolyl compounds were generally found to be difficult to crystallize when pure and often refused to crystallize, even with a seed, if other impurities present exceed a few per cent of the total mixture.

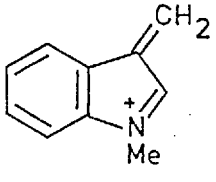
These problems were largely avoided by careful purification of the dipeptide (20) following preparation by the D.C.C. method. The protected dipeptide (20) was highly insoluble in non-polar and polar solvents once it had crystallized but was highly soluble in acetone, ethyl acetate and dichloromethane whilst still an oil. Accordingly concentration of the dipeptide in one of these solvents followed by standing in the cold and crystallization of the dicyclohexylurea

allowed isolation of reasonably pure material after removal of solids by filtration.

The L-proline methyl ester used to prepare the dipeptide (20) was obtained from L-proline by a standard Fischer esterification using thionyl chloride in methanol.[9] The amino acid ester was obtained as the hydrochloride salt from this reaction and was stored as such to prevent self condensation and formation of cyclo-L-prolyl-L-prolyl. The free ester was liberated directly before use by treatment of the salt with ammonia in cold dichloromethane. In one preparation L-proline ethyl ester hydrochloride was used. The salt was obtained by the literature method[10] (ethanol saturated with hydrochloric acid gas) and crystallized as large colourless prisms on standing for several months. The melting point of the solid, which hitherto had only been reported as an oil was determined as 115-117°C.



(27)



m/e 144

(28)

With the preparation of cyclo-L-prolyl-L-tryptophyl, (23), the next step of the synthesis, the selective alkylation of the indole nitrogen atom in order to produce compound (10), was attempted. The acidities of the indolic and amidic NH groups were expected to be similar. This was confirmed when, in an exploratory experiment, dioxopiperazine (23) was treated with one equivalent of sodium hydride in dimethylformamide followed by a large excess of iodomethane. Two compounds were isolated following preparative thin layer chromatography (p.l.c.); one of these was the starting material, identified from melting point and chromatographic comparison with (23), and the other was

apparently the product arising from dimethylation of (23), i.e. structure (27). Thus the ^{1}H n.m.r. indicated the absence of both the indolic and amidic NH resonances and the presence of two methyl groups. The mass spectrum of the compound confirmed the assignment of structure (27) by exhibiting a molecular ion at 311 mass units and a base peak at 144 mass units. The latter corresponds to stable fragment (28); this fragmentation is generally very characteristic of cyclo-dipeptides containing tryptophan.[11]

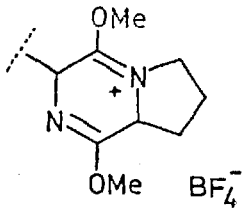
The isolation of the dimethylated structure (27) rather than a mixture of the two possible mono-alkylated compounds was somewhat surprising and the initial conclusion drawn was that selective mono-alkylation was not feasible. Consequently protection of the secondary amide nitrogen atom of dioxopiperazine (23) against alkylation by conversion to the imino ether derivative (24) was considered (scheme 2). This has previously been carried out in good yield with simple dioxopiperazines using Meerwein salt.[12,13] The dioxopiperazine (23) was treated with an excess of trimethyloxonium tetrafluoroborate and initially t.l.c. indicated slow formation of a less polar compound whilst after longer periods a second less polar compound was also detected. However before all the starting material had disappeared numerous other by-products began to be formed leading to rather complex product mixtures. Puzzlingly the proportion of starting material remaining at any time during the reaction varied and on occasions actually increased between consecutively withdrawn t.l.c. samples. The reason for this was to become clear when the nature of the by-products was determined.

Several repetitions of the reaction gave the same result. Isolation of the initially formed material when most of (23) still remained gave the desired imino ether (24) in poor yield suggesting that the reaction was being diverted from its proper path after an induction period. Some

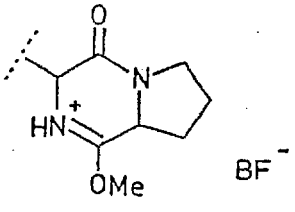
of the problems with this reaction were thought to arise from the fact that trimethyloxonium tetrafluoroborate is only sparingly soluble in the reaction solvent (dichloromethane); at the start of the reaction the Meerwein salt was a reasonably free flowing, easily stirred white solid but it was soon transformed to a brown sludge. This effect may have arisen from complex formation between the Meerwein salt and the tertiary amide function of the starting material (23) or the desired product (24).[14] It has been reported that treatment of amides with Meerwein salts initially gives the imino ether which can rearrange to the N-alkyl amide in the presence of an excess of reagent.[14] Accordingly, treatment of a dilute dichloromethane solution of the dioxopiperazine (23) with one equivalent of Meerwein salt, added in small amounts over a long period, was investigated. This procedure, however, merely slowed the reaction down and eventually gave similar results as before. The reaction was also tried at 40°C and 0°C; in the former case no improvement in Meerwein salt solubility or product composition was detected whilst in the latter case the reaction proceeded slowly but quite cleanly to give a mixture of starting material and the two products previously observed.

Careful purification by column chromatography and p.l.c. of these reaction mixtures gave a pure, crystalline sample of the imino ether (24) in very low yield. The ^{1}H n.m.r. spectrum of this compound exhibited a singlet at τ 6.25 consistent with the new methoxy group whilst the amide NH proton of the starting material was no longer visible. The solid phase (Nujol mull) i.r. spectrum of the product indicated the loss of the secondary amide carbonyl stretching vibration at 1660 cm^{-1} whilst the tertiary amide carbonyl stretching vibration was still present at 1690 cm^{-1}. In addition two very strong bands appeared at 1620 and 1710 cm^{-1} corresponding to the imino ether function.[14]

The problems of reagent solubility were eventually overcome by using triethyloxonium tetrafluoroborate, which is readily soluble in dichloromethane, instead of the trimethyl salt which is not. When a solution of the dioxopiperazine (23) was stirred with an excess of triethyloxonium tetrafluoroborate t.l.c. of aliquots indicated gradual disappearance of starting material and quite clean formation of a less polar product. After approximately half of the starting material had reacted a second less polar compound also began to appear and gradually increased in quantity until all of (23) had been consumed. This second product was chromatographically identical to the second product formed in the reaction of (23) with trimethyloxonium tetrafluoroborate. During the reaction a yellow oil separated out of solution. T.l.c., after work-up, indicated that it contained the same two products as the solution and it was presumed that the oil was an insoluble complex between the Meerwein salt and tertiary amide function, as in structure (29), or a protonated imino ether function as in structure (30). Both types of complex are known to be formed under these conditions.[14] The oil decomposed with gas evolution when treated with aqueous sodium hydrogen carbonate and became soluble in dichloromethane which is consistent with both possibilities.



(29)

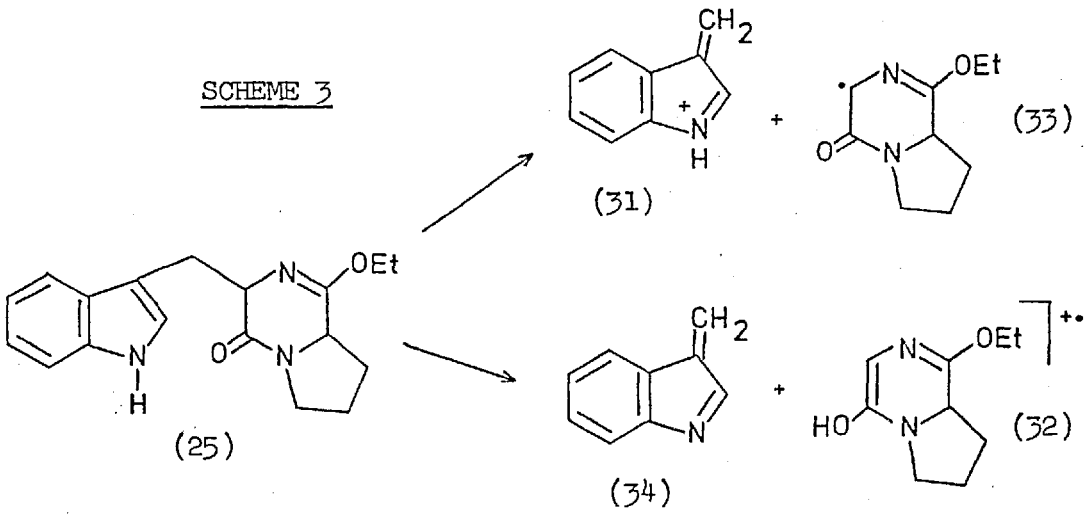


(30)

Isolation of the two major products of the reaction was performed by p.l.c. The more polar of the two was obtained as a foam which crystallized from acetone/light petroleum and was found to be the desired imino ether (25). The i.r. spectrum was very similar to that of the

homologous imino ether (24) as was the ^1H n.m.r. spectrum which exhibited the expected ethoxy signals at τ 5.85 (quartet) and τ 8.80 (triplet). The u.v. spectrum of (25) was that of a typical indole chromophore[11] (a coalesced triplet at 275, 282 and 290 n.m.) and the mass spectrum contained the expected molecular ion at 311 mass units. The base peak was at 130 mass units corresponding to the normal fragmentation to give the ion (31) but a very intense peak was also observed at 182 mass units. A metastable peak at 107 mass units corresponding to the fragmentation of the molecular ion to the ion of mass 182 was observed confirming that it was not derived from an impurity. The only reasonable fragment of

SCHEME 3



this mass appears to be structure (32) resulting from rearrangement of (25) in the manner shown (scheme 3). The transition state of this transformation involves an 8-membered ring which would normally be unfavourable; however the configuration of many of the atoms in (25) are fixed by rings or π -systems and this reduction in the degrees of rotational freedom around the axes of the bonds involved in the transition state may assist the rearrangement. Examination of a model of (25) suggested that whilst the termini of this cyclic transition state (i.e. the hydrogen atom on the indole nitrogen and the amide oxygen atom) could

approach without strain, a planar stereoelectronic requirement for a concerted process was not met. Possibly the energetically excited, electron deficient species which the imino ether (25) becomes in the mass spectrometer does not rearrange *via* the normal ground state molecular orbitals and the rearrangement depicted in scheme 3 does become viable.

The less polar product from the reaction of dioxopiperazine (23) with Meerwein salt was obtained as a foam which crystallized from acetone/light petroleum. The ^{1}H n.m.r. spectrum of the compound did not contain an ethoxy group and integration showed it to possess the same number of protons as the starting material. Also absent were the singlet signals present in the starting material at τ 1.3 and 3.0 corresponding to the hydrogen atoms at positions 1 and 2 of the indole nucleus respectively. Only four aromatic protons were observed and these resonated between τ 2.8 and 3.5 compared with 5 aromatic protons absorbing between τ 2.3 and 3.1 in the starting material. The upfield shift of the aromatic protons in the product suggested that the slightly electron deficient benzene ring of the indole system of (23) had become electron rich. A single D_2O exchangeable proton was located at τ 4.9; this proton exchanged rapidly implying that it was not part of the secondary amide function which in the starting material resonates at approximately the same position but only exchanges slowly with D_2O in the absence of a base catalyst. Most important of all a doublet (J = 7Hz) was observed at τ 4.5 which integrated to a single proton. All other signals appeared between τ 5.9 and 8.3 as a complex system of superimposed multiplets.

The mass spectrum of the product revealed that it was isomeric with the starting material and the fragmentation pattern also appeared identical although the relative intensities of the peaks were different and a much stronger molecular ion was present.

The u.v. spectrum of the compound was recorded and maxima were observed at 297 and 242 n.m. which is very different from that of an indole chromophore (c.f. the u.v. spectrum of (25)). Addition of acid caused no immediate change in the spectrum but addition of base resulted in rapid loss of the originally observed bands and their replacement by maxima identical in position and shape to those of the starting material (23). This change, which was complete in 20 minutes, was not reversible. It was found later that addition of acid also caused reversion to an indole chromophore but that the reaction was much slower, taking hours rather than minutes.

Secondary amides normally exhibit a strong carbonyl stretching band in their i.r. spectra in the range 1700-1665 cm^{-1} in solution and 1680-1630 cm^{-1} in the solid phase whilst tertiary amides absorb in the range 1670-1630 cm^{-1} independent of the state of the sample.[17] Thus the i.r. spectrum of dioxopiperazine (23), which contains both a secondary and a tertiary amide group, possesses strong peaks at 1675 and 1655 cm^{-1} when recorded as a Nujol mull. The i.r. spectrum of the least polar product of the Meerwein alkylation reaction exhibited a single, strong carbonyl stretching band at 1660 cm^{-1} in both solution and the solid phase indicating the absence of a secondary amide group.

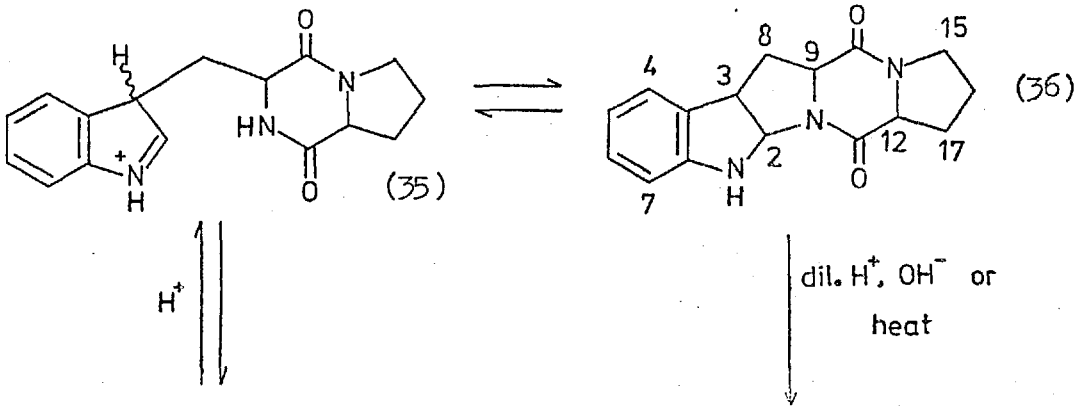
These observations led to the assignment of structure (36) to the new compound. In the ^{1}H n.m.r. spectrum the doublet at τ 4.5 corresponds to the methine proton at position 2, in good agreement with the chemical shift of the analogous proton in the known structure (41) which is quoted as τ 4.3 (J = 7Hz).[22] The properties of the indoline (41) have not been fully reported (for example no u.v. or mass spectra are given) but it was stated that the compound was unstable and reverted to the relevant tryptophan derivative upon treatment with acid or base.

A comparison of the u.v. spectrum of indoline (36) with that of N-methyl-o-toluidine[18] shows good agreement:

	N-methyl-o-toluidine	(36)
λmax (log ε)	244(4.1)	242(3.78)
	294(3.4)	297(3.31)

The close similarity of the mass spectrum of compound (36) with that of the starting material (23), presumably means that under the conditions of the mass spectrometer inlet system (36) thermally rearranges back to (23).

The ^{13}C n.m.r. spectrum of compound (36) was consistent with the structure assigned; the spectrum is discussed on p.210 along with the spectra of some of its derivatives and closely related compounds.

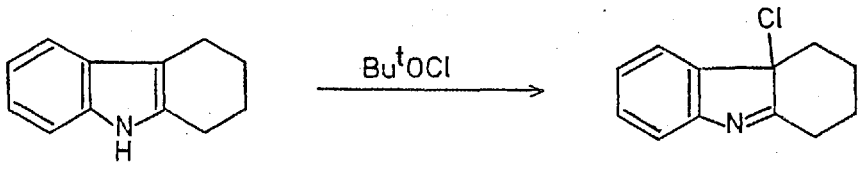


(23) SCHEME 4 (23)

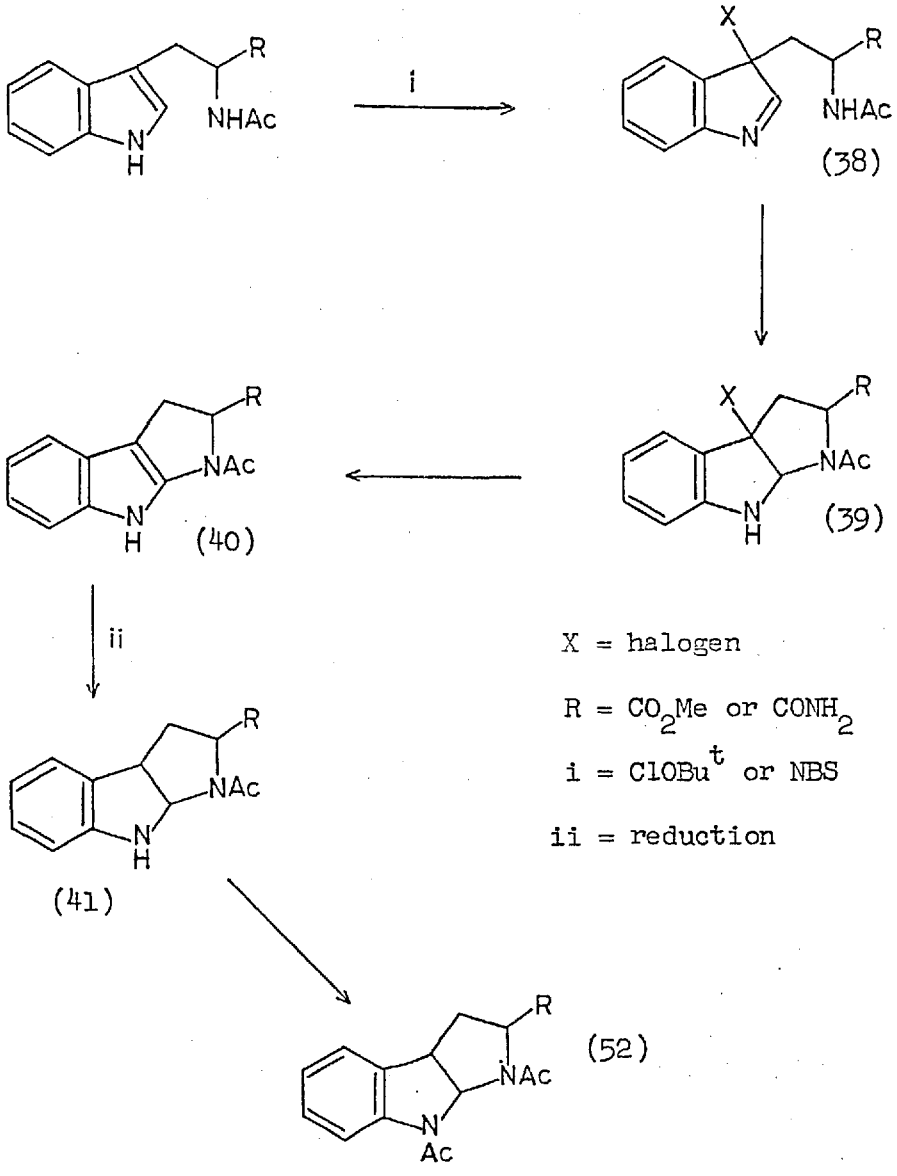
Compound (36) is presumably formed during the alkylation of dioxopiperazine (23) with Meerwein salts via protonation by tetrafluoroboric acid, a by-product of the reaction, according to the mechanism shown in scheme 4. The reaction is driven to completion at suitably low pH by N-protonation of the product (36).

The suggested mode of formation and instability towards base of the indoline (36) explain the observed course of the reactions of

SCHEME 5



SCHEME 6

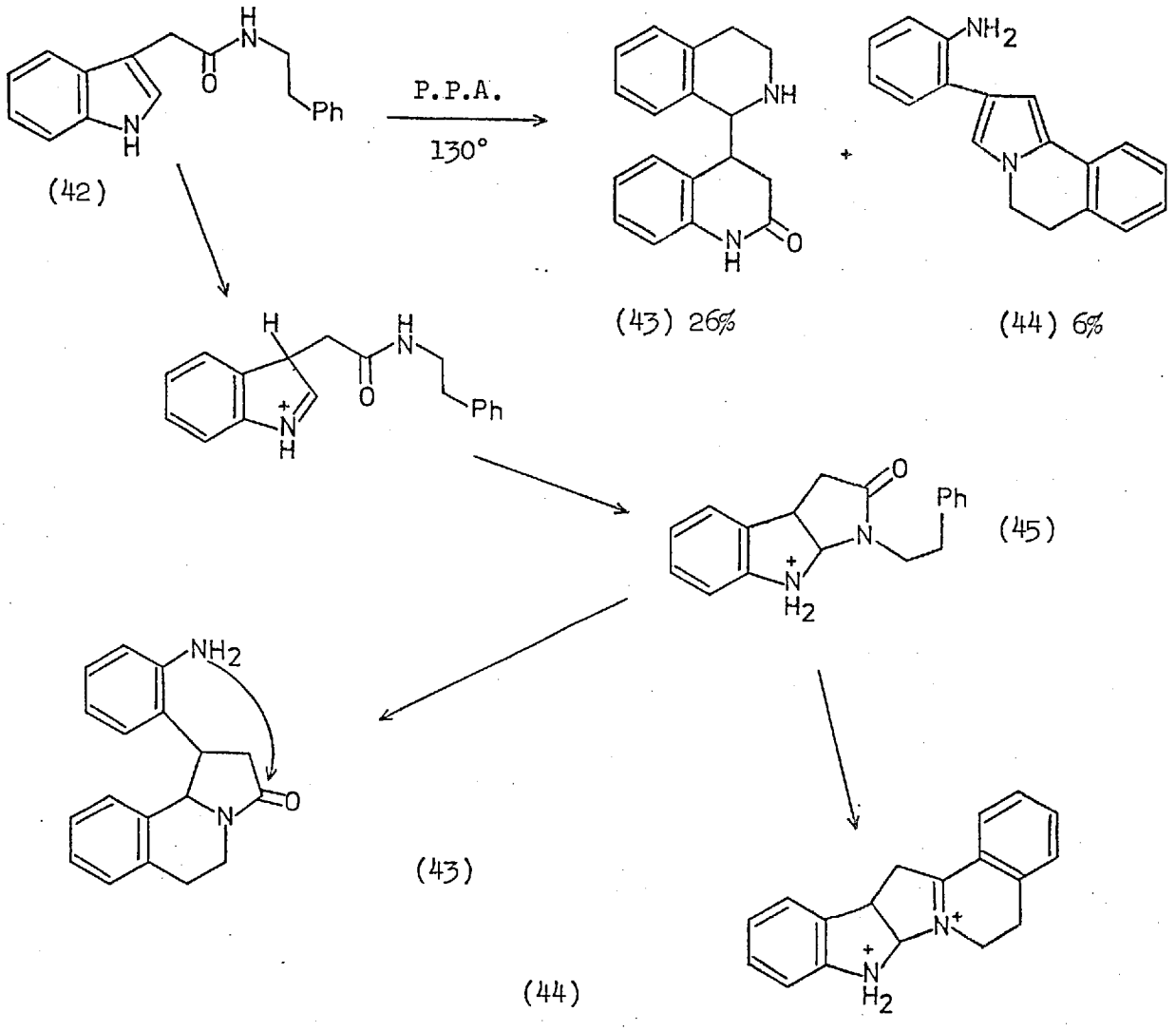


dioxopiperazine (23) with Meerwein salts. At the start of the reaction alkylation proceeds smoothly to give the imino ethers (24) or (25) but gradually the concentration of tetrafluoroboric acid builds up until the pH is sufficiently low to convert the starting material to the indoline (36). The apparent variation in the relative proportions of starting material remaining at any time during the reaction can be explained by decomposition of (36) to (23) during work-up of the aliquots extracted for t.l.c. The amount of decomposition depends upon the speed of work-up (vide infra, p. 131). Also the long reaction times unnecessarily used allowed decomposition of the imino ethers which after isolation were found to be unstable compounds and slowly decomposed when in solution or in the gummy state prior to crystallization.

Indoles are known to protonate preferentially at position 3 under suitably strong acid conditions to give protonated indolenines of the type (35)[19] but such species have never been isolated and have only been observed spectroscopically (u.v., ^{1}H n.m.r.). Similarly indoles react with electrophiles at position 3 rather than on nitrogen to give, as isolable intermediates, indolenines such as (37) as shown in scheme 5.[20] Many examples also exist of the intramolecular trapping of indolenine intermediates[21] and a particularly pertinent example is illustrated in scheme 6.[22] As far as the writer is aware, however, there are no examples of the isolation of an intramolecularly trapped indolenine produced by simple protonation of an indole at position 3. A report exists[23] of the isolation of two compounds whose formation can be rationalized by a mechanism involving intramolecular trapping of a protonated indole. This is summarized in scheme 7; the intermediate (45) was not isolated nor was any attempt made to detect it by inspection of the ^{1}H n.m.r. or u.v. spectra of the precursor (42) under strongly acidic conditions. By analogy with the behaviour of the indoline (36), compound (45) should

be sufficiently stable to attain observable concentrations at room temperature.

SCHEME 7



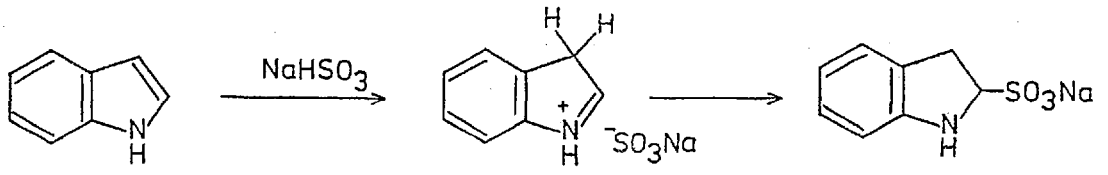
In order to prove that indoline (36) is formed by acid catalysed cyclization of the dioxopiperazine (23) as shown in scheme 4 two experiments were carried out. In the first a sample of (23) was dissolved in trifluoroacetic acid and the ^1H n.m.r. spectrum was recorded. Allowing for solvent shifts and the fact that the N-protonated form was presumably being observed, the spectrum obtained was similar to that of indoline (36) in deuteriochloroform. Thus a doublet (J = 6Hz) was present at τ3.9 and the aromatic protons appeared at τ2.8 as a broad singlet.

When the solution was poured into an excess of aqueous sodium hydrogen carbonate solution and rapidly extracted into dichloromethane, a quantitative yield of (36) was obtained with no trace of starting material.

In the second experiment the reaction between dioxopiperazine (23) and triethyloxonium tetrafluoroborate was repeated in dichloromethane containing a well stirred suspension of dry calcium carbonate. The carbonate was present to remove tetrafluoroboric acid as it was formed and so prevent protonation of the starting material to give indoline (36). T.l.c. indicated that as the reaction proceeded, only one major product was formed. At the completion of the reaction a good yield of the imino ether (25) was obtained and none of (36) was observed.

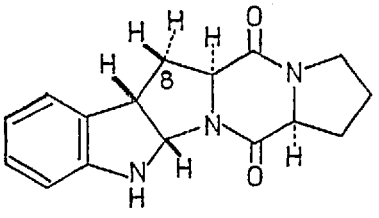
The ability of indole to form an insoluble bisulphite complex is not a well known reaction and has not found application to indole derivatives.[24] However it does represent an example of the protonation of an indole followed by quenching of the indolenine (scheme 8). The formation of the complex is surprising given the pH of a saturated solution of bisulphite (5). Normally a somewhat lower pH would be required to protonate indole (pKa =-3.6) but presumably a small amount

SCHEME 8

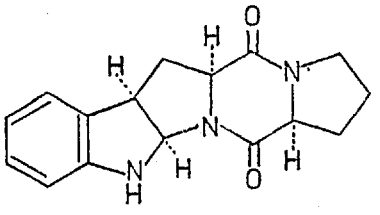


of protonation does occur and the reaction is driven to completion by removal of the bisulphite complex from the reaction medium as it crystallizes out. Given the poor crystallinity of the cyclo-L-prolyl-L-tryptophyl derivatives encountered in this work, the formation of a bisulphite complex with cyclo-L-prolyl-L-tryptophyl, (23), was investigated

as an alternative purification procedure. However no complex could be formed under any of the conditions tried, possibly because of hindrance by the side chain of the indole or possibly because the indole nucleus in (23) is a much weaker base than indole itself (an alkyl group in position 3 reduces the basicity of an indole by a factor of several thousand in the pK value[19]).



(46)



(47)

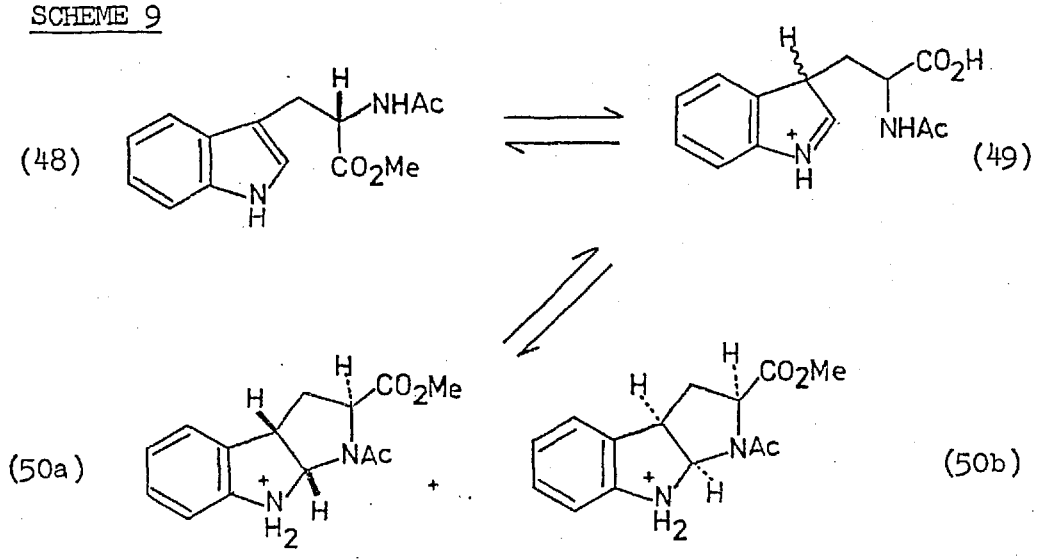
It will have been immediately noticed that formation of the indoline (36) from the dioxopiperazine (23) by the route suggested (scheme 4) should give rise to two isomers, (46) or (47), depending upon whether protonation occurs on the upper or lower face of (23) as written. These two structures are diastereoisomers and, consequently, would be expected to possess quite different physical properties. However the samples of (36) isolated appeared homogeneous on t.l.c. and crystallized in one form to a sharp melting point. Furthermore crude samples of (36) derived from pure dioxopiperazine (23) appeared as a single compound and not as a mixture of isomers when their ^{1}H and ^{13}C n.m.r. spectra were recorded. This suggests that protonation of (23) is occurring preferentially upon one face of the molecule or that of the two possible protonated species represented by structure (35) one cyclizes to (36) far more rapidly than the other. The stereospecificity of this reaction is discussed further below (p.125).

In order to differentiate between structures (46) and (47) it is necessary to identify and measure the coupling constants of the protons at position 8 in the ^1H n.m.r. spectrum of compound (36) and to compare them with the values predicted by the Karplus equations. Unfortunately the protons of interest are obscured by signals from the rest of the molecule and it is not possible to measure their coupling constants. In an attempt to overcome this the ^1H n.m.r. spectrum of (36) was recorded in the presence of a lanthanide shift reagent but this did not produce sufficient separation of signals for the required measurements to be made, possibly because of the multiple coordination sites available to the shift reagent.

The isolation of (36) in such high yield and from such mild conditions might, it was thought, be important in its implications for peptide chemistry and biochemical processes where tryptophan is a common and important molecule. In order to test the generality of the rearrangement giving (36), the effect of acid on other tryptophan derivatives was investigated. The first system examined was N-acetyl-L-tryptophan methyl ester, (48), which was prepared by Fischer esterification of L-tryptophan[25] followed by Schotten-Baumann acetylation.

Dissolution of compound (48) in trifluoroacetic acid followed by rapid pouring into an excess of saturated bicarbonate and extraction with dichloromethane under identical conditions to the formation of (36) from (23) gave back unchanged starting material. Similarly rapid quenching with solid sodium hydrogen carbonate, pouring into a large volume of water or buffer, or evaporation of the acid under reduced pressure afforded in each case starting material only. However the ^1H n.m.r. spectrum of (48) in trifluoroacetic acid solution indicated that approximately half of (48) had been converted to a compound whose spectrum was consistent with the desired products, the indolines (50a) and (50b), shown in scheme 9.

SCHEME 9



A new peak was observed at τ 4.0 (doublet, J = 6Hz) which was assigned to the methine proton at position 2 by analogy with the spectrum of (36) in trifluoroacetic acid. The acetyl methyl group had split into two peaks and a number of unassignable small multiplets were superimposed upon the signals of the starting material. The methoxy signal remained a singlet but slightly broadened. When the ^{1}H n.m.r. spectrum of L-tryptophan methyl ester was recorded in trifluoroacetic acid and compared with its spectrum in D_2O no change was observed. This indicated that the acid was not strong enough to protonate the indole nucleus to give an indolenine to any noticeable extent and also that the amino function was fully protonated and so unable to quench any trace of indolenine formed. This is consistent with the reported basicity of 3-substituted indoles; skatole requires 9 M. aqueous sulphuric acid before it will protonate completely.[19] The implication of these observations is that it is improbable that the compound whose ^{1}H n.m.r. spectrum was observed when (48) was dissolved in trifluoroacetic acid was the simple protonated indole, the indolenine (49), but rather the predicted products (50a) and (50b).

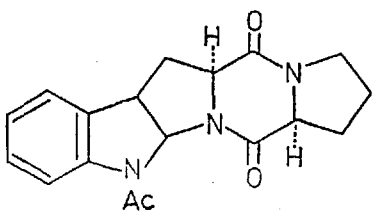
The u.v. spectrum of the tryptophan derivative (48) was recorded in trifluoroacetic acid as was that of the cyclized dioxopiperazine (36).

Unfortunately this acid is not a good u.v. solvent because of its rather high cut-off at approximately 265 n.m. Despite this the spectrum of (36) exhibited a weak maximum at 280 n.m. which is assigned to the protonated form of the indoline. The spectrum of (48) possessed maxima at 287 and 277 n.m. appearing above the solvent cut-off and the latter absorption was overlaid by another band at slightly lower wavelength. The maxima at 287 and 277 n.m. were of the shape and position characteristic of an indole chromophore. These observations are consistent with the tryptophan derivative (48) forming a mixture of the indolines (50a) and (50b) and starting material in trifluoroacetic acid.

The ^{1}H n.m.r. spectrum of compound (48) was also recorded in concentrated sulphuric acid. In this solvent (48) appeared perfectly stable and the spectrum was unchanged after 20 hours. In contrast, in trifluoroacetic acid decomposition was complete after two hours and examination of the mixture indicated a large number of coloured, acid and base soluble compounds whose structures were not investigated. The ^{1}H n.m.r. spectrum of (48) in sulphuric acid showed no evidence for the formation of the indolines (50a) and (50b) but did indicate that a mixture of two new compounds had been formed and that no starting material remained. Thus two acetyl methyl groups of equal intensity were present along with a broadened methoxyl signal. However aqueous work-up of the solution gave back unchanged starting material. 3-Alkyl indoles are known to be fully protonated at position 3 in 18 M. sulphuric acid[19] and comparison of the ^{1}H n.m.r. spectra of skatole and (48) in concentrated sulphuric acid show features in common which suggest that the latter exists in the indolenine form illustrated by structure (49). Presumably cyclization to (50a) and (50b) under these conditions does not occur because the acid conditions are sufficiently strong to protonate the amide function as well, so reducing its nucleophilicity. If this is so then it may explain the

stability of the acetyl tryptophan (48) in sulphuric acid and of tryptophan methyl ester in trifluoroacetic acid; in both cases the amino group is fully protonated and unable to react. The observation of two compounds in the ^{1}H n.m.r. spectrum of (48) in sulphuric acid can be explained if it is assumed that protonation occurs from both sides of the indole nucleus to give (49) as a pair of diastereoisomers.

Since the indolines (50a) and (50b) could be detected but not isolated from acid solutions of (48), attempts were made to trap them in situ. The method chosen was to dissolve the tryptophan derivative (48) in trifluoroacetic acid and add acetic anhydride in the hope that this would acetylate the indoline nitrogen atom of (50a) and (50b), even under the acidic conditions. To check the viability of this scheme, cyclo-L-prolyl-L-tryptophyl, (23), was dissolved in trifluoroacetic acid and acetic anhydride was added. After 10 minutes the reaction was submitted to aqueous work-up and the N-acetyl derivative of (36), compound (51), was isolated along with some other unidentified products. Acetyl indoline (51) could also be prepared in quantitated yield by simply



(51)

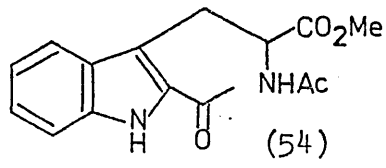
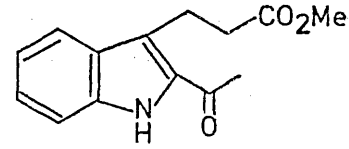
dissolving the indoline (36) in acetic anhydride followed by evaporation of the solvent. The structure of (51) was confirmed by its ^{1}H n.m.r. and ^{13}C n.m.r. spectra. In the former the new acetyl signal appeared at τ 7.35 and the proton at what was previously position 7 of the indole nucleus had experienced a shift down field to τ 1.9. This effect has been reported previously for N-acetylindolines.[27] The methine proton at position 2 had also moved down field to a new position at τ 3.75 but still resonated as a doublet (J = 6Hz). The ^{13}C n.m.r. spectrum of (51)

possessed two more carbon atoms than that of the desacetyl compound (36) and their chemical shifts were consistent with the incorporation of an N-acetyl group.

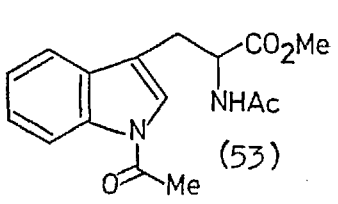
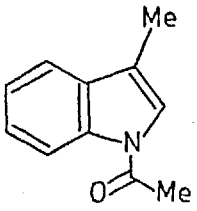
The trapping experiment was then carried out. N-Acetyl-L-tryptophan methyl ester, (48), was dissolved in trifluoroacetic acid and an equal volume of acetic anhydride was immediately added. When after 10 minutes the reaction was worked up, t.l.c. indicated the absence of the starting material and the formation of two major products. The reaction was also performed in an n.m.r. tube; the ^{1}H n.m.r. spectrum of a trifluoroacetic acid solution of (48) was monitored following the addition of a drop of acetic anhydride. Gradual disappearance of starting material and its presumed tautomers (50a) and (50b) was observed along with the formation of the two major products. Isolation of the products by p.l.c. gave a pair of compounds whose ^{1}H n.m.r. and mass spectral data indicated the incorporation of an acetyl group but were not consistent with the desired product, the acetyl indoline (52), which is a known compound[22] previously prepared by acetylation of the indoline (41) with acetic anhydride and pyridine (see scheme 6).

The ^{1}H n.m.r. spectrum of the more polar reaction product exhibited an exchangeable proton at τ 0.6 attributable to an indole NH group, a 5 proton multiplet between τ 2.1 and 3.0 which collapsed to 4 protons following deuterium exchange, a one proton double triplet at τ 5.0 collapsing to a triplet after deuterium exchange and methyl singlets at τ 7.95, 7.35 and 6.30, the last of these superimposed upon a 2 proton doublet. The spectrum is therefore in good agreement with a structure derived from ring acetylation of the starting material. The position of substitution is deduced from the fact that the aromatic proton at position 2 of the indole nucleus,[25] which in the starting material resonates as a doublet at τ 3.15 collapsing to a singlet following

deuterium exchange, is absent in the product. Therefore the more polar product of the reaction is identified as 2,N^b-diacetyl-L-tryptophan methyl ester, (54), and this is confirmed by comparison of its u.v. spectrum with that of 2-acetylindole-3-propionic acid:

	 (54)	
λ max(log ε)	236(4.20) 312(4.27)	235(3.92) 310(4.19)

The less polar reaction product possessed an ^{1}H n.m.r. spectrum similar to that of the starting material. The most noticeable differences between the spectra were the absence in the product of an indolic NH group and the presence of a methyl singlet at τ 7.40 which suggested that acetylation had taken place upon the indole nitrogen atom to give N,N'-diacetyl-L-tryptophan methyl ester, (53). The chemical shifts of the aromatic protons support this assignment; the proton corresponding to position 2 appeared as a singlet at τ 2.65 in the product whereas it was seen at τ 3.1 in the starting material split to a doublet (J = 2.5Hz) by the indole NH group. The lowering of the chemical shift is attributed to inductive and through space interaction with the carbonyl group. Even more pronounced is a peri-peri interaction with the proton in position 7 of (53) which resonated at τ 1.5. A similar effect was observed in the ^{1}H n.m.r. spectrum of the acetyl indoline (51). N-Acetylation was confirmed by comparison of the u.v. spectrum with that of N-acetyl skatole:[26]

	 (53)	
λ max (log ε)	239 (4.26) 262 (3.90) 270 (3.90) 291 (3.83) 299 (3.86)	240 (4.1) - - 290 (3.9) 300 (3.8)

Addition of n-propylamine to the u.v. cell resulted in aminolysis of the acetyl indole and regeneration of the spectrum of N^b-acetyltryptophan methyl ester confirming that it was the indole nitrogen which had undergone acetylation. The product was stable to the addition of acid.

A possible sequence of events explaining the formation of the 1- and 2-acetylated tryptophan derivatives (53) and (54) rather than the indoline (52) is shown in scheme 10. The 2-acetyl indole probably arises by direct electrophilic substitution of the starting material (48). Such reactions are well known; for example treatment of the methyl ester of indole-3-acetic acid with acetyl chloride in the presence of zinc chloride at room temperature gave a 90% yield of the 2-acetyl compound after only 5 minutes reaction.[28] The formation of the 1-acetyl derivative (53) is less easy to rationalize but three routes are possible:

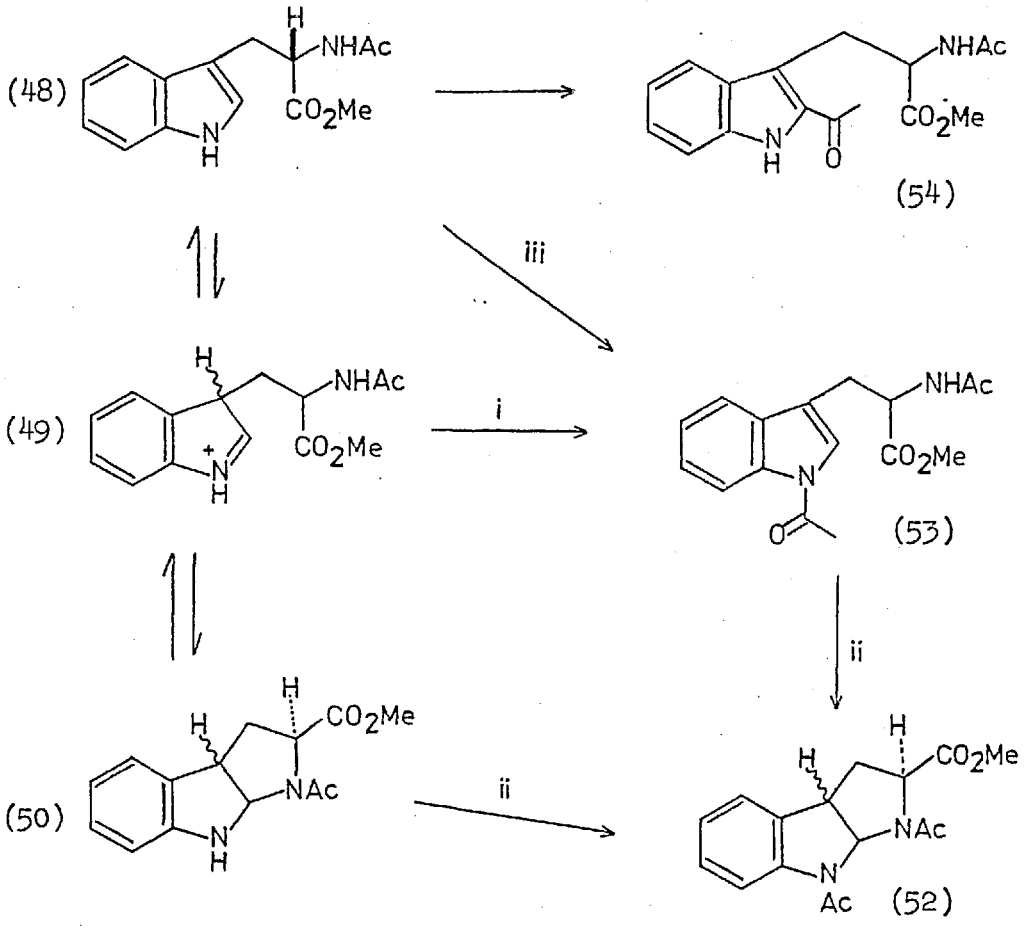
(i) The protonated form of N-acetyltryptophan methyl ester, i.e. (49), is directly acetylated on nitrogen.

(ii) The indoline (50) is acetylated to give (52) which opens up under the acidic conditions to give the isolated product (53).

(iii) Direct N-acetylation of tryptophan derivative (48) occurs.

Route (iii) is ruled out by the fact that under acidic conditions indoles undergo electrophilic substitution at the 2 or 3 positions but

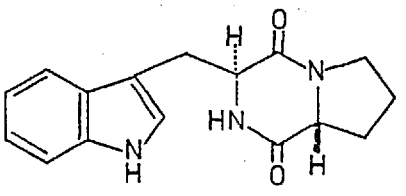
SCHEME 10



not on nitrogen.[29] Route (i) is unlikely because it is known that in trifluoroacetic acid the concentration of the protonated form of 3-alkyl-indoles is very low[19] (for example the ^{1}H n.m.r. spectrum of methyl tryptophanate in trifluoroacetic acid described above exhibits no signs of protonation) and the rate of acetylation of indolenine (49) would not be expected to be vastly greater than that of the indoline (50) which is present in much greater quantity.

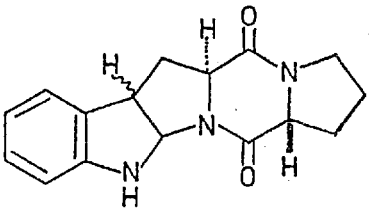
The validity of route (ii) is supported by an experiment carried out with the acetylated derivative of the indoline (36), compound (51). Inspection of the u.v. spectrum of (51) in aqueous acidic ethanol revealed

a gradual change in the observed maxima over a period of several hours. The spectrum of the acetyl indoline was replaced by one similar to that of the 1-acetyl indole (53) and is presumably due to the formation of the 1-acetyl derivative of cyclo-L-prolyl-L-tryptophyl, (23). It would therefore appear that the relative instability of the indoline (50) compared with (36) is repeated in the acetate series.



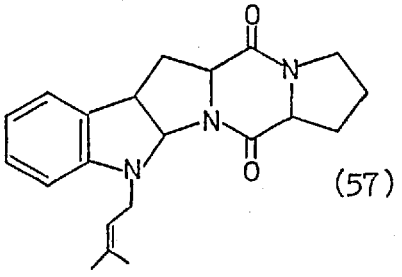
(55)

(55a) = optical antipode

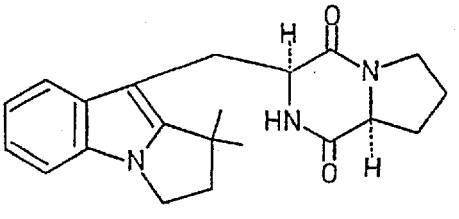


(56)

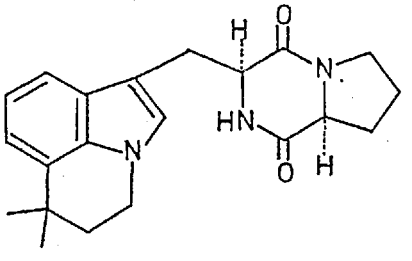
Four other experiments were carried out in the attempt to extend the acid catalysed cyclization reaction. In the first cyclo-D-prolyl-L-tryptophyl, (55), was dissolved in trifluoroacetic acid and the ^{1}H n.m.r. spectrum was recorded. The spectrum contained no evidence for cyclization to give the expected product (56) or even protonation of the indole nucleus of (55) and was very similar to the spectrum of the same compound in deuteriochloroform. The same result was obtained in the second and third experiments when the substituted indoles (58) and



(57)



(58)



(59)

(59) were dissolved in trifluoroacetic acid. No signs of the formation of indolines analogous to (36) or of protonated intermediates analogous to indolenines (49) or (35) were observed in the ^{1}H n.m.r. spectra. In contrast when cyclo-L-prolyl-L-(N^{a}-(3',3'-dimethyl)allyl)tryptophyl, (10), was dissolved in trifluoroacetic acid quantitative conversion to the indoline (57) occurred. Like the indoline (36), compound (57) appeared to be a single diastereoisomer..

Summarising these results; in trifluoroacetic acid:

(a) The cyclo-L-prolyl-L-tryptophyl compounds (23) and (10) are in equilibrium with the indolines (36) and (57) respectively and the position of equilibrium favours the latter compounds to the extent that none of the former is detectable.

(b) The tryptophan derivative (48) is in equilibrium with the indoline (50) and the two compounds are present at equilibrium in a ratio of approximately 1:1.

(c) If cyclo-D-prolyl-L-tryptophyl, (55), is in equilibrium with the indoline (56) then the position of equilibrium favours the former compound to the extent that none of the latter is detectable.

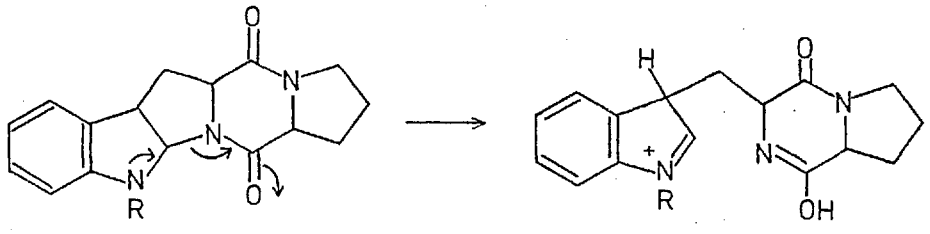
(d) The cyclo-L-prolyl-L-tryptophyl derivatives (58) and (59), like (55) above, do not cyclize to give detectable quantities of the indolines analogous to (56), (57) or (36).

(e) The indolines (36) and (57) are apparently formed as single diastereoisomers.

Possible explanations for these observations will now be considered.

The relative positions of the equilibria between the indolines (57), (36) and (50) and the indoles (10), (23) and (48) respectively can be rationalized by either of two independent lines of reasoning. One is that the opening of the indolines (36) and (57) to give indolenines of the type (35) proceeds via a transition state which involves what is

essentially a cis-elimination as shown in structure (60), whereas the opening of indoline (50) can proceed through a transition state involving a more favourable trans-elimination mechanism as in structure (61). Models show that this argument applies irrespective of which particular diastereoisomer compound (60) happens to be (e.g. structures (46) or (47)). Consequently the transition state for the opening of indolines (36) or (57) is of relatively high energy compared to (50) whilst the transition state energies of the reverse reaction - i.e. ring closure of indolenines (35) or (49) - are not necessarily affected by these considerations.



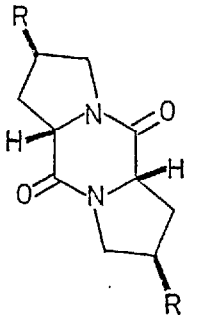
(60) (35)

(61)

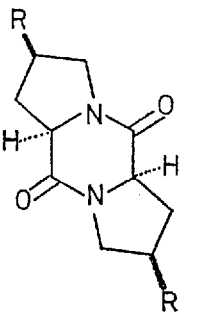
The second line of reasoning relates to the fact that the secondary amide groups of the indolyl dioxopiperazines (10) and (23) are more nucleophilic than the amide group of the N-acetyltryptophan derivative (48). This arises from the constraints forced upon the amide groups by the dioxopiperazine ring which not only holds them in the higher energy cis-conformation[30] but also reduces the planarity of the N—C=O system, decreasing π-overlap and consequently inhibiting delocalization of electron density from the nitrogen atom to the carbonyl group. As a result the secondary amide nitrogen atom in compounds (10) and (23) has more

amino character than the corresponding nitrogen atom in (48) in which the amide function is free to adopt the more stable trans-conformation and enjoys maximum delocalization of the nitrogen lone pair into the carbonyl π-system.

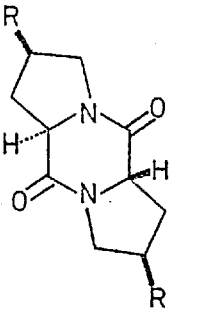
The failure to observe formation of the indoline (56) following acid treatment of cyclo-D-prolyl-L-tryptophyl, (55), may be explained in terms of the instability of the former. It has been reported that cyclo-L-prolyl-L-prolyl, (62), epimerizes very slowly on treatment with aqueous base to give a mixture of starting material and its enantiomer cyclo-D-prolyl-D-prolyl, (63). Despite the fact that for the epimerization to proceed both asymmetric centres must invert, no cyclo-L-prolyl-D-prolyl, (64), or its enantiomer were observed.[32] Similarly when



(62) R = H
(65) R = OH



(63) R = H
(66) R = OH



(64) R = H
(67) R = OH

cyclo-L-prolyl-D-prolyl, (64), was treated with aqueous base it rapidly epimerized to give a racemic mixture of dioxopiperazines (62) and (63). The same is true for the 3-hydroxyproline analogues (65), (66) and (67) which, when treated with base, give a mixture of the diastereoisomers (65) and (66) with no trace of (67) present.[33] The conclusion to be drawn from these facts is that a cis-fused dioxopiperazine such as (62), (63), (65) or (66) is thermodynamically much more stable than the trans-fused compound such as (64) or (67). Models of dioxopiperazines (62)

and (64) reveal the reason for this; in the cis-fused ring system the amide groups are able to adopt a near planar conformation with maximum delocalization of the nitrogen lone pair electrons into the carbonyl π-systems whereas in the trans-fused system the two 5-membered rings force the dioxopiperazine ring into a boat conformation which twists the amide bonds out of planarity. In the formation of indoline (56) from cyclo-D-prolyl-L-tryptophyl, (55), the former incorporates two 5-membered rings fused to the dioxopiperazine ring in the same fashion as in (64) and (67). This high energy arrangement is presumably so unstable relative to the starting material that the equilibrium position of the reaction is pushed right over towards the latter.

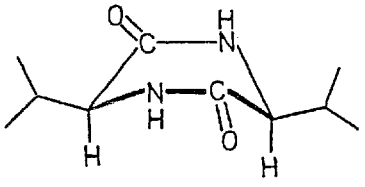
The failure of the pyrrolo-indole derivative (58) to cyclize in trifluoroacetic acid can be reasonably explained on steric grounds. For cyclization to occur the secondary amide group must attack position 2 of the protonated indole nucleus (c.f. structure (107), X = H, p. 166). However position 2 is substituted by what is effectively a tertiary-butyl group which can slow down the rate of ring closure to the point where loss of the acid proton from position 3 occurs much more rapidly. Consequently no indoline formation occurs.

The failure of the pyridino-indole derivative (59) to cyclize in trifluoroacetic acid has not been rationalized. Theoretically it would be expected that in this acid (59) would form an indoline by intramolecular quenching of an indolenine rather more readily than either (10) or (23). This is because an N-alkyl substituent increases the basicity of an indole[19] and also because formation of an indoline would require rehybridization of the indole nitrogen atom from sp^2 to sp^3 so releasing strain in the fused 6-membered ring.

Formation of apparently only one diastereoisomer when indolyl-dioxopiperazines (23) and (10) cyclize in acid to the indolines (36) and

(57) respectively must be due to one of two reasons; either protonation occurs preferentially on one face of the indole molecule or one of the diastereoisomers is thermodynamically more stable than the other.

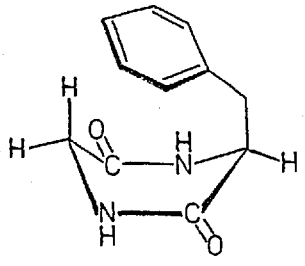
Possible factors producing selective protonation from one side of the molecule can be understood by consideration of the conformation adopted by the dioxopiperazine ring and the indole nucleus. It has been shown that simple dioxopiperazines adopt a planar conformation if possible. This allows maximum planarity of the amide bonds, and hence maximum delocalization of the π-system, along with minimization of the contact between the two amide π-systems across the molecule. Thus cyclo-glycyl-glycyl adopts a planar conformation.[34] The presence of substituents on the dioxopiperazine ring alters this situation. Substitution at positions 2 and 5 results in the adoption of a boat conformation which allows both substituents to be equatorially disposed. This minimizes the steric interaction of the two substituents and at the same time maximizes planarity of the amide bonds. Thus cyclo-L-leucyl-L-leucyl adopts the



(68)

conformation illustrated in structure (68).[35] The introduction of an aromatic ring into the side chain of the dioxopiperazine dramatically changes the conformation. It has been shown that in these cases the aromatic ring folds over the dioxopiperazine nucleus due to an interaction between the amide bonds and the aromatic ring and this produces an enthalpy of stabilization of between 3 and 4 kcal/mol.[36] Thus cyclo-glycyl-histidyl, cyclo-glycyl-phenylalanyl, cyclo-glycyl-tryptophyl, cyclo-glycyl-tyrosyl, cyclo-L-alanyl-L-tyrosyl, cyclo-L-tyrosyl-L-valyl and cyclo-L-leucyl-L-

tyrosyl all adopt a folded conformation.[36] The conformation of the dioxopiperazine ring itself is also affected by the presence of an aryl substituent; in the case of cyclo-glycyl-phenylalanyl the dioxopiperazine ring adopts the most stable boat shape shown in structure (69)[37] whilst in cyclo-L-alanyl-L-phenylalanyl the alanine methyl group causes the dioxopiperazine ring to become planar, as shown in structure (70), because of hindrance between it and the phenyl group.



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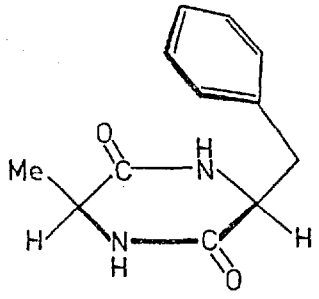
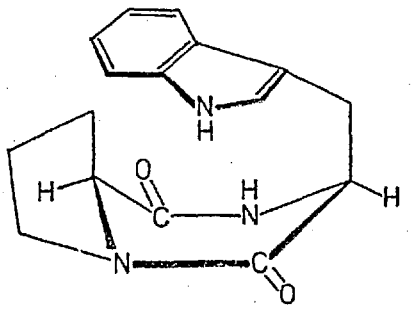


(70)

It was originally thought that this folded conformation might furnish an explanation for the observed selectivity of stereochemistry in the formation of indolines (36) and (57) since such a conformation might result in preferential protonation from one side of the indole nucleus. However this reasoning appears to be invalidated by the finding that aryl dioxopiperazines containing a proline unit are exceptional and do not exist in a folded conformation! The reason for this is that the fused 5-membered ring derived from proline forces the dioxopiperazine ring into a boat conformation and disallows the adoption of a planar one. Consequently the folded form of cyclo-L-prolyl-L-tryptophyl, structure (71), is destabilized due to a steric interaction between the proline ring and the aryl group with the result that the indole nucleus swings away. This effect has been demonstrated by comparison of the ^{1}H n.m.r. spectra of cyclo-L-phenylalanyl-L-prolyl, (72), and its trans isomer cyclo-D-phenylalanyl-L-prolyl. The latter compound is of course free to



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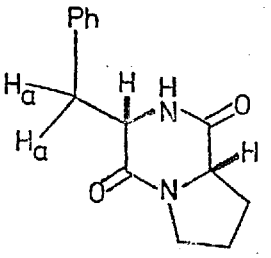
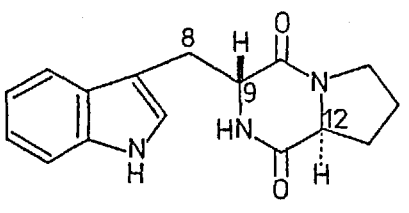


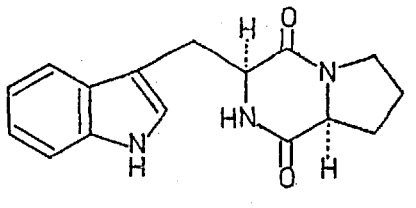
(72)

adopt a folded conformation since no steric interference between the proline ring and the benzene system can occur. The chemical shift of the alanyl methine proton reflects this by appearing approximately 1 p.p.m. to higher field compared with the corresponding proton in the cis dioxopiperazine (72). This shift arises because in the folded conformation the proton points into the aromatic ring and experiences a shielding effect from the ring current. Comparison of the J values arising from coupling between the methylene protons marked a in (72) and the adjacent methine proton confirms that whilst in the trans isomer a folded conformation exists, in the cis isomer - i.e. (72) - the benzene ring has moved away.

The same result has been obtained for cyclo-L-prolyl-L-tryptophyl, (23), and its trans isomer, cyclo-L-prolyl-D-tryptophyl, (55a).[3] In the



(55a)



(23)

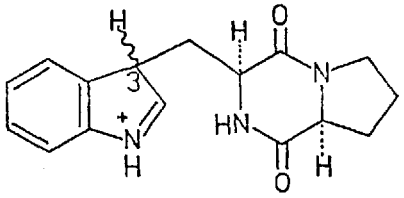
^{1}H n.m.r. spectrum of (23) and (55a) the protons at position 8 constitute the AM part of an AMX system with the adjacent methine proton at position 9

providing the X part. The J_{AX} and J_{MX} values are 4 and 10 Hz for (23) whilst for (55a), which is free to adopt a folded conformation, they are 4 and 6 Hz. Models of (23) demonstrate that the J values are only consistent with a non-folded conformation with the indole nucleus swung away from the dioxopiperazine ring.

Measurement of the relevant coupling constants in all the cyclo-L-prolyl-L-tryptophyl derivatives prepared in the work described in this thesis showed good agreement with the literature results. All cis dioxopiperazines (i.e. those derived from L-amino acids) had corresponding J_{AX} and J_{MX} values close to 4 and 10 Hz whilst the trans dioxopiperazines had values near to 4 and 6 Hz indicating a folded conformation for the latter. Also the methine proton at position 12 consistently appeared approximately 1 p.p.m. higher in the spectra of trans compounds due to the shielding effect of the aromatic nucleus in the folded conformation.

Although the idea that a folded conformation of (23) and (10) might account for the stereospecific formation of the indolines (36) and (57) appears to be untenable, it is possible that a folded conformation could be adopted by the protonated form of these molecules, e.g. structure (35).

(35)



In this form the carbon atom at position 3 is rehybridized to the sp^3 configuration and so allows more freedom for the dioxopiperazine ring to swing over the aromatic nucleus whilst avoiding steric congestion with the proline derived 5-membered ring. The molecule could then enjoy a stabilizing interaction between the π-systems. Furthermore the attraction between the amide π-systems and the benzene ring should be enhanced

because of the increased electron deficiency of the latter. There is also some evidence[36] that in trifluoroacetic acid dioxopiperazine rings become more planar due to protonation of the amide oxygen atoms which serves to increase the contribution of the imino ether structure to the amide molecular orbitals. This has not been studied for proline derived dioxopiperazine rings but obviously a slight reversion to planarity would increase the time spent in a folded conformation.

The alternative explanation for the stereospecific formation of (36) and (57) under acid conditions is that they are the products of an equilibrium reaction and that of the two possible diastereoisomers which can be formed in each case, one is more stable than the other. The degree of stabilization would not have to be large; calculations show that if two possible products are in equilibrium and one is more stable by only 4 kcal/mol. then in the final state that compound will constitute 99.9% of the mixture.[39] Inspection of models of the diastereoisomeric indolines (46) and (47) suggest that (46) may be the more stable product; compound (47) is an almost planar molecule whilst the plane of (46) is curved into a hemisphere allowing contact between the amide π systems and the benzene ring. The ensuing interaction may result in stabilization of this isomer.

In an experiment described below it was shown that the indoline (57) is in equilibrium with the parent indole (10) in trifluoroacetic acid and so exclusive formation of a more stable diastereoisomer is possible. However it has also been found that irreversible reaction of cyclo-L-prolyl-L-tryptophyl derivatives with electrophiles also proceeds with selectivity of attack on one side of the molecule, which suggests that factors such as a folded conformation may also be involved.

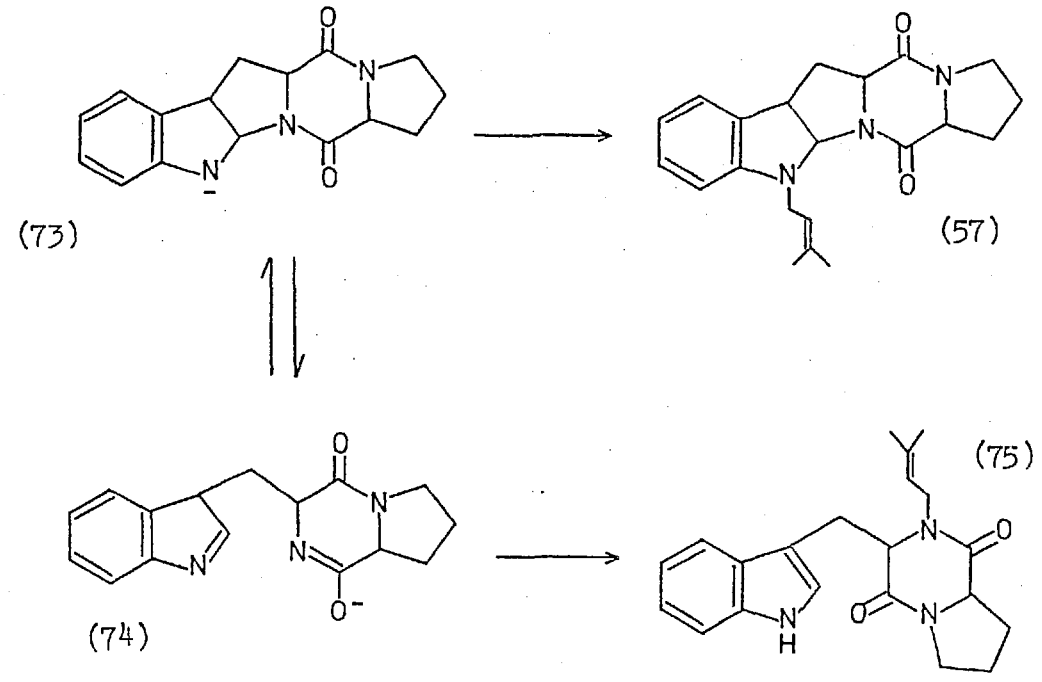
The quantitative formation of the indoline (36) from cyclo-L-prolyl-L-tryptophyl, (23), and its ease of conversion back to the starting material suggested that the cyclic intermediate could furnish an effective

means of protecting the secondary amide function during the synthetic elaboration of compound (23). Also, since the indole nucleus has been converted to an indoline the way is open for substitution of the benzo-ring in positions 5 and 7 which is normally difficult in indole chemistry.

The potential synthetic utility of compound (36) led to the optimization of the reaction conditions for its preparation. Dissolution of cyclo-L-prolyl-L-tryptophyl, (23), in trifluoroacetic acid followed by addition of water and extraction into dichloromethane gave variable conversion and frequently much starting material remained. Attempted reclamation of the acid by distillation under reduced pressure gave compound (36) as a glass which rapidly opened up to give back (23) when a solvent was added, presumably due to the catalytic effect of small amounts of residual trifluoroacetic acid. The most efficient procedure was found to be very slow addition of a trifluoroacetic acid solution of (36) to a well stirred solution of excess aqueous sodium hydrogen carbonate; in this manner quantitative conversion of (23) to (36) was possible.

For the envisaged route to brevianamide A (scheme 1) it was necessary to selectively alkylate the indole nitrogen of the dioxopiperazine (23) and this had led to the attempted preparation of the imino ethers (24) and (25) in order to protect the secondary amide function. The subsequent isolation of the indoline (36) appeared to make this unnecessary and accordingly conditions for the alkylation of (36) with dimethylallyl bromide to give cyclo-L-prolyl-L-(N^a-(3',3'-dimethyl)allyl)tryptophyl, (10), were worked out.

The results described above showed that under mildly acidic conditions indoline (36) slowly reverted to compound (23) and that under basic conditions this occurred more rapidly. Since dimethylallyl bromide is unstable to acidic conditions and the acid catalysed alkylation of amines is difficult it was necessary to attempt the alkylation of compound (36)



under basic conditions. The optimum procedure for this reaction was found to be rapid addition of a solution of (36) in dimethylformamide along with a large excess of dimethylallyl bromide to a well stirred suspension of one equivalent of sodium hydride in dimethylformamide. Under these conditions it was hoped that the anion (73) initially formed would be alkylated to give the indoline (57) before it could open up to give the alternative anion (74). In the event this worked satisfactorily and no cyclo-L-prolyl-L-(N^b-(3',3'-dimethyl)allyl)-tryptophyl, (75), was ever observed as a product of this reaction.

Small scale reactions (i.e. 50mg) afforded two products following p.l.c. and these were identified as the expected compounds, the allyl-indoline (57) and the allylindole (10). On a slightly larger scale (i.e. 1g) an 85% yield of crystalline (10) was obtained following column chromatography and none of its indoline isomer (57). In subsequent reactions the relative amounts of (10) and (57) varied considerably and on one occasion (57) was the major product. The reason for this variation lies in the mode of work-up; the initial product of the reaction is

most probably (57) resulting from straightforward alkylation of the anion (73) and this species is stable under the basic reaction conditions.

During work-up, dichloromethane was commonly used as an extracting solvent and was found to be sufficiently acidic to catalyse the ring opening of the indoline to give the allylindole (10). The acidity of the solvent was also sufficient to induce polymerization of the excess of dimethylallyl bromide present resulting in the crude product mixtures being transformed from a yellow oil into a black tarry gum. Various measures were tried to combat this polymerization and the most effective procedure was found to be rapid work-up using base washed dichloromethane and reduced pressure evaporation of the solvent and the excess of dimethylallyl bromide present. The last stage required warming which served to remove almost all of the bromide and polymerize the rest. During the polymerization process the pH of the mixture became sufficiently low (presumably due to HBr formation) for all of the indoline (57) to be converted to the allylindole (10) so that column chromatography of the black oil obtained gave pure, crystalline (10) only.

On one occasion the crude reaction extract was treated with ammonia gas to remove the residual alkenyl bromide. This worked well except that some tetra(3,3-dimethylallyl)ammonium bromide was formed which proved difficult to remove from the product. It was interesting to find that after this procedure very little of the allylindole (10) was present in the product mixture which was composed mainly of the indoline (57).

In larger scale reactions (10-16g) the combined yields of (10) and (57) were reduced to around 50%; probably this was due to inefficient stirring and local overheating. The by-products were not identified.

The structures of the reaction products (10) and (57) were assigned on the basis of their spectral data. Thus the more polar reaction product was identified as cyclo-L-prolyl-L-(N^a-(3',3'-dimethyl)allyl)tryptophyl,

(10), and possessed an ^{1}H n.m.r. spectrum which differed from that of cyclo-L-prolyl-L-tryptophyl, (23), in not containing an indole NH proton but instead exhibiting signals consistent with a 3,3-dimethylallyl unit bonded to nitrogen. The mass spectrum of compound (10) incorporated the correct molecular ion at 351 mass units and the base peak at 198 mass units corresponded to the characteristic fragmentation of the 8,9 bond[11] to give an indolyl methylene fragment. This fragmentation confirmed that alkylation had occurred on the indole nitrogen atom and not on the dioxopiperazine ring. The u.v. spectrum of compound (10) was that of a typical indole chromophore (maxima at 278, 287 and 292 n.m.) and the band at longest wavelength had become a shoulder which is characteristic of an N-substituted indole.[31] The ^{13}C n.m.r. spectrum of the product was very similar to that of the starting material (23) except that new signals corresponding to the dimethylallyl group were superimposed. The chemical shift of the methylene carbon atom of the new side chain was 43.7 which is consistent with it being bonded to nitrogen.[104]

The structure of the less polar reaction product, indoline (57), was assigned mainly on the basis of its ^{1}H n.m.r. and u.v. spectra by analogy with its precursor, compound (36). In the ^{1}H n.m.r. spectrum no indolic NH resonance was visible and the aromatic protons appeared between τ 2.95 and 3.80 compared with the range τ 2.30-3.10 in the indole (23). This upfield shift reflects the increase in electron density in the benzo-ring of the indole nucleus as it becomes part of an indoline system. A one proton doublet (J = 7Hz) was observed at τ 4.20 corresponding to the methine proton at position 2; the low shift is due to the two flanking nitrogen atoms as in compounds (36), (41),[22] (51)[22] and (52). No deuterium exchangeable protons were present whilst signals corresponding to a 3,3-dimethylallyl unit bonded to nitrogen were observed. The u.v. spectrum was similar to that of the starting material, (36), with maxima at 306 and 256 n.m. (c.f. 297 and 242 in the starting material).

The spectrum was unchanged by addition of base but aqueous acid caused gradual replacement of the spectrum by that of the more polar product, (10), over several hours. The change was not reversed when the solution in the u.v. cell was made alkaline. This observation supports the supposition that compound (57) is the initial alkylation product and that it isomerises to compound (10) under the acidic work-up conditions. The ^{13}C n.m.r. spectrum of (57) was similar to that of the starting material except that, as with (10), signals assignable to a 3,3-dimethylallyl group bonded to nitrogen were present. The ^{13}C n.m.r. spectra of both compounds are considered in more detail below (p. 208) along with those of related compounds.

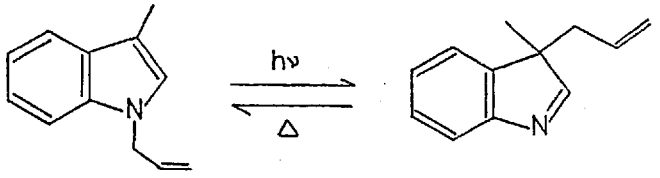
The relationship between compounds (10) and (57) was confirmed by brief treatment of the former with trifluoroacetic acid. When the ^{1}H n.m.r. spectrum of (10) was recorded in trifluoroacetic acid it was, apart from differences attributable to solvent effects, identical with that of (57). Quenching of the solution by treatment with an excess of sodium hydrogen carbonate solution gave a quantitative yield of (57).

Rearrangement of cyclo-L-prolyl-L-(N^a-(3',3'-dimethyl)allyl)tryptophyl, (10)

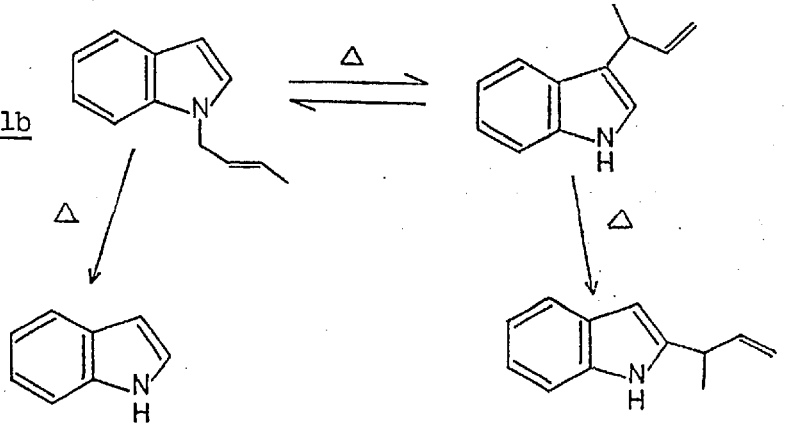
Possible biosynthetic routes leading to the insertion of a 1',1'-dimethylallyl group into position 2 of the indole nucleus in dioxopiperazines derived from L-tryptophan and either L-alanine or L-proline were discussed in the review section. One of the hypotheses proposed was that a 3',3'-dimethylallyl group rearranges into position 2 from the indole nitrogen atom[40] and some model studies to test the viability of this reaction have been reported. The first attempt at this type of rearrangement was in 1965[41] when the isomerization of N-allylskatole under acidic and thermal conditions was studied. No products were isolated from these

reactions and it was reported that "decomposition and polymerization" took place. Since this work the rearrangement of N-allylskatole has been reinvestigated; photolysis under unspecified conditions is said to give 3-allyl-3 methylindolenine which thermally reverts back to the starting material[42] (scheme 11a). In contrast to this result is the report that photolysis of N-crotylindole under a variety of conditions gave no reaction whatsoever whilst thermolysis at temperatures in the region 450°-470°C gave a mixture of indole and 3-(1'-methyl)allylindole as major reaction products. The latter compound was found to be in equilibrium with the starting material under the reaction conditions and also underwent a further irreversible 1,2 shift to give 2-(1'-methyl)-allylindole[43] (scheme 11b).

SCHEME 11a



SCHEME 11b



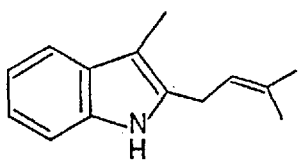
Italian workers have rearranged a variety of 3-alkyl-1-allylindoles under acidic conditions to give the corresponding 3-alkyl-2-allylindole derivatives.[44] These results are summarized in tables 1 and 2.

Table 1

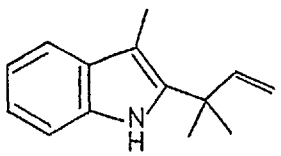
Acid catalysed rearrangement of 1-(3',3'-dimethyl)allylskatole

Acid/ solvent	Temp. (°C)	Reaction time (hr)	Yield*	
			(76)	(77)
T.F.A.	Reflux (70°)	1	70	30
T.F.A.	ambient	24	50	50
T.F.A.	0°C	144	35	65
BF_3Et_2O/Et_2O	ambient	24	50	50
$SnCl_4$/hexane	"	72	65	35
$AlCl_3$/hexane	"	72	75	25

* ratios obtained from g.l.c. trace



(76)

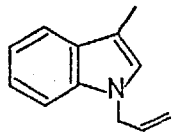
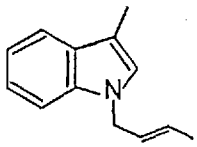
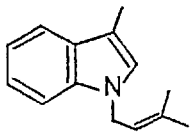
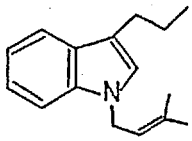
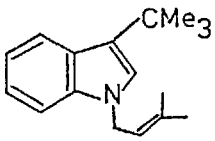


(77)

Most recently the rearrangement of N-allyl and N-trans-crotyl-indole to 3-allyl and 3-(1'-methyl)allylindole with a variety of Lewis acids has been investigated and the results for N-allylindole are summarized in table 3.[45] In the case of N-crotylindole, heating to reflux in benzene for 2 hours in the presence of aluminium chloride gave a 43% isolated yield of 3-(1'-methyl)allylindole.

Table 2

Rearrangement of various substrates in trifluoroacetic acid at room temperature for 48 hours (unless otherwise stated)

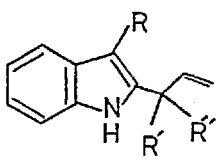
Substrate	Amount recovered* (%)	% Yield of rearranged products* (a)	(b)	% Yield of other products* (unidentified)
	100	none	none	trace
	36	36	14	14
	trace	50	50	trace
ditto (+)	(**)	32	68	trace
ditto (++)	(**)	65	35	trace
	3	45	52	trace
	20	none	10	70

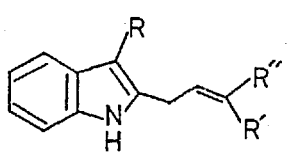
(*) g.l.c. assay

(**) not stated

(+) at 70°C for 1 hour

(++) at 0°C for 7 days

(a) 

(b) 

R = alkyl; R',R'' = H or Me

Table 3

Rearrangement of N-allylindole

Catalyst	Solvent (at reflux)	Reaction time (hr)	% Yield*	
			Starting material	3-allylindole
$ZnCl_2$	benzene	24	100	trace
$ZnCl_2$	tetralin	20	68	14**
$SbCl_5$	benzene	0.5	-	"tarry matter"
$AlCl_3$	benzene	1	15	85
$TiCl_4$	benzene	3	74	26

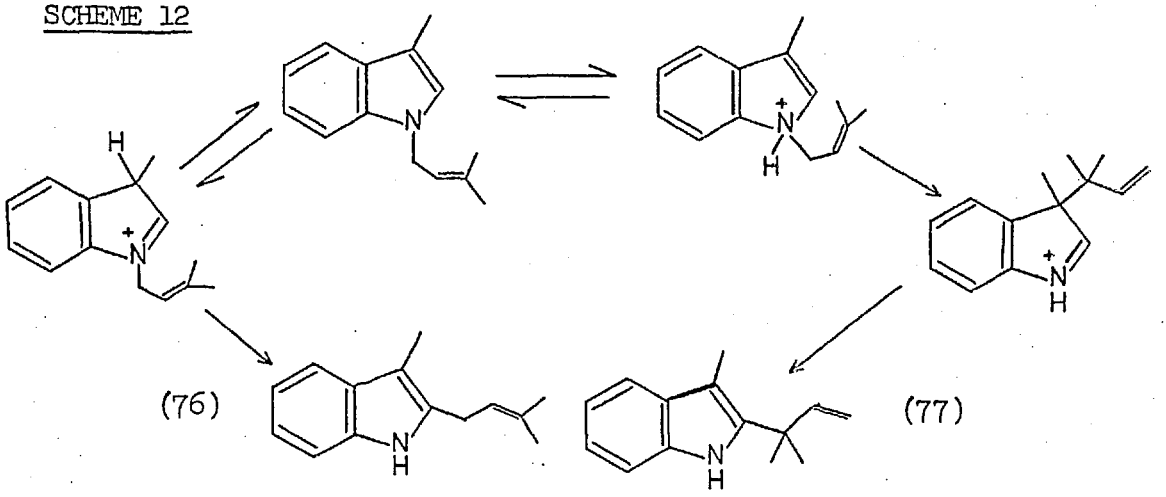
* g.l.c. assay

** also present in the product mixture were indole (4%), 3-propylindole (9%) and unidentified materials (5%)

At the outset of the research described in this thesis only the unsuccessful 1965[41] report and a preliminary account of the Italian work[44a] had appeared.

Despite the high temperatures, the mechanism of the thermal rearrangement of N-allylskatole derivatives was considered to involve a concerted Claisen type rearrangement[43] in order to account for the observed inversion of the allyl group. The acid catalysed rearrangements are rationalised *via* concerted charge induced shifts by analogy with the thermal rearrangements of N-allylanilines in the presence of Lewis acids.[46] The proposed mechanisms are shown in scheme 12. The route leading to the inverted product (77) requires N-protonation followed by a Claisen rearrangement. Some doubt is cast on this mechanism by the demonstration that indoles protonate exclusively at position 3 and not on nitrogen[19] and also by inspection of models which

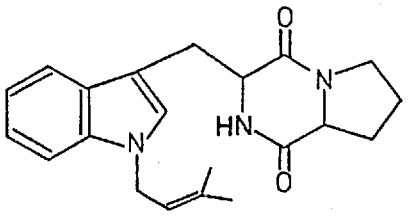
SCHEME 12



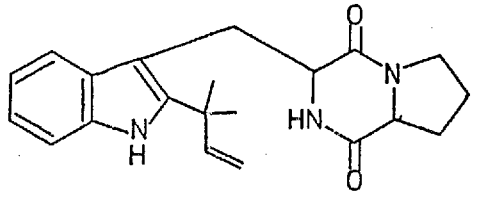
which indicate that even when the indole nitrogen possesses a tetrahedral configuration the termini of the proposed electrocyclic transition state are far apart. The second step of the rearrangement, the 1,2-shift of 3,3'dialkylindolenines under acidic conditions, is well exemplified.[47]

Crossover experiments using isotopically labelled N-allylindoles have been carried out to show that the acid catalysed rearrangements are intramolecular.[44b] Whilst these experiments confirm that the allyl group of rearranged products remains bonded to the original indole nucleus, they do not prove that the reactions are concerted; the rearrangement could proceed *via* initial cleavage of the C-N bond followed by recombination of the ion-pair to give back starting material or the observed products. The work described below suggests that the latter is the case.

The results of the literature work described led to the investigation of the rearrangement of *cyclo*-L-prolyl-L-(N-(3',3'-dimethyl)allyl)-tryptophyl, (10), under various acidic, basic, thermal and photolytic conditions in the hope that this would furnish a biomimetic synthetic route to *cyclo*-L-prolyl-L-(2-(1',1'-dimethyl)allyl)tryptophyl, (5), (*i.e.* desoxybrevianamide E).

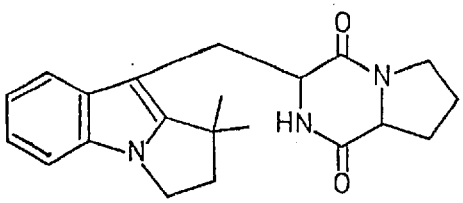


(10)

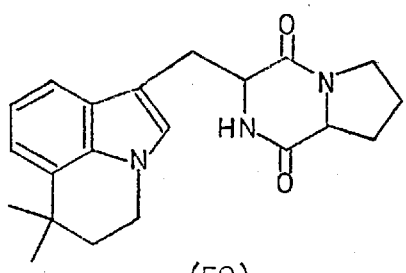


(5)

Treatment of the allylindole (10) with boron trifluoride etherate in dichloromethane, carbon tetrachloride, or in the absence of solvent for periods in the order of twelve hours at room temperature resulted in complete disappearance of starting material and the formation of two major products. On t.l.c. these compounds were slightly more polar than the starting material and travelled very closely in all solvent systems tried and on all adsorbents used (silica, alumina and silver nitrate impregnated silica). Separation was eventually achieved by multiple elution p.l.c. (up to ten "dips") on thin silica plates.



(58)



(59)

The more polar reaction product was identified as the pyrroloindole derivative (58) and the less polar compound as the isomer (59). Both reaction products are seen to result from intramolecular Friedel-Crafts alkylation of the aromatic nucleus. No products bearing the unsaturated allylic side chain or an unsubstituted indole nitrogen atom were detected.

The assignment of structure (58) to the more polar product was made on the basis of its ^{1}H n.m.r. spectrum which indicated the loss

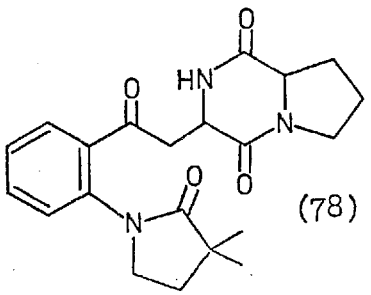
of the vinylic proton and methyl groups of the dimethylallyl group of (10) along with the proton at position 2 of the indole nucleus, which normally resonates as a singlet at approximately τ 3.[48] The methyl groups of the product appeared as singlets at τ 8.34 and 8.42 and the methylene group adjacent to the indole nitrogen atom had moved upfield to τ 6.98 as a result of the removal of unsaturation. No indole NH appeared in the ^{1}H n.m.r. spectrum and this evidence along with the u.v. spectrum, which was typical for an N-substituted indole, suggested that the nitrogen atom was substituted. The mass spectrum possessed the correct molecular ion at 351 mass units with the base peak at 198 mass units, corresponding to characteristic cleavage of the bond between positions 8 and 9 previously observed for dioxopiperazines derived from tryptophan.[11] No fragmentation corresponding to loss of the C_5 unit of the allyl group was detected supporting the conclusion that it had cyclized. Compound (58) was initially isolated as a glass but crystallized from acetone/light petroleum following repeated chromatography.

The less polar reaction product was assigned structure (59) from its ^{1}H n.m.r. spectrum which indicated that whilst only four aromatic protons remained, the proton corresponding to position 2 of the indole nucleus was still present at τ 3.01.[48] The absence of signals corresponding to vinylic protons and the indole NH suggested that the C_5 unit was bonded to the indole nitrogen atom and the benzene ring of the nucleus. The u.v. spectrum supported this conclusion by exhibiting maxima corresponding to the chromophore of an N-substituted indole. Also the mass spectrum was very similar to that of compound (58), again implying that the allyl group had cyclized.

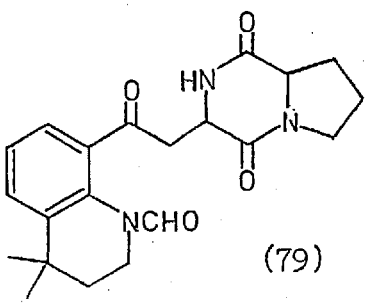
The assignment of the structure (58) to the more polar reaction product and (59) to the less polar compound rather than the other way round was based solely on ^{1}H n.m.r. evidence relating to the absence

or presence of a signal at approximately $\tau 3$, corresponding to the proton at position 2 of the indole nucleus. Since this signal was partially overlaid by those of other aromatic protons, which made interpretation difficult, and because the compound with structure (58) was required for experiments described below further evidence for the structural assignments was sought.

Urk's reagent is an ethanolic acidic solution of p-dimethylamino-benzaldehyde which condenses rapidly with 3-unsubstituted indoles to give mauve, or occasionally yellow, dyes.[49] With 3-substituted-2-unsubstituted indoles colours are also produced but take longer to form and sometimes require heating before doing so. Thus it was found that compound (58) did not give a colour with Urk's reagent whilst compound (59) did so slowly. Given the poor separation of (58) and (59) on t.l.c., this reagent also gave a useful method for assessing the amount of (59) contaminating a sample of (58) by spraying a chromagram of the mixture.



(78)



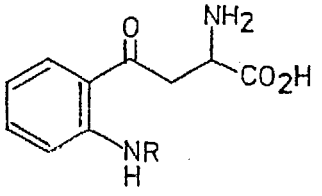
(79)

The correctness of the structural assignments was also confirmed by degradation. Ozonolysis of indoles normally results in cleavage of the bond joining positions 2 and 3 to yield a dicarbonyl compound. Thus ozonolysis of compounds with structures (58) and (59) would be expected to give compounds (78) and (79) respectively. When compounds (58) and (59) were treated with one equivalent of ozone at -78°C followed by reduction with hydrogen in the presence of palladium to destroy the

intermediate ozonides, a single compound was formed in each case. It was found that if more than one equivalent of ozone was used, over-oxidation apparently occurred and mixtures were formed. In the case of the pyrrolo-indole (58) the oxidation product was a glass which could not be induced to crystallize. The ^{1}H n.m.r. spectrum of this material did not contain a resonance near τ 2.0, such as is seen in the spectrum of dimethylformamide[50] and when it was treated with aqueous ethanolic hydrochloric acid for two days no change occurred. In contrast N-formylkynurenine, (80), one of the products obtained by photo-oxidation of tryptophan,[51] hydrolyses with loss of the formyl group to give kynurenine, (81), on standing for 18 hours at room temperature in the presence of 1 M. hydrochloric acid or 0.5 M. sodium hydroxide. The mass spectrum of (78) was also consistent with the assigned structure.

(80) R = CHO

(81) R = H



As expected, the oxidation product of the pyridino-indole (59) exhibited a signal at τ 0.90 in its ^{1}H n.m.r. spectrum corresponding to the formyl proton of compound (79) and ruling out structure (78).

Later experiments, which will be discussed below, showed that singlet oxygen was also capable of affecting the cleavage of the indole nucleus. Thus when oxygen was bubbled through a solution of compounds (58) and (59) in methanol containing methylene blue, under light from a tungsten lamp, clean and quantitative conversion to the o-amino-acetophenone derivatives (78) and (79) occurred. This represents an extremely mild and high yield method for degrading an indole nucleus and, surprisingly, had not been reported in the literature at the time

the experiment was carried out. Since then a paper has appeared recommending the reaction for just this purpose.[53]

Final, unequivocal, confirmation of the structures (58) and (59) and their correct assignment to the two rearrangement products was obtained from the ^{13}C n.m.r. spectra which are considered in a later section (p. 209).

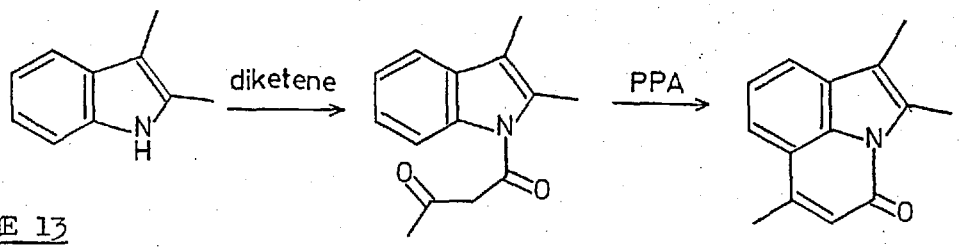
Variation of the reaction conditions for the rearrangement of allylindole (10) in boron trifluoride etherate was also investigated. At lower temperatures (e.g. 0°C) the reaction was much slower. After three days at 0°C t.l.c. of the reaction mixture indicated that all starting material had vanished and the ^{1}H n.m.r. spectrum showed that the only major products were again compounds (58) and (59). However at this lower temperature the proportion of isomer (59) formed was greatly increased. Raising the temperature to 70°C resulting in a faster reaction with all the starting material consumed after only one hour. The major products were still compounds (58) and (59) but now the former was the predominant isomer present. At yet higher temperatures (e.g. 125°C) compounds (58) and (59) were still formed and comprised the major components of the mixture but several other compounds also began to appear in substantial amounts. The structures of these minor products were not rigorously investigated since, from their ^{1}H n.m.r. spectra, none of them possessed vinylic protons and did not appear related to the desired product, the 2-allylindole (5).

It was noticed that the proportions of compounds (58) and (59) formed in different preparations varied according to the state of purity of the boron trifluoride etherate used, all other things being equal. Normally these reactions were carried out under anhydrous conditions using dry solvents and boron trifluoride etherate freshly distilled from calcium hydride but in one reaction where unpurified

boron trifluoride etherate was used the proportion of compound (58) formed was increased. Consequently a series of control reactions were carried out where known quantities of water were added, in the belief that this was the most likely impurity. It was found that as the amount of water added was increased from zero through 1 to 2% (v/v) the proportion of the pyrrolo-indole (58) in the product mixture also increased at the expense of the pyridino-indole (59). When the amount of water present was raised to 5, and even 10%, no further alteration in product ratio was observed. The rate enhancing properties of water and, of course, heat in Friedel-Crafts reactions are well known[56] but the effect upon product ratios observed here is not understood.

It was found that by carrying out the rearrangement reaction in refluxing carbon tetrachloride (70°C) in the presence of 1% water it was possible to reduce the proportion of compound (59) formed to less than 15% leaving its isomer (58) as the only major product. In this way it was possible to isolate the pyrrolo-indole (58) in the pure crystalline state in greater than 50% yield following column chromatography of the crude reaction mixture. The yield could be further increased by careful p.l.c. of the mother liquors followed by crystallization. The difficulty of crystallization of these compounds has already been mentioned; in the case of compound (58) no product could be induced to crystallize from the crude reaction mixture until after it had been chromatographed. Even then crystallization always ceased when the proportion of (58) fell to less than half the mixture and further chromatography was required.

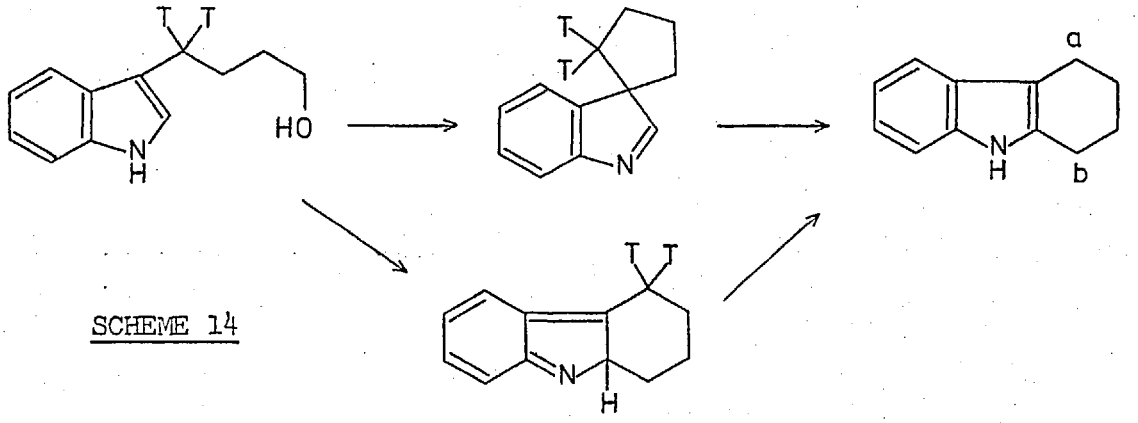
Whilst being disappointing from the point of view of a synthesis of cyclo-L-prolyl-L-[2-(1',1'-dimethyl)allyl]tryptophyl, (5), the formation of compounds (58) and (59) are of some interest because they represent products resulting from direct electrophilic substitution



SCHEME 13

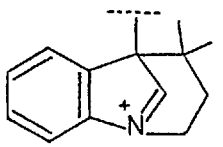
at positions 2 and 7 of the indole nucleus, which are rare reactions. The only examples of direct substitution into position 7 of an indole nucleus involve similar intramolecular electrophilic attack such as shown in scheme 13.[55] In this reaction, substitution occurs solely at position 7 because position 2 is blocked. Direct electrophilic substitution into position 2 of an indole is a contentious reaction; empirically it is found that the most reactive sites for substitution are position 3 closely followed by position 2 with the benzo-ring positions all rather less reactive. It has been proposed that the fast rate of 2-substitution does not reflect the inherent reactivity of this site but rather that of position 3 and that all substitution reactions of the pyrrole ring of the indole nucleus proceed *via* initial substitution at position 3 followed by rearrangement into position 2 if the original site of attack is already occupied.[29] This has been proven for some reactions and an example is shown in scheme 14. In the reaction the tritium atoms of the 4-indolyl-butan-1-ol precursor were found to be distributed equally between positions *a* and *b* in the tetrahydrocarbazole product. If direct 2-substitution occurred, instead of *ipso* attack at position 3 followed by the Wagner-Meerwein rearrangement, then all of the label would of course be located at *a*.[59]

In the Friedel-Crafts cyclization of the allylindole (10) to give compounds (58) and (59), models reveal that initial attack at position 3 is not possible, involving as it does the intermediacy of the σ-complex



SCHEME 14

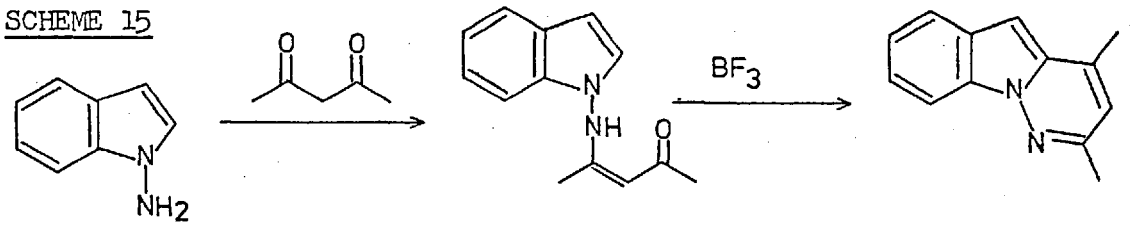
(82) which contradicts Bredt's rule. Thus the pyrrolo-indole (58) must be formed by direct substitution at position 2 and this conclusion is supported by the isolation of approximately equal amounts of compounds (58) and (59) which indicates that positions 2 and 7 are of equal reactivity. Normal intermolecular electrophilic substitution where initial ipso-attack at position 3 can occur would yield only the 2-substituted product.



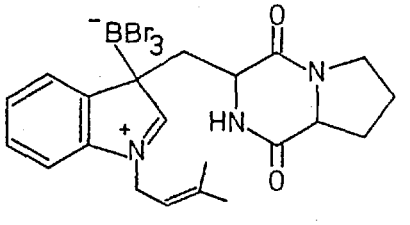
(82)

The formation of the pyrrolo-indole (58) from the allylindole (10) represents an unequivocal case of direct 2-substitution of an indole nucleus. The only other examples of this known to the writer are the Friedel-Crafts acylation of indoles,[28] an intramolecular example of which is shown in scheme 15.[57]

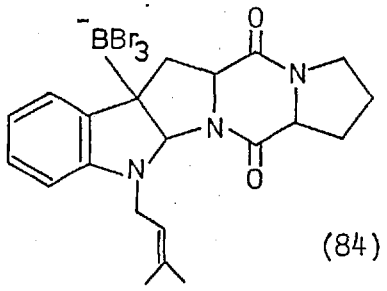
SCHEME 15



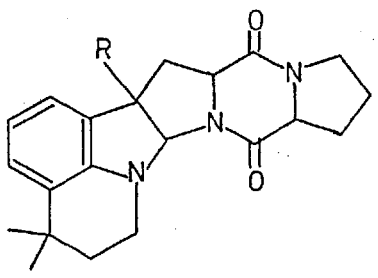
Following the failure of the rearrangement of the N-allylindole (10) to give the desired product, the 2-allylindole (5), in the presence of boron trifluoride etherate, alternative Lewis acids were considered. The first alternative used was boron tribromide, a much stronger Lewis acid than boron trifluoride.[54] Treatment of a dichloromethane solution of compound (10) with boron tribromide for 30 hours gave a single major product along with numerous more polar by-products. The major product was isolated by p.l.c. and found to be compound (59) and no trace of its isomer (58) was detected. The most rational explanation of this result is that Friedel-Crafts alkylation is again occurring but that here boron tribromide is a sufficiently strong Lewis acid to complex position 3 of the indole as well as the allylic double bond. In the case of boron trifluoride, the Lewis acid is weaker and can only complex the olefin. As a result the reacting species with a boron tribromide is the complex (83) or possibly even the cyclized form (84) in which the new indoline nitrogen atom would also be complexed to boron. Cyclization would then give structure (85) which on work-up gives the observed product, compound (59), possibly <u>via</u> the cyclized form (86). The latter is known to be



(83)



(84)



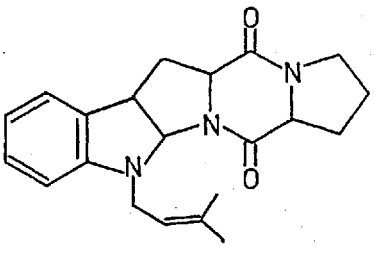
(85) R = BBr_3

(86) R = H

unstable (c.f. its non-formation in trifluoroacetic acid, p.121) and would be expected to open immediately to give (59). Further evidence for this route is the reaction rate, which is much slower than the boron trifluoride catalysed rearrangement even though boron tribromide is a much stronger Lewis acid; in the reaction with boron tribromide cyclization is occurring into an electron deficient benzene ring (deficient because of coordination to the Lewis acid) whereas in the boron trifluoride reaction substitution occurs more rapidly into the free indole nucleus.

Partial confirmation of this rationalization was obtained from two experiments. In the first a u.v. spectrum of the allylindole (10) was recorded in boron trifluoride etherate; this indicated that the indole chromophore was intact and therefore that the boron trifluoride was not strong enough to coordinate position 3 of the indole to any great extent to give an indolenine structure like (83), or to induce cyclization to give an indoline analogous to compound (84). It was not possible to record a spectrum of compound (10) in boron tribromide because of the highly corrosive nature of the solution. In the second experiment the rearrangement of the N-allylindoline (57) in boron trifluoride etherate was investigated. Room temperature rearrangement of the allylindole (10) in anhydrous boron trifluoride etherate gave a mixture of compounds (58) and (59) in the ratio 3:2 approximately.

(57)



Rearrangement of allylindoline (57) under identical conditions but for 24 hours instead of 18 gave a mixture of compounds (10), (58), and (59) in the ratio 3:4:5. None of the starting material was detected. The

product ratio is rationalized as follows: allylindoline (57) is very slowly converted to the pyridino-indoline (86) which opens to the more stable indole (59); concurrently (57) is also slowly opening up to give the allylindole (10) which can cyclize relatively rapidly to give a mixture of compounds (58) and (59). The isolation of the indole (10) from the product mixture requires, according to this scheme, that the opening of the indoline (57) is slow in boron trifluoride etherate and that residual (57) is lost during work-up by opening to (10). The latter transformation was observed in the preparation of (10) *via* (57) where the acidity of commercial dichloromethane was found to be sufficient. What this experiment demonstrates is that the rate of Lewis acid catalysed Friedel-Crafts cyclization of allylindoline (57) to give compound (85) is slow, supporting the intermediacy of structures (83) or (84) in the boron tribromide catalysed reaction.

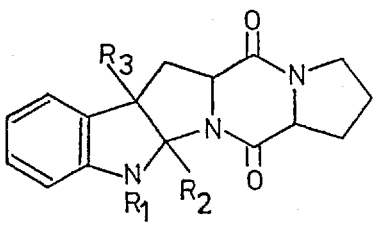
The rearrangement of *cyclo*-L-prolyl-L-(N-(3',3'-dimethyl)allyl) tryptophyl, (10), in the presence of stannic chloride was also investigated. Addition of stannic chloride to a solution of (10) in dichloromethane resulted in the immediate precipitation of a white complex. This complex was insoluble and stable in water and in dilute or concentrated hydrochloric acid but slowly decomposed in aqueous sodium hydrogen carbonate solution to give carbon dioxide, a heavy white precipitate (presumably stannic oxide) and extractable organic material.

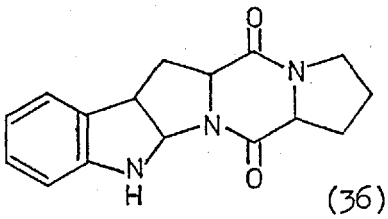
Samples of the complex removed shortly after the start of the reaction and destroyed with sodium hydrogen carbonate gave back organic material which was found to be identical with the starting material. If the complex was left to stir in dichloromethane for several hours before destruction it yielded a mixture of organic compounds which contained no starting material. The relative proportions of the

products formed appeared unchanged by refluxing the complex in dichloromethane or chloroform.

Separation of the mixture by p.l.c. revealed that the two major products of the reaction were identical with compounds (58) and (59). The only other compound present in substantial amount was an isomeric substance (mass spectrum) travelling much faster on t.l.c. than either (58) or (59). The ^{1}H n.m.r. spectrum of this material exhibited no indole N-H proton or vinylic signals, revealing that the product did not contain a "rearranged" allyl group. Also absent from the spectrum was the proton in position 2 of the indole nucleus of the starting material and the four aromatic protons present resonated between τ 2.80 and 3.55, reminiscent of the cyclized structures (36) and (57). The u.v. spectrum of the new product was also very similar to those of (36) and (57), suggesting a part structure (87). The proton corresponding to R^2 in structure (87) and resonating at approximately τ 4.0 in compounds (36) and (57) was absent from the ^{1}H n.m.r. spectrum suggesting substitution at this position. The presence of a single readily

(87)



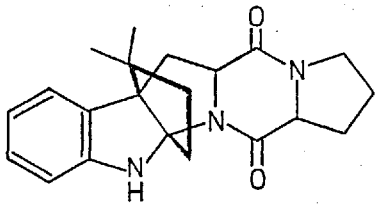


(36)

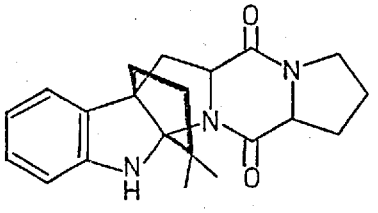
exchangeable proton at τ 4.5 indicated that the N-atom was unsubstituted and this was confirmed by the i.r. spectrum.

These observations clearly indicated that the five carbon allylic group of compound (10) had left the nitrogen atom and become fixed to position 2 of the part structure (87). The absence of vinyl signals in the ^{1}H n.m.r. spectrum also suggested that the other end of the allyl group was fixed and the most reasonable position appears to be

position 3 of what was the indole nucleus. On this basis structures (88) or (89) can be assigned to this product. The u.v. spectrum of the new compound whilst similar in appearance to (36) and (57) was unaffected by addition of acid or base indicating that it is not free to rearrange to an indole; this is consistent with the presence of two



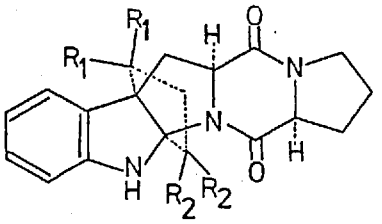
(88)



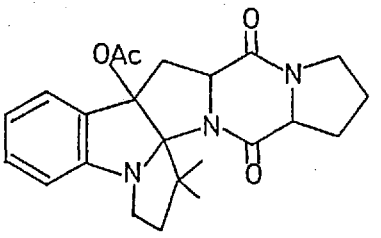
(89)

substituents in position 3. In the ^{1}H n.m.r. spectrum, the methyl group of the product resonated at τ 8.85 and 9.30; models of both (88) and (89) show that the molecules are extremely rigid and that in both cases the two methyl groups are each held in different environments. Thus in (89) one of the methyl groups points into the π-system of the amide bond whilst in (88) one of the methyl groups point into the π-system of the benzene ring. In both cases the methyl group occupies an area of space corresponding to the shielding region of the π-systems and so both structures can account for the high chemical shift of the upfield methyl group. The ^{13}C-spectrum was also recorded (vide infra p.211) but afforded no clue to distinguish between the two possible structures. Mechanistic considerations discussed below suggest that the more likely structure is (88) but two observations imply that the correct structure might be (89). The first of these was the complete failure of the rearrangement product to undergo acetylation when treated with acetic anhydride under identical conditions used for preparation of the acetylindoline (51) from the indoline (36). This is attributable to steric hindrance in structure (89). The second observation is the similarity between the ^{1}H n.m.r. spectra of the product and compound

(115) which was isolated from the oxidation products of compound (58) (p.173). The methyl groups of compound (115) resonate at τ 8.44 and 9.03 and a model shows a very close similarity in the environment of the relevant methyl groups to those of (89). Deshielding by the acetoxy group in (115) could account for the down field shifts relative to (89).



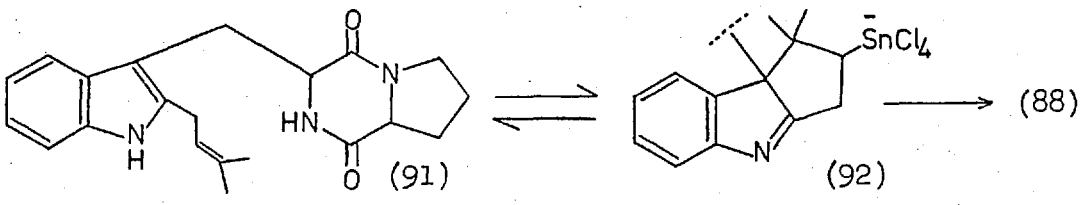
(90)



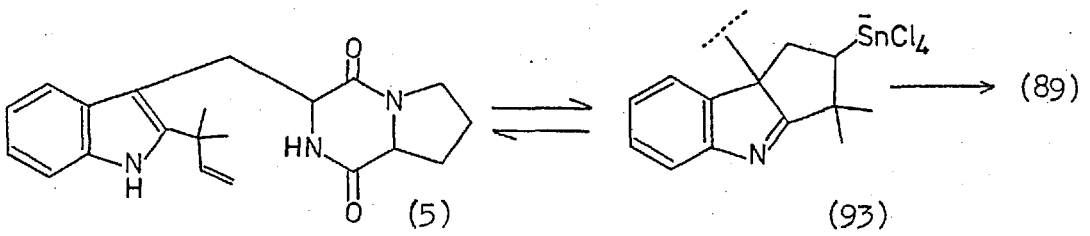
(115)

As with compounds (36), (57) and (51), no evidence could be found for the presence of two stereoisomers in the product assigned structures (88) or (89). By analogy, the new fused 5-membered ring may be either cis or trans to the cis-fused dioxopiperazine ring. Applying similar arguments to those used for compounds (36) and (57), the all cis-structure (90) is preferred since here the plane of the molecule is curved into a hemisphere allowing contact between the aromatic π -system and the amide π-systems. This conclusion rests upon the assumption that the reaction is an equilibrium and allows formation of the most stable product. The formation of compound (88) or (89) can be rationalized by schemes 16 or 17 respectively; if these reactions are equilibria (i.e. if the intermediate Lewis acid stabilized anions (92) and (93) are not quenched until work-up) then the product would be expected to possess the stereochemistry shown in structure (90). If the reactions are not equilibria then the fact that the isolated product is apparently a single isomer requires that electrophilic attack by the allylic double bond of (5) or (91) is occurring preferentially on one side of the indole nucleus. Evidence that the reaction is an equilibrium is supplied by the model studies quoted at the beginning of this section. These

SCHEME 16



SCHEME 17



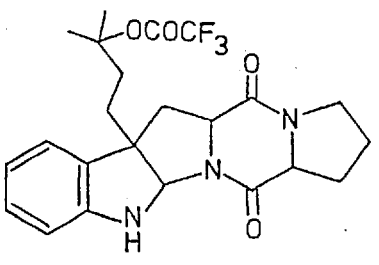
demonstrated that N-dimethylallylindoles rearrange in the presence of stannic chloride to give 2-isopentenylindoles which were stable in the reaction medium. Therefore it must be presumed that the intermediates corresponding to (92) or (93) can be formed but collapse back to the isolated product because no side chain is present to trap them.

The formation of a compound with structure (88) or (89) was of no use for the preparation of desoxybrevianamide E, (5). However it did suggest that the allyl group of compound (10) could migrate as well as undergo Friedel-Crafts cyclizations because of the necessity to invoke the desired products (5) or (91) as intermediates.

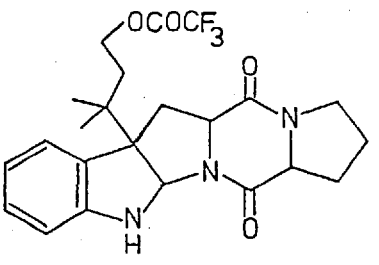
The rearrangement of cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)-tryptophyl, (10), in trifluoroacetic acid was next investigated. Dissolution of compound (10) in trifluoroacetic acid followed by immediate work-up gave a quantitative yield of the indoline (57) as described above. Prolonged stirring of the solution until compounds (10) and (57) had disappeared followed by aqueous work-up gave a complex mixture of compounds which was shown to include the pyrrolo-indole (58) as the

major product. Three other products were present which on t.l.c. travelled very close together and close to (58). These compounds were not separated and the ^{1}H n.m.r. spectrum of the mixture indicated the absence of vinyl protons in all of them. This, along with the chemical shifts of the methyl groups, ruled out the presence of the desired product, (5), in the mixture.

A fifth major product of the reaction was present which was much less polar on t.l.c. than the rest. Isolation of this material gave a glass which was shown to be contaminated with a small amount of the compound assigned structures (88) or (89). The u.v. spectrum contained the same chromophore as compounds (36), (57) and (88) (or (89)) suggesting that the product might have arisen from a similar intramolecular cyclization. This was supported by the ^{1}H n.m.r. spectrum; thus the proton corresponding to position 2 appeared as a singlet at τ 4.75 and four aromatic protons resonated between τ 2.80 and 3.60. The multiplicity of the signal at τ 4.75 implies that position 3 is substituted and this was confirmed by the observation of no irreversible change in the u.v. spectrum when acid or base was added to the u.v. cell. A single exchangeable proton was observed at τ 4.80 which was assigned to the



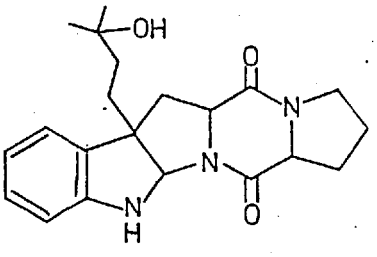
(94)



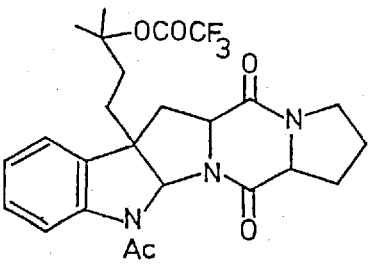
(95)

indoline NH group and two methyl groups appeared as a sharp singlet at τ 8.52. The mass spectrum of the new substance appeared to exhibit a molecular ion at 351 mass units, isomeric with starting material. However the i.r. spectrum possessed carbonyl stretching absorptions at 1665 cm^{-1} and 1780 cm^{-1}; the latter signal is typical for a trifluoro-

acetic acid ester[60] and led to the assignment of structure (94) to the reaction product. The ^{13}C spectrum is in agreement with this (see p.211). A possible alternative structure, (95), was ruled out by the absence of a signal in the ^{1}H n.m.r. spectrum corresponding to the methylene group adjacent to the ester oxygen. The absence of a molecular ion peak in the mass spectrum is presumably due to ready loss of trifluoroacetic acid.



(96)



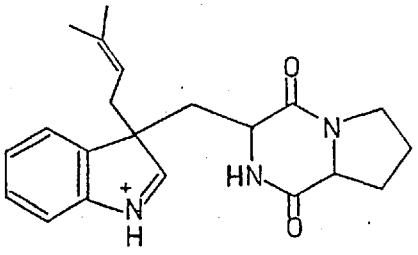
(98)

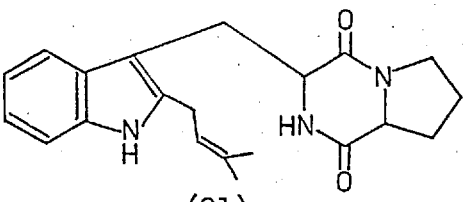
Treatment of the trifluoroacetate (94) with acetic anhydride under identical conditions to those used for acetylation of the indoline (36) gave a single, unstable compound whose ^{1}H n.m.r. spectrum was in good agreement with structure (98). Thus the singlet due to the proton in position 2 had moved downfield to τ 4.10 and the proton in position 7 had moved down to τ 1.90, whilst a methyl singlet appeared at τ 7.35 confirming acetylation of the unhindered indoline nitrogen atom.

Compound (94) was an unstable compound and readily hydrolysed during attempts to crystallize it; it was also found impossible to separate by p.l.c. the small amount of compound (88) or (89) present. Accordingly (94) was hydrolysed to the alcohol (96), which, being more polar and stable, was more readily purified. Treatment of compound (94) with sodium hydrogen carbonate in aqueous tetrahydrofuran gave clean hydrolysis to the alcohol (96): this differed from its precursor only in the presence of a new exchangeable proton in the ^{1}H n.m.r. spectrum at τ 6.40 and in the absence of the carbonyl stretch at 1780 cm^{-1} in its i.r. spectrum. The mass spectrum of the alcohol (96)

gave the expected molecular ion at 369 mass units. Unfortunately (96) also failed to crystallize although it readily gave a powder on trituration with ether and it was also unstable, turning purple on standing. It is not known if this is an oxidative, photolytic or thermal process.

(97)





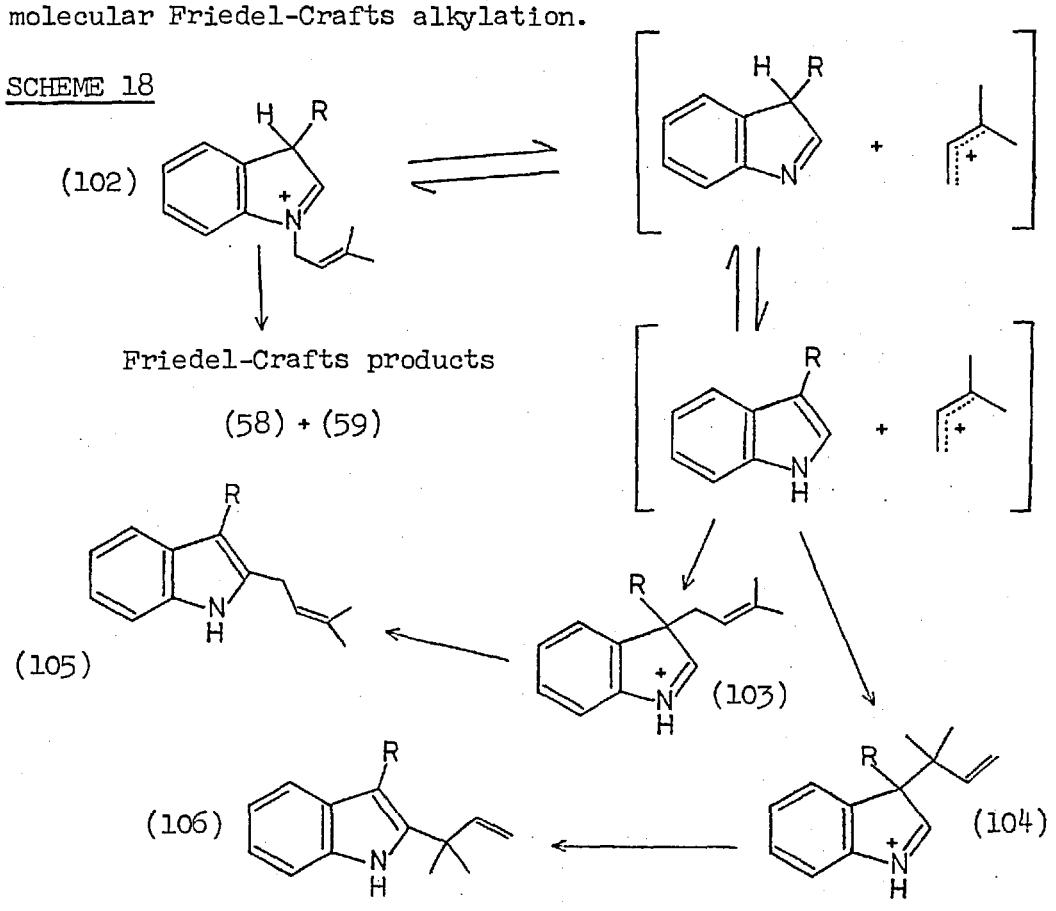
(91)

Formation of compound (94) is of mechanistic interest since it suggests a non-concerted rearrangement pathway. The most reasonable route to (94) is *via* initial cleavage of the allyl group followed by recombination either at nitrogen, to regenerate starting material, or at the electron-rich position 3 to give the intermediate (97). Ring closure by attack of the dioxopiperazine nitrogen atom on the protonated indolenine of (97) and addition of trifluoroacetic acid across the double bond of the allylic group gives the observed product. Indolenines disubstituted in position 3 are known to rearrange readily to 2,3-disubstituted indoles with migration of the most electron rich substituent.[47] In the case of the intermediate (97), the dioxopiperazine ring traps the indolenine and inhibits the rearrangement but in its absence the expected product would be (91). The addition of trifluoroacetic acid across the double bond may also contribute towards the trapping of compound (94); in its absence (91) might form and react further to give the stannic chloride rearrangement product (88) *via* (92) as shown in scheme 16.

It has already been mentioned (p. 139) that the proposed mechanism of the rearrangement of N-allylindoles to 2-allylindoles is *via* a 1,2-shift. The isolation of compound (94) suggests that the correct mechanism may be non-concerted and may proceed through structures analogous

to (97). Such a mechanism would explain some of the product ratios observed when N-dimethylallyl-3-alkylindoles are rearranged in acid. In table 2 it was seen that increasing the bulk of the 3-alkyl-substituent resulted in a decrease in the proportion of product bearing an inverted allyl group. In the case of 3-t-butyl-N-dimethylallylindole, no 3-t-butyl-2-(1',1'-dimethyl)allylindole was isolated whilst only a low yield of 3-t-butyl-2(3',3'-dimethyl)allylindole was obtained. Apart from starting material, the other products were not isolated but were reported as being "saturated products" - possibly resulting from intramolecular Friedel-Crafts alkylation.

SCHEME 18



The proposed rationalization for these published results and the results reported in this thesis is shown in scheme (18). Protonation of the N-allylindole (102) results in cleavage of the allyl group which can recombine on nitrogen or at position 3 at similar rates. Simultaneously, but more slowly, Friedel-Crafts cyclization of (102) can

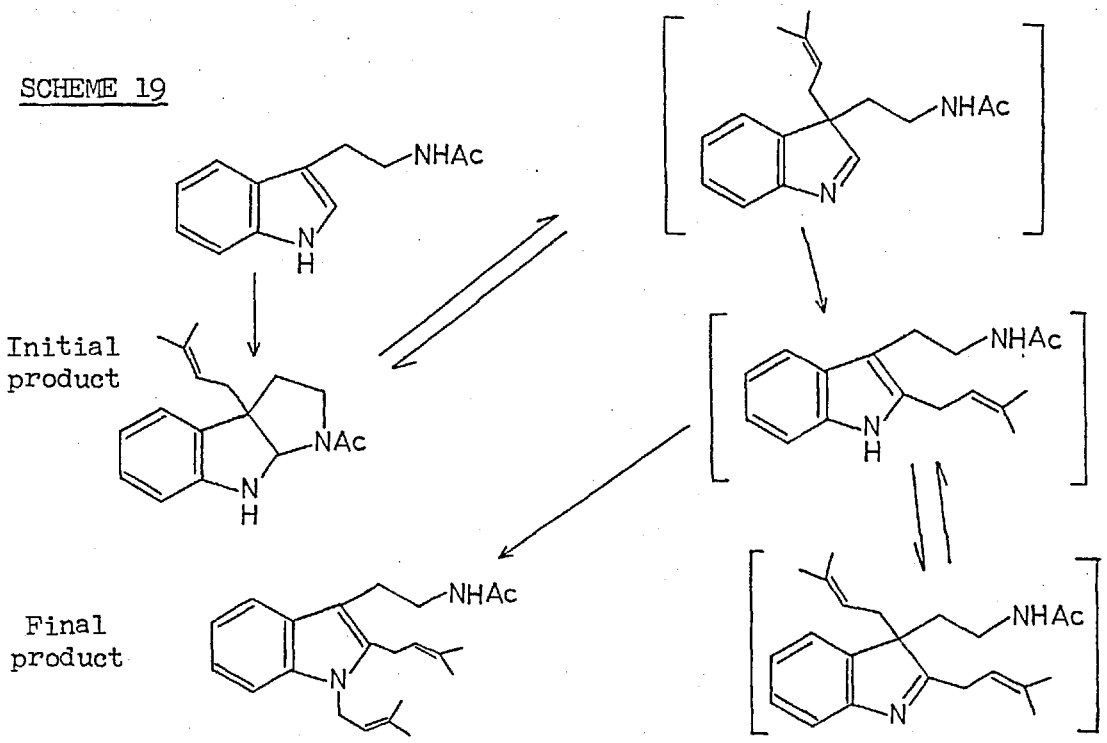
occur at positions 2 or 7. Following cleavage, recombination at position 3 gives the indolenines (103) and (104), the rate declining as the size of R is increased. Increasing the size of R also reduces the proportion of indolenine (104) formed so that after rearrangement compound (105) predominates over its isomer (106). When R is a dioxopiperazine ring, intramolecular trapping of the indolenines can occur at any stage - e.g. in (103) or (104). The isolation of compound (94) and none of its isomer (95) demonstrates how hindrance of the allyl group by the 3-substituent controls the direction of recombination as in the case of 3-t-butylindoles.

These mechanistic proposals suggest that the least polar stannic chloride rearrangement product should possess the structure (89) rather than (88) since these products are probably derived from the intermediates (91) or (5) corresponding to (105) and (106) in scheme (18). Thus steric hindrance by the dioxopiperazine ring during recombination should inhibit formation of (5) relative to (91).

Finally two other comments can be made. Firstly, the formation of the pyrrolo-indole (58) confirms that the indoline (57) must be in equilibrium with the allylindole (10) in trifluoroacetic acid. Therefore it is probably that (57) exists in the more stable diastereoisomeric form, postulated as structure (46) on p.130. Secondly the spectral and chromatographic data for compounds (94) and (96) indicate that they are single diastereoisomers. This implies that if the mechanism proposed in scheme (18) is correct then recombination of the allyl group occurs preferentially from one face of the molecule. The reason for this selectivity is best attributed to a semi-folded conformation of the indolyl dioxopiperazine. The conformations of dioxopiperazines bearing an aryl substituent were discussed on p.127 where it was concluded that cyclo-L-prolyl-L-tryptophyl derivatives

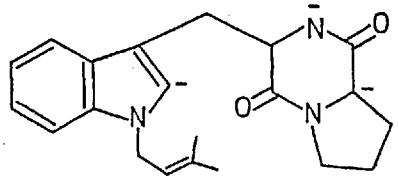
do not exist in a fully folded conformation but that the indole nucleus is swung slightly away from the dioxopiperazine ring due to hindrance by the proline system. The coupling constants of the methylene protons in position 8 of these compounds are consistent with such a partially folded conformation with one amide π-system in contact with the aromatic nucleus.

SCHEME 19



The formation of the trifluoroacetate (94) is analogous to the behaviour of N-acetyltryptamine with dimethylallyl bromide in sodium acetate buffered aqueous acetic acid.[58] In the reaction, which is illustrated in scheme 19, substitution in position 3 of N-acetyltryptamine by a dimethylallyl group leads initially to an indoline analogous to compound (94). Under the reaction conditions the initial product is in equilibrium with its presumed precursor, the 3-dimethylallylindolenine, which can undergo a Wagner-Meerwein rearrangement to a 2-dimethylallylindole. Since both positions 2 and 3 of this compound are substituted, further substitution can only occur upon the nitrogen atom to give the observed final product.

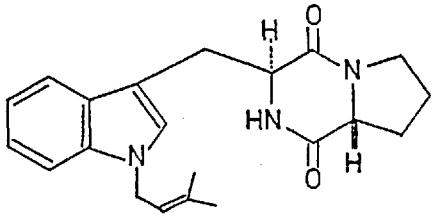
The rearrangement of cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)-tryptophyl, (10), in the presence of zinc chloride and aluminium chloride was also investigated. With freshly sublimed aluminium chloride in dichloromethane, no reaction occurred after eight days. With zinc chloride in dichloromethane a very slow reaction took place at room temperature which was accelerated by heating. After prolonged refluxing in chloroform, all of the starting material was consumed and a mixture of three products identical to that obtained with stannic chloride, was formed and in approximately the same ratios.



(99)

The rearrangement of compound (10) in the presence of base was next investigated. It has been shown that the proton at position 2 of indoles is acidic[61] and it was thought that treatment of (10) with a sufficiently strong base might generate the trianion (99) which could rearrange to the 2-allylindole (5) and its epimer around position 12. The driving force for this reaction is the formation of a less basic product. Treatment of a tetrahydrofuran solution of compound (10) with slightly more than three equivalents of butyl lithium at 0°C gave an orange solution. T.l.c. indicated that after 30 minutes little starting material remained and a complex mixture of more polar products had formed. The mixture was left for 18 hours at room temperature after which time almost all of the starting material had disappeared. P.l.c. of the product allowed isolation of the only major component, which was isolated in 26% yield. The spectral data of this substance were consistent with the structure (100) although the possibility of

the presence of its enantiomer is not ruled out. The p.m.r. spectrum of compound (100) was similar to that of compound (10) except that the proton at position 12 had moved upfield by approximately 1 p.p.m. Furthermore, the proton at position 9 had changed from a double doublet (J = 4,14 Hz) to a broad triplet (J = ca 4 Hz). These two changes are in accord with epimerization at the 12-position which allows the molecule to adopt a fully folded conformation in which the proton in position 12 points into the shielding sector of the aromatic nucleus and the dihedral angles between the protons at positions 8 and 9 are approximately 60°. The i.r., u.v. and mass spectra of compound (100) were all very similar to those of the starting material but the polarity on t.l.c. was very much greater. This effect is general for dioxopiperazines;[62] with very few exceptions trans-dioxopiperazines travel slower on g.l.c. or t.l.c. than their cis-isomers.

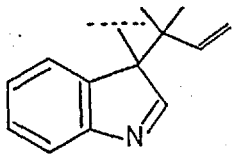


(100)

The failure to observe any rearrangement products from this reaction may be due to non-formation of the 2-lithioindole salt or possibly the transition state energy for the rearrangement is too high. Models of the trianion (99) do in fact show that the degree of overlap between the anion orbital and the olefin π-system is small. In view of the instability of the system to base at room temperature, no attempt was made to heat the trianion.

Photolytic and thermal rearrangements of the N-allylindole (10) were also studied. The concerted [3,3] shift of the N-allyl group into

positions 7 and 3 of the indole nucleus are both theoretically thermally allowed processes whilst the [2,3] rearrangement into position 2 is a photo-induced reaction. Therefore one of the expected products of a concerted thermal rearrangement of compound (10) would be the 3,3-disubstituted indolenine (101). Such structures are known to rearrange under thermal[43] and acidic[47] conditions via a Wagner-Meerwein type shift to give, in this case, desoxybrevianamide E, (5).

(101) 

When compound (10) was heated in refluxing decalin (190°C) for 22 hours unchanged starting material was recovered. However when refluxing xylene was used as solvent some epimerization at position 12 to give compound (100) was observed after $2\frac{1}{2}$ hours. This was assumed to result from basic impurities in the xylene which had previously been dried with sodium. Refluxing compound (10) for $1\frac{1}{2}$ hours in ethylene glycol gave complete equilibration with compound (100) along with the formation of small amounts of polar products presumed to arise from alcoholysis of the dioxopiperazine. No signs of the desired rearrangement was observed. When (10) was heated alone for four minutes under nitrogen at 220°C t.l.c. indicated that the residual material was mainly starting material along with a small amount of (100). Raising the temperature to 325°C for a further two minutes resulted in extensive decomposition, charring, and evolution of fumes. T.l.c. showed the presence of small amounts of compounds (10) and (100) and indicated the presence of much polymeric material. Apart from (10) and (100) no other discrete products could be detected.

Attempted photolytic rearrangement of compound (10) under mild conditions (i.e. using a medium pressure lamp and Pyrex filter) was

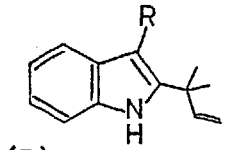
also unsuccessful. Photolysis in dichloromethane resulted in polymer formation by primary products which inhibited further photolysis. The gradual decomposition of (10) and the formation of unidentifiable, inseparable mixtures was observed.

In conclusion it has not been found possible to extend the reported rearrangement of N-allylindoles to 2-allylindoles to cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)tryptophyl,(10). Some of the products isolated from acid catalysed rearrangement of compound (10) suggest that the reaction is not concerted as had been proposed for simple N-allylindoles.[44b] Instead the pathway may involve initial cleavage of the allyl group followed by recombination at position 3 of the indole. Subsequent rearrangement yields the isolated 2-allylindoles (scheme 18).

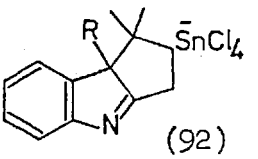
The failure of (10) to yield the expected products (5) and (91) is attributed to two reasons.

(a) Steric congestion during recombination of the allyl group and the indole at position 3. This slows down the rearrangement to the point where recombination on to the indole nitrogen atom and subsequent Friedel-Crafts cyclization to give compounds (58) and (59) can compete.

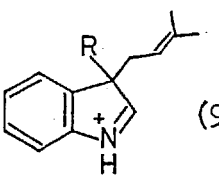
(b) The dioxopiperazine ring traps out intermediates such as structures (92), (93) and (97) which would otherwise decompose to the desired products desoxybrevianamide E, (5), and its isomer compound (91).



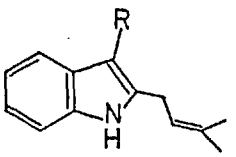
(5)



(92)



(97)



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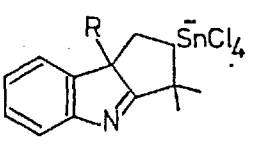
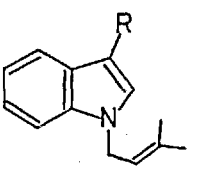
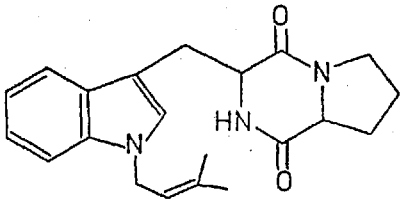


(93)

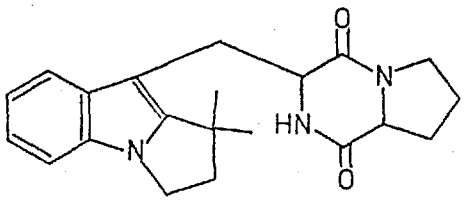


(10)

Attempted conversion of cyclo-L-prolyl-L-(3',3'-dimethyl-3',4',5'-H-pyrrolo[1,2-a])tryptophyl, (58), into cyclo-L-prolyl-L-(2-(1',1'-dimethyl)allyl)tryptophyl, (5).



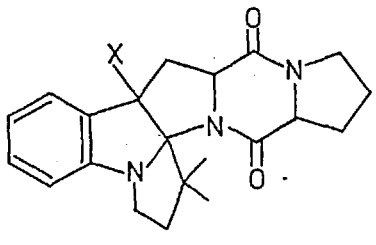
(10)



(58)

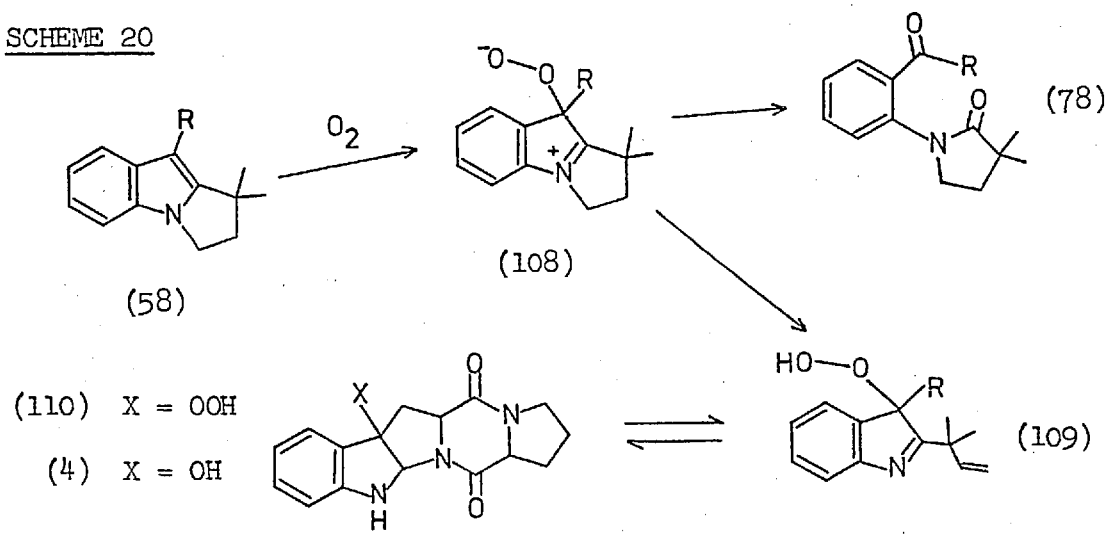
Although the attempted conversion of cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)tryptophyl, (10), into desoxybrevianamide E, (5), had not been achieved, one of the major products of the attempted rearrangement was the pyrrolo-indole (58), which could be prepared in good yield using hot, moist boron trifluoride etherate. It was decided, therefore, to attempt the cleavage of the carbon-nitrogen bond of compound (58) to give desoxybrevianamide E or a simple derivative. The main approaches to this problem involved either nucleophilic attack at position 5' with the indole nucleus as a leaving group, or proton abstration at position 4' and elimination of the indole nucleus via an E2 mechanism - a process analogous to the Hofmann elimination. In order to convert the indole nucleus into a good leaving group, use was made of the electron rich position 3 which can be protonated or coordinated to an electrophile (or oxidizing agent) to give structures of the general type (107).

(107)



The first experiment tried was oxidation with molecular oxygen. Treatment of many 2-alkyl- or 2,3-dialkylindoles with ground state molecular oxygen, either on its own or in the presence of radical initiators or platinum oxide, has been found to give stable 3-hydroperoxyindolenines.[63] However no accounts of successful autoxidation of 3-alkylindoles have been published, perhaps indicating that these compounds are resistant to autoxidation. 1,2,3-Trisubstituted indoles cannot form stable 3-hydroperoxyindolenines and on treatment with oxygen are transformed instead to o-acetamido-acetophenones,[29] presumably via a 3-hydroxyindolenine such as compound (107), X = OOH. It was hoped that reaction of pyrrolo-indole (58) with oxygen would lead to the hydroperoxyindolenine (109) (scheme 20) by way of the zwitterion (108).

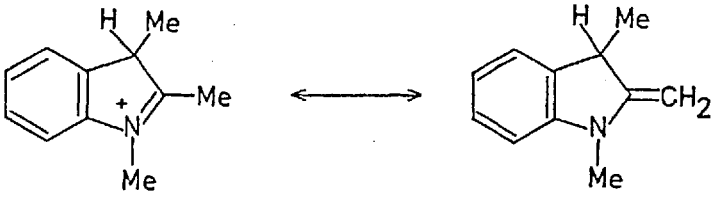
SCHEME 20



By analogy with earlier work described in this thesis, the indolenine (109) would be expected to cyclize to give structure (110), mild reduction of which would yield brevianamide E, (4). In the event, when autoxidation of compound (58) was attempted in ethyl acetate with oxygen in the presence of Adam's catalyst or dibenzoyl peroxide, no reaction occurred. For other reasons, related to a different synthetic route to the brevianamides, the autoxidation of cyclo-L-prolyl-L-tryptophyl, (23),

and cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)tryptophyl, (10), was also attempted but both gave back unchanged starting materials. This is in agreement with the previously observed lack of reactivity of 3-alkylindoles with ground state oxygen. The failure of compound (58) was surprising considering the easy oxidation of other 2,3-dialkylindoles and possible reasons were sought. As has been noted, indoles react with electrophiles almost exclusively at position 3,[21] even when already substituted there, to give an intermediate indolenine. Similarly, in acid indoles protonate preferentially at position 3[19] and consequently a relationship between reactivity and basicity might be expected. Such a relationship is apparent in the reactivity of indoles towards ground state oxygen. It is generally found that the presence of an alkyl group in position 2 of an indole results in an increase in basicity whilst an alkyl group in position 3 causes a decrease. This correlates with the observed reactivity of 2-alkyl- and 2,3-dialkylindoles and the lack of reactivity of 3-alkylindoles and indole itself towards autoxidation. This correlation is summarized in table 4 where it is seen that for many of the indoles more basic than indole itself reports exist of their autoxidation whilst for all the indoles less basic than indole no such reports are known.

SCHEME 21



A possible contributory reason for the increased activity of 2-substituted indoles is hyperconjugation (scheme 21); thus 2-ethylindole is a weaker base than 2-methylindole. Extending this idea to compound (58), no reaction with ground state oxygen in this case may

be due to the reactivity of position 3, as reflected in its basicity, not being enhanced in the usual way by a 2-substituent since hyperconjugation is not possible. Position 3 may also be sterically hindered in compound (58), slowing down the autoxidation reaction further.

Table 4

Correlation between basicity of substituted indoles and their reactivity towards autoxidation

Substituent	pKa*	Reacts with ground state oxygen?
2-Me	-0.28	yes
2-Et	-0.41	not recorded
1,2,3-triMe	-0.66	yes
2,3-diMe	-1.49	not recorded
none	-3.62	yes**
3-Bu^t	-3.84	no
3-Et	-4.25	no
3-nPr	-4.34	no
3-Me	-4.55	no

*calculated from u.v. spectra of the indole derivative in acid[19e]

**vigorous conditions required[90]

Since no reaction was observed with ground state oxygen, the more reactive singlet state species was tried. It has been reported that singlet oxygen forms unstable 3-hydroperoxyindolenines with indoles which decompose thermally to produce o-amino-acetophenone derivatives, presumably via an intermediate dioxetane.[65] If the reaction is carried out at a low enough temperature the intermediate 3-hydroperoxyindolenines can be isolated.[64] The reaction appears to be applicable to all indoles irrespective of the degree and position of substitution. It was hoped that treatment of the pyrrolo-indole (58) with singlet oxygen would

result in formation of compound (108) (scheme 20), which could either cyclize to a dioxetane by attack of the hydroperoxy anion at position 2 and hence decompose to the o-amino-acetophenone derivative (78), or open up to give the desired indolenine (109). It was felt that steric hindrance by the dimethyl group of the intermediate (108) might inhibit intramolecular attack leading to the dioxetane and that the latter route would predominate. In the event, treatment of compound (58) with singlet oxygen at room temperature gave a quantitative yield of the o-amino-acetophenone derivative (78), whilst repetition of the reaction at -78°C gave back unchanged starting material.

Oxidation of the pyrrolo-indole (58) with m-chloroperbenzoic acid was also attempted in the hope that the expected intermediate (107), X = O^-, would rearrange to brevianamide E, (4). The reaction was carried out at -95°C and allowed to warm to -25°C by which time all the peracid had been consumed. The major product of the reaction was again found to be the o-amino-acetophenone derivative (78) which is consistent with the results of peracid oxidation of indoles recorded in the literature.[66]

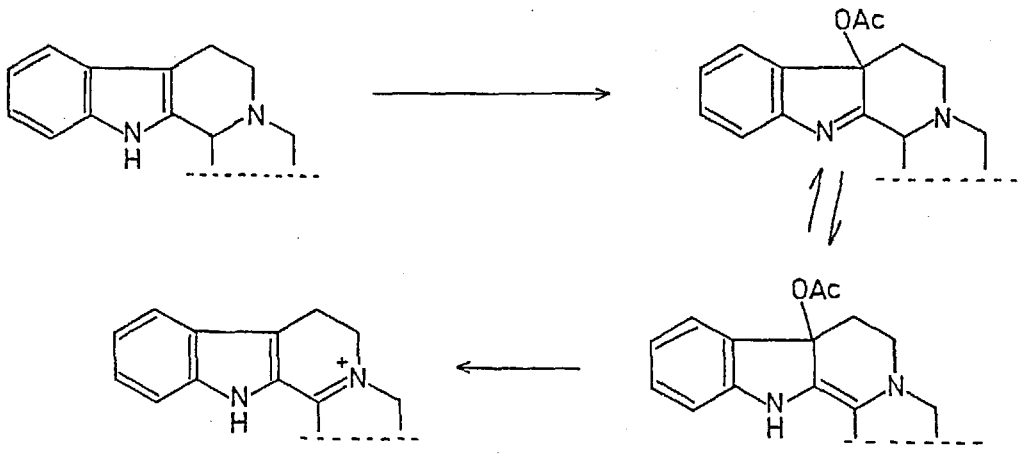
Oxidation of the pyrrolo-indole (58) with mercuric acetate was next investigated. No reports of reaction of indoles with mercuric acetate are known but it was predicted that an initial adduct (107), X = HgOAc would be formed and that in the presence of the acetoxy counter-ion loss of a proton in position 4' could occur leading to a structure analogous to (109) in scheme 20. Alternatively, acetate could attack the intermediate (107) at position 5' and displace the indole.

When one equivalent of mercuric acetate dissolved in methanol was stirred with compound (58), a white precipitate was gradually formed and the vapour of acetic acid could be detected above the reaction solution. However investigation of a sample of the reaction mixture by t.l.c. indicated the presence of the starting material only. This

was confirmed when after 9 days the reaction mixture was worked-up to give a single compound identical with the pyrrolo-indole (58). The observed changes during the reaction were presumed to be due to reversible complex formation.

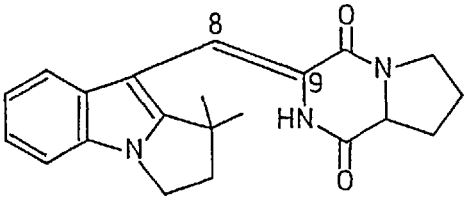
Since no net reaction with mercuric acetate was observed a stronger oxidant, lead tetraacetate, was tried. With this reagent some indoles give isolable 3-acetoxyindolenines which can rearrange under acidic conditions[67] (scheme 22). It was hoped that reaction of compound (58) with lead tetraacetate would give the intermediate (107), X = OAc and that the acetate counter-ion present would either attack nucleophilically at position 5' or promote loss of the proton at position 4' so as to achieve the desired C-N bond cleavage.

SCHEME 22



When the pyrrolo-indole (58) was treated with one equivalent of lead tetraacetate in dichloromethane under anhydrous conditions all the oxidant immediately dissolved and the solution became yellow. After two hours a considerable precipitate had appeared (presumed to be sugar of lead) but tests with starch/iodide indicated that some oxidant was still present in the solution. After 15 hours no lead tetraacetate remained and t.l.c. revealed that a complex mixture of products was present.

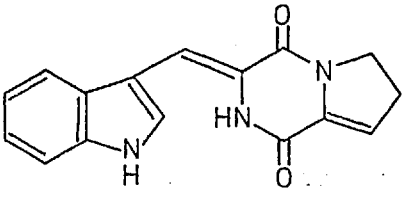
On leaving, the product mixture appeared to simplify and a major product, less polar than the starting material, was formed. Work-up of the reaction mixture after 5 days gave a low yield of the major product which was isolated by p.l.c. This compound was highly crystalline and recrystallization from acetone/light petroleum gave yellow needles which analysed as $C_{21}H_{23}N_3O_2$. This formula, which was confirmed by mass spectroscopy (molecular ion at 349 mass units) suggested that net dehydrogenation of compound (58) with the introduction of a double bond had occurred. The mass spectrum exhibited an extremely strong molecular ion which was also the base peak implying that the new double bond was present between positions 8 and 9. This conclusion was based upon the observation that dioxopiperazines derived from tryptophan normally undergo ready cleavage of the 8,9-bond[11] and consequently suggests structure (111) for the reaction product. This is supported by its u.v. spectrum which is similar to that of compound (112), an artefact obtained by hydrolysis of telomycin:[68]



(111)

λ max 349 nm

log ε 4.35

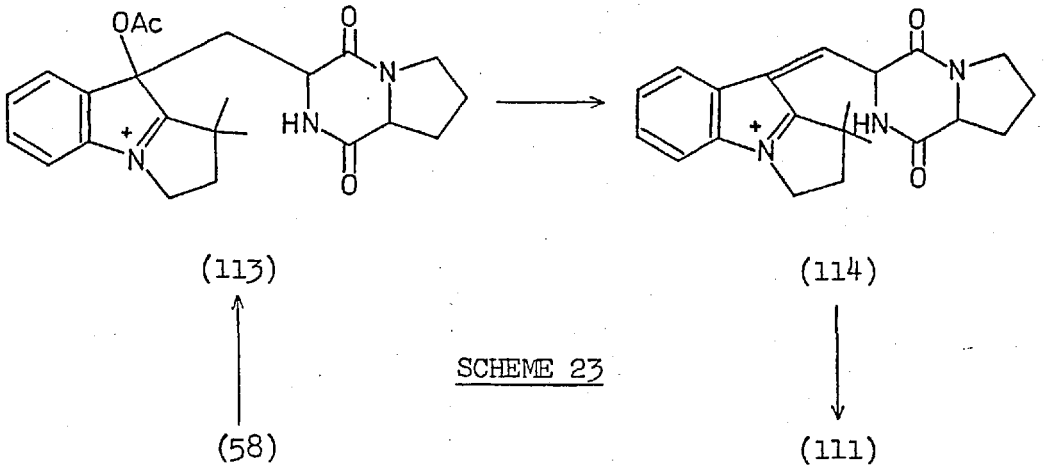


(112)

λ max 339 nm

log ε 4.34

The stereochemistry around the double bond of compound (111) is not known. The formation of this product is rationalized by the route shown in scheme 23; initial reaction with lead tetraacetate leads to intermediate (113) which can eliminate acetic acid to yield (114). Loss of the acidic proton at position 9 then gives the observed product.

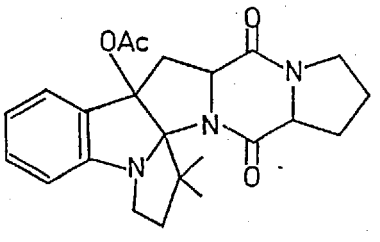


SCHEME 23

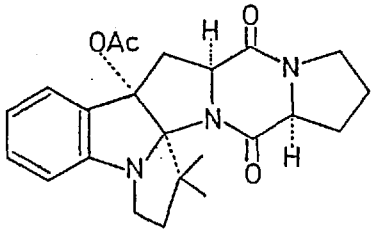
The reaction of compound (58) with lead tetraacetate in acetic acid was also investigated. When a solution of compound (58) in acetic acid was treated with one equivalent of lead tetraacetate, t.l.c. indicated a slow disappearance of starting material with the formation of two major products. The more polar of these substances travelled with approximately the same R_F as the starting material whilst the other product was very much less polar. Following an aqueous work-up and purification by p.l.c., the two products were isolated as glasses which solidified when triturated with ether to give amorphous powders. Attempted crystallization of the more polar product failed but the second product crystallized from acetone/light petroleum.

The less polar compound was assigned structure (115) on the basis of its spectral and microanalytical data. Thus elemental analysis of the product corresponded to a molecular formula $C_{23}H_{27}N_3O_4$ which is in agreement with the molecular ion observed at 409 mass units in the mass

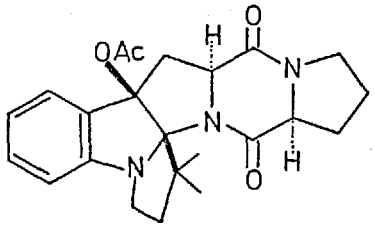
(115)



spectrum. The u.v. spectrum of compound (115) was similar to those of the related structures (57), (36) and (94) suggesting that the indole chromophore of the starting material had been converted into the indoline ring system of those compounds. In the ^{1}H n.m.r. spectrum of compound (115) no exchangeable protons were observed and the characteristic proton corresponding to position 2 at ca τ 4 in compounds (57), (36) and (94) was absent. This confirms that position 2 and both N-H groups of the starting material have been substituted, as in structure (115). The acetate methyl group resonated as a singlet at τ 8.06 and the geminal groups were seen at τ 8.44 and 9.03. This large non-equivalence can be rationalized by inspection of a model of structure (115) which shows that one of the methyl groups is held in the shielding region of the amide carbonyl group causing the observed upfield shift. The ^{13}C n.m.r. spectrum of compound (115) was recorded and assigned by comparison with the spectra of other, similar structures isolated in this work (see p.211). The spectrum obtained was fully consistent with the proposed structure.



(116)

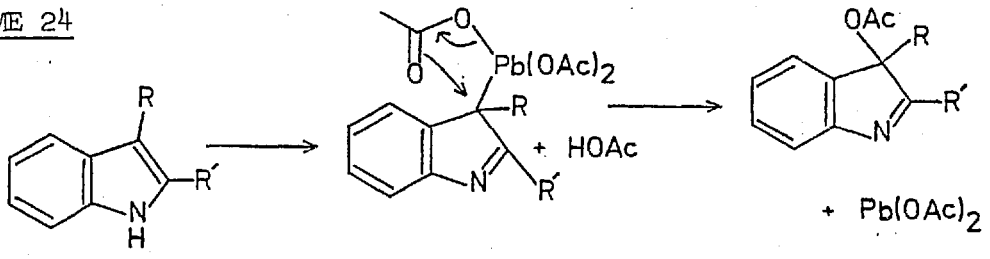


(117)

The mechanism of formation of compound (115) may reasonably be regarded as proceeding via normal acetoxylation of position 3 of the pyrrolo-indole (58) to give (113)[67] (scheme 23) followed by nucleophilic attack of the dioxopiperazine ring nitrogen atom upon the iminium system. The mechanism implies that compound (115) should be formed as a mixture of isomers (116) and (117) depending upon which side of the molecule acetoxylation occurs. The chromatographic and spectral properties of (115) suggested, however, that it is a single compound

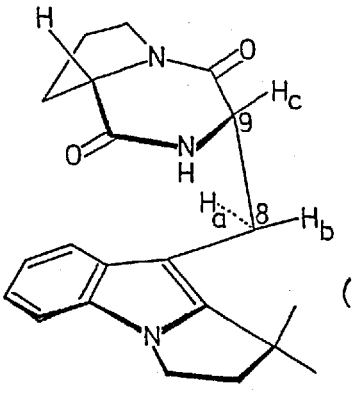
although they do not provide sufficient unambiguous information to assign either isomeric structure. It has been proposed[29] that the oxidation of indoles by lead tetraacetate is initiated by formation of a carbon-lead bond at position 3 followed by intramolecular displacement by acetate (scheme 24). If correct then this irreversible process would control the stereochemistry of the product and formation

SCHEME 24



of either (116) or (117) would depend upon which face of compound (58) lead tetraacetate attacked. It has been mentioned previously (p.127) that many aryldioxopiperazines exist in a folded conformation with the exception of cis-aryldioxopiperazines derived from proline. It was also suggested that cyclo-L-prolyl-L-tryptophyl derivatives may exist in a partially folded conformation allowing some interaction of one amide π-system of the dioxopiperazine ring with the indole nucleus whilst at the same time avoiding steric congestion between the latter and the prolyl 5-membered ring. Such a partially folded conformation could explain the apparent selectivity of attack of the lead tetraacetate molecule upon the pyrrolo-indole, (58). The coupling constants of the proton in position 9 with those in position 8 of (58) (4 and 11 Hz) confirm that the molecule does not exist in a fully folded conformation (for which the Karplus equations require both coupling constants to be ca 4 Hz) but are consistent with a partially folded conformation. Further evidence for such a conformation is the non-equivalence of the geminal methyl groups of compound (58), which indicate that the environments on the two faces of the molecule are substantially different.

Inspection of models of (58) show that a conformation can be adopted (structure 118) where the dihedral angles between proton c and protons a and b are approximately 180° and 70°C which would result in J values in the observed region. In this conformation the carbonyl function of the secondary amide group can interact with the aromatic system to produce the stabilization to maintain the conformation without steric hindrance between the indole nucleus and the dioxopiperazine ring.

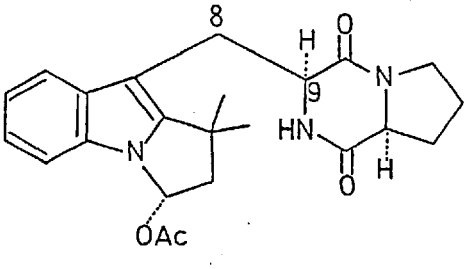


(118)

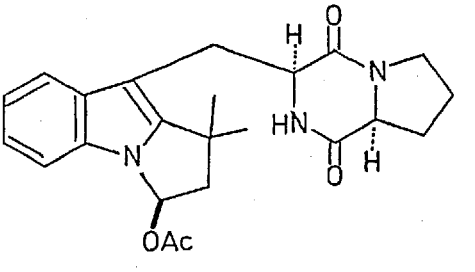
dihedral angle for $H_c C^9-C^8 H_b$ = ca 70°

dihedral angle for $H_c C^9-C^8 H_a$ = ca 180°

The more polar product from the lead tetraacetate oxidation of compound (58) was found to be a mixture of the diastereoisomers (119a) and (119b). The two compounds travelled very close on t.l.c. and although they could be partially resolved on an analytical scale, no separation was possible using multiple elution p.l.c. Diastereoisomers (119a) and (119b) were also never obtained crystalline and consequently were never separated.



(119a)



(119b)

The mass spectrum of the mixture of diastereoisomers (119) exhibited the correct molecular ion at 409 mass units and this fragmented to ions of mass 349, 256 (base peak) and 196. The heaviest of these fragments

corresponds to simple elimination of acetic acid whilst the base peak results from the ready cleavage of the bond joining positions 8 and 9, characteristic of tryptophan derived dioxopiperazines.[11] The predominant fate of the base peak is loss of acetic acid to give the second most abundant ion in the spectrum at 196 mass units. Metastable peaks were observed corresponding to the fragmentations 409→256 and 256→196. The mass of the base peak and its structural assignment confirm that acetoxylation has occurred on the indole half of the molecule and not in the dioxopiperazine ring, whilst the ready loss of acetic acid from the base peak implies that the acetyl group is present at position 4' or 5'.

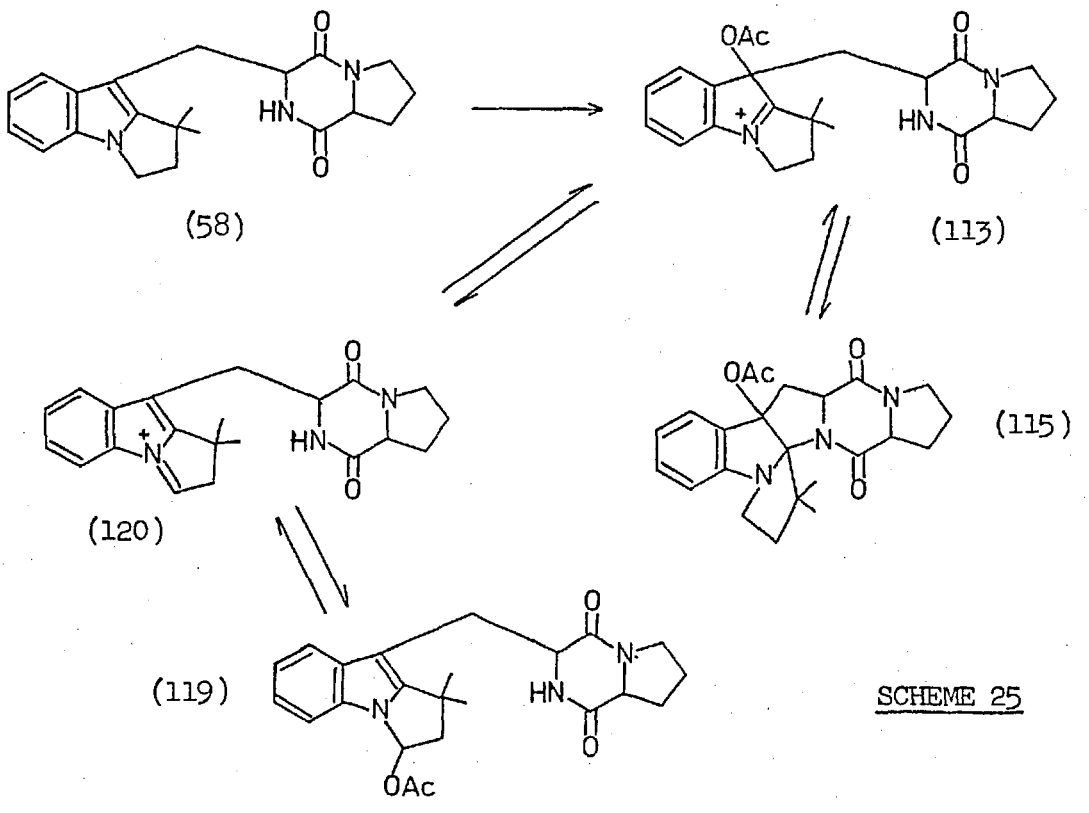
The i.r. spectrum of (119) confirmed the presence of a simple acetate by exhibiting a carbonyl stretch at 1750 cm^{-1} and a carbon-oxygen stretch at 1230 cm^{-1}. The u.v. spectrum indicated that the indole chromophore was still present and that the nitrogen atom of the indole was still substituted since the lowest energy absorption at 291 n.m. appeared as an inflexion rather than as the distinct band which is observed for N-unsubstituted indoles.[31]

The ^{1}H n.m.r. spectrum of (119) showed plainly that it was a mixture of diastereoisomers; two acetyl signals were seen at τ 7.91 and 7.78 and two pairs of non-equivalent methyl groups between τ 8.30 and 8.40, whilst the methines at position 5' appeared as the X parts of two ABX systems. The chemical shifts of the protons assigned to position 5' (ca τ 3.8) were consistent with the expected value of a methine proton flanked by a nitrogen atom and an oxygen atom predicted by Shoolery's rules, confirming that acetoxylation had occurred at position 5' rather than position 4'. The relative proportions of the two isomers, as determined from their ^{1}H n.m.r. spectra, varied in different preparations and at different stages during work-up in any given preparation, indicating that they are in ready equilibrium.

Since compounds (119a) and (119b) could not be separated, the ester was hydrolysed to the diastereoisomeric alcohols one of which crystallised and was characterized. The hydrolysis reaction, which is described more fully in the following section, was a remarkably facile process under acidic conditions and even occurred slowly on silica t.l.c. plates. Presumably the mechanism involves the intermediacy of the iminium ion (120) shown in scheme 25 and its ease of formation accounts for the variation in ratio of (119a) to (119b) and for the failure to separate them by p.l.c. on silica.

The relative proportions of compounds (119) and the indoline (115) isolated from a number of preparations varied by as much as ± 10% whilst the absolute yield of the mixture (119) was on average ca 60-70% and of compound (115) ca 20%; thus it is seen that the reaction is reasonably clean and of synthetic utility.

The formation of compounds (119a) and (119b) is rationalized by the route shown in scheme 25. By analogy with the known mode of reaction



SCHEME 25

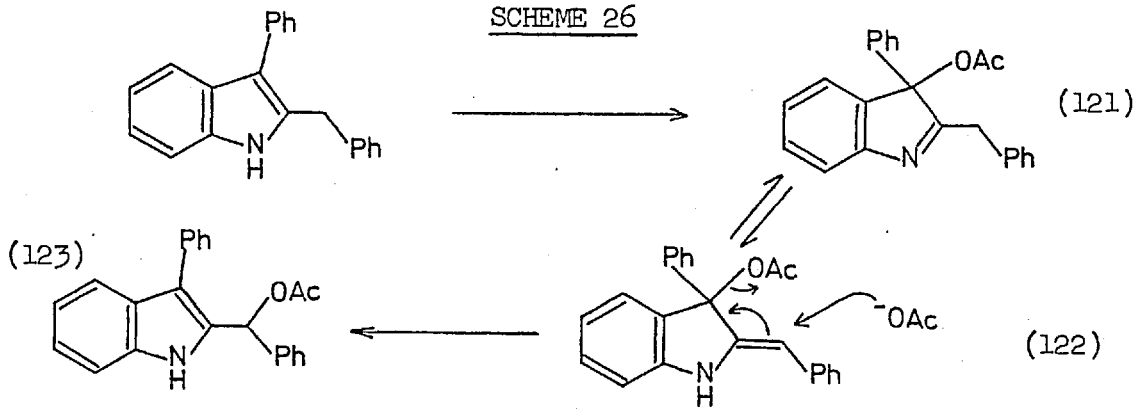
of lead tetraacetate with indoles,[69] the reaction is probably initiated by attack at position 3 of the indole nucleus of compound (58) to give the indolenine (113). It is proposed that this intermediate is in equilibrium with the other major product of the reaction, compound (115), and with the iminium structure (120) formed by proton loss at position 5'. Qhenching of the latter by acetate then leads to compounds (119a) and (119b). It is possible that the same equilibrium is set up in the reaction of compound (58) with lead tetraacetate in dichloromethane; however in that reaction the concentration of acetate present is low, prejudicing the equilibrium between (120) and (119). The intermediacy of the iminium structure (120) is supported by the reactions of compounds (119a) and (119b) and other 5'-substituted analogues and also by comparison with the chemistry of derivatives of the alkaloid eburnamine. These reactions are the subject of the following section.

Confirmation that an equilibrium exists between the indoline (115) and the indoles (119a) and (119b) under the reaction conditions was obtained by dissolving a pure sample of compound (115) in acetic acid. After 15 hours t.l.c. of the solution revealed that a mixture of starting material and a more polar compound had been formed. The solution was left a further 8 days before the reaction was worked-up. Isolation of the two compounds by p.l.c. and comparison of the more polar products ^{1}H n.m.r. and i.r. spectra with those of (119) indicated that they were the same mixture of diastereoisomers (119a) and (119b). The ratio of compound (115) to (119) isolated from the reaction mixture was approximately 2:1. When (119) was dissolved in acetic acid, none of compound (115) was observed and none of the iminium species (120) could be trapped out by addition of better nucleophiles than acetate. This implies that the position of equilibrium between (115) and (119) is in favour of the latter compound and, since not all of (115) was converted to (119) after

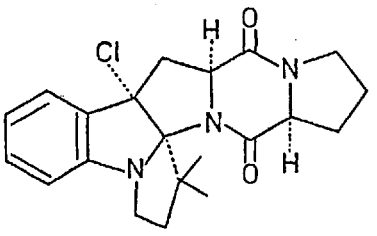
9 days, that the rate of equilibration is slow. Transformation of compound (115) to (119) in acetic acid was accelerated by the addition of a catalytic amount of trifluoroacetic acid.

The formation of the mixture of diastereoisomers (119a) and (119b) is a reaction without direct precedent in indole chemistry. However an example does exist of an analogous reaction which is shown in scheme (26).[69] In this transformation the intermediate indolenine (121) is presumed to be in equilibrium with the enamine (122) which is quenched by acetate to give the product, (123). A similar course of action is followed by the intermediate (113) in scheme 25 except that the 2-substituent has no free protons and cannot equilibrate; instead the iminium (120) is formed.

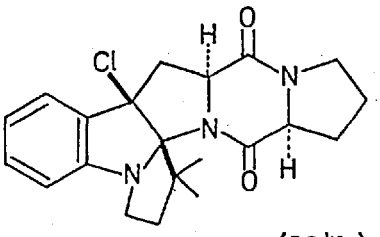
SCHEME 26



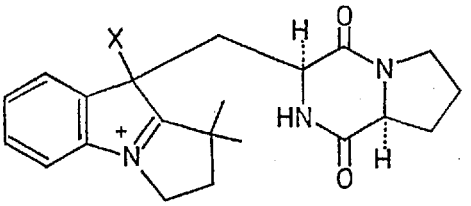
The reaction of the pyrrolo-indole (58) with tertiary butyl hypochlorite in dichloromethane at -10°C was also briefly investigated. The major products of this reaction appeared to possess the structures (124a) and (124b) identified from their ^{1}H n.m.r. spectra, which exhibited similarities with that of compound (115). Thus the geminal methyl groups of (124a) and (224b) resonated as pairs of singlets at τ 8.34 and 8.86 and τ 8.38 and 8.92. These products clearly arise from trivial ring closure of structure (107), X = Cl, and no signs of the desired cleavage of the C-N bond were observed. Precedent exists for this reaction; for examples see scheme 5[20] and scheme 6.[22]



(124a)



(124b)



(107)

The reactivity of the pyrrolo-indole (58) towards concentrated sulphuric acid was also investigated. It was predicted that the initial intermediate formed would be structure (107), X=H, and to confirm this the u.v. spectrum of compound (58) was recorded in concentrated sulphuric acid. Comparison of the spectrum obtained with those of 1,1-dimethylindolium perchlorate,[19] 1,2,3-trimethylindole,[19] 1,2,3-trimethylindolenium sulphate,[19] 2,3,3-trimethylindolenium chloride,[19] N-methylaniline hydrochloride[91] and other compounds isolated in this work (see table 5) indicated that compound (58) exists entirely as the indolenium structure (107), X=H, in this medium and not as an N-protonated indole or indoline. Somewhat surprisingly compound (58) was very stable to concentrated sulphuric acid and after 7 days at room temperature no change whatsoever had occurred. It had been hoped that bisulphate anion might be a sufficiently good nucleophile under these conditions to attack position 5' and so effect the desired bond cleavage.

One further attempt was made to cleave the C-N bond of compound (58) and this involved treatment of a collidine solution of (58) with lithium iodide. The rationale behind the attempted reaction was that iodide ion might nucleophilically displace the indole nucleus at position 5'. By way of precedent, anhydrous and moist lithium iodide

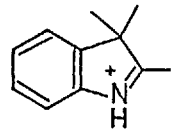
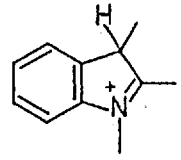
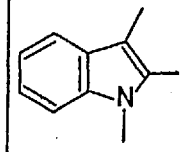
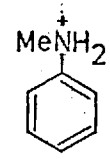
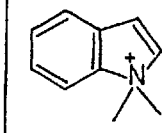
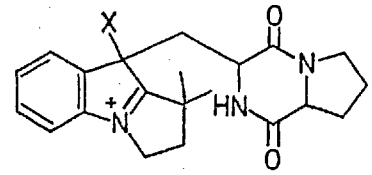
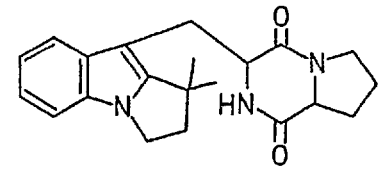
Structure			(107) X = H	(58)		(57)		
Solvent	$^{N}/_{10}$ HCl	6M.H_2SO_4	18M.H_2SO_4	EtOH	EtOH	EtOH	1N.HCl	EtOH
λ max (log ε)	-	-	-	-	-	^{209}(4.37)	-	^{215}(4.19)
	^{229}(4.00)	^{231}(3.71)	^{233}(3.67)	^{227}(4.47)	^{230}(4.54)	-	^{243} (-)	^{250}(3.94)
	^{235}(3.95)	^{238}(3.69)	^{238}(3.70)	^{278}(3.65)	^{279}(3.77)	^{256}(3.91)	^{253} (-)	^{278}(2.54)
	^{275}(3.91)	^{275}(3.74)	^{285}(3.72)	^{284}(3.70)	^{286}(3.82)	-	^{260} (-)	^{282}(2.52)
	-	-	-	^{292}(3.65)	^{293}(3.80)	^{306}(3.28)	^{263} (-)	^{288}(2.36)

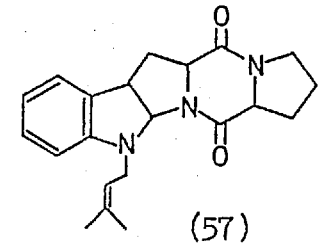
Table 5



(107) X = H



(58)

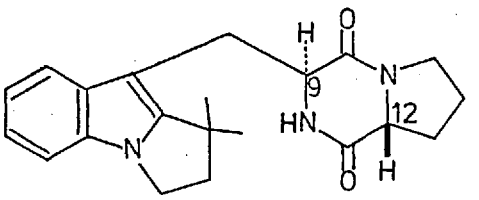


(57)

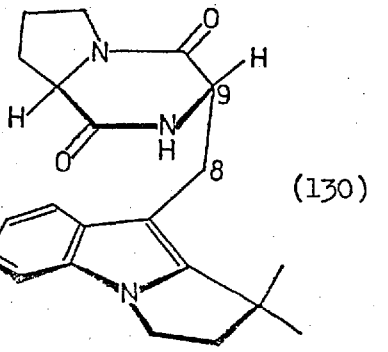
have both been used in refluxing pyridine, lutidine or collidine to cleave esters, the products being an alkyl iodide and the lithium salt of the acid.[70]

When a sample of the pyrrolo-indole (58) was refluxed in dry collidine with lithium iodide trihydrate for two hours, partial conversion to a new, more polar compound was observed by t.l.c. However after 12 hours the relative proportions of the product and compound (58) had become static and no further reaction was detected. A similar experiment was performed under anhydrous conditions using lithium iodide from which the water of crystallization had been removed and the same result was obtained. Following work-up and p.l.c. purification, starting material and the new product were isolated in the ratio 1:5. The product was a glass which solidified on trituration with light petroleum to yield an amorphous powder. Crystallization was achieved from acetone/light petroleum.

The u.v. spectrum of the new compound was that of an N-substituted indole[31] indicating that the desired bond cleavage had not occurred. The mass spectrum possessed a molecular ion at 351 mass units showing that the product was isomeric with the starting material (58). Comparison of the mass spectrum with that of (58) revealed that they were extremely similar whilst the i.r. spectra of the two compounds were almost superimposable. The ^{1}H n.m.r. spectrum of the product also resembled that of compound (58) with two important differences; the proton assigned to position 12 in (58) had moved upfield by ca 1 p.p.m. whilst the proton assigned to position 9, previously observed as a double doublet (J=4 and 11 Hz), had become a broadened triplet (J=4 Hz). By analogy with the product obtained by base treatment of cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)tryptophyl, (10), these observations suggested that the product was the result of base catalysed epimerization



(129)



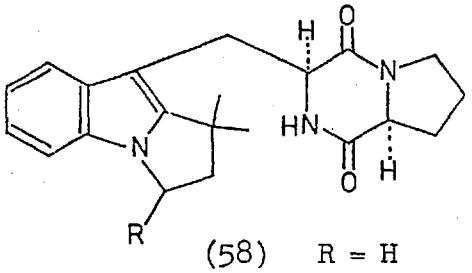
(130)

around positions 9 and/or 12 and possessed the structure (129), or its enantiomer. As discussed previously, cis-substituted aryl dioxopiperazines normally adopt a folded conformation but hindrance by the proline ring presents this in cyclo-L-prolyl-L-tryptophyl derivatives. Epimerization of compound (58) at position 9 or 12 allows a folded conformation to be assumed by the resulting trans-substituted aryl dioxopiperazine as shown in structure (130). The changes in the ^{1}H n.m.r. spectrum of the product compared with compound (58) arise from shielding of the proton at position 12 by the indole nucleus into which it is directed in the folded conformation. The folded conformation also alters the dihedral angles between the methylene group protons in position 8 and the methine proton at position 9 to approximately 50° which is consistent with the new coupling constants.

Confirmation that a base catalysed (or thermal) equilibration of compounds (58) and (129) was occurring was obtained by refluxing the pyrrolo-indole (58) alone in colidine with the result that partial conversion to the isomer (129) was observed. The same result was obtained by treatment with other bases, e.g. potassium t-butoxide in methanol.

The epimerization reaction is discussed in more detail on p. 191 where it is shown that under basic conditions cyclo-L-prolyl-L-tryptophyl derivatives epimerize at position 12 only, to give the thermodynamically more stable trans-substituted dioxopiperazine ring.

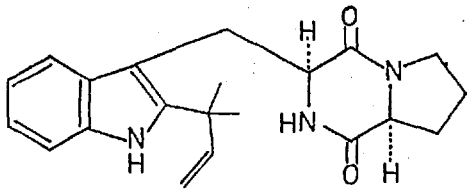
Attempted conversion of cyclo-L-prolyl-L-(5'-(R,S)-acetoxy-3',3'-dimethyl-3',4',5'H-pyrrolo[1,2-a])tryptophyl, (119), into cyclo-L-prolyl-L-(2-(1',1'-dimethyl)allyl)tryptophyl, (5).



(58) R = H

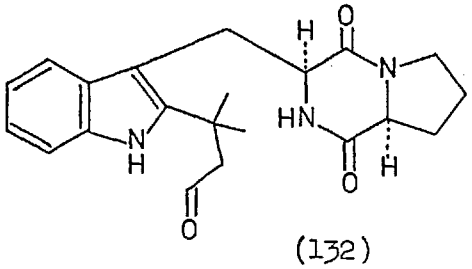
(119) R = OAc

(131) R = OH

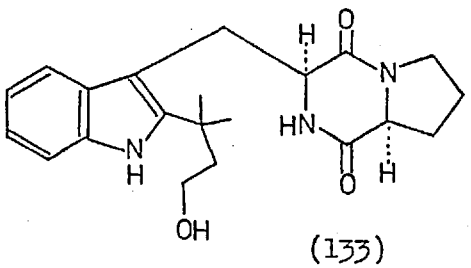


(5)

The preceding section described efforts made to convert the pyrrolo-indole (58) into desoxybrevianamide E, (5), by cleavage of the C-N bond of the fused pyrrole ring. This was not achieved but functionalization of position 5' was observed in the form of compound (119). The latter may be viewed as an aminol which has been trapped as the acetate. It was thought that if the acetate (119) could be hydrolysed to the alcohol (131) then this compound should be in equilibrium with the aldehyde (132). Reduction of the aldehyde would then lead to the alcohol (133) which on dehydration would give desoxybrevianamide E, (5). Accordingly a search for the optimum conditions for the hydrolysis of the acetate (119) was instigated.



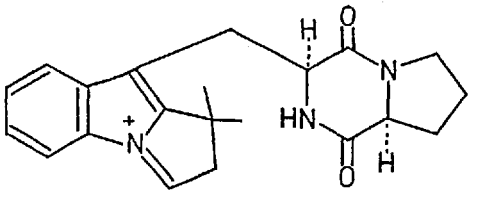
(132)



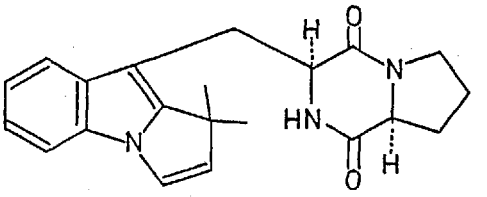
(133)

Base hydrolysis of compound (119) using aqueous, ethanolic potassium hydroxide was found to be a messy reaction, presumably due to competitive attack upon the dioxopiperazine ring. Treatment of the acetate with

boron tribromide followed by addition of water[71] resulted in formation of the desired alcohol (131) contaminated with large amounts of a by-product which was later identified as the olefin (150). Treatment of the acetate with aqueous acetonitrile containing a catalytic amount of trifluoroacetic acid gave a good yield of the alcohol (131) although some of the olefin (150) was also formed if the reaction was left too long or too much acid was present. The ease of hydrolysis of compound (119) suggests the intermediacy of the imine (120) rather than a normal acid catalysed ester hydrolysis mechanism and this is borne out by the reactions of compound (131) and its derivatives described below.



(120)



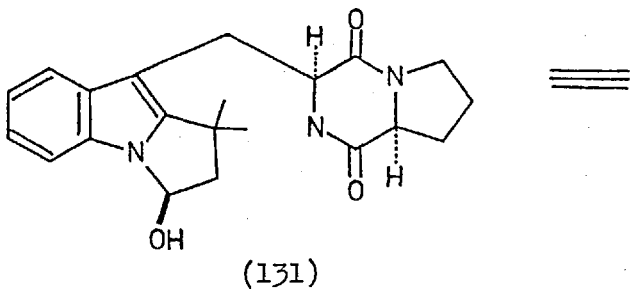
(150)

On t.l.c. the alcohol (131) was rather more polar than the acetate (119). It also appeared to travel as a single compound and showed no signs of resolving into two components corresponding to the diastereoisomeric alcohols epimeric around position 5'. The general appearance of overlapping signals in the ^{1}H n.m.r. spectrum of compound (131) suggested however that it was indeed a mixture of two isomers. No aldehyde proton was observed indicating that the spectrum was not that of a mixture of compounds (131) and (132).

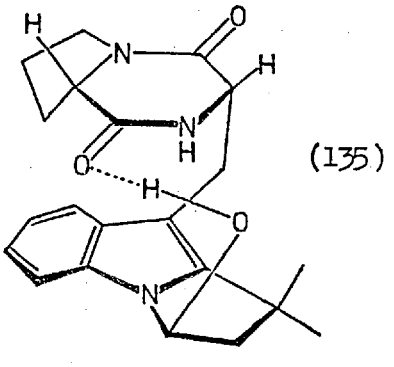
When crystallization of compound (131) was attempted, only one of the diastereoisomeric alcohols crystallized out and the ^{1}H n.m.r. spectrum of the pure isomer confirmed its structure as the alcohol (131); thus no acetate methyl group resonance was visible and the methine proton at position 5' resonated at τ 4.62 compared with τ 3.66 in the starting material. This methine proton was coupled to the hydroxyl proton at

τ 4.31 and following rapid deuterium exchange appeared as a double doublet (J=4 and 6 Hz) consistent with it being the X part of an ABX system. The AB part, corresponding to the methylene protons at position 4', was clearly visible as a pair of double doublets centred around τ 7.28 and 7.60 (J=6 and 14 Hz and 4 and 14 Hz respectively). These relationships were confirmed by decoupling experiments. An AMX system was also partially visible, corresponding to the methylene and methine protons at positions 8 and 9. One of the methylene protons (the A part) resonated as a double doublet at τ 7.03 (J=9 and 14 Hz) whilst the methine proton appeared as a doublet (J=9 Hz) at τ 5.84. The M part of the system was lost under other signals around τ 6.5.

The secondary amide proton of the alcohol (131) appeared as a slowly exchanging,[72] broad singlet at τ 1.67 in the ^{1}H n.m.r. spectrum. This is at somewhat lower field than is observed in other similar compounds in this series; in structures (119), (58), (23), etc. the signal is observed around τ 4. The low field position in this case is attributed to hydrogen bonding between the new alcohol function and the secondary amide system. The i.r. spectrum of the alcohol (131) exhibits amide carbonyl stretching bands at 1670 and 1685 cm^{-1} in carbon tetrachloride, compared with 1685 and 1695 cm^{-1} for the acetate (119) in the same solvent, supporting the proposal that hydrogen bonding to the amide oxygen atom does occur. Models of the alcohol (131) confirm that



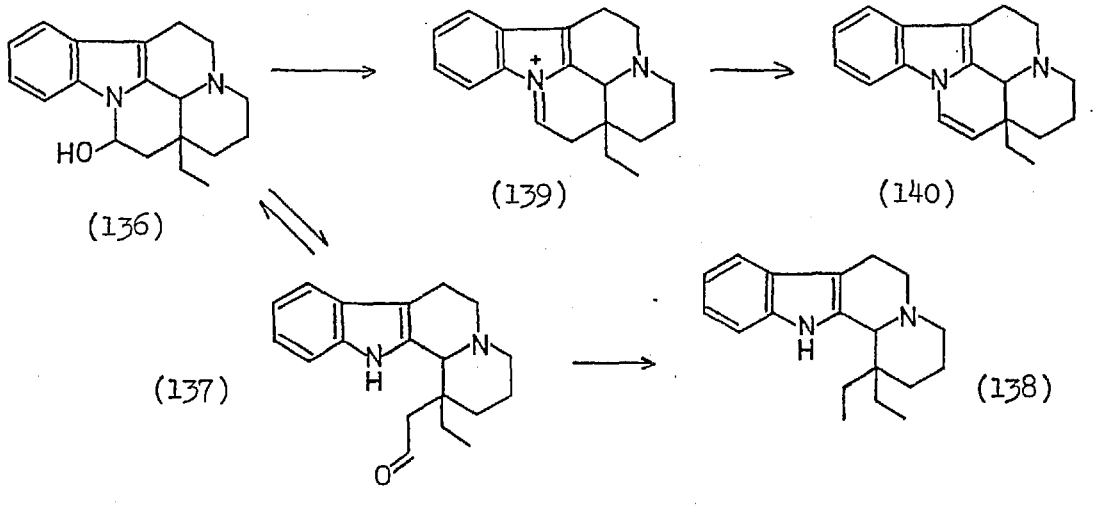
(131)



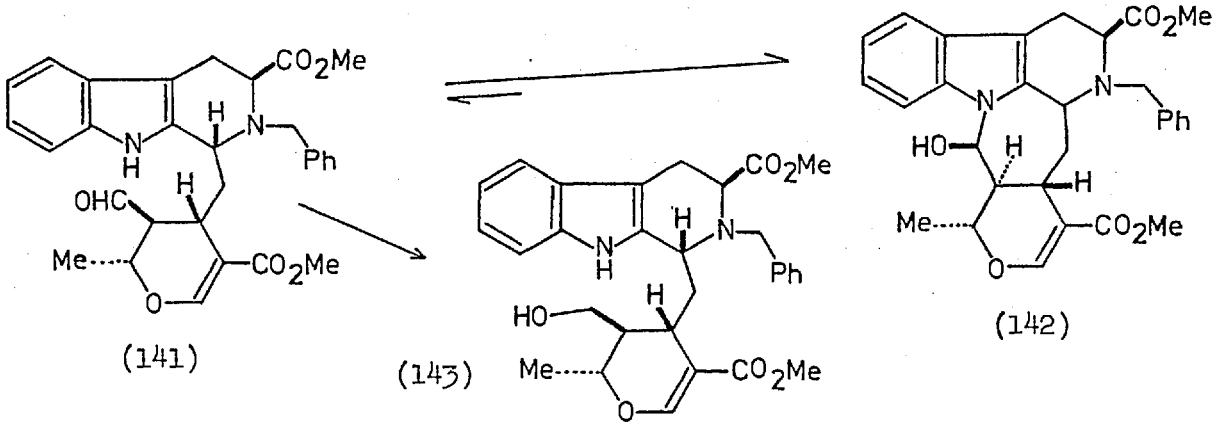
(135)

hydrogen bonding between the secondary amide carbonyl group and the hydroxyl proton is sterically feasible and furthermore show that comfortable hydrogen bonding is only possible for one of the diastereoisomeric alcohols - the compound with the absolute configuration shown in structure (131). A representation of the hydrogen bonded species is shown in structure (135); in this conformation the secondary amide π-system is lying above the aromatic ring whilst the tertiary amide function is swung away. This is very similar to the partially folded conformation suggested for compounds (58) and (10) used to explain their stereoselective reactivity with electrophiles. The coupling constants observed for the AMX system of the methylene/methine protons of positions 8 and 9 of structure (131) (J_{AX} = 9 Hz and J_{MX} = 0 Hz) correlate well with the values predicted by the Karplus equations for the relevant dihedral angles of 10° and 90° observed in models of structure (135).

No evidence that the isomeric aldehyde (132) exists in equilibrium with the alcohol (131) is forthcoming from the u.v., i.r., ^{1}H n.m.r. or ^{13}C n.m.r. spectra of the latter. This is in agreement with the observations reported for compounds bearing similar structural features. Thus the alkaloid eburnamine, (136), also exists predominantly in the aminol form and none of the open chain aldehyde (137) has been detected.[73] Most of the reactions of eburnamine involving the aminol system can be

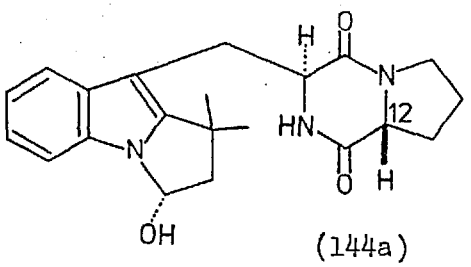


rationalized by the intermediacy of the imine (139). The only recorded reaction of eburnamine which must involve the aldehyde tautomer is Wolf-Kischner reduction to give the derivative (138). Another analogous system is the synthetic alkaloid (142) which exists in the form shown[74] rather than as the aldehyde (141). However reduction of compound (142) with sodium borohydride in isopropanol gave the alcohol (143) which must involve the intermediacy of aldehyde (141).

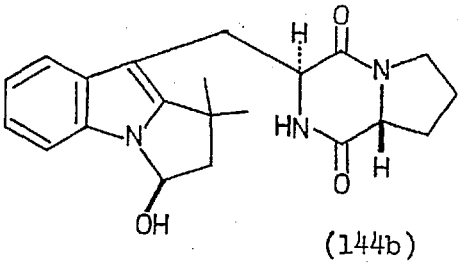


With these precedents in mind, it was hoped that reduction of the aminol (131) via its presumed tautomer (132) would yield the alcohol (133) and accordingly various methods of reduction were investigated. Treatment of compound (131) with sodium borohydride in isopropanol resulted in slow but clean and complete conversion to two new compounds, both more polar on t.l.c. than the starting material. Their ^{1}H n.m.r. spectra were very similar and suggested that the 5'-hydroxypyrrolo-indole part structure had remained intact in both compounds. Thus the proton at position 5' was visible in both spectra at ca $\tau 4.6$ and the geminal methyl groups appeared as non-equivalent singlets at $\tau 8.25$ and 8.45 for both compounds; the corresponding geminal methyl groups in an open chain structure such as the desired product (133) would probably resonate as a singlet. Further inspection of the ^{1}H n.m.r. spectra indicated that the proton corresponding to position 12 of the starting material was no longer present at $\tau 5.2$ and the mass spectra of the two

compounds revealed that they were not reduction products but instead were both isomeric with the starting material. On the basis of these observations the two products were identified as the diastereoisomers (144a) and (144b), resulting from base catalysed epimerization around position 12. Their greater polarity than the starting material is



(144a)



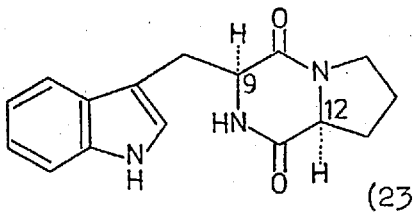
(144b)

consistent with the pattern generally observed for cis- and trans-substituted dioxopiperazines[62] and the disappearance of the proton at τ5.2 assigned to position 12 is explained by the fact that the trans-substituted dioxopiperazines (144a) and (144b) are free to adopt a fully folded conformation over the indole nucleus. In this conformation the proton at position 12 is directed into the shielding region of the indole nucleus and experiences an upfield shift where it is superimposed upon signals from other protons in the molecule. The secondary amide protons of compounds (144a) and (144b) appear underneath the aromatic signals in the ^{1}H n.m.r. spectrum. Whilst this is not at such low field as in compound (131), it is approximately 1 p.p.m. lower than the corresponding protons in the acetates (119a) and (119b) or the pyrrolo-indole (58). This suggests that the hydroxyl proton is hydrogen bonded to the secondary amide carbonyl group of the dioxopiperazine, as proposed for the alcohol (131). Models confirm that this is sterically feasible for both isomers (144a) and (144b). It is of interest to note that the non-equivalence of the geminal methyl groups in compounds (144a) and (144b) is greatly enhanced relative to the cis-isomer (131); in the former pair the methyl group separation

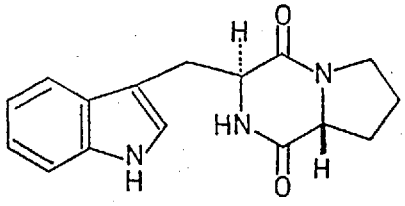
is 10 Hz at 60 MHz whilst for compound (131) the value is 4 Hz. This may reflect the degree of overlap between the indole nucleus and dioxopiperazine ring in the folded conformations of these compounds.

The optical rotations of compounds (144a) and (144b) were found to be very low (5° and 9°) which suggested that epimerization might have occurred at both positions 9 and 12 and consequently that compounds (144a) and (144b) were both pairs of diastereoisomers travelling homogeneously on t.l.c. like compound (131). In order to determine if this was so, cyclo-L-prolyl-L-tryptophyl, (23), was treated with sodium borohydride in isopropanol under identical conditions to compound (131). A single more polar compound was formed which from micro-analytical and spectral data was found to be isomeric with the starting material. An identical product was also formed when compound (23) was treated with potassium t-butoxide in refluxing methanol. In the ^{1}H n.m.r. spectrum the proton assigned to position 12 in compound (23) had suffered an upfield shift confirming that epimerization to a trans-substituted dioxopiperazine had occurred. If epimerization of compound (23) had taken place at both positions 9 and 12 then the product would be optically inactive, being a mixture of the enantiomers cyclo-L-prolyl-D-tryptophyl, (55a) and cyclo-D-prolyl-L-tryptophyl, (55). Compound (55a) is recorded in the literature[3] as possessing a rotation of -101° and a melting point of 204-6°C, although incorrect analytical figures were supplied. The product of the epimerization of the dioxopiperazine (23) was found to be optically active with a rotation of +120° and a melting point of 191-3°C, demonstrating that it was compound (55) and that epimerization had occurred at position 12 only. By extension of these observations, the products of borohydride "reduction" of the alcohol (131) - i.e. compounds (144a) and (144b) - possess the S-configuration at position 9 and the R configuration at position 12,

resulting from epimerization of the latter position only. Similarly the base catalyzed epimerization products of compounds (10) and (58), compounds (100) and (129) respectively, are assigned the R and S configurations at positions 12 and 9 respectively.

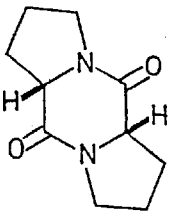


(23)

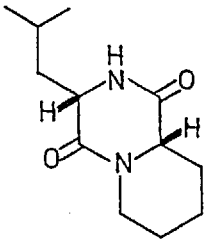


(55)

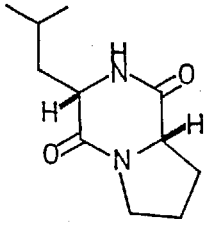
(55a) = optical antipode



(62)



(145)



(146)

Since the above work was carried out, two publications have reported investigations into the rates of epimerization of dioxopiperazines. In one[32] the rates of epimerization of cyclo-L-prolyl-L-prolyl, (62), cyclo-L-pipecolyl-L-seryl (145), and cyclo-L-prolyl-L-seryl, (146), in the presence of caustic soda were studied. It was found that the unusually easy epimerization of position 12 of compounds (23), (10), (58) and (131) also occurs in the dioxopiperazines (62) and (146), but only at the prolyl asymmetric centre, whilst epimerization at the pipecolyl and seryl asymmetric centres was very slow, the rate being comparable to the rate of hydrolysis of the amide bonds.

In the second report[75] it was found that cyclo-L-prolyl-L-tryptophyl, (23), is epimerized to cyclo-D-prolyl-L-tryptophyl, (55) when treated with refluxing triethylamine in ethanol or with aqueous, ethanolic sodium hydroxide. Both of these reports are consistent with the results of base treatment of compounds (131), (23), (10) and (58) observed in this work.

It is of interest to note that base treatment of the alcohol (131) resulted in complete conversion to compounds (144a) and (144b). Similarly base catalysed epimerization of the dioxopiperazines (23), (58) and (10) resulted in the trans-substituted dioxopiperazine ring predominating at equilibrium. This is in accord with the generally observed rule that trans-substituted dioxopiperazine rings are more stable than the cis-substituted isomers.[32] The exception to this is cyclo-prolyl-prolyl, as noted on p. 124.

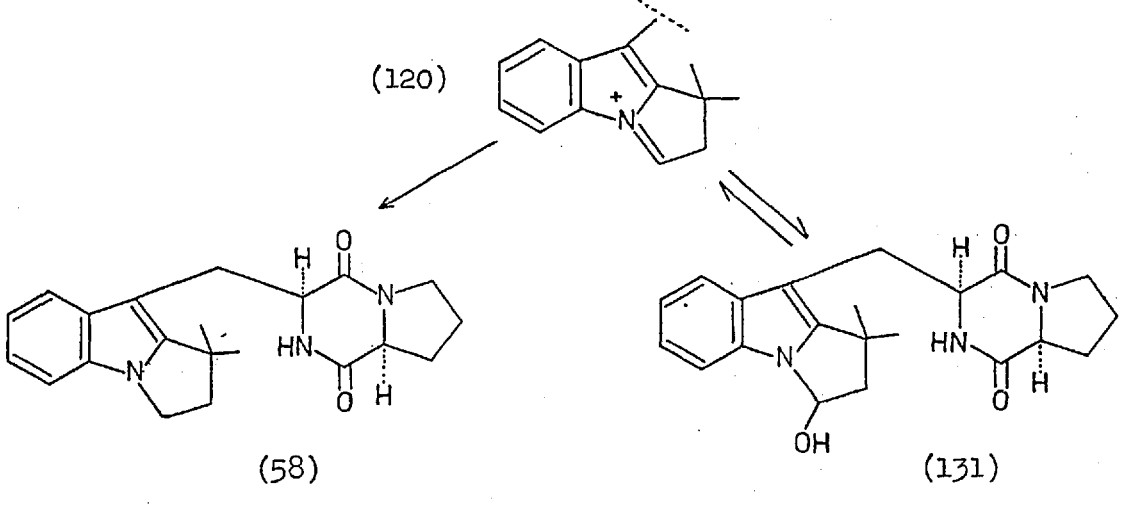
Whilst treatment of the alcohol (131) with sodium borohydride in isopropanol gave complete conversion into the diastereoisomeric alcohols (144a) and (144b), no trace of reduction products was detected. This implies that the concentration of the aldehyde (132) present in equilibrium with (131) is so low that the rate of alcoholysis of the borohydride is rapid relative to the rate of reduction. The precedent for this reaction - sodium borohydride reduction of the alkaloid (141) - took place in isopropanol under unspecified conditions and rate. Presumably the reaction did not fail in this instance because the aminol system of structure (142) is part of a 7-membered ring which would be expected to equilibrate readily with the aldehyde form (141).

Attempts were made to reduce compound (131) with sodium borohydride at lower pH in the hope that removal of the basic borohydride alcoholysis products would inhibit epimerization of the dioxopiperazine ring. However it was found that lowering the pH of an isopropanol solution of sodium borohydride below 10 caused rapid and complete decomposition of the borohydride. Consequently alternative reducing agents which are not too basic but which are capable of reducing aldehyde functions were sought.

Calcium borohydride[76] and zinc borohydride[77] are complex metal hydrides whose use as reducing agents in organic synthesis is not well

known. Both are reported to reduce under neutral conditions and zinc borohydride is claimed to be of similar reducing power to sodium borohydride whilst calcium borohydride is said to be more active. With cyclo-L-prolyl-L-tryptophyl, (23), calcium borohydride in tetrahydrofuran did not cause epimerization of the dioxopiperazine ring but instead resulted in extensive decomposition and formation of a number of unidentified compounds. Treatment of the alcohol (131) with the same reagent gave a similar result; no epimerization of position 12 to give compounds (144a) and (144b) was observed but t.l.c. indicated the formation of many products of similar or higher R_F than the starting material. Since the desired product, the alcohol (133), would almost certainly be more polar than the starting material and because of the small quantities of the latter available, rigorous identification of the reaction products was not attempted; however the ^1H n.m.r. spectrum of the crude reaction mixture confirmed that no discernible amounts of the alcohol (133) had been formed.

Treatment of compound (131) with zinc borohydride in ether/dichloromethane resulted in neither epimerization or reduction to the alcohol (133) and the only compound formed in any amount was identified as the pyrrolo-indole (58). It was presumed that zinc borohydride is a sufficiently good Lewis acid to cause formation of the iminium (120) which is then rapidly reduced to the observed product.

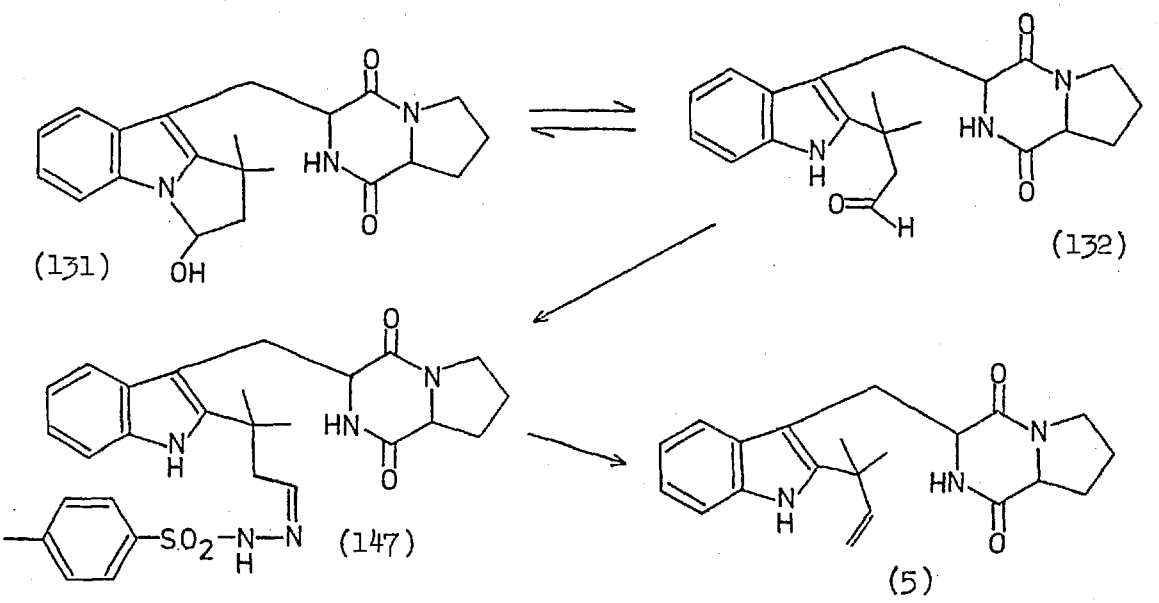


The apparent resistance of the aminol (131), or rather its tautomer the aldehyde (132), to reduction by hydrides demanded the use of alternative methods. Aldehydes are known to be reduced by catalytic hydrogenation[78] and by sodium in liquid ammonia[79] and accordingly these reagents were tried. Atmospheric pressure hydrogenation of the aminol (131) in ethanol in the presence of Adam's catalyst and acetic acid resulted in slow formation of the pyrrolo-indole (58), presumably because acid catalysed dehydration of the starting material occurs to give the intermediate iminium (120), which is then rapidly reduced. This interpretation was supported by the fact that when the hydrogenation reaction was repeated in the presence of sodium hydrogen carbonate rather than acetic acid, no reaction whatsoever was observed even after extended periods.

Before the reduction of the aminol (131) was attempted with sodium in liquid ammonia, the stability of the dioxopiperazine ring system towards the reaction conditions were investigated. It was found that cyclo-L-prolyl-L-tryptophyl, (23), was unaffected by dissolution in ammonia, ammonia containing dissolved sodium or ammonia containing sodium to which acetone had been added. These experiments indicated that the dioxopiperazine ring is not reduced under the reaction conditions, nor is it epimerized or cleaved by the basic species present (viz. ammonia or ketone reduction products). With the stability of the rest of molecule assured, the reduction of the aminol system of compound (131) was attempted. A sample of the aminol was dissolved in liquid ammonia at its boiling point and excess sodium was slowly added until the blue colour of the dissolved sodium became permanent. Excess sodium was rapidly destroyed by addition of acetone and the resulting sodium salts were quenched by addition of ammonium chloride. Following work-up and p.l.c. two compounds were isolated, one of which was found to be

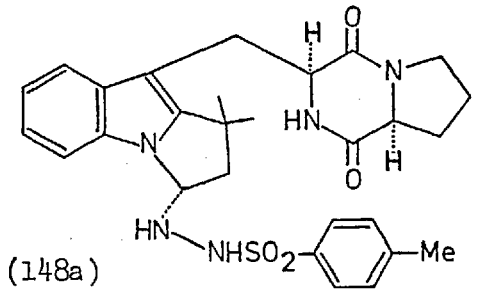
starting material and the other compound (58). Formation of the pyrroloindole (58) implies either that the desired alcohol (133) is being formed but cyclizes or that reduction of the iminium (120) is occurring.

The failure to observe reduction of the aminol (131) was attributed to the low concentration of the aldehyde tautomer (132) presumed to be in equilibrium and so it was decided to try and trap out the aldehyde as a simple derivative such as the hydrazone or dithioacetal. Conversion of the aldehyde (132) into the tosyl hydrazone (147) would, it was hoped, allow the preparation of desoxybrevianamide E, (5), via a Bamford-Stevens reaction.[80]

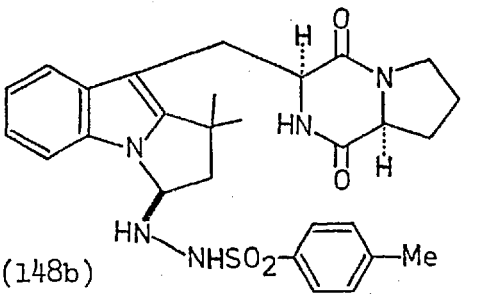


Preparation of the hydrazone (147) was attempted by treatment of a solution of the acetate (119) with an excess of tosyl hydrazine hydrochloride in the presence of a catalytic amount of trifluoroacetic acid. T.l.c. indicated the immediate disappearance of the starting material and the formation of two, more polar products which were isolated by p.l.c. following an aqueous work-up. The ^{1}H n.m.r. spectrum of the less polar product confirmed the presence of a tosyl group (AA'BB' quartet centred around τ 2.5 and a methyl singlet at τ 7.6) as did the

i.r. spectrum (sulphonamide S = O stretch at 908 and 1160 cm^{-1}). However the absence of an indolic NH resonance suggested that the compound was not the desired hydrazone (147). The presence in the ^1H n.m.r. spectrum of a pair of non-equivalent methyl singlets implied, by analogy with structures (58), (131) and (144), that the product still possessed the pyrrolo-indole part structure of the starting material and that the tosyl hydrazine had simply displaced the acetate, presumably *via* the iminium (120), to give structure (148). The proton at position 5', seen at τ 4.62 in (131) and 3.66 in (119) was not visible in the ^1H n.m.r. spectrum of the product but was concealed under signals at *ca* τ 5.5. Application of Shoolery's rules show that this is consistent with the proposed structure (148).



(148a)



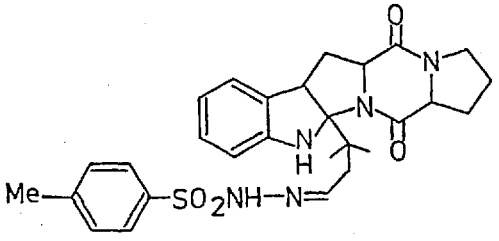
(148b)

The more polar product of the reaction possessed ^1H n.m.r. and i.r. spectra very similar to that of the less polar product. The two products of the reaction were therefore identified as the diastereoisomeric hydrazides (148a) and (148b), epimeric around position 5'.

Initially some difficulty was experienced in the separation of the hydrazides (148a) and (148b) because of their thermal instability. Mild warming of methanolic solutions of these compounds (for example during removal of solvent *in vacuo*) was found to result in partial or even complete decomposition, as did attempted crystallization from hot carbon tetrachloride. When a sample of either isomer was heated *in vacuo* at 160°C for 5 minutes, complete and clean decomposition to a slightly less polar compound occurred which was shown to be identical

to compound (58). The fate of the tosyl group in these reactions was not investigated. The mass spectra of compounds (148a) and (148b) were identical and exhibited extremely weak molecular ions at 535 mass units. Weak fragment peaks were also visible corresponding to loss of tolyl and tosyl groups and strong peaks were present at 351 and 198 (base peak) mass units. Below the fragment at 351 mass units the spectra were virtually identical to that of compound (58) (M.W. 351) superimposed by peaks at 155 and 91 mass units which can be assigned to the toluene-p-sulphonyl and tropylium ions respectively. The spectra are therefore consistent with rapid decomposition of compound (148) in the mass spectrometer, possibly prior to volatilization of the sample.

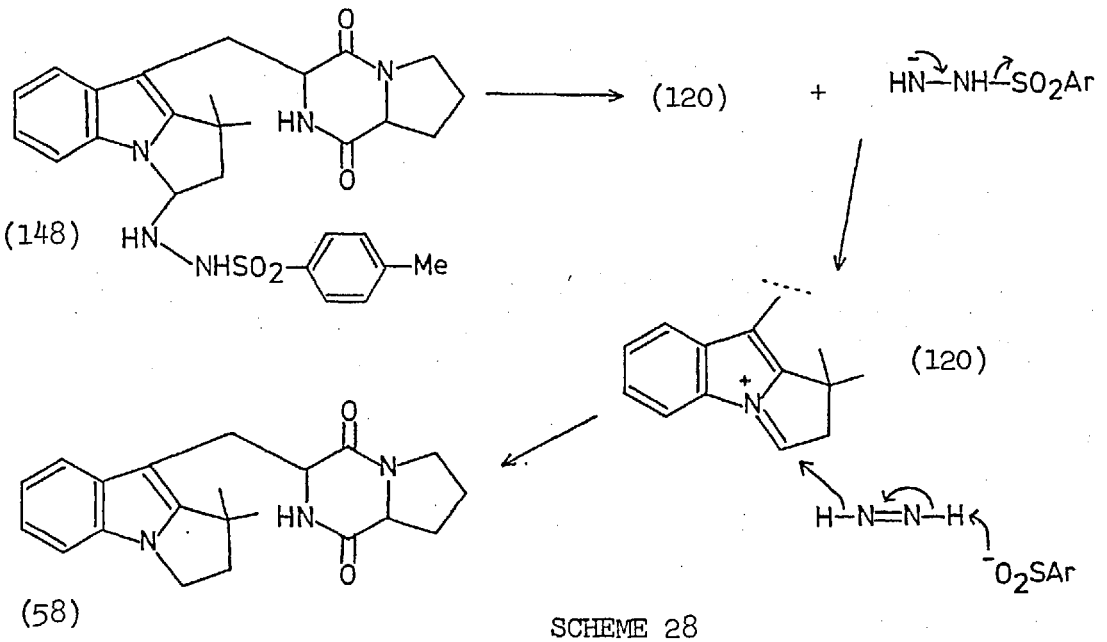
The easy decomposition of the hydrazide (148) to the pyrroloindole (58) is perhaps not too surprising in view of the ready conversion of benzenesulphonyl hydrazides into the parent hydrocarbon on warming with alkali[81] (e.g. scheme 27). A plausible mechanism for such transformations is shown in scheme (28). The mechanism is



(149)

supported by the fact that tosyl hydrazine has been used to generate diimine (N_2H_2), either by the action of heat[82] or in the presence of base,[83] under conditions comparable to those described for the decomposition of compound (148). Furthermore diimine is known to be a good reducing agent for polarized double bonds such as that in the iminium (120).[84,92]

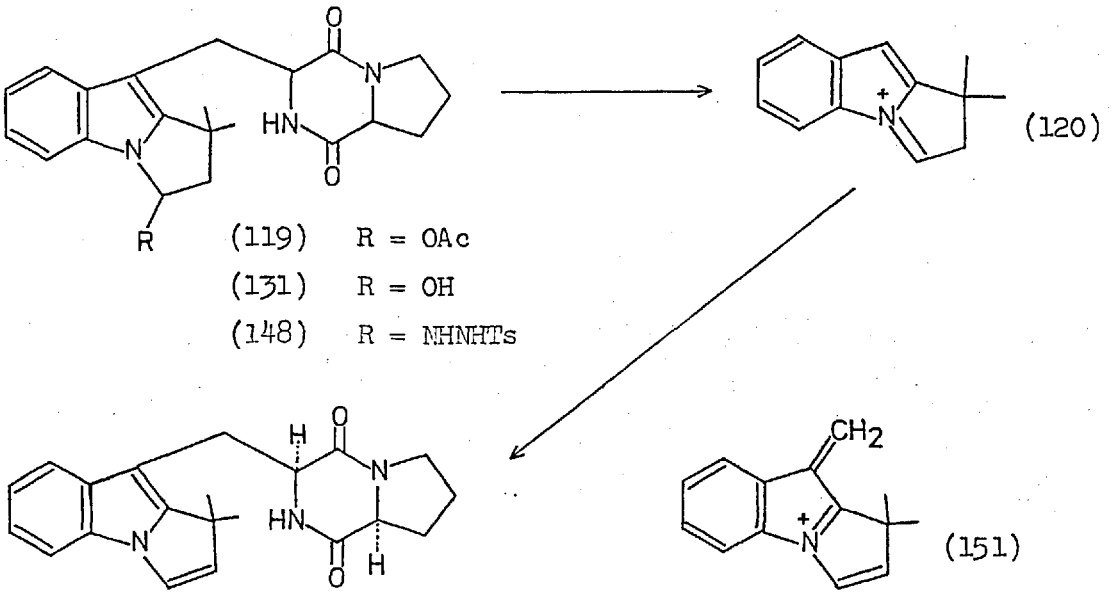
SCHEME 27 $PhSO_2NHNHPh \longrightarrow C_6H_6 + N_2 + PhSO_2H$



SCHEME 28

Following the failure of the attempted preparation of the hydrazone (147), alternative reaction conditions were investigated. Thus the acetate (119) was treated with tosyl hydrazine hydrochloride using trifluoroacetic acid as solvent. It was hoped that any of the hydrazone (147) present in equilibrium with the hydrazine (148) would undergo protonation of the indole nucleus at position 3 and hence cyclize to give the indoline (149), by analogy with the formation of compounds (36) and (57) from (23) and (10).

In the event, rapid disappearance of the starting material was observed but none of the desired compound was detected. Following aqueous work-up the only major product present was isolated by p.l.c. and identified as the enamine (150). The product is consistent with protonation and elimination of the acetoxy group of (119), or the tosyl hydrazine moiety of compound (148), to give the iminium intermediate (120), which in the reaction medium is encouraged to deprotonate to yield compound (150). This was found to be a general reaction for any derivative of the pyrrolo-indole (58) bearing a substituent in position 5' capable of becoming a leaving group following protonation.



(119) R = OAc
(131) R = OH
(148) R = NHNHTs

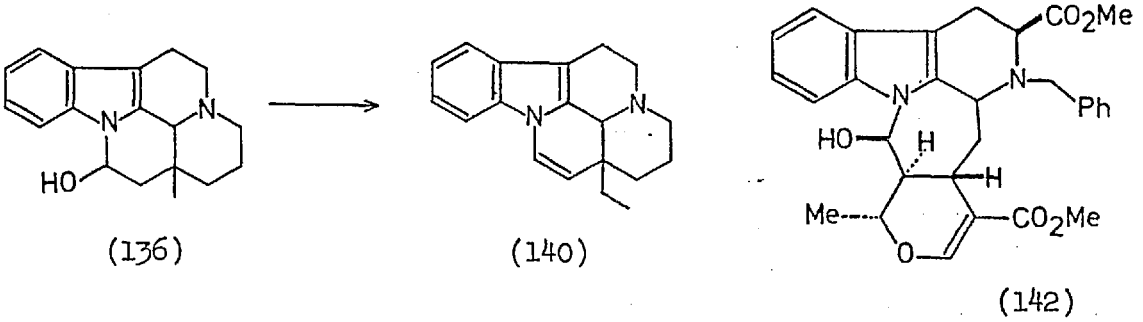
(120)

(150)

(151)

Thus compound (150) was formed when compounds (148), (131) and (119) were dissolved in trifluoroacetic acid. Compound (150) was isolated in rather low yield from these reactions and this was attributed to the instability of the enamine system to acidic conditions in the presence of potential nucleophiles. It was found later that the enamine could be prepared in quantitative yield from the alcohol (131) by refluxing in benzene in the presence of tosic acid and removal of the water produced by distillation of the azeotrope.

The assignment of structure (150) to the product was made on the basis of its ^{1}H n.m.r. spectrum which exhibited an AB quartet centered around τ 3.4 corresponding to the new olefinic protons. The mass spectrum possessed the correct molecular ion at 349 mass units and the base peak was of mass 196, attributed to the characteristic indolylmethylene unit (151).

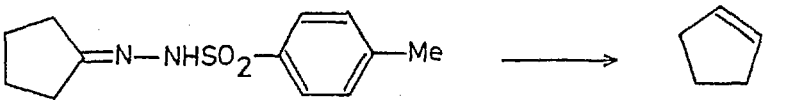


(136)

(140)

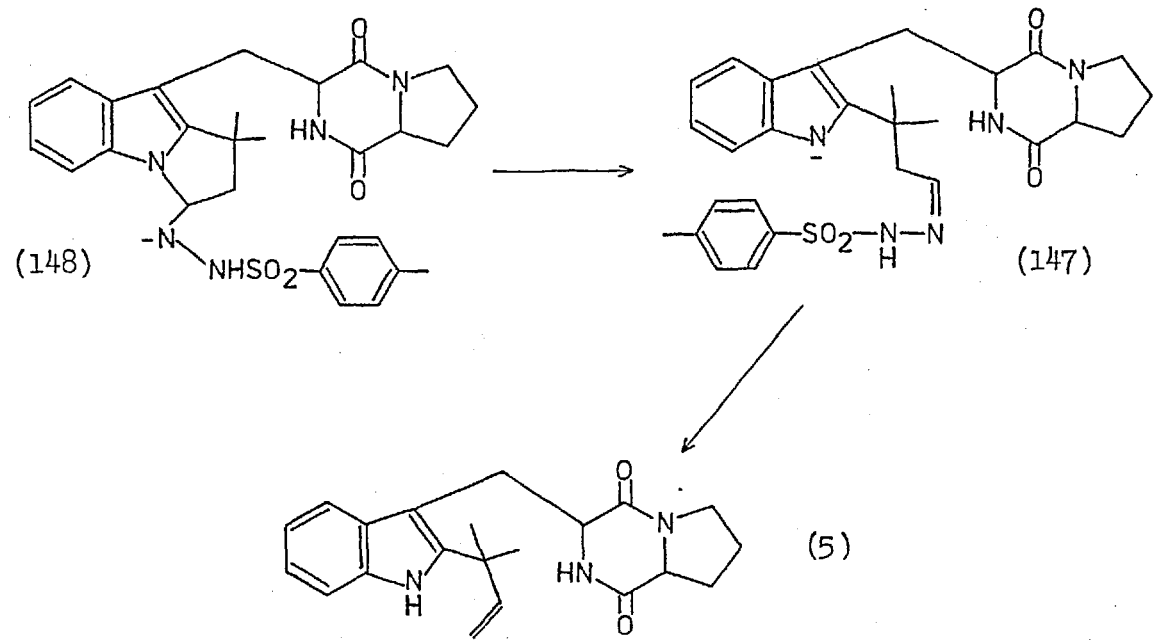
(142)

Formation of the enamine (150) has some precedent; eburnamine, (136), eliminates water when heated in acetic acid to give eburnamenine,[73] (140), and the descarbomethoxy-desbenzyl derivative of compound (142) is reported to eliminate water in trifluoroacetic acid to give the analogous enamine.[85]



SCHEME 29

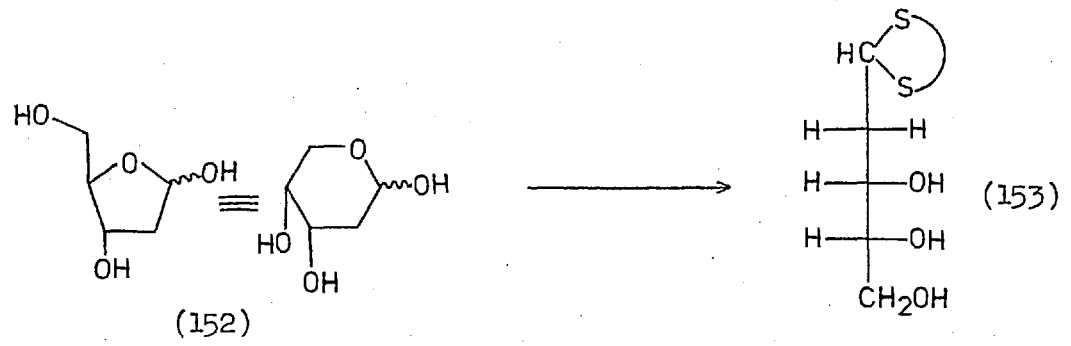
The original Bamford-Stevens reaction required strong heating of a tosyl hydrazone with a base (typically a sodium alkoxide) to yield the olefin[80] (Scheme 29). Recent modifications of the reaction have utilized strong alkyl lithium bases at room temperature, to the same end.[86] It seemed reasonable that if the hydrazide (148) was treated in this way, the resulting anion might equilibrate with the anion of the hydrazone (147) and hence decompose to the desired olefin, desoxybrevianamide E, (5). The observed instability of the hydrazide to heat and the instability of the dioxopiperazine ring to normal



bases prohibited the use of the original Bamford-Stevens conditions; however it was hoped that using the modified alkyl lithium base system, the desired reaction could occur.

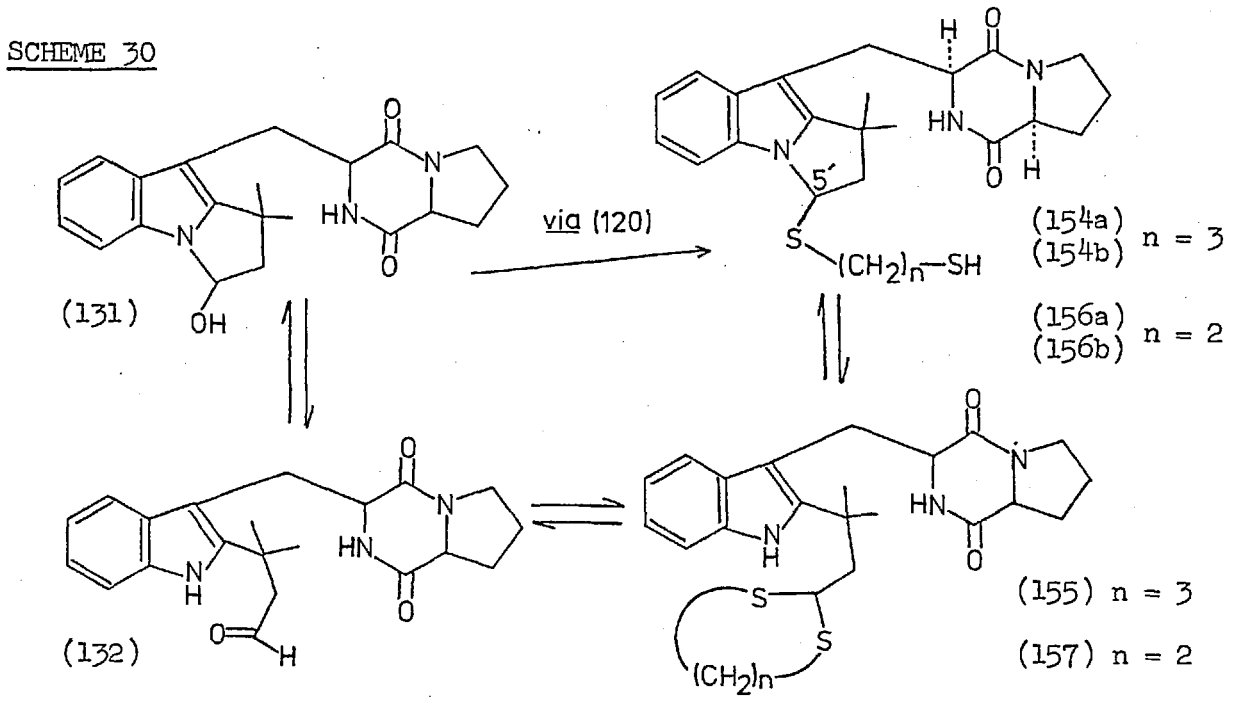
When a suspension of the hydrazide (148) in ether was treated with four equivalents of methyl lithium, initially at -10°C and then at room temperature, t.l.c. indicated slow disappearance of starting material and the formation of no discrete products. After all the starting material had been consumed, the ^{1}H n.m.r. spectrum of the worked-up reaction mixture was recorded. This was consistent with the t.l.c. evidence, i.e. decomposition and polymerization of the hydrazide and formation of no single product in any quantity. The reaction presumably failed due to the instability of the dioxopiperazine ring to the basic conditions and the presence in the reacting molecule of a number of protons of similar acidity to those whose removal was required, thus initiating many side reactions.

Following the failure of hydrazone formation by the aminol (131), an alternative mode of trapping out the aldehyde function of its tautomer (132) was sought. The open chain aldehyde tautomer of a reducing sugar such as 2-desoxyribose has been trapped out as its phenyl hydrazone,[87] the same principle behind the method which failed here. It has also been trapped out as its dithioacetal.[87,88] Thus treatment of 2-desoxyribose (152) with a dithioalkane under conditions of acid catalysis gave[89] the dithioacetal (153). Since, under acidic conditions, a thiol is rather more nucleophilic than nitrogen, it was felt that a dithiol might be capable of trapping out the aldehyde (132) as its dithioacetal where hydrazone formation had failed (scheme 30). Dithioacetals have been successfully converted to olefins by treatment with deactivated Raney-nickel or nickel boride as shown in scheme 31[93] and application of this reaction to compound (155) or (156) would lead to desoxybrevianamide E, (5).

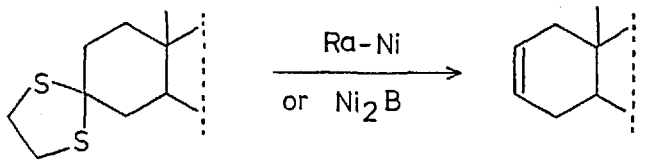


(152) (153)

SCHEME 30



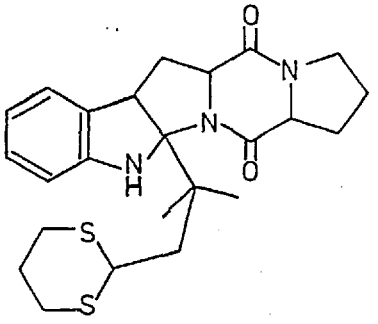
SCHEME 31



Treatment of the alcohol (131) with propanedithiol in dichloromethane in the presence of a catalytic amount of trifluoroacetic acid resulted in rapid disappearance of starting material and the formation of two, less polar compounds. Following an aqueous work-up and p.l.c., the two products were identified as the sulphides (154a) and (154b), epimeric around position 5'. The structural assignments were made by inspection of the spectral data and their comparison with spectra of the hydrazides (148). Thus neither of the sulphides possessed an

indolic NH signal in their ^{1}H n.m.r. spectra and a double doublet at τ5.5 was observed corresponding to the methine at position 5'. The methyl groups appeared as non-equivalent singlets at τ 8.2 and 8.3; this is consistent with their being attached to a 5-membered ring rather than to a free side chain, as in the dithioacetal (155) where they would probably resonate as a singlet. A thiol stretching band was also detected at 2450 cm^{-1} in the i.r. spectra of both products.

(158)



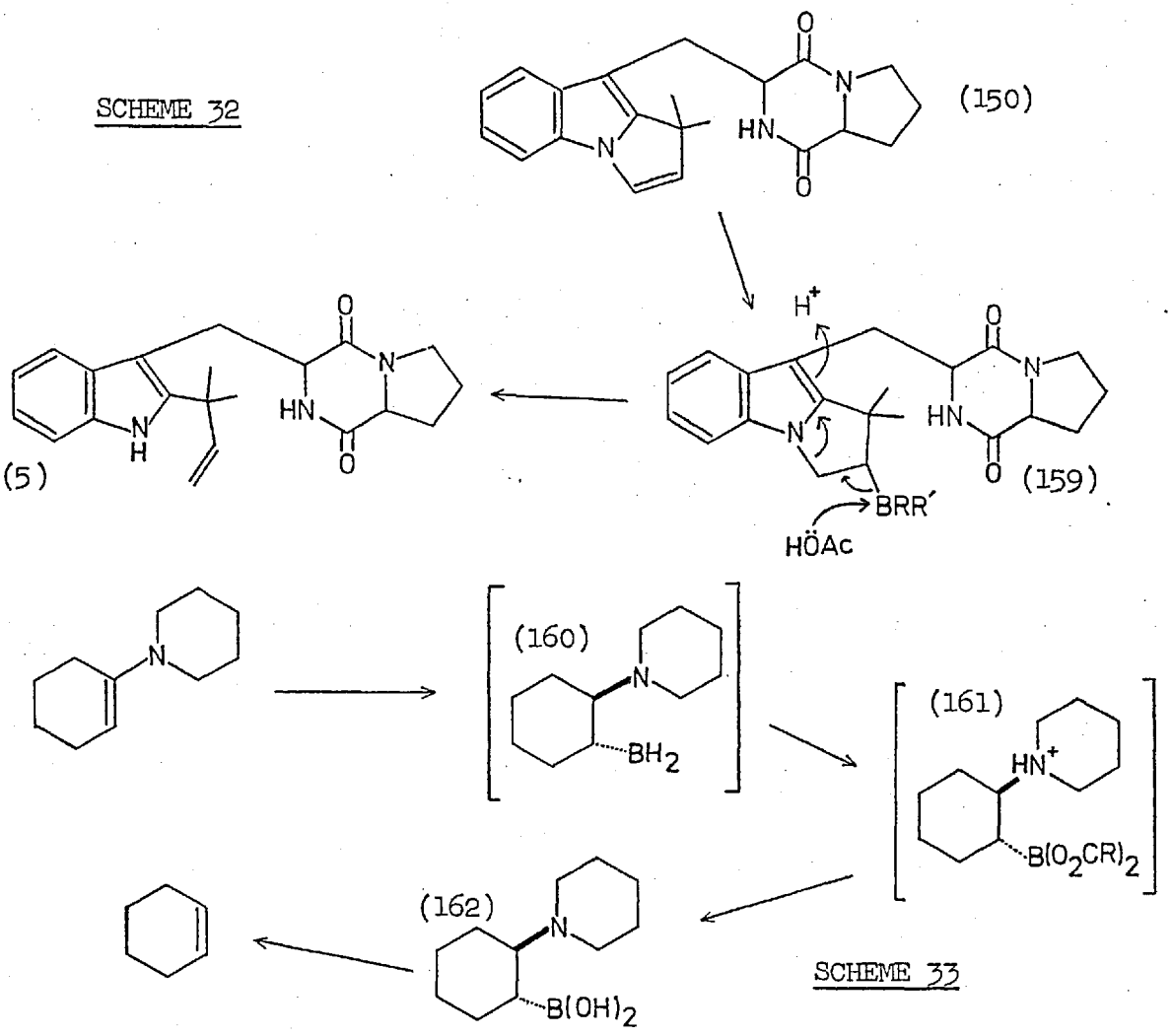
It was felt that since sulphur is a better nucleophile than nitrogen under basic conditions, the thiol group of compound (154) should compete effectively with the indole nitrogen for position 5' to give the dithioacetal, (155). Accordingly compound (154) was treated with triethylamine in dichloromethane but after 18 hours no change had occurred indicating that if an equilibrium exists between compounds (154) and (155) then it does not favour the latter. By analogy with the acid treatment of the hydrazide (148), compound (154) was also treated with trifluoroacetic acid. It was hoped that the acid would protonate position 3 of the indole, converting the nitrogen atom into a good leaving group and facilitating attack of the thiol upon position 5' to give either compound (155) or the indoline (158). In the event, however, the reaction failed and the major products were the enamine (150) and the aminol (131). These are presumably formed from the iminium intermediate (120), the latter being produced on work-up.

The reaction of the aminol (131) with a dithiol was repeated using ethanedithiol rather than propanedithiol and with boron trifluoride as the catalyst instead of trifluoroacetic acid. The new catalyst resulted in a much cleaner reaction but unfortunately the only products were the two thiols (156a) and (156b), epimeric around position 5'. The two compounds were identified from their spectral data which were very similar to their homologues, compounds (154a) and (154b). It had been hoped that the stability of a 5-membered ring containing two sulphur atoms would be greater than that of the corresponding 6-membered ring derived from propanedithiol, so that an equilibrium between the dithioacetal (157) and compound (156) would favour the former.

The action of heat upon the thiols (154a) and (154b) was investigated. Heating to reflux in dioxan, benzene or tetrahydrofuran resulted in extensive decomposition in all cases, suggesting perhaps that elimination of the dithiol to give the iminium (120) was occurring. To combat this, or at least to direct the fate of the intermediate (120), compounds (155a) and (155b) were heated in ethanedithiol at 82°C. Under these conditions no reaction occurred implying either that this temperature is not high enough to convert the thiol (156) into the dithioacetal (157), or that if an equilibrium exists between the two then the former compound is favoured.

The final attempt to prepare desoxybrevianamide E, (5), to be described in this thesis was <u>via</u> the route shown in scheme 32. The intermediate in this pathway, structure (159), results from electrophilic attack of diborane upon the enamine system of compound (150) where the residues R and R' are either a proton or a second pyrroloindole unit. The precedent for this reaction is illustrated in scheme 33; reduction of the piperidine enamine of cyclohexanone with diborane

SCHEME 32



SCHEME 33

gave the presumed intermediate organoborane (160) which when heated with a carboxylic acid produced a high yield of cyclohexene. Treatment of the organoborane with a carboxylic acid at room temperature followed by addition of water gave the boronic acid (162), probably *via* the ester (161). Compound (162) was an isolable, crystalline solid which decomposed on heating to yield cyclohexene.[94,95]

When the enamine (150) was treated with one equivalent of diborane in tetrahydrofuran, t.l.c. of the product following thermolysis with acetic acid indicated that much of the starting material remained and that a number of products had been formed. Repetition of the reaction with several equivalents of diborane gave a similar result; the major component of the product mixture was compound (150) and of the many

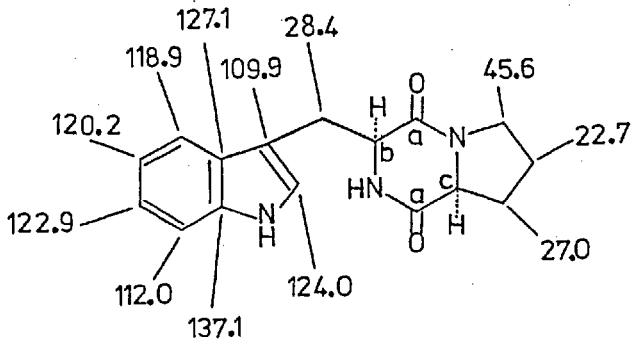
products formed, no one predominated. Because of the small amount of enamine available and its lengthy preparation, the minor products of the reaction were not investigated but it is probable that at least some of them result from reduction of the amide and indole systems as well as the enamine function. Amide groups are readily reduced by diborane and steric hindrance of the enamine system of compound (150) by the geminal methyl groups may reduce the rate of desired reaction to the point where alternative reduction pathways can compete effectively.

^{13}C n.m.r. spectra of cyclo-prolyltryptophyl derivatives

The ^{13}C n.m.r. spectral assignments for 7 indole and 6 indoline derivatives isolated in the course of the work described in this thesis are given in tables 6 and 7. The spectra were all recorded in deuteriochloroform and chemical shifts are quoted in parts per million from tetramethylsilane. The spectral assignments of the indolyl carbon atoms of compounds in table 6 are based upon the data reported for methyl substituted indoles which is summarised in tables 8 and 9.[87,96,97,98] Assignments for the dioxopiperazine rings of compounds in tables 6 and 7 are made by comparison with the values reported for cyclo-L-tryptophyl-L-tryptophyl, cyclo-glycyl-L-tryptophyl, cyclo-L-leucyl-L-tryptophyl and related compounds.[37,100,101] No ^{13}C n.m.r. spectra of proline derived dioxopiperazines have been reported and consequently assignments in the prolyl rings of compounds in tables 6 and 7 were made by comparison with the published spectra of proline containing peptides.[99] The spectra described in tables 6 and 7 were all recorded on a Jeol FX 60 spectrometer in fully decoupled, partial off-resonance and alternately pulsed ("Gated") modes; the multiplicities of the signals in the non-fully decoupled spectra were in all cases consistent with the assignments made.

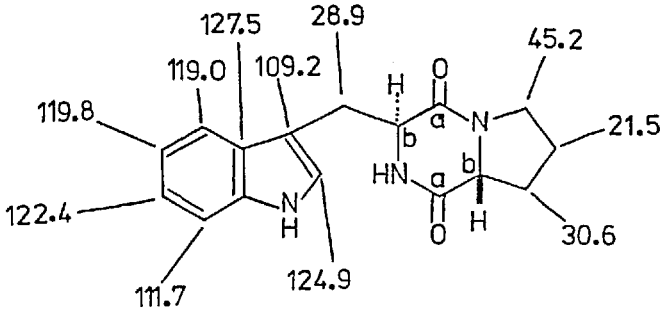
Table 6

(23)



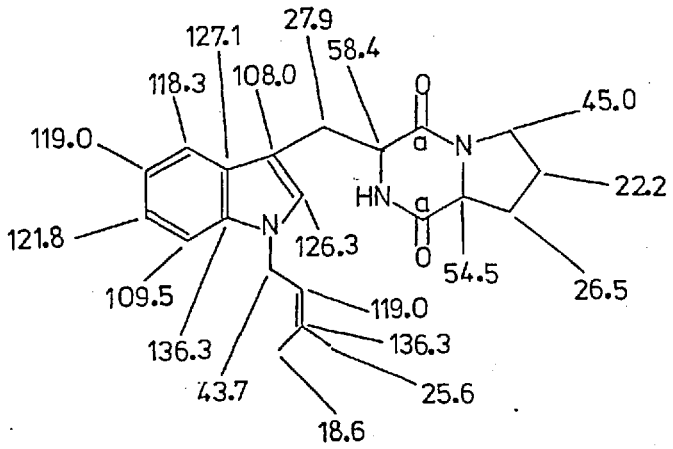
a = 169.9, 166.0
b = 59.4
c = 54.9

(55)



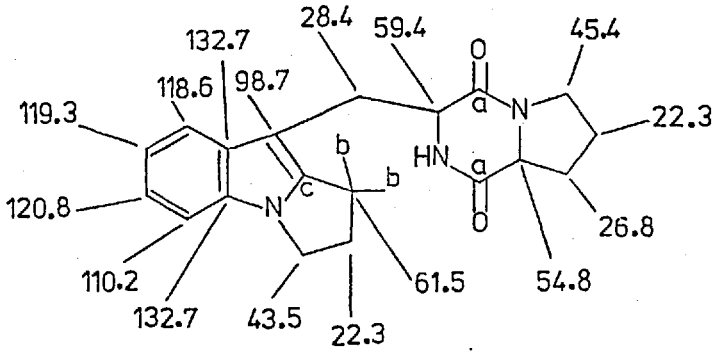
a = 170.3, 166.3
b = 58.3
c = 58.1

(10)



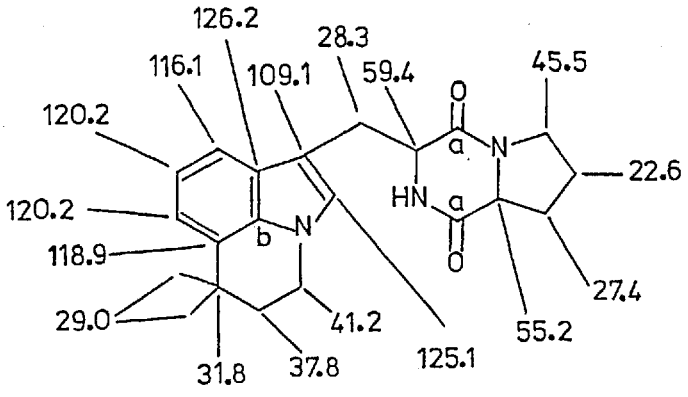
a = 168.9, 165.2

Table 6 (Continued)



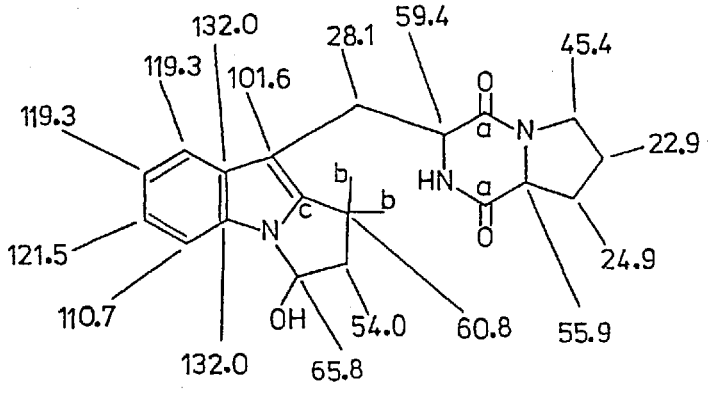
(58)

a = 169.8, 166.1
b = 26.8
c = 143.1



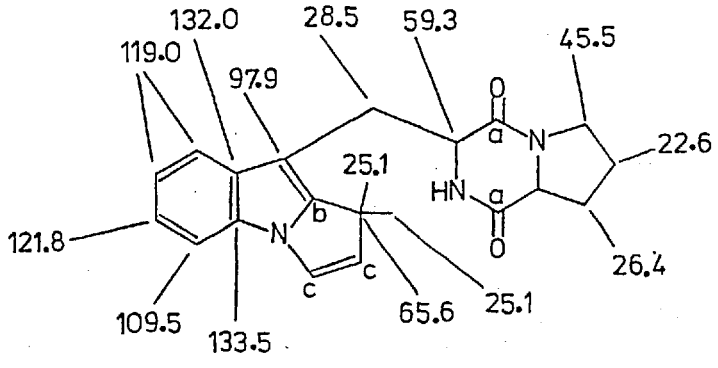
(59)

a = 169.3, 165.8
b = 131.2



(131)

a = 170.6, 166.5
b = 28.1
c = 143.9

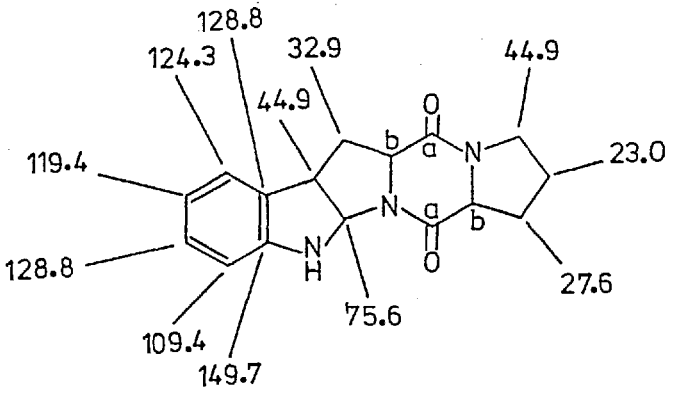


(150)

a = 169.5, 165.7
b = 145.5

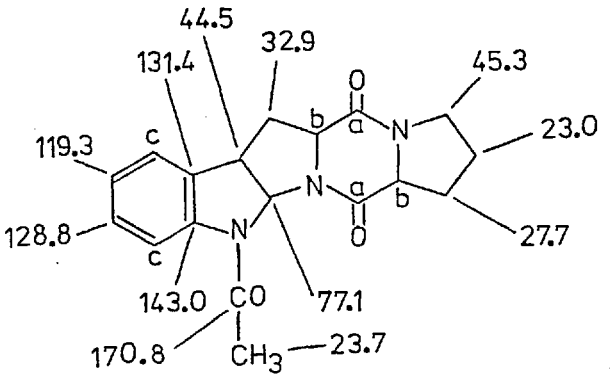
Table 7

(36)



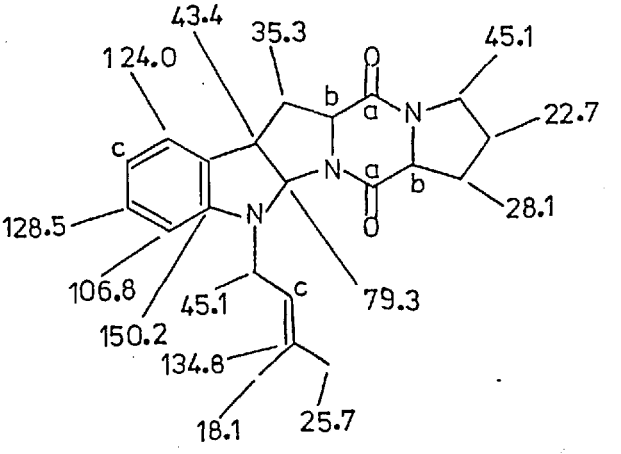
a = 167.2, 166.3
b = 60.5, 59.6

(51)



a = 166.9, 165.6
b = 60.5 59.9
c = 125.1 123.7

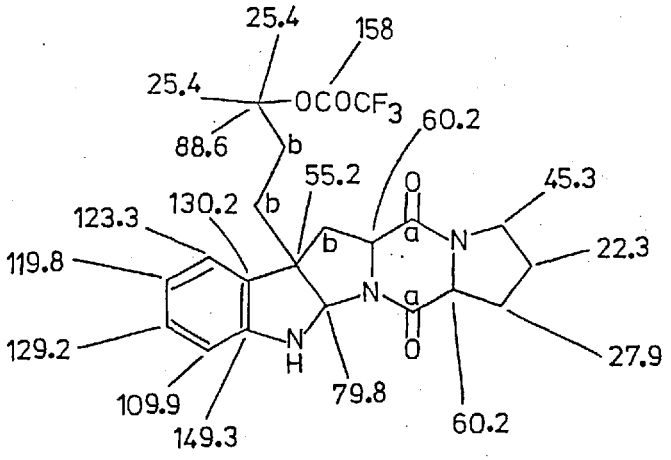
(57)



a = 165.9 165.7
b = 60.7 58.9
c = 120.6 118.0

Table 7 (Continued)

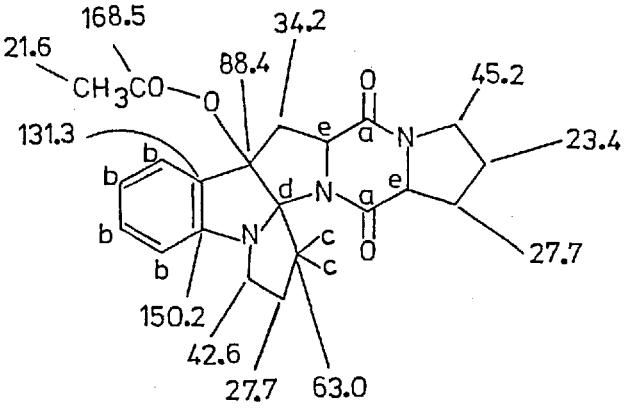
(94)



a = 167.2, 165.9

b = 39.1, 35.7, 32.4

(115)



a = 166.5, 165.6

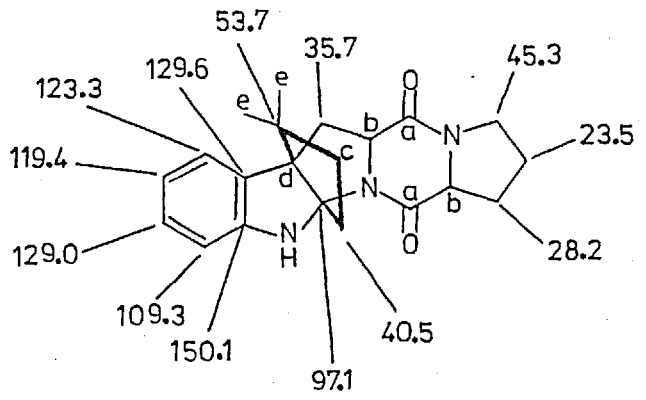
b = 131.3, 130.9, 127.6, 121.2

c = 24.8, 30.3

d = 100.2

e = 59.6, 60.8

(88)



a = 167.0, 166.5

b = 61.1, 62.1

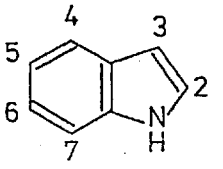
c = 43.8

d = 70.4

e = 23.5, 28.2

Table 8

^{13}C shieldings of indole and some methyl derivatives (p.p.m. from T.M.S.)



Substituent	C_2	C_3	C_4	C_5	C_6	C_7
H	125.2	102.6	121.3	120.3	122.3	111.8
2-Me	[135.7]	100.4	(120.0)	(119.9)	121.1	110.9
3-Me	122.7	[111.4]	(119.4)	(119.6)	122.3	111.7
4-Me	124.2	101.1	[130.2]	120.1	122.2	109.3
5-Me	125.0	102.1	120.8	[128.8]	123.7	111.3
6-Me	124.3	102.3	(120.7)	(121.9)	[131.5]	111.6
7-Me	124.8	103.0	(118.9)	(120.3)	122.7	[120.9]
1,2-diMe	[138.1]	100.0	(120.0)	(119.6)	120.7	109.2
2,3-diMe	[131.4]	[106.8]	118.4	119.3	121.1	110.7
2,7-diMe	[135.1]	101.0	117.7	119.8	121.7	[119.9]
2,3,5-triMe	[131.2]	[106.3]	118.1	[127.8]	122.5	110.2
2,5,7-triMe	-	100.5	117.1	[128.9]	123.2	[118.9]

[ ] = substituted carbon

( ) = reverse assignment possible

Table 9

Methyl substituent effects in indole ring systems (p.p.m.)

Substituent	C_2	C_3	C_4	C_5	C_6	C_7
2-Me	[10.5]	-2.2	-1.3	-0.4	-1.2	-0.9
3-Me	-2.5	[8.8]	-1.9	-0.7	0.0	-0.1
4-Me	-1.0	-1.5	[8.9]	-0.2	-0.1	-2.5
5-Me	-0.2	-0.5	-0.5	[8.5]	1.4	-0.5
6-Me	-0.9	-0.3	(-0.6)	(1.6)	[9.2]	-0.2
7-Me	-0.4	0.4	(-2.4)	(0.0)	0.4	[9.1]

[ ] = substituted

( ) = tentative

The ^{13}C n.m.r. spectra of the cis- and trans-substituted dioxopiperazines (23) and (55) show only small differences except for signals due to carbon atoms at positions 12 and 17. This presumably reflects the different folded conformations of the two molecules; in compound (55) a fully folded conformation causes shielding of positions 12 and 17, resulting in the observed upfield shift of these carbon atoms compared with compound (23), which is not free to adopt a completely folded conformation.

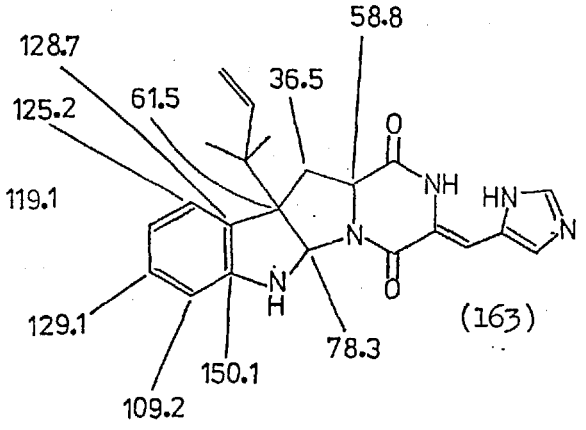
Application of the substituent parameters quoted in table 9 to the N-allylindole (10) allows prediction of the ^{13}C chemical shifts in its Friedel-Crafts cyclization products, compounds (58) and (59). The calculated and found values are as follows:

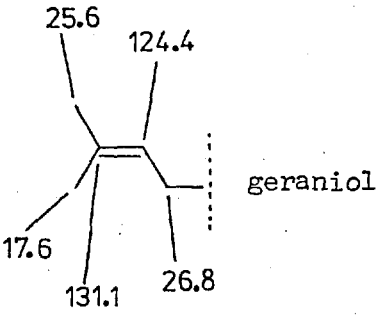
		C_2	C_3	C_4	C_5	C_6	C_7
(10)		126.3	108.0	118.3	119.0	121.8	109.5
(58)	calculated:	136.8	105.8	117.0	118.6	120.6	108.6
	found:	143.1	98.7	118.6	119.3	120.8	110.2
(59)	calculated:	125.9	108.4	115.9	119.0	122.2	118.6
	found:	125.1	109.1	116.1	120.2	120.2	118.9

Reasonably close agreement between the calculated and observed values is obtained, confirming the structural assignments of compounds (58) and (59). The poorest agreement occurs for those carbon atoms close to the point of substitution; this effect has been noted previously.[97]

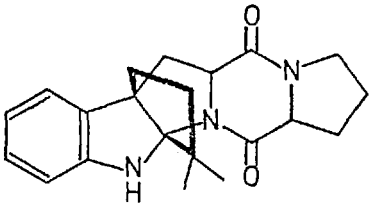
The assignments in the aromatic rings of the compounds in table 7 were made largely by analogy with those of the natural product roquefortine, (163), whose ^{13}C n.m.r. spectrum has been reported.[102] The chemical shifts of the aromatic carbon atoms of compound (115) do not correlate with those of the other indolines in table 7, presumably because of the perturbing effect of the acetoxy group. The signals corresponding to the N-dimethylallyl groups of compounds (10) and (57)

were assigned by comparison with the ^{13}C n.m.r. spectrum of geraniol and related compounds.[103,104]

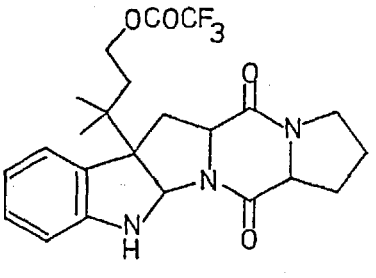




The chemical shifts, and multiplicities in off-resonance spectra, of the signals corresponding to carbon atoms at positions 2 and 3 of the indoline system of the compounds in table 7 confirm the positions of substitution of the rearranged dimethyl allyl unit in compounds (88), (94) and (115). Thus compound (94) is seen to bear the trifluoroacetoxylated dimethylallyl group at position 3 and in the configuration shown, whilst the alternative structure, (95), is ruled out. Similarly compound (88) is confirmed as bearing a substituent at both positions 2 and 3, although it cannot be distinguished from structure (89). In



(89)



(95)

the spectrum of compound (94), the presence of a trifluoroacetoxy group is verified by the observation of the carbonyl resonance at approximately 158 p.p.m. as a quartet (J_{CCF} = 41 Hz), split by coupling with the trifluoromethyl group. The signals corresponding to the trifluoromethyl

carbon atom, seen as a quartet (J_{CF} = 283 Hz) centred around 114 p.p.m. in the ^{13}C n.m.r. spectrum of trifluoroacetic acid, were lost underneath other peaks in the spectrum of compound (94).

EXPERIMENTAL

Melting points were determined on a Kofler hot stage apparatus and are uncorrected.

Infra-red (i.r.) spectra were recorded for Nujol mulls applied to sodium chloride plates or for solutions in sodium chloride cells on a Pye-Unicam SP 1000 spectrophtometer unless otherwise stated.

Ultra-violet (u.v.) spectra were recorded for ethanolic solutions in silica cells with a Pye-Unicam SP 800 instrument, unless otherwise stated.

Proton nuclear magnetic resonance (^{1}H n.m.r.) spectra were recorded for solutions in deuteriochloroform ($CDCl_3$) with tetramethylsilane (T.M.S.) as internal reference. All 60 MHz spectra were recorded on a Varian T60 instrument and 100 MHz spectra were recorded on a Jeol MH 100 spectrometer. The following abbreviations are used in connection with n.m.r. data:

s = singlet

d = doublet

dd = double doublet

t = triplet

q = quartet

m = multiplet

b = broadened

exch. = disappears on addition of D_2O

Coupling constants are quoted as J values in Hz.

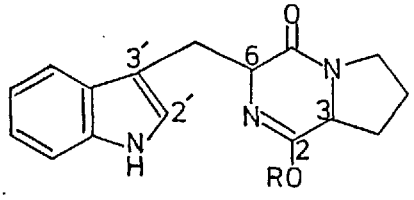
Mass spectra were recorded on an A.E.I. MS9 or by the Physical and Chemical Measurements Unit (P.C.M.U.), Harwell.

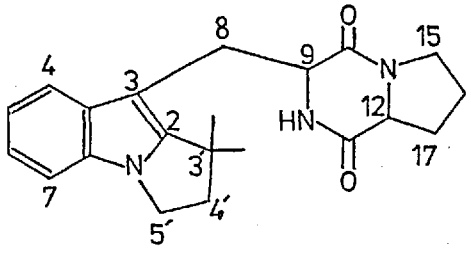
Thin layer chromatography (t.l.c.) was carried out on Kieselgel GF_{254} (Merck) and, unless otherwise stated, chromatograms were developed in chloroform or dichloromethane containing 1-10% methanol (v/v). Chromatograms were visualized using medium and low pressure mercury

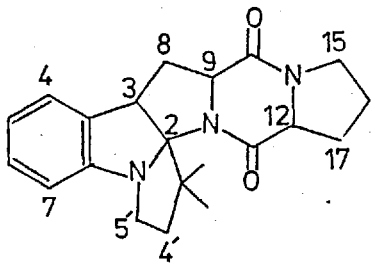
u.v. sources, iodine vapour, sulphuric acid or Urk's reagent.[49] Preparative scale t.l.c. (p.l.c.) was carried out on plates spread to a depth of 1 mm ("thick plates") or 0.25 mm ("thin plates") under similar conditions.

Dichloromethane and carbon tetrachloride were purified by distillation from calcium hydride. Boron trifluoride etherate and dimethylformamide were distilled from calcium hydride under reduced pressure. Benzene and ether were dried over sodium wire and for special purposes ether and tetrahydrofuran were redistilled from lithium aluminium hydride. Light petroleum refers to the fraction of b.pt. 40-60°C unless otherwise stated. Extracts were dried over anhydrous sodium sulphate and were evaporated under reduced pressure on a Buchi rotary evaporator.

The numbering systems used in spectral assignments are given below:







Cyclo-L-prolyl-L-tryptophyl, (23). L-proline (25g, 0.217 mol) was dissolved in methanol (250 ml) and cooled to 5°C. Thionyl chloride (20 ml, 32.8g, 0.276 mMol) was added at a rate sufficiently slow to maintain a temperature between 5 and 10°C. After completion of addition the solution was stirred for one hour at 5°C and 3 hours at 40°C. Evaporation gave an oil which was triturated with ether (3 x 70 ml) and dried *in vacuo* to give a quantitative yield of L-proline methyl ester hydrochloride as a white solid. L-tryptophan (50g, 0.246 mol) was dissolved in 1N sodium hydroxide solution (250 ml) and stirred in ice-salt. Carbobenzoxy chloride (61g, 0.35 mol) and 1N sodium hydroxide (250 ml) were simultaneously added dropwise over 15 minutes. The resulting emulsion was stirred in ice for 75 minutes and extracted with petroleum ether (3 x 250 ml). The aqueous phase was neutralized with 2N hydrochloric acid and the precipitated white solid was filtered off and washed successively with water and petroleum ether. Drying *in vacuo* over phosphorous pentoxide gave pure N-carbobenzoxy-L-tryptophan (80.3g, 0.238 mol, 96%). L-Proline methyl ester hydrochloride (24g, 0.145 mol) was dissolved in dichloromethane (200 ml) and cooled in ice-salt. Ammonia gas was bubbled through the solution for 30 minutes and the liberated ammonium chloride was filtered off. The filtrate was evaporated to remove residual ammonia and the oil obtained was taken up in dichloromethane (300 ml). N-Carbobenzoxy-L-tryptophan (46g, 0.137 mol) and dicyclohexylcarbodiimide (28g, 0.152 mol) were added and the mixture was stirred for 23 hours. The white suspension was filtered and the filtrate was washed with 2N hydrochloric acid (200 ml), saturated sodium hydrogen carbonate solution (200 ml) and water (200 ml). Drying and evaporation gave a white foam, homogeneous on t.l.c., of N-carbobenzoxy-L-tryptophyl-L-proline methyl ester (49.3g, 0.111 mol, 80% based on N-carbobenzoxy-L-tryptophan). The foam was

taken up in the minimum of ethyl acetate and left to stand in the cold. The small amount of dicyclohexylurea which crystallized out was filtered off and the filtrate was evaporated to give a foam, which was used in the next stage. N-Carbobenzoxy-L-tryptophyl-L-proline methyl ester (17g, 37.9 mmol) was dissolved in methanol (200 ml) and palladized charcoal (1g containing 5% palladium) and acetic acid (10 drops) were added. The suspension was hydrogenated at atmospheric pressure until t.l.c. indicated the disappearance of starting material. The reaction mixture was filtered and evaporated to give a foam which was taken up in dichloromethane (100 ml) and treated with saturated sodium hydrogen carbonate solution (100 ml). The organic phase was separated, washed with water (100 ml) and dried. Evaporation gave a foam which solidified on trituration with ether to yield cyclo-L-prolyl-L-tryptophyl, (23), (8.20g, 29.0 mmol, 77%) homogeneous by t.l.c. Crystallization from acetone gave a sample m.pt. 173-4°C (lit.[3] 174°C) and $[\alpha]_D^{24}$ = -99° (c = 1.2, AcOH) (lit.[3] -101°).

(3S, 6S)-2-methoxy-[3,4-a]-tetrahydropyrrolo-6-(3'-indolyl)-methyl-pyrazin-5-one, (24). Cyclo-L-prolyl-L-tryptophyl, (23), (2.83g, 10 mmol) was stirred in dichloromethane (50 ml) under nitrogen. Trimethyloxonium tetrafluoroborate (4g, 27 mmol) was added and the resulting suspension was refluxed for 8 hours. After cooling, the mixture was decomposed with saturated sodium hydrogen carbonate solution (50 ml). The organic phase was separated, washed with water (25 ml), dried and evaporated to give a brown oil. The reaction was repeated at room temperature, 0°C, under more dilute conditions and using a smaller excess of trimethyloxonium salt but in each case the same result was obtained, as indicated by t.l.c. Purification of a sample of these reaction mixtures by column chromatography followed by p.l.c. gave the pyrazinone, (24), m.pt.

98-100°C: ν max (Nujol mull) 3260, 1710, 1690, 1620 cm^{-1}: λ max, log ε; 290, 3.70; 282, 3.77; 274, 3.73; 222, 4.56 nm: τ(60 MHz) 1.70 (1H, s, exch., NH), 2.10-3.00 (5H, m, aromatic), 5.70 (1H, m, C^6H), 4.25 (3H, s, CH_3O), 4.30-4.90 (3H, m, C^3H and N-CH_2), 7.80-9.0 (6H, m, CH_2-CH_2, CH_2-C^6H): Found; C, 68.62%; H, 6.41%; N, 14.24%. $C_{17}H_{19}N_3O_2$ requires C, 68.67%; H, 6.44%; N, 14.13%.

(3S,6S)-2-ethoxy-[3,4-a]-tetrahydropyrrolo-6-(3'-indolyl)-methyl-pyrazin-5-one, (25). (a) Cyclo-L-prolyl-L-tryptophyl, (23), (1.4g, 5 mmol) was dissolved in dichloromethane (30 ml) and stirred at room temperature under nitrogen. Triethyloxonium tetrafluoroborate (1.2g, 6 mmol) dissolved in dichloromethane (20 ml) was added and the combined solutions were stirred for 24 hours during which time more triethyl-oxonium salt (1g) was added at intervals. The solution was treated with potassium carbonate (24g) in water (50 ml) and following cessation of bubbling the layers were separated. The aqueous phase was extracted with dichloromethane (2 x 40 ml) and the combined organic phases were washed with water (3 x 25 ml). Drying and evaporation gave a yellow oil which was purified by p.l.c. to give the pyrazinone (25) and the indoline (36). For the pyrazinone (25): m.pt. 128-130°C (acetone/light petroleum): ν max (Nujol mull) 3260, 1710, 1690, 1620 cm^{-1}: λ max, log ε ; 290, 3.62; 282, 3.69; 275, 3.65; 221, 4.87 nm: τ (60 MHz) 1.80 (1H, s, exch., NH), 2.30-3.00 (5H, m, aromatic), 5.60-6.10 (3H, q superimposed upon m, J = 7 Hz, OCH_2CH_3 and C^6H), 6.10-7.05 (3H, m, N-CH_2 and C^3H), 7.75-9.00 (9H, t superimposed upon m, J = 7 Hz, CH_3CH_2O, CH_2-CH_2 and C^3H-CH_2): m/e 311, 24% (M^+); 182, 98%; 153, 10%; 130, 100%; 103, 5%; 77, 5%; 70, 7%; m* 107 (311⟶182): Found; C, 69.53%; H, 6.77%; N, 13.44%. $C_{18}H_{21}N_3O_2$ requires C, 69.43%; H, 6.80%; N, 13.49%.

(b) Cyclo-L-prolyl-L-tryptophyl, (23), (50 mg, 0.18 mmol) was stirred in dichloromethane (5 ml) at room temperature and under nitrogen. Dry calcium carbonate (100 mg) and triethyloxonium tetrafluoroborate (100 mg) in dichloromethane (5 ml) were added and the mixture was stirred for 7 hours, during which time more calcium carbonate (100 mg) and triethyloxonium salt (40 mg) were added. The mixture was poured into saturated sodium hydrogen carbonate solution (40 ml) and when gas evolution ceased the organic phase was removed and washed with saturated sodium hydrogen carbonate solution (20 ml), water (2 x 20 ml) and dried. Following evaporation and p.l.c., the pyrazinone (25) was isolated in 63% yield, physical data as above.

Indoline (36). Cyclo-L-prolyl-L-tryptophyl, (23), (17g, 0.06 mol) was dissolved in trifluoroacetic acid (50 ml) and the solution was added very slowly to a well stirred suspension of sodium hydrogen carbonate (60g) in water (100 ml). When the addition was complete the mixture was extracted with dichloromethane (2 x 150 ml). The combined organic extracts were washed with water (100 ml), dried and evaporated to give the indoline (36), homogeneous by t.l.c., as a foam. Trituration with ether gave a white solid (16g, 94%) and crystallization of a sample from acetone/light petroleum gave m.pt. 187-188°C: $[\alpha]_D^{21}$-414° (C = 0.97, EtOH): ν max (Nujol) 3405, 3320, 1660 cm^{-1}: ν max (CCl_4) 3400, 3310, 1670 cm^{-1}: λ max, log ε; 297, 3.31; 242, 3.78; 207, 4.15 nm: τ (100 MHz) 2.80-3.50 (4H, m, aromatic), 4.50 (1H, d, J = 7 Hz, $C^2\underline{H}$), 4.90 (1H, bs, exch., N$\underline{H}$), 5.85-6.17 (3H, m, $C^9\underline{H}$, $C^{12}\underline{H}$, $C^3\underline{H}$), 6.40-6.65 (2H, m, $C^{15}\underline{H}_2$), 7.25-8.25 (6H, m, $C^8\underline{H}_2$, $C^{16}\underline{H}_2$-$C^{17}\underline{H}_2$): ^{13}C n.m.r. given on p.210 m/e 283, 84% (M+); 153, 67%; 130, 100%; 117, 71%; 70, 51%; molecular ion, mass 283.1318; $C_{16}H_{17}N_3O_2$ requires 283.1321: Found; C, 68.08%; H, 6.13%; N, 14.91%. $C_{16}H_{17}N_3O_2$ requires C, 67.83%; H, 6.05%; N, 14.83%.

Acetylindoline (51). The indoline (36) (100 mg, 0.35 mmol) was stirred in acetic anhydride (10 ml) for 40 minutes. The solvent was removed *in vacuo* at room temperature to give a quantitative yield of the acetylindoline (51) as a white solid, homogeneous on t.l.c. Recrystallization from acetone/light petroleum gave a pure sample, m.pt. 253-257°C: ν max (Nujol) 1670, 1660 cm^{-1}: λ max, log ε; 283, 3.33; 276, 3.36; 244 nm: τ (100 MHz) 1.90 (1H, d, J = 8 Hz, $C^{7}\underline{H}$), 2.6-2.96 (3H, m, aromatic), 3.75 (1H, d, J = 6 Hz, $C^{2}\underline{H}$), 5.72-6.15 (3H, m, $C^{9}\underline{H}$, $C^{12}\underline{H}$, $C^{3}\underline{H}$), 6.44 (2H, bt, J = ca 6 Hz, $C^{15}\underline{H}_2$), 7.35 (3H, s, $C\underline{H}_3CO$), 7.40-8.20 (6H, m, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$, $C^{8}\underline{H}_2$): ^{13}C n.m.r. spectrum given on p.210: Found; C, 66.33%; H, 5.90%; N, 13.02%. $C_{18}H_{17}N_3O_3$ requires C, 66.40%; H, 5.89%; N, 12.92%.

N^{b}-Acetyl-L-tryptophan methyl ester, (48). Methanol (40 ml) was stirred at -10°C whilst thionyl chloride (3.6 ml) was added dropwise. When the addition was complete, L-tryptophan (4g) was added and the resulting heavy white precipitate was stirred at -10°C for two hours and allowed to warm to room temperature. After $2\frac{1}{2}$ hours at room temperature all solids had dissolved and the solution was stirred for 48 hours. Addition of ether (135 ml) produced a dense white precipitate which was filtered off, washed with ether and dried *in vacuo* to give L-tryptophan methyl ester hydrochloride (4.2g, 90% lit. yield[25a]). The ester (3g, 11.8 mmol) was dissolved in water (50 ml) and acetic anhydride (1.3 ml, 13.8 mmol) and sodium acetate (0.8g, 12.1 mmol) in water (10 ml) were added. The mixture was shaken for 10 minutes and the white, oily precipitate formed was extracted with dichloromethane (20 ml). The organic extract was washed with 2N hydrochloric acid (10 ml), water (2 x 10 ml) and dried. Evaporation gave N^{b}-acetyl-L-tryptophan methyl ester as a solid (2.6g, 10 mmol, 84%), m.pt. 147-150°C (lit.[25b] 152.5°C).

N,N'-diacetyl-L-tryptophan methyl ester, (53 and N^b,2-diacetyl-L-tryptophan methyl ester, (54). N^b-Acetyl-L-tryptophan methyl ester, (48), (110 mg) was stirred in redistilled acetic anhydride (5 ml) at room temperature and trifluoroacetic acid (4 ml), freshly distilled from phosphorous pentoxide, was added to the suspension. All solids dissolved and a yellow solution was obtained which after 10 minutes was slowly added to a well stirred suspension of sodium hydrogen carbonate (20g) in water. On completion of addition the solution was extracted with dichloromethane (25 ml) and the separated organic layer was washed with water (2 x 20 ml) and dried. Evaporation yielded a solid residue which after multiple elution p.l.c. on thick plates gave the acetate (53) (36 mg, 31%) as the less polar product and the acetate (54) (64 mg, 58%) as the more polar product. Compound (53) was isolated as a glass and was crystallized from ethyl acetate/light petroleum to give light yellow needles, m.pt. 156-8°C: ν max (Nujol) 3290, 3110, 1740, 1705, 1650 cm^{-1}: λ max, log ε; 299, 3.86; 291, 3.83; 270 sh, 3.90; 262, 3.90; 239, 4.26 nm: τ (60 MHz) 1.4-1.6 (1H, m, $C^7\underline{H}$), 2.2-2.75 (4H, m, aromatic), 3.55 (1H, bd, J = ca 8 Hz, exch., N$\underline{H}$), 4.75-5.2 (1H, dt, J = 8 Hz and 6 Hz, collapses to t, J = 6 Hz, with D_2O, $C^9\underline{H}$), 6.25 (3H, s, O$C\underline{H}_3$), 6.70 (2H, d, J = 6 Hz, $C^8\underline{H}_2$), 7.40 (3H, s, $C\underline{H}_3CON^a$), 8.0 (3H, s, $C\underline{H}_3CON^bH$): m/e; 302, 6% (M^+); 243, 17%; 201, 19%; 172, 11%; 130, 100%; 43, 27%: Found; C, 63.30%; H, 5.99%; N, 9.36%. $C_{16}H_{18}N_2O_4$ requires C, 63.56%; H, 6.00%; N, 9.27%. Compound (54) was isolated as a glass which crystallized from acetone/light petroleum to give colourless prisms, m.pt. 202-204°C: ν max (Nujol) 3230, 3080, 1745, 1658, 1650 cm^{-1}: λ max, log ε; 312, 4.27; 236, 4.20 nm: τ (60 MHz) 0.6 (1H, bs, exch., indole N$\underline{H}$), 2.1-3.0 (5H, m, 1 exch., aromatic and amide N$\underline{H}$), 4.8-5.2 (1H, dt, collapses to t with D_2O, $C^9\underline{H}$), 5.8-6.65 (5H, s superimposed upon d, $C^8\underline{H}_2$ and O$C\underline{H}_3$), 7.35 (3H, s, C^2-CO$C\underline{H}_3$), 7.95 (3H, s,

$NHCOCH_3$): m/e; 302, 5% (M^+); 243, 5%; 184, 31%; 172, 100%; 130, 16%; 43, 34%: Found; C, 63.32%; H, 6.00%; N, 9.11%. $C_{16}H_{18}N_2O_4$ requires C, 63.56%; H, 6.00%; N, 9.27%.

Cyclo-L-prolyl-L-(N^a-(3',3'-dimethyl)allyl)tryptophyl, (10).

Dimethylallyl bromide was prepared from 3-methyl-but-1-ene-3-ol by the method of Crombie et al.[105] and stored over 4A molecular sieve, under nitrogen at 0°C until required. Sodium hydride (3.53 mmol as a 60% emulsion in mineral oil) was suspended in dimethylformamide (5 ml) under nitrogen and a solution of the indoline (36) (1g, 3.53 mmol) and dimethylallylbromide (6.4g, 35 mmol) in dimethylformamide (30 ml) was rapidly added. The mixture was stirred for 1 hour and then poured into water (50 ml) containing sodium hydrogen carbonate (1.5g). The solution was extracted with dichloromethane (50 ml) and the organic phase was washed with water (25 ml) containing sodium hydrogen carbonate (0.5g). The combined aqueous phases were extracted with dichloromethane (25 ml) and the combined organic extracts were dried and evaporated to give a dark brown oil. Column chromatography of this material on silica (50g) using dichloromethane/methanol (97:3) as eluent gave compound (10) in 85% yield as a crystalline solid. Recrystallization from acetone/light petroleum gave m.pt. 147-150°C: $[\alpha]_D^{21}$ -99° (c = 1.06, EtOH): ν max (Nujol) 3220, 1685, 1640 cm^{-1}: ν max (CCl_4) 3360, 1670 cm^{-1}: λ max, log ε; 278 sh, 3.60; 288, 3.63; 292 sh, 3.56: τ (100 MHz) 2.55-3.05 (4H, m, aromatic), 3.10 (1H, s, $C^2\underline{H}$), 4.10 (1H, s, exch., $N\underline{H}$), 4.72 (1H, t, J = 7 Hz, vinyl $\underline{H}$), 5.45 (2H, d, J = 7 Hz, indole N-$C\underline{H}_2$-CH), 5.75 (1H, dd, X part of AMX system, J_{XM} = 3 Hz, J_{XA} = 10 Hz, $C^9\underline{H}$), 6.06 (1H, bt, J = ca 6 Hz, $C^{12}\underline{H}$), 6.23-6.62 (3H, m superimposed upon dd, M part of AMX system, J_{MX} = 3 Hz, J_{MA} = 15 Hz, $C^8\underline{H}$ and $C^{15}\underline{H}_2$), 6.97-7.24 (1H, dd, A part of AMX system, J_{AM} = 15 Hz, J_{AX} = 10 Hz, $C^8\underline{H}$), 7.6-8.4

(10 H, s and s superimposed upon m, $C\underline{H}_3$, $C\underline{H}_3$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$): ^{13}C n.m.r. spectra given on p.208: m/e; 351, 5% (M^+); 198, 61%; 130, 100%; 73, 20%; 69, 35%; 41, 34%; m* 85.3 (198→130); 111.8 (351→198); molecular ion, mass 351.1940; calculated for $C_{21}H_{25}N_3O_2$, 351.1947: Found; C, 72.07%; H, 7.00%; N, 12.24%. $C_{21}H_{25}N_3O_2$ requires C, 71.77%; H, 7.17%; N, 11.96%.

N-(3',3'-dimethyl)allylindoline (57). Cyclo-L-prolyl-L-(N^a-(3',3'-dimethyl)allyl)tryptophyl, (10), (200 mg) was dissolved in trifluoroacetic acid (5 ml) and immediately added dropwise and with vigorous stirring to saturated sodium hydrogen carbonate solution(150 ml). The addition was made over a period of 10 minutes and when complete the solution obtained was extracted with dichloromethane (30 ml). The organic phase was washed with saturated sodium hydrogen carbonate solution (100 ml) and water (2 x 100 ml) and dried. Evaporation gave a glass which crystallized on standing to yield a quantitative yield of the product (57), homogeneous on t.l.c. Recrystallization from acetone/light petroleum gave rosettes of needles (88 mg) m.pt. 128-133°C: $[\alpha]_D^{19.5}$ -348° (c = 0.93, EtOH): ν max (Nujol) 1605, 1665 cm^{-1}: ν max (CCl_4) 1670, 1600 cm^{-1}: λ max, log ε; 209, 4.37; 256, 3.91; 306, 3.28 nm: τ(100 MHz) 2.95-3.80 (4H, m, aromatic), 4.20 (1H, d, J = 7 Hz, $C^2\underline{H}$), 4.90 (1H, t, J = 7 Hz, vinyl $\underline{H}$), 5.76-6.25 (5H, m superimposed upon d, J = 7 Hz, indole N-$C\underline{H}_2$, $C^9\underline{H}$, $C^{12}\underline{H}$, $C^3\underline{H}$), 6.34-6.58 (2H, m, $C^{15}\underline{H}_2$), 7.40-8.45 (12H, s and s superimposed upon m, $C\underline{H}_3$, $C\underline{H}_3$, $C^8\underline{H}_2$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$): ^{13}C n.m.r. spectrum given on p.210: m/e; 351, 13% (M^+); 198, 79%; 130, 100%: Found; C, 71.78%; H, 7.11%; N, 11.74%. $C_{21}H_{25}N_3O_2$ requires C, 71.77%; H, 7.17%; N, 11.96%.

Cyclo-L-prolyl-L-(3',3'-dimethyl-3',4',5'H-pyrrolo[1,2-a])tryptophyl, (58). Freshly distilled boron trifluoride etherate (100 ml) and carbon tetrachloride (100 ml) were heated to reflux under nitrogen.

Cyclo-L-prolyl-L-(N^a-(3',3'-dimethyl)allyl)tryptophyl, (10), (6.33g) and water (1 ml) were added and the mixture was refluxed for one hour. The black solution obtained was cooled and slowly added to sodium hydrogen carbonate (125g) suspended in water (200 ml) with efficient stirring. When gas evolution had ceased and all black tars had dissolved the mixture was extracted with dichloromethane (100 ml, 4 x 50 ml). The combined organic layers were washed with saturated sodium hydrogen carbonate solution (200 ml) and water (2 x 200 ml) and dried. Evaporation gave a brown foam which was purified by column chromatography on silica (200g) using dichloromethane/methanol (98:2) as eluent. The major fraction gave compound (58) (5.37g, 86%) as a yellow foam, which from t.l.c. and ^{1}H n.m.r. evidence was contaminated with approximately 15% of its isomer, compound (59). Crystallization of the product from acetone/light petroleum gave pure (58) (0.677g, 11%) and further chromatography of the mother liquors followed by crystallization from acetone/light petroleum gave more of (58)(0.920g, 15%). For compound (58): m.pt. 145-147.5°C: $[\alpha]_D^{22}$-85° (c = 1.06, EtOH): ν max (Nujol) 3200, 1675, 1655 cm^{-1}: ν max (CCl_4) 3380, 3210, 1675 cm^{-1}: λ max, log ε; 227, 4.47; 278 sh, 3.65; 284, 3.70; 292, 3.65 nm: τ (100 MHz) 2.18-3.01 (4H, m, aromatic), 4.25 (1H, bs, exch., N$\underline{H}$), 5.58 (1H, dd, X part of AMX system, J_{XM} = 3 Hz, J_{XA} = 10 Hz, C$^9\underline{H}$), 5.88 (1H, bt, J = ca 7 Hz, C$^{12}\underline{H}$), 6.17-6.54 (3H, m, C$^8\underline{H}$ (M part of AMX system), C$^{15}\underline{H}_2$), 6.80-7.20 (3H, dd superimposed upon t; X part of AMX system, J_{XA} = 10 Hz, J_{XM} = 15 Hz, C$^8\underline{H}$; J = 8 Hz, C$^{5'}\underline{H}_2$), 7.37-7.75 (3H, t superimposed upon m, J = 8 Hz, C$^{4'}\underline{H}_2$, C$^{16}\underline{H}$?), 7.75-8.48 (9H, s and s superimposed upon m, C$\underline{H}_3$, C$\underline{H}_3$, C$^{16}\underline{H}$, C$^{17}\underline{H}_2$): ^{13}C n.m.r. spectrum given on p.209: m/e; 351, 5% (M^+); 198, 100%; 143, 9%; 41, 11%; 56, 14%: Found; C, 71.69%; H, 7.27%; N, 11.85%. $C_{21}H_{25}N_3O_2$ requires C, 71.77%; H, 7.17%; N, 11.96%.

o-Amino-acetophenone (78). A procedure based upon the method of Meyer et al. was used.[106] An ozone/oxygen mixture containing approximately 5% ozone was bubbled through dichloromethane (10.0 ml) at -78°C for 10 minutes at a gas flow rate of 1 litre/hour. This procedure allowed reproducible preparation of a blue solution containing ca 0.14 mmol of ozone (determined by iodine titration). To the above solution at -78°C was added a sample of cyclo-L-prolyl-L-(3',3'-dimethyl-3',4',5'H-pyrrolo[1,2-a])tryptophyl, (58), (40 mg, 0.11 mmol) dissolved in dichloromethane (5 ml). The blue colour of the solution was immediately discharged and it was allowed to warm to room temperature prior to evaporation of the solvent. The residual glassy material, which gave a positive starch/iodide test, was dissolved in ethyl acetate and shaken under hydrogen in the presence of palladium (14 mg, 10% on carbon) for 45 minutes. The resulting solution gave a negative test with starch/iodide paper and following filtration was evaporated to give a yellow glass. Following purification by p.l.c. the product, a colourless glass (11 mg) gave a white solid when crystallization from acetone/light petroleum was attempted. Under the microscope this solid appeared amorphous and a m.pt. 82-92°C was determined. The reaction was repeated using singlet oxygen instead of ozone. The pyrrolo-indole (58) (44 mg) was dissolved in methanol (75 ml) and methylene blue (ca 0.5 mg) was added. The solution was cooled in water and illuminated with a 300 watt tungsten lamp for 15 hours whilst oxygen was bubbled through slowly. P.l.c. of the product following evaporation gave the o-amino-acetophenone derivative (78) as a colourless glass (33 mg). For this material, $[\alpha]_D^{19.5}$ -56° (c = 1.07, EtOH): ν max (CCl_4) 3280, 1705, 1680 cm^{-1}: λ max, log ε; 281, 3.0; 225, 4.11; 211, 4.13 nm: τ (60 MHz) 2.20-2.83 (4H, m, aromatic), 2.80-3.60 (1H, exch., very bs, NH), 5.35 (1H, bd, J = ca 8 Hz, $ArCOCH_2CH$), 5.80 (1H, m, NH-CO-CH),

6.0-7.0 (4H, m, $ArCOCH_2$-CH-CO-N-CH_2), 7.25-8.20 (8H, m, CMe_2-CH_2-CH_2-N, NH-CO-CH-CH_2-CH_2-CH_2N), 8.46 (3H, s, Me), 8.60 (3H, s, Me): m/e; 383, 32% (M^+); 366, 9%; 351, 8%; 217, 35%; 216, 46%; 189, 51%; 174, 100%; 70, 86%; 41, 4%; m* 350 (383→366).

Pyridino-indole (59). Cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)-tryptophyl, (10), (508 mg) was stirred in dichloromethane (50 ml) and boron tribromide (5.5 ml) was added. After 52 hours the solution was poured into water (50 ml) and sodium hydrogen carbonate was added with stirring until gas evolution had ceased. The mixture was extracted with dichloromethane (50 ml) and the organic extract was washed with water (50 ml) and dried. Evaporation gave a brown glass (469 mg) which was purified by p.l.c. to give compound (59) (123 mg, 24%) as a glass which failed to crystallise but which formed an amorphous powder when triturated with light petroleum. For (59), m.pt. 70-85°C: $[\alpha]_D^{27}$ -161° (c = 1.14, EtOH): ν max (CCl_4) 3490, 3240, 1680 cm^{-1}: λ max, log ε; 223, 4.34; 284 sh, 3.53; 290, 3.64; 300, 3.62 nm: τ (100 MHz) 2.56-3.08 (3H, m, aromatic), 3.01 (1H, s, C^2H), 4.02 (1H, bs, exch., NH), 5.62 (1H, bdd, X part of AMX system, J_{XA} = 10 Hz, J_{XM} = ca 4 Hz, C^9H), 5.80-6.56 (6H, m, $C^{12}H$, indole N-CH_2, C^8H (M part of AMX system), $C^{15}H_2$), 7.02 (1H, dd, X part of AMX system, J_{AX} = 10 Hz, J_{AM} = 14 Hz, C^8H), 7.58-8.45 (6H, m, $C^{16}H_2$, $C^{17}H_2$, indole N-CH_2-CH_2), 8.65 (6H, s, CH_3, CH_3): ^{13}C n.m.r. spectrum given on p.209: m/e; 351, 29% (M^+); 198, 100%; 143, 18%; 109, 22%; 70, 21%; 56, 64%; 41, 24%; molecular ion, mass 351.1947; calculated for $C_{21}H_{25}N_3O_2$, 351.1947.

Rearrangement of N-allylindoline (57). The N-allylindoline (57) (72 mg) was stirred in freshly distilled boron trifluoride etherate (10 ml) for 24 hours and poured into saturated sodium hydrogen carbonate

solution (100 ml). Sodium hydrogen carbonate was added with stirring until bubbling ceased and the mixture was extracted with dichloromethane (50 ml). The organic extract was washed with saturated sodium hydrogen carbonate solution (100 ml) and water (100 ml) and dried. Evaporation and purification by p.l.c. gave the pyrrolo-indole (58) (12 mg, 17%), the pyridino-indole (59) (16 mg, 22%) and the N-allyl-indole (10) (9 mg, 13%).

Rearrangement of cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)tryptophyl, (10) with stannic chloride. To the allylindole (10) (530 mg) in dichloromethane (35 ml) was added stannic chloride (2.2 ml). The resulting flocculent suspension was stirred for 22 hours after which time t.l.c. indicated that no starting material remained. The reaction mixture was slowly added to water (100 ml) containing an excess of sodium hydrogen carbonate and stirred until gas evolution ceased and all of the white organo-tin complex had decomposed and dissolved (about 1 hour). Insoluble tin oxide was removed by filtration and the resulting liquid phases separated. The aqueous layer was extracted with dichloromethane (25 ml) and the combined organic layers were washed with water (50 ml) and dried. Evaporation gave a yellow foam (487 mg) which was purified by multiple elution p.l.c. on thick silica plates to give the indoline (88) (or (89)) (103 mg, 21%) as a colourless glass, and a mixture of the pyrrolo-indole (58) and the pyridino-indole (59) (265 mg). The latter mixture was separated by multiple elution p.l.c. on thin silica plates to give compound (58) (123 mg, 25%) and compound (59) (58 mg, 12%) as colourless glasses. Compound (88) (or (89)) refused to crystallize but gave an amorphous powder on trituration with light petroleum for which, $[\alpha]_D^{23}$ -303° (c = 1.04, EtOH): ν max (CCl_4) 3430, 1675 cm^{-1}: λ max, log ε; 229, 3.91; 244, 3.80; 296, 3.42 nm: τ (100 MHz) 2.80-3.55 (4H, m, aromatic),

4.50 (1H, bs, exch., NH), 5.97 (2H, bt, J = ca 8 Hz, $C^{9}H$, $C^{12}H$), 6.30-6.63 (2H, m, $C^{15}H_2$), 7.45-8.70 (10H, m, $C^{8}H_2$, $C^{16}H_2$, $C^{17}H_2$, CMe_2CH_2-CH_2), 8.85 (3H, s, CH_3), 9.30 (3H, s, CH_3): ^{13}C n.m.r. spectrum given on p.211: m/e; 351, 100% (M^+); 198, 63%; 185, 49%; 170, 46%; 143, 38%; 70, 37%; 56, 56%; 41, 30%; molecular ion, mass 351.1940; calculated for $C_{21}H_{25}N_3O_2$, 351.1947.

Rearrangement of cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)-tryptophyl, (10), in trifluoroacetic acid. The allylindole (10) (238 mg) was stirred in trifluoroacetic acid (10 ml) at room temperature and under nitrogen for 17 hours after which time t.l.c. indicated no starting material remained. The solution was slowly dropped into a suspension of sodium hydrogen carbonate (15g) in water (25 ml) and, when gas evolution had ceased, dichloromethane (25 ml) was added. Sufficient water was added to dissolve excess sodium hydrogen carbonate and the two liquid phases were separated. The aqueous layer was extracted with dichloromethane (10 ml) and the combined organic layers were washed with water (3 x 15 ml) and dried. Evaporation gave a yellow glass which was purified by multiple elution p.l.c. to yield the pyrrolo-indole (58) (34 mg, 14%), the trifluoroacetoxyindoline (94) (51 mg, 21%) and a third substance (82 mg, 34%) which was homogeneous on t.l.c. but was a mixture of compounds (see discussion). Compound (94), which was unstable and refused to crystallize, gave $[\alpha]_D^{24}$ -275° (c = 0.8, EtOH): ν max (CCl_4) 3400, 1780, 1665 cm^{-1}: λ max, log ε; 295, 3.29; 241, 3.70 nm; τ(100 MHz) 2.80-3.60 (4H, m, aromatic), 4.75 (1H, s, $C^{2}H$), 4.80 (1H, bs, exch., NH), 5.96 (2H, bt, J = ca 8 Hz, $C^{9}H$, $C^{12}H$), 6.35-6.60 (2H, m, $C^{15}H_2$), 7.20-8.40 (10 H, m, $C^{16}H_2$, $C^{17}H_2$, $C^{8}H_2$, CMe_2CH_2-CH_2), 8.52 (6H, s, CH_3,CH_3): ^{13}C n.m.r. spectrum given on p.211: m/e; 351, 67%; 282, 35%; 198, 20%; 185, 15%; 143, 35%; 130, 67%; 99, 47%; 93, 44%; 69, 50%; 56, 100%; 45, 73%.

Hydrolysis of the trifluoroacetoxyindoline, (94). Compound (94) (150 mg) and sodium hydrogen carbonate (600 mg) were stirred together in a mixture of water (5 ml) and tetrahydrofuran (5 ml) for 48 hours after which time t.l.c. indicated clean conversion to a more polar compound. The reaction mixture was poured into water (25 ml) and extracted with dichloromethane (10 ml, 15 ml). The combined organic extracts were washed with water (25 ml) and dried. Evaporation and purification by multiple elution p.l.c. on thin silica plates gave the alcohol (96) (66 mg, 55%) as a glass which failed to crystallize. Trituration with ether gave an amorphous solid which turned blue on standing. For (96), ν max (CH_2Cl_2) 3600, 3410, 1665 cm^{-1}: 2.84-3.50 (4H, m, aromatic), 4.73 (1H, s, $C^2\underline{H}$), 5.96 (2H, bt, $C^{12}\underline{H}$, $C^9\underline{H}$, X part of AMX, J_{XM} = ca 7 Hz), 6.30-6.65 (3H, bt, J = ca 6 Hz, superimposed upon bs, exch., $C^{15}\underline{H}_2$, O$\underline{H}$), 7.32 (1H, dd, M part of AMX system, J_{MX} = 7 Hz, J_{MA} = 13 Hz, $C^8\underline{H}$), 7.58-8.70 (9H, m, $C^{16}\underline{H}_2$ $C^{17}\underline{H}_2$, CMe_2-$C\underline{H}_2$-$C\underline{H}_2$, $C^8\underline{H}$), 8.85 (6H, s, $C\underline{H}_3$, $C\underline{H}_3$): m/e; 369, 3% (M^+).

Rearrangement of cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyltryptophyl, (10), in the presence of zinc chloride. Dichloromethane (5 ml) and zinc chloride (2.4g) were stirred together for 40 minutes under anhydrous conditions in order to dissolve some of the inorganic salt. Compound (10) (50 mg) was added and the mixture was stirred for 16 bours during which time a small amount of brown/red oily matter separated. The reaction mixture was poured into water (40 ml) and extracted with dichloromethane (10 ml). The brown/red oil was insoluble in dichloromethane, water and saturated sodium bicarbonate solution but dissolved slowly in dilute hydrochloric acid, suggesting that it was an organo-zinc complex. The acid solution was extracted with dichloro-

methane (10 ml) and the two organic extracts were washed with water (2 x 10 ml) and dried to yield a colourless glass (48 mg) whose spectral and chromatographic properties indicated it to be mainly the starting material (10). The reaction was repeated: compound (10) (50 mg) was dissolved in dichloromethane (5 ml) and refluxed with zinc chloride (1g) under anhydrous conditions for 17 hours. The reaction mixture was poured into water (40 ml) containing dilute hydrochloric acid solution (10 ml) and extracted with dichloromethane (2 x 10 ml). The organic extracts were washed with water (2 x 10 ml) and dried; evaporation gave a colourless glass (42 mg) which from chromatographic and spectral data was found to be mainly the starting material (10). The reaction was repeated: compound (10) (62 mg) and zinc chloride (1.6g) were refluxed together in chloroform under anhydrous conditions for 16 hours after which time the mixture was poured into water (15 ml) containing dilute hydrochloric acid solution (10 ml). The solution was extracted with dichloromethane (15 ml, 10 ml) and the combined extracts were dried and evaporated to give a brown glass (68 mg) which was shown to contain a mixture of compounds (58), (59) and (88) by spectral and chromatographic comparison with the products of the rearrangement of compound (10) with stannic chloride; the relative proportions of the products were the same in both reactions.

Rearrangement of cyclo-L-prolyl-L-(N-(3',3'-dimethyl)allyl)-tryptophyl, (10), in the presence of strong base. Compound (10) (72 mg) was dissolved in dry tetrahydrofuran (10 ml) under nitrogen and stirred in ice. Butyl lithium (0.1 ml of 2.1M solution in hexane) was added slowly using a syringe. During the first half of the addition the solution became yellow and in the second half, it became red,

rapidly changing to orange. The solution was stirred at room temperature and under nitrogen for 18 hours after which time t.l.c. indicated that most of the starting material had disappeared. The reaction mixture was poured into water (30 ml) and extracted with dichloromethane (20 ml, 10 ml), addition of sodium chloride being necessary to induce phase separation. The combined organic extracts were washed with dilute hydrochloric acid solution (2 x 15 ml) and water (30 ml) and dried. Evaporation gave a brown glass (58 mg) which was purified by multiple elution p.l.c. on thin silica plates to give the product, cyclo-D-prolyl-L-(N-(3',3'-dimethyl)allyl)tryptophyl, (100), in 26% yield as a colourless glass, which resisted attempts at crystallization. For (100), ν max (CCl_4) 3320, 1670 cm^{-1}: λ max, log ε; 223, 4.46; 278 sh, 3.67; 288, 3.70; 296 sh, 3.63 nm: τ (100 MHz) 2.30-3.15 (5H, m, aromatic), 3.80 (1H, bs, exch., NH), 4.75 (1H, bt, J = ca 6 Hz, vinyl H), 5.40 (2H, d, J = 61 Hz, indole N-CH_2), 5.83 (1H, bt, J = ca 4 Hz, C^9H), 6.15-6.90 (4H, m, C^8H, $C^{12}H$, $C^{15}H_2$), 7.20 (1H, dd, J = 4, 14 Hz, C^8H), 7.40-8.40 (10H, s and s superimposed upon m, CH_3, CH_3, $C^{16}H_2$, $C^{17}H_2$): m/e; 351, 5% (M^+); 198, 92%; 130, 100%; m* 85.3 (198→130); molecular ion, mass 351.1938; calculated for $C_{21}H_{25}N_3O_2$, 351.1947.

Thermal rearrangement of cyclo-L-prolyl-L-(3',3'-dimethyl)allyl)-tryptophyl, (10). Compound (10) (10 mg) was refluxed in decalin (10 ml) under nitrogen for 21 hours, at the end of which time evaporation of solvent under reduced pressure gave back unchanged starting material identified by spectral and chromatographic comparison. The reaction was repeated; compound (10) (42 mg) was refluxed in xylene (5 ml) for 2 hours, resulting in partial conversion to compound (100), identified by spectral and chromatographic means. The reaction was repeated;

compound (10) was refluxed in ethylene glycol for 25 hours and the solvent was removed by distillation under reduced pressure. The glassy residue was shown to contain approximately equal amounts of compounds (10) and (100) by spectral and chromatographic means. The same result was obtained when compound (10) (24 mg) was heated alone under nitrogen at 220-230°C for 4 minutes. Repetition of this reaction at 325°C for 2 minutes resulted in extensive decomposition of compound (10) to give a complex mixture from which no products were isolated.

Reaction of cyclo-L-prolyl-L-(3',3'-dimethyl-3',4',5'H-pyrrolo-[1,2-a])tryptophyl, (58), with mercuric acetate. Compound (58) (35 mg, 0.1 mmol) was dissolved in methanol (5 ml) and a solution of mercuric acetate (34.6 mg, 0.109 mmol) in methanol (4 ml) was added. The solution was stirred for 9 days at the end of which period it smelt strongly of acetic acid and a white precipitate had formed. The solvent was evaporated and the residue was taken up in dichloromethane (15 ml) and washed with water (10 ml). Drying and evaporation gave a yellow waxy solid which by spectral and chromatographic comparison was found to be identical with the starting material.

Oxidation of compound (58) with lead tetraacetate in dichloromethane. Compound (58) (49 mg, 0.14 mmol) and lead tetraacetate (74.6 mg, 0.17 mmol, previously dried in vacuo) were dissolved in dichloromethane (10 ml) and stirred for 15 hours. At the end of this time a considerable precipitate had formed and tests with starch/iodide indicated that no oxidant remained. T.l.c. of the mixture indicated that a major product, less polar than the starting material, had been formed along

with a large number of more polar compounds. On standing the reaction mixture appeared to simplify with the proportion of more polar products declining. After 5 days the reaction mixture was poured into water (25 ml) and extracted with dichloromethane (15 ml, 5 ml). The combined organic extracts were washed with saturated sodium hydrogen carbonate solution (10 ml) and water (2 x 15 ml) and dried. Evaporation and purification by multiple elution p.l.c. on thin silica plates gave the major product as a yellow glass (8.9 mg, 18%) which readily crystallized from acetone/light petroleum to give light yellow needles (5.7 mg) m.pt. 217-218°C (sub.): ν max (CCl_4) 3360, 1696, 1672, 1631 cm^{-1}: λ max, log ε; 234, 4.48; 284, 3.92; 349, 4.35 nm; addition of one drop dilute NaOH solution to u.v. cell gave λ max, log ε; 242, 4.45; 284 sh, 3.89; 353, 4.36 nm; neutralization with 2 drops dilute HCl solution to the u.v. cell gave back the original spectrum: m/e; 349, 100% (M^+); 334, 7%; 294, 5%; 182, 5%; 169, 11%; 168, 16%; 70, 13%; m* 319 (349→334), 248 (349→294): Found; C, 72.27%; H, 6.77%; N, 12.19%. $C_{21}H_{23}N_3O_2$ requires C, 72.18%; H, 6.63%; N, 12.03%.

Oxidation of compound (58) with lead tetraacetate in acetic acid. Compound (58) (67 mg, 0.19 mmol) and lead tetraacetate (100 mg 0.22 mmol, dried in vacuo) were stirred together in acetic acid (10 ml) for 13 hours. The solvent was evaporated and the residual brown gum was taken up in dichloromethane (15 ml) and water (25 ml). The phases were separated and the aqueous layer was extracted with dichloromethane (5 ml). The combined organic extracts were washed with saturated sodium hydrogen carbonate solution (10 ml) and water (2 x 20 ml) and dried. Evaporation gave a brown glass (83 mg) which on t.l.c. was seen to be a mixture of compounds (119) and (115). Separation of the two isomers by multiple

elution p.l.c. on thin silica plates gave compound (115) as the less polar material (13.7 mg, 18%) as a colourless glass and compound (119) as the more polar material (38 mg, 49%) also as a colourless glass. Both compounds gave white amorphous solids on trituration with ether but only compound (115) crystallized when crystallization from acetone/light petroleum was attempted. For compound (119); ν max (CCl_4) 3360, 1750, 1680, 1230 cm^{-1}: λ max, log ε; 226, 4.60; 280 sh, 3.83; 286, 3.86; 294 sh, 3.80 nm: τ (100 MHz) 2.30-3.05 (4H, m, aromatic), 3.80 (1H, mixture of dd, $C^{5'}\underline{H}$), 4.65 (1H, bs, exch., N$\underline{H}$), 5.70 (1H, bd, J = ca 9 Hz, $C^{9}\underline{H}$), 6.03 (1H, m, $C^{12}\underline{H}$) 6.20-6.70 (3H, m, $C^{8}\underline{H}$, $C^{15}\underline{H}_2$), 6.90-7.30 (1H, mixture of dd, $C^{8}\underline{H}$), 7.30-7.60 (1H, m, $C^{4}\underline{H}$) 7.60-8.40 (5H, m, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$, $C^{4'}\underline{H}$), 7.91 and 7.78 (3H, s, CH_3CO), 8.30-8.40 (6H, 4s, $\underline{CH}_3$, $\underline{CH}_3$): m/e; 409, 4% (M^+); 349, 9%; 256, 100%; 196, 86%. For compound (115); m.pt. 222-225°C: ν max (CCl_4) 1755, 1690, 1235 cm^{-1}: λ max, log ε; 256, 3.88; 299.5, 3.25 nm: τ (100 MHz) 2.31-3.23 (4H, m, aromatic), 6.02 (2H, bt, $C^{9}\underline{H}$, $C^{12}\underline{H}$), 6.27 (2H, bt, $C^{15}\underline{H}_2$), 6.50 (2H, bt, indoline N-$\underline{CH}_2$), 6.76-8.24 (8H, m, $C^{8}\underline{H}_2$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$, indoline N-CH_2-$\underline{CH}_2$), 8.06 (3H, s, $\underline{CH}_3CO$), 8.44 (3H, s, $\underline{CH}_3$), 9.03 (3H, s, $\underline{CH}_3$): ^{13}C n.m.r. spectrum given on p.211: m/e; 409, 60% (M^+); 394, 36%; 334, 31%; 243, 28%; 201, 52%; 198, 100%; 182, 59%; m* 380 (409→394), 283 (394→334), 166 (243→201), 99 (394→198); molecular ion, mass 409.1994; calculated for $C_{23}H_{27}N_3O_4$, 409.2001: Found; C, 67.52%; H, 6.55%; N, 10.38%. $C_{23}H_{27}N_3O_4$ requires C, 67.46%; H, 6.65%; N, 10.38%.

Equilibration of compounds (119) and (115). Compound (115) (6.7 mg) was dissolved in acetic acid (2 ml) and allowed to stand for 8 days before the solvent was removed by vacuum sublimation at room temperature. The ^1H n.m.r. spectrum of the crude product showed

compounds (115) and (119) to be present in the ratio 2:1 as estimated from the integral trace. Separation by t.l.c. gave compound (115), 1.4 mg, and compound (119), 0.6 mg. The i.r. and ^{1}H n.m.r. spectra of the latter were superimposable upon those of previously isolated samples of compound (119).

Epimerization of cyclo-L-prolyl-L-(3',3'-dimethyl-3',4',5'H-pyrrolo[1,2-a])tryptophyl, (58). Lithium iodide hydrate (100 mg) was dried by heating at 100°C, under vacuum and over phosphorous pentoxide for 15 hours. Compound (58) (39 mg) was refluxed in collidine (3 ml, freshly distilled (130°C/13 mm) from caustic soda) in the presence of anhydrous lithium iodide (85 mg) for 21 hours. The dark brown solution was poured into dilute hydrochloric acid solution (25 ml) and extracted into dichloromethane (3 x 20 ml). The combined extracts were washed with dilute hydrochloric acid solution (30 ml) and with water (30 ml) and dried. Evaporation gave a glassy solid consisting of a mixture of compounds (58) and (129). The two compounds were separated by p.l.c. on thin silica plates to give compound (58) (5.5 mg, 14%) and compound (129) (26 mg, 67%). The reaction was repeated; compound (58) (7 mg) and lithium iodide hydrate (10 mg) were refluxed together in collidine (3 ml) for 15 hours and worked-up as above to give a mixture of compounds (58) and (129) as before. The reaction was repeated in the absence of lithium iodide and the same result was obtained. Compound (129) was isolated as a colourless glass which crystallized on standing, m.pt. 205-208°C: ν max (CCl_4) 3220, 1670 cm^{-1}: λ max, log ε ; 227, 4.54; 277, 3.75; 283, 3.79; 292, 3.75 nm: τ (60 MHz) 2.35-3.04 (4H, m, aromatic), 3.35 (1H, bs, exch., NH), 5.80 (1H, q becoming t following exch., J = 4 Hz, C^9H), 6.20-7.22 (4H, m, $C^{12}H$, $C^{15}H_2$, C^8H), 7.42-7.85 (3H, m,

$C^{8}\underline{H}$, $C^{5'}\underline{H}_2$), 7.85-8.60 (12H, s and s superimposed upon m, $C\underline{H}_3$, $C\underline{H}_3$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$, $C^{4'}\underline{H}_2$): m/e; 351, 6% (M^+); 198, 100%; 156, 3%; 130, 5%; m* 123 (198→156), 111.75 (351→198), 82.25 (198→130); molecular ion, mass 351.1947; calculated for $C_{21}H_{25}N_3O_2$, 351.1947.

Hydrolysis of cyclo-L-prolyl-L-(3',3'-dimethyl-5'-(R,S)-acetoxy-3',4',5'H-pyrrolo[1,2-a])tryptophyl, (119). Compound (119) (140 mg, 0.34 mmol) was dissolved in acetonitrile (15 ml) and water (6 ml) and trifluoroacetic acid (0.3 ml) were added. The solution was stirred overnight and concentrated under reduced pressure and at room temperature to yield an aqueous oily mixture which was treated with saturated sodium hydrogen carbonate solution (25 ml). When bubbling ceased, the mixture was extracted with dichloromethane (2 x 15 ml) and the combined organic extracts were washed with water (20 ml) and dried. Evaporation gave compound (131) as a brown glass (110 mg, 0.30 mmol, 88%) which was homogeneous by t.l.c. The product was isolated as a mixture of diastereoisomers, epimeric about position 5'; crystallization from acetone/light petroleum gave a single isomer, m.pt. 165-167°C and recrystallization from ethyl acetate gave a sample ν max ($CHCl_3$) 3300 (broad), 1675 cm^{-1}: ν max (Nujol) 3310, 3220, 1690, 1670 cm^{-1}: λ max, log ε; 280 sh, 3.86; 286, 3.89; 293 sh, 3.85 nm: τ (100 MHz) 1.67 (1H, bs, exch., $N\underline{H}$), 2.30-3.00 (4H, m, aromatic), 4.32 (1H, bs, exch., $O\underline{H}$), 4.62 (1H, bdd, becoming dd following exch., X part of ABX system, J_{XA} = 4 Hz, J_{XB} = 6 Hz, $C^{5'}\underline{H}$), 5.84 (1H, bd, X part of AMX system, J_{XM} = 0 Hz, J_{XA} = 9 Hz, $C^{9}\underline{H}$), 6.10 (1H, m, $C^{12}\underline{H}$), 6.16-6.65 (3H, m, $C^{8}\underline{H}$, M part of AMX system, $C^{15}\underline{H}_2$), 7.03 (1H, dd, A part of AMX system, J_{AX} = 9 Hz, J_{AM} = 14 Hz), 7.28 (1H, dd, B part of ABX system, J_{BA} = 14 Hz, J_{BX} = 6 Hz, $C^{4'}\underline{H}$), 7.60 (1H, dd, A part of ABX system, J_{AB} =

14 Hz, J_{AX} = 4 Hz, $C^{4'}\underline{H}$) 7.65-8.45 (10H, s and s superimposed upon m, $C\underline{H}_3$, $C\underline{H}_3$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$): ^{13}C n.m.r. spectrum given on p.209: m/e; 367, 7% (M^+); 349, 11%; 214, 100%; 196, 84%; 158, 19%; m* 331.9 (367→349), 124.8 (367→214), 179.5 (214→196); molecular ion, mass 367.1873; calculated for $C_{21}H_{25}N_3O_3$, 367.1895: Found; C, 68.59%; H, 6.76%; N, 11.39%. $C_{21}H_{25}N_3O_3$ requires C, 68.64%; H, 6.86%; N, 11.44%.

Attempted reduction of cyclo-L-prolyl-L-(3',3'-dimethyl-5'-hydroxy-3',4',5'H-pyrrolo[1,2-a])tryptophyl, (131) with sodium borohydride. Compound (131) (160 mg) was dissolved in isopropanol (10 ml) and sodium borohydride (100 mg) was added. The suspension was stirred in the dark and under nitrogen for 41 hours at the end of which time t.l.c. indicated that some starting material remained. More sodium borohydride (100 mg) was added and the reaction was continued for a further 18 hours before the solvent was evaporated and the residue was taken up in dichloromethane (25 ml) and water (25 ml). Sodium chloride was added to encourage separation of the phases and the organic phase was washed with water and dried to give a light yellow glass (146 mg). Multiple elution p.l.c. on thin silica plates allowed separation of two compounds, both more polar than the starting material, identified as compounds (144a) and (144b). The more polar compound was isolated as a colourless glass (65 mg, 41%) which crystallized from acetone/light petroleum, m.pt. 240-243°C: $[\alpha]_D^{20}$ + 9° (C = 1.17, EtOH): ν max (CCl_4) 3430, 3270, 1675, 1650 cm^{-1}: λ max, log ε; 226, 4.51; 279, 3.73; 285, 3.78; 291, 3.73 nm : τ(60 MHz) 2.20-3.05 (5H, m, 1H exch., aromatic and $N\underline{H}$), 4.60 (1H, m, becoming bt following exch., $C^{5'}\underline{H}$), 5.43 (1H, bs, exch., $O\underline{H}$), 5.70 (1H, m, becoming bt following exch., $C^{9}\underline{H}$), 6.25-6.90 (4H, m, $C^{8}\underline{H}$, $C^{9}\underline{H}$, $C^{15}\underline{H}_2$), 6.90-7.90 (3H, m, $C^{4'}\underline{H}_2$, $C^{8}\underline{H}$), 7.90-8.50 (10H, s and

s superimposed upon m, $C^{\underline{H}}_3$, $C^{\underline{H}}_3$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$): m/e; 367, 9% (M^+); 349, 3%; 214, 100%; 196, 18%; 158, 13%; 130, 5%; m* 116.6 (214 → 158), 179.6 (214 → 196), 332 (367 → 349). The less polar product was isolated as a glass (21 mg, 13%) which crystallized from acetone/light petroleum, m.pt. 135 – 137°C: $[\alpha]_D^{23}$ + 5° (c = 1.03, EtOH): ν max 3390, 3260, 1670 cm^{-1}: λ max, log ε; 226, 4.50; 280, 3.74; 286, 3.79; 293, 3.75 nm: τ(60 MHz) 2.25-3.05 (4H, m, aromatic), 3.10 (1H, bd, exch., $N\underline{H}$), 4.65 (1H, m, $C^{5'}\underline{H}$), 5.65 (2H, m becoming 1H, bt, following exch., $O\underline{H}$, $C^{9}\underline{H}$), 6.18-6.85 (4H, m, $C^{8}\underline{H}$, $C^{12}\underline{H}$, $C^{15}\underline{H}_2$), 6.85-7.75 (3H, m, $C^{8}\underline{H}$, $C^{4'}\underline{H}_2$), 7.75-8.85 (10H, s and s superimposed upon m, $C^{\underline{H}}_3$, $C^{\underline{H}}_3$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$): m/e 367, 11% (M^+); 349, 2%; 214, 100%; 196, 9%; 158, 14%; 130, 6%; m* 116.6 (214 → 158), 179.6 (214 → 196), 332 (367 → 349).

Epimerization of cyclo-L-prolyl-L-tryptophyl, (23). Compound (23) (111 mg) was dissolved in isopropanol (5 ml) and stirred with sodium borohydride (58 mg) in the dark, under nitrogen for 18 hours. Evaporation of solvent gave a white solid which was taken up in water (25 ml) and dichloromethane (25 ml). The phases were separated and the organic layer was washed with water (25 ml) and dried. Evaporation gave a glass (62 mg) which was purified by p.l.c. to give cyclo-D-prolyl-L-tryptophyl, (55), as a colourless glass (38 mg, 34%) which crystallized from acetone/light petroleum as rosettes (15 mg). The reaction was repeated; compound (23) (842 mg) was refluxed in methanol (15 ml) containing potassium tertiary butoxide (1 mg) for 3 hours. The solution was cooled and diluted with dichloromethane (25 ml) and washed with 2N hydrochloric acid solution (25 ml), saturated sodium hydrogen carbonate solution (25 ml) and water (25 ml) and dried. Evaporation gave a white foam (586 mg). Purification by p.l.c. gave compound (55) (250 mg, 30%) and

starting material (83 mg, 10%). For compound (55), m.pt. 191-193°C: $[\alpha]_D^{20.5}$ + 120° (c = 1.3, AcOH): ν max ($CHCl_3$) 3490, 3410, 1670 cm^{-1}: ν max (Nujol) 3260, 1675, 1650 cm^{-1}: λ max, log ε ; 220, 4.37; 273, 3.66; 279, 3.69; 289, 3.63 nm: τ (100 MHz) 1.50 (1H, bs, exch., indole NH), 2.40-3.10 (5H, m, 1H exch., amide NH, aromatic), 3.20 (1H, s, C^2H), 5.84 (1H, bs, becoming bt following exch., J = ca 4 Hz, C^9H), 6.40-7.22 (4H, m, $C^{12}H$, C^8H, $C^{15}H_2$), 7.40 (1H, m, C^8H), 7.90-8.90 (4H, m, $C^{16}H_2$, $C^{17}H_2$): ^{13}C n.m.r. spectrum given on p.208: Found; C, 67.80%; H, 6.07%; N, 14.70%. $C_{16}H_{17}N_3O_2$ requires C, 67.83%; H, 6.05%; N, 14.83%.

Reduction of cyclo-L-prolyl-L-(3',3'-dimethyl-5'-hydroxy-3',4',5'H-pyrrolo[1,2-a])tryptophyl, (131) with sodium in liquid ammonia. Compound (131) (17 mg, 0.046 mmol) was dissolved in ammonia (5 ml) at its boiling point. Sodium metal (ca 3.7 mg, 0.16 mmol) was added in two portions to the solution. As the first portion was added, it rapidly dissolved to give a blue colour which disappeared quickly. The second portion did likewise until approximately half of it had dissolved, at which point the blue colour of the solution became permanent. Excess sodium was rapidly quenched by addition of acetone (1 ml) followed by ammonium sulphate (ca 200 mg). The ammonia solvent was allowed to evaporate and the residual solid was taken up in dichloromethane (20 ml) and water (20 ml). Separation of the layers and drying and evaporation of the organic phase gave a yellow glass which was purified by p.l.c. to give starting material (9.3 mg, 55%) as the more polar product and compound (58) (2.0 mg, 12%) as colourless glasses. Compound (58) crystallized on standing to give m.pt. 139-145°C. A mixed m.pt. determination with an authentic sample of compound (58) (m.pt. 146-148°C) gave m.pt.

142-147°C. The i.r. spectrum (CCl_4) of the isolated sample of compound (58) was identical with that of an authentic sample.

Reaction of cyclo-L-prolyl-L-(3',3'-dimethyl-5'-acetoxy-3',4',5'H-pyrrolo 1,2-a)tryptophyl, (119), with tosylhydrazine. Compound (119) (425 mg, 1.04 mmol) and tosylhydrazine (1.2g, 6.45 mmol) were stirred together in dichloromethane (7 ml) to yield a suspension which was cooled in ice. Trifluoroacetic acid (0.3 ml) was added causing most of the tosylhydrazine to dissolve. After one hour's stirring in ice/water the reaction mixture was poured into ice cold 2N hydrochloric acid solution (50 ml) and extracted with dichloromethane (2 x 15 ml). The combined organic extracts were washed with ice cold 2N hydrochloric acid (50 ml), ice cold saturated sodium hydrogen carbonate solution (50 ml) and ice cold water (50 ml). Drying and evaporation gave a brown glass (583 mg). Multiple elution p.l.c. gave the isomeric hydrazides (148a) and (148b) as colourless glasses which solidified on trituration with light petroleum. For the more polar isomer, yield 65 mg, 12%, $[\alpha]_D^{23°}$ -130° (c = 1.2, EtOH): ν max ($CHCl_3$) 3360, 3250, 1665, 1160, 908 cm^{-1}: λ max, log ε ; 229, 4.50; 278, 3.68; 286, 3.73; 293, 3.69 nm: τ (60 MHz) 2.05-3.00 (10h, AA' BB' system superimposed upon m, 2H exch., indolyl aromatic, tosyl aromatic, NH-NH), 3.12 (1H, bs, exch., amide NH), 5.10-5.75 (2H, m, $C^{5'}H$, $C^{9}H$), 5.75-6.20 (1H, m, $C^{12}H$), 6.20-6.90 (3H, m, $C^{15}H_2$, $C^{8}H$), 7.20-7.90 (6H, s superimposed upon m, tosyl CH_3, $C^{8}H$, $C^{4'}H_2$), 7.90-8.70 (10H, s and s superimposed upon m, CH_3, CH_3, $C^{16}H_2$, $C^{17}H_2$): m/e; 535, extremely weak (M^+); 351, 14%; 198, 100%; 155, 14%; 91, 50%. For the less polar isomer, yield 103 mg, 19%, $[\alpha]_D^{19.5}$ -78° (c = 0.96, EtOH), ν max ($CHCl_3$) 3360, 3250, 1665, 1160, 908 cm^{-1}: λ max, log ε ; 229, 4.50; 278, 3.68; 286, 3.73; 293, 3.69 nm:

τ(60 MHz) 2.03-3.0 (10H, AA' BB' system superimposed upon m, 2H, exch., indolyl aromatic, tosyl aromatic, NH-NH), 3.51 (1H, bs, exch., amide NH), 5.32-5.80 (2H, m, $C^{5'}H$, $C^{9}H$), 5.80-6.20 (1H, m, $C^{12}H$), 6.20-6.95 (3H, m, $C^{15}H_2$, $C^{8}H$), 7.0-7.60 (3H, m, $C^{8}H$, $C^{4'}H_2$), 7.62 (3H, s, tosyl CH_3), 7.65-8.70 (10H, s and s superimposed upon m, CH_3, CH_3, $C^{16}H_2$, $C^{17}H_2$): m/e; 535, extremely weak (M^+); 351, 14%; 198, 100%; 155, 14%; 91, 50%.

Cyclo-L-prolyl-L-(3',3'-dimethyl-3'H-pyrrolo[1,2-a])tryptophyl, (150). Cyclo-L-prolyl-L-(3',3'-dimethyl-5'-acetoxy-3',4',5'H-pyrrolo-[1,2-a])tryptophyl, (119), (129 mg, 0.31 mmol) and tosylhydrazine (276 mg) were stirred together in trifluoroacetic acid (5 ml) for 25 min. The solution was added dropwise to sodium hydrogen carbonate (7g) suspended in water (50 ml) over 5 minutes. When gas evolution ceased, the solution was extracted with dichloromethane (2 x 20 ml) and the combined organic extracts were washed with 2N hydrochloric acid solution (30 ml) and water (30 ml) and dried. Evaporation gave a brown glass which was purified by multiple elution p.l.c. on thick silica plates to give compound (150) (21 mg, 19%) as a glass which solidified on trituration with light petroleum. The reaction was repeated; cyclo-L-prolyl-L-(3',3'-dimethyl-5'-hydroxy-3',4',5'H-pyrrolo[1,2-a])trypto-phyl, (131), (134 mg, 0.36 mmol) was refluxed in benzene (20 ml) in the presence of a crystal of tosic acid mono-hydrate in a Soxhlet assembly containing 4A molecular sieve in the upper chamber. After 5 hours the solution was evaporated and the residue was taken up in dichloromethane (10 ml) and washed with saturated sodium hydrogen carbonate solution (10 ml) and water (10 ml) and dried. Evaporation gave a brown foam (122 mg, 0.35 mmol, 97%) which t.l.c. indicated to be compound (150) in

greater than ca 95% purity. Purification by p.l.c. gave a sample (70 mg) for which $[\alpha]_D^{19.5}$ -30° (c = 0.84, EtOH): ν max (CCl_4) 3390, 1680 cm^{-1}: λ max, log ε; 220, 4.25; 232 sh, 4.14; 245 sh, 3.90; 313, 3.86; 325 sh, 3.74; 341, 3.34 nm: τ (100 MHz) 2.37-3.00 (4H, m, aromatic), 3.40 (2H, ABq, J = 6 Hz, vinyl H's), 4.27 (1H, bs, exch., NH), 5.65 (1H, dd, X part of AMX system, J_{XA} = 10 Hz, J_{XM} = 4 Hz, C^9H), 5.97 (1H, bt, J = ca 7 Hz, $C^{12}H$), 6.10-6.52 (3H, m, C^8H, $C^{15}H_2$), 7.00 (1H, dd, A part of AMX system, J_{AM} = 14 Hz, J_{AX} = 10 Hz, C^8H), 7.60-8.30 (4H, m, $C^{16}H_2$, $C^{17}H_2$), 8.36 (3H, s, CH_3), 8.43 (3H, s, CH_3): ^{13}C n.m.r. spectrum given on p.209: m/e; 349, 7% (M^+); 196, 100%; molecular ion, mass 349.1793; calculated for $C_{21}H_{23}N_3O_2$, 349.1790.

Preparation of sulphides (154) and (156). Cyclo-L-prolyl-L-(3',3'-dimethyl-5'-hydroxy-3',4',5'H'pyrrolo[1,2-a])tryptophyl, (131), (112 mg, 0.3 mmol) was dissolved in dichloromethane (10 ml) with propane dithiol (341 mg, 3.3 mmol) and trifluoroacetic acid (267 mg) was added. The mixture was stirred for 90 minutes and poured into saturated sodium hydrogen carbonate solution (50 ml). The solution was extracted with dichloromethane (10 ml) and the organic phase was washed with water (50 ml) and dried. Evaporation gave an oil consisting of the product and excess dithiol. Purification by multiple elution p.l.c. on thin silica plates gave the major products, compounds (154a) and (154b) as glasses which solidified on trituration with light petroleum. The more polar isomer was obtained in 23% yield (32 mg) and gave τ (100 MHz) 2.40-3.05 (4H, m, aromatic), 4.20 (1H, exch., NH), 5.35-5.65 (2H, m, C^9H, $C^{5'}H$), 5.80-6.05 (1H, m, $C^{12}H$), 6.10-6.56 (3H, m, C^8H, $C^{15}H_2$), 6.65-6.96 (1H, dd, X part of AMX system, J_{XA} = 11 Hz, J_{XM} = 14 Hz, C^8H), 6.97-7.55 (6H, m, $C^{4'}H_2$, $HSCH_2CH_2CH_2S$), 7.80-8.50 (13H, s and s

superimposed upon m, 1H exch., $\underline{H}SCH_2C\underline{H}_2CH_2S$, $C\underline{H}_3$, $C\underline{H}_3$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$): the ^1H n.m.r. spectrum of the less polar isomer, which was obtained in 13% yield (18 mg), was very similar except that the amide NH appeared τ 3.55. For a mixture of the two isomers, m/e; 457, 5% (M^+); 349, 21%; 304, 13%; 196, 100%; molecular ion, mass 457.1869; calculated for $C_{24}H_{31}N_3O_2S_2$, 457.1857; fragment 304, mass 304.1215; calculated for $C_{17}H_{22}NS_2$, 304.1193. The reaction was repeated using ethane-dithiol in the presence of boron trifluoride etherate; compound (131) (141 mg, 0.38 mmol), ethane-dithiol (472 mg) and boron trifluoride etherate (117 mg) were stirred together in dichloromethane (10 ml) for 60 minutes and poured into saturated sodium hydrogen carbonate solution (50 ml) and extracted with dichloromethane (25 ml). The organic extract was washed with water (25 ml) and dried. Evaporation gave an oily glass (165 mg) which was purified by multiple elution p.l.c. on thin silica plates to give the sulphides (156a) and (156b) as colourless glasses. The more polar compound was isolated in 9% yield (15 mg) and gave ν max ($CHCl_3$) 3360, 2460 (SH), 1670 cm^{-1}: τ (100 MHz) 2.40-3.04 (4H, m, aromatic), 3.56 (1H, bs, exch., N$\underline{H}$), 5.35-65 (2H, m, $C^{5'}\underline{H}$, $C^9\underline{H}$), 5.90-6.10 (1H, m, $C^{12}\underline{H}$), 6.10-6.65 (3H, m, $C^{15}\underline{H}_2$, $C^8\underline{H}$), 6.86 (1H, dd, J = 9, 12 Hz, $C^8\underline{H}$), 6.97-7.23 (5H, m, $SC\underline{H}_2C\underline{H}_2S$, $C^{4'}\underline{H}$), 7.23-7.52 (1H, dd, J = 4, 13 Hz, $C^{4'}\underline{H}$), 7.68-8.50 (11 H, s and s superimposed upon m, 1H exch., $C\underline{H}_3$, $C\underline{H}_3$, $C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$, S$\underline{H}$): m/e; 443, 1% (M^+); 349, 5%; 290, 5%; 196, 100%; 94, 21%; molecular ion, mass 443.1704; calculated for $C_{23}H_{29}N_3O_2S_2$, 443.1702. The less polar product was isolated in 18% yield (31 mg) and gave ν max ($CHCl_3$) 3360, 2450, 1670 cm^{-1}: τ (100 MHz) 2.40-3.00 (4H, m, aromatic), 4.21 (1H, bs, exch., N$\underline{H}$), 5.36-5.60 (2H, m, $C^{5'}\underline{H}$, $C^9\underline{H}$), 5.85-6.10 (1H,m, $C^{12}\underline{H}$), 6.20-6.60 (3H, m, $C^{15}\underline{H}_2$, $C^8\underline{H}$), 6.66-6.92 (1H, dd, J = 10, 14 Hz), 7.00-7.32 (5H, m, $C^{4'}\underline{H}$, $SC\underline{H}_2C\underline{H}_2S$), 7.32-7.55 (1H, dd, J = 4, 12 Hz, $C^{4'}\underline{H}$), 7.55-8.50 (11H, s and s superimposed upon m, 1H exch., S$\underline{H}$, $C\underline{H}_3$, $C\underline{H}_3$,

$C^{16}\underline{H}_2$, $C^{17}\underline{H}_2$): m/e; 443, 2% (M^+); 349, 5%; 290, 17%; 288, 15%; 230, 25% ; 196, 100%; 94, 7%; molecular ion, mass 443.1714; calculated for $C_{23}H_{29}N_3O_2S_2$, 443.1702.

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