SYNTHESIS AND BIOSYNTHESIS OF PYRROLIZIDINE ALKALOIDS.

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

by

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March 1982

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SUMMARY

This work is divided into five parts: (a) Synthesis of macrocyclic diester alkaloid analogues, (b) Synthesis of a macrocyclic diester alkaloid and elucidation of its absolute stereochemistry, (c) Structural studies, (d) Synthetic approaches to pyrrolizidine bases, and (e) Biosynthetic studies.

(a) Synthesis of Macrocyclic Diester Alkaloid Analogues.

Treatment of (+)-retronecine with a series of substituted glutaric anhydrides resulted in the formation of mixtures of 7- and 9- monoesters of (+)-retronecine. Cyclisation of these mixtures by the Corey-Nicclaou method led to the formation of 13-substituted 1,2-didehydrocrotalanines. 1,2-Didehydrocrotalanine, 13,13-dimethyl, 13,13-diphenyl, (13R)- and (13S)-13-methyl, and 13,13-tetramethylene-1,2-didehydrocrotalanine were synthesised by this method.

(b) Synthesis of a Macrocyclic Diester Alkaloid and Elucidation of its Absolute Stereochemistry.

A mixture of the 7- and 9- monoesters of (+)-retronecine was formed from (+)-retronecine and dicrotalic anhydride. This mixture was cyclised by the Corey-Nicolaou method to yield the pyrrolizidine alkaloid dicrotaline and its C-13 epimer. The absolute configuration at C-13 of both these alkaloids was determined by correlation with mevalonolactone.

(c) Structural Studies.

The plant Crotalaria globifera was shown to contain two pyrrolizidine alkaloids. One was shown to be trichodesmine. The other is believed to be a new pyrrolizidine alkaloid and has been named globiferine. A structure has been proposed for this alkaloid.

(d) Synthetic Approaches to Pyrrolizidine Bases.

Synthetic approaches to the pyrrolizidine base crotanecine based on 1,3-dipolar cycloaddition reactions and intramolecular cyclisations on iminium ions have been investigated.

(e) Biosynthetic Studies.

Evidence has been obtained for the incorporation of L-isoleucine and valine into the pyrrolizidine alkaloids trichodesmine and globiferine in C.globifera.

PUBLICATION

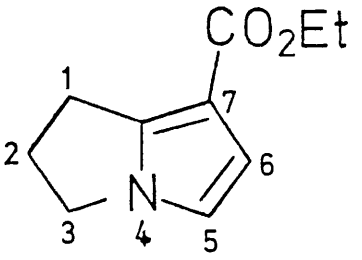
Some of the work described in chapters 2 and 3 has been published:

Synthesis and Stereochemistry of Dicrotaline, a Macrocyclic Pyrrolizidine Alkaloid. J.A.Devlin and D.J.Robins, J.Chem.Soc., Chem. Commun., 1981, 1272.

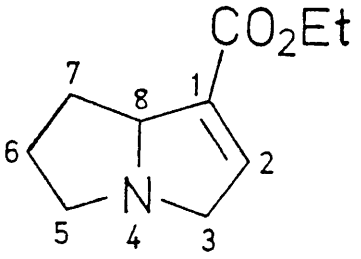
Pyrrolizidine Alkaloid Analogues. Synthesis of Eleven-membered Macrocyclic Diesters of Retronecine. J.A.Devlin, D.J.Robins and S.Sakdarat., J. Chem. Soc. Perkin Trans. 1. 1982, in the press.

NOTE ON NOMENCLATURE

Pyrrolizidine compounds with one or two double-bonds are named as derivatives of 1H- or 3H-pyrrolizine in accordance with Chemical Abstracts nomenclature.



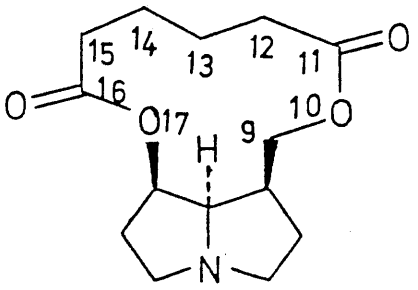
Ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate



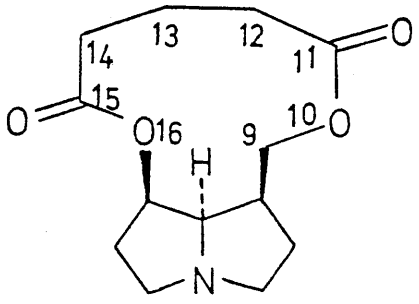
Ethyl 5,6,7,8-tetrahydro-3H-pyrrolizine-1-carboxylate

Fully saturated compounds are named as pyrrolizidine derivatives. Stereochemistry of substituents is indicated by the α and β nomenclature to conform with usual practice in ths field. In the case of racemic material, a single enantiomer is represented where appropriate in order to specify the relative stereochemistry of the structure.

For macrocyclic diester alkaloids the numbering scheme proposed by Culvenor et al., is used (C.C.J. Culvenor, D.H.G.Crout, W.Klyne, W.P.Mose, J.D.Renwick, and P.M.Scopes, J.Chem.Soc. (C), 1971, 3653.



Senecanine

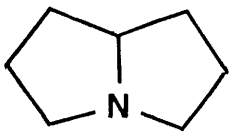


Crotalanine

CHAPTER 1
INTRODUCTION

GENERAL

The pyrrolizidine alkaloids constitute a large group of naturally occurring compounds. They contain the 1-azabicyclo [3.3.0.] octane nucleus (1) or a closely related system e.g.(2)



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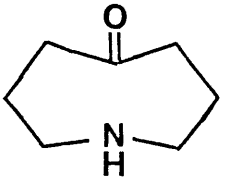

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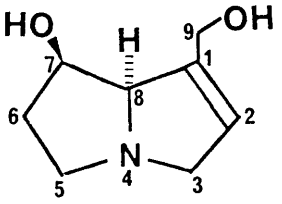
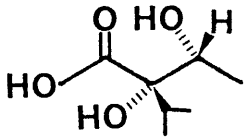


(3)

These secondary metabolites are most commonly found in the higher plant families Boraginaceae, Compositae, and Leguminosae.[1]

The alkaloids occur as amino-alcohols (necines), such as retronecine (3) or as esters of these alcohols.

The esterifying acids, with the exception of the most simple examples such as acetic or angelic, are unique[1] to the pyrrolizidine alkaloids. These necic acids (usually C_6-C_{10}) are often highly branched and oxygenated and can be present as mono-acids such as (+)- trachelanthic acid (4) or di-acids such as trichodesmic acid (5) .



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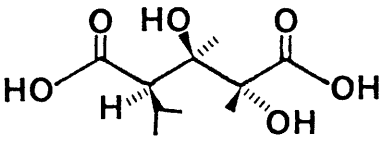
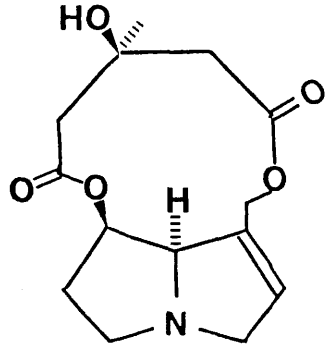


(5)

One of the most interesting types of alkaloid is the macrocyclic diester structure formed by combination of a di-acid with (+)- retronecine (3). These cyclic diesters are typified by dicrotaline (6), which contains an 11-membered ring and retrorsine (7) which has a 12-membered ring.



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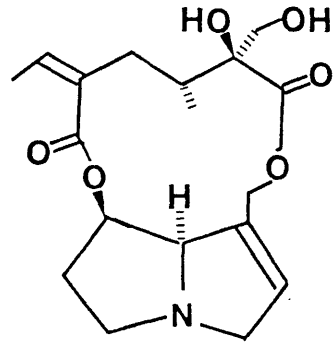

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Cyclic diesters with 13-[2] and 14-membered rings[3] have also been isolated, as well as cyclic diesters based on amino-diols other than retronecine.[4] A review[5] contains the structures of all pyrrolizidine alkaloids isolated up to 1981. This review in combination with previous ones in the same series[6,7] and others[8,9] and Specialist Reports[10] gives a broad coverage of the study of these alkaloids. A review[13] is also available on general pyrrolizidine chemistry.

It is not the purpose of this introduction to review further the pyrrolizidine alkaloids but to cover briefly some aspects of their study which are relevant to work presented in this thesis. These aspects are outlined in the following sections.

1.1 COMMERCIAL IMPORTANCE OF THE PYRROLIZIDINE ALKALOIDS.

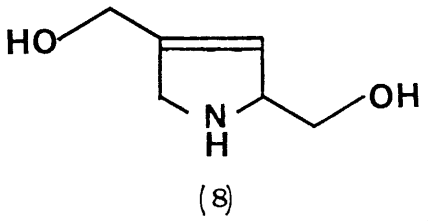
The principal economic importance of the alkaloids derives from their hepatotoxic[1] and carcinogenic[16] properties. These are manifest in both the poisoning of humans[12,14] and of livestock.[1,15] The initial discovery of these alkaloids came about during early chemical investigations of plant species which exhibited hepatotoxic activity.

There is also increasing interest in pyrrolizidine derivatives which show some potential as chemotherapeutic agents in tumour inhibition.[16]

1.2 HEPATOTOXIC ACTIVITY OF THE PYRROLIZIDINE ALKALOIDS.

The hepatotoxic nature of the pyrrolizidine alkaloids has long been recognised;[1] however, this behaviour is not shown by all members of the class. In order to exhibit this toxic action, the alkaloid must possess the following features;[16,20] (a) 1,2-unsaturation in the necine portion giving rise to a primary allylic grouping as in retronecine (3), and (b) esterification of this alcohol. It is also found that (c) esterification of the C-7 hydroxyl group and (d) substitution at the α-position of the C-9 esterifying acid increases the toxicity of the alkaloid.

The sufficiency of conditions (a) and (b) has been demonstrated by MATTOCKS.[19] He fed substituted 3,4-didehydropyrrolidines of the type (8) to test animals and showed that the histological changes (principally megalocytosis) within the liver were analagous to those caused by pyrrolizidine alkaloids.



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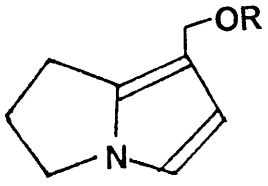

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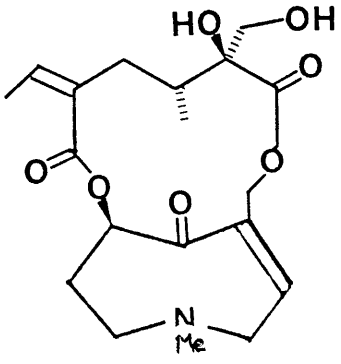
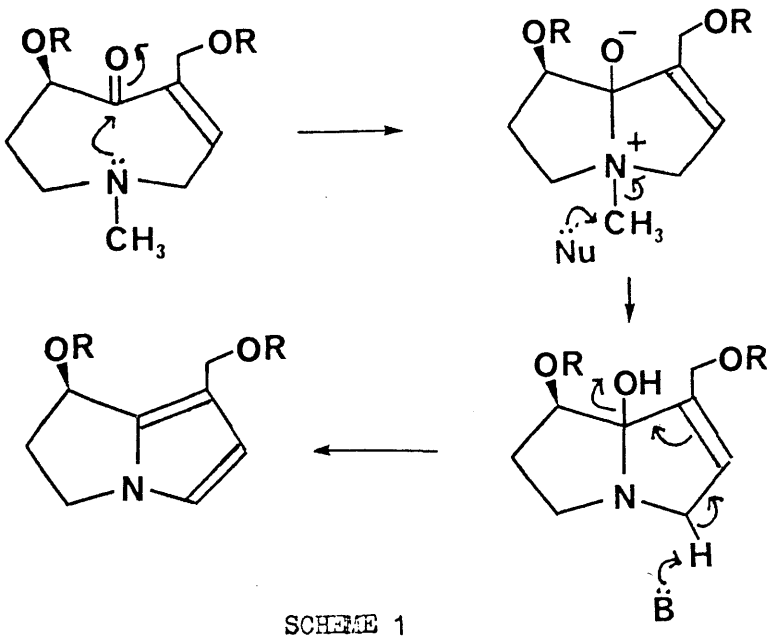


(10)

The liver damage is not however caused directly by the alkaloids. Pyrrole derivatives e.g. (9) are formed in the liver by oxidation of the pyrrolizidine nucleus with hepatic oxidases.[8] These pyrrole derivatives are then believed to act as alkylating agents. Some of the alkaloids of the otonecine group e.g. senkirkine (10) also exhibit hepatotoxic activity. Although they do not contain a retronecine type structure, a mechanism can readily be formulated to show how the otonecine nucleus can be converted to a pyrrole.



SCHEME 1

On consideration of the four structural features needed for toxic action, one would expect the macrocyclic diester alkaloids based on retronecine to exhibit marked hepatotoxic activity. This is indeed the case and these alkaloids are discussed further in the next section.

1.3 THE MACROCYCLIC DIESTERS BASED ON RETRONECINE

These constitute one of the largest groups of pyrrolizidine alkaloids and are distributed through a wide range of plant species, although they are found mainly in Senecio (fam. Compositae) and Crotalaria (fam. Leguminosae) species.[1] They occur most frequently with 11- or 12- membered rings. All the diesters containing retronecine so far tested are toxic. Thus the macrocyclic diester alkaloids are of interest principally for their hepatotoxic properties. It has been suggested[20] that the conformation of the macrocyclic ring plays an important part in determining the toxic behaviour of these alkaloids. The conformations of several 11- and 12- membered cyclic diesters have been established by X-ray crystallography.[21] The 11- membered rings adopt a conformation such that both ester carbonyl groups are syn-parallel and directed below the plane of the macro-ring, except for trichodesmine where the ester groups are reported to be anti-parallel. Pyrrolizidine alkaloids containing 12- membered rings exist in a conformation with the carbonyl groups anti-parallel. This difference in disposition of the macrocyclic rings is illustrated in the p.m.r. spectra of the two classes of compound. The geminal C-9 protons of these esters give rise to a characteristic AB quartet. The difference in the chemical shifts of these protons is helpful in determining ring size. In 11- membered rings, the difference in chemical shifts is typically 0 to 0.73 ppm while in 12- membered rings, the range is usually 1.25 to 1.53 ppm.[1]

It seems possible therefore, that there is a relationship between ring conformation and the chemical shift difference of the C-9 protons. This may be significant with regard to the toxic properties of the alkaloids. The conformation of the bonds around C-9 may have a marked effect on the ease of oxidation of the pyrroline ring or on the rate of hydrolysis of the ester function. The rate of production of the toxic pyrrole metabolite might therefore be influenced by these factors. There has been some debate[23,24] on the precise relationship between structure, conformation, and toxicity

in these alkaloids. When ROBINS and SAKDARAT[25] constructed an analogue of the 11-membered retronecine diesters the possibility emerged for the synthesis of analogues with specific structural features. Study of the ratios of pyrrole formation and ester hydrolysis of these analogues together with their toxicity might lead to a better understanding of the relationship between alkaloid structure, conformation and toxicity. Some work in this direction has been carried out with the synthesis of a series of 11-membered diester alkaloids. This work is detailed in CHAPTER 2. The development of the method of ROBINS and SAKDARAT into a synthesis of dicrotaline (6) the simplest of the natural 11-membered macrocyclic alkaloids also appeared feasible. Dicrotaline (6) had been reported in 1944 in Crotalaria dura and C. globifera by MARIAS.[26] Seeds of C. dura* were obtained and found to contain dicrotaline [26,27] although the stereochemistry at C-13 in the acid portion was not known. The synthesis of dicrotaline and elucidation of the stereochemistry at C-13 in the alkaloid is described in CHAPTER 3.

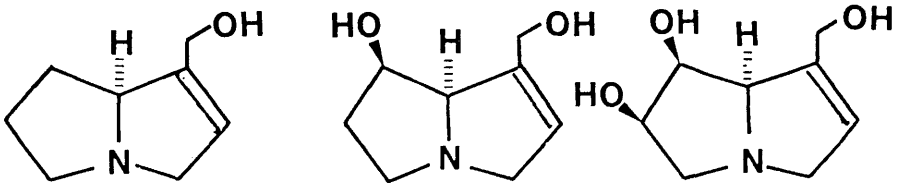
Seeds of C. globifera* were also examined for their alkaloid content in the expectation that they would yield a further sample of dicrotaline.[26] However, it was found that C. globifera did not contain dicrotaline, but two other pyrrolizidine alkaloids were present. The isolation and identification of these alkaloids is described in CHAPTER 4.

1.4 SYNTHESIS OF THE NECINE BASES.

The necine bases provide many attractive synthetic targets, and the chemical literature abounds with synthesis of these molecules.[13] Unfortunately, most of this work has been directed towards the simple saturated bases. The more challenging unsaturated and more highly oxygenated bases have been largely ignored. It is the routes applicable to these more complex bases which are summarised in this section.

*We are grateful to Mr. B.D.Schrire, Curator, Natal Herbarium, and Dr. C.H.Sirton, Botanical Research Institute, Pretoria, for obtaining seeds of Crotalaria dura and Crotalaria globifera.

Examples of such bases are supinidine (11), retronecine (3) and crotanecine (12).

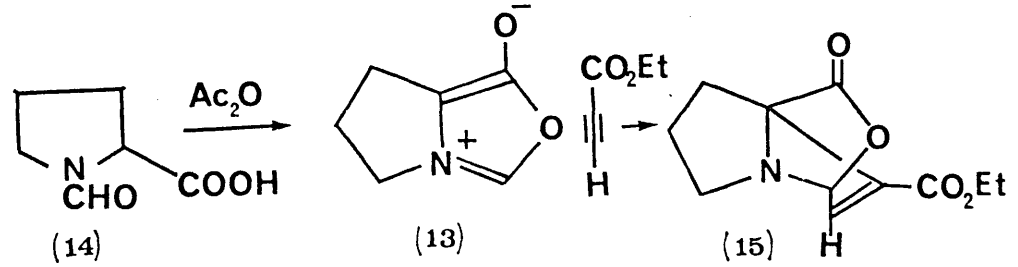


(11) (3) (12)

The three following approaches to the synthesis of the pyrrolizidine nucleus represent a selection of the more powerful methods which have been used for the synthesis of these natural bases.

1.4.1 THE 1,3-DIPOLAR CYCLOADDITION REACTION

This reaction was developed principally by HUISGEN and co-workers,[28] and has been adapted by PIZZORNO and ALBONICO[29] and later by TUFARIELLO et al[30] for the synthesis of the necines. The route of PIZZORNO and ALBONICO (SCHEME 2) involved the addition of ethyl propiolate to the 1,3-dipole of the oxazolium-5-oxide (13) formed by dehydration of N-formyl-L-proline (14). Spontaneous decarboxylation of the adduct (15) gave ethyl-2,3-dihydro-1H-pyrrolizine-7-carboxylate (16) in good yield.



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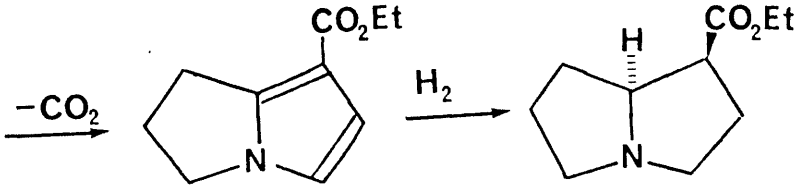
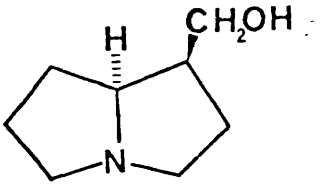


(16) (17)

SCHEME 2

Catalytic hydrogenation of this material (16) afforded ethyl endo-pyrrolizidine-1-carboxylate (17). This could then be converted into (±)-isoretronecanol (18) (hydride reduction) or (±)-trachelenthamidine (19) (epimerisation plus reduction). The scope of this reaction has been greatly enhanced by ROBINS and SAKDARAT.[31] They developed a method for the conversion of endo-pyrrolizidine-1-carboxylates into their 1,2-didehydro analogues. This procedure is illustrated in their synthesis of (±)-supinidine (11) (Scheme 3). A phenylseleno group was introduced α to the ester function of (17). The product was then reduced to the alcohol. Thermal elimination of the derived selenoxide gave (±)-supinidine (11).



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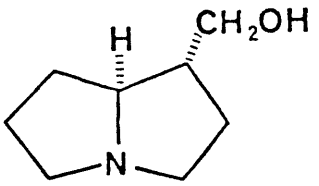

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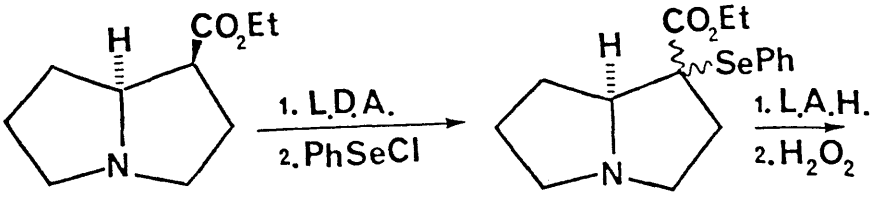

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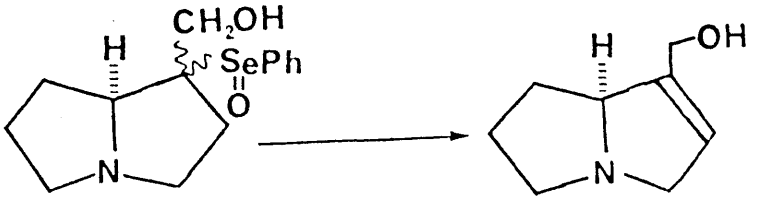
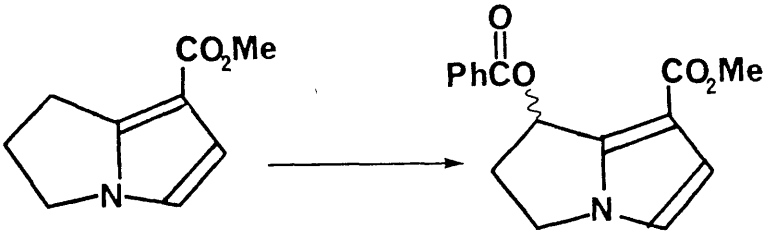


(11)

SCHEME 3

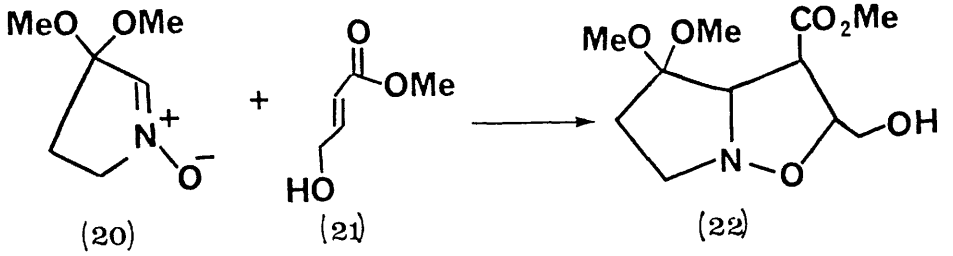
BOHLMANN et al[32] have reported a route to the 7-hydroxynecines by perester oxidation of the activated 1-position of the 2,3-dihydro-1H-pyrrolizine-7-carboxylates available by the method of PIZZORNO and ALBONICO (SCHEME 4).



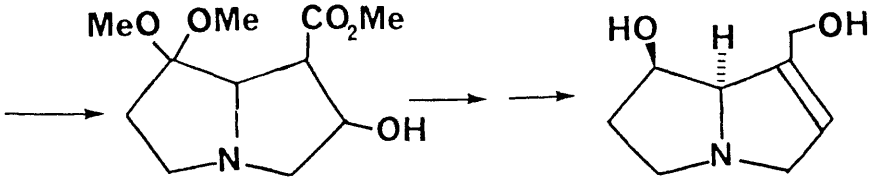
SCHEME 4

An alternative route to the necine bases using 1,3-dipolar cycloaddition has been taken by TUFARIELLO and LEE [33] in their synthesis of (±)-retronecine (SCHEME 5).

In this case the 1,3-dipole is provided by the pyrroline-1-oxide (20). Addition of this dipole to methyl-γ-hydroxycrotonate (21) gave the oxazole derivative (22). Mesylation, followed by hydrogenolysis of the N-O bond produced the pyrrolizidine ester (23). Dehydration, reduction and hydrolysis steps then yielded (±)-retronecine (3).



(20) (21) (22)



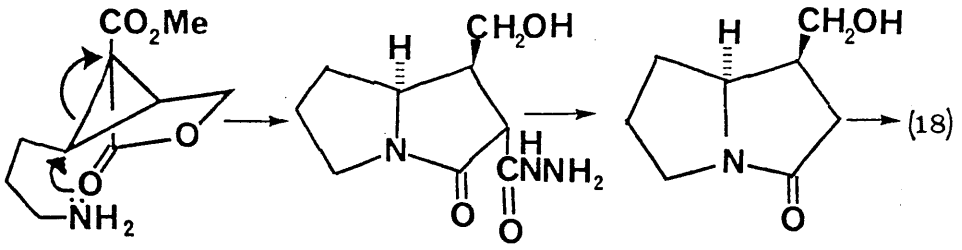
(23) (3)

SCHEME 5

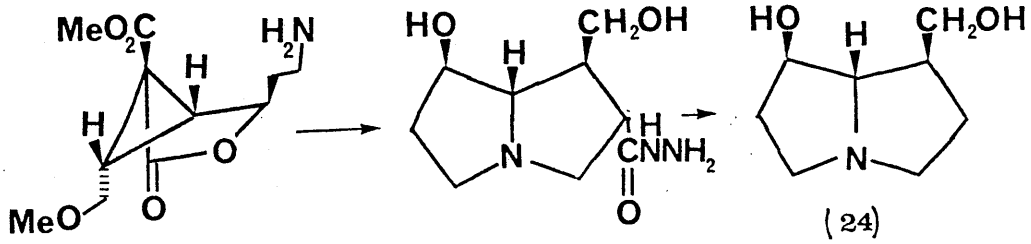
Development of the synthetic strategy based on N-formyl proline for the synthesis of crotanecine (12) has been investigated. This work is discussed in CHAPTER 5.

1.4.2. INTRAMOLECULAR OPENING OF CYCLOPROPANES

This reaction has been used by DANISHEFSKY et al in the synthesis of (±)-isoretronecanol[34] (18) (SCHEME 6), and (±)-hastanecine[35](24) (SCHEME 7). While it offers the possibility for construction of more elaborate necine structures, this method suffers from the need to prepare rather complex cyclopropanes as intermediates.



SCHEME 6

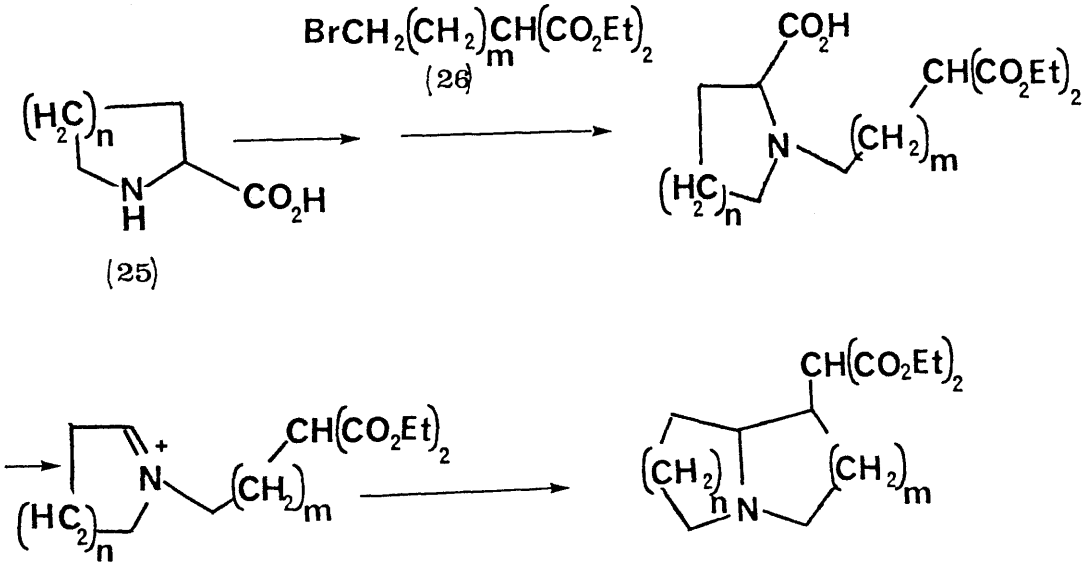


SCHEME 7

1.4.3. INTRAMOLECULAR NUCLEOPHILIC ATTACK ON IMINIUM IONS DERIVED FROM α-(TERTIARYAMINO) ACIDS.

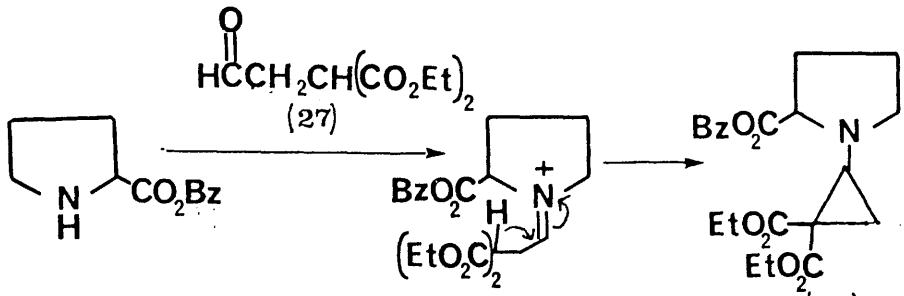
This particularly powerful method for generating 1-azobicyclo-[x,z,o] alkanes has been developed principally by RAPOPORT and co-workers[36] at Berekley. Their general approach is shown in SCHEME 8.

It involves the N-alkylation of cyclic α-amino acids such as (25) with a side chain of suitable length carrying a potentially nucleophilic carbon atom, e.g. a substituted malonate ester of the type (26). The amino acid is decarbonylated by heating with phosporous oxychloride. The resulting iminium salt is dissolved in water and cyclisation is effected by adjusting the pH of the solution to an appropriate value.



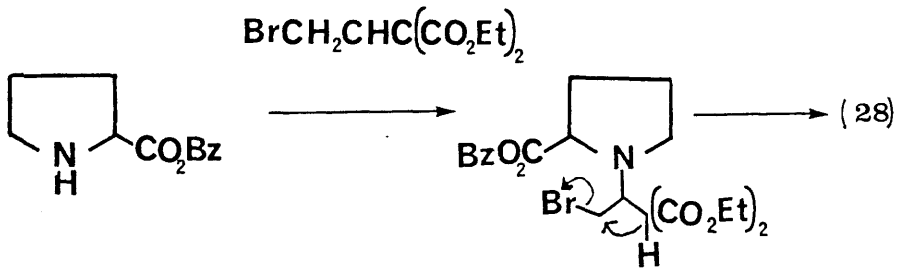
SCHEME 8

Unfortunately, this method has major drawbacks when applied to the synthesis of the 1-azabicyclo[3.3.0] octane system of the necine bases. The required alkyl bromide (2-bromoethylmalonate) readily cyclised to a cyclopropane derivative under the reaction conditions.[37] Attempts to avoid this complication by utilising the aldehydomalonate (27) also failed due to the cyclisation of the first iminium ion formed. (SCHEME 9)



SCHEME 9

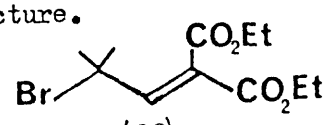
Alkylation with 2-bromoethylidemalonate also failed to give desired product due to cyclopropane formation, this time preceded by 1,4 addition to the $\alpha\beta$ -unsaturated system (SCHEME 10).



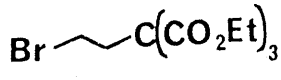
SCHEME 10

This problem of cyclopropane formation was overcome by using substituted bromides of the type (29). However, the pyrrolizidines formed from these more highly substituted bromides are becoming further removed from the natural bases. The synthesis of the 3-unsubstituted heterocycles was eventually achieved by utilising the triester (30).

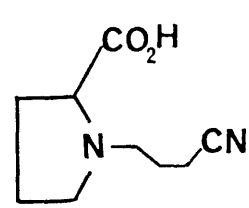
Prior to the publication of RAPOPORT'S work, this general approach had been considered for inclusion in this present work. The intermediate (31) was synthesised and some exploratory attempts were made to cyclise this compound to a pyrrolizidine structure.



(29)



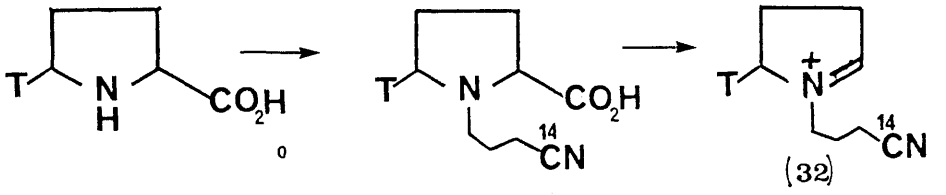
(30)



(31)

However, after the publication of the above work, it was considered inappropriate to continue this approach from a synthetic point of view.

Work on the production of iminium ions was not totally abandoned, as it was envisaged that the synthesis of a doubly labelled form (32) (SCHEME 11) could be carried out. This material would be of considerable value in the study of necine biosynthesis (CHAPTER 1 section 5.2). This work is included in Chapter 5.

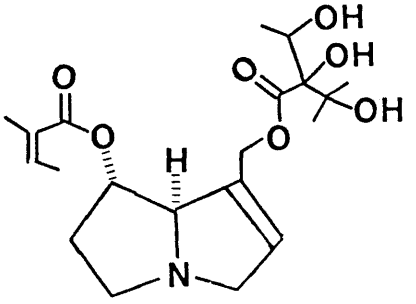


(32)

SCHEME 11

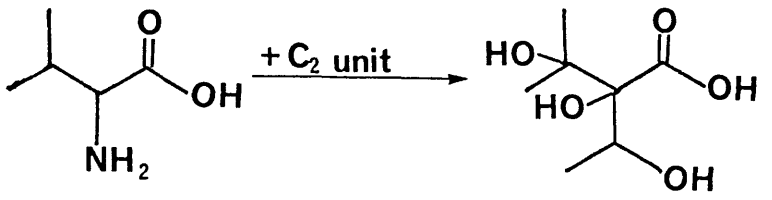
1.5.1. BIOSYNTHESIS - THE ACIDS

Cursory examination of the C_{10} necic acid structures (the largest group) suggests terpenoid derivation. More detailed examination reveals that the mode of coupling and pattern of oxygenation is inconsistent with biogenesis from mevalonate. It has been shown[38] that mevalonate is not a direct precursor of the necic acids tested. CROUT[39] has investigated the biosynthesis of heliosupine (33) in Cynoglossum officinale plants.

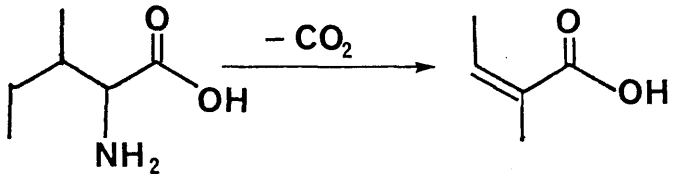


(33)

He has shown that valine (34) is specifically incorporated into echimidinic acid (35) and that isoleucine (36) is specifically incorporated into angelic acid (37).



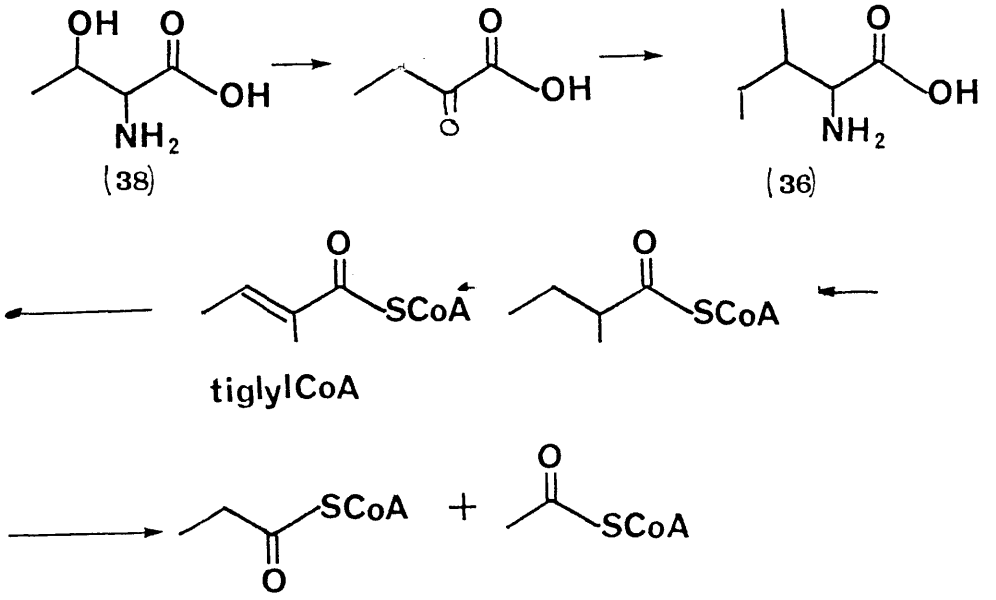
(34) (35)



(36) (37)

CROUT has suggested that angelic acid (37) is formed by isomerisation of tiglic acid, a known intermediate on the metabolic pathway from isoleucine (36) to acetyl Co A. (SCHEME 12)

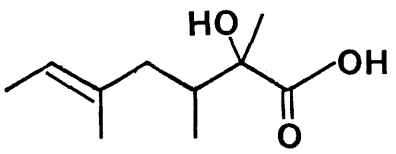
Evidence for this conversion was obtained by McGAW and Woolley.[40] They fed 1-^{14}C tiglic acid to C. officinale plants and found that it is incorporated specifically into angelic acid.



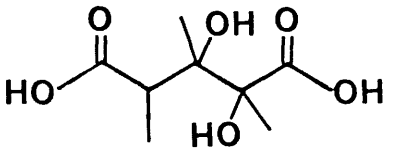
(38) (36)

SCHEME 12

CROUT and co-workers have also shown[41] that isoleucine (36) and threonine (38) are incorporated into the left hand C_5 unit of senecic acid (39).



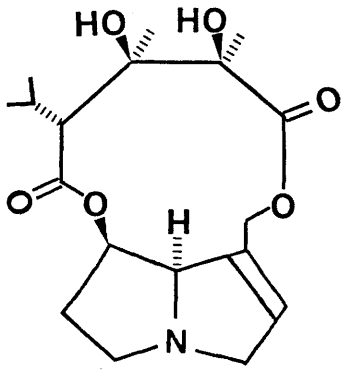
(39)



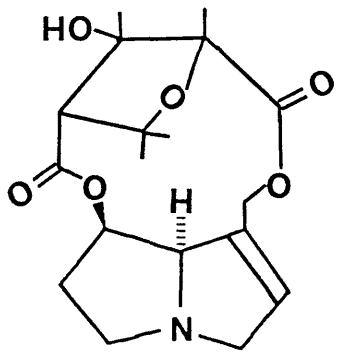
(40)

Further work[41] by these workers showed that the labelling pattern of senecic acid obtained by feeding [2-^{14}C] and [6-^{14}C] isoleucine to Senecio magnificus plants is consistent with biosynthesis from two isoleucine molecules with loss of both carboxyl carbons. ROBINS and CROUT[42] have shown that threonine and isoleucine are specific precursors for monocrotalic acid (40).

On the basis of the above feeding results, it was considered that valine and isoleucine are likely precursors for the acid portions of two related alkaloids trichodesmine (46) and globiferine (41) (proposed structure) which were isolated from Crotalaria globifera plants in the course of the present work (CHAPTER 4). The preliminary feeding experiments are described in CHAPTER 6.



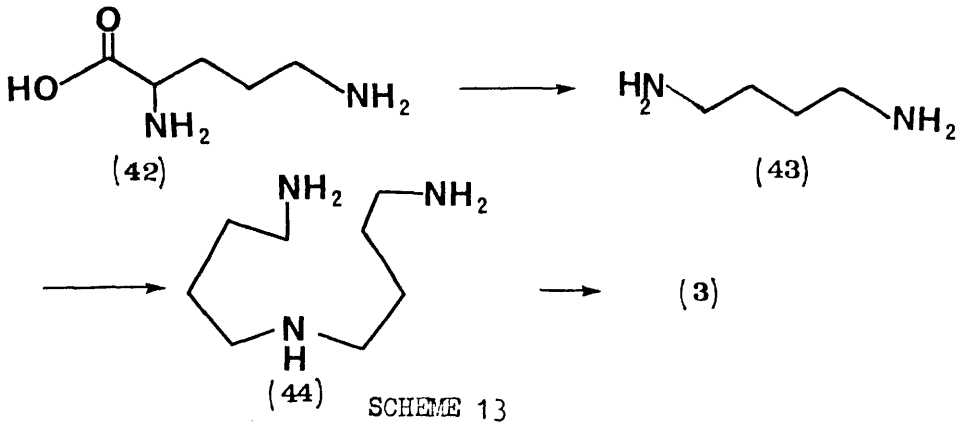
(46)



(41)

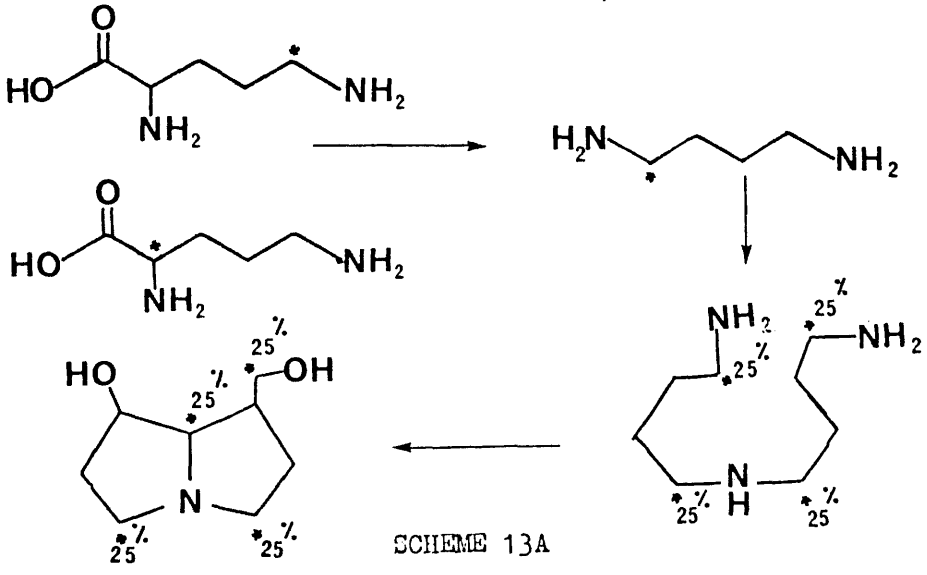
1.5.2. BIOSYNTHESIS-THE BASES.

Retronecine (3), the most common of the necine bases, is the only one on which biosynthetic studies have been carried out. The known biosynthetic pathway is outlined in scheme 13.



SCHEME 13

In 1962 BYERRUM and NOWACKI[43] fed [2-^{14}C] ornithine (42) to Crotalaria spectabilis plants, which produce the retronecine based alkaloid monocrotaline. They obtained radioactive monocrotaline which on degradation was shown to be labelled only in the retronecine portion. Later work by BOTTOMLEY and GEISSMAN[44] confirmed this result. They fed [2-^{14}C] - and [5-^{14}C] ornithine (42) to Sececio douglasii plants and found that the radioactivity was confined to the basic portion of the alkaloid. Further degradation of the retronecine showed that 25% of the total radioactivity of the retronecine was associated with the hydroxy methyl carbon (C-9). The same result was obtained with [1,4-^{14}C] putrescine.[44] From these results BOTTOMLEY and GEISSMAN inferred that two molecules of ornithine are used to form retronecine via a mechanism in which C-2 and C-5 of ornithine become equivalent, presumably via putrescine, at least in the biosynthesis of the right hand ring. They further postulated that a symmetrical C4-N-C4 unit derived from two putrescine molecules is generated later in the sequence (SCHEME 13A).

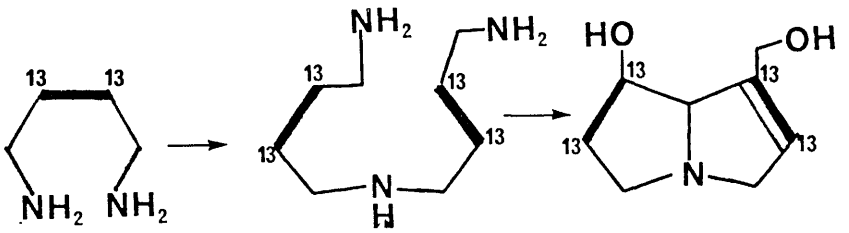


SCHEME 13A

ROBINS and SWEENEY[45] provided further evidence for this pathway by similar experiments with precursors used by BOTTOMLEY and GEISSMAN. They degraded the radioactive retronecine by osmium tetroxide/periodate oxidation to yield C-9 as formaldehyde. They showed that in all experiments about a quarter of the total radioactivity was located at C-9. Chromic acid oxidation of retronecine yielded β-alanine corresponding to C-(5+6+7) of retronecine which contained a further quarter of the total radioactivity in each experiment.

KHAN and ROBINS[46] fed [1,4-$^{13}C_2$] putrescine to Senecio isatideus plants. The ^{13}C.M.R. spectrum of the resultant retronecine showed equal enhancement (corresponding to a 1% ^{13}C enrichment) of the four signals corresponding to C-3, C-5, C-8, and C-9. These results are in agreement with the proposed biosynthetic pathway (SCHEME 13A).

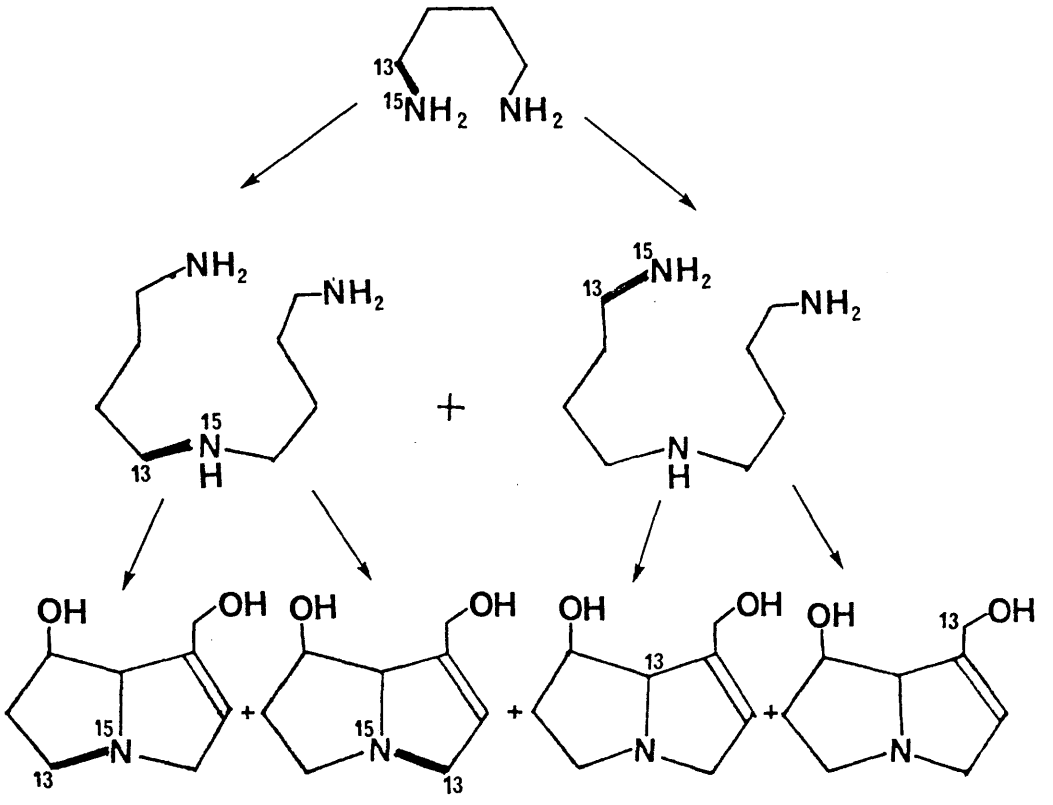
KHAN and ROBINS then fed doubly-labelled [2,3-$^{13}C_2$] putrescine to S.isatideus plants. The C.M.R. spectrum of retronecine obtained showed two pairs of doublets corresponding to C-1/C-2 and C-6/C-7 again supporting the proposed biosynthetic path (SCHEME 13B).



SCHEME 13B

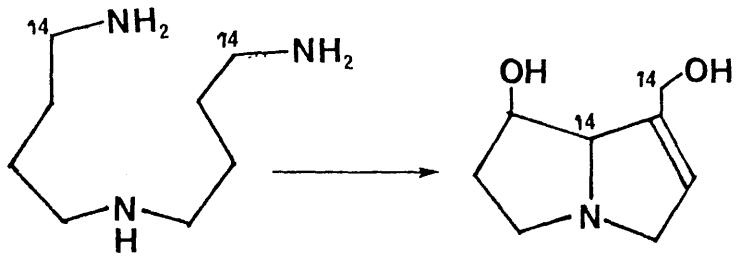
In a further double labelling experiment[47] [1-amino ^{15}N;1-^{13}C] putrescine was fed to S. isatideus. As before the C.M.R. spectrum showed enhancement of the signals for C-3, C-5, C-8 and C-9 of retronecine. The C.M.R. spectrum also showed two doublets of equal intensity corresponding to C-3/N-4, and C-5/N-4. These doublets were also observed in the

^{15}N magnetic resonance spectrum. The enrichment factor for these doublets was half of the total enrichment factor for the C-3, C-5, C-8 and C-9 signals indicating equal amounts of $[^{13}C-^{15}N]$ and $[^{13}C-^{14}N]$ species associated with C-5 and C-3 (SCHEME 13C).



SCHEME 13C

This is very convincing evidence for the existence of a C4-N-C4 symmetrical intermediate such as homospermidine (44). A later independent experiment by GRUE-SORENSEN and SPENSER[48] confirmed this result. KHAN and ROBINS[49] also fed homospermidine (44) double-labelled with ^{14}C in the terminal carbon positions. On degradation about half of the alkaloid radioactivity was located at C-9 and the β-alanine degradation product C-(5+6+7) was inactive. This is consistent with the proposed pathway (SCHEME 13D).



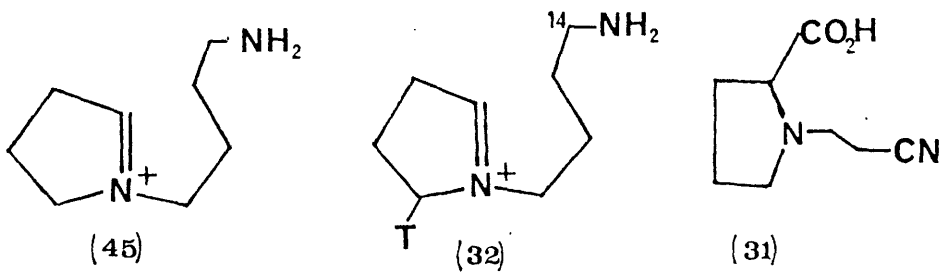
(44) (3)

SCHEME 13D

Finally, the identity of homospermidine as an intermediate was proved by an intermediate trapping experiment. [5-^{14}C]-Ornithine was fed to S. . isatideus plants. The plants were harvested 24 hours later and extracted with trichloroacetic acid. Inactive homospermidine was added to the extract and the homospermidine was isolated as its phenylthiourea derivative. This material was re-crystallized to constant specific radioactivity and shown to retain 0.5% of the original radioactivity fed.

The biosynthetic pathway between homospermidine(44) and retronecine(3) (SCHEME 13) has still to be investigated.

A possible intermediate on this path is the iminium ion (45), which might be formed by the conversion of one of the primary amino groups of (44) into an aldehyde followed by condensation to give the Schiff's base (45). Subsequent oxidation of the remaining amino function of this species followed by nucleophilic attack by the α-carbon on the iminium ion would then generate the 1-azabicyclo-[3.3.0.] octane skeleton. To test this postulate in feeding experiments, the doubly labelled species (32) is required. Therefore, the synthesis of unlabelled (45) was investigated. This work is described in CHAPTER 5.



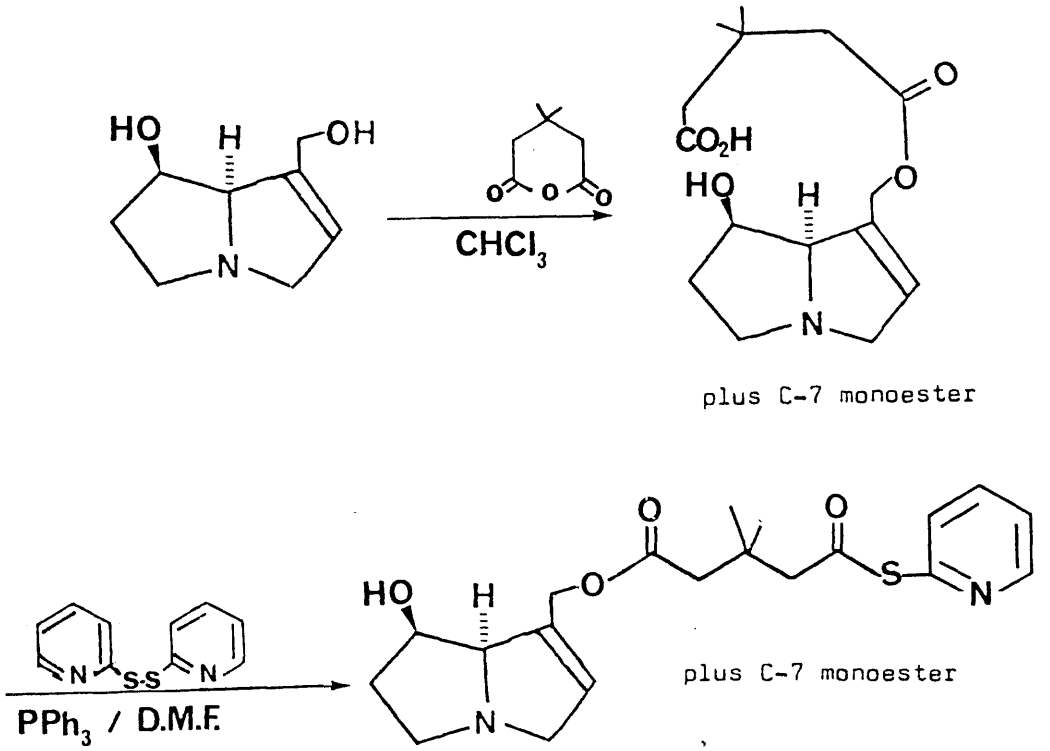
(45) (32) (31)

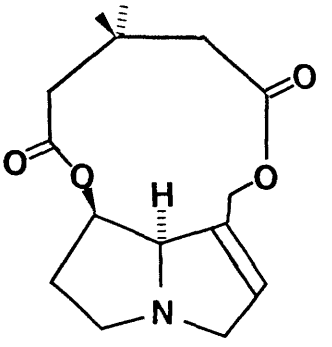
CHAPTER 2

SYNTHESIS OF 11-MEMBERED MACROCYCLIC DIESTER ALKALOID ANALOGUES

2.1 INTRODUCTION

One of the major synthetic challenges in the field of pyrrolizidine alkaloid chemistry is the synthesis of macrocyclic diester alkaloids based on retronecine (3). The synthesis of the simple diester alkaloids is relatively straightforward, but at the outset of this work none of the natural macrocyclic alkaloids had been synthesised. The synthesis of an analogue was achieved in 1979 by ROBINS and SAKDARAT.[25] Their synthesis of 13,13-dimethyl-1,2-didehydrocrotalarine (47) is outlined in SCHEME 14.





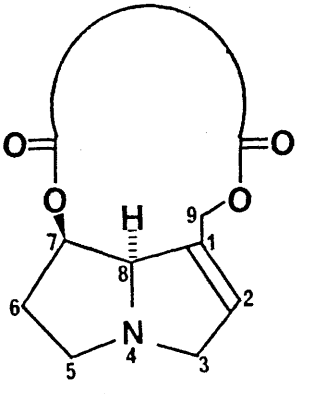
(47)

SCHEME 14

It was decided to investigate their method further with the intention of using it in the synthesis of some of the natural alkaloids. It was anticipated that this work might give information relevant to the structure/conformation/toxicity relationships discussed in CHAPTER 1.3.

2.2 SOME NOTES ON THE PROTON MAGNETIC RESONANCE AND MASS SPECTRA OF THE MACROCYCLIC DIESTER ALKALOIDS.

2.2.1.P.M.R. SPECTRA.

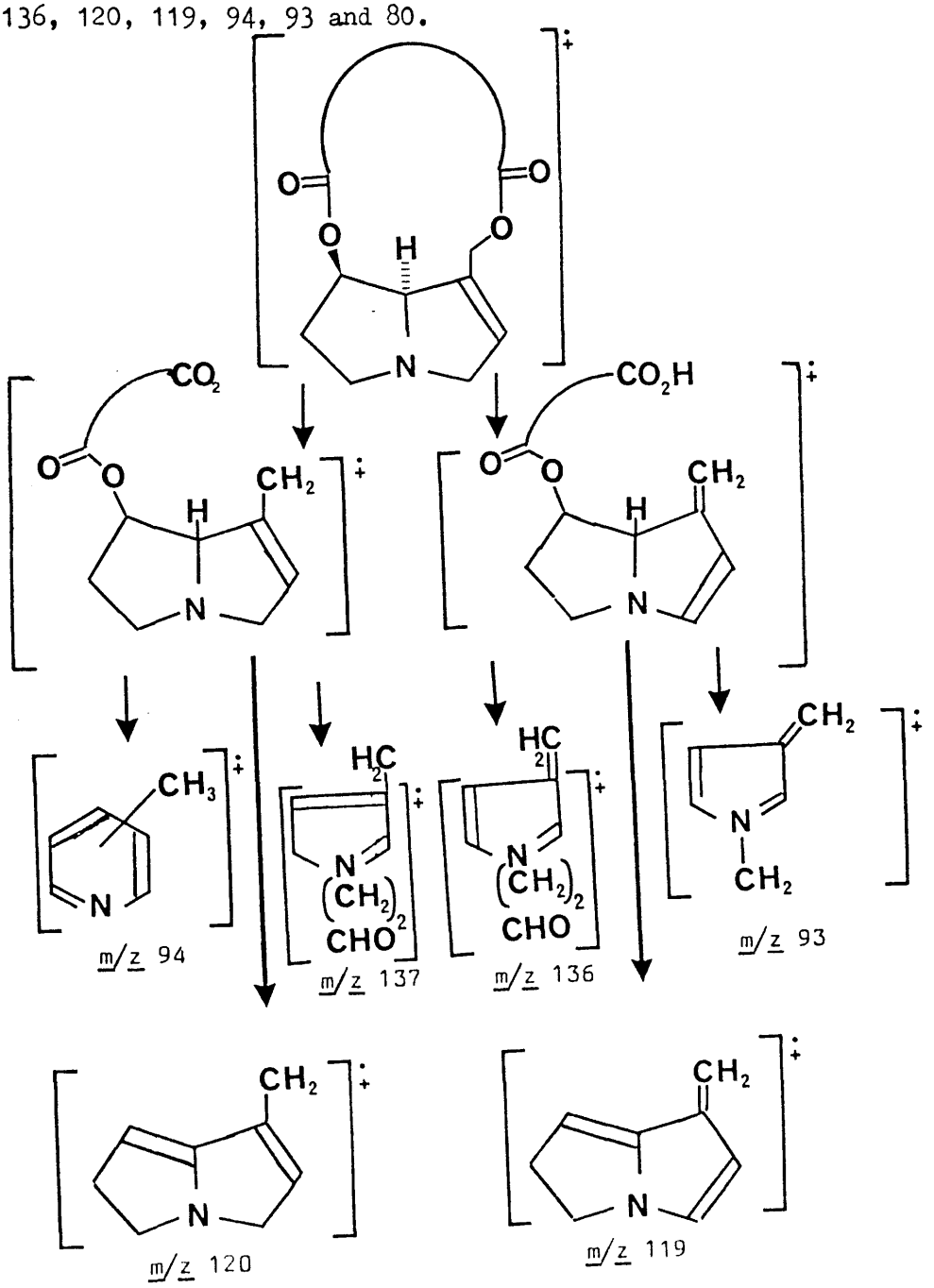


The signals due to the protons at C-7 and C-9 of retronecine give the most useful information about the state of esterification of the molecule.[1] When the C-7 and C-9 hydroxyl groups are not esterified these C-H protons give rise to signals in the region

δ 4.0-4.3 ppm. Esterification at C-7 of retronecine shifts the H-7 signal to around δ 5.05 ppm. Esterification at C-9 gives rise to a variety of signals due to the protons at C-9 depending on the extent of magnetic non-equivalence of these diastereotopic protons. In the simplest case where the protons are nearly magnetically equivalent they give rise to a broad singlet at about δ 4.8 ppm. As the degree of non-equivalence becomes greater, this signal resolves into an AB quartet distributed more or less symmetrically about the chemical shift which would be expected if the protons were equivalent. The difference in magnetic environments of the C-9 protons is largest in the case of the macrocyclic diester alkaloids due to the restriction of rotation about the C-1/C-9 bond. The difference in the chemical shifts of these two protons at C-9 is conveniently represented as $\Delta\delta$ H-9. The occurence of this AB quartet with a $\Delta\delta$ H-9 of 0.4 ppm or greater is considered to be convincing evidence for the existence of a macrocyclic diester (CHAPTER 1.3). The appearance of this quartet is characteristic, see figs. 1 and 2 (CHAPTER 2), figs. 3 and 4 (CHAPTER 3) and fig.7 (CHAPTER 4). The downfield doublet is usually very sharp, indicating that the C-9 downfield proton is in or nearly in the plane of the C-1/C-2 double bond (and thus deshielded). The upfield doublet is broadened by additional allylic coupling, so that it is sometimes difficult to identify without the aid of decoupling experiments.

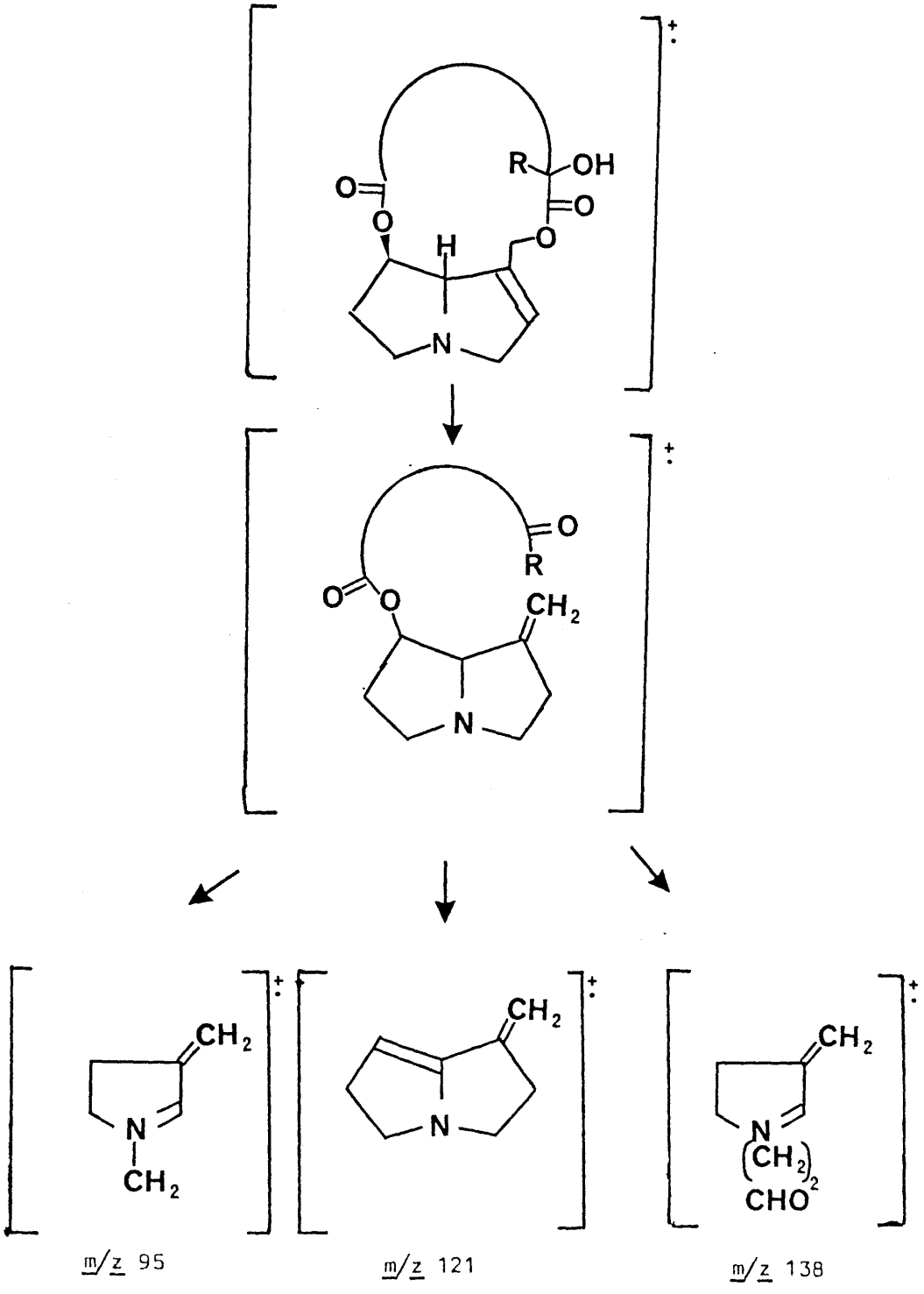
2.2.2 MASS SPECTRA

The mass spectrum is particularly useful in identifying the base portion of pyrrolizidine alkaloids.[1] Two characteristic fragmentation patterns of a macrocyclic diester of retronecine are shown in SCHEME 15. These give rise to the diagnostic fragments m/z 137, 136, 120, 119, 94, 93 and 80.



SCHEME 15

If there is a hydroxyl group α to the allylic ester carbonyl, the fragmentation is as shown in SCHEME 16.



SCHEME 16

NOTE ON SYNTHESIS.

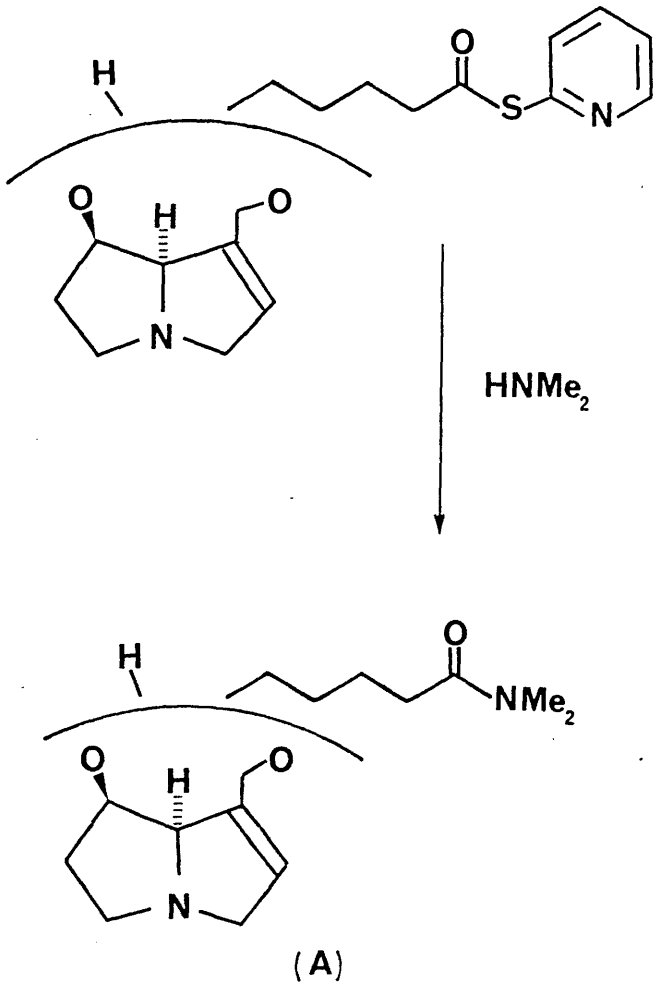
In each of the following syntheses of macrocyclic diesters a small-scale preliminary experiment was carried out to establish that the reaction of each anhydride with (+)-retronecine did produce the expected monoesters. The molecular ion, i.r. spectrum, and p.m.r. spectrum of the obtained materials were consistent with the proposed structures.

2.3 13,13-DIMETHYL-1,2-DIDEHYDROCROTALANINE (47)

As a starting point for this work the synthesis of (47) was repeated. A chloroform solution of (+)-retronecine was treated with one equivalent of 3,3-dimethylglutaric anhydride. After 12 hours at room temperature the solvent was removed at reduced pressure to leave a mixture of the C-9 and C-7 monoesters as a gum. This mixture was dissolved in dimethylformamide (D.M.F.). Triphenylphosphine and 2,2′-dipyridyldisulphide were added and the solution left at room temperature to allow the thioester to form. The cyclisation was achieved by heating the thioester solution at high dilution. Conditions of high dilution were obtained by adding the thioester solution over a period of several hours to refluxing D.M.F. The product was isolated by removal of solvent followed by acid/base recycling. The crude base was purified by preparative t.l.c. (yield 49%). The spectral and analytical data obtained for this material were identical with those obtained for a sample of (47) provided by the previous workers.

It was considered that the yield of 49% obtained was rather low for such a simple system. The conditions used for the cyclisation (refluxing D.M.F.) were also thought to be too extreme to be suitable for use in the synthesis of more complex systems.

Significant amounts (10-30%) of amide by-products were produced during the reaction. This indicated a breakdown of the D.M.F. to give dimethylamine which in turn attacked the activated ester.(SCHEME 17)



(A)

SCHEME 17

Spectral data of these by-products were consistent with the presence of the mixture (A). In particular, an amide absorption was observed at 1690 cm^{-1} in the i.r. spectrum. The molecular ion at m/z 324 in the mass spectrum is correct for the formula $C_{17}H_{28}N_2O_4$. The dimethylamide methyl singlets were observed at δ 3.00 and 2.90 ppm. These singlets had a total integral of six times that of the pyrrolizidine H-2 proton.

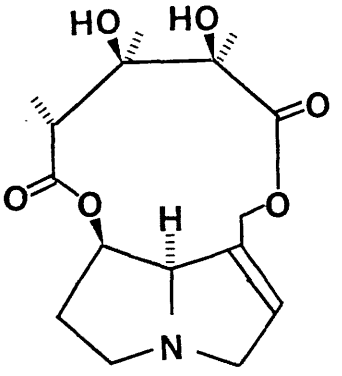
In the course of several experiments using D.M.F. as solvent no reproducible pattern could be observed in the proportions of these by-products produced. It was considered that very small changes in pH or trace quantities of impurities in the reaction mixture were producing large changes in the composition of the product mixture. In order to eliminate the problem of solvent breakdown, 1,2-dimethoxyethane (D.M.E.) was tested as an alternative solvent. This improved the reaction yields to over 60%.

In an attempt to increase the yield still further it was decided to omit the isolation of the monoester mixture. This would involve either carrying out the monoesterification in D.M.E. or carrying out the final cyclisation in chloroform. The latter alternative was chosen as this would allow the reaction to be carried out at a lower temperature and thus permit the reaction to be applied to less stable macrocyclic products. This modification consistently gave yields of over 80%.

It was found that for reactions on a 0.5 mmol or larger scale, higher yields could be obtained by vacuum column chromatography of the total concentrated reaction product mixture. For smaller quantities acid/base recycling followed by preparative t.l.c. gave better results.

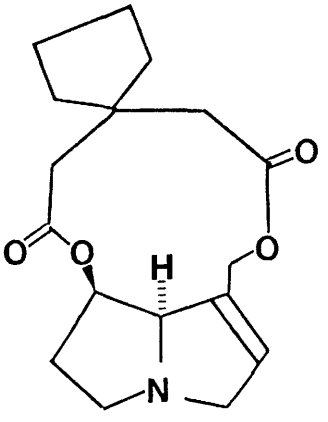
The most interesting aspect of 11-membered macrocyclic diester (47) is its p.m.r. spectrum. The key feature of this spectrum is the AB quartet due to the C-9 protons. (δ 5.32 and δ 4.08, J 12Hz). The large difference in the chemical shift of these protons of δ 1.24ppm would previously have been taken as a strong indication of a 12-membered macrocyclic diester (see CHAPTER 1.3). However, as this molecule can only contain an 11-membered ring, the large chemical shift difference may indicate that the conformation of the diester is significantly different from that of the other 11-membered

macrocycles so far studied. As the crystal structure of this molecule would be of interest, a series of crystalline derivatives of (47) was prepared. The picrate, hydrobromide and picrolonate derivatives could not be crystallised in a form suitable for X-ray analysis. The perchlorate salt of (47) was obtained in a suitable crystalline form by the slow diffusion of methanol vapour into a saturated aqueous solution of the salt. The X-ray analysis of this derivative is being carried out but is not yet available. A further sample of (47) prepared as its hydrobromide has been tested for its hepatotoxic properties.[49] It has been shown to have a greater toxicity than the simple retronecine acyclic diesters, and a comparable toxicity with monocrotaline (48), which is a common 11-membered alkaloid.



(48)

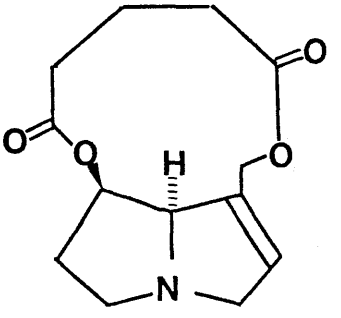
2.4 13,13-TETRAMETHYLENE-1,2-DIDEHYDROCROTALANINE (49)



(49)

To test the scope of this reaction it was repeated with a different anhydride, 3,3-tetramethyleneglutaric anhydride. The cyclisation was carried out as before with chloroform as solvent throughout. This yielded an oil which was characterised as its picrate. The high resolution mass spectrum of the free base showed a molecular ion corresponding to $C_{17}H_{23}NO_4$. In addition a typical fragmentation pattern for a retronecine macrocyclic diester was observed,[1] with peaks at m/z 137, 136, 120, 119, 94, 93 and 80. The i.r. spectrum of (49) showed a saturated ester carbonyl absorption at 1 730 cm^{-1}, with no evidence of any hydroxyl or carboxylic acid groups present. The p.m.r. spectrum contained an AB quartet at δ 5.33 and 4.10 ppm (J 13Hz) due to the geminal C-9 protons. The above spectral data are taken as convincing evidence that the macrocyclic diester (49) has been formed.

2.5 1,2-DIDEHYDROCROTALANINE (50)



(50)

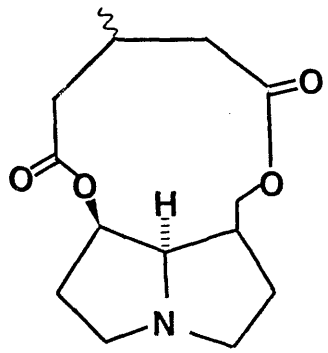
The synthesis of 1,2-didehydrocrotalanine was of particular interest as it is the parent compound of the 11-membered macrocyclic diester series.

The usual method of cyclisation could not be used in this case, as the monoester mixture obtained by the reaction of retronecine with glutaric anhydride was not soluble in chloroform, D.M.E.,acetonitrile, or chlorobenzene. The desired cyclisation was eventually achieved by allowing the monoester mixture to be slowly dissolved into a solution of triphenylphosphine and 2,2-dipyridyldisulphide by the refluxing D.M.E. solvent.

It was subsequently discovered that if the monoester suspension was rapidly stirred for several hours in a chloroform solution containing 2.5 molar equivalents of triphenylphosphine and 2,2′-dipyridyldisulphide a homogenous solution of the activated thioesters was obtained.

After cyclisation, the reaction mixture was worked up in the usual manner to yield an oil in about 75% yield which was characterised as its picrate. High resolution mass spectrometry indicated a molecular formula of $C_{13}H_{17}NO_4$ for the free base. The characteristic fragmentation pattern of a macrocyclic retronecine diester was also observed in the mass spectrum of (50). The i.r. spectrum showed a saturated ester carbonyl absorption at 1 732 cm^{-1}. There was no evidence of any hydroxyl or carboxylic acid groups. The p.m.r. spectrum showed an AB quartet at δ 4.95 and 4.34, J 12Hz. The difference in chemical shifts of the C-9 protons of 0.61 ppm, in combination with the other spectral data, is considered sufficient evidence to establish the synthesis of the macrocyclic pyrrolizidine analogue (50).

2.6 13R, 13S-METHYL-1,2-DIDEHYDROCROTALANINE (51+52)



(51)+(52)

This mixture was chosen as a synthetic target partly because it is a close model for dicrotaline (6) and its C-13 epimer and also because it would be of interest to compare the $\Delta\delta$ H-9 values for the two isomers.

As in the previous case the monoester mixture was found to be insoluble in the usual cyclising solvents. This problem was overcome by forming the activated thioester in D.M.F. (as in 2.2 method 1) and by slowly adding this solution to a 100 fold excess of refluxing D.M.E.. It was hoped that by keeping the D.M.F. concentration low amide formation would be avoided.

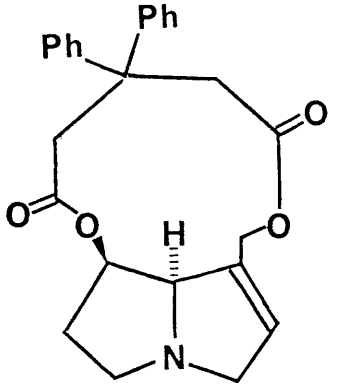
Significant amounts of the amide by-products were however found in the reaction mixture. A mixture of (13R)- and (13S)- methyl-1,2-didehydrocrotalanine was obtained from the reaction mixture by preparative t.l.c.. No t.l.c. system could be found to separate the two isomers. Spectral data were therefore obtained for the mixture of the free bases.

High resolution mass spectrometry indicated a molecular formula of $C_{14}H_{19}NO_4$. The i.r. spectrum again revealed an absorption at 1 732 cm^{-1} corresponding to saturated

esters with no absorptions due to hydroxyl or carboxylic acid groups.

The 90 MHz p.m.r. spectrum of this mixture gave little information due to the extensive overlapping of signals. With a 360 MHz spectrum however it was possible to resolve each signal clearly. As the two isomers were not present in equal proportions it was possible to assign the signals of the separate isomers by comparing the integrals and chemical shifts of each signal. By this means the mixture was identified as a 2:1 mixture of the two isomers. The C-9 protons of the major component gave rise to an AB quartet (δ 4.81 and 4.30 ppm, J 12Hz) with a $\Delta\delta$ H-9 of 0.51ppm. The AB quartet (δ 5.02 and 4.03 ppm, J 12 Hz) due to the C-9 protons of the minor component showed a $\Delta\delta$ H-9 value of 0.99 ppm.

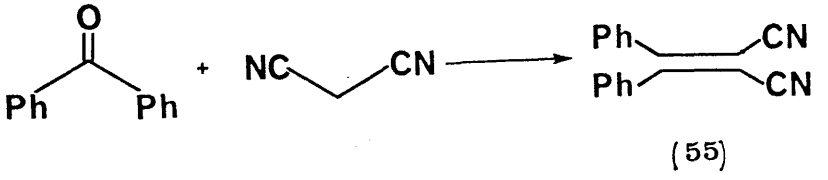
2.7 13,13-DIPHENYL-1,2-DIDEHYDROCROTALANINE (53).



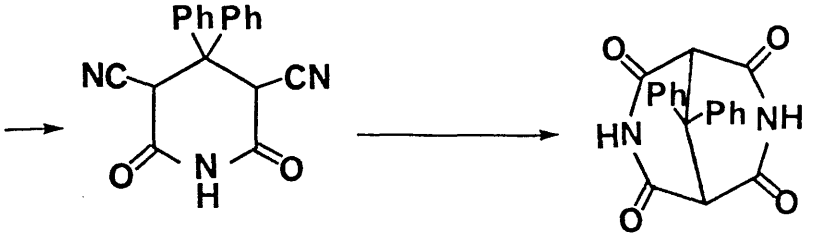
(53)

3,3-Diphenylglutaric anhydride (54) was prepared by the method of BRUICE and BRADBURY[50] (SCHEME 18). Benzophenone was treated with a solution of malononitrile in benzene in the presence of acetic acid. The condensation was driven to completion by azeotropic removal of water. The resulting 1,1-dicyano-2,2-diphenylethene (55) was treated with an ethanolic solution

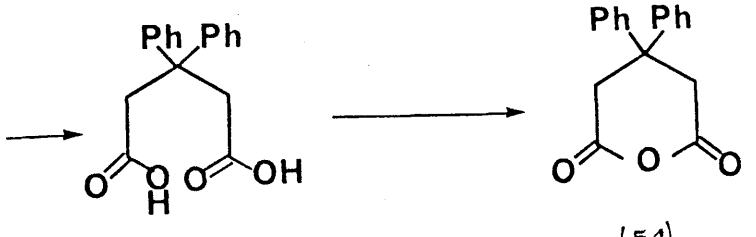
of sodiomalononitrile. Acidification yielded 3,3-diphenyl-2,4-dicyanoglutarimide (56). This imide was heated at reflux in a mixture of acetic and sulphuric acids to give the diimide (57) of 2,2-diphenylpropane-1,1,3,3-tetracarboxylic acid. Basic hydrolysis yielded the tetracarboxylic acid which was decarboxylated <u>in situ</u> by further heating at reflux with excess sulphuric acid. The 3,3-diphenylglutaric acid (58) thus obtained was converted into the anhydride (54) by treatment with acetic anhydride. Analytical and spectral data for this material (54) were the same as those reported by the above workers.[50]



(55)



(56) (57)



(58) (54)

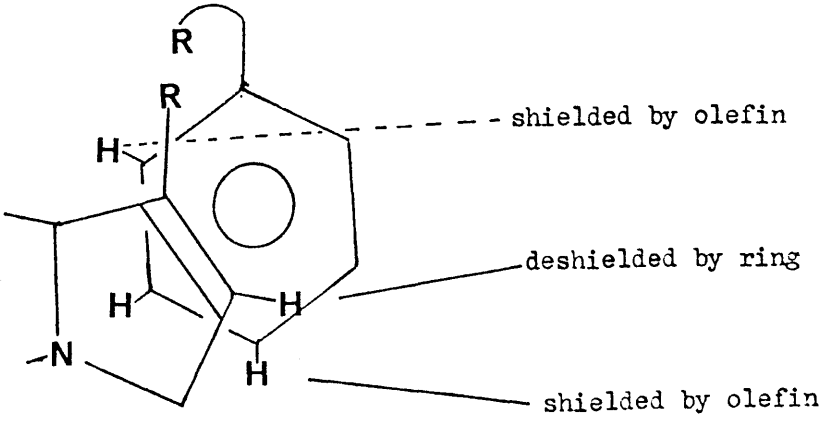
SCHEME 18

The anhydride (54) was treated with (+)-retronecine as before and the thioester formation and cyclisation carried out in chloroform as in chapter 2.2.method 3. Isolation of the product by either column chromatography or acid/base recycling and preparative t.l.c. yielded a colourless oil in about 70% yield. This oil began to darken within a few minutes of isolation. Subsequent purification by t.l.c. yielded the same oil in about 60% overall yield, but again it started to decompose almost immediately. Attempts to prepare stable derivatives of this material such as the hydrochloride, picrate or methiodide resulted in the formation of black gums. Spectra of freshly prepared samples of the free base were therefore taken as rapidly as possible. The high resolution mass spectrum of this base showed a molecular ion corresponding to $C_{25}H_{25}NO_4$. The characteristic pyrrolizidine fragmentation pattern was also clearly visible in the mass spectrum. The i.r. spectrum revealed an absorption at 1 740 cm^{-1}, corresponding to a saturated ester carbonyl. No absorptions were observed which could be attributed to carboxylic acid or hydroxyl groups. These spectroscopic data were considered to be convincing evidence for the formation of (53). The p.m.r. spectrum at first seemed at variance with this result. The characteristic AB quartet of the C-9 protons was not present. The signal for these protons was visible as a broad singlet at δ 4.45 ppm. The magnetic near equivalence of the C-9 protons is generally considered to be evidence for free rotation about the C-1/C-9 bond, thus excluding the existence of a macro-ring. It was considered however on the grounds of the m.s. and i.r. data that the cyclic diester had been formed and that the C-9 protons were "accidently" equivalent. This may indicate that the alkaloid has adopted an unusual conformation. The following p.m.r. spectral information supports this

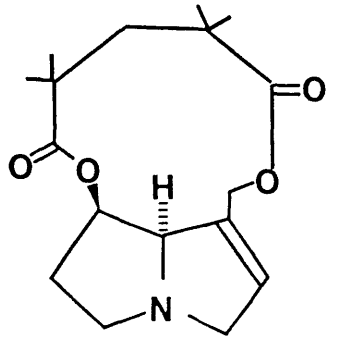
suggestion. While the chemical shift of the C-7 proton is typical (δ 5.05 ppm), that of the C-9 protons is atypically low (δ 4.45ppm) compared to the usual value (δ 4.7-4.9 ppm). The chemical shift of the C-2 proton of δ 5.38 ppm is also unusual. The chemical shift of this olefinic proton is generally in the region of δ 5.9 ppm. Furthermore multiplets were observed at δ 7.65 and δ 8.51 ppm, each corresponding to a single proton. The remaining aromatic protons give rise to an eight proton complex at δ 7.23 ppm. Decoupling experiments showed that the signal at δ 8.51 ppm is a doublet (J 7Hz), (ortho-coupling with one of the protons at δ 7.23) and meta-coupling (J 2Hz) to the proton at δ 7.65 ppm. The signal at δ 7.65 was shown to be a distorted triplet (J 8Hz) (ortho-coupling with two protons at δ 7.23 ppm).

The above data are considered to show that the two phenyl groups constrain the macro-ring to adopt a conformation in which one of the aromatic rings is close enough to the retronecine nucleus to influence and be strongly influenced by this nucleus.

A possible partial conformation consistent with these data is shown below. The lack of an AB quartet for the C-9 protons does not therefore preclude the assignment of this compound as the macrocyclic diester (53).



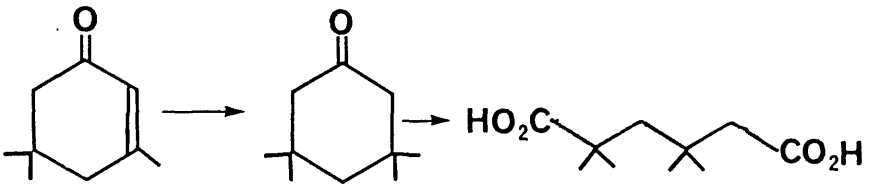
2.8 ATTEMPTED SYNTHESIS OF 12,12,14,14-TETRAMETHYL-1,2-DIDEHYDROCROTALANINE (59).



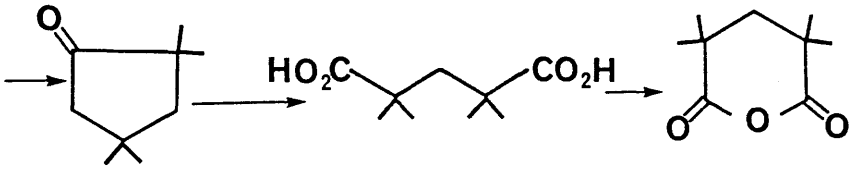
(59)

This compound was selected as a synthetic target, as its hepatotoxicity would be worthy of investigation. It might have a higher toxicity than the dimethyl analogue (47) and many of the natural alkaloids, due to the hindered α-position of both the C-7 and C-9 esters. This steric hindrance should reduce the rate of detoxification of the base by ester hydrolysis. 2,2,4,4-Tetramethylglutaric anhydride (60) was prepared by the method of HALL.[51] (SCHEME 19)

3,3,5,5-Tetramethylcyclohexanone (62) was prepared by the copper (1) catalysed 1,4 addition of methyl magnesium bromide to isophorone (3,5,5-trimethylcyclohex-2-enone) (61). Nitric acid oxidation of this compound produced 2,2,4,4-tetramethyl adipic acid (63). Destructive distillation from barium oxide yielded 2,2,4,4-tetramethylcyclopentanone (64). Nitric acid oxidation of this ketone gave 2,2,4,4-tetramethylglutaric acid (65) which was converted into its anhydride (60) by treatment with acetic anhydride.



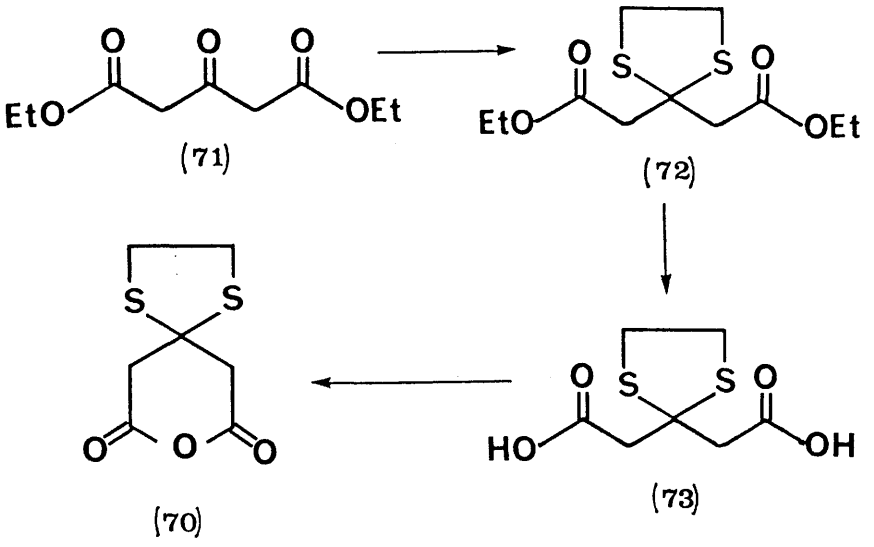
(61) (62) (63)



(64) (65) (60)

SCHEME 19

Reaction of the anhydride (60) with (+)-retronecine in chloroform gave a quantitative yield of the C-9 monoester (66). No C-7 monoester was present as shown by the absence of a signal around δ 5.05 in the p.m.r. spectrum. The ratio of the signals at δ 5.70 and δ 4.70 ppm (corresponding to H-2 and H-9 when C-9 is esterified) was found to be precisely 1:2. The 2-pyridyl thioester was formed in the usual fashion. The intramolecular cyclisation was attempted in a series of solvents with progressively higher boiling points. No cyclised material was detected in any case. The imidazole thioester (67) has been reported[52] to be 100 times more effective in acid activation than the pyridyl thioester. No cyclisation was detected in several attempts using the imidazole thioester.



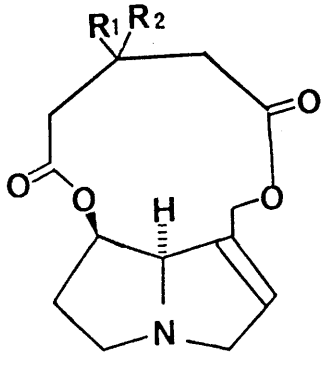
SCHEME 20

The structures (70)-(73) were confirmed by p.m.r. and i.r. spectra and by elemental analyses.

The anhydride (70) was reacted with (+)-retronecine in the usual manner to yield a mixture of the C-7 and C-9 monoesters in a 1:1 ratio (established by p.m.r.). All attempts to cyclise the ω -hydroxy acid via the 2-pyridyl of imidazole thioesters resulted in the production of complex mixtures of products, none of which could be identified.

2.9 CONCLUSIONS

Six 11-membered macrocyclic diesters of (±)-retronecine (46)-(51) have been synthesised.



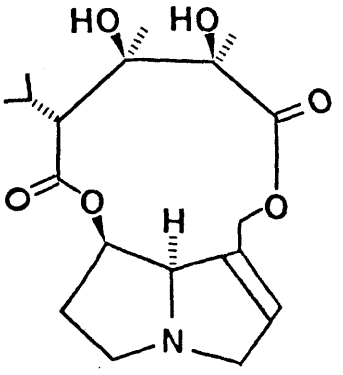
$R_1=R_2=CH_3$ (47)
R_1;R_2=tetramethylene (49)
$R_1=R_2=H$ (50)
$R_1=H$, $R_2=CH_3$ (51) + (52)
$R_1=CH_3$, $R_2=H$
$R_1=R_2=Ph$ (53)

The $\Delta\delta$H values for these analogues are tabulated below.

ANALOGUE	$\Delta\delta$H (ppm)
(47)	1.25
(49)	1.23
(50)	0.62
(51) and (52)	0.99 and 0.51
(53)	0

Before this work and the work of ROBINS and SAKDARRAT[25] there had appeared to be a sharp division between the $\Delta\delta$ H-9 values of the 11-membered macrocyclic diesters (0-0.73 ppm) and those of the 12-membered diesters (1.25-1.53). It now seems reasonable to postulate that there is a wider range of $\Delta\delta$ H-9 values which reflect the different conformations of the alkaloids. The major factor governing the conformation of the macro-ring may be the ring size, although it has been demonstrated that substituents on the ring (at least in the 13 position) also have a significant bearing on the conformation adopted. As the above series of analogues is now readily available, (a 100mg sample of any one can be conveniently prepared in two days) the investigation of their structure/conformation/ toxicity relationships is now feasible. As the $\Delta\delta$H value reflects the conformation of the alkaloid in solution there may be no obvious correlation between the $\Delta\delta$ H value and the crystal structure of the material. A case in point is trichodesmine[53](46) which has been reported to have its ester carbonyls antiparallel in the solid state (i.e. the typical conformation of the 12-membered diesters), while in chloroform solution its $\Delta\delta$ H-9 value is 0.74 ppm, a value typical of the 11-membered macrocycles. Further information about the conformation of these alkaloids in solution may

be obtainable by carrying out N.O.E. experiments on these molecules and observing the p.m.r. spectra.



(46)

fig. 1

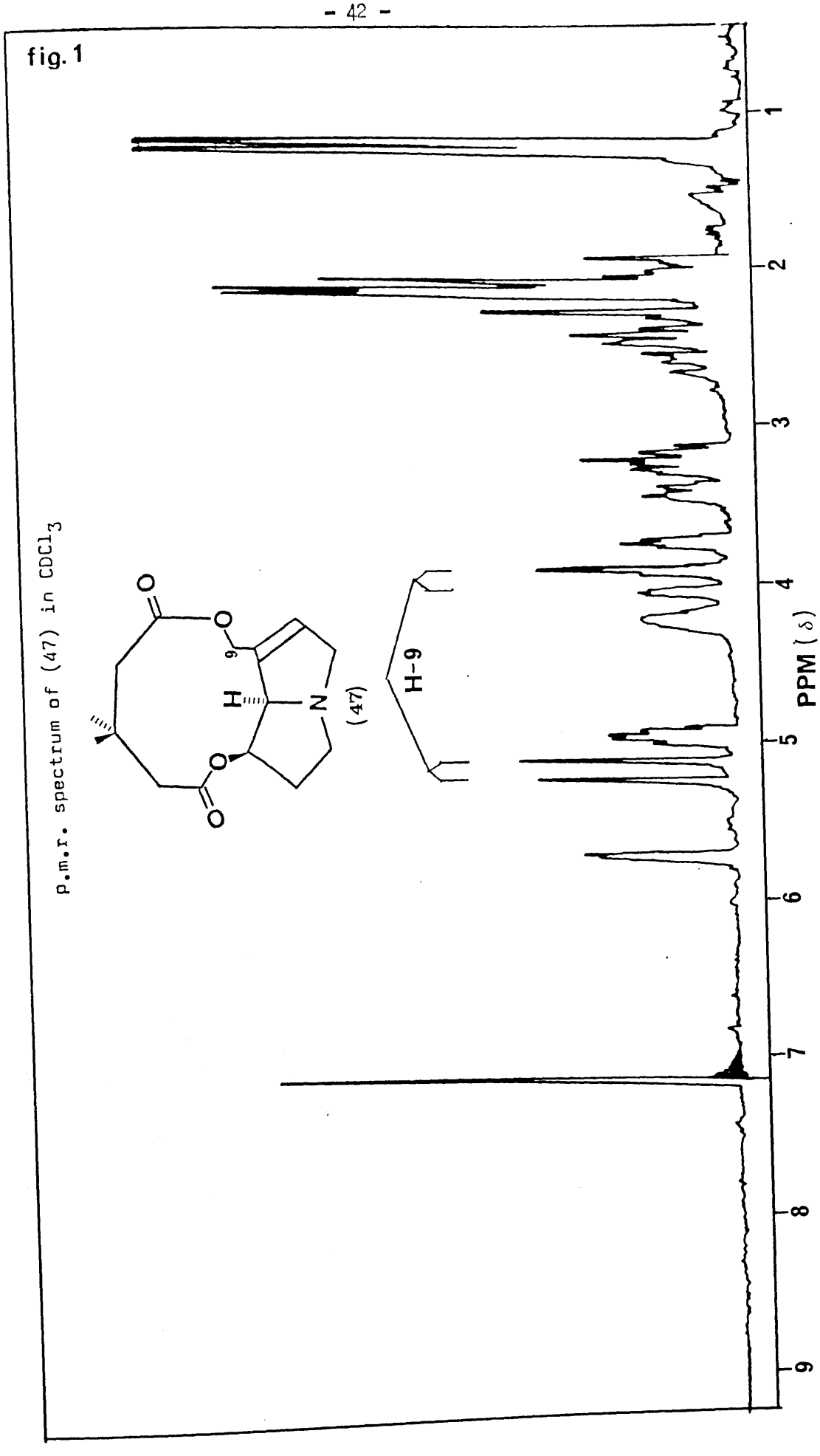
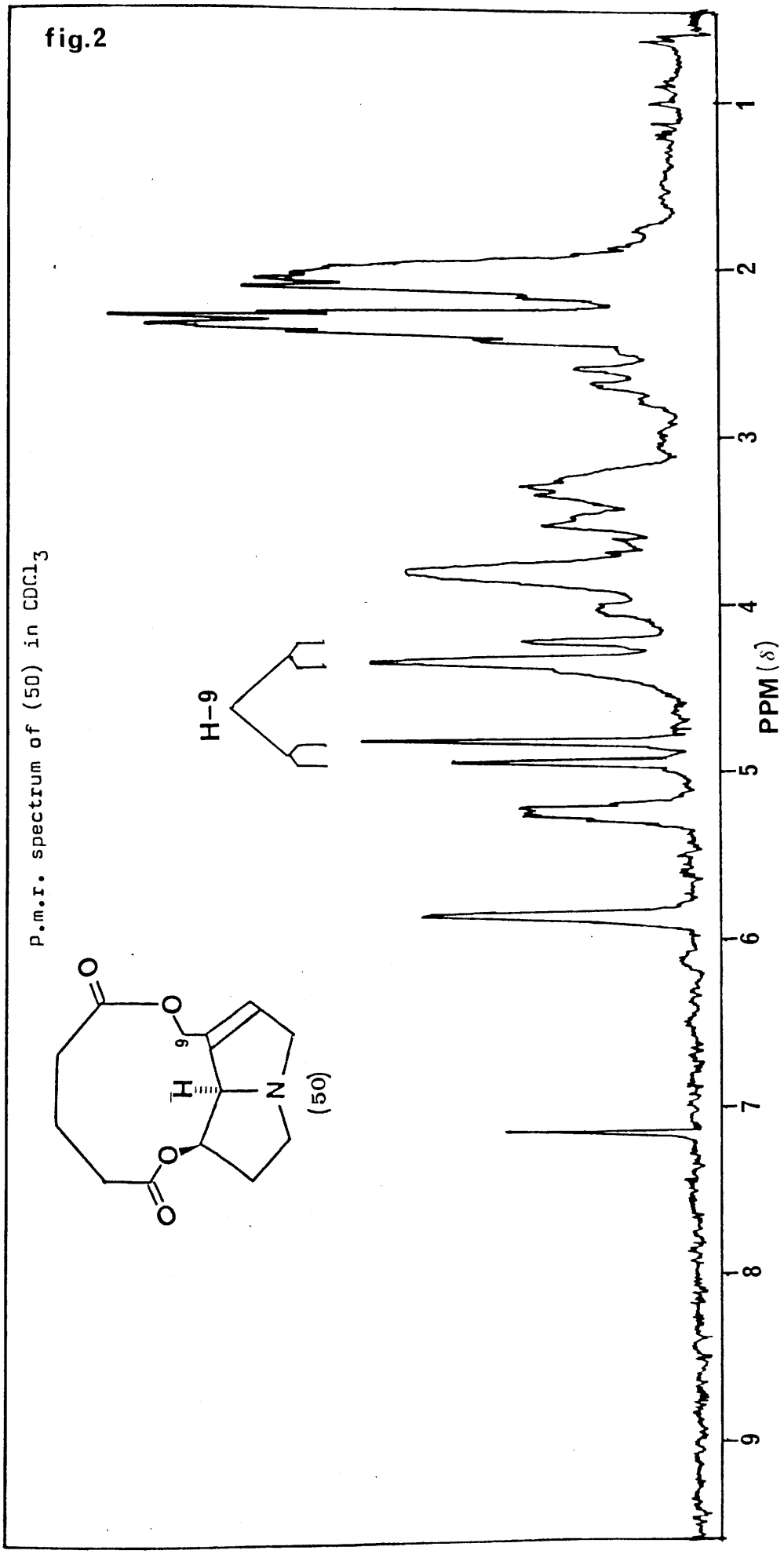


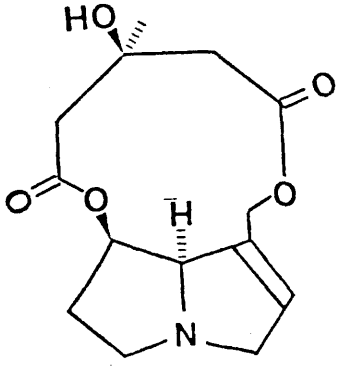
fig.2



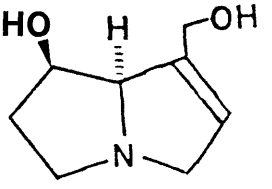
CHAPTER 3

THE SYNTHESIS AND ABSOLUTE CONFIGURATION OF DICROTALINE.

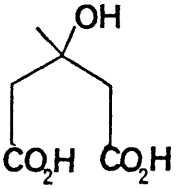
3.1 INTRODUCTION Dicrotaline (6) was isolated from Crotalaria dura (WOOD and EVANS), by MARAIS in 1944.[26] It was shown to be a macrocyclic diester of retronecine (3). The diacid was found to have the molecular formula $C_6H_{10}O_5$. The identity of this acid as 3-hydroxy-3-methylglutaric acid (75) was proved by the synthesis of ADAMS and VAN DUUREN.[27]



(6)



(3)

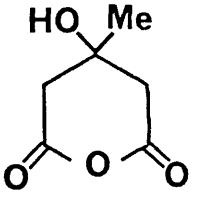


(75)

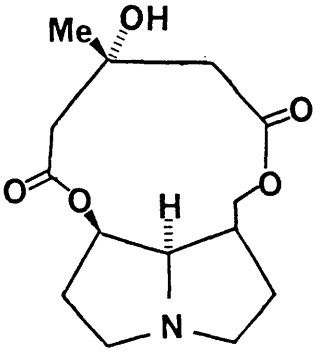
Reconstruction of dicrotaline from (+)-retronecine (3) and dicrotalic acid (75) would constitute a total synthesis as (±)-retronecine has previously been synthesised by several groups of workers (CHAPTER 1.4), and resolved.[54]

It was envisaged that this reconstruction could be achieved by cyclisation of the monoesters obtained from the reaction of (+)-retronecine with dicrotalic anhydride (76) as described for the macrocyclic pyrrolizidine alkaloid analogues in CHAPTER 2. As dicrotalic acid is not symmetrically substituted about C-3 it was anticipated that the ring closure would lead to a mixture of C-13 epimers as in the case of 13-methyl-1,2-didehydrocrotalanine (51) + (52).

Although no separation of isomers was possible in that case it was considered that the two different orientations of the hydroxyl group at C-13 might lead to sufficiently different polarities of dicrotaline (6) and its epimer (77), which would allow the isomers to be separated.



(76)



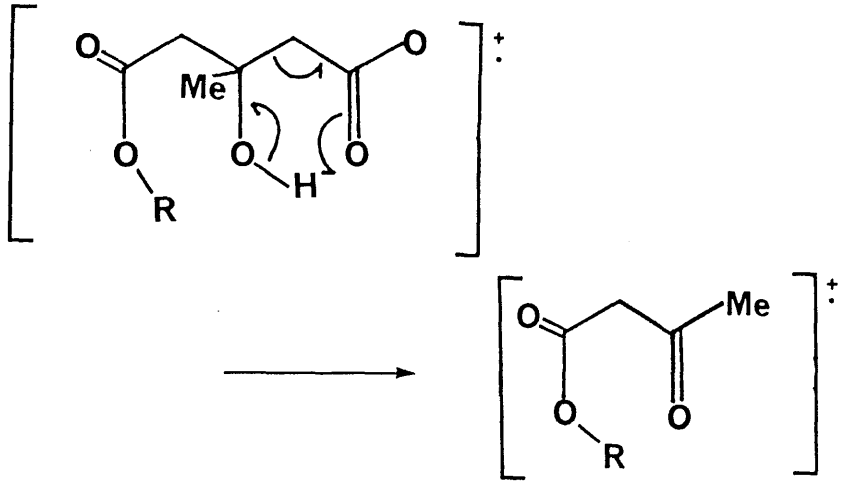
(77)

3.2 CHARACTERISATION OF NATURAL DICROTALINE.

Before this synthesis was undertaken it was necessary to obtain an authentic sample of dicrotaline for comparison purposes. No spectral data had previously been reported for this alkaloid.

Seeds of Crotalaria dura were extracted and the alkaloidal component was investigated. Analytical t.l.c. showed that this consisted of a single compound. Spot tests showed that the alkaloid contained a 3-pyrroline structure.[55]

The crude alkaloid was purified by preparative t.l.c. The following spectral analysis showed that the compound was dicrotaline. The molecular ion in the high resolution mass spectrum of the alkaloid was consistent with the formula required for dicrotaline. The mass spectrum also contained a peak at M-59, corresponding to the loss of $C_2H_3O_2$. This is most conveniently explained by the McLafferty rearrangement shown in SCHEME 21.



SCHEME 21

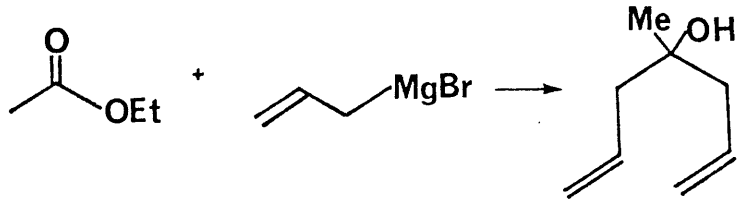
This rearrangement confirms the position of the hydroxyl group at C-13. The mass spectrum also contained the typical fragmentation pattern for a pyrrolizidine diester.

The p.m.r. spectrum of dicrotaline showed a methyl singlet at δ 1.40 ppm and an AA' BB' multiplet at δ 2.67 and 2.42 ppm corresponding to the α-protons of the diacid, in addition to the usual signals for retronecine. An AB quartet due to the C-9 protons was observed at δ 5.40 and δ 4.16 ppm, J 12Hz. The difference in the chemical shifts of the C-9 protons (1.24 ppm) is the largest observed for a natural 11-membered macrocyclic diester of retronecine. It is interesting to note that the $\Delta\delta$ H-9 value is the same as that of the 13,13-dimethyl analogue (47) discussed in the previous chapter.

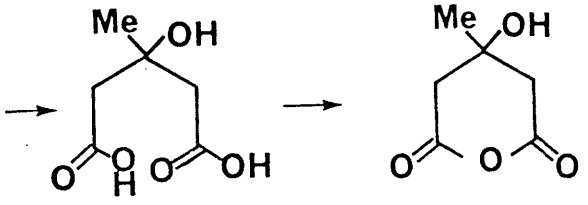
The analysis of the alkaloid hydrochloride was consistent with the molecular formula of dicrotaline hydrochloride. Dicrotaline hydrochloride has been reported to exist in two crystalline forms.[26] The melting point and the appearance of the crystals prepared in this work were similar to those of the form reported with the lower melting point.

3.3 SYNTHESIS OF DICROTALINE AND ITS C-13 EPIMER.

Dicrotalic acid (75) was synthesised by a modified version of the route of KLOSTERMAN and SMITH[56] outlined in SCHEME 22. Addition of allyl magnesium bromide to ethyl acetate yielded 4-hydroxy-4-methylhepta-1,6-diene (78). Ozonolysis of the diene (78) followed by oxidative cleavage of the ozonide gave dicrotalic acid (75). The anhydride was formed by treatment of the acid (75) with thionyl chloride in diethyl ether.



(78)

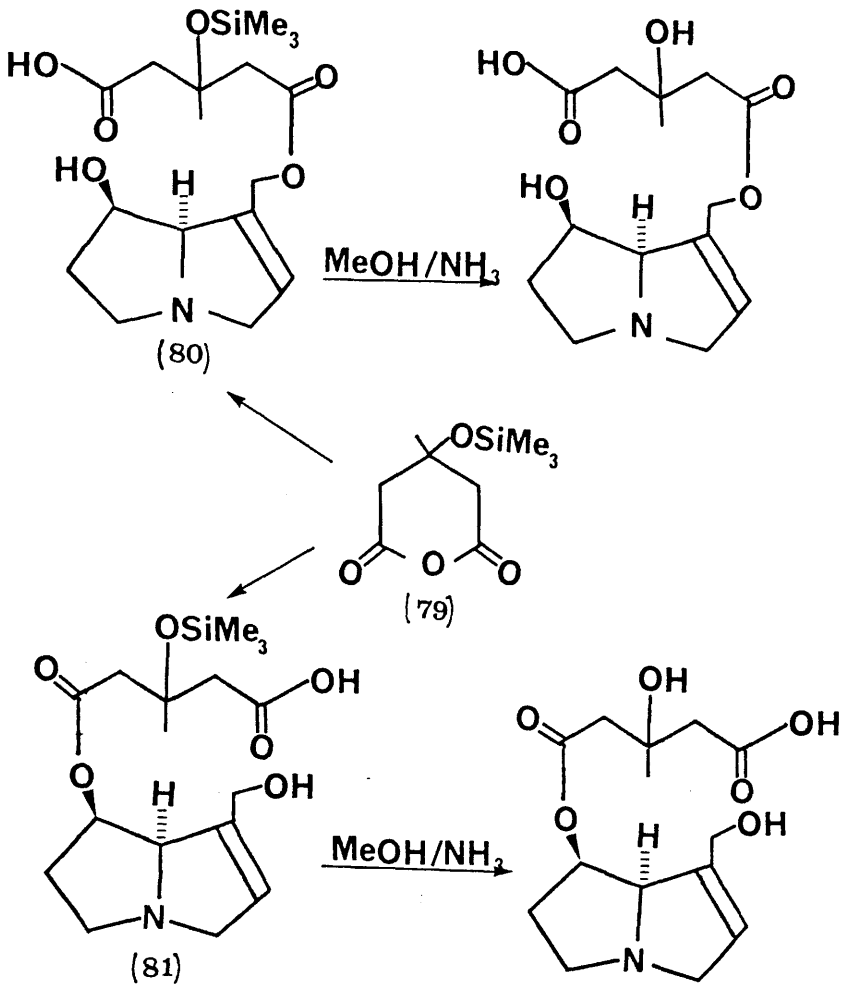


(75)

(76)

SCHEME 22

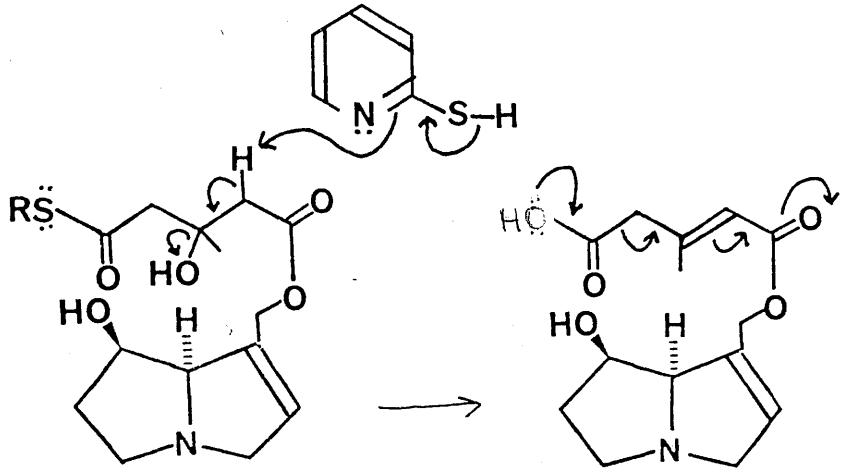
The tertiary alcohol was protected as its trimethylsilyl ether by treatment with trimethylsilyl chloride and pyridine in ether. The protected anhydride (79) was treated with (+)-retronecine in chloroform to give a mixture of the two C-9 monoester diastereomers (80) and the corresponding C-7 monoesters (81) in about equal proportions judged from the p.m.r. spectrum. Spectral data were also obtained for the deprotected monoester mixture (SCHEME 23).



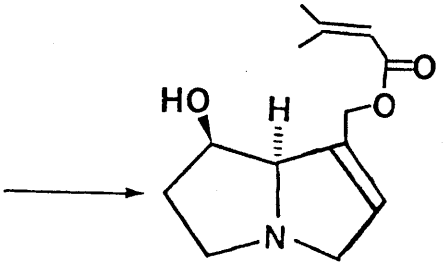
SCHEME 23

One equivalent each of triphenylphosphine and 2,2′-dipyridyldisulphide were added to a chloroform suspension of the protected monoesters. As the thioesters formed they passed into solution. Cyclisation of the thioesters and work up of the products were carried out in the usual manner. Analytical t.l.c. showed that the reaction product consisted of three components. Several solvent systems were tried before one was found which gave clear separation of the three components. The most polar component gave rise to an elongated spot which could not be further resolved.

The most polar component was identified as a mixture of the C-7 and C-9 monoester of retronecine with 3-methylbut-2-enoic acid (82) and (83). These structures were assigned on the basis of a molecular ion at m/z 235 in the mass spectrum, and absorptions in the i.r. and u.v. spectra corresponding to an $\alpha\beta$ -unsaturated ester. A possible mechanism for the formation of these by-products is shown in SCHEME 24.



plus C-7 monoester



(83)

plus C-7 monoester

(82)

SCHEME 24

The least polar component of the mixture was shown to be identical with a sample of authentic dicrotaline by comparison of high resolution mass spectra, p.m.r. and i.r. spectra. No depression of melting point was obtained by a mixed melting point of the two hydrochlorides of natural and synthetic material.

The third component was identified as the C-13 epimer of dicrotaline. The mass spectrum of "epidicrotaline" (77) was closely similar to that of dicrotaline (6). The i.r. spectra of the two bases were also very similar. In addition to the usual retronecine signals, the p.m.r. spectrum of epidicrotaline showed a methyl singlet at δ 1.40 ppm, and a broad singlet at δ 2.53 ppm corresponding to the four α-protons of the diacid. The AB quartet due to the C-9 protons was observed at δ 5.17 and δ 4.19 ppm, J 12Hz.

Several times during the silylation of dicrotalic anhydride the pyridine hydrochloride did not precipitate from solution. On these occasions the subsequent cyclisations proceeded in lower yield, presumably due to the presence of the conjugate acid.

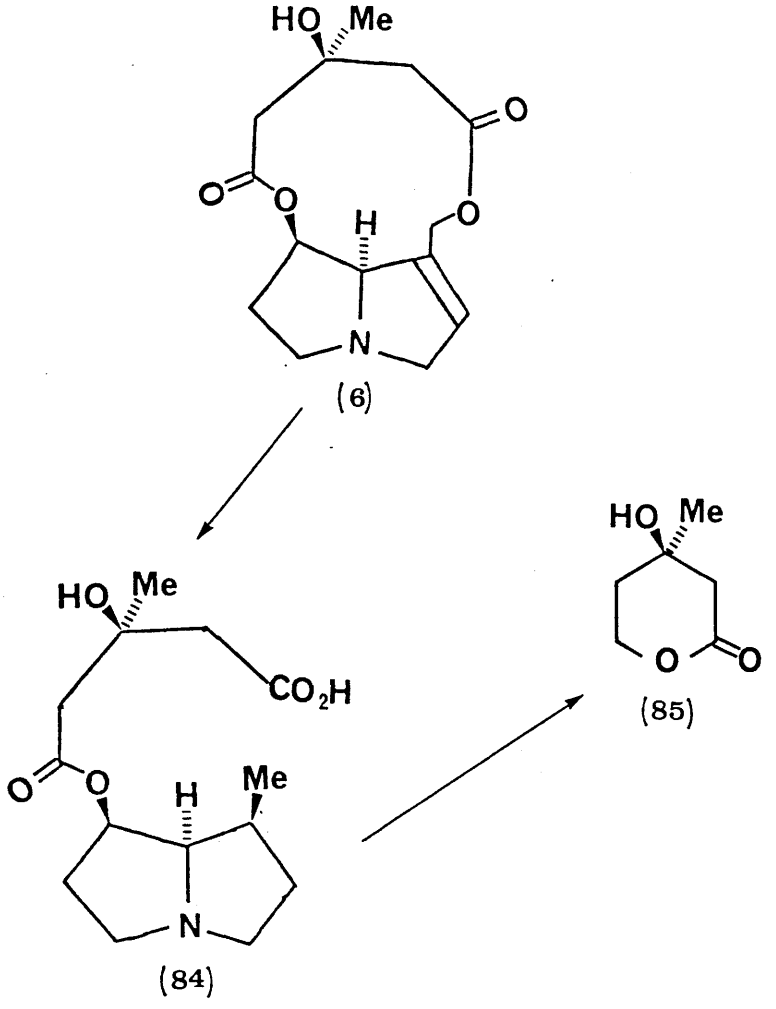
It had been thought that the principal function of the trimethylsilyl group was to render the monoester mixture more soluble in the chosen solvent and thus increase the rate of thioester formation. So an alternative method of solubilising the monoester was sought to avoid making the trimethylsilyl ether.

It was discovered that addition of an excess of triphenylphosphine to a suspension of the monoester in chloroform greatly increased the rate of thioester formation. If two and a half equivalents each of triphenylphosphine and 2,2′-dipyridyldisulphide were added to a rapidly stirring suspension of the non protected monoesters, thioester formation was complete in a few hours. Treatment of this solution in the usual fashion gave a product mixture essentially the same as that obtained from the protected monoesters, in similar yield.

3.4 ABSOLUTE CONFIGURATION AT C-13 IN DICROTALINE AND EPIDICROTALINE.

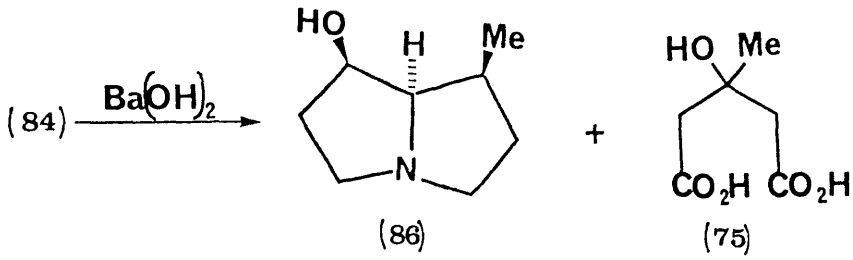
In the previous section, the synthesis of dicrotaline and epidicrotaline was described. The absolute configuration at C-13 of each epimer was not known.

Dicrotaline (6) was shown to have the (S) configuration at C-13 by conversion into optically active mevalonolactone (SCHEME 25) by a sequence of two selective reactions.



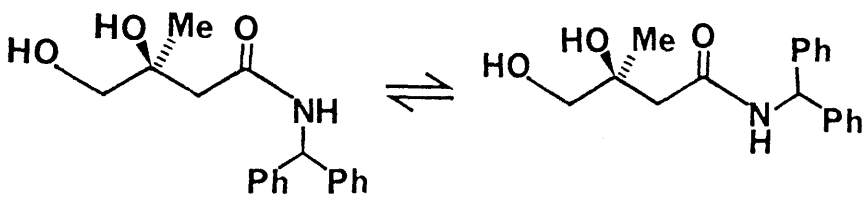
SCHEME 25

Hydrogenolysis of dicrotaline (6) over PtO_2 in acetic acid gave a single compound (84). This formulation was established by basic hydrolysis of the ester (84) to yield dicrotalic acid (75) and (-)-retronecanol (86) (SCHEME 26) which were identical with authentic material.



SCHEME 26

Treatment of the ester (84) with sodium in liquid ammonia followed by acidification and continuous extraction of the acid solution with methylene chloride yielded (R)-mevalonolactone (85). The crude lactone had an $[\alpha]_D^{22}$ in ethanol of -20° (lit.[57] -23°) Spectral data (p.m.r.,i.r.) for this material were closely similar to those of authentic (±)-mevalonolactone. The mevalonolactone (85) was converted into its benzhydrylamide (87).



(87)

The rotation and melting point of this material were identical with those reported for the benzhydrylamide of (R)-mevalonolactone.[57]

The p.m.r. spectrum of racemic benzhydrylamide obtained from (±)-mevalonolactone showed two singlets at δ 6.20 and 6.12 ppm due to the benhydrylic proton of the two amide rotomers. Treatment of the deuteriochloroform solution of the benzhydrylamide of (±)-mevalonolactone with 0.35 equivalents of the chiral shift reagent $Eu(hfc)_3$ resulted in the splitting of the two signals for the benzhydrylic proton into a pair of broad doublets, arising from the two diastereomeric

complexes formed.[59] Similar treatment of the benzhydrylamide of (-)-mevalonolactone obtained in this work did not result in a splitting of the signals for the benzhydrylic proton. Thus, no detectable racemisation occurred during the degradation. Addition of 10% of the benzhydrylamide of (±)-mevalonolactone to the (-)-derivative did result in a detectable splitting of the signals due to the benzhydrylic proton. Thus the limit of detection of racemisation is ca 10%.

Epidicrotaline was degraded in the same way to yield (S)-mevalonolactone which was also converted into its benzhydrylamide. Again, using the chiral europium shift reagent, no trace of R-mevalonolactone was observed.

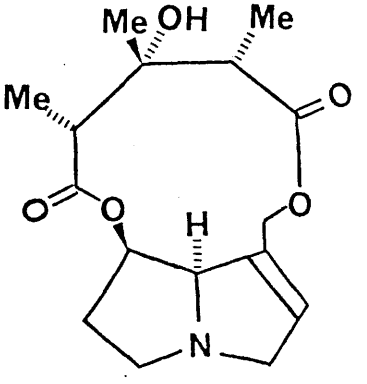
There are no reports of hydrogenolysis of pyrrolizidine alkaloids leading to racemisation of any chiral centre in the alkaloid.[10] The cleavage shown in SCHEME 25 is the only one reasonable under the reaction conditions. Sodium in liquid ammonia is known to reduce esters but not carboxylic acids.[58] No racemisation of mevalonic acid or its lactone has been observed under any of the reaction conditions used in this procedure.[57] It is considered therefore that the two degredations described above have proved that dicrotaline has the (13S) absolute configuration.

3.5 CONCLUSIONS.

Dicrotaline (6) and its C-13 epimer (77) have been synthesised. The absolute configuration of each epimer has been established. These compounds may prove to be useful in structure/conformation/toxicity studies discussed on CHAPTER 2. N.O.E. through space decoupling experiments of these two isomers might give information on the conformation of these molecules in solution, particularly as their configurations are now known. Study of Feiser models of the two structures shows that in a number of the more likely conformations several protons, (particularly the methyl, hydroxyl and α-protons

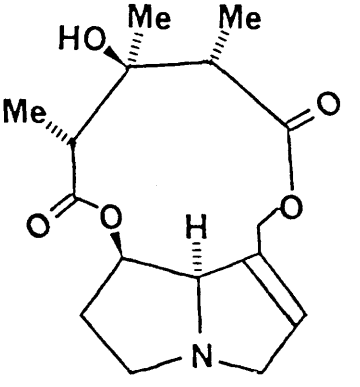
of the diacid, and the C-7, C-8 and C-9 protons of retronecine) are close enough to interact sufficiently to allow N.O.E. studies to be valuable. It would also be of value to study the conformation of dicrotaline in buffered aqueous solutions. Under these conditions the conformation of dicrotaline should be close to its conformation *in vivo*.

Finally, now that this method of cyclisation has been applied to the synthesis of dicrotaline, the synthesis of some other 11-membered macrocyclic pyrrolizidine diesters (such as crispatine (88) and fulvine(89)[60]) may be possible. The absolute stereochemistry of the acid portions of these alkaloids may also be determined in a manner similar to that used for dicrotaline.



(88)

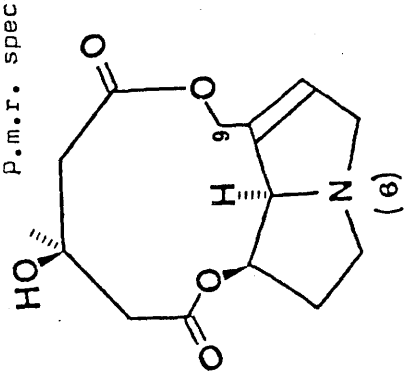
(or opposite mode of attachment of the acid moiety)



(89)

fig. 3a

P.m.r. spectrum of authentic dicrotaline (6) in $CDCl_3$



(6)

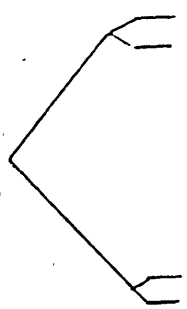


fig. 3b

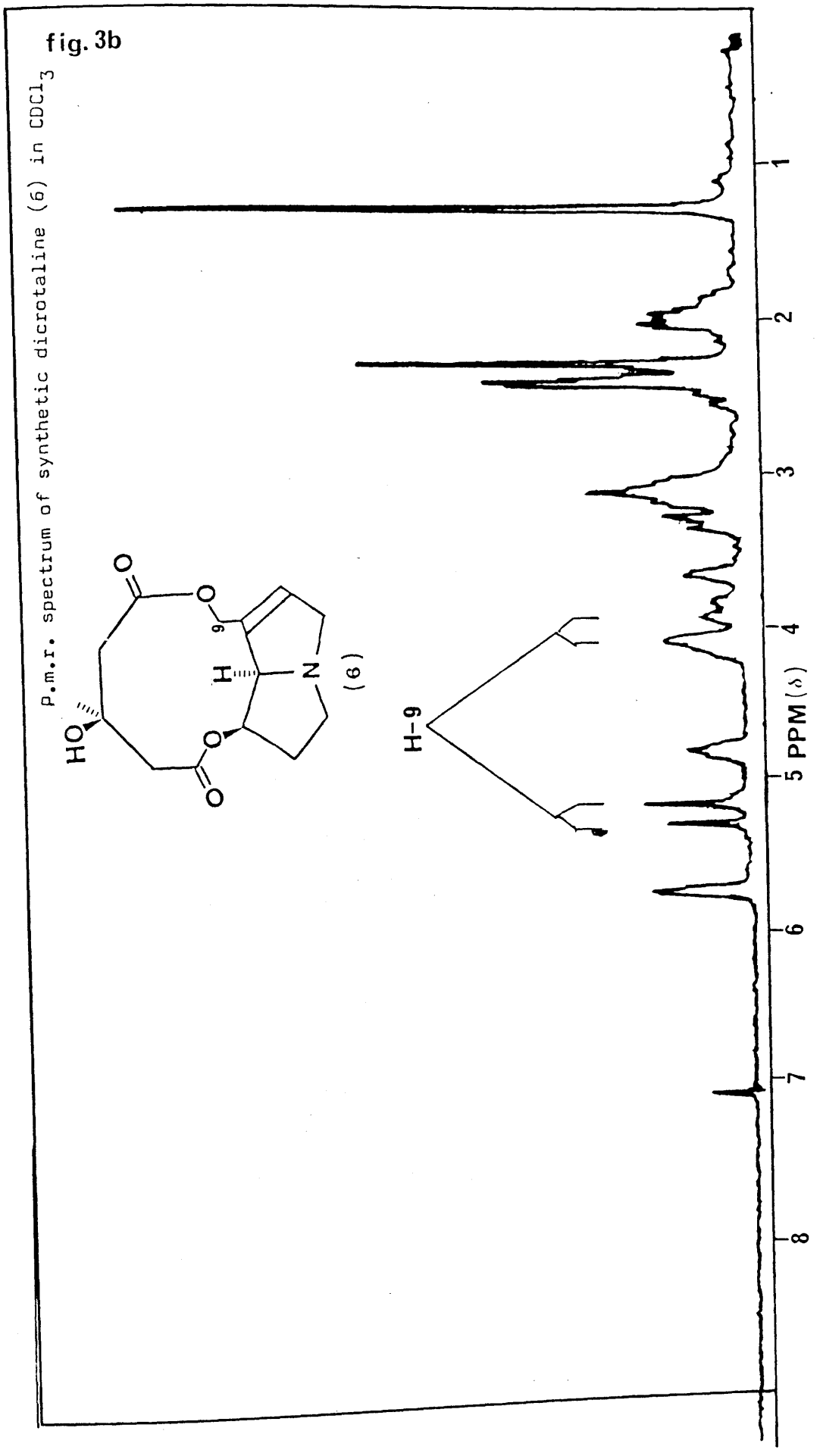
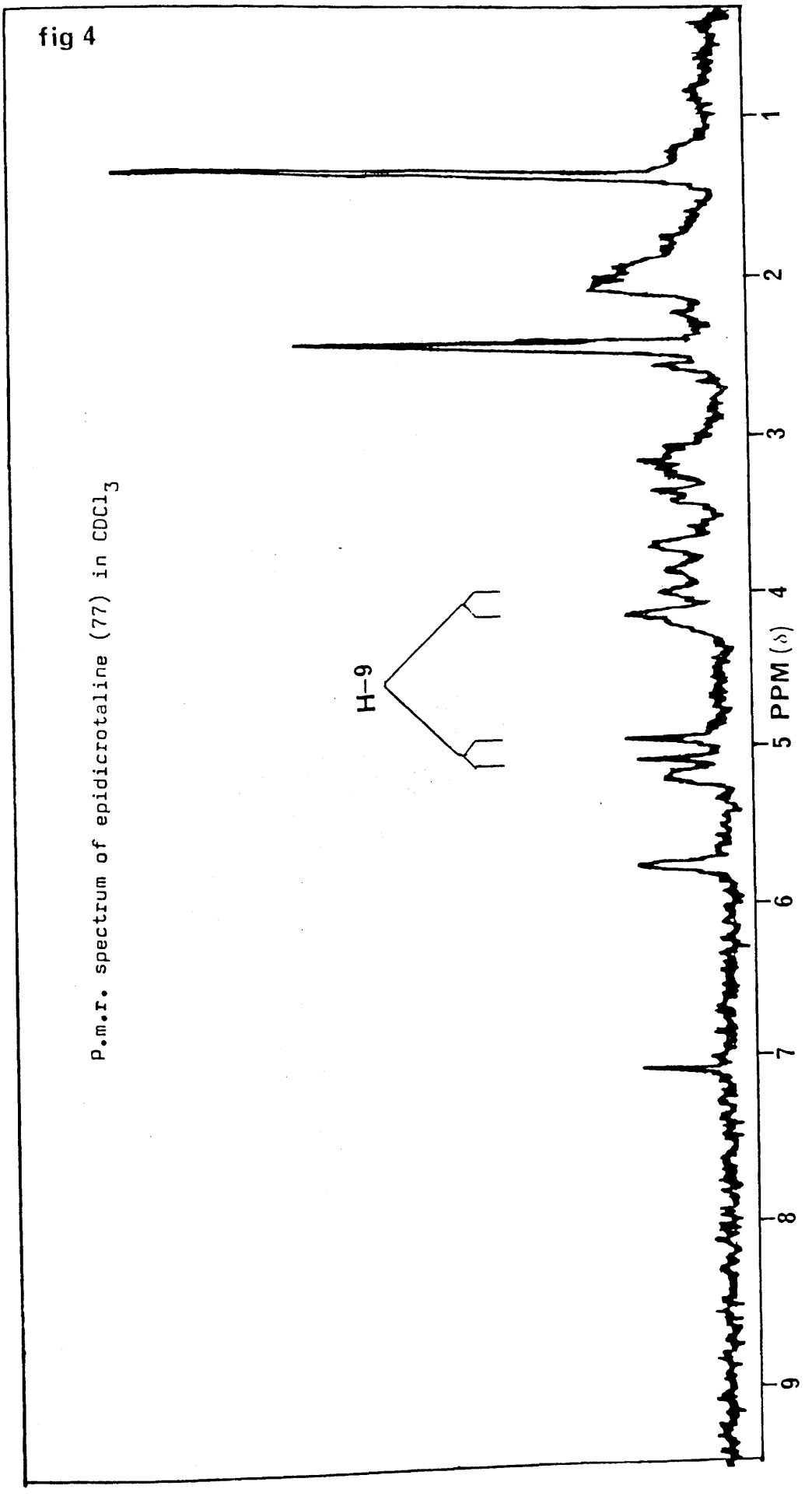

P.m.r. spectrum of synthetic dicrotaline (6) in $CDCl_3$
HO
O
O
O
O
H
N
9
(6)
H-9
1
2
3
4
5 PPM (δ)
6
7
8

fig 4


P.m.r. spectrum of epidicrotaline (77) in $CDCl_3$
H-9
1
2
3
4
5 PPM (δ)
6
7
8
9

I.R. Spectra of Dicrotaline Hydrochlorides (KBr disc).

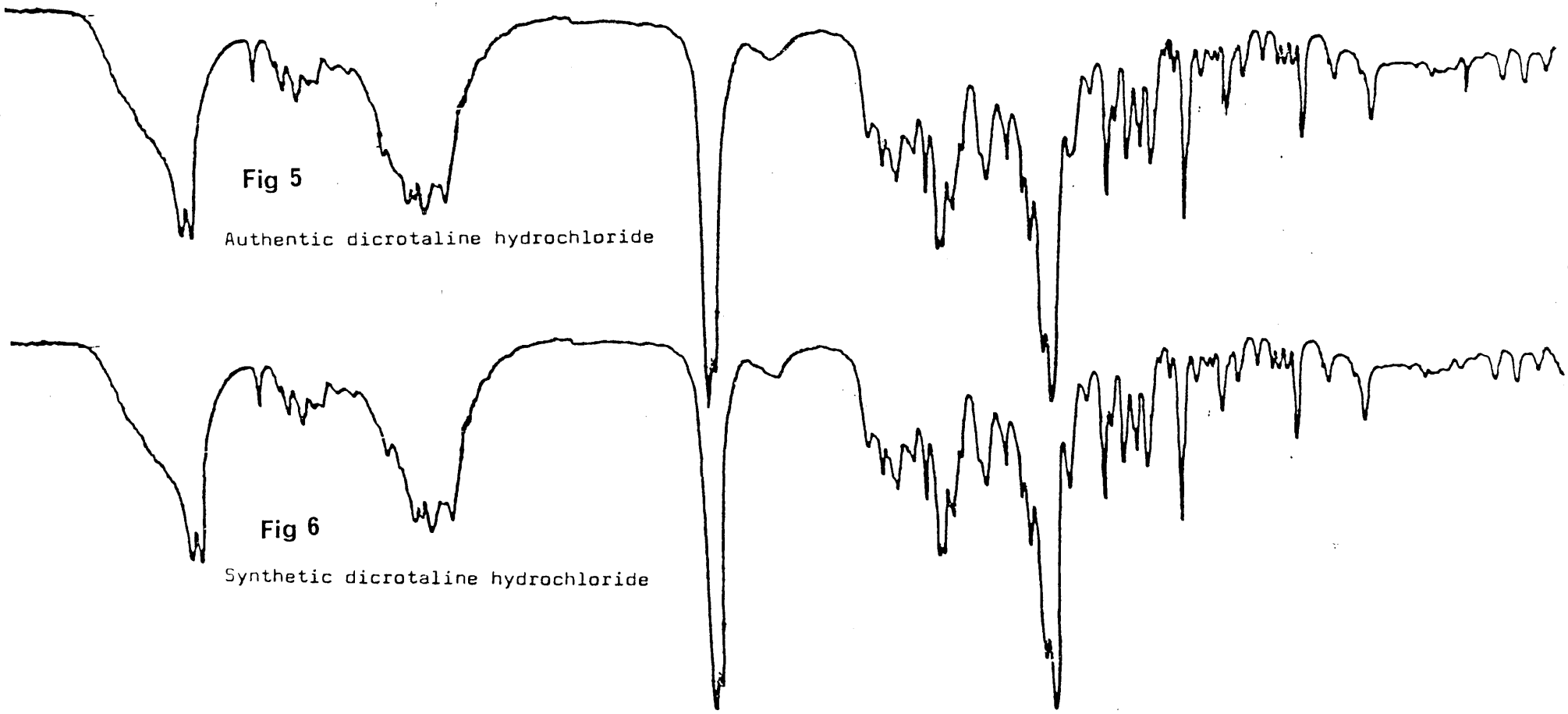


Fig 5

Authentic dicrotaline hydrochloride

Fig 6

Synthetic dicrotaline hydrochloride

CHAPTER 4.

ISOLATION OF PYRROLIZIDINE ALKALOIDS FROM CROTALARIA GLOBIFERA.

4.1 INTRODUCTION.

Crotalaria globifera (E.Mey.) has been reported to contain the pyrrolizidine alkaloid dicrotaline.[26]

As a sample of natural dicrotaline was required for comparison with the synthetic material prepared in CHAPTER 3, seeds of C. globifera were obtained and their alkaloid content examined. It was found that the seeds did not contain dicrotaline, but rather two other alkaloids were present.

4.2 IDENTIFICATION OF THE ALKALOIDS.

The basic extract of C. globifera was shown by analytical t.l.c. to consist of two components. Each of these components gave a positive spot test for a didehydropyrrolidine moiety. Thus the alkaloids were most probably derivatives of a 1,2-didehydropyrrolizidine base.

The alkaloids were separated and purified by preparative t.l.c. The more polar component was a white crystalline solid. The mass spectrum of this material confirmed that it was a pyrrolizidine alkaloid. The fragmentation pattern of the alkaloid was typical of a macrocyclic diester of retronecine.[1] High resolution mass spectrometry showed that the molecular formula of the alkaloid was $C_{18}H_{27}NO_6$.

If it is assumed that the necine base is retronecine (3) then this would require that the acid is an eight carbon unit. Esterifying acids with eight carbon atoms are common among the pyrrolizidine alkaloids.[5]

The i.r. spectrum of this alkaloid showed that the only carbonyl groups present in the molecule are saturated ester carbonyls. The p.m.r. spectrum contained all the features expected for a macrocyclic diester of retronecine. In addition to the usual signals for retronecine, the p.m.r. spectrum contained two sharp methyl singlets corresponding to two methyl tertiary carbinols, and two methyl doublets corresponding to an isopropyl group. Integration of the complex signals between δ 1.5 and δ 3 ppm in the spectrum showed that there were signals for two protons superposed on the retronecine proton signals in that region. The AB quartet at δ 4.45 and δ 5.18 ppm due to the C-9 protons of retronecine confirmed that this is a macrocyclic derivative of retronecine. The $\Delta\delta$ H-9 value of 0.73 is in the range generally accepted to be diagnostic of an 11-membered macro-ring. Although $\Delta\delta$ H-9 values have now been shown not to be infallible in determining ring size, in this case the macro-ring must be 11-membered. This is because the p.m.r. spectrum requires the presence of two methyl groups and an isopropyl group on the ring; therefore a simple carbon count shows that a 12-membered macro-ring is not a possible structure.

The structural requirements for the molecule established at this point in its investigation are shown in diagram A.

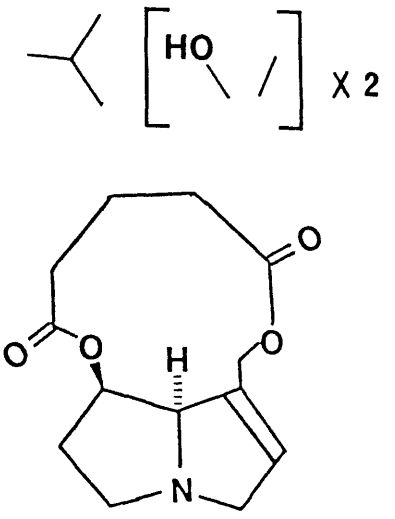
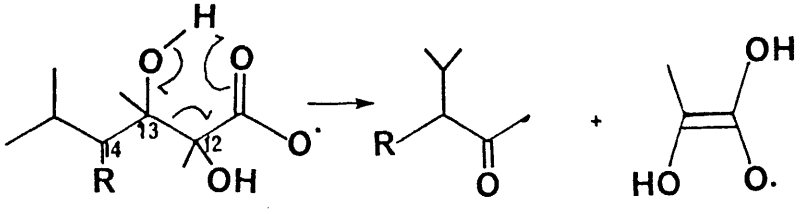


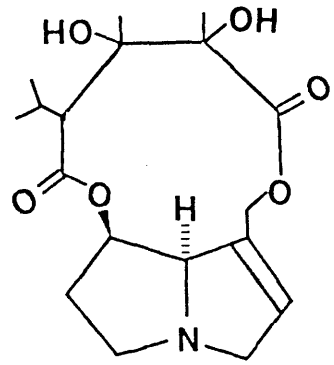
DIAGRAM A.

Further study of the mass spectrum of this alkaloid showed that it contained a M-89 fragment corresponding to loss of $C_3H_5O_3$. It was considered that this fragmentation came about by a McLafferty rearrangement of the type described in CHAPTER 3. If this is the case then the positions of all the groups on the macro-ring are established (SCHEME 27). This rearrangement requires that there is a hydroxyl group on C-13. The position of the methyl group next to this hydroxyl is therefore also established. Furthermore, for this fragmentation to give rise to a fragment with mass M-89,C-12 must carry a hydroxyl and a methyl group. This in turn means that the isopropyl group can only be on C-14.



SCHEME 27

On the basis of this argument, structure (90) is proposed for this alkaloid.



(90)

This structure satisfies all the requirements of the m.s., i.r. and p.m.r. spectra obtained for the alkaloid. None of the preceding spectral data gives any information about the stereochemistry at C-12, C-13 or C-14.

The above structure was recognised as being that of trichodesmine or a stereoisomer. Trichodesmine has been isolated from Heliotropum arguziodes[61], Trichodesma incanum[7] and Crotalaria rubiginosa[62]. Its structure has been determined by X-ray crystallography.[53]

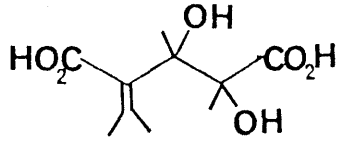
The alkaloid obtained in this work was shown to be identical with authentic trichodesmine by spectral comparison of the alkaloids and their picrates.

The faster running component of the alkaloid mixture was obtained as a white amorphous solid. Initial spectral analysis of this material indicated that it is a new pyrrolizidine alkaloid for which the name globiferine is proposed. As only a small quantity of globiferine was obtained (~10mg) no attempt was made to crystallize or further purify it in case the material decomposed. It was anticipated that the alkaloid could be fully characterised when

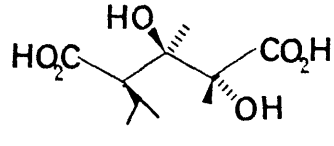
further quantities became available by extraction of plants of C. globifera grown from the remaining seeds. The high resolution mass spectrum of globiferine was typical of a retronecine macrocyclic diester. Its molecular formula was found to be $C_{18}H_{25}NO_6$. As the molecular formula had only two protons less than that of trichodesmine, and the alkaloids occur in the same plant, it was considered likely that the structures of the two alkaloids would be similar.

As the fragmentation pattern of globiferine showed that the necine portion of the molecule is retronecine, the difference in structure between this new alkaloid and the trichodesmine is due to the difference in the structures of the respective esterifying acids.

The p.m.r. spectrum of globiferine revealed the expected signals for the retronecine nucleus. The AB quartet due to the C-9 protons showed the unusually large $\Delta\delta$ H-9 value of 1.06 ppm. This is the second largest difference in H-9 chemical shifts recorded for a naturally occuring 11-membered macrocyclic diester of retronecine; dicrotaline possesses the largest. The spectrum also contained four sharp methyl singlets in two pairs. One pair at δ 1.42 and δ 1.36 ppm approximately corresponded to the two tertiary methyl groups in trichodesmine while the other pair were at lower field at δ 1.68 and 1.61 ppm. It was considered that these lower field signals may have been due to isopropylidene methyl groups. If this were the case then a possible structure for the necic acid would be (91) i.e. a didehydro analogue of trichodesmic acid (5) .



(91)

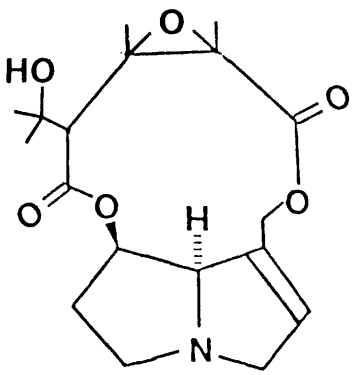


(5)

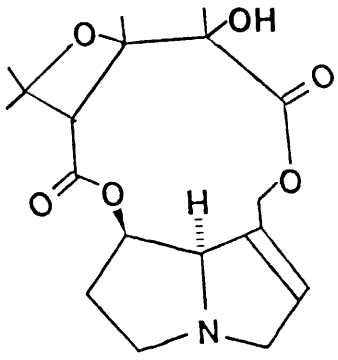
The i.r. spectrum of the molecule however did not contain an absorption corresponding to a carbon-carbon double bond conjugated with a carboxyl group, nor was any signal detected for an unsaturated ester carbonyl. Furthermore there was no indication of an α, β-unsaturated ester apparent in the u.v. spectrum. It was considered possible, though unlikely, that the structure (91) was still valid and that the α, β-unsaturation was not evident to i.r. and u.v. investigation due to steric influences causing the olefinic and carbonyl bonds to be twisted out of conjugation. This was shown not to be the case as the c.m.r. spectrum contained signals for only four sp^2 hybridised carbons, corresponding to the two ester carbonyl carbons and the two olefinic carbons of retronecine.

It was now evident that globiferine did not posess an extra olefinic linkage and so its unsaturation relative to trichodesmine could only be due to the presence of a cyclic ether in its structure. This was confirmed by re-examination of the p.m.r. spectrum. D_2O exchange experiments showed that globiferine contained only one exchangeable proton.

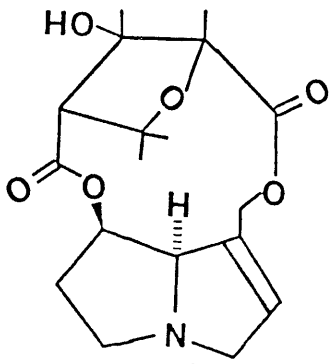
As the p.m.r. spectrum of globiferine indicated that the molecule contained two tertiary methyl groups adjacent to oxygen, and that the two isopropyl methyls were deshielded (which in this case must be by oxygen) there are only three possible structures (discounting stereoisomers) for the alkaloid. These structures (92), (93) and (94) are shown below.



(92)



(93)

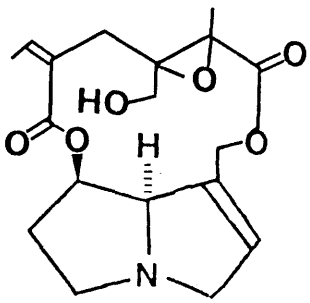


(94)

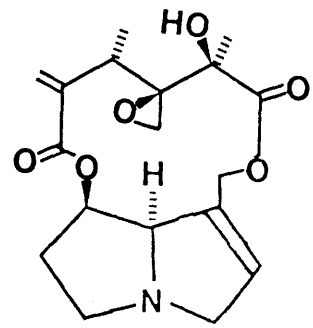
It is difficult to differentiate between these three structures by spectroscopic means. Structure (92) has been referred to as grantaline, a component of C. grantiana (Harvey). The original work by CULVENOR and SMITH has not been published and so details of spectra and structural elucidation are not available.[1]

Structure (94) has been tentatively assigned to the alkaloid globiferine pending further investigation. The reasons for this assignment are outlined below.

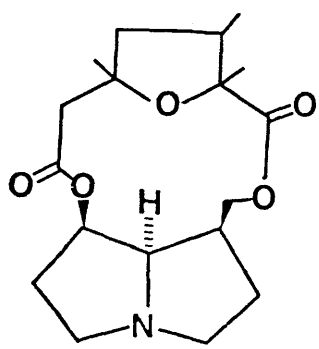
1. Precedent. While epoxides and tetrahydrofuran structures occur very commonly as secondary metabolites, there are few if any examples of naturally occuring oxetanes. More specifically there are examples of epoxides in the pyrrolizidine alkaloids such as erucifoline[63](95) and swazine[64](96), and examples of tetrahydrofuran derivatives such as nemorensine[65](97) and retroisesenine[66](98).



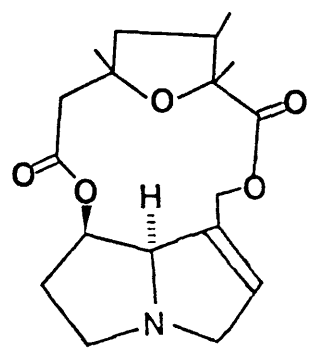
(95)



(96)



(97)

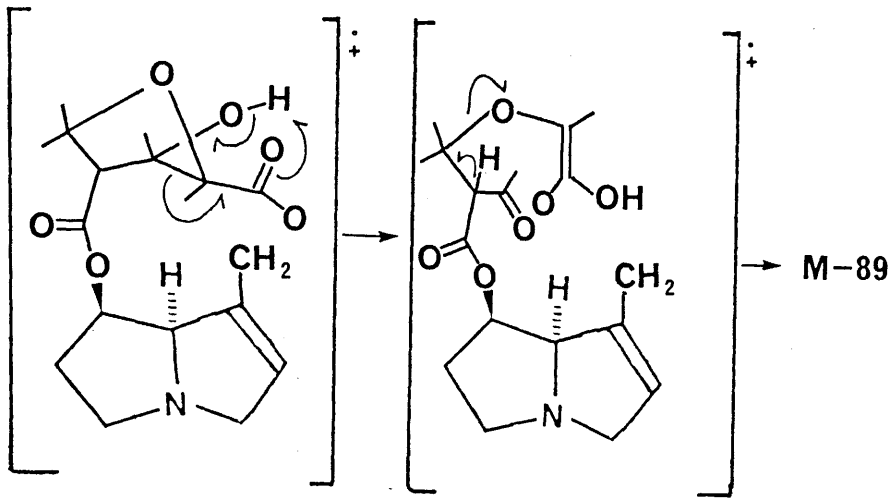


(98)

There are no known pyrrolizidine alkaloids containing the 4-membered ether structure. This would indicate that structures (92) and (94) are the most likely.

2. Mass spectral data. Globiferine gives rise to a strong M-89 fragment in the mass spectrum. The 11-membered macrocyclic diesters trichodesmine, monocrotaline, and incanine also give rise to strong M-89 fragments. In these three cases this has been attributed to a McLafferty rearrangement involving the hydroxyl group at C-13.[67] Of the three possible structures of globiferine only (94) posesses the C-13 hydroxyl required for this rearrangement. Although even in this case the McLafferty rearrangement does not result immediately in a fragmentation, the proposed intermediate contains an enol ether which

could reasonably break down to yield the M-89 fragment.

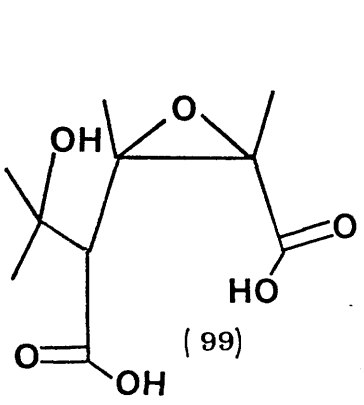


3. P.m.r. spectral data. Although all three structures could conceivably give rise to the p.m.r. spectrum obtained, variation of solvent and variable temperature studies indicate that there is no free rotation about the bond joining the isopropyl group to the macro-ring. This information was obtained by observing spectra of globiferine in deuteriochloroform d-4 methanol, d-6 benzene, d-6 acetone and d-6 D.M.S.O. at temperatures from 10°C to 60°C. In each case the 4 methyl groups remained sharp and distinct. This would tend to rule out structure (92).

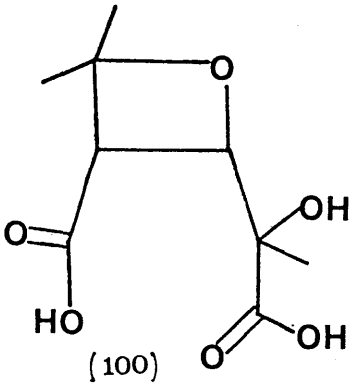
4. C.m.r. spectral data. As the c.m.r. spectrum of retronecine has not been unambiguously assigned[10] it was not possible to assign the signals of globiferine with absolute certainty. However the 9 signals downfield from 75ppm were easily identified. None of these signals was due to any of the quaternary carbons of the acid. As epoxide carbons absorb at higher fields (e.g. 20ppm for ethylene oxide) than those

of other ethers, this indicates that the acid does not contain an epoxide.

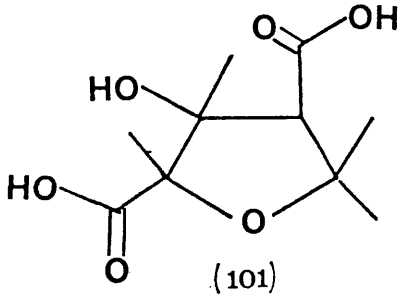
4.3 Further work. The above structural assignment is only tentative. In order to determine the structure with any certainty, larger samples of the alkaloid must be obtained and chemically degraded to produce a sample of the necic acid. The alternative structures of the acid (99)—(101) should be easily distinguishable.



(99)



(100)



(101)

Apart from spectroscopic methods simple chemical tests could be used to differentiate between the possible structures. For example an acid with structure (99) would yield a methyl ether on treatment with sodium methoxide, whereas structures (100) and (101) would not.

Treatment of (99) with $LiAlH_4$ would yield a tetra-ol while compounds (100) and (101) would yield triols. The triol derived from compound (100) would be subject to periodate cleavage while that derived from (101) would not.

Hydrolysis of the alkaloid may not be straightforward as the possible structures of the acid or the lactones derived from it may be interchangeable under

the conditions of acid or base hydrolysis. These problems could be avoided if the whole alkaloid was reduced with $LiAlH_4$ to yield the alcohols as described above.

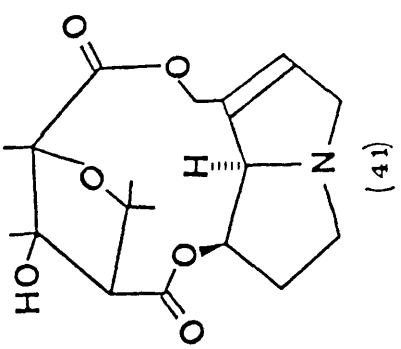
Summary and Conclusions.

Seeds of Crotalaria globifera have been shown not to contain dicrotaline as previously described. Instead two other alkaloids are present. One has been identified as the known pyrrolizidine alkaloid, trichodesmine. The second alkaloid is believed to be a new pyrrolizidine alkaloid, and has been named globiferine. A tentative structure for globiferine as been assigned.

Extraction of more that 100 C. globifera plants at the end of the growing season in 1981 yielded negligible amounts of pyrrolizidine alkaloids. Attempts will be made to obtain more seeds of C. globifera to complete the structural identification of globiferine.

Fig. 7

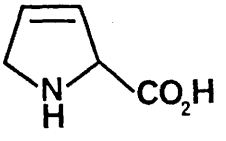
P.m.r. spectrum of globiferine (41) in $CDCl_3$



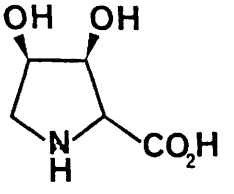
(41)

In that case it was shown that on hydrogenation the hydrogen added to the face of the molecule opposite to that occupied by the 6-hydroxyl group. This result indicated that the stereochemistry at C-8 in crotanecine could be controlled by the configuration at C-7 and C-6. Pyrroles very similar to (103) have been synthesised by several groups of workers utilising the 1,3-dipolar cycloadditions of electron deficient acetylenes (in particular ethyl propiolate) to oxazolium-5-oxides formed by dehydration of substituted N-formyl prolines (CHAPTER 1.4). This reduced the synthetic sub-goal to an appropriately substituted proline.

It was considered that the required cis 3,4-dihydroxy proline (106) could be derived from 3,4-didehydroproline (107) by cis glycolation (SCHEME 28).



(107)



(106)

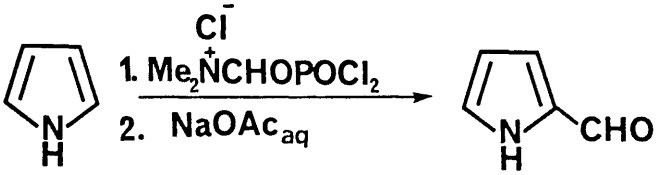
SCHEME 28

5.3 SYNTHESIS OF 3,4-DIHYDROXYPROLINE

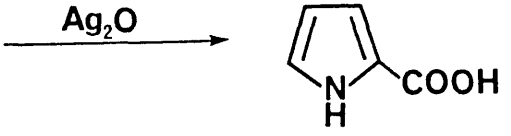
3,4-Didehydroproline has been synthesised by ROBERTSON and WITKOP as a by-product during their 1,4 reduction of pyrrole-2-carboxamide with phosphonium iodide in hydroiodic acid.[70] The acid was later synthesised by CORBELLA et al who reported that the method of ROBERTSON and WITKOP gave erratic results and was unsuitable for the reduction of pyrrole-2-carboxylic acid.[71] The method of CORBELLA et. al

required large amounts of hydrogen iodide which was not conveniently available during this work. It was therefore decided to investigate the first method further to see if it could be modified to reduce pyrrole-2-carboxylic acid in acceptable yield.

Pyrrole-2-carboxylic acid was prepared as shown in SCHEME 29.



(108)



(109)

SCHEME 29

Pyrrole was treated with the Schiff's base derived from the treatment of D.M.F. with phosphoryl oxychloride. The intermediate was hydrolysed with aqueous sodium acetate to yield the aldehyde (108).[72] Silver oxide oxidation gave the acid (109).[73] Phosphonium iodide was prepared by hydrolysis of a mixture of P_2I_4 and white phosphorus.[74] Hydroiodic acid was prepared by treatment of aqueous iodine with hydrogen sulphide and HI gas was obtained by treating red phosphorus with iodine in hydr iodic acid.

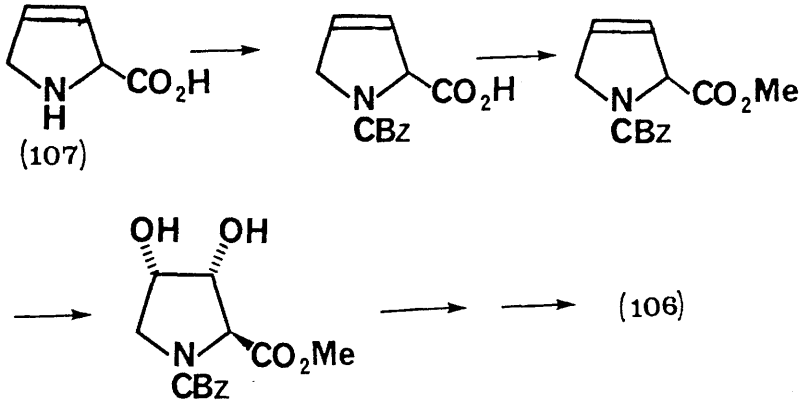
Reduction of pyrrole-2-carboxylic acid with PH_4I in hydroiodic acid following the method of ROBERTSON and WITKOP afforded 3,4-didehydroproline (107) in 70%

yield. On subsequent runs the yield of this reaction was found to be consistently between 70 and 80%. This procedure was found to be satisfactory with none of the shortcomings reported by previous workers. The only drawback to the method was the rather hazardous preparation of phosphonium iodide. It was later found that if a saturated solution of iodine in 80% hydroiodic acid was dropped into a 50/50 mixture of iodine and red phosphorous, and the vapour produced bubbled into glacial acetic containing 10% w/v anhydrous hypophosphorus acid, a solution of hydrogen iodide was produced containing a thick semi-crystalline mass. This mixture was found to reduce the pyrrole-2-carboxylic acid to 3,4-didehydroproline in 80% yield. It was believed that the solid produced was phosphonium iodide, indicating that some P_2I_4 was being formed during the production of HI. This belief was supported by the fact that the solid obtained could be co-sublimed with PH_4I to give cubic crystals which seemed identical with sublimed PH_4I.

5.3.1 GLYCOLATION OF 3,4-DIDEHYDROPROLINE

As the two secondary hydroxyl groups of crotanecine have a cis- relationship it was required that the two hydroxyl groups of its substituted proline should be cis.

It was decided to produce the cis glycol by osmium tetroxide oxidation of the double bond. The reaction sequence (a variation of the procedure of ROBERTSON et al) for the glycolation of (107) is shown in SCHEME 30.

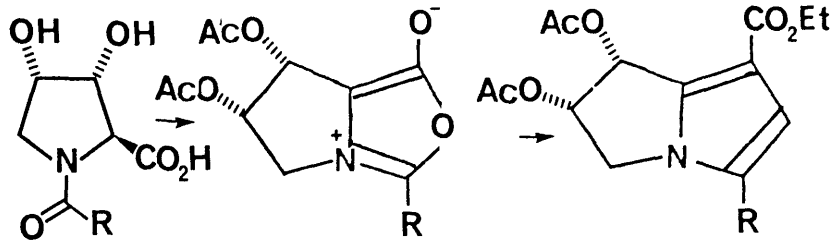


SCHEME 30

The amino group was protected as the benzyl urethane derivative and the carboxylic acid was protected as the methyl ester. (The p.m.r. spectrum of this protected amino acid showed two sharp singlets for the ester methyl group. This was attributed to a substantial energy barrier to free rotation about the urethane N-C bond.) Oxidation of the double bond with osmium tetroxide in pyridine followed by reductive cleavage of the osmate ester[75] yielded the protected 3,4-dihydroxyproline. Hydrogenolysis of the carbobenzyloxy group and ester hydrolysis gave the cis dihydroxy amino acid (106) in good overall yield.

5.4 ATTEMPTED 1,3-DIPOLAR CYCLOADDITIONS.

It had been anticipated that the formation of the required oxazolium oxide (110) and/or the addition of ethyl propiolate to it (SCHEME 31) might be complicated by the presence of the 3-hydroxy group.



(110)

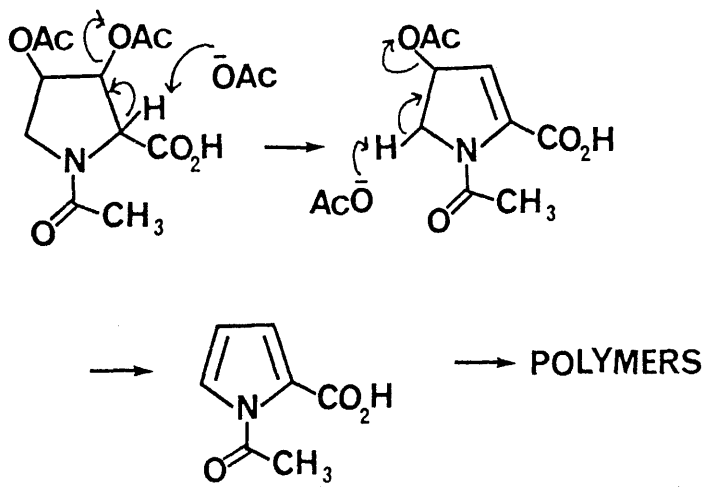
SCHEME 31

A model experiment was therefore carried out by heating 3,4-dihydroxy proline in acetic anhydride in the presence of ethyl propiolate. After heating at reflux for one hour the reaction mixture was cooled and concentrated to a thick black polar gum which could not be analysed by t.l.c. Residual solvent and ethyl propiolate were removed by leaving the gum at 0.05 mmHg for 10 hours. The p.m.r. spectrum of the gum showed that the desired pyrrole (SCHEME 31, R=Me) had not been formed as no signals were observed which corresponded to an O-ethyl group. Furthermore the aromatic region of the spectrum was very complex, (the desired product should give rise to a distinct methyl singlet with a small allylic coupling). The main features of the p.m.r. spectrum were three broad singlets in the aromatic region and a broad singlet at δ 2.4 ppm. All attempts to purify or analyse this gum were unsuccessful. (The experiment was repeated in the absence of ethyl propiolate to yield a gum with the same spectral characteristics as before. Attempts to purify this material were again unsuccessful.) Shorter reaction times and lower reaction temperatures resulted in a mixture of starting material (easily isolated by acid extraction and ion exchange chromatography) and varying amounts of the same gum which became resinous on handling or storage.

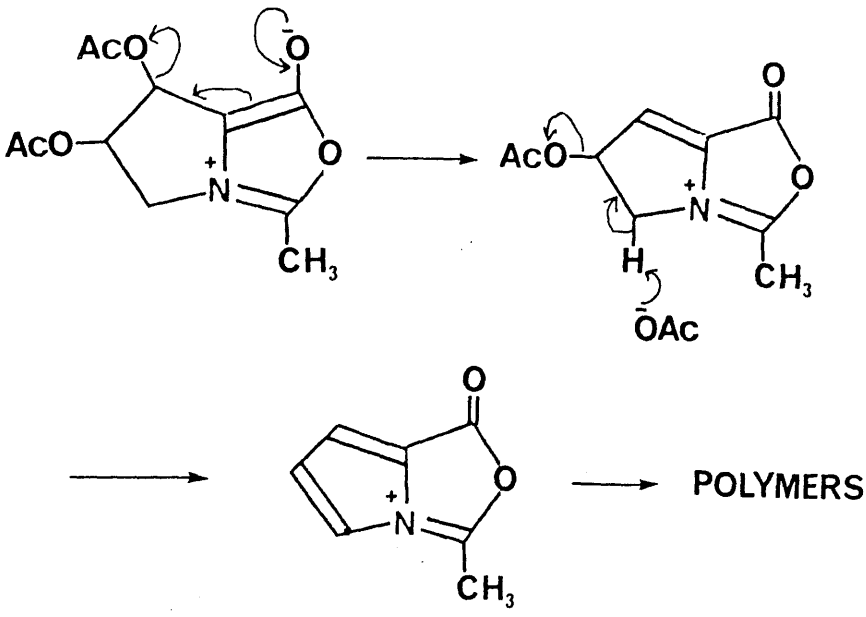
In an attempt to further investigate the reaction product, the p.m.r. spectrum of a solution of 3,4-dihydroxy proline in trifluoroacetic anhydride was recorded on consecutive sweeps. The first spectrum showed a rapid decrease in the signals corresponding to 3,4-dihydroxy proline and the appearance of 3 broad singlets in the aromatic region. The second sweep (about 4 minutes from mixing) gave no spectrum as a dark red resinous material had coated the inside of the sample tube.

The most likely explanation for the appearance of the three aromatic signals is the formation of a 2-substituted pyrrole or a pyrrole like structure.

This could come about in two ways, either elimination before formation of the oxazoluim oxide (e.g. SCHEME 32A) or elimination after formation (e.g. SCHEME 32B).



SCHEME 32A

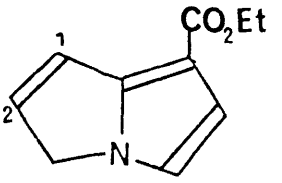


SCHEME 32B

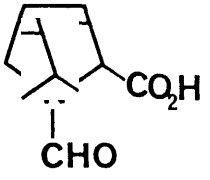
No further attempts were made to identify the products of this reaction. Several hydroxyl protecting groups were considered as a possible solution to the elimination problem. Ester groups were discounted as they could be eliminated easily as in the case of acetate. Acid sensitive groups were also rejected as it was felt they would not survive the reaction conditions, also it would not be possible to N-formylate the amino acid if it contained acid sensitive groups.

Attempted formation of the methylene acetal resulted in the formation of polymeric material. Several attempts to prepare methyl and benzyl ethers were unsuccesful and resulted in loss of starting material.

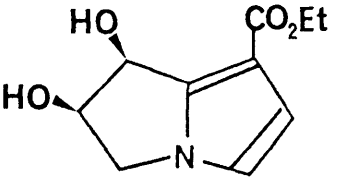
It was then decided to attempt the cyclisation with N-formyl-3,4-didehydroproline (111). If this cyclisation was successful selective oxidation of the product (112) at the pyrrolizine 1,2-bond might yield the required intermediate (103).



(112)



(111)



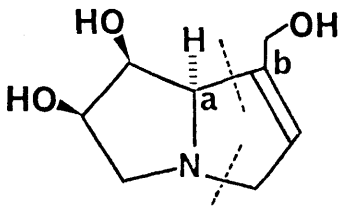
(103)

N-Formyl-3,4-didehydroproline (111) was prepared and treated with acetic anhydride and ethyl propiolate. No homogeneous material was obtained from this reaction under a variety of reaction conditions. At this point it was decided that the 1,3-dipolar route would not be of any further use unless it could be modified to avoid the problems of elimination caused by the presence of the hydroxyl group in the 3-position of proline. It may be possible to devise a milder method for dehydrating the N-formyl acid to produce the oxazolium oxide. If, for example, dicyclohexylcarbodiimide could be used for the dehydration step then the hydroxyl groups could safely be protected as their tetrahydropyranyl or trimethylsilyl ethers.

It was discovered during the literature search for properties of molecules with structures similar to (112) that a synthesis of (112) in good yield already exists.[76] This suggests that it may be useful at a future date to study selective oxidation of (112) as a possible route to crotanecine.

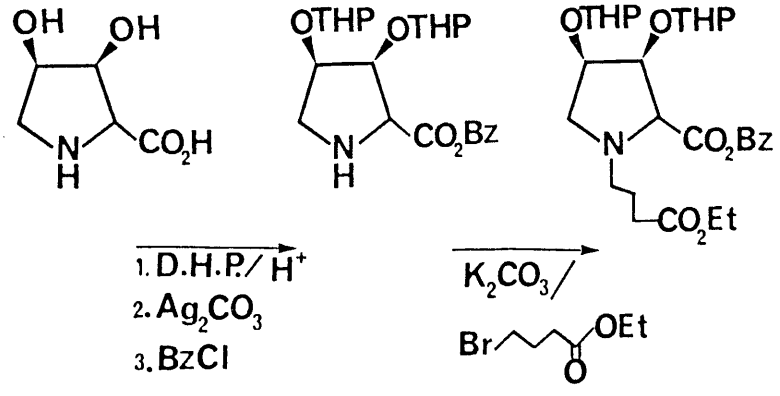
5.5 THE INTRAMOLECULAR CYCLISATION ROUTE- SYNTHETIC STRATEGY.

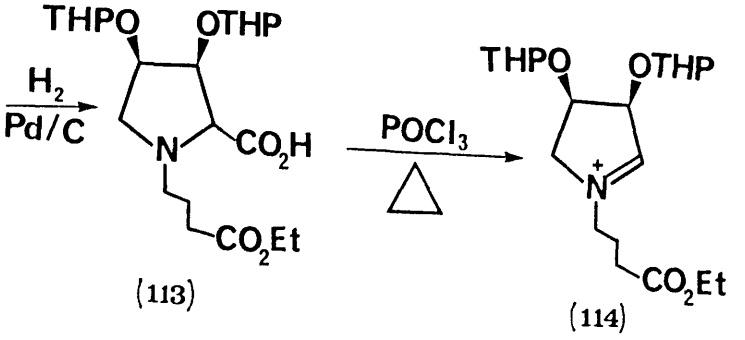
Study of the crotanecine structures (12) suggested cleavage of bond a/b as a reasonable retrosynthetic dislocation.



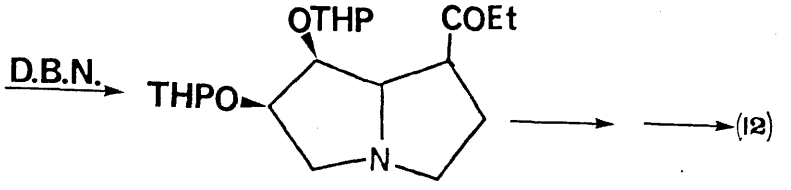
(12)

This dislocation was chosen as it required that carbon b should be capable of becoming an anion or equivalent and that carbon a sould be capable of becoming a cation or equivalent. Generation of a carbonium ion equivalent α to a nitrogen by formation of a Schiff's base is well known. Consideration of this basic synthetic strategy and of the elaboration required to provide the appropriate oxidation levels at the various carbons of crotanecine led to the postulation of the following synthetic route.



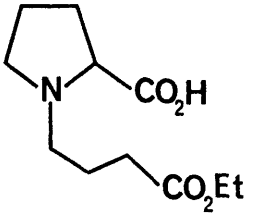


(113) (114)



(115)

Selective O- protection of cis 3.4-dihydroxyproline followed by N-alkylation with ethyl 4-bromobutanoate, and selective deprotection of the carboxylic acid was expected to give the α tertiary amono acid (113). Decarbonylation of the acid with $POCl_3$[36] to yield the iminium ion (114) and treatment with a non-nucleophilic base (e.g. D.B.N.) was then expected to effect cyclisation by attack of the ester enol on the iminium ion. This intermediate (115) could then be transformed into (12) as described in CHAPTER 5.2. As this type of intramolecular cyclisation had not previously been reported it was decided to try the cyclisation on a simpler system first. The proline derivative (116) was chosen as a suitable model system.



(116)

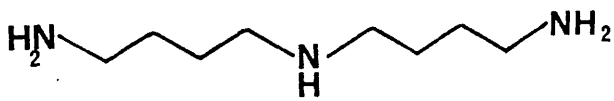
This was prepared as follows. The benzyl urethane of L-proline was prepared by the standard Schotten-Baumann proceedure. The N-protected amino acid was then converted into its tertiary butyl ester by treatment with freshly prepared 2-methylprop-1-ene introduced as a liquid into a methylene chloride solution containing a catalytic amount of P.T.S.A. An alternative method for the preparation of the tertiary butyl ester by transesterification with tertiary butyl acetate was found to give lower yields. Hydrogenolysis of the N-protecting group afforded the amino ester. This was alkylated with ethyl 4-bromobutanoate. (The bromo ester was obtained by standard methods from γ-butyrolactone). Removal of the tertiary butyl group with hydrogen bromide in acetic acid yielded the amino acid (116).

At this point RAPOPORT and co-workers published a synthesis of diethyl pyrrolizidine-1,1-dicarboxylate by the approach proposed in this chapter.[36] It was considered that although the proposed route to crotanecine was still feasible, it would be more useful to investigate the utility of this method for the synthesis of labelled compounds for use in biosynthetic studies.

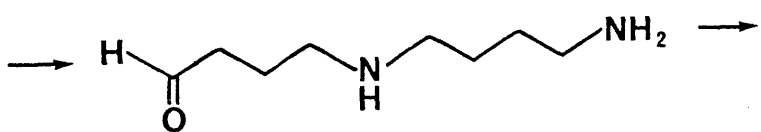
5.6 ATTEMPTED SYNTHESIS OF IMINIUM IONS.

Much of the biosynthetic pathway to retronecine has been elucidated by ROBINS and co-workers. It has been shown (CHAPTER 1.5) that the triamine homospermidine (44) is a specific precursor for retronecine. The course of the biosynthetic transformation from homospermidine to retronecine has not yet been discovered.

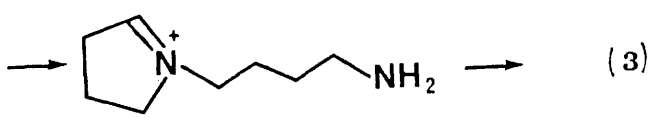
It can be postulated (SCHEME 33) that oxidation of one of the primary amino groups to an aldehyde (117) followed by intramolecular attack by the central secondary amine would give the Schiff's base (45).



(44)



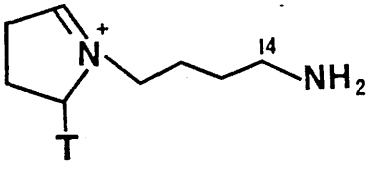
(117)



(45) (3)

SCHEME 33

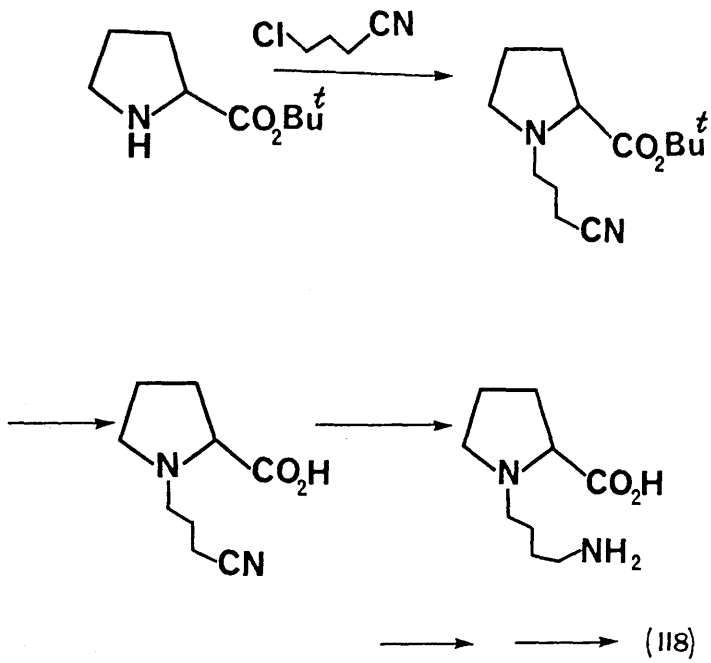
The status of (45) as a biosynthetic intermediate could be investigated if it could be synthesised in an isotopically labelled form and fed to plants producing retronecine. In order to determine whether (45) is a specific precursor it would have to be doubly labelled in such a way that if it were incorporated it could be shown that it had been incorporated intact. The labelling pattern shown in (118) was considered to be suitable for the proposed feeding experiments.



(118)

It was thought reasonable that such a molecule could be synthesised in a manner analogous to that proposed for the syntheis of (114) in CHAPTER 5.5. The synthesis of unlabelled (118) was undertaken in order to determine whether it was stable and if so to find the most efficient route for preparation of the labelled material.

The proposed synthetic route is shown on SCHEME 34



SCHEME 34

This route was chosen as it could easily be adapted to the synthesis of labelled (118). Tritiated proline is commercially available, and the required 4-chlorobutyronitrile could be obtained by treatment of 1-bromo-3-chloropropane with ^{14}C cyanide ion.

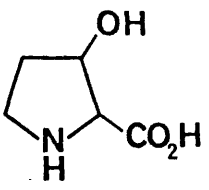
Treatment of tertiary butyl prolinate with 4-chlorobutyronitrile in toluene at reflux in the presence of potassium carbonate gave the tertiary amino ester in high yield. Treatment of the ester with trifluoroacetic acid in methylene chloride failed to yield the desired free amino acid. On addition of trifluoroacetic acid the solution turned dark brown. Removal of the solvent left an intractable tar. In a further attempt to cleave the ester it was treated with hydrogen bromide in acetic acid. On gentle warming of the solution a white crystalline solid precipitated from solution. Analysis of this solid showed it to be ammonium bromide.

Unfortunately time did not allow this approach to be investigated further. Although this work could not be taken very far in the time available, it is felt that the synthesis of the required labelled molecule should be investigated further. Deprotection of the ester has turned out to be unexpectedly difficult. However, there are several very mild methods available (e.g. PTSA in benzene[77]) for cleavage of tertiary butyl esters. Reduction of the nitrile should afford the required primary amine. Catalytic hydrogengenation would be the method of choice although hydrogenation of nitriles is complicated by formation of dimeric primary amines produced by attack on the intermediate imine by the primary amine. This can be avoided by carrying out the hydrogenation in acetic anhydride which traps the primary amine as an amide as it is formed. Hydrolysis would then yield the required amine.

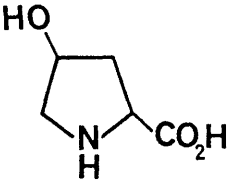
It will probably be necessary to keep the iminium ion in acid solution to avoid attack by the primary amine on the imine to form a D.B.N. type structure.

If the synthesis of the required iminium ion can be achieved and if it is shown to be an intermediate in retronecine biosynthesis, then further investigation could be carried out by synthesising intermediates with tritium on various positions of the proline ring.

The tritium could be specifically introduced to chosen prochiral position in the ring by T^- reduction of the tosyl derivatives of either of the resolved enantiomers of the available (119)[78] or (120)[31,39].



(119)



(120)

5.7 CONCLUSIONS.

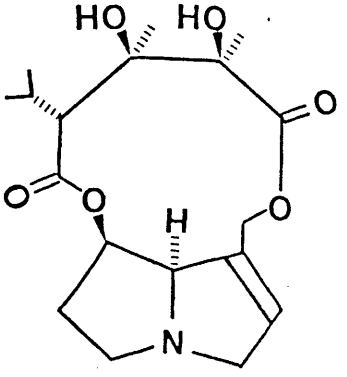
Synthetic routes to crotanecine have been devised, and the problems encountered in tackling these have been discussed. Iminium ions may be intermediates in necine biosynthesis, and may also be useful synthetic intermediates for necines. Routes for the synthesis of these species have been investigated.

CHAPTER 6.

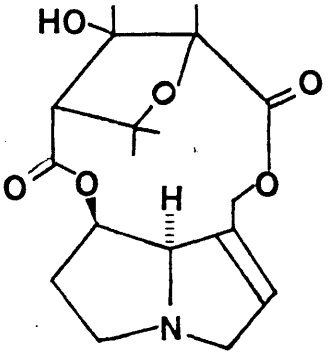
FEEDING EXPERIMENTS ON CROTALARIA GLOBIFERA PLANTS.

6.1 INTRODUCTION

In CHAPTER 4 the isolation of two pyrrolizidine alkaloids from Crotalaria globifera was described. One of these alkaloids was shown to be trichodesmine (46). It was proposed that the other alkaloid named "globiferine" has the structure (41).



(46)

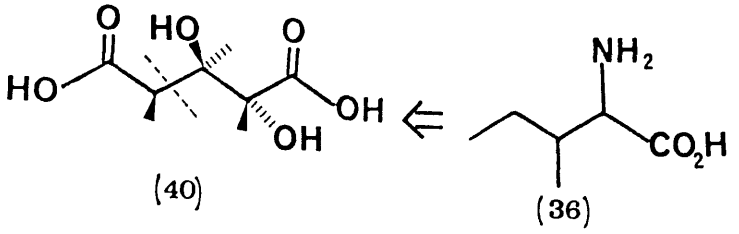


(41)

It was considered that an investigation into the biosynthesis of these alkaloids would be of value for several reasons. The biosynthesis of trichodesmic acid (28) has not been investigated although that of the closely related monocrotalic acid (40) has.[42] Labelling experiments may be able to show a biosynthetic relationship between trichodesmine (46) and globiferine (41). These experiments may also be of assistance in verifying the structure of globiferine (41).

6.2 FEEDING EXPERIMENTS.

ROBINS et al have shown that L-isoleucine (36) is a specific precursor of monocrotalic acid (40).[42] Their results indicate that L-isoleucine (36) is incorporated into the right hand C_5 unit of monocrotalic acid (40) (SCHEME 35).

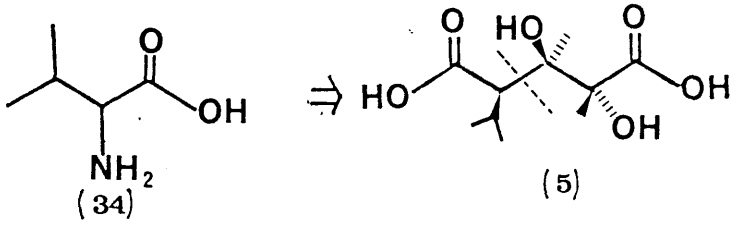


(40) (36)

SCHEME 35

As trichodesmic acid (5) contains the same C_5 unit it seemed likely that L-isoleucine (36) is a precursor of this acid.

Inspection of the remaining C_5 unit suggested that it could be derived from valine (34) (SCHEME 36). Valine has been shown to be incorporated into several necic acids (CHAPTER 1.5.1).



(34) (5)

SCHEME 36

It was therefore decided to feed isotopically labelled isoleucine and valine to C.globifera plants.

Because of the practical problems associated with introducing material into whole plants it is often difficult to determine accurately how much of the labelled compound is actually taken into the plant. There are a number of other uncertainties associated with the use of percent incorporations as criteria for the measurement of relative efficiencies of compounds tested as biosynthetic precursors of a metabolite. A better measure of the relative efficiencies of a range

of possible precursors may be obtained by "double labelling" experiments. The prospective precursor is fed admixed with a known proportion of a standard compound. This compound should be a known precursor of the target molecule. If the precursor and the standard are labelled with different isotopes then the ratio of these isotopes in the target molecule will give a measure of the efficiency of the test compound as a precursor relative to the standard compound. By this method the relative efficiencies of a range of test precursors may be established.

Putrescine (1,4-diaminobutane) was chosen as a standard in the following experiments as it has been shown to be efficiently incorporated into retronecine (3), the necine base of the two alkaloids in question. The incorporations of [1-^{14}C]acetate, L-[U-^{14}C] isoleucine, DL-[4-^{14}C] valine and [1,4-^{14}C] putrescine into trichodesmine and globiferine were measured.

Solutions of the labelled materials were prepared as shown in TABLE 1. The solutions were prepared by mixing together aqueous solutions of the appropriate compounds. The resulting solutions were evaporated to dryness and then redissolved in a small volume of sterile water. The mixtures for feeding were prepared so that the ratio of $^{3}H/^{14}C$ was 8:1. In the case of DL-valine the solution was made up to give a $^{3}H/^{14}C$ ratio of 8:1 with respect to L-valine. Acetate was fed as a control experiment as it has been shown that acetate is not a specific precursor of the necic acids. ^{14}C putrescine was fed to determine how closely the incorporation of ^{3}H from ^{3}H-putrescine reflected the actual incorporation of putrescine. Each solution was fed to thirty 3 month old C.globifera plants by the xylem pricking method.[45] The plants were harvested after seven days and their alkaloids extracted as described in CHAPTER 4. Between 1 and 2 mg of

alkaloid was obtained in each experiment. The radioactivity of the total alkaloid extract in each case was measured. The results obtained are shown in TABLE 2. The alkaloid mixture was then diluted with a mixture of trichodesmine and globiferine obtained from previous work (CHAPTER 4). The two alkaloids were separated by t.l.c. and the radioactivity of the individual alkaloids measured. These results are shown in TABLE 3.

TABLE 1

FEED	PRECURSOR	RADIOACTIVITY (μCi)	STANDARD	RADIOACTIVITY (μCi)	$^{3}H/^{14}C$
1	1-^{14}C acetate	12.5	1,4-^{3}H putrescine	100	8:1
2	L U-^{14}C isoleucine	12.5	"	100	8:1
3	DL 4-^{14}C valine	25	"	100	4:1
4	1,4-^{14}C putrescine	12.5	"	100	8:1

TABLE 2

FEED	TOTAL RADIOACTIVITY (μ Ci)		$^{3}H/^{14}C$ RATIO.
	^{3}H	^{14}C	
1	0.48	1.85×10^{-3}	260:1
2	0.33	1.65×10^{-2}	20:1
3	0.40	3.17×10^{-2}	12.6:1
4	0.45	6.52×10^{-2}	6.9:1

TABLE 3

FEED	$^{3}H/^{14}C$ RATIO GLOBIFERINE	$^{3}H/^{14}C$ RATIO TRICHODESMINE
1	300:1	305:1
2	20:1	22:1
3	12:1	12:1
4	7:1	7:1

6.3 DISCUSSION

The results from feeding [1,4-^{14}C] putrescine indicate that the incorporation of tritium from [1,4-^{3}H] putrescine gives a good measure of the incorporation of putrescine into the alkaloids. The slight decrease in the $^{3}H/^{14}C$ ratio can be explained by the loss of some ^{3}H during oxidation of the amino groups on the retronecine biosynthetic pathway.

The large increase in the $^{3}H/^{14}C$ ratio obtained from the [1-^{14}C] acetate feed is in agreement with other results that acetate is not a specific precursor of the necic acids.[38] It is interesting to note that the $^{3}H/^{14}C$ ratio increased still further after chromatographic purification of the alkaloids. This indicates that an acetate derived material had been extracted along with the alkaloids. It has been noted previously that terpenoid material can be co-extracted with pyrrolizidine alkaloids and that subsequent removal of this material is difficult on a small scale.[38]

The $^{3}H/^{14}C$ ratio obtained from the valine feed suggests that this amino acid is an efficient precursor of both the alkaloids studied in this work. Valine appears to be incorporated into the alkaloids with an efficiency similar to that of putrescine. It was assumed by analogy with isoleucine [42] that only the L-isomer of valine would be incorporated even though a racemate was fed to the plant.

L-isoleucine also appears to be a specific precursor of the two alkaloids. The incorporation of this amino acid as measured by the increase in the $^{3}H/^{14}C$ ratio is somewhat less efficient than that of valine.

The values obtained from valine and isoleucine feeds are not directly comparable as [4-^{14}C] valine would be expected to retain all of its radioactivity if incorporated while [U-^{14}C] isoleucine must lose 1/6th of its activity as its carboxyl group is lost.

It has been suggested by CROUT that the biosynthesis of the pyrrolizidine macrocyclic diesters proceeds *via* an acyclic intermediate in which two C_5 units are linked to the necine base.[80] These two C_5 units then cyclise intramolecularly to form the cyclic diester.

If this is the pathway followed in this case then it can be postulated that valine could be linked to the necine nucleus virtually unchanged while isoleucine may have to undergo several transformations (e.g. oxidation and decarboxylation) before it can be linked to the base. There would therefore be a greater likelihood of the isoleucine derived moiety being lost to the carbon pool of the plant before it could be incorporated. This would result in a lower incorporation of isoleucine than of valine.

Chromatographic separation of the alkaloids showed that the amino acids had been incorporated with similar ratios into each alkaloid. This would seem to indicate either that one of the alkaloids is derived from the other or that their biosynthetic pathways "fork" from a common route some time after both amino acid derivatives have been linked to the necine base. It seems unlikely that two separate biosynthetic pathways could give rise to identical $^{3}H/^{14}C$ ratios found for the alkaloids.

6.4 FURTHER WORK

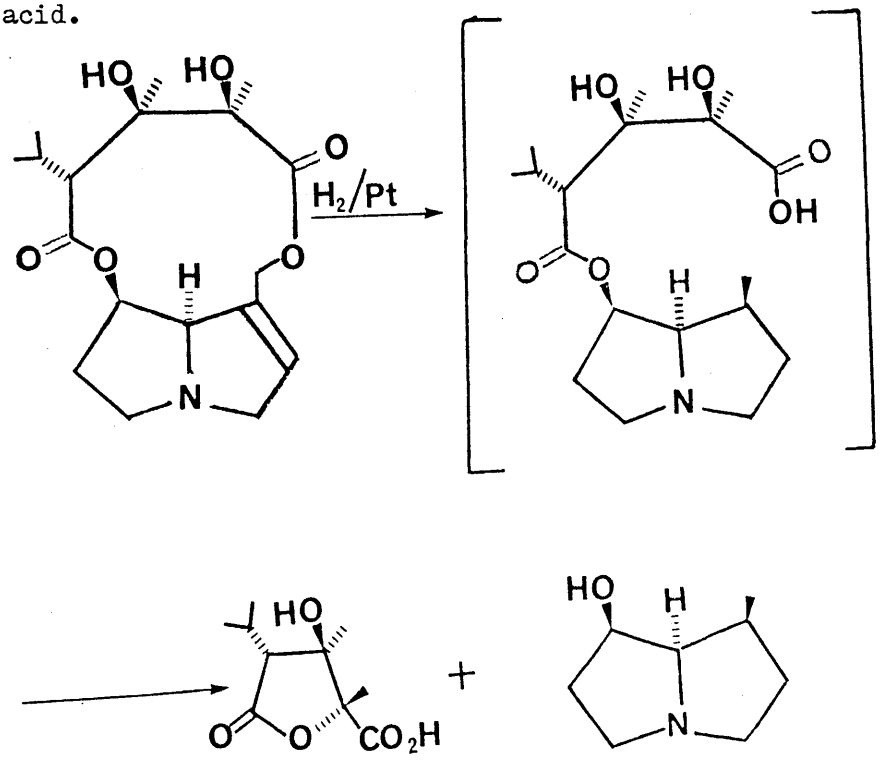
The above results only constitute a preliminary investigation into the biosynthesis of the esterifying acids of trichodesmine and globiferine. The alkaloid content of the plants at the stage in their development when these experiments were carried out was very low. A typical value of the alkaloid content was 0.05% of live weight. The rate of alkaloid production at this time must therefore have been very low. In order to obtain the best results from feeding experiments the precursors should be administered when the rate of alkaloid production is at its highest. It is suggested

therefore that before further feeding experiments are carried out the alkaloid content of the plants should be measured throughout a full growing season. This would determine at what stage in the plant's development it would be best to carry out the experiments and over what length of time.

Once sufficient quantities of labelled alkaloid have been obtained specific degradations may then be carried out.

[To avoid repetition the following proposed degradation scheme will refer only to trichodesmine; the same scheme could with some modification be applied to globiferine.]

The most important degradation would be the cleavage of the alkaloid to necic acid and necine base portions. This should establish that most of the ^{14}C (amino acid) radioactivity is confined to the acid portion and most of the ^{3}H (putrescine) radioactivity is confined to the retronecine portion. This could most conveniently be carried out by catalytic hydrogenolysis (SCHEME 37) to yield retronecanol and the γ lactone of trichodesmic acid.



SCHEME 37

The lactone could then be degraded to determine the location of the radioactive carbons. The following SCHEME 38 is a suggested specific degradation of the lactone.

Treatment of the lactone with lithium aluminium hydride would give the tetrahydroxy compound (A). Oxidation of (A) with sodium periodate would yield the β-ketoalcohol (B) plus formaldehyde (corresponding to C-1) and acetaldehyde (corresponding to C-2+C-6). The aldehydes could be isolated as their dimedone derivatives. Isolation of acetaldehyde as its bisulphite addition product would allow it to be regenerated. Treatment of the acetaldehyde with phenyl magnesium chloride followed by oxidation with pyridinium chlorochromate then basic aqueous iodine would yield triiodomethane (corresponding to C-6) and benzoic acid (containing C-2).

Oxidation of β-ketoalcohol (B)with permanganate would give the β-ketoacid (C). Decarboxylation of (C) by heating with aqueous acid would produce CO_2 corresponding to C-5) which could be trapped as $BaCO_3$. The remaining ketone (D) would yield triiodomethane (corresponding to C-7) on treatment with basic aqueous iodine. Bromination of the acid (E) by the Hell-Volhard-Zelinsky method would yield an α-bromo acid. This bromo acid could readily be hydrolysed with aqueous NaOH to the α-hydroxy acid (F). Cleavage of this acid with periodate would yield CO_2 (corresponding to C-3) and aldehyde (G). Bromination of (G) followed by hydrolysis and periodate oxidation would yield formic acid (corresponding to C-4) and acetone (corresponding to C-8+C-9+C-10).

These degradations could be carried out on a convenient scale as compounds B-G are readily available and could be used to dilute the radioactive samples as necessary.

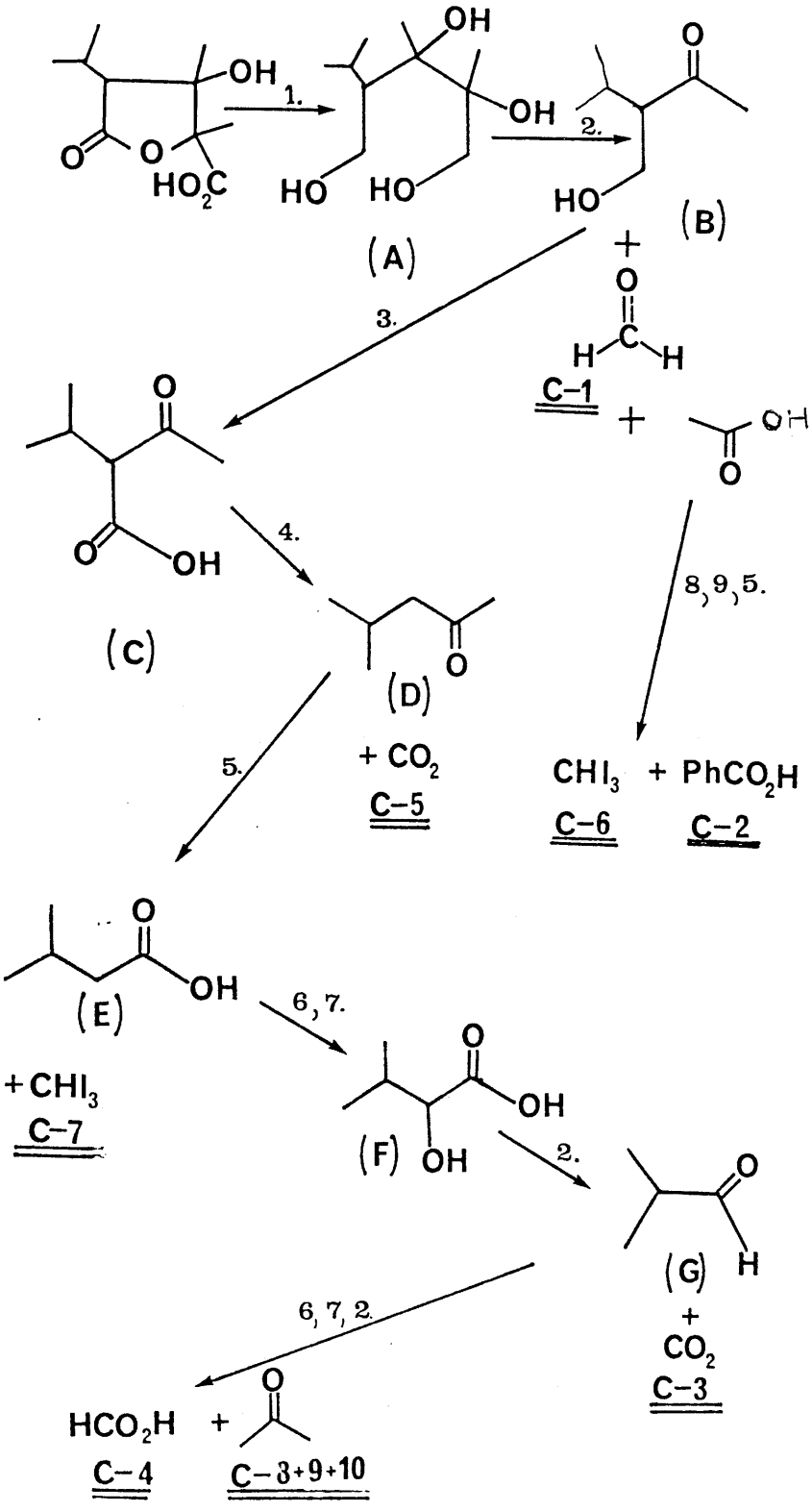
If radioactive samples of each alkaloid with sufficiently high specific incorporation from feeding experiments could be obtained, it would be of value to re-administer the alkaloids separately to C.globifera plants to test if one was a precursor of the other. It would be of further interest to establish if retronecine esterified at C-7 with valine was incorporated into the alkaloid.

6.5 CONCLUSIONS

Evidence has been obtained that valine and L-isoleucine are efficient precursors of the pyrrolizidine alkaloids obtained from C.globifera. It is assumed that these amino acids are precursors of the esterifying acids of trichodesmine and globiferine. The results suggest that trichodesmine and globiferine have a common biogenesis. It has been shown that $[1,4\text{-}^{3}H]$ putrescine is incorporated into these alkaloids with little loss of tritium.

REAGENTS FOR SCHEME 38

1. $LiAlH_4$
2. $NaIO_4$
3. $KMnO_4$
4. H_3O^+
5. $NaOH/I_2$
6. Br_2/P_4
7. OH^-
8. PhMgCl
9. P.C.C.


1.
2.
(A)
(B)
3.
C-1
(C)
4.
(D)
+ CO2
C-5
8,9,5.
CHI3 + PhCO2H
C-6
C-2
5.
(E)
+ CHI3
C-7
6,7.
(F)
2.
(G)
+
CO2
C-3
6,7,2.
HCO2H
C-4
C-3+9+10

SCHEME 38

CHAPTER 7 EXPERIMENTAL

7.1 General Notes

All melting points are uncorrected and were taken with a Kofler hot-stage apparatus. Optical rotations were measured with a Perkin-Elmer 141 Polarimeter. Infra red spectra were determined with Perkin-Elmer 197, 257, or 580 Infra red Spectrophotometers or a Perkin-Elmer 225 Grating Infra red Spectrophotometer. Nuclear magnetic resonance spectra were determined with Varian T-60, Perkin-Elmer R32, or Varian XL-100 spectrometers. All n.m.r. spectra were determined for solutions in deuteriochloroform unless otherwise indicated, with tetramethylsilane as internal standard. Mass spectra were obtained with an AEI MS 12 spectrometer.

Thin layer chromatography (t.l.c.) of alkaloids was carried out on Kiesselgel G (Merk). The alkaloids were located by oxidation with o-chloranil, followed by treatment with Ehrlich's reagent.[55]

Diethyl ether, tetrahydrofuran and dimethoxyethane (D.M.E.) were dried over lithium aluminium hydride and distilled prior to use. N,N-Dimethylformamide (D.M.F.) was dried over barium oxide and distilled at reduced pressure. Chloroform was dried by passing through grade 1 basic alumina. Organic solutions were dried with anhydrous magnesium sulphate and solvents were evaporated off under reduced pressure below 40 ^{o}C.

7.2 EXPERIMENTAL TO CHAPTER 2

(+)-Retronecine (3)- A supply of (+)-retronecine was obtained by hydrolysis of retorsine extracted from Senecio isatideus plants as described by ROBINS and SWEENEY.[79]

Reaction of 3,3-dimethylglutaric anhydride with (+)-retronecine (3)- (+)-Retronecine (15.5 mg, 0.1 mmol) was dissolved in chloroform (5 ml). To this was added a solution of 3,3-dimethylglutaric anhydride (14.2 mg, 0.1 mmol) in chloroform (5 ml). This solution was left at room temperature for 12 hours, after which time the solvent was removed to leave a foam in quantitative yield which collapsed to a gum on storage. This gum was a mixture of 7- and 9-O-(hydrogen 3,3-dimethylglutaryl)-retronecine; $\nu_{max.}$ ($CHCl_3$) 3 300, 3 000, and 1 726 cm^{-1}; δ (CD_3OD) 4.52 (m, H-7 of C-7 ester), 4.71 (s, H-9 of C-9 ester). From the integration for these signals the ratio of the C-7 monoester to C-9 monoester varied from 1:2 to 1:7 on different runs.

13,13-Dimethyl-1,2-didehydrocrotalanine (47)- Method one (typical run): (+)-Retronecine (50 mg, 0.32 mmol) was dissolved in chloroform (5 ml) 3,3-dimethylglutaric anhydride (46 mg, 0.32 mmol) in chloroform (5 ml) was added and the reaction mixture stirred at room temperature for 12 hours. The solvent was removed and the resulting oil dissolved in D.M.F. (15 ml) under argon. 2,2'-Dipyridyldisulphide (88 mg, 0.4 mmol) and triphenylphosphine (105 mg, 0.4 mmol) were added and the solution stirred at room temperature for 12 hours. The resulting yellow solution was diluted with D.M.F. (10 ml) and added via a syringe to D.M.F. (15 ml) at reflux under argon over a period of 6 hours. The reaction mixture was heated at reflux for a further 20 hours. The mixture was cooled and the solvent was removed to yield an oil which was dissolved in 1M sulphuric acid (10 ml). The acidic solution was washed with chloroform (2x10 ml) and made strongly basic with concentrated sodium hydroxide solution (10 ml). The basic solution was extracted with chloroform (4x10 ml) and the extracts dried, filtered and concentrated to yield an oil which was purified by preparative t.l.c. (silica GF_{254}, $CHCl_3/MeOH/NH_3$=85/14/1)

to give 13,13-dimethyl-1,2-didehydrocrotalanine (47) (44 mg, 49%) as an oil (rf 0.06). $[\alpha]_D^{22}$ +42.5° (c 4.40, $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 735 and 1 655 cm^{-1}; δ 1.18 (3 H, s, Me), 2.03 and 2.22 (4 H, ABq, J 13.5 Hz, H-12 and H-14), 2.10-2.40 (2 H, complex, H-6), 2.50-3.10 (2 H, complex, H-5), 3.30-3.89 (2 H, complex, H-3), 4.35 (1 H, m, H-8), 5.14 (1 H, m, H-7), 4.08 and 5.32 (2 H, ABq, J 12 Hz, H-9), 5.88 (1 H, m, H-2); m/z 279 (M^+), 137, 136, 120, 119, 94, 93 and 80 (Found M^+, 279.1469. $C_{15}H_{21}NO_4$ requires M, 279.1470). The picrate had m.p. 191-192 °C (EtOH) (Found: C, 49.45; H, 4.85; N, 11.3%. $C_{21}H_{24}N_4O_{11}$ requires C, 49.6; H, 4.7; N, 11.0%). The picrolonate had m.p. 232-234 °C (decomp.) ($CHCl_3$) (Found: C, 55.4; H, 5.45; N, 12.8%. $C_{25}H_{29}N_5O_9$ requires C, 55.25; H, 5.35; N, 12.9%). The hydrobromide had m.p. 208-210 °C (EtOH) (Found: C, 50.1; H, 5.85; N, 3.8%. $C_{15}H_{22}BrNO_4$ requires C, 50.0; H, 6.1; N, 3.9%).

N,N-Dimethylamides of 7- and 9-O-(hydrogen 3,3-dimethylglutaryl)-retronecine (A). During t.l.c. purification of (47) a second more polar component was isolated; (rf 0.3-0.4; silica, $CHCl_3$/MeOH/NH_3=85/14/1). This material accounted for between 10 and 30% of the total basic product. The t.l.c. behaviour of this product indicated that it was not a single compound, but no attempt was made to separate the components of the presumed mixture. $\nu_{max.}$ ($CHCl_3$) 3 300, 1 725 and 1 690 cm^{-1}; δ 1.00 (6 H, br, s, Me_2C), 2.90 (3 H, s, MeN), 3.00 (3 H, s, MeN), 5.70 (m, H-2 of C-7 ester), and 5.81 (m, H-2 of C-9 ester) (plus usual complex pattern for retronecine); m/z 324 (M^+), 137, 136, 120, 119, 113, 99, 94, and 93 (Found M^+, 324.2044. $C_{17}H_{28}N_2O_4$ requires M, 342.2050).

Method two (Typical run). (+)-Retronecine (62 mg, 0.4 mmol) and 3,3-dimethylglutaric anhydride (57 mg, 0.4 mmol) were dissolved in chloroform (10 ml) and stirred at room temperature for 12 hours. The chloroform was removed under reduced pressure and the resulting colourless oil was dissolved in 1,2-dimethoxyethane (D.M.E.)(10 ml). To this solution was added triphenylphosphine (131 mg, 0.5 mmol) and 2,2 -dipyridyldisulphide (110 mg, 0.5 mmol). The solution was stirred under argon for a further 12 hours. The yellow solution was taken up into a 10 ml syringe and added to D.M.E. (50 ml) at reflux under argon over a period of 4 hours. Reflux was continued

for a further 10 hours after addition was complete. The solvent was removed and the resulting oil dissolved in chloroform (2 ml) and passed through a silica column (6 g HF_{254}, 1 cm diameter) under a negative pressure of 18 mm Hg with eluent 5% v/v methanol/chloroform to yield 13,13-dimethyl-1,2-didehydrocrotalanine (72 mg, 64%).

Method three (Typical run). As in method two with the following alterations. The chloroform solution of monoester was not concentrated but triphenylphosphine and 2,2 -dipyridyldisulphide were added directly to it. After 12 hours at room temperature this solution was added to refluxing chloroform (50 ml) and worked up as before in method two. Yield=84%.

Reaction of 3,3-tetramethyleneglutaric anhydride with (+)-retronecine- (+)-Retronecine (15.5 mg, 0.1 mmol) and 3,3-tetramethyleneglutaric anhydride (16.8 mg, 0.1 mmol) were dissolved in chloroform (5 ml). After 18 hours at room temperature the solvent was removed to give a mixture of the C-7 and C-9 monoesters as an oil in quantitative yield. $\nu_{max.}(CHCl_3)$ 3 300, 3 010 and 1 725 cm^{-1}; δ (CD_3OD) 4.50 (m, H-7 of C-7 ester), 4.68 (s, H-9 of C-9 ester), 5.60 (m, H-2 of C-7 ester), and 5.72 (m, H-2 of C-9 ester). From the integration of these signals the ratio of C-7 to C-9 monoester was 1:3.

13,13-Tetramethylene-1,2-didehydrocrotalanine (49)- (+)-Retronecine (50 mg, 0.325 mmol) was dissolved in chloroform (10 ml), 3,3-tetramethyleneglutaric anhydride (55 mg, 0.325 mmol) was added and the reaction mixture left at room temperature for 16 hours. 2,2 -Dipyridyldisulphide (88 mg 0.4 mmol) and triphenylphosphine (105 mg, 0.4 mmol) were added to the resulting solution, the solution was then stirred for a further 12 hours at room temperature. The chloroform solution was added over a period of 3 hours to refluxing chloroform (50 ml) under an argon atmosphere. When addition was complete, refluxing was continued for a further 10 hours. The chloroform solution was allowed to cool and was then concentrated to a volume of 10 ml and extracted with 1M sulphuric acid (5x1 ml). The acid washings were titrated to pH 10 with 5M sodium hydroxide solution and then poured into chloroform (50 ml). The mixture was then stirred vigorously while finely ground anhydrous potassium carbonate was added until the chloroform was dry. The solution was filtered and concen-

trated to yield an oil which was purified by t.l.c. (silica GF_{254}, $CHCl_3/MeOH/NH_3$=85/14/1) to give 13,13-tetramethylene-1,2-didehydrocrotalanine (49) (60 mg, 60%) as an oil (rf 0.62), $[\alpha]_D^{22}$ +45.1° (c 1.0 in $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 730, and 1 675 cm^{-1}; δ 1.2-2.7 (16 H, complex), 3.2-3.9 (2 H, complex, H-3), 4.32 (1 H, m, H-8), 5.13 (1 H, m, H-7), 4.10 and 5.33 (2 H, ABq, J 13 Hz, C-9), and 5.89 (1 H, m, H-2); m/z 305 (M^+), 137, 136, 120, 119, 94, 93, and 80 (Found: M^+, 305.1622. $C_{17}H_{23}NO_4$ requires M, 305.1626). The picrate had a m.p. 205-208 °C (decomp.) (EtOH) (Found: C, 51.4; H, 4.95; N, 9.95%. $C_{23}H_{26}N_4O_{11}$ requires C, 51.7; H, 4.85; N, 10.3%).

Reaction of (+)-Retronecine with glutaric anhydride- (+)-Retronecine (15.5 mg, 0.1 mmol) and glutaric anhydride (11.4 mg, 0.1 mmol) were dissolved in chloroform (5 ml). After 2 hours precipitation was complete. The solvent was decanted from the precipitated oil which was dried under vacuum to leave a dense gum. This was a mixture of the C-7 and C-9 monoesters of retronecine in a 1:1 ratio; $\nu_{max.}$ ($CHCl_3$) 3 310, and 1 720 cm^{-1}; δ $(CD_3)_2SO$ 4.50 (m, H-7 of C-7 ester), 4.62 (s, H-9 of C-9 ester), 5.70 (m, H-2 of C-7 ester), and 5.80 (m, H-2 of C-9 ester).

1,2-Didehydrocrotalanine (50)- Method one (+)-Retronecine (78 mg, 0.5 mmol) was dissolved in chloroform (10 ml) and glutaric anhydride (57 mg, 0.5 mmol) was added. The solution was stirred overnight after which time an oil had precipitated. This oil was deposited on the inner surface of the lower end of a reflux condenser by applying it as a suspension in diethyl ether and removing the solvent in a stream of nitrogen. This condenser was then placed in the neck of a 250 ml r.b. flask. The top of the condenser was sealed with a rubber septum cap and the system flushed with a stream of argon. A solution of triphenylphosphine (131 mg, 0.5 mmol) and 2,2'-dipyridyldisulphide (110 mg, 0.5 mmol) in D.M.E. (100 ml) was introduced into the flask by syringe. The solution was then refluxed under argon for 12 hours. All of the deposited monoester had been dissolved into the solution after 3 hours. The refluxing solution took on a deepening yellow colour as the reaction progressed. After 12 hours the

solution was cooled, concentrated at reduced pressure and the resulting gum purified by column chromatography as before. This yielded 1,2-didehydrocrotalanine (50)(52 mg, 41.4%) as a gum; rf 0.52 (silica GF_{254}, $CHCl_3$/MeOH/NH_3=85/15/1), $[\alpha]_D^{22}$ +39.0° (c 1.0, $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 732 and 1 605 cm^{-1}; δ 1.90-2.20 (4 H, complex, H-6 and H-13), 2.22-2.49 (4 H, complex, H-12 and H-14), 2.50-2.83 and 3.20-3.45 (2 H, complex, H-5), 3.45-4.01 (2 H, complex, H-3), 4.41 (1 H, m, H-8), 4.34 and 4.96 (2 H, ABq, J 12 Hz, H-9), 5.32 (1 H, m, H-7), 5.97 (1 H, m, H-2); m/z 252 (M^+), 137, 136, 120, 119, 94, 93, and 80 (Found: M^+, 251.1156. $C_{13}H_{17}NO_4$ requires M, 252.1157). The picrate had m.p. 210-212 °C (decomp.) (EtOH) (Found: C, 47.3; H, 4.05; N, 11.4%. $C_{19}H_{20}N_4O_{11}$ requires C, 47.5; H, 4.15; N, 11.65%).

Method two. Glutaric anhydride (11.4 mg, 0.1 mmol) in chloroform (5 ml) was added to a solution of (+)-retronecine (15.5 mg, 0.1 mmol) in chloroform (5 ml). This mixture was vigorously stirred at room temperature for 6 hours. After this time the monoester had precipitated as a fine suspension of droplets. To this stirring suspension was added triphenylphosphine (65.6 mg, 2.5 equiv.) and 2,2'-dipyridyldisulphide (55 mg, 2.5 equiv.) and stirring continued for a further 12 hours after which time the solution was a clear yellow with no suspended material. The solution was then added in portions (2 ml) to refluxing chloroform (50 ml) in an argon atmosphere over a period of 3 hours. The solution was heated at reflux for a further 8 hours then cooled, concentrated and the resulting oil dissolved in methylene chloride (15 ml). This solution was extracted with M/10 hydrochloric acid (3x5 ml) and the acid extract washed once with methylene chloride (5 ml). The aqueous solution was then basified with concentrated ammonia solution and extracted with methylene chloride (3x5 ml). The organic layers were combined and shaken with 5M sodium hydroxide solution (5 ml) till colourless then washed twice with water, dried, filtered, concentrated and purified by preparative t.l.c. (silica GF_{254}, $CHCl_3$/MeOH/NH_3= 85/14/1) to yield 1,2-didehydrocrotalanine (20 mg, 74%).

Reaction of (+)-retronecine with 3-methylglutaric anhydride- (+)-Retronecine (15.5 mg, 0.1 mmol) and 3-methylglutaric anhydride (12.8 mg, 0.1 mmol) were dissolved in chloroform (10 ml) and stirred at room temperature overnight. During this time the monoester mixture precipitated out of solution. The solvent was decanted and the residue washed with chloroform and dried under vacuum to give the monoester in quantitative yield; $\nu_{max.}$ (nujol) 3 300, 3 000, and 1 730 cm^{-1}; δ (CD_3OC) 1.16 (3 H, d, J 8 Hz, Me), 4.70 (2 H, s, H-9), and 6.79 (1 H, br, s, H-2). In this case only the C-9 monoester had been formed.

Synthesis of a mixture of (13-R and 13-S) 13-methyl-1,2-didehydrocrotalanine (51)+(52)- (+)-Retronecine (78 mg, 0.5 mmol) was dissolved in chloroform (5 ml), to this solution was added 3-methylglutaric anhydride (64 mg, 0.5 mmol) in chloroform (5 ml). After 4 hours precipitation of the monoester was complete. The solvent was removed and the precipitate dissolved in D.M.F. (1 ml). Triphenylphosphine (131 mg, 0.5 mmol) and 2,2'-dipyridyldisulphide (110 mg, 0.5 mmol) were added and the solution stirred for 16 hours at room temperature. The solution was then added dropwise over a period of 6 hours to refluxing D.M.E. (100 ml) under an argon atmosphere. After addition the solution was heated at reflux for a further 8 hours, then cooled, concentrated at reduced pressure and purified by preparative t.l.c. to yield a mixture of (13-R and 13-S) 13-methyl-1,2-didehydrocrotalanines (40 mg, 30%) which could not be separated under a variety of t.l.c. systems; rf 0.55, $\nu_{max.}$ ($CHCl_3$) 1 732 and 1 634 cm^{-1}; δ (360 MHz) (major isomer) 1.11 (3 H, d, J 7 Hz, Me), 4.53 (1 H, m, H-8), 4.30 and 4.81 (2 H, ABq, J 12 Hz, H-9), 5.39 (1 H, m, H-7), and 5.95 (1 H, d, J 0.1 Hz, H-2); (minor isomer) 1.23 (3 H, br, s, Me), 4.49 (1 H, m, H-8), 4.03 and 5.20 (2 H, ABq, J 12 Hz, H-9), 5.15 (1 H, m, H-7), and 5.92 (1 H, br, s, H-2); the ratio of major to minor isomers was 2:1 (from integration): m/z 265 (M^+), 137, 136, 121, 122, and 93 (Found: M^+, 265.1322. $C_{14}H_{19}NO_4$ requires M, 265.1312).

A second more polar component was separated by preparative t.l.c. (rf 0.25) as an oily mixture of the N,N-dimethylamides of 9-O-(hydrogen (3R)- and (3S)-3-methylglutaryl) retronecine

(40 mg, 25%); $\nu_{max.}$ ($CHCl_3$) 3 300, 1 725, and 1 685 cm^{-1}; δ 1.00 (3 H, d, J 7 Hz, MeC), 2.90 (3 H, s, MeN), 2.98 (3 H, s, MeN), 4.70 (2 H, br, s, H-9), and 5.82 (1 H, br, s, H-2) (plus the usual complex signals for retronecine); m/z 310 (M^+), 137, 136, 120, 119, 94, 93, and 80 (Found: M^+, 310.1890. $C_{16}H_{26}N_2O_4$ requires M, 310.1892).

Reaction of 3,3-diphenylglutaric anhydride with (+)-retronecine- (+)-Retronecine (15.5 mg, 0.1 mmol) and 3,3-diphenylglutaric anhydride (26.6 mg, 0.1 mmol) were dissolved in chloroform (5 ml). After 6 hours at room temperature the solvent was removed to yield a mixture of 7- and 9-O-(hydrogen 3,3-diphenylglutaryl) retronecine as an oil; $\nu_{max.}$ (nujol) 3 220, 1 735, 1 500, and 910 cm^{-1}; δ (CD_3OD) 4.74 (m, H-9 of C-9 ester), 5.00 (m, H-7 of C-7 ester), 5.55 (m, H-2 of C-9 ester), and 5.60 (m, H-2 of C-7 ester). From integration the ratio of C-9 to C-7 monoester was 7:1.

13,13-Diphenyl-1,2-didehydrocrotalanine (53)- (+)-Retronecine (155 mg, 1 mmol) and 3,3-diphenylglutaric anhydride (266 mg, 1 mmol) were dissolved in chloroform (10 ml). This solution was left at room temperature for 12 hours. To this solution was added 2,2'-dipyridyldisulphide (220 mg, 0.1 mmol) and triphenylphosphine (262 mg, 0.1 mmol). After 6 hours this solution was taken up in a syringe and added over 4 hours to chloroform (50 ml) at reflux under argon. Heating was continued for 2 hours after addition was complete. The solvent was removed to leave an oil. This was dissolved in 1M hydrochloric acid (15 ml). The acid solution was washed with chloroform (3x10 ml). The aqueous layer was then basified by adding concentrated ammonia (10 ml). This solution was extracted with chloroform (3x10 ml). The organic layers were combined and washed with 1M sodium hydroxide (5 ml) and water (2x10 ml), dried, filtered and concentrated to leave an oil which was purified by preparative t.l.c. (silica, $CHCl_3$/MeOH/NH_3=85/14/1) rf 0.60. Yield (300 mg, 74%); $\nu_{max.}$ (CCl_4) 1 740, 1 578, 1 450, and 1 424 cm^{-1}; δ 1.95 (2 H, complex, H-6), 2.50-2.95 (1 H, complex, H-3), 3.38-3.58 (4 H, complex, H-6), 3.95-4.13 (4 H, complex, H-3, H-5, and H-8), 4.45 (2 H, s, H-9), 5.05 (1 H, complex, H-7), 5.40 (1 H, s, H-2), 7.32 (8 H, complex ArH), 7.62 (1 H, complex, ArH), and 8.51 (1 H, complex, ArH);

m/z 403.1764. $C_{25}H_{25}NO_4$ requires M, 403.1784).

1,1-Diphenyl-2,2-dicyanoethylene (55)- Benzophenone (100 mg, 0.55 mol), ammonium acetate (7.7 g, 0.10 mol) and glacial acetic acid (24 g) were added to benzene (150 ml). The mixture was refluxed for 12 hours with azeotropic removal of water. On cooling the product crystallised out. The diphenyldicyanoethylene was filtered and crystallised from aqueous ethanol. Yield 80 g (70%) m.p. 138-139 °C (lit.[50] 140 °C); $\nu_{max.}$ (disc) 2 214, 1 798, 1 733, 1 449, 780, 758, and 703 cm^{-1}; (Found: C, 83.24; H, 4.44; N, 12.17%. $C_{16}H_{10}N_2$ requires C, 83.45; H, 4.38; N, 12.16%).

3,3-Diphenyl-2,4-dicyanoglutamide (56)- Sodium ethoxide (7 g Na, 400 ml EtOH) was added to malononitrile (20 g, 0.3 mol). To this solution was added 1,1-diphenyl-2,2-dicyanoethylene (46 g, 0.2 mol). The solution was stirred for 3 hours. It was then diluted with water (750 ml) filtered and the filtrate acidified with concentrated hydrochloric acid (100 ml). The acidified solution was extracted with ether (5x200 ml). The ether solution was dried over anhydrous magnesium sulphate, filtered and concentrated to yield 3,3-diphenyl-2,4-dicyano-glutarimide. This was crystallised from aqueous ethanol. Yield 25 g, m.p. 210-211 °C (lit.[50] 210-211 °C). $\nu_{max.}$ (disc) 2 170, 1 710, 1700, 750 cm^{-1};(Found: C, 72.57; H, 3.50; N, 13.44%. $C_{19}H_{11}N_3O_2$ requires C, 72.84; H, 3.51; N, 13.42%).

Diimide of 2,2-diphenyl-1,1,3,3-tetracarboxylic acid (57)- 3,3-Diphenyl-2,4-dicyanoglutarimide (3.95 g, 0.125 mol) was refluxed for 10 hours in a mixture of water (40 ml) acetic acid (40 ml) and concentrated sulphuric acid (28 ml). The solution was diluted with water (200 ml). On cooling the diimide crystallised out of solution. Yield 3.5 g (84%) m.p. 320 °C (lit.[50] 330 °C sub); $\nu_{max.}$ (disc) 3 310, 1 725, 1 453, 1 285 cm^{-1}; (Found: C, 68.31; H, 4.33; N, 8.27%. $C_{19}H_{14}N_2O_4$ requires C, 68.26; H, 4.19; N, 8.38%).

3,3-Diphenylglutaric acid (58)- Diimide (57) (0.44 g, 1.32 mmol) was refluxed in 10% sodium hydroxide solution (30 ml) for 24 hours. The solution was cooled and 50%

sulphuric acid (30 ml) was added Reflux was continued for a further 3 hours. Acetic acid (10 ml) was added and the solution refluxed for 3 hours. The solution was filtered while hot. On cooling 3,3-diphenylglutaric acid crystallised from the filtrate. It was recrystallised from water. Yield 0.25 g, (70%), m.p. 158-159 °C (lit.[50] 159 °C); $\nu_{max.}$ (disc) 3 450, 1 722, 1 709, 1 500 and 700 cm^{-1} (Found: C, 71.78; H, 5.55%. $C_{17}H_{16}O_4$ requires C, 71.83; H, 5.63%).

3,3-Diphenylglutaric anhydride (54)- 3,3-Diphenylglutaric acid (284 mg, 1 mmol) was heated at reflux in acetic anhydride for 1 hour. Removal of solvent left 3,3-diphenylglutaric anhydride which was crystallised from benzene. Yield 250 mg, (13%), m.p. 149-150 °C (lit.[50] 147-148 °C); $\nu_{max.}$ (disc) 1 810, 1 773, 1 755, 1 080 and 700 cm^{-1}; δ 3.25 (4 H, s, $-CH_2-$), 7.25 (10 H, complex, Ph), (Found: C, 76.67; H, 5.20%. $C_{17}H_{14}O_3$ requires C, 76.69; H, 5.26%).

3,3,5,5-Tetramethylcyclohexanone (62)- Methyl bromide (100 g, 1.05 mol) in anhydrous ether (250 ml) was added to magnesium turnings (24 g, 1 mol) in anhydrous ether (500 ml) at such a rate as to maintain a gentle reflux. After addition was complete cuprous chloride (0.99 g, 0.01 mol) was added and the mixture stirred while redistilled isophorone (65 g, 0.69 mol) was added dropwise over a period of 1 hour, during which time the flask temperature was maintained between 10 and 15 °C by means of an ice/water bath. The mixture was then refluxed for 1 hour and left at room temperature overnight. The mixture was poured onto ice (800 g) in glacial acetic acid (100 ml). The ether layer was separated and the aqueous layer was extracted with ether (2x50 ml). The organic layers were combined, washed with 10% sodium bicarbonate solution (3x50 ml) and water (2x 25 ml), then dried, concentrated and distilled at a pressure of 10 mm Hg to yield 3,3,5,5-tetramethylcyclohexanone (83 g, 77%) as an oil, b.p. 194-196 °C (lit.[51] 195-196 °C); $\nu_{max.}$ ($CHCl_3$) 2 960 and 1 710 cm^{-1}; δ 1.0 (12 H, s, $-CH_3$), 1.7 (2 H, s, $-CH_2-$), 2.2 (4 H, s, $-CH_2-$).

2,2,4,4-Tetramethyladipic acid (63)- A mixture of 50% nitric acid (250 ml) and ammonium metavanadate (1 g) was heated to 65 °C while being vigorously stirred. To this mixture 3,3,5,5-tetramethylcyclohexanone (62) (73 g, 0.47 mol) was added dropwise while the reaction mixture was maintained at a temperature of 60-70 °C by means of a cooling bath. The mixture was stirred for a further 30 minutes at this temperature. After cooling to room temperature the 2,2,4,4-tetramethyladipic acid (63) was filtered off and washed with water till the filtrate was neutral. Crystallisation from water gave the pure acid (91 g, 95%). m.p. 121-123 °C (lit.[51] 121-123 °C); $\nu_{max.}$ (disc) 2 980, 1 700, 1 190 cm^{-1} (Found: C, 59.30; H, 8.88%. $C_{10}H_{18}O_4$ requires C, 59.40; H, 8.91%).

2,2,4,4-Tetramethylcyclopentanone (64)- 2,2,4,4-Tetramethyladipic acid (70 g, 0.35 mol) was mixed with manganese carbonate (10 g) and barium oxide (10 g). This mixture was distilled with a small bunsen flame to give 2,2,4,4-tetramethylcyclopentanone (15 g, 30%) as the distillate; $\nu_{max.}$ ($CHCl_3$) 1 740 cm^{-1}; δ 1.05 (6 H, s, $-CH_3$), 1.10 (6 H, s, $-CH_3$), 1.70 (2 H, s, $-CH_2-$), 2.15 (2 H, s, $-CH_2-$).

2,2,4,4-Tetramethylglutaric acid (65)- 2,2,4,4-Tetramethylcyclopentanone (64) (5 g, 35 mmol) was oxidised in a solution of ammonium metavanadate (0.1 g) in 50% nitric acid (20 ml) as for 3,3,5,5-tetramethylcyclohexanone above, to yield 2,2,4,4-tetramethylglutaric acid (4.6 g 70%). m.p. 196-197 °C (Ex H_2O) (lit.[51] 197-198 °C); $\nu_{max.}$ (disc) 1 700, 1 190 cm^{-1}; δ (CD_3OD) 1.30 (12 H, s, $-CH_3$), 2.05 (2 H, s, $-CH_2-$) (Found: C, 57.47; H, 8.60%. $C_9H_{16}O_4$ requires C, 57.45; H, 8.51%).

2,2,4,4-Tetramethylglutaric anhydride (60)- 2,2,4,4-Tetramethylglutaric acid (65) (1.88 g, 10 mmol) was dissolved in redistilled acetic anhydride (10 ml). This solution was heated at reflux for 1 hour. The solvent was removed under reduced pressure to leave 2,2,4,4-tetramethylglutaric anhydride as a gummy solid which crystallised from benzene as needles in quantitative yield. m.p. 88-91 °C (lit.[51] 91-92 °C); $\nu_{max.}$ (disc) 1 790, 1 750 and 1 050 cm^{-1}; δ 1.4 (12 H, s, $-CH_3$), 1.85 (2 H, s, $-CH_2-$); (Found: C, 63.31; H, 8.31%. $C_9H_{14}O_3$ requires C, 63.53; H, 8.23%).

Reaction of 2,2,4,4-Tetramethylglutaric anhydride (60) with (+)-retronecine- 2,2,4,4-Tetramethylglutaric anhydride (60) (17 mg, 0.1 mmol) was dissolved in chloroform (5 ml) and added to a solution of retronecine (15.5 mg, 0.1 mmol) in chloroform (5 ml). This solution was left at room temperature for 12 hours after which time the solvent was removed at reduced pressure to leave 7-O-(hydrogen 2,2,4,4-tetramethylglutaryl) retronecine solid glass; $\nu_{max.}$ (nujol) 3 300, 1 730, 1 380 cm^{-1}; δ 1.10 (6 H, s, $-CH_3$), 1.21 (6 H, s, $-CH_3$), 4.70 (2 H, s, H-9), 6.79 (1 H, s, H-2).

Attempted cyclisation of 7-O-(hydrogen 2,2,4,4-tetramethyl-glutaryl) retronecine (66)- This cyclisation was attempted via the 2-pyridine thioester in the usual fashion in the following solvents: chloroform, D.M.E., and D.M.F. at reflux for varying lengths of time. In each case the only homogeneous material obtained was starting material. The cylisation was similarly attempted via the S-imidazole derivative (67) with the same solvents. No reaction was obtained.

Ethylenedithioketal of diethyl-3-oxoglutarate (72)- Dimethyl-3-oxoglutarate (71) (2 g, 10 mmol) was dissolved in ethane-1,2-dithiol (4.7 g, 50 mmol). To this was added BF_3 etherate (1 ml). The solution was left at room temperature for 5 days. The reaction mixture was dissolved in ethyl acetate (10 ml) and washed with5M sodium hydroxide (4x5 ml) then brine (2x10 ml). The organic layer was dried, filtered and concentrated to leave the ethylene dithioketal of diethyl-3-oxoglutarate. Crystallisation from methanol yielded crystals (2.5 g, 90%). m.p. 168-170 °C; $\nu_{max.}$ (disc) 2 990, 1 735, 1 370, 1 335, 1 183 cm^{-1}; δ 1.3 (6 H, t, $-CH_3$), 3.3 (8 H, s, $-CH_2-$), 4.2 (4 H, q, $-CH_2-CH_3$) (Found: C, 47.33; H, 6.40; S, 23.23%. $C_{11}H_{18}O_4S_2$ requires C, 47.48; H, 6.47; S, 23.02%).

Ethylenedithioketal of 3-oxoglutaric acid (73)- Diethyl-3-oxoglutarate ethylenedithioketal (72) (1.4 g, 5 mmol) was dissolved in ethanol (60 ml). to this solution was added 30% sodium hydroxide solution (30 ml). The solution was stirred at room temperature for 72 hours, after which time it was cooled in a salt/ice bath and acidified to pH3 with concentrated

hydrochloric acid. The acid solution was extracted with chloroform (3x20 ml). The combined chloroform extracts were washed with water (10 ml), dried, filtered and concentrated to leave a solid. The thioketal crystallised from water as plates (1 g, 90%). m.p. 210-213 °C; $\nu_{max.}$ (disc) 3 400, 1 710, 1 420, 1 395, 1 255, 1 220 cm^{-1}; δ ($CDCl_3$/MeOH d_4) 3.30 (4 H, s) 3.35 (4 H, s) (Found: C, 37.64; H, 4.37; S, 28.92%. $C_7H_{10}O_4S_2$ requires C, 37.83; H, 4.50; S, 28.82%).

Ethylenedithioketal of 3-oxoglutaric anhydride (70)- Thioketal (73) (1 g, 4.5 mmol) was dissolved in acetic anhydride (10 ml). This solution was heated at reflux for 1 hour. The solvent was removed and the semi-solid residue was crystallised from dry benzene to yield the ethylenedithioketal of 3-oxoglutaric anhydride (70) (0.67 g, 73%). m.p. 150-151 °C; $\nu_{max.}$ (disc) 1 810, 1 780, 1 160, 1 140, 1 075 cm^{-1}; δ 3.30 (4 H, s), 3.40 (4 H, s) (Found: C, 41.37; H, 4.08; S, 31.20%. $C_7H_8O_3S_2$ requires C, 41.17; H, 3.93; S, 31.37%).

Reactions of the ethylenedithioketal of 3-oxoglutaric anhydride (70) with (+)-retronecine- (+)-Retronecine (15.5 mg, 0.1 mmol) and (70) (20.4 mg, 0.1 mmol) were dissolved in chloroform (5 ml). After 2 hours precipitation of the monoester mixture as an oil was complete; $\nu_{max.}$ (nujol) 3 350, 1 745, 1 580 cm^{-1}; δ (DMSO d_6) 5.9 (H-2, C-9 monoester), 4.75 (H-9, C-9 monoester), 5.80 (H-2, C-7 monoester), 5.08 (H-7, C-7 monoester). Integration of these signals showed that the ratio of C-7 to C-9 monoester was 1:2. Cyclisation of the ω-hydroxy acid was attempted in the usual fashion to yield no homogeneous product.

7.3 EXPERIMENTAL TO CHAPTER 3

Natural Dicrotaline - Dried seeds of Crotalaria dura (3 g) were ground to a fine powder. This powder was ground repeatedly with methanol until the methanol extract was colourless. The combined methanol extracts were concentrated at room temperature . The residue was dissolved in 2% citric acid (10 ml). The acid solution was washed with chloroform (3x10 ml) then basified with concentrated ammonia (2 ml). The basic solution was extracted with chloroform (4x10 ml). The chloroform extracts were dried, filtered and concentrated to yield an oil. Preparative t.l.c. (silica GF_{254} 0.25 mm, $CHCl_3$/MeOH/NH_3=80/15/1) yielded dicrotaline (10 mg, 0.33%), rf 0.68; $\nu_{max.}$ ($CHCl_3$) 3 690, 3 610, 1 730, 1 445, 1 260, 1 168, and 1 075 cm^{-1}; δ 1.95-2.28 (2 H, complex, H-6), 2.42 (2 H, s, $-CH_2-$), 2.67 (2 H, br, s, $-CH_2-$), 2.25-2.8 (1 H, complex, H-5), 3.10-4.10 (3 H, complex, H-3, and H-5), 4.32 (1 H, m, H-8), 5.01 (1 H, m, H-7), 4.16 and 5.40 (2 H, ABq, J 12 Hz, H-9), 5.94(1 H, br, s, H-2); m/z 281 (M^+), 238, 222, 179, 137, 136, 120, 119, 94, 93, and 80 (Found: M^+, 281.1274. $C_{14}H_{19}NO_5$ requires M, 305.1626). The hydrochloride had m.p. 210-211 °C (decomp.) (lit.[26] 200 °C (decomp.) and 258-260 °C (decomp.))$[\alpha]_D^{20}$ +25.7° (c 2.06, H_2O) (Found: C, 52.75; H, 6.62%. $C_{14}H_{20}NO_5Cl$ requires C, 52.91; H, 6.34%).

4-Hydroxy-4-methylhepta-1,6-diene (78)- Allyl bromide (30.25 g, 0.25 mol) in ether (10 ml) was added dropwise over 30 minutes to a rapidly stirring mixture of magnesium turnings (6.0 g, 0.25 mol) and iodine (0.1 g) in ether (80 ml). After addition was complete the solution was stirred at room temperature for a further 15 minutes. Ethyl acetate (8.8 g, 0.1 mol) was added in portions to this solution over 15 minutes. The solution was stirred at room temperature for a further 30 minutes and then poured onto a mixture of crushed ice and water (100 g). The precipitate which formed was dissolved by adding 5N hydrochloric acid (30 ml). The organic layer was separated and the aqueous layer was extracted with ether (3x50 ml). The combined organic layers were dried, filtered and concentrated to an oil, which was distilled at 60 °C/15 mm Hg (lit.[56] 56-57 °C /14 mm Hg) to yield 4-hydroxy-4-methylhepta-1,6-diene (78)

(8.5 g, 68%); $\nu_{max.}$ (film) 3 410 and 1 640 cm^{-1}; δ 1.1 (3 H, s, $-CH_3$), 2.1 (2 H, br, s, $-CH_2-$), 2.3 (2 H, br, s, $-CH_2-$), 5.0-6.0 (6 H, complex, olefinic protons).

3-Hydroxy-3-methylglutaric acid (dicrotalic acid) (75)- 4-Hydroxy-4-methylhepta-1,6-diene (78) (6.3 g, 50 mmol) in dry ethyl acetate (120 ml) was treated with ozone at -78 °C until a blue colour persisted in the mixture. The mixture was allowed to warm to room temperature and nitrogen was bubbled through it until a negative test was obtained with starch/iodide paper. The reaction mixture was concentrated to give a colourless viscous oil. This was dissolved in 98% formic acid (100 ml) and 27% hydrogen peroxide solution (40 ml) was added. After a few minutes a vigorous exothermic reaction occured. When this reaction had ceased (ca 1 hour) the mixture was concentrated to dryness to give 3-hydroxy-3-methylglutaric acid (75) in quantitative yield; m.p. 108-109 °C (ether/pet. ether) (lit.[26] 108-109 °C); $\nu_{max.}$ (disc) 3 250, 1 710 and 1 150 cm^{-1}; δ (acetone d_6) 1.4 (3 h, s, $-CH_3$), 2.7 (4 H, br, s, $-CH_2-$), 8.2 (3 H, br, s, CO_2 H+OH) (Found: C, 44.64; H, 6.13%. $C_6H_{10}O_5$ requires C, 44.4; H, 6.22%).

3-Hydroxy-3-methylglutaric anhydride (dicrotalic anhydride) (76)- Dicrotalic acid (75) (1.6 g, 5 mmol) was dissolved in anhydrous ether (75 ml) and redistilled thionyl chloride (5 g, 42 mmol) was added. The reaction mixture was left at room temperature overnight. The resultant crystals of dicrotalic anhydride (76) were filtered off and recrystallised from benzene to yield needles (1.2 g, 83%); m.p. 99-100 °C; $\nu_{max.}$ (disc) 3 570, 1 810, 1 768 and 1 755 cm^{-1}; δ 1.5 (3 H, s, $-CH_3$), 2.9 (4 H, br, s, $-CH_2-$), 5.1 (1 H, s, OH). (Found: C, 49.84; H, 5.52%. $C_6H_8O_4$ requires C, 50.0; H, 5.60%).

3-Methyl-3-trimethylsilyloxyglutaric anhydride (79)- Solutions of trimethylchlorosilane (108 mg, 1 mmol) in ether (1 ml) and pyridine (79 mg, 1 mmol) in ether (1 ml) were added to dicrotalic anhydride (76) (144 mg, 1 mmol) dissolved in anhydrous ether (10 ml). The mixture was left at room temperature for 10 hours, during which time pyridine hydrochloride crystallised out of solution. The pyridine hydrochloride was

filtered off and the reaction mixture concentrated to give 3-trimethylsilyloxy-3-methylglutaric anhydride (79) in quantitative yield as a colourless oil; $\nu_{max.}$ (film) 1 800, 1 750, 1 250, and 1 070 cm^{-1}; δ (pyridine d_5) 0.10 (9 H, s, Si-CH_3), 1.32 (3 H, s, -CH_3), 3.13 (4 H, ABq, J, 16 Hz -CH_2-).

(+)-Retronecine (3)- Retronecine was prepared by hydrolysis of retorsine obtained from Senecio isatideus plants. In a typical run retorsine (1 g) was heated at reflux in a solution of barium hydroxide (5 g) in water (150 ml) for 2 hours. The cooled solution was acidified to pH 3 with 5M hydrochloric acid, filtered through celite, and continuously extracted with ether for 24 hours. The acid solution was then percolated through a column of Dowex 1-X8 (OH^- form), and the eluent collected until neutral. This eluent was concentrated to dryness and the residue extracted several times with boiling acetone. Evaporation of the acetone to give prisms, m.p. 119-121 °C (lit.[79] 120-121 °C); $\nu_{max.}$ (disc) 3 320 and 1 660 cm^{-1}; δ 1.80-2.10 (2 H, complex, H-6), 2.80-3.30 (2 H, complex, H-5), 3.50-3.90 (3 H, complex, H-3, H-8), 4.30 (3 H, m, H-7, H-9), 5.72 (1 H, m, H-2) (Found: C, 61.68; H, 8.40; N, 8.88%. $C_8H_{13}O_2N$ requires C, 61.94; H, 8.39; N, 9.03%).

Esterification of (+)-retronecine (3) with 3-methyl-3-trimethylsilylglutaric anhydride (79)- Retronecine (15.5 mg, 0.1 mmol) in chloroform (5 ml) was added to dicrotalic anhydride trimethylsilyl ether (79) (21.6 mg, 0.1 mmol) in chloroform (5 ml). This solution was left under an atmosphere of argon for 12 hours at room temperature, during which time an oil precipitated from solution. The chloroform solvent was decanted and the oil was shown to be a mixture of C-9 monoester (80) and C-7 monoester (81) in a ratio of 1:1; δ (pyridine d_5) 4.7 (s, H-9 of (80)), 5.1 (m, H-7 of (81)), 5.7 (br, s, H-2 of (80)+(81)). The mixture of monoesters was dissolved in methanolic ammonia solution (5 ml). This solution was stirred at room temperature for 4 hours. Removal of solvent at reduced pressure left an oil which was washed twice with acetone to give a mixture of 7- and 9-O-(hydrogen 3-hydroxy-3-methylglutaryl) retronecine. $\nu_{max.}$ (film) 3 400, 3 000 and 1 735 cm^{-1}; δ (CD_3OD) 5.85 (m, H-2 of C-9 monoester), 4.80

(s, H-9 of C-9 monoester), 5.72 (m, H_2 of C-9 monoester), 4.60 (m, H-7 of C-9 monoester) (Found m/z 299.13685 (M^+). $C_{14}H_{21}NO_6$ requires M 299.13687).

Synthesis of dicrotaline (6) and epidicrotaline (77)- Retronecine (155 mg, 1 mmol) in chloroform (5 ml) was added to dicrotalic anhydride trimethylsilyl ether (216 mg, 1 mmol) in chloroform (5 ml). The mixture was left at room temperature for 12 hours under argon. During this time an oil precipitated from solution. To this suspension was added triphenylphosphine (262 mg, 1 mmol) and 2,2'-dipyridyldisulphide (220 mg, 1 mmol). The resulting mixture was stirred under argon for 6 hours to give a yellow solution. The solution was taken up in a syringe and added dropwise over a period of 4 hours to chloroform (30 ml) at reflux under argon. After addition was complete, heating was continued for 8 hours. The cooled solution was concentrated to leave a clear red gum which was dissolved in methylene chloride (10 ml). The methylene chloride solution was extracted with 0.1M hydrochloric acid (5x5 ml). The aqueous solution was then basified to pH 10 with concentrated ammonia solution and extracted with methylene chloride (5x5 ml). The organic extracts were washed with 5M sodium hydroxide (5 ml), brine (2x5 ml) and water (2x5 ml). The organic solution was dried, filtered and concentrated to yield an oil (260 mg). Analytical t.l.c. (silica, $CHCl_3$/MeOH/NH_3=80/15/1) showed 3 components, all giving positive spot tests for 1,2-didehydropyrrolizidine derivatives. Preparative t.l.c. of the mixture gave the following results; component 1. rf 0.40 (80 mg), component 2. rf 0.61 (69 mg), component 3. rf 0.68 (71 mg). The most polar component was a mixture of 7-(82) and 9-O-(3-methylbut-2-enoyl) retronecine (83). $\lambda_{max.}$ (film) (EtOH) 222 nm; $\nu_{max.}$ (film) 3 510, 1 710 and 1 640 cm^{-1}; m/z 235 (M^+). The middle component was the C-13 epimer of dicrotaline (77), $\nu_{max.}$ ($CHCl_3$) 3 700, 2 915, 2 830, 1 730, 1 600, 1 260 and 1 170 cm^{-1}; δ 1.41 (3 H, s, $-CH_3$), 1.80-2.20 (1 H, complex, H-5), 3.15-4.10 (3 H, complex, H-5, H-3), 4.35 (1 H, complex, H-5), 4.19 and 5.17 (2 H, ABq, J 12 Hz, H-9), 5.35 (1 H, complex, H-7), 5.92 (1 H, br, s, H-2). m/z 281, (M^+), 238, 222, 179, 137, 136, 120, 119, 94, 93, 80. (Found M^+ 281.1254. $C_{14}H_{20}NO_5Cl$ requires M 281.1263). The

hydrochloride had m.p. 158-161 °C (decomp.); $[\alpha]_D^{20}$ +29.6° (c 0.1, H_2O) (Found: C, 53.13; H, 6.44; N, 4.12%. $C_{14}H_{20}NO_5Cl$ requires C, 52.91; H, 6.34; N, 4.41%).

Component 3 was dicrotaline (6); $\nu_{max.}$ ($CHCl_3$) 3 690, 3 610, 1 730, 1 605, 1 445, 1 260, 1 168, and 1 075 cm^{-1}; δ 1.40 (3 H, d, $-CH_3$), 1.95-2.28 (2 H, complex, H-6), 2.42 (2 H, s, $-CH_2-$), 2.25-2.80 (1 H, complex, H-5), 3.10-4.10 (3 H, complex, H-3, H-5), 4.32 (1 H, m, H-8), 5.01 (1 H, m, H-7), 4.16 and 5.40 (2 H, ABq, J 12 Hz, H-9), 5.94 (1 H, br, s, H-2); m/z 281 (M^+), 238, 222, 179, 138, 136, 120, 119, 94, 93, 80. (Found M^+ 281.1238. $C_{14}H_{19}NO_5$ requires M 281.1263). The hydrochloride had m.p. 211-212 °C (decomp.) (lit.[26] 200 °C (decomp.)); $[\alpha]_D^{20}$ +25.2° (c 0.20, H_2O) (lit.[26] +25.7°, H_2O) (Found: C, 52.85; H, 6.30; N, 4.64%. $C_{14}H_{20}NO_5Cl$ requires C, 52.91; H, 6.34; N, 4.41%.)

Synthesis of dicrotaline, 2nd method- (+)-Retronecine (62 mg, 0.4 mmol) and dicrotalic anhydride (57.5 mg, 0.4 mmol) were dissolved in chloroform (25 ml). After 4 hours triphenylphosphine (262 mg, 1 mmol) and 2,2'-dipyridyldisulphide (220 mg, 1 mmol) were added to the monoester suspension. The mixture was stirred vigorously for 8 hours to leave a homogeneous solution of the activated thioesters. Cyclisation and work up were carried out in the usual manner to yield dicrotaline (36 mg, 32%) and epidicrotaline (41 mg, 36%). Spectral data for these compounds were identical with those of the previously synthesised materials.

Preparation of (-)-retronecanol (86)- (+)-Retronecine (15.5 mg, 0.1 mmol) was dissolved in 0.01M hydrochloric acid (1 ml). PtO_2 (1 mg) was added and the mixture stirred under hydrogen for 48 hours. The solution was filtered through celite and basified by the addition of 1M ammonium hydroxide. The basic solution was continuously extracted with ether for 72 hours. The ethereal extract was dried over anhydrous potassium carbonate, filtered and concentrated to yield a gum. The crude retronecanol was crystallised from petroleum ether (b.p. 60-80 °C) m.p. 95-96 °C (lit.[79] 95-95 °C); $[\alpha]_D^{20}$ -91°

(c 1, EtOH) (lit.[79] -91.1°); (Found: C, 68.13; H, 10.80; N, 10.01%. $C_8H_{15}NO$ requires C, 68.04; H, 10.70; N, 9.91%).

Preparation of 7-O-(hydrogen (3RS)-3-hydroxy-3-methylglutaryl) retronecanol- (-)-Retronecanol (86) (12.7 mg, 0.1 mmol) and dicrotalic anhydride (14.4mg, 0.1 mmol) were dissolved in chloroform (10 ml). After 3 hours precipitation of the ester was complete. The solvent was decanted to leave 7-O-(hydrogen (3RS)-3-hydroxy-3-methylglutaryl) retronecanol as an oil. $\nu_{max.}$ (film) 3 200 and 1 735 cm^{-1}; δ (CD_3OD) 1.20 (3 H, d, J 7 Hz, $-CH_3$), 1.36 (3 H, s, $-CH_3$), 5.38 (1 H, m, H-7); m/z 285.1560 (M^+).

Hydrogenolysis of epidicrotaline (77)- PtO_2 (2 mg) was added to a solution of epidicrotaline (20 mg, 0.07 mmol) in acetic acid (5 ml). The mixture was stirred rapidly for 24 hours under an atmosphere of hydrogen (1 atm.). The catalyst was filtered off and the filtrate concentrated to leave 7-O-(hydrogen (3R)-3-hydroxy-3-methylglutaryl) retronecanol as an oil (20 mg), $\nu_{max.}$ (film) 3 200 and 1 735 cm^{-1}; δ (CD_3OD) 1.20 (3 H, d, J 7 Hz, $-CH_3$), 1.32 (3 H, s, $-CH_3$), 5.38 (1 H, m, H-7); m/z 285.1558 (M^+).

Sodium/liquid ammonia reduction of 7-O-(hydrogen (3R)-3-hydroxy-3-methylglutaryl) retronecanol- 7-O-(hydrogen (3R)-3-hydroxy-3-methylglutaryl) retronecanol (28.5 mg, 0.1 mmol) was dissolved in methanol (1 ml). Liquid ammonia (10 ml) was added and the solution was stirred rapidly. A small piece of sodium was suspended in the stirring ammonia solution until the reaction mixture was a uniform dark blue. The sodium was removed and methanol (1 ml) was added to the solution. The ammonia was allowed to evaporate and the residue was dissolved in 1M hydrochloric acid (10 ml). Continuous extraction of this solution with methylene chloride for 48 hours yielded (S)-mevalonolactone as an oil (9 mg 69%); $[\alpha]_D^{20}$ +19° (c 0.18, EtOH) (lit.[57] +23°); δ 1.35 (3 H, s, $-CH_3$), 1.90 (2 H, m, H-4), 2.45 and 2.70 (2 H, ABq, J 18 Hz, H-2), 3.45 (1 H, br, s, -OH), 4.47 (2 H, m, H-5); m/z 130 (M^+).

Hydrolysis of 7-O-(hydrogen (3R)-3- hydroxy-3-methylglutaryl) retronecanol- The product of the hydrogenolysis of epidicrotaline (28.5 mg, 0.1 mmol) was dissolved in 1M barium hydroxide (1 ml). The solution was heated at reflux for 1 hour. The cooled solution was made up to 5 ml with water and CO_2 was bubbled through the solution until precipitation had stopped. The solution was filtered through celite and the pH adjusted to pH 3 by addition of 1M hydrochloric acid. The acid solution was continuously extracted with ether for 24 hours. The ethereal solution was dried and concentrated to yield dicrotalic acid which was recrystallised from ether/pet. ether (12 mg, 74%). This material was identical with dicrotalic acid (i.r. and n.m.r. spectra and mixed m.p.) previouslv prepared. The acid solution was basified bv adding concentrated ammonia solution(1 ml). Continuous extraction of this solution with ether for 72 hours yielded (-)-retronecanol (10 mg, 70%). This material was shown to be identical (i.r. and n.m.r. spectra etc) with that obtained by hydrogenolysis of (+)-retronecine.

Benzhydrylamide of (S)-mevalonolactone- (S)-Mevalonolactone (6.5 mg, 0.05 mmol) and benzhydrylamine (30 mg) were placed in a small screw top sample bottle. The cap was tightly replaced and the mixture heated on a steam bath for 2 hours. The mixture was dissolved in chloroform (2 ml) and washed with 1M hydrochloric acid (3x2 ml) and5% sodium bicarbonate (2x2 ml). The chloroform solution was dried, filtered and concentrated to give an oil. The benzhydrylamide crystallised from benzene/hexane (12 mg, 76%); m.p. 99-100 °C (lit.[79] 98-99 °C); $[\alpha]_D^{20}$ +2.7° (c 0.6, EtOH) (lit.[57] +2.7); δ ($CDCl_3$/ CD_3OD) 1.17 (3 h, s, $-CH_3$), 1.60 (2 H, m, $-CH_2-$), 2.17 and 2.49 (2 H, ABq, J 12 Hz, $-CH_2$), 3.71 (2 H, m, $-CH_2OH$), 6.12 and 6.20 (1 H, two singlets, benzylic H (rotomers)), 7.18 (10 H, s, Ar).

Hydrogenolysis of dicrotaline (6)- Dicrotaline was hydrogenolysed to give 7-O-(hydrogen (3S)-3-hydroxy-3-methylglutaryl) retronecanol (84) as an oil in quantitative yield. $\nu_{max.}$ 3 200 and 1 730 cm^{-1}; δ (CD_3OD) 1.14 (3 H, d, J 7 Hz, $-CH_3$), 1.33 (3 H, s, $-CH_3$), 5.37 (1 H, m, H-7); m/z 285.1557 (M^+).

Preparation of (R)-mevalonolactone (85)- Treatment of the ester (84) with sodium in liquid ammonia as described for the ester obtained from hydrogenolysis of epidicrotaline gave (R)-mevalonolactone as an oil in 75% yield; $[\alpha]_D^{20}$ -20° (c 0.14, EtOH) (lit.[57] -23°); δ 1.35 (3 H, s, $-CH_3$), 1.90 (2 H, m, H-4), 2.45 and 2.70 (2 H, ABq, J 18 Hz, H-2), 3.45 (1 H, br, s, -OH), 4.47 (2 H, m, H-5); m/z 130 (M^+).

Benzhydrylamide of (R)-mevalonolactone (87)- This derivative was prepared as described for (S)-mevalonolactone; $[\alpha]_D^{20}$ -2.7°(c 0.5, EtOH) (lit.[57] -2.7°); m.p. 99-100 °C (lit.[57] 98-99 °C); δ (CD_3OD) 1.17 (3 H, s, $-CH_3$), 1.60 (2 H, m, $-CH_2-$), 2.17 and 2.49 (2 H, ABq, J 12 Hz, $-CH_2-$), 3.71 (2 H, m, $-CH_2OH$), 6.12 and 6.20 (1 H, two singlets, benzylic H (rotomers)), 7.18 (10 H, s, Ar).

P.m.r. specroscopy of mevalonolactone benzhydrylamides- Addition of $Eu(hfc)_3$ (0.35 equivalents) to a deuteriochloroform solution of racemic mevalonolactone benzhydrylamides resulted in the splitting of the singlets due to the benzyl proton into two pairs of broad doublets. Similar treatment of each of the enantiomers obtained by degradation of dicrotaline and epidicrotaline did not result in any splitting of the benzylic signals. Due to the broadening of the t.m.s. signal the lock of the spectrometer was not consistent enough to allow assignment of signals for the individual enantiomers.

7.4 EXPERIMENTAL TO CHAPTER 4

Extraction of alkaloids from Crotalaria globifera- Dried seed husks of Crotalaria globifera (50 g) were ground to a fine powder. This powder was continuously extracted with ethanol for 48 hours. The solution was concentrated to leave a dark syrup. The extract was dissolved in 2% citric acid (150 ml) and washed with methylene chloride (5x50 ml). The acid layer was basified with concentrated ammonia solution (20 ml) and extracted with methylene chloride (5x50 ml). The combined methylene chloride extracts were concentrated to leave an oil (38 mg). Analytical t.l.c. of this oil (silica, $CHCl_3$/MeOH/NH_3=80/15/1) showed that it contained two pyrrolizidine alkaloids rf 0.68 and 0.75. Preparative t.l.c. of the mixture yielded the pure alkaloids.

The faster running alkaloid (22 mg, 0.044%) was characterised as follows; m.p. 159-161 ^{o}C;$[\alpha]_D^{20}$ +36.5^{o} (c 0.4, EtOH); $\nu_{max.}$ (disc) 3 440, 1 738, 1 627 and 1 109 cm^{-1}; δ 0.94 (3 H, d, J 9 Hz, $-CH_3$), 1.01 (3 H, d, J 9 Hz, $-CH_3$), 1.34 (3 H, s, $-CH_3$), 1.38 (3 H, s, $-CH_3$), 1.62 (1 H, d, =CH-), 1.9-2.7 (4 H, complex, H-5, H-6, =CH-), 3.0-4.0 (3 H, complex, H-3, H-5), 4.43 (1 H, m, H-8), 5.06 (1 H, m, H-7), 4.45 and 5.18 (2 H, ABq, J 13 Hz, H-9), 5.98 (1 H, br, s, H-2); m/z 353 (M^+) 264, 222, 220, 138, 137, 136, 121, 120, 119, 95, 94, 93, 80. (Found M^+ 353.1870. $C_{18}H_{27}NO_6$ requires M 353.1838). This material was identical with a sample of authentic trichodesmine.

The following data were obtained for the more polar component (10 mg, 0.02%); $\nu_{max.}$ (disc) 3 440, 1 734, 1 729, 1 232, and 1 145 cm^{-1}; δ 1.36 (3 H, s, $-CH_3$), 1.42 (3 H, s, $-CH_3$), 1.61 (3 H, s, $-CH_3$), 1.68 (3 H, s, $-CH_3$), 1.9-4.0 (7 H, complex, H-3, H-5, H-6, CH), 4.30 (1 H, m, H-8), 4.97 (1 H, m, H-7), 4.20 and 5.26 (2 H, ABq, J 12 Hz, H-9), 6.18 (1 H, br, s, H-2); δ (^{13}C) 21.237, 21.444, 25.916, 30.273, 33.859, 48.486, 53.317, 61.793, 75.601, 77.009, 78.259, 80.181, 81.916, 131.875, 136.909, 169.208, 173.111; m/z 351 (M^+) 262, 220, 138, 137, 136, 121, 120, 129, 95, 94, 93, 80. (Found M^+ 351.1679. $C_{18}H_{25}NO_6$ requires M 351.1682).

7.5 EXPERIMENTAL TO CHAPTER 5

Pyrrole-2-aldehyde (108)- D.M.F. (40 g, 0.55 mol) was cooled to 0 °C and phosphoryloxychloride (85 g, 0.55 mol) was added with stirring over 10 minutes. The solution was stirred for a further 30 minutes at room temperature. The mixture was cooled to 5 °C and methylene chloride (150 ml) was added. Pyrrole (33.5 g, 0.5 mol) was added dropwise at such a rate that the temperature did not rise above 10 °C. After addition was complete the solution was heated at reflux for 30 minutes. The solution was stirred at room temperature for 1 hour and then sodium acetate trihydrate (400 g) in water (500 ml) was added and the mixture heated at reflux for 30 minutes. After cooling the organic layer was separated and the aqueous layer extracted with methylene chloride (3x100 ml). The combined organic layers were washed with sodium carbonate solution (3x 100 ml), dried and concentrated to give an oil which solidified on standing. Crystallised from petroleum ether (b.p. 30-40 °C) yielded pyrrole-2-aldehyde (43 g, 90%); m.p. 46-48 °C (lit.[72] 44-45 °C); $\nu_{max.}$ (disc) 1 710 cm^{-1}; (Found: C, 63.12; H, 5.48; N, 14.77%. C_5H_5NO requires C, 63.13; H, 5.30; N, 14.74%).

Pyrrole-2-carboxylic acid (109)- A suspension of silver oxide was prepared by adding a solution of silver nitrate (70 g) in water (500 ml) to a rapidly stirring solution of sodium hydroxide (35 g) in water (500 ml). Pyrrole-2-aldehyde (108) (20 g, 0.21 mol) in 50% aqueous methanol (200 ml) was added and the suspension stirred at room temperature for 2 hours. The suspension was filtered and the residue washed with boiling water (200 ml). The combined filtrate and washings were extracted with ether (2x100 ml). The aqueous layer was cooled to 0 °C and acidified to congo red with concentrated hydrochloric acid, and extracted with ether (4x100 ml). The ether solution was dried, filtered and concentrated to yield a purple powder which was crystallised from water to yield pyrrole-2-carboxylic acid (22 g, 94%); m.p. 200 °C (lit.[73] 204 °C (decomp.)); (Found: C, 53.81; H, 4.53; N, 12.35%. $C_5H_5NO_2$ requires C, 54.04; H, 4.50; N, 12.61%).

Phosphonium Iodide- White phosphorus (25 g) was dissolved in carbon disulphide (30 ml) under an atmosphere of argon. Iodine (44 g) was added in portions with stirring. The solvent was removed with a stream of argon and the residue ground to a fine powder. This powder was placed in a three necked flask, fitteded with a dropping funnel and an air condenser arranged to collect the sublimed product. A stream of argon was passed through the system and water added dropwise to the powder. Phosphonium iodide was collected as it sublimed from the reaction mixture.

3,4-Didehydroproline (107)- Constant boiling hydroiodic acid (60 ml) was saturated with HI gas at -25 ^{o}C over 2 hours. Pyrrole-2-carboxylic acid (109)(10 g) and phosphonium iodide (10 g) were added and the flask stoppered securely. The reaction mixture was shaken at room temperature for 6 hours. The resultant solution was poured onto crushed ice (50 g) and the aqueous solution concentrated to dryness at 0.5 mm Hg. The residue was dissolved in water (25 ml) and percolated through a cation resin (DOWEX 50-W80) (150 ml). The column was washed with water until the eluent was neutral then eluted with 2M ammonium hydroxide solution (500 ml). The basic solution was concentrated to dryness to leave a yellow-brown mass which was crystallised from aqueous ethanol to yield 3,4-didehydroproline (7 g, 70%); m.p. 234-236 ^{o}C (lit.[70] 234-236 ^{o}C); $\nu_{max.}$ (disc) 3 420, 1 640, and 1 620 cm^{-1}; δ (CF_3CO_2H) 4.55 (2 H, complex, $-CH_2-$), 5.60 (1 H, complex, =CH-) and 6.30 (2 H, s, olefinic); (Found: C, 53.02; H, 6.24; N, 12.44%. $C_5H_7NO_2$ requires C, 53.07; H, 6.24; N, 12.44%.

Method two A saturated solution of iodine in 80% hydroiodic acid was added dropwise to a 50/50 mixture of iodine and red phosphorus. The vapour produced was bubbled into a solution of glacial acetic acid containing 10% anhydrous hypophosphorous acid. After 2 hours a thick semi-crystalline mass had been deposited at the bottom of the solution. Addition of pyrrole-2-carboxylic acid (1 g per 20 ml original solution) followed by vigorous shaking at room temperature for 5 hours yielded after the usual work up 3,4-didehydroproline in 80% yield.

N-Carbobenzyloxy-3,4-didehydroproline – 3,4-Didehydroproline (1.13 g, 10 mmol) was dissolved in 1M sodium hydroxide solution (10 ml). This solution was poured into a beaker containing water (50 ml). The beaker was fitted with a magnetic stirrer and pH electrode. The solution was cooled to 0 °C and carbobenzyloxychloride (2 ml) in ether (10 ml) added. 1M Sodium hydroxide solution was added at such a rate to keep the pH of the solution at 9-10. After addition the solution was extracted with ether (3x50 ml) and the aqueous layer acidified to pH 2 with concentrated hydrochloric acid. The precipitated oil was dried and concentrated to yield N-carbobenzyloxy-3,4-didehydroproline as a colourless gum (2.35 g, 95%); rf 0.25 (silica, methanol); $\nu_{max.}$ (film) 3 450, 1 710, 1 620 and 1 500 cm^{-1}; δ 4.4 (2 H, complex, $-CH_2-$), 5.2 (3 H, complex, =CH- and Bz), 6.0 (2 H, complex, olefinic) and 7.4 (5 H, s, Ph).

N-Carbobenzyloxy-3,4-didehydroproline, Methyl ester – N-Cbz-3,4-didehydroproline (2.6 g, 10 mmol) was dissolved in methanol (10 ml) and dropped onto an excess of ethereal diazomethane. Excess diazomethane was destroyed by addition of formic acid. The solution was concentrated to give N-Cbz-3,4-didehydroproline methyl ester in quantitative yield as an oil. rf 0.34 (silica, chloroform), 0.76 (silica, ethanol); $\nu_{max.}$ (film) 1 755, 1 710 and 1 415 cm^{-1}; δ 3.7 (3 H, d, $-CH_3$), 4.4 (2 H, complex, $-CH_2-$), 5.3 (1 H, complex, =CH-), 5.4 (2 H, s, Bz), 7.5 (5 H,s, Ph).

N-Carbobenzyloxy-3,4-dihydroxyproline, Methyl ester

N-Cbz-3,4-didehydrproline methyl ester (1 g, 3.7 mmol) was dissolved in dry pyridine (15 ml). Osmium tetroxide (1 g) was added and the solution stirred at room temperature for 24 hours. A solution of sodium bisulphite (1.8 g) in water (30 ml) and pyridine (20 ml) was added to the reaction mixture. The resulting solution was extracted with chloroform (3x60 ml) and the organic layer washed with brine (20 ml). The organic solution was dried and concentrated to give N-Cbz-3,4-dihydroxyproline methyl ester which was crystallised from ethanol (0.9 g, 80%); m.p. 113-115 °C; $\nu_{max.}^{(nujol)}$

3 630, 1 760 and 1 710 cm^{-1}; δ 3.7 (3 H, d, $-CH_3$), 3.7 (2 H, complex, $-CH_2-$), 4.3 (2 H, complex, =CH(O)), 5.2 (2 H, s, Bz), 7.2 (5 H, s, Ph).

3,4-Dihydroxyproline (106)- N-Cbz-3,4-dihydroxyproline methyl ester (2.97 g, 10 mmol) was dissolved in methanol (10 ml) containing 1 drop of concentrated hydrochloric acid. Platinum on charcoal (200 mg) was added and the mixture stirred under an atmosphere of hydrogen for 4 hours. The reaction mixture was filtered and the methanol solution poured into 6M hydrochloric acid (30 ml). This solution was heated at reflux for 2 hours then the methanol removed by distillation. The solution was worked up by the same method used for 3,4-didehydroproline (107)to yield 3,4-dihydroxyproline (1 g, 68%); m.p. 240-242 °C (decomp.) (lit.[70] 241-242 °C (decomp.)). (Found: C, 41.01; H, 6.15; N, 9.00%. $C_5H_9NO_4$ requires C, 40.8; H, 6.2; N, 9.5%).

Attempted 1,3-Dipolarcycloaddition with 3,4-Dihydroxyproline- 3,4-Dihydroxyproline (106)(147 mg, 1 mmol) was dissolved in acetic anhydride (10 ml) and ethyl propiolate (490 mg, 5 mmol) added. The solution was heated at reflux for 2 hours then cooled and concentrated to leave an intractable black tar. The reaction was repeated without addition of ethyl propiolate. This also resulted in the formation of a black tar. Repetition of the reaction at temperatures between 80 °C and reflux resulted in formation of the black tar. Shorter reaction times gave a mixture of starting material and the same black tar. The only spectral data obtained for this tar were the following p.m.r. signals; δ (CD_3OD) 7.0 (br, s) 6.9 (br, s), 6.5 (br, s), 2.0 (br, s).

N-Formyldidehydroproline (111) A mixture of acetic anhydride (10 ml) and 98% formic acid (10 ml) was stirred at 50 °C for 3 hours. A solution of 3,4-didehydroproline (1.13 g, 10 mmol) in 98% formic acid (5 ml) was added and the mixture stirred at room temperature for 10 hours. The solution was concentrated to dryness to leave an amorphous solid. This solid was placed in a soxhlet extraction thimble and extracted with ethyl acetate (10 ml) for 12 hours. The ethyl acetate

solution was cooled and N-formyldidehydroproline crystallised out (1.3 g, 88%); m.p. 155-156 °C; $\nu_{max.}$ (nujol) 3 000, 1 730 and 1 675 cm^{-1}; δ (CD_3OD) 4.3 (1 H, complex, H-1), 4.5 (1 H, complex, H-1), 5.2 (1 H, complex, =CH-), 6.0 (2 H, br, s, olefinic), 8.4 (1 H, s, CHO). (Found: C, 51.11; H, 4.80; N, 9.60%. $C_6H_7NO_3$ requires C, 51.00; H, 4.96; N, 9.82%).

Attempted 1,3,-Dipolarcycloaddition with N-formyl-3,4-didehydroproline (111) Treatment of N-formyl-3,4-didehydroproline as described for 3,4-dihydroxyproline above yielded either recovered starting material or no homogeneous product.

N-Carbobenzyloxy-4-proline- L-Proline (10.1 g, 0.1 mol) was treated with carbobenzyloxy chloride as for 3,4-didehydroproline above. The resultant N-Cbz-proline was crystallised from carbon tetrachloride in quantitative yield; m.p. 77 °C (lit.[11] 76-77 °C); $\nu_{max.}$ 3 000, 1 760, 1 654, 1 440 and 1 240 cm^{-1}. (Found:C, 62.83; H, 6.08; N, 5.62%. $C_{13}H_{15}NO_4$ requires C, 62.65; H, 6.02; N, 5.62%).

L-Proline Tertiarybutyl ester - N-Cbz-L-proline (2.5 g, 10 mmol) was dissolved in methylene chloride (100 ml) and p-toluenesulphonic acid (100 mg) added. The solution was cooled to 0 °C and liquid 2-methylpropene (40 g) added. After stirring at room temperature for 16 hours the solution was washed with saturated sodium carbonate solution, dried and concentrated to yield a thick oil. This oil was dissolved in methanol (20 ml) and 10% platinum on charcoal (150 mg) added. The resulting suspension was rapidly stirred under an atmosphere of hydrogen for 4 hours. The solution was filtered and concentrated to yield L-proline tertiarybutyl ester as an oil (1.5 g, 86%). (Found M^+ 171.2400, $C_9H_{17}NO_2$ requires M 171.2405).

N-(3-ethoxycarbonylpropyl) proline (116) Ethyl-4-bromobutyrate (1.95 g, 10 mmol) and L-proline tertiarybutyl ester (1 g, 6 mmol) were dissolved in benzene (10 ml). Anhydrous potassium carbonate (2 g) was added and the mixture heated at reflux under nitrogen for 36 hours. The solution was cooled and washed with water (2x2 ml). The organic layer was poured

into a 10% solution of hydrogen bromide in glacial acetic acid and stirred at room temperature for 3 hours. The solution was concentrated at a pressure of 0.5 mmHg to leave a gum which was dissolved in water and purified by ion exchange chromatography as described for 3,4-didehydroproline above to yield a gum (0.916 g, 66%); $\nu_{max.}$ (film) 3 400, 1 730 and 1 600 cm^{-1} δ (CD_3OD) 1.35 (3 H, t), 2.2 (4 H, complex), 2.5 (4 H, complex) 3.9 (1 H, m), 4.2 (2 H, q).

N-(3-cyanopropyl) proline tertiarybutyl ester

4-Bromobutyronitrile (1.48 g, 10 mmol) and L-proline tertiatybutyl ester (1 g, 6 mmol) were dissolved in benzene (10 ml). Anhydrous potassium carbonate (2 g) was added and the mixture heated at reflux under nitrogen for 36 hours. The solution was cooled, washed with water (2x2 ml), dried over potassium carbonate and concentrated to leave an oil (2.3 g, 98%); $\nu_{max.}$ ($CHCl_3$) 2 980, 2 250, 1 730 and 1 360 cm^{-1}. (Found: M^+ 238.3325, $C_{13}H_{22}N_2O_2$ requires M 238.3312).

7.6 EXPERIMENTAL TO CHAPTER 6

Radiochemical methods. Radiochemicals were purchased from The Radiochemical Centre, Amersham and Le Commissariat a l'Energie Atomique, Service des Molecules Marquees, Gif-Sur-Yvette.

Samples for feeding were prepared as aqueous solutions with a ratio of ^{3}H activity to ^{14}C activity of 8:1. Radioactivity was determined for toluene/methanol solutions with a Philips Scintillation Counter.

Feeding methods. The radioactive solutions were fed to batches of thirty 3 month old C. globifera plants by the xylem pricking method.[45] The plants were harvested after seven days and the alkaloids extracted as desribed in CHAPTER 4. The radioactive extract was diluted with cold trichodesmine adn globiferine then purified by t.l.c.

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