SYNTHESIS AND BIOSYNTHESIS OF PYRROLIZIDINE ALKALOIDS AND THEIR ANALOGUES.

Thesis presented in part fulfilment of the requirement for the degree of Doctor of Philosophy.

by

MICHAEL RODGERS

Department of Chemistry
The University,
Glasgow.

January 1990.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ACKNOWLEDGEMENTS

I would like to thank Dr. D.J. Robins for all of the help he has given over the past three years.

I am grateful to the technical staff at the University of Glasgow, particularly Isobel Freer (root culture laboratory), Dr. D. Rycroft and J. Gall (n.m.r spectroscopy).

Thanks also to Liz Hughes for the excellent typing of this thesis.

To my friends and colleagues in the Henderson Lab. and in Lab. 311, I send thanks for the help and entertainment over the years, and a special thanks to my Mum and Dad who supported and encouraged me throughout my University career.

This thesis is dedicated to Susan M. Reid, who helped me all the way through from lab work to proof reading, and who is a very special person.

Some of the work reported in this volume has been accepted for publication:-

(a) "Synthesis of Macrocyclic Diesters of Platynecine"
D.J. Robins and M. Rodgers, J. Chem. Soc., Perkin Trans. 1, 1989, 2437.

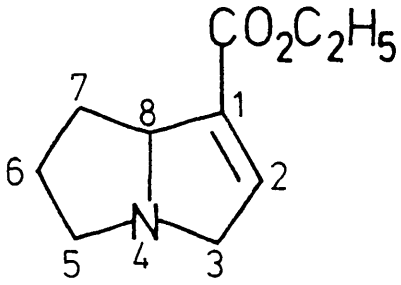
(b) "Biosynthesis of the Seco-Pyrrolizidine Base, Otonecine"
H.A. Kelly, E.K. Kunec, D.J. Robins and M. Rodgers, J. Chem. Res (S), 1989, 358.

ABBREVIATIONS

br	-	broad
CDI	-	N,N-carbonyl diimidazole
COSY	-	correlation spectroscopy
d	-	doublet
DBN	-	1,5-diazabicyclo[4.3.0]non-5-ene
DBU	-	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	-	Dicyclohexylcarbodiimide
DEPT	-	distortionless enhancement by polarisation transfer
DIBAL	-	di-isobutylaluminium hydride
DMAP	-	4-N,N-dimethylamino pyridine
DMF	-	N,N-dimethylformamide
DME	-	1,2-dimethoxyethane
Im	-	imidazole
i.r.	-	infra red
MEM	-	methoxyethoxy methyl
MOM	-	methoxy methyl
MTM	-	methane thio methyl
n.m.r.	-	nuclear magnetic resonance
s	-	singlet
t	-	triplet
TBDMS	-	tertiary butyldimethylsilyl
THF	-	tetrahydrofuran
t.l.c	-	thin layer chromatography
u.v.	-	ultra violet

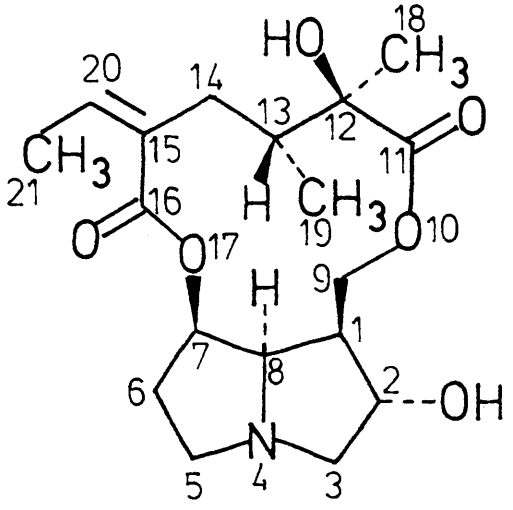
NOTES ON NOMENCLATURE

Pyrrolizidine compounds with one or two double-bonds are named as derivatives of 1H- or 3H-pyrrolizine in accordance with Chemical Abstracts nomenclature e.g., ethyl 5,6,7,8-tetrahydro-3H-pyrrolizine-1-carboxylate.



Fully saturated compounds are named as pyrrolizidine derivatives. The stereochemistry of substituents is indicated by the α and β nomenclature conforming with the usual practice in this field.

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). For example, rosmarinine is shown.



SUMMARY

The work presented in this thesis is divided into three sections: (a) Biosynthesis of otonecine; (b) Synthesis of macrocyclic pyrrolizidine diesters; and (c) Synthetic analogue studies.

(a) Biosynthesis of Otonecine

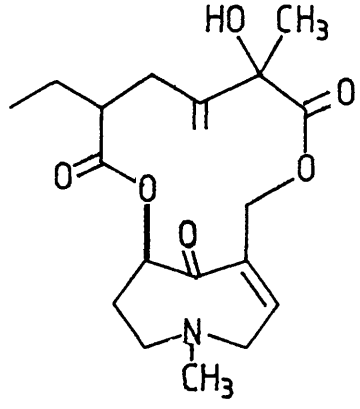
The biosynthesis of otonecine, the base portion of the pyrrolizidine alkaloid emiline (A), has been studied in *Emilia flammea* (family Compositae) plants and hairy root cultures. It was observed that [1,4-^{14}C] putrescine dihydrochloride was incorporated into emiline with specific activities of >4% for hairy root cultures and <1% for plants. The incorporation of [1,9-^{14}C] homospermidine trihydrochloride with comparable specific incorporation to [1,4-^{3}H] putrescine indicates the conversion of putrescine via the symmetrical C_4-N-C_4 intermediate, homospermidine. Incorporation of ^{3}H-labelled retronecine into emiline in *E. flammea* hairy root cultures is indicative of the intermediacy of retronecine in the emiline biosynthesis.

The stereochemistry of the enzymic processes on the pathway was investigated by feeding (R)-[1-^{2}H]-, (R)-[2-^{2}H]- and (S)-[2-^{2}H] putrescine dihydrochloride to *E. flammea* hairy root cultures. The labelling patterns obtained in emiline, as determined by ^{2}H n.m.r. spectroscopy, were consistent with the following. The oxidation of putrescine to 4-aminobutanal occurs with the loss of the pro-S hydrogen. The aldehyde and another molecule of putrescine condense to give the corresponding imine, which after reduction (on the C-si face) affords homospermidine. Two further oxidations each take place with loss of the pro-S hydrogens generating the dialdehyde, which after Mannich-type cyclisation produces 1α-formyl-8α-pyrrolizidine. Reduction to

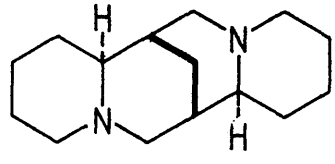
trachelanthamidine proceeds by the delivery of a hydride equivalent on the C-re face of the carbonyl group. The hydroxylation at C-7 in otonecine occurs with retention of configuration.

Retronecine was shown to be an efficient precursor for otonecine biosynthesis using ^{3}H-labelling experiments.

The plant *Adenocarpus decorticans* was shown, by spectroscopic and comparative studies, to contain the quinolizidine alkaloid, sparteine (B).



(A)



(B)

(b) Synthesis of Macrocyclic Pyrrolizidine Diesters

A number of optically active 10- and 11-membered dilactones containing (-)-platynecine were prepared by lactonisation via the pyridine-2-thiolesters with different anhydrides. These are the first synthetic macrocyclic pyrrolizidine diester analogues which contain (-)-platynecine.

(c) Synthetic Analogue Studies

The diamines cis and (+)-trans-1,2-Bis(aminomethyl)-cyclopropane were made from acyclic precursors. Along with cis- and trans-buten-2-ene-1,4-diamines, these will be tested for anti-fungal and enzyme inhibitory activity.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Pyrrolizidine Alkaloids.

The group of naturally occurring compounds, known as the pyrrolizidine alkaloids, have long been the subject of great interest. Their wide distribution among a number of unrelated plant families (e.g. Boraginaceae, Compositae and Leguminosae) is one notable feature.[1,2] More important is the remarkable range of biological activities that these natural products exhibit, including cytotoxicity and carcinogenicity.[3] Consumption of these alkaloids by both humans and animals has become a major health problem. For instance, an outbreak of veno-occlusive disease in Afghanistan was attributed to the presence of seeds from *Heliotropium popovii* (fam. Boraginaceae) in wheat flour.[4] Ironically, several herbal remedies and teas contain toxic pyrrolizidine alkaloids. Extracts of both coltsfoot (*Tussilago farfara* fam. Compositae) and comfrey (*Symphytum officinale* fam. Boraginaceae) are carcinogenic.[5] The toxicity of the alkaloids will be discussed later.

The pyrrolizidine alkaloids are found mainly in plants, although some species of butterflies and moths store the alkaloids for defensive purposes or for pheromone production, after feeding on the plant leaves.[6]

Almost all of the alkaloids are derivatives of 1-methyl pyrrolizidine (1) rather than pyrrolizidine (2) itself. Hydroxylated derivatives of base (2) form the amino-alcohol components of the alkaloids known as necines. The base can also be unsaturated, typically at the 1,2-positions. In fact, the most common pyrrolizidine

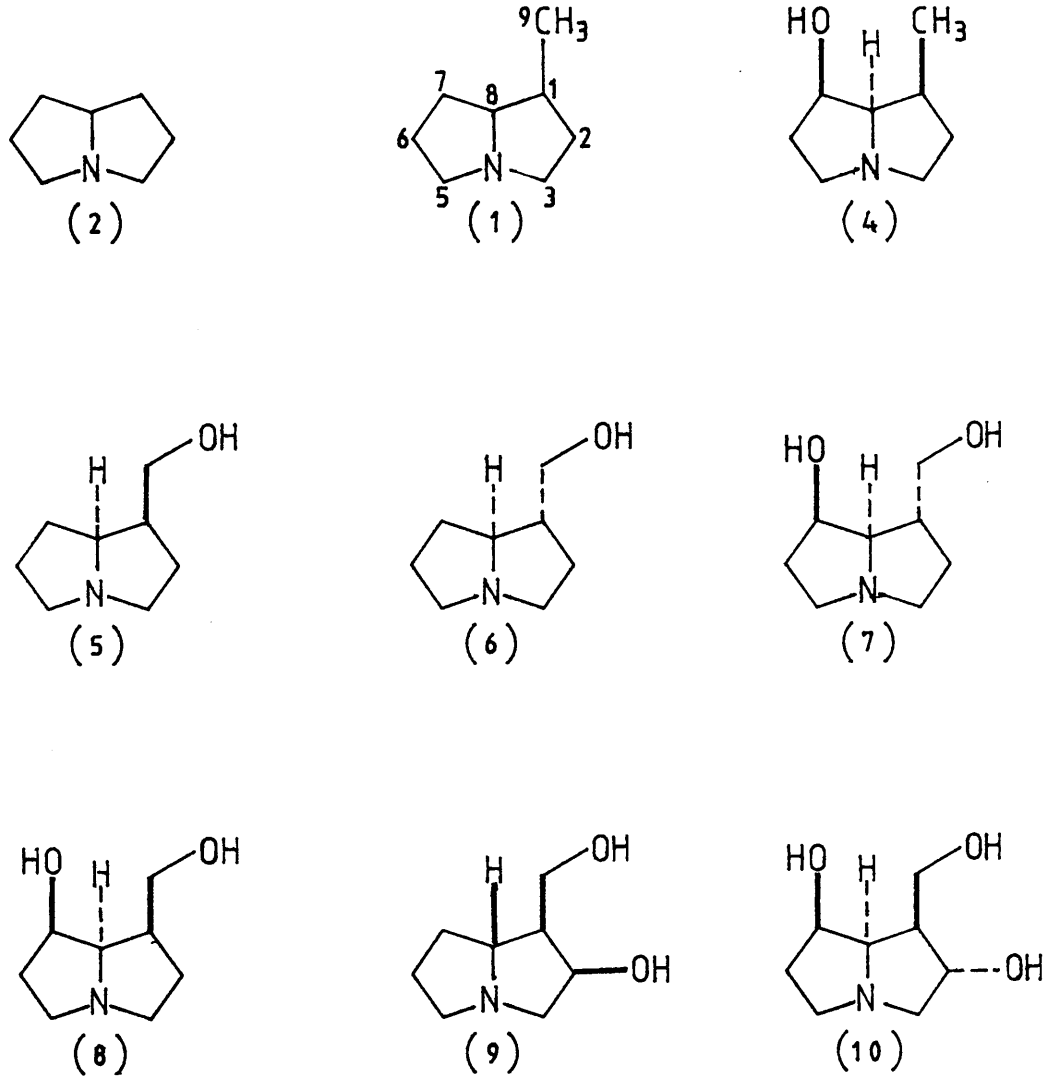


Figure 1. Some saturated pyrrolizidine bases.

base is the unsaturated diol, retronecine (3). A number of necine structures are shown in Figures 1 and 2.

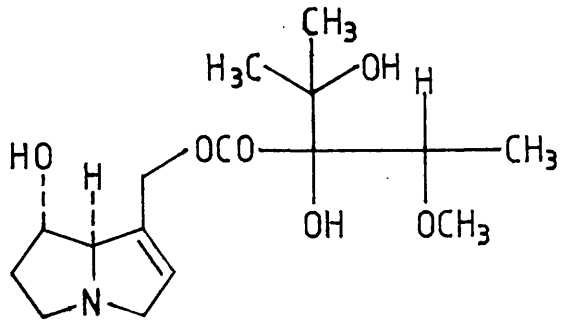
The alkaloids themselves are mostly found as esters or diesters of the necine bases. The esterifying acids are known as the necic acids. For instance, these may be monoesters such as europine (15), diesters such as echimidine (16), or macrocyclic diesters such as retrorsine (17). Necines are rarely isolated as free alcohols from plants. A group of substituted amino pyrrolizidines has also been isolated, loline (18) being a typical example.

More information on the structure, sources and pharmacology of pyrrolizidine alkaloids can be found in the books by Bull et al.[7] and by Mattocks.[3]

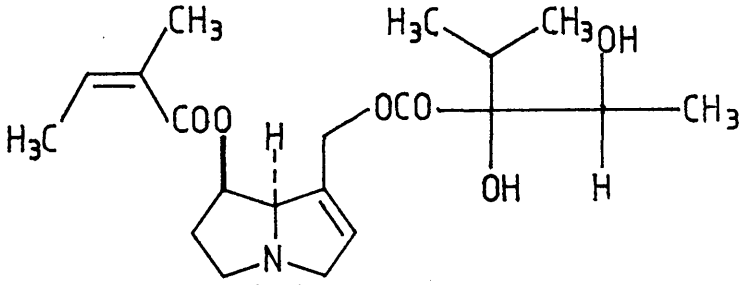
1.2 Metabolism and Toxicity

The metabolism of pyrrolizidine alkaloids has been shown to be closely linked to their toxicity towards animal cells. The evidence supports the theory that it is not the alkaloids themselves which are toxic, but their metabolites. For example, the alkaloids do not cause local damage, either to the skin or elsewhere. Almost invariably the liver sustains the most damage after contact with a toxic pyrrolizidine alkaloid. Cell damage on this scale must be due to some highly reactive species and the pyrrolizidine alkaloids are certainly not chemically reactive.

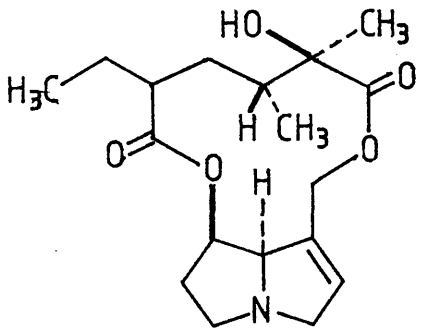
Culvenor noticed in 1962 that the effects of pyrrolizidine alkaloids on cell nuclei bore some resemblance to the effects of biological alkylating agents.[8] It was Mattocks who later reported the most convincing evidence that toxicity was due to pyrrolic derivatives



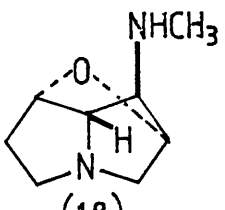
(15)



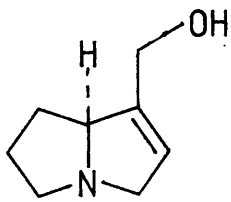
(16)



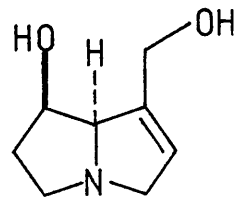
(17)



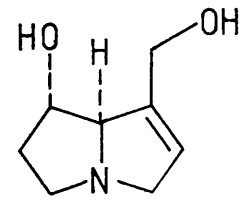
(18)



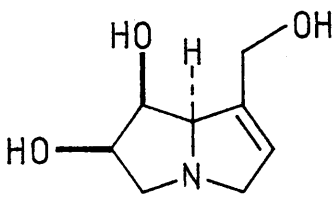
(11)



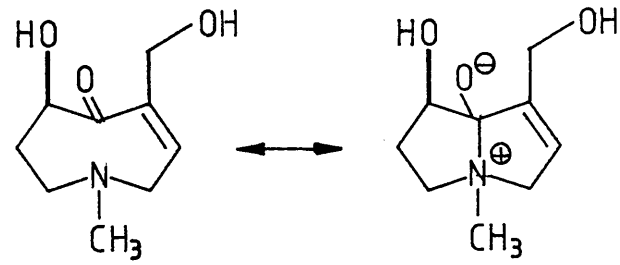
(3)



(12)



(13)

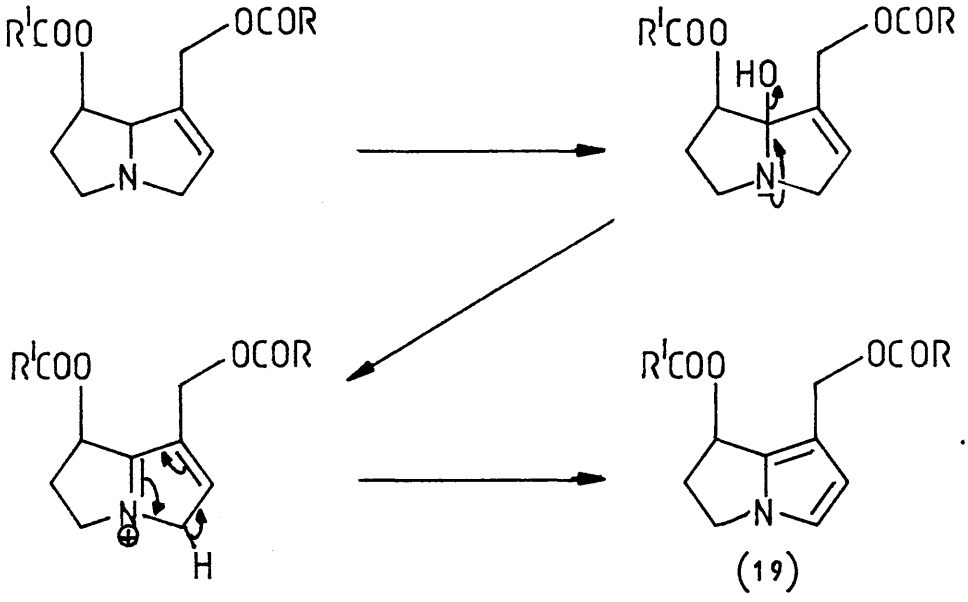


(14)

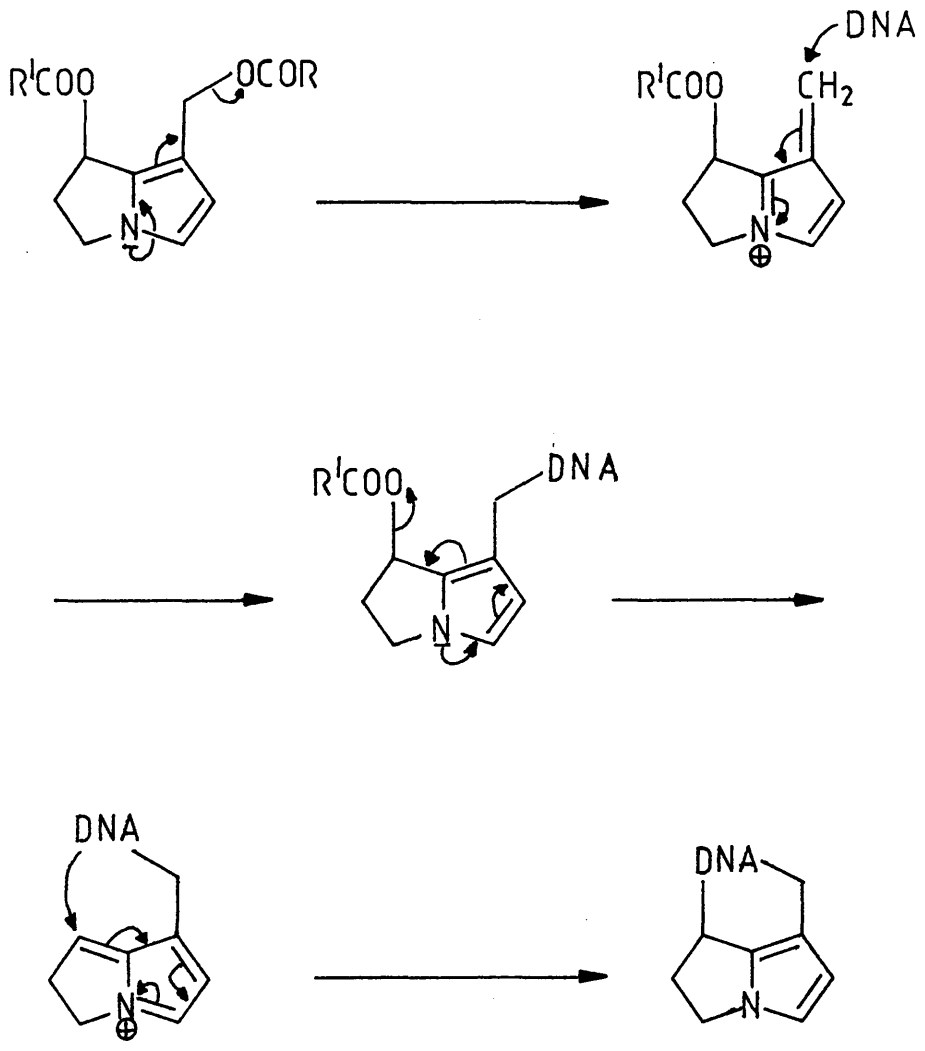
Figure 2. Some unsaturated necines.

of the alkaloids.[9] Scheme 1 shows a possible metabolic route to tne pyrrole (19). Mattocks found evidence for these pyrrolic materials in the liver of rats fed upon pyrrolizidine alkaloids. He also noted their presence in large quantities in the lungs.

These pyrroles, such as (19), can then act as bifunctional alkylating agents. A process has been postulated whereby a DNA molecule can become covalently bound to a pyrrolizidine alkaloid metabolite (Scheme 2).



Scheme 1.



Scheme 2.

For exactly the above reasons, the saturated pyrrolizidine alkaloids are largely non-toxic. They cannot easily be dehydrogenated to pyrrolic materials and, hence, cannot undergo a process such as shown in Scheme 2.

A very important property of certain pyrrolizidine alkaloids is their anti-tumour activity. The best known example is indicine N-oxide

(20) which has undergone clinical trials with favourable results.[10] In addition, this alkaloid shows less of the usual pyrrolizidine alkaloid toxicity.

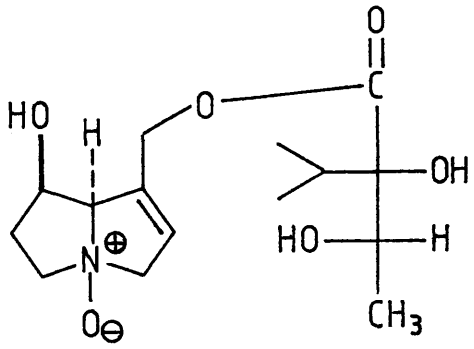
It was noted by Schoental and Mattocks that the nature of the alkaloid acid portion also had an effect on toxicity.[11] It was later shown that bulky carbon or hydroxy substituents at the α-position of the acid portions create steric hindrance around the ester groups[12] and that this inhibits esterase activity.[13] This, in turn, increases toxicity.[13] Such observations explain why macrocyclic diesters are generally much more toxic than simple diesters.

A result of the comprehensive studies carried out on the alkaloids and their analogues is that a general structure activity relationship has begun to appear. It seems that for a pyrrolizidine alkaloid to be toxic, it must contain:

1. An unsaturated ring.
2. At least one hydroxyl attached to the unsaturated ring via one carbon atom.
3. At least one of the hydroxyls should be esterified.
4. For increased toxicity, the acid portion should have a branched chain.

This is shown graphically in Figure 3.

Models such as this continue to be extended and adapted by further synthetic and biological studies.



(20)

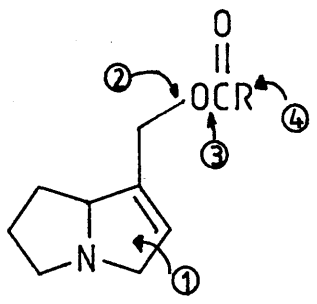


Figure 3.

1.3 Aims of Project

The methods by which pyrrolizidine alkaloids are biosynthesised by plant systems is a subject of great interest and importance. It is known that the base portions are derived from the amino acid ornithine (21)[1], but there is a lack of detailed work on most of the necines. In fact, only retronecine biosynthesis has been studied in any great detail. A review of the pyrrolizidine alkaloid biosynthetic area is given in Chapter 2.

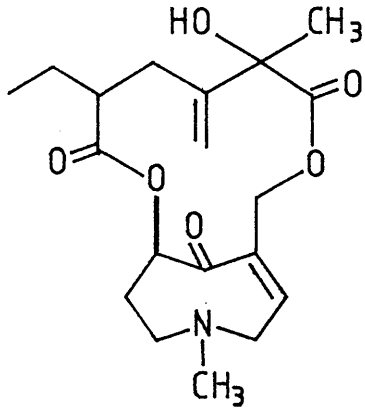
To extend the available knowledge, it was decided to make the base otonecine (14) the subject of a more detailed biosynthetic study. This diol (14) is the base portion of the alkaloid emiline (22), and presumably derives from ornithine (21), *via* putrescine (23). The results of the investigation are given in Chapter 5.

There is, in contrast, a great deal of work available on the chemical synthesis of pyrrolizidine bases (Chapter 3). For this work diols (24) and (25) were chosen. The reasons for their selection and the outcome of the effort is given in Chapter 6.

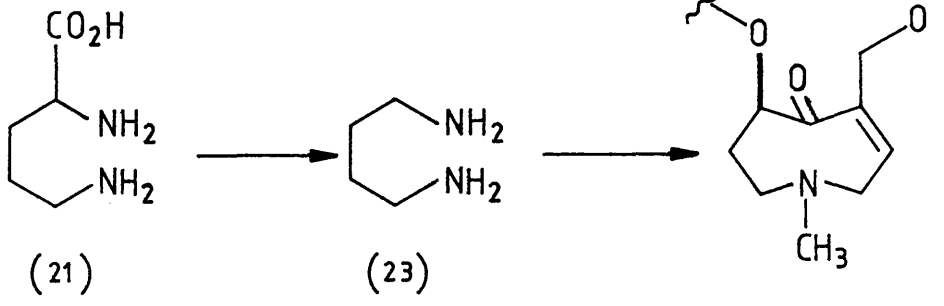
The synthetic problems in constructing the macrocyclic diesters of pyrrolizidine bases have received little attention. This area is reviewed in Chapter 4. Such a position is surprising given the toxicity and other biological activities of the natural macrocyclic compounds. To help to remedy this, a set of new macrocyclic esters of the diol platynecine (8) was produced. This is discussed in Chapter 8.

Finally, analogues of pyrrolizidine alkaloid precursors are a useful tool in the investigation of biological pathways and may be

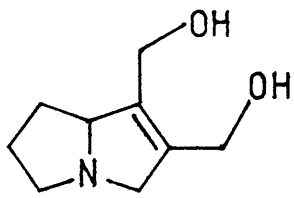
useful as biologically active molecules in their own right. The synthesis of some such compounds is described in Chapter 7.



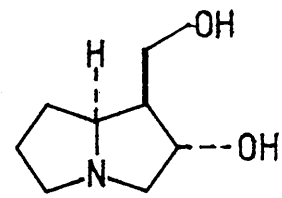
(22)



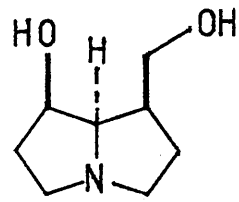
(21) (23)



(24)



(25)



(8)

CHAPTER 2

THE BIOSYNTHESIS OF PYRROLIZIDINE ALKALOIDS.

2.1 The Biosynthesis of Pyrrolizidine Bases.

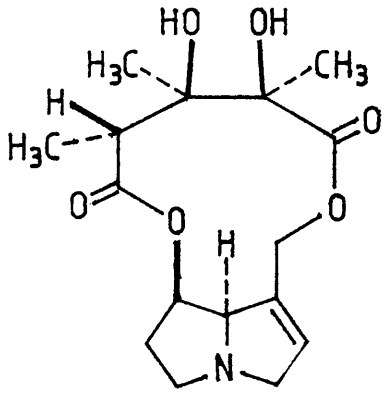
It was Sir Robert Robinson who first suggested that the pyrrolizidine ring is derived from the condensation of two ornithine (20) residues.[14] This was later supported by the work of Nowacki and Byerrum, who fed [2-^{14}C] ornithine, [1-^{14}C] acetate and [1-^{14}C] propionate to *Crotalaria spectabilis* which produces monocrotaline (26).[15] As with all of the early labelling experiments, the alkaloid was isolated and the location of the radioisotope was established after hydrolysis. They found that the ornithine gave rise to label mainly in the retronecine moiety, whereas the other two precursors resulted in a labelled necic acid portion.

Bottomley and Geissman, in 1963 reported the results that they obtained by feeding [1,4-^{14}C] putrescine (27), [2-^{14}C]ornithine and [5-^{14}C]ornithine to *Senecio douglasii* .[16] The alkaloids in this plant are all esters of retronecine (3). After feeding, the plants were extracted and the alkaloid mixture produced was hydrolysed to give ^{14}C-labelled retronecine. Table 1 shows the amounts of radiolabel present in the retronecine after each experiment.

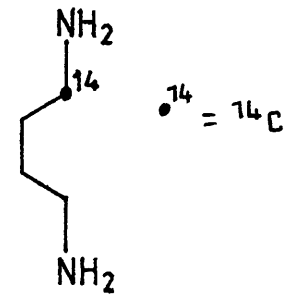
Table 1 : Incorporation of Radioactive Precursors into the Alkaloids of Senecio douglasii.

Precursor	Total incorporation into alkaloid. (%)	Percentage of total activity found in: Acids.	Retronecine.
[1,4-^{14}C]-putrescine	0.18	5.0	98
[2-^{14}C]-ornithine	0.30	1.4	94
[5-^{14}C]-ornithine	0.75	2.4	94

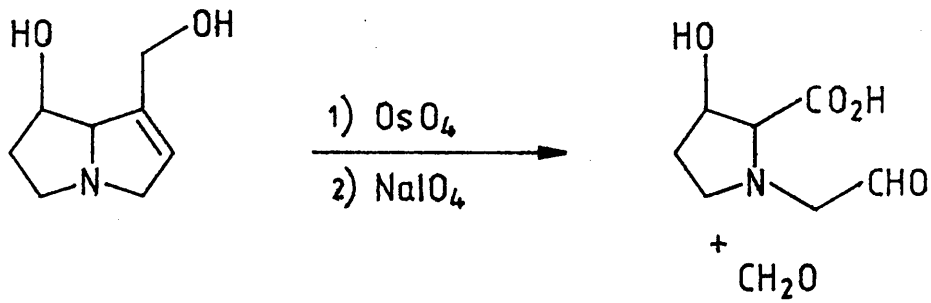
Note: Total incorporation = $\left(\frac{\text{Total radioactivity in alkaloid}}{\text{Total radioactivity in precursor}} \times 100\right)\%$



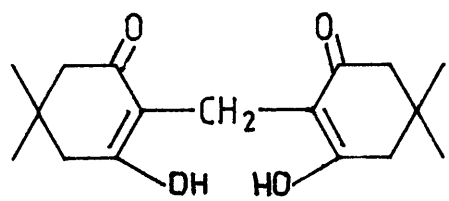
(26)



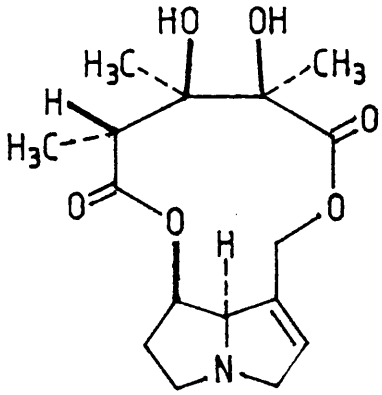
(27)



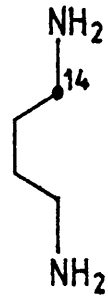
Dimedone



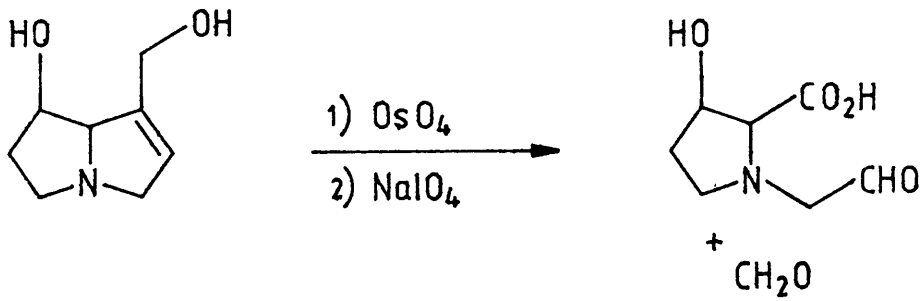
Scheme 3

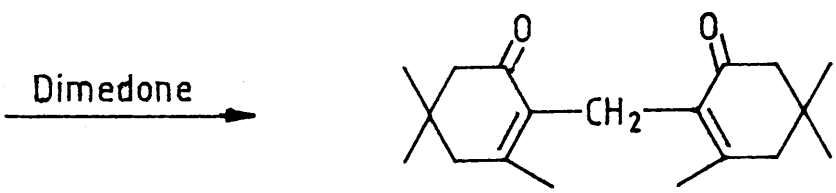


(26)



(27)

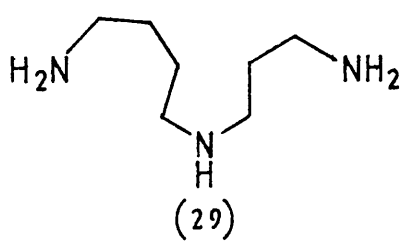




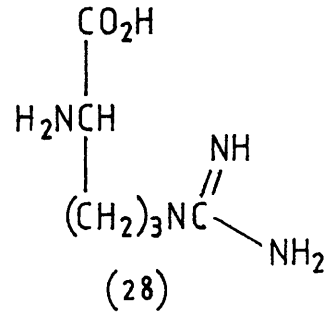
Scheme 3

This confirms the results of Nowacki and Byerrum[15], that ornithine is incorporated into retronecine. In order to identify the labelled positions, Bottomley and Geissman degraded their retronecine samples using osmium tetroxide, followed by sodium periodate, Scheme 3. This isolated the $-CH_2OH$ group of retronecine as formaldehyde which was trapped in its dimedone derivative. The activity found in each of the formaldehyde dimedone samples was found to be a quarter of that of the entire retronecine sample. That is to say that the C-2 and C-5 of at least one of the ornithine (21) units become equivalent.

Work reported by Robins and Sweeney in 1979 showed that in addition to ornithine (21) and putrescine (23), the compounds arginine (28), spermidine (29) and spermine (30) are good precursors for retronecine (3) in *S. isatideus*.[17] This plant produces the retronecine derived alkaloid retrorsine(17). The results of these experiments are shown in Table 2. Clearly putrescine (23) is a better precursor for retronecine than ornithine (21), supporting the theory of Geissman and Crout[18] that putrescine comes after ornithine in retronecine biosynthesis. The degradative sequence for retronecine (4) was extended by Robins and Sweeney using a modified

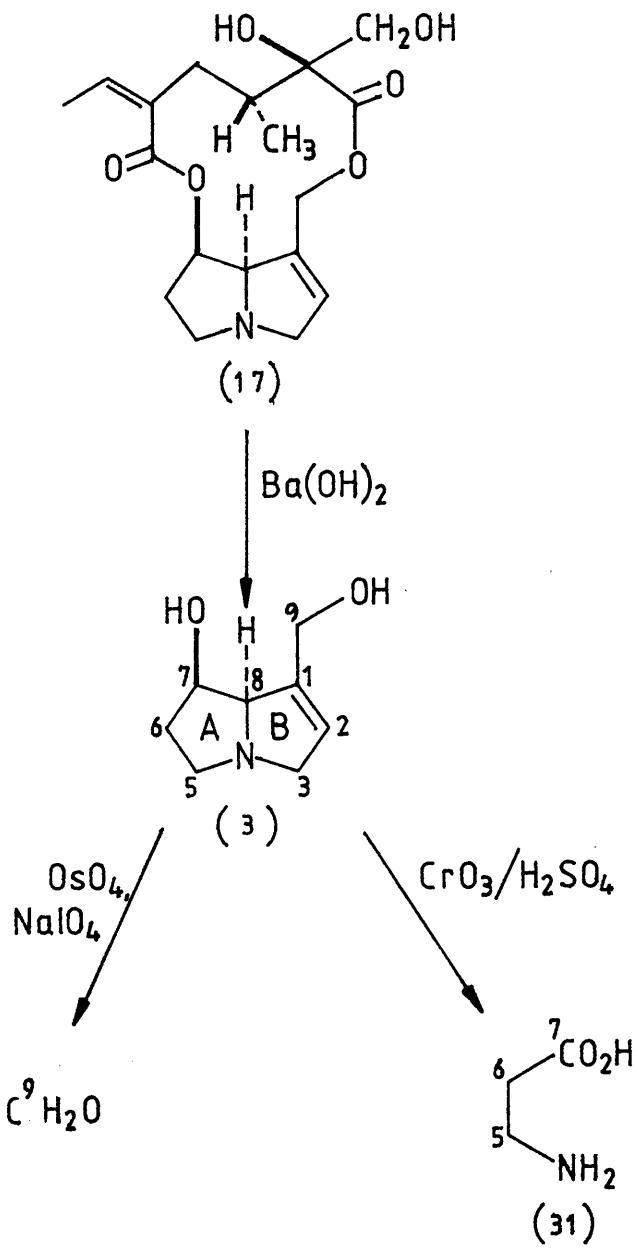


(29)



(28)

(30) $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$


HO
CH2OH
O
H
CH3
O
O
O
H
N
(17)
Ba(OH)2
HO
H
9
OH
7
8
1
6
A
B
2
N
5
3
(3)
OsO4,
NaIO4
CrO3/H2SO4
C9H2O
7
CO2H
6
5
NH2
(31)

Scheme 4.

Kuhn-Roth oxidation.[19] This procedure gave β-alanine (31) (isolated as its 2,4-dinitrophenyl derivative) containing carbon atoms C-5, C-6 and C-7 from retronecine. Degradation of each

Table 2 : Incorporation of Precursors into Retrorsine (17) in S. isatideus.

Expt.	Precursor	Total % Incorporation	% Radioactivity in Acid	Base
1	L-[U-^{14}C]arginine	0.46	6	99
2	DL-[2-^{14}C]ornithine	0.25	5	97
3	[1,4-^{14}C]putrescine	1.6	1	94
4	[1,4-^{14}C-tetramethylene] spermine	5.2	1	103
5	[1,4-^{14}C-tetramethylene] spermidine	2.0	1	95

of the retronecine samples in experiments 1-5 gave β-alanine containing between 22 and 25% of the total ^{14}C base activity. In particular, the result of experiment 2 showed that the C-2 and C-5 positions of ornithine (21) become equivalent in the formation of the A ring of retronecine (3). It was suggested that a C_4-N-C_4 symmetrical intermediate could be formed from two molecules of putrescine (23).

The main difficulty at this point was the absence of other methods for identifying the positions of labelled atoms within the base portion of the alkaloids. This problem was largely solved with the use of ^{13}C nuclear magnetic resonance (n.m.r) spectroscopy and the fact that large enough incorporations of labelled precursors

could be obtained in certain plant species.

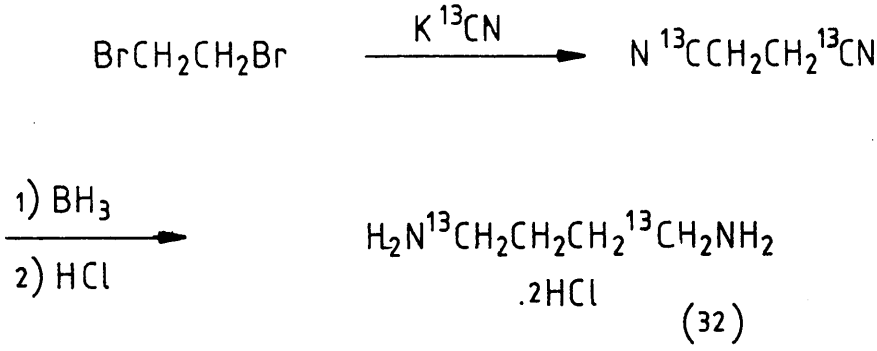
The first major report on the incorporation of ^{13}C-labelled precursors came from Khan and Robins in 1981. They fed two precursors, [1,4-$^{13}C_2$]putrescine (32) and [2,3-$^{13}C_2$]putrescine (33) to *S. isatideus* and examined the retronecine derived from them by ^{13}C n.m.r. spectroscopy. Scheme 5 shows how [1,4-$^{13}C_2$]putrescine (32) was made. Reaction of 1,2-dibromoethane with potassium [^{13}C]cyanide gave [1,4-$^{13}C_2$]succinonitrile. Reduction followed by acidification gave [1,4-$^{13}C_2$]putrescine (32) dihydrochloride.

Isolation of retrorsine (17) from *S. isatideus* plants fed with precursor (32), followed by hydrolysis gave a sample of ^{13}C-labelled retronecine. The proton decoupled ^{13}C n.m.r spectrum [^{13}C-{^{1}H} n.m.r spectrum] showed enriched signals for C-3, C-5, C-8 and C-9 of retronecine (Scheme 6).

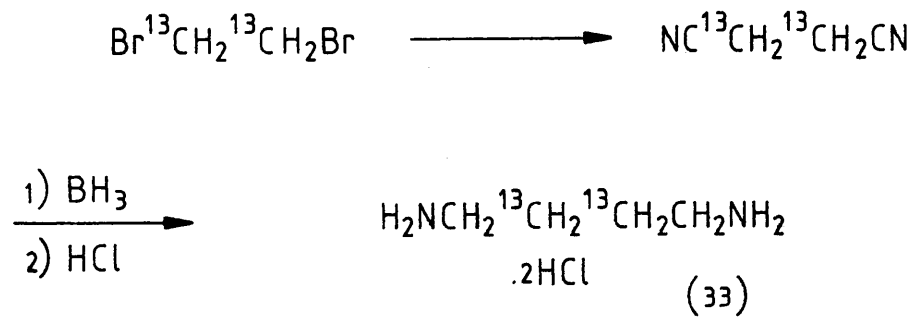
The precursor [2,3-$^{13}C_2$]putrescine (33) was made in an analagous fashion to precursor (32), using [1,2-$^{13}C_2$]-1,2-dibromo ethane (Scheme 5b). Retronecine was extracted from *S. isatideus* fed with this labelled material (33). The ^{13}C-{^{1}H} n.m.r spectrum showed a pair of doublets (J 71 Hz) for C-1 and C-2 and a pair of doublets (J 34 Hz) for C-6 and C-7 of retronecine (Scheme 6).

In both experiments, equal enrichment was observed for the four labelled sites. These labelling patterns are consistent with the formation of a later C_4-N-C_4 intermediate in retronecine biosynthesis.

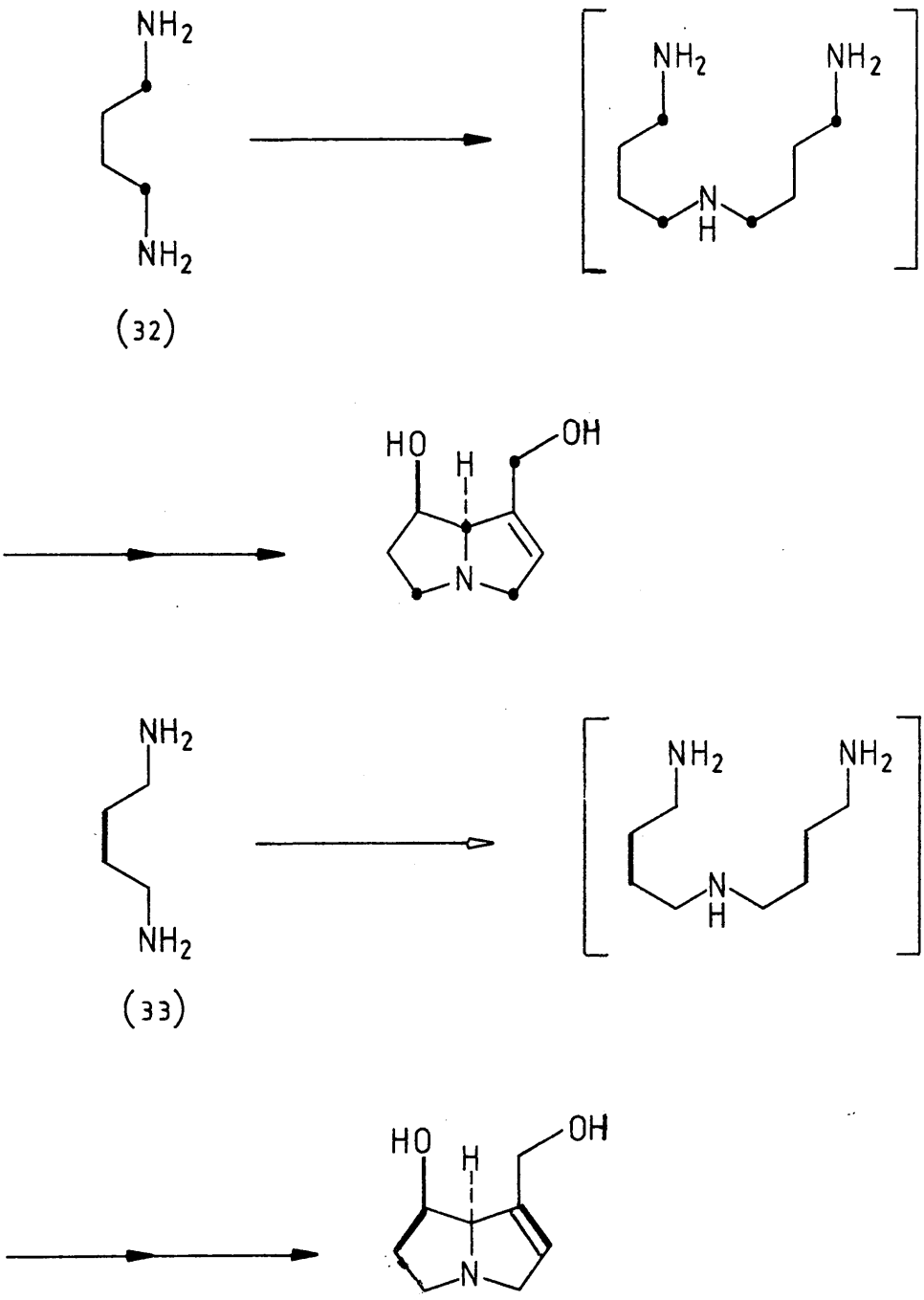
In another report, Robins made and fed [1,2-$^{13}C_2$]putrescine (34) to *S. isatideus*.[20] Treatment of the sodium derivative of



Scheme 5a



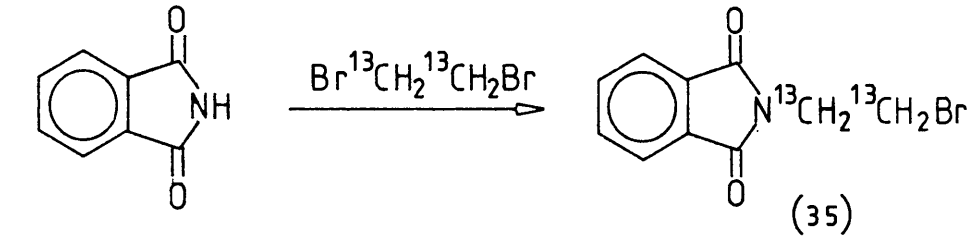
Scheme 5b


NH2
NH2
NH2
NH2
N
H
(32)
HO
H
OH
N
NH2
NH2
NH2
NH2
N
H
(33)
HO
H
OH
N
• – 13C single label
— – 13C—13C double label

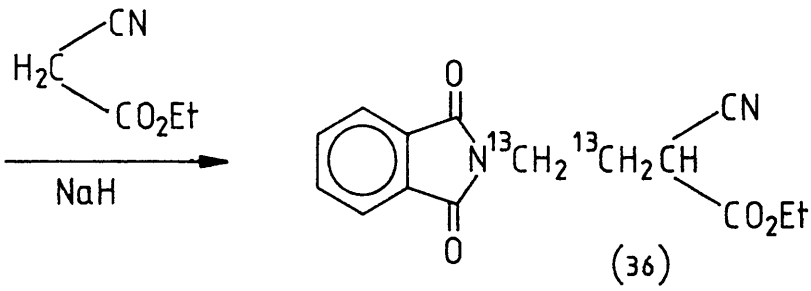
Scheme 6

ethylcyanoacetate with [1,2-$^{13}C_2$]-1-bromo-2-phthalimido ethane (35) gave the adduct (36). Removal of the ethoxycarbonyl group using NaCl in DMSO/water, followed by hydrogenation and acidic hydrolysis gave the ^{13}C-labelled putrescine (34) (Scheme 7). The ^{13}C-{^{1}H} n.m.r spectrum of the retronecine derived from this experiment showed eight doublets with four pairs of coupling constants with the distinctive labelling pattern shown in Scheme 7. (The natural abundance singlet signals were also present). This reinforced the idea of a later symmetrical intermediate, because each of the doublets had equal intensity.

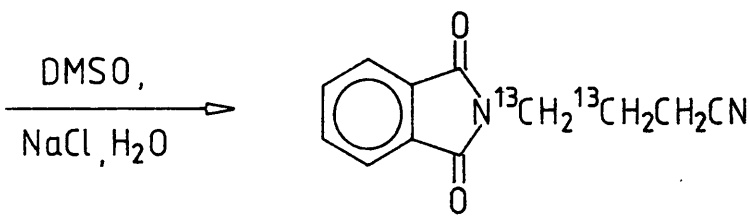
The key experiment in proving the involvement of the symmetrical later intermediate came in independent reports from Grue-Sørensen and Spenser[21] and Khan and Robins.[22] The first group made [1-amino-^{15}N, 1-^{13}C]putrescine (37) and administered it to *Senecio vulgaris*, a plant which produces several retronecine (3) based alkaloids. The precursor was synthesised by treatment of N-(3-bromopropyl)phthalimide (38) with potassium [^{13}C,^{15}N] cyanide, with subsequent Raney nickel reduction and acidic hydrolysis (Scheme 8). After extraction of the alkaloids and hydrolysis to obtain the retronecine base portion, the positions of the labels were examined using the ^{13}C-{^{1}H} n.m.r spectrum. There were four equally enriched signals due to ^{13}C labels at C-3, C-5, C-8 and C-9. However, the presence of coupling within the signals was the important feature. From the difference spectrum (that is, the spectrum minus the natural abundance spectrum) it was clear that each of the signals due to C-3 and C-5 consisted of a doublet superimposed on a singlet. The doublets, both of equal intensity, were due to ^{13}C-^{15}N species while the singlets were due

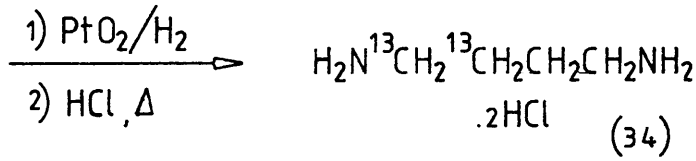


(35)

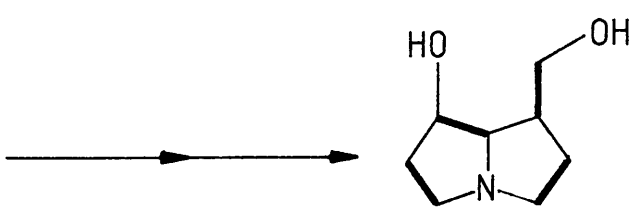


(36)

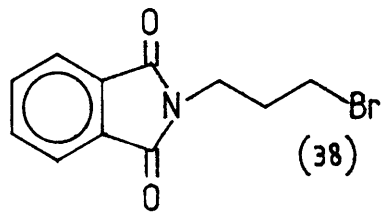




(34)

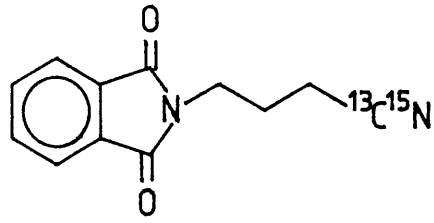


Scheme 7



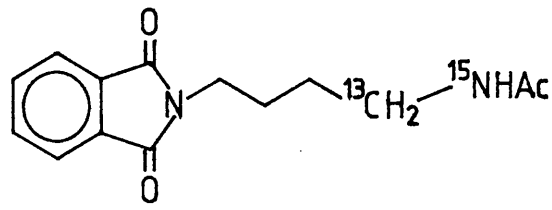
(38)

$K^{13}C^{15}N$



Raney Ni

H_2, Ac_2O

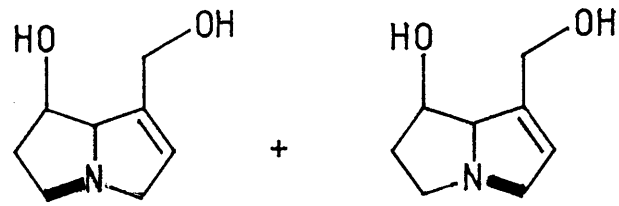


$H^{\oplus}$, Δ

$H_2NCH_2CH_2CH_2{}^{13}CH_2{}^{15}NH_2$

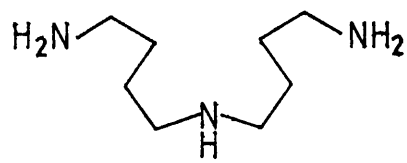
$.2HCl$

(37)



(39) + (40)

Scheme 8

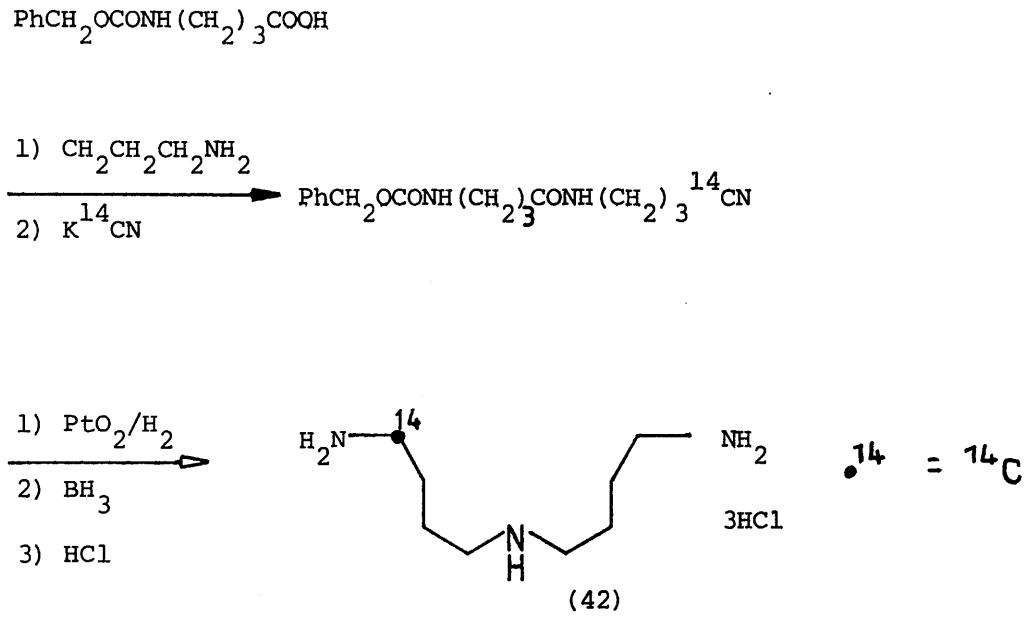


(41)

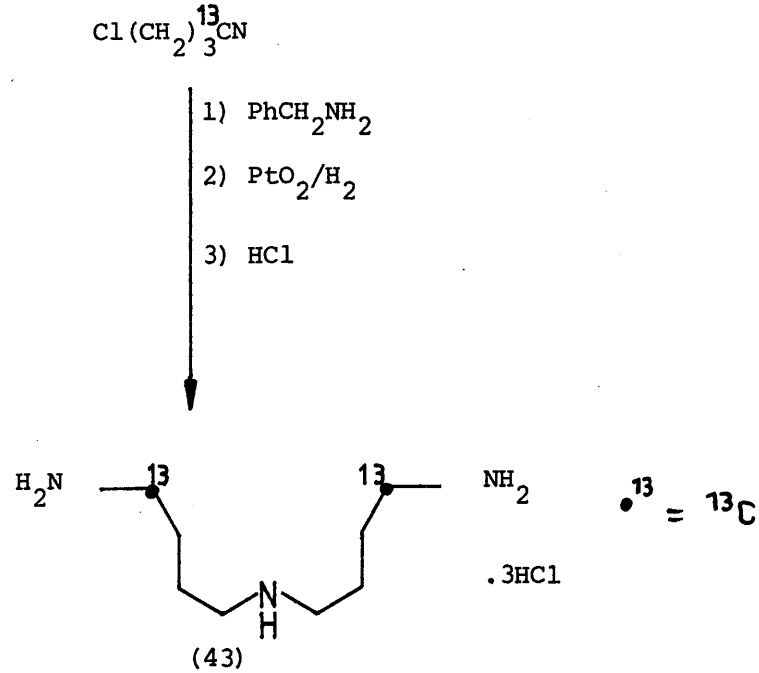
to ^{13}C-^{14}N species. The formation of equal amounts of the two labelled species (39) and (40) is strong support for a later C_4-N-C_4 intermediate.

Similar results were obtained by Khan and Robins, but they went a step further and identified this later intermediate as N-(4-aminobutyl)-1,4-diaminobutane (41) (homospermidine).[22] This triamine is a known plant constituent[23] but its involvement, although speculated, had yet to be proven. These workers did this using a ^{14}C-labelled homospermidine in a feeding experiment. They made the precursor by first condensing the N-benzyloxycarbonyl derivative of 4-aminobutanoic acid with 3-bromopropylamine. Treatment of the amide with potassium [^{14}C]cyanide followed by hydrogenation and reduction gave [1,9-^{14}C]homospermidine (42) as its trihydrochloride (Scheme 9). When this was fed to *S. isatideus*, the retronecine extracted was ^{14}C labelled. Treatment of the sample with OsO_4-$NaIO_4$ gave formaldehyde (trapped as its dimedone derivative) containing 44% of the retronecine activity. Modified Kuhn-Roth oxidation gave β-alanine (31) (isolated as its 2,4-dinitrophenyl derivative) with 2% of the retronecine activity. These results imply intact incorporation of the homospermidine (41) into retronecine (3). An intermediate trapping experiment confirmed that homospermidine (41) is probably a normal intermediate in retronecine (3) biosynthesis.

The complete labelling pattern for retronecine (3) was reported by Rana and Robins, who made and fed [1,9-$^{13}C_2$]homospermidine (43) to *S. isatideus*.[24] The precursor (43) was prepared by the reaction of two equivalents of 4-chloro[1-^{13}C]butanenitrile with benzylamine followed by catalytic reduction (Scheme 10). After



Scheme 9.



Scheme 10.

feeding the plants were extracted giving retrorsine (17) which was then hydrolysed to yield a sample of ^{13}C-labelled retronecine. The important feature of the ^{13}C-{^{1}H} n.m.r spectrum was the presence of two doublet signals (J 6 Hz) surrounding the natural abundance singlet signals. This observation was due to a geminal ^{13}C-^{13}C coupling between the C-8 and C-9 nuclei. This was further evidence to support the intact incorporation of homospermidine (41) into retronecine.

Kelly and Robins recently reported the use of ^{13}C-labelled precursors in the elucidation of the biosynthetic pathway to rosmarinine (44), produced by *Senecio pleistocephalus*.[25] They fed [1-^{13}C]putrescine (45) and [2,3-$^{13}C_2$]putrescine (33) to *S. pleistocephalus* and examined the labelling patterns of the derived rosmarinine (44) by ^{13}C n.m.r spectroscopy. Precursor (45) was made by the reaction of N-(3-bromopropyl)phthalimide (38) with sodium [^{13}C]cyanide, followed by hydrogenation and acidic hydrolysis. The result of feeding precursor (45) was the observation of equal enrichments of the signals in the ^{13}C-{^{1}H} n.m.r spectrum for C-3, C-5, C-8 and C-9. Feeding the doubly labelled precursor (33) produced a similar result to that for retronecine (3). The ^{13}C-{^{1}H} n.m.r spectrum for the derived rosmarinine showed two equally enriched pairs of doublets corresponding to C-1 and C-2 (J 35.2 Hz) and C-6 and C-7 (J 35.5 Hz).

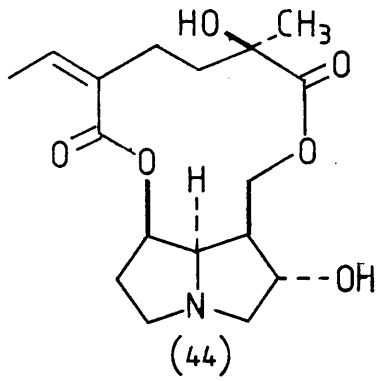
These workers also fed the doubly labelled [1-amino-^{15}N,1-^{13}C] putrescine (37). After extraction of the derived rosmarinine, the ^{13}C-{^{1}H} n.m.r spectrum showed two equally intense doublets with different coupling constants around the C-3 and C-5 enriched signals.

Such an observation is consistent with the presence of the symmetrical intermediate (homospermidine (41)) in the biosynthetic pathway. To confirm this, they went on to feed [1,9-$^{13}C_2$] homospermidine (43). The labelled rosmarinine produced showed enriched ^{13}C-{^{1}H} n.m.r spectral signals only for the C-8 and C-9 positions. In this case the geminal coupling constant is zero. It seems clear that the biosynthesis of rosmarinine (44) also takes place from two molecules of putrescine (23) via homospermidine (41).

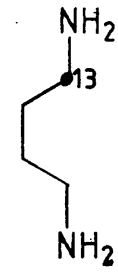
The next major question was how could homospermidine (41) be converted into pyrrolizidine alkaloids. It was known that diamine oxidase enzymes could oxidise amino groups, such as those in triamine (41). It was possible that the dialdehyde (46) so formed could cyclise to the iminium species (47) and then cyclise again to give the stereoisomeric 1-formylpyrrolizidines (48) (Scheme 11). Biological reduction of these aldehydes (48) could yield 1-hydroxymethyl pyrrolizidines (49), which are known compounds.

The first evidence in support of this came from Robins in 1982.[26] He successfully converted homospermidine (41) into (±)-trachelanthamidine (6) using diamine oxidase and alcohol dehydrogenase enzymes under physiological conditions. Also, Rana and Leete reported the incorporation of (±)-[3,5-^{14}C]trachelanthamidine (50) and (±)-[5-^{3}H]isoretronecanol (51) into riddelline (52) in *Senecio riddelli*.[27] They noted that (±)-trachelanthamidine (6) was a better precursor for the retronecine (3) portion of riddelline (52) than (±)-isoretronecanol (5).

In similar experiments, Kunec and Robins fed (±)-[5-^{3}H] trachelanthamidine (50) and (±)-[5-^{3}H] isoretronecanol (51) to

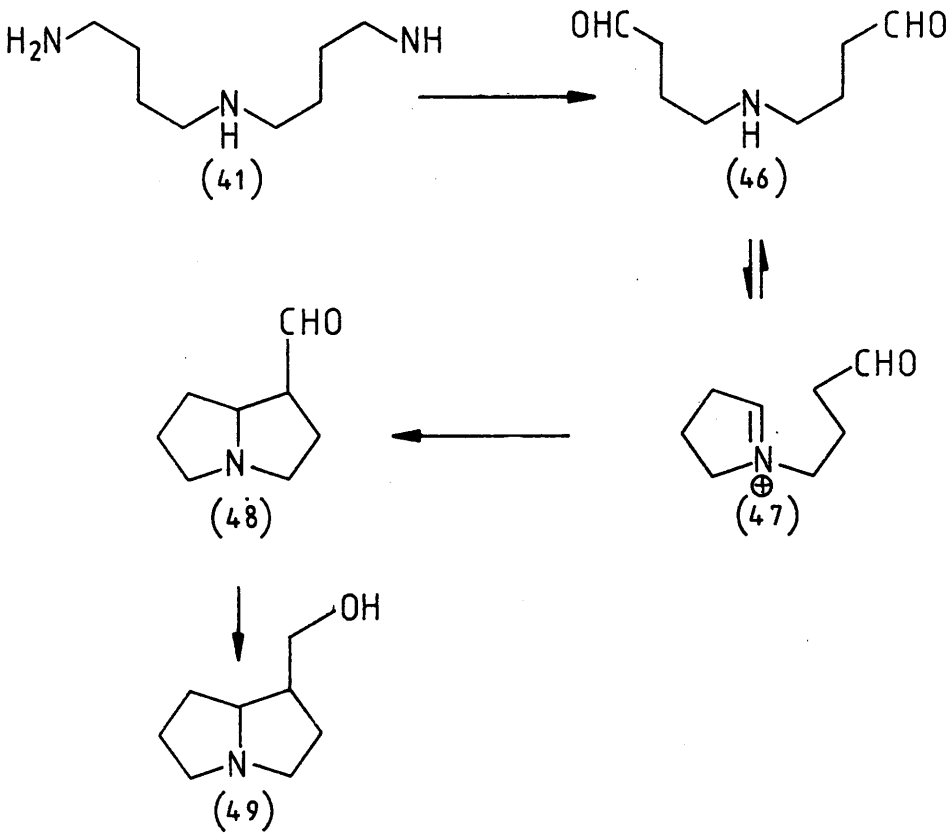


(44)



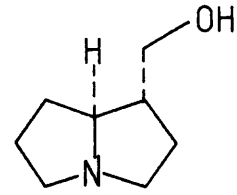
$^{13}\bullet = {}^{13}C$

(45)

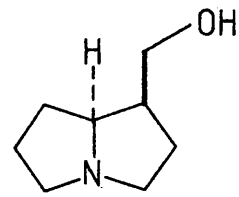


(41) (46) (47) (48) (49)

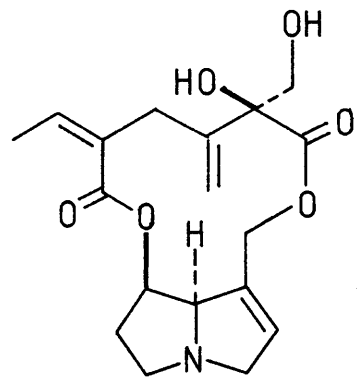
Scheme 11



(±)-(6)



(±)-(5)



(52)

S. isatideus and *S. pleistocephalus*.[28] These two precursors were made by the method of Pizzorno and Albonico[29] (see Chapter 3 for further details), starting from N-formyl-[5-^{3}H]-L-proline. It was shown that trachelanthamidine is a much better precursor for retrorsine biosynthesis, by some 20 times, than isoretronecanol. Conversely, isoretronecanol is incorporated into rosmarinine more than 34 times more efficiently than is trachelanthamidine.

The last results indicate that trachelanthamidine is a precursor for retronecine (3) while isoretronecanol is a precursor for rosmarinine (44).

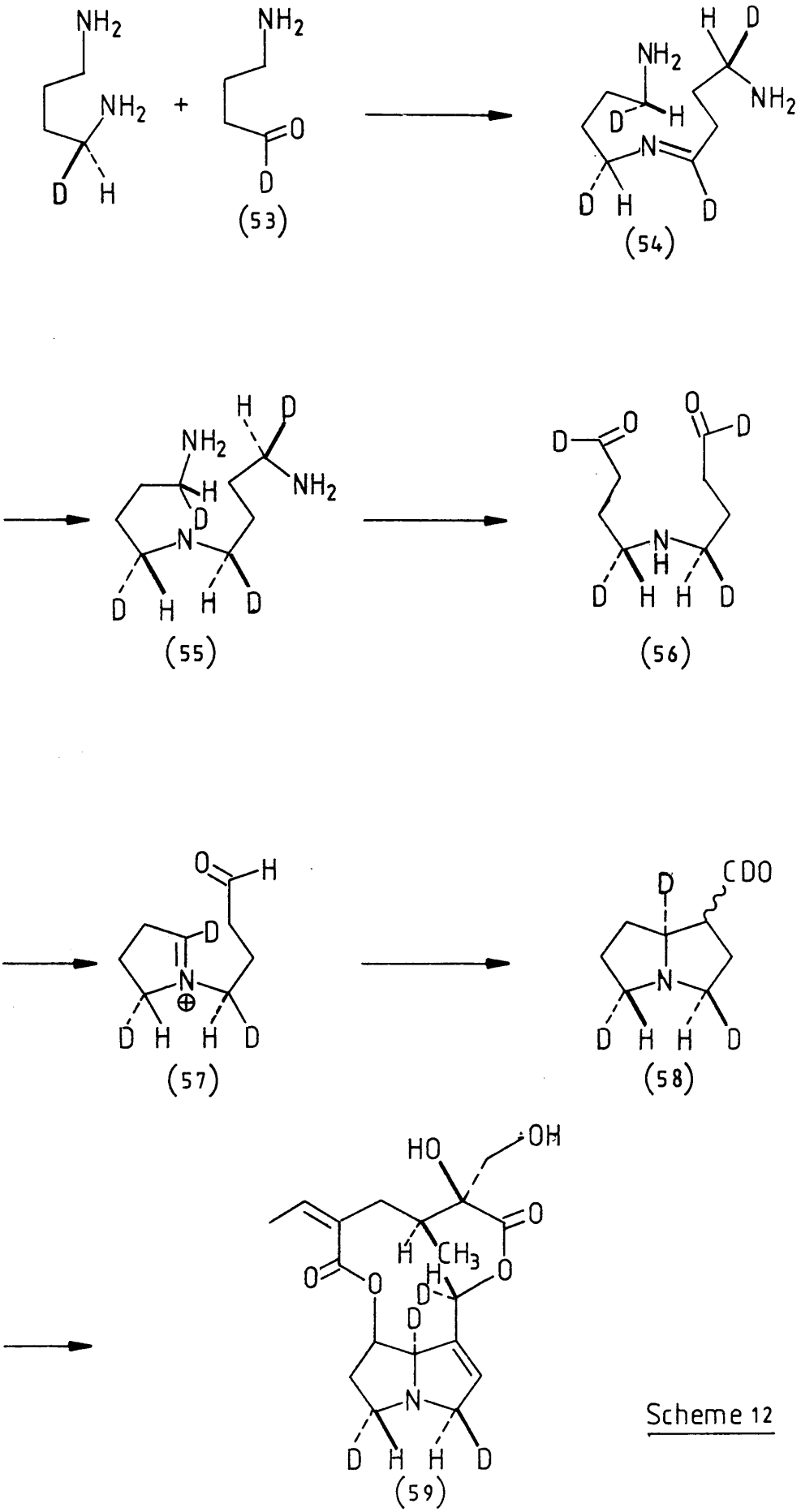
In all of the previous reports, no mention was made of the absolute stereochemistry of the processes involved. It was not until Robins and Sweeney showed that retronecine (3) in *S. isatideus* is derived from L-ornithine only, that this changed.[30]

The best way to obtain the stereochemical details of the biosynthetic pathways was to feed enantiomerically deuteriated precursors and to examine the labelling patterns by ^{2}H n.m.r spectroscopy. Rana and Robins[31] made the (1R)-[1-^{2}H]- and (1S)-[1-^{2}H]-putrescines, according to the method of Richards and Spenser[32], and fed them to *S. isatideus*. In the (1R)-[1-^{2}H] putrescine experiment the ^{2}H-{^{1}H} n.m.r spectrum of the derived retrorsine showed signals corresponding to deuterium at C-3β, C-5α, C-8α and C-9 proS as in (59). This suggested the following speculated mechanism. The initial oxidation of putrescine (23) to 4-aminobutanal (53) by the enzyme diamine oxidase is known to proceed via the stereospecific removal of the pro-S hydrogen from

the methylene group of the primary amine.[33] Coupling of the aldehyde (53) with a molecule of (1R)-[1-^{2}H]putrescine gives the imine (54) which is stereospecifically reduced by hydride attack on the si-face to give the labelled homospermidine (55). If the reduction was carried out from the opposite face then the derived retrorsine would have ^{2}H labels at C-3α, C-3β, C-5α and C-5β, which is not observed. Two more oxidations removing the pro-S hydrogens give the dialdehyde (56) which cyclises to imine (57). This in turn cyclises by attack on the re-face of the iminium ion giving only the 8α-pyrrolizidine aldehyde (58). Stereospecific reduction from the re-face of the carbonyl group leads to the labelling pattern shown in (59) (Scheme 12).

The (1S)-[1-^{2}H]putrescine experiment confirms this explanation. The retrorsine extracted had signals in the ^{2}H-{^{1}H} n.m.r spectrum corresponding to deuterium at the C-3α and C-5β positions. Similar results were obtained by Grue-Sorensen and Spenser.[32a]

An extension to the knowledge on the stereochemistry of the biosynthesis was reported recently by Kunec and Robins.[34] They made the (2R)-[2-^{2}H]- (60) and (2S)-[2-^{2}H]-putrescines (61) and fed them to *S. isatideus*. The ^{2}H-{^{1}H} n.m.r spectrum of retrorsine (62) derived from the (2R)-[2-^{2}H]-putrescine feed showed peaks corresponding to deuterium at C-2 and C-6α. The spectrum for the (2S)-[2-^{2}H]-putrescine feed showed deuterium at the C-6β and C-7α positions in retrorsine (63). There are two important features here (Scheme 13). Firstly, the hydroxylation at C-7 to form retronecine (3) occurs with retention of configuration, since there was no loss of ^{2}H when (2S)-[2-^{2}H] putrescine was fed. This observation also discounts

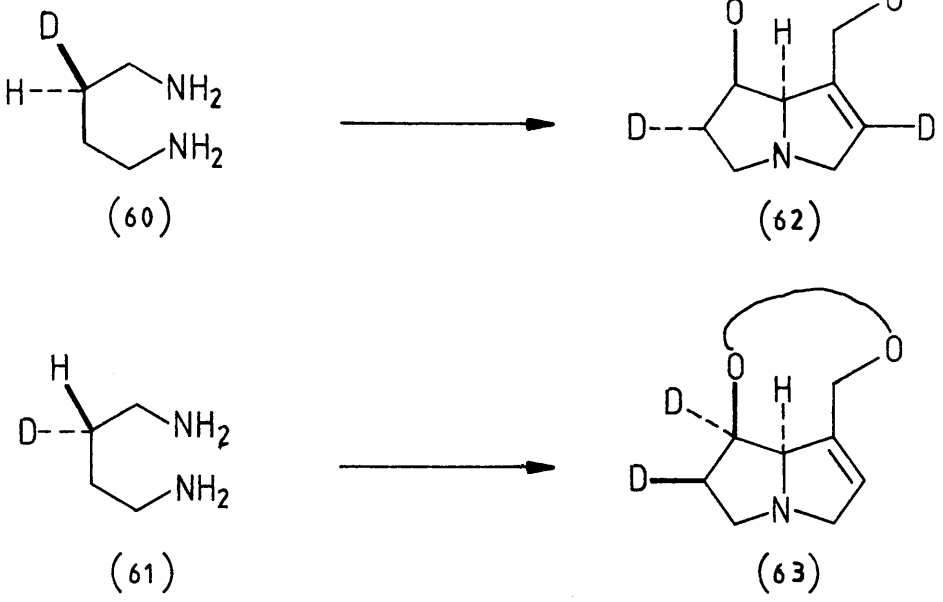


Scheme 12

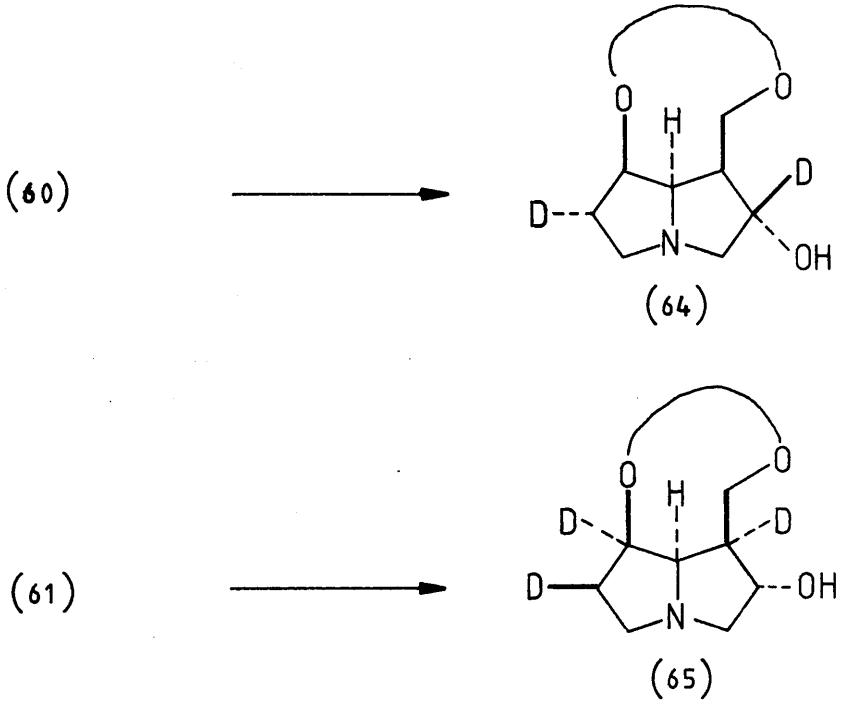
the presence of a keto or enol intermediate in this step. Secondly, the formation of the 1,2-double bond involves removal of the pro-S hydrogen, of the carbon which later becomes C-2 of retronecine and retention of the pro-R hydrogen.

In a related paper, Kelly and Robins fed the (1R)-[1-^{2}H]-,(1S)-[1-^{2}H]-,(2R)-[2-^{2}H]-,and (2S)-[2-^{2}H]-putrescines to *S. pleistocephalus*.[35] Feeding (1R)-[1-^{2}H]-putrescine gave rosmarinine which showed ^{2}H labelling at C-3β, C-5α, C-8α, and C-9 pro-S. Feeding the enantiomeric putrescine gave labelling only at C-5β and C-3α. These patterns of isotopic labelling are analogous to those observed for retrorsine (59), and can be explained in the same way. Administration of the (2R)-[2-^{2}H]-putrescine (60) gave the isotopically labelled rosmarinine (64) with ^{2}H atoms at C-2β and C-6α. The enantiomeric putrescine (61) gave rosmarinine (65) labelled at C-6β, C-1α and C-7α (Scheme 14). This observation allows a further extension of the stereochemical details. The pyrrolizidine aldehyde (66) is formed from the iminium species (67) by loss of the pro-R hydrogen and retention of the pro-S hydrogen. The ionic species (68) (or an equivalent) then cyclises to form the aldehyde (66) (Scheme 15).

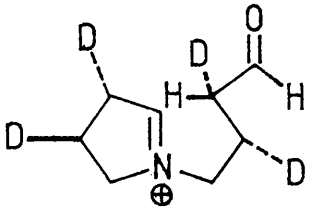
The presence of an iminium species of some sort had often been suggested but it was not until the report of Kelly and Robins that evidence of its intermediacy was presented.[36] These workers postulated that the N-(4-aminobutyl)-1,2-didehydropyrrolidinium ion (69) was an intermediate. They made a ^{14}C-labelled version (70) of the ion by the route shown. Reaction of pyrrolidine with [1-^{14}C]-4-chlorobutanenitrile followed by hydrogenation and acidification gave the diamine salt (71). Oxidation with mercuric(II)



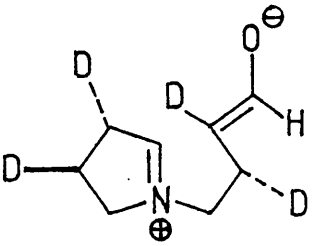
Scheme 13



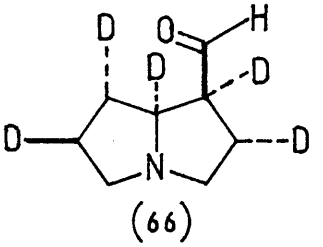
Scheme 14



(67)

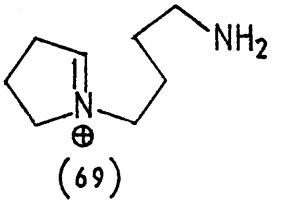


(68)



(66)

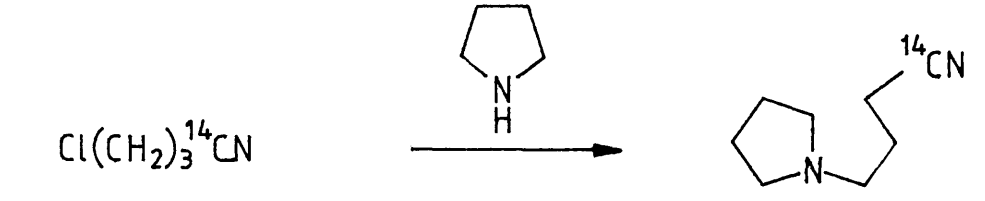
Scheme 15

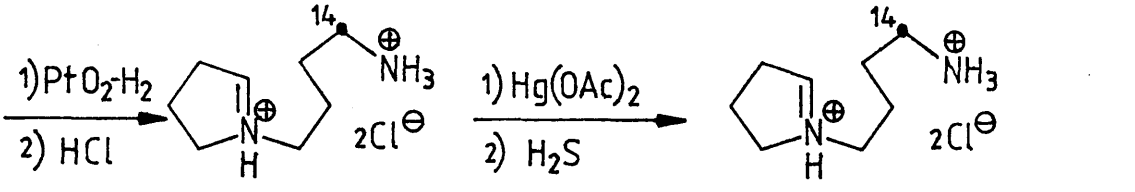


(69)

Note: No molecules of labelled putrescine contain more than one ^{2}H atom. The labelling patterns depicted in Schemes 12, 13, 14 and 15 are therefore composite representations of all the ^{2}H -labelled species that are present.

acetate gave the ^{14}C-labelled iminium ion (70). Feeding of this precursor along with ^{3}H-labelled putrescine (^{3}H : ^{14}C ratio 5:1) to *S. pleistocephalus* gave rosmarinine with a ^{3}H : ^{14}C ratio of 2:9. A similar result was observed when the mixture was given to *S. isatideus*. These results indicate that the iminium ion (69) is a better intermediate than putrescine (23) itself. An intermediate trapping experiment confirmed the presence of species (69) in the plant.





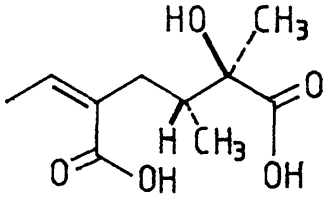
(71) (70) • ^{14} = ^{14}C

2.2 The Biosynthesis of Pyrrolizidine Necic Acids.

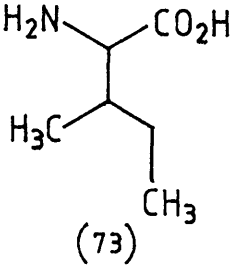
There is no work on the biosynthesis of necic acids contained in this volume, therefore, a detailed review of the literature is not presented. The following is a brief summary of the major features of the biosynthetic pathways to the necic acids.

In general, necic acids contain ten carbon atoms, are monobasic or dibasic acids, with various numbers of hydroxyl and unsaturated groups. There are also various stereoisomers known. A typical example is senecic acid (72). It was previously believed that these acids were derived from mevalonate, since they can be easily divided (on paper) into two isoprene units. However, it was later shown that acetate and mevalonate are not specific precursors for the necic acids.[37] It was later shown, by Crout and others that the acids originate from branched chain amino acids, particularly isoleucine (73).[37,38] Crout *et al.* have also published a proposed mechanism for the coupling reactions.[39]

Work on the necic acids was reviewed by Robins in 1982[1] and synthetic routes to them are reviewed regularly.[2]



(72)



(73)

2.3 The Use of Analogues in Biosynthetic Studies.

Analogues of known biosynthetic precursors can be useful in the elucidation of the pathways. The observation that an organism can successfully deal with one modified precursor and not another may be useful in establishing the mechanisms involved. Figure 4 shows a model biogenetic pathway A to B to C and so on to Z. Introducing a modified precursor A^{1} can have one of two effects. If the organism mistakenly recognises the precursor A^{1} as a normal metabolite then it can be transformed into a modified natural product Z^{1}. Of more interest is the situation where a different precursor A^{11} is only recognised by some of the enzymes on the pathway. This may cause a halt in the overall biosynthesis to Z^{11} and may result in the production of a new product D^{11}. Another possibility is that the modified precursor may completely block one or more steps, to the detriment of the whole system. The latter is the basis of one type of enzyme inhibitory action.

The most simple type of analogue is the isotopically labelled compound. The common examples incorporating ^{2}H, ^{3}H and ^{14}C generally have little or no effect on the biosynthesis and are, therefore, used to produce isotopically labelled natural products. The use of

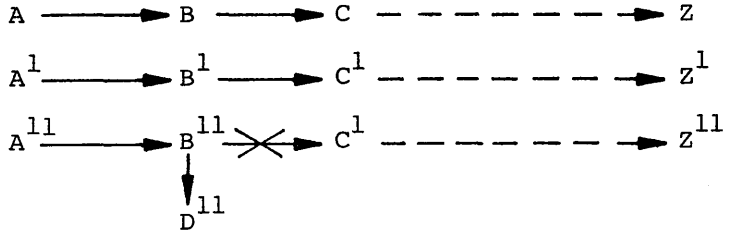


Figure 4.

^{19}F-labelled precursors has also become popular. However, precursors

containing ^{19}F labels tend to be toxic to the organisms to which they are administered, although some organisms can adapt to ingestion of ^{19}F-labelled materials without being destroyed.[40]

A more drastic change to the precursor may produce a more severe effect upon the living system. For instance, the substitution of a CH_3 group for an H or a double bond for a single bond. The effects of such materials in vivo can be examined to see if a pattern can be established relating the presence of a functional group to a particular biological action. Thus, a mechanism for the biological activity may be deduced.

2.4 Conclusions

The biosynthesis of pyrrolizidine alkaloids has been extensively studied particularly the biosynthesis of retronecine (3) containing alkaloids.

Chapter 5 describes the first investigations into the biosynthesis of the seco-pyrrolizidine base, otonecine (14).

In Chapter 7, the synthesis and biological activity of some analogues of 1,4-butanediamine (putrescine) (23) are discussed.

CHAPTER 3

SYNTHESIS OF NECINE BASES

3.1 Introduction.

There are many syntheses available for the pyrrolizidine bases, although most of these are for the simpler 1-hydroxymethyl pyrrolizidines or for retronecine (3). It is only recently that the synthetic problems of the more complex pyrrolizidine bases have been addressed. Producing the correct stereochemistry and functionalisation of such small molecules presents a particularly challenging task.

There are several synthetic reviews available[41] and new syntheses are discussed annually.[2] The following section is a selected review of syntheses which have direct relevance to the work undertaken or which are typical of the syntheses of necines.

3.2 A Selected Review

The synthetic routes fall into several broad categories depending on the structural features of the starting molecules. Each category generally takes the form of an initial observation by one group, followed by elaboration by other groups.

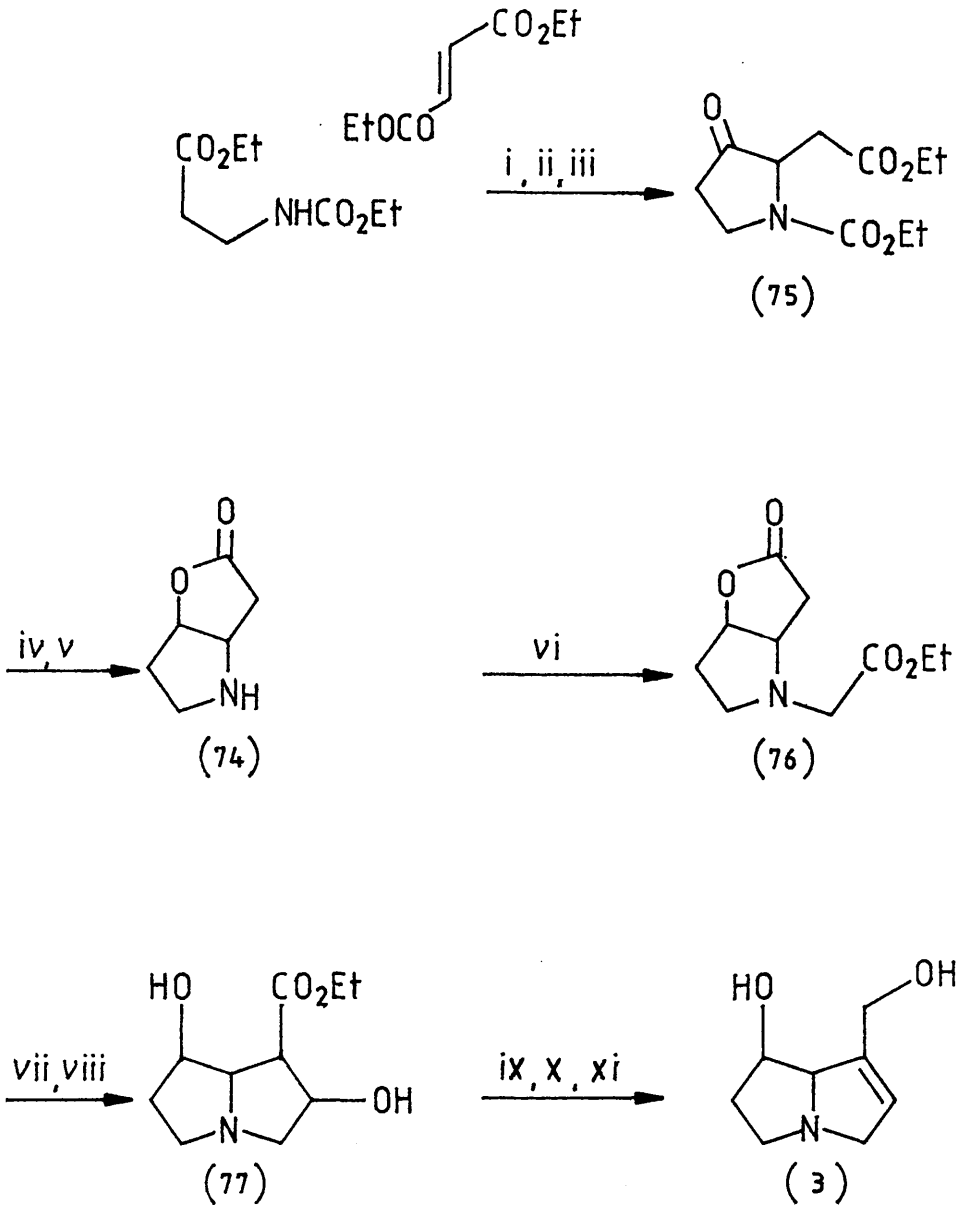
The first synthesis of the base (+)-retronecine (3) was reported by Geissman and Waiss in 1962.[42] This involved the production of the (±) lactone (74), which has since proved to be a key intermediate in many other syntheses. The route starts, as shown in Scheme 16, from ethyl 3-*N*-(carboxyethyl)propanoate and diethyl fumarate, to give the pyrrolidone (75),[43] which after reduction and hydrolysis of the carbamate gave the (±)-lactone (74). The second ring of the pyrrolizidine nucleus was formed by base induced cyclisation of the *N*-alkylated lactone (76) giving the diol-ester (77) which was converted

into (±)-retronecine (3) as shown. The enantiomers formed were separated via their d-camphorate salts, giving (+)-retronecine in an overall yield of around 5%.

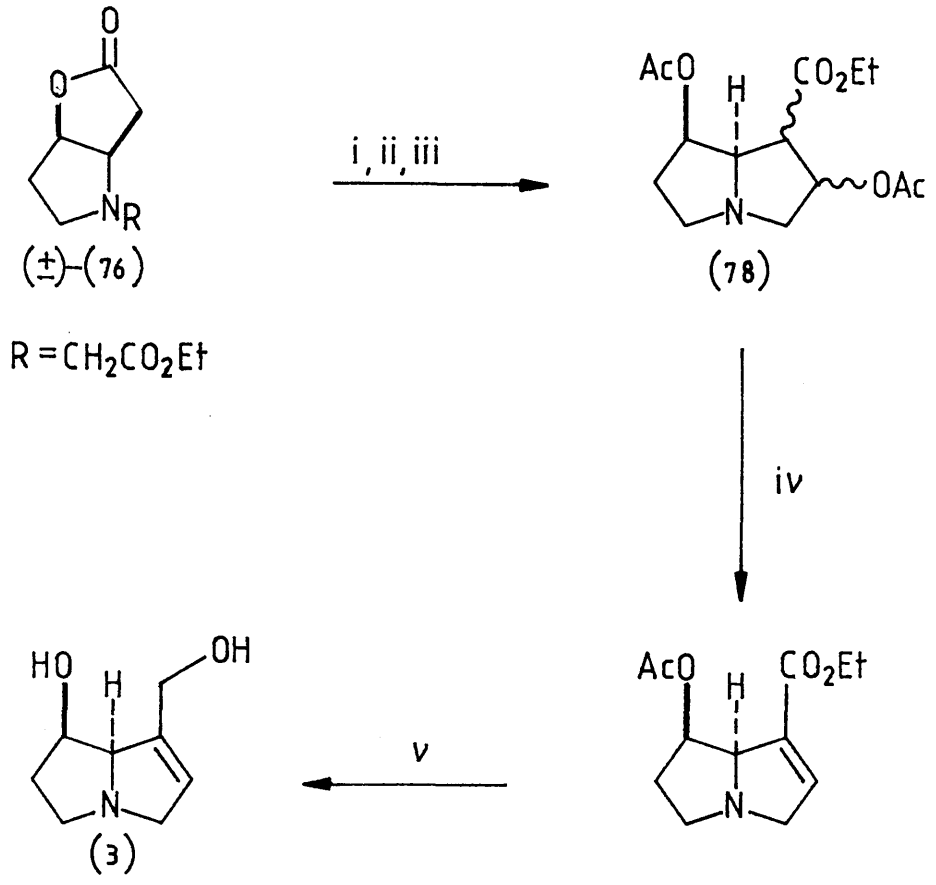
Narasaka *et al.* reported a significant improvement on this route in 1982.[44] Their modifications are shown in Scheme 17, and gave an overall yield of 15%. Starting from Geissmann's lactone (74), cyclisation was carried out on ester (76), followed by reduction and acylation to give the di-acylated ester (78) which after elimination of acetic acid and reduction gave (±)-retronecine (3).

The synthetic potential of the lactone (74) was obviously apparent. If it was possible to produce an optically active version then the way would be open to the total synthesis of optically active retronecine (3) and other pyrrolizidines. However, it was not until 1983 that this occurred. Rüeger and Benn reported the synthesis of the optically active lactone (74),[45] and, soon after, the first syntheses of (+)-retronecine (3), (-) platynecine (8), (+)-croalbinecine (79)[46] and (+)-crotanecine (13).[47] The method used, starting from N-benzyloxycarbonyl-(2S, 4R)-4-hydroxyproline (80), is fairly lengthy and no yield of (+)-lactone was quoted. The material obtained was reported, however, to have high optical purity.

The crucial steps in their synthesis of (+)-lactone (74) are the homologation of the acid and the transposition of the oxygen function from C-4 to C-3 of the hydroxyproline with inversion of stereochemistry. The acid (80) was converted into its homologue by a Wolff rearrangement on the related diazoketone (80a). The oxygen function at the 4-position was removed by pyrolysis of the xanthate ester giving mostly the 3-pyrroline derivative (81). After ester hydrolysis, the lactone (82)



Scheme 16: Reagents : (i) Na, (ii) HCl/H_2O, (iii) EtOH/HCl, (iv) Pt/H_2, (v) $Ba(OH)_2$ (vi) $BrCH_2CO_2Et$, (vii) KOEt, (viii) $H_2/Pt/AcOH$, (ix) $Ba(OH)_2$, (x) EtOH/HCl, (xi) $LiAlH_4$



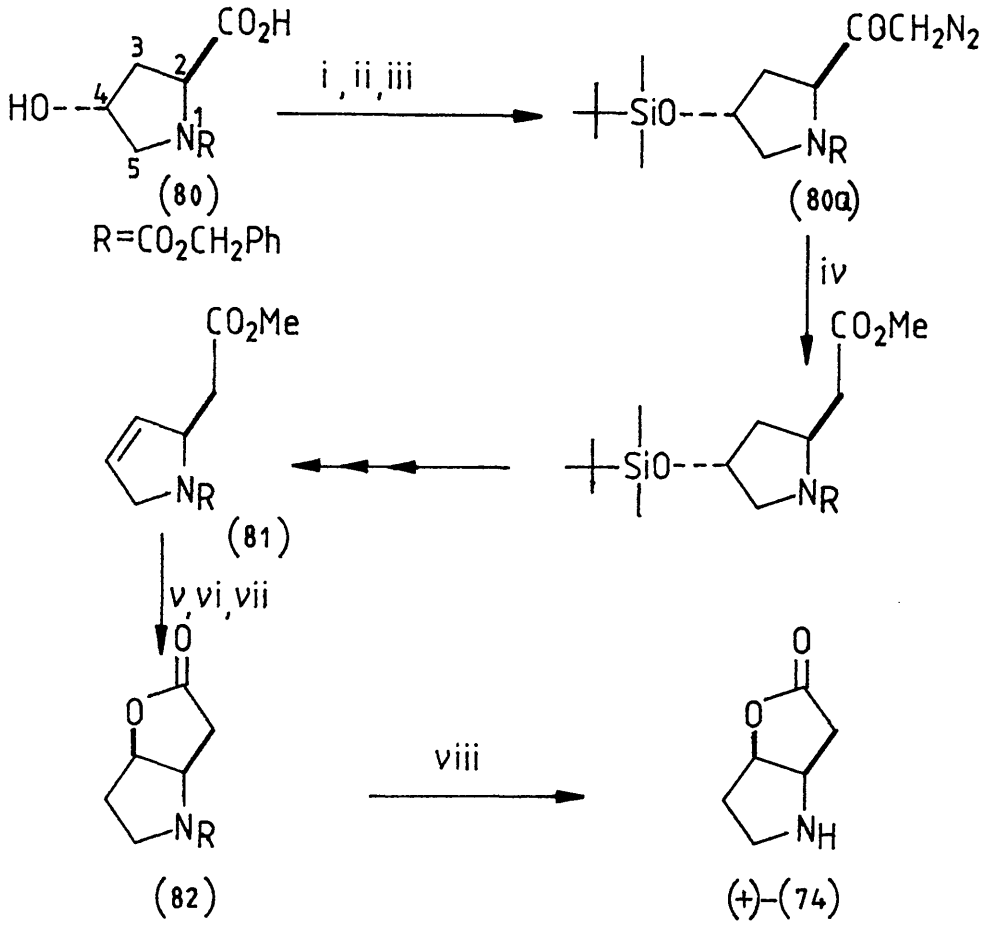
Scheme 17 ; Reagents : (i) NaOEt/EtOH, (ii) $NaBH_4$, (iii) Ac_2O/pyr/DMAP, (iv) $KOBu^t$, (v) Bu^i_2AlH

was formed via the phenylselenium species. The S-stereochemistry of the 2-position induced the 4R-stereochemistry at the new C-O bond. Hydrogenolysis of the carbamate gave the (+)-lactone (74) with >99% optical purity (Scheme 18).

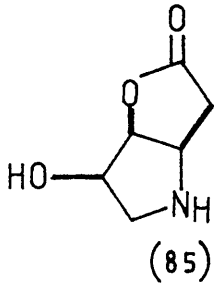
The conversion of the (+)-lactone (74) into optically active necines was carried out as shown in Scheme 19. (+)-Retronecine (3) was made by a similar method to that of Narasaka et al.[44], using slightly different reagents. (+)-Croalbinecine (79) and its C-1 epimer (83) were made by retaining the C-2 hydroxyl group of the intermediate (77). Simple reduction of ester (77) gave (+)-croalbinecine (79), whereas isomerisation, lactonisation, then reduction gave the C-1 epimer (83). (-)-Platynecine (8) arose from the complete reduction of the ketone in keto-ester (84). Lactonisation and reduction gave the optically pure diol (8).

The synthesis of (+)-crotanecine was carried out using the 4-hydroxylactone (85).[47] This was made by a similar route to that for lactone (74), involving a rather lengthy reaction sequence. The conversion into (+)-crotanecine (13) was carried out in an analogous fashion to (+)-retronecine (3).

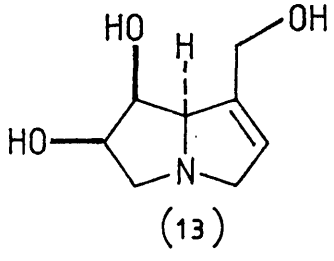
At around the same time, Buchanan et al., reported a more convenient synthesis of the optically active lactone (74).[48] As shown in Scheme 20, this route started from readily available 2,3-O-isopropylidene-D-erythrose (86) which was converted, via the oxime into the cyanomethanesulphonate (87). A Blaise reaction[49] using methyl bromoacetate gave a mixture of β-keto ester isomers (88a,b) then base induced cyclisation yielded a mixture of pyrrolidine derivatives (89a,b).[50] Reduction by $NaCNBH_3$ gave rise to only one isomer (90).



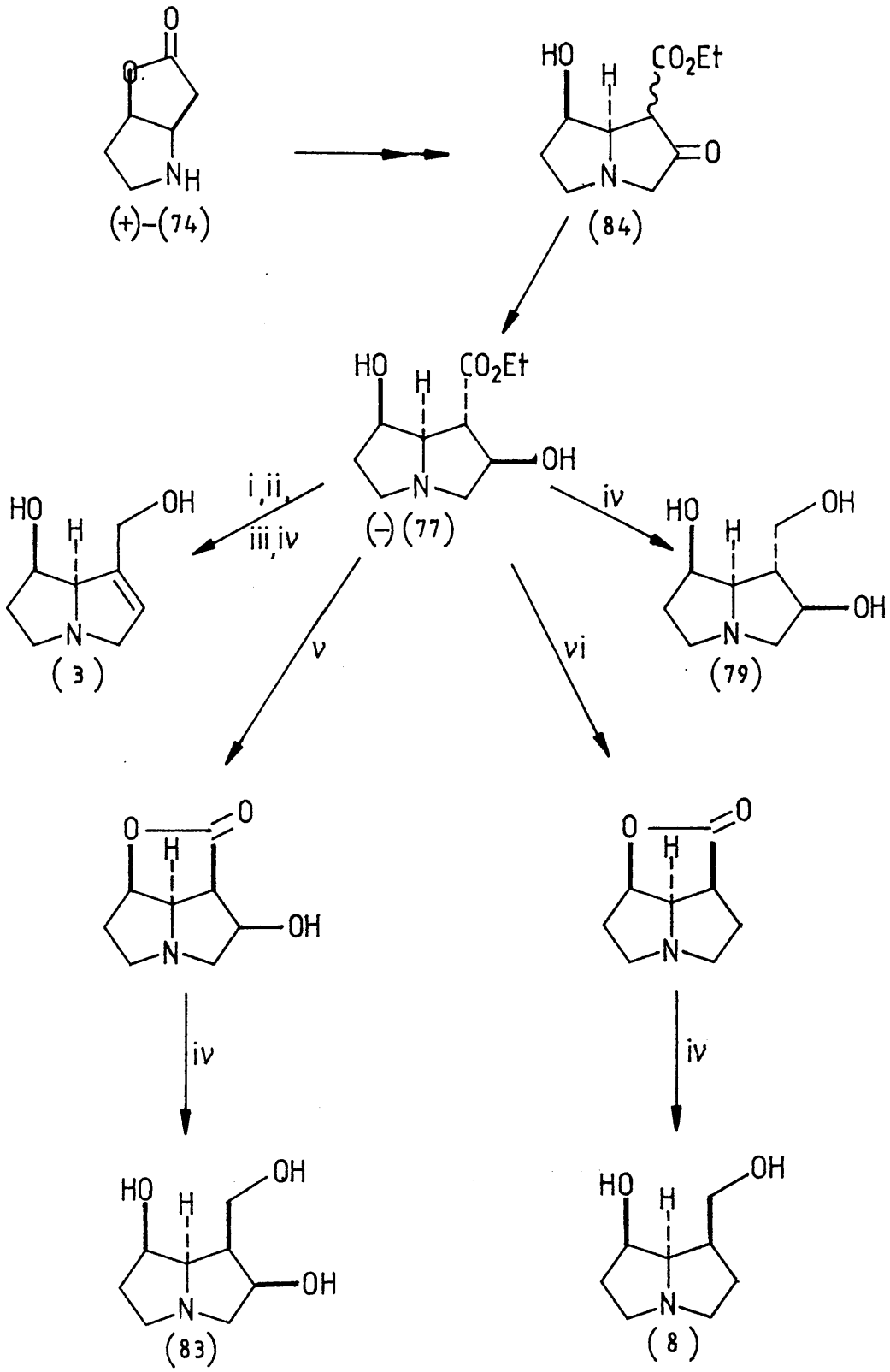
Scheme 18 : Reagents : (i) TBDMS-Cl, DMF, imidazole, (ii) Bu^tOCOCl, Et_3N (iii) CH_2N_2, (iv) MeOH, Ag_2O, (v) MeOH, H_2O, Na_2CO_3, (vi) PhSeCl, (vii) Bu^n_3SnH, (viii) 5% Pd/C, EtOH, HCl.



(85)



(13)

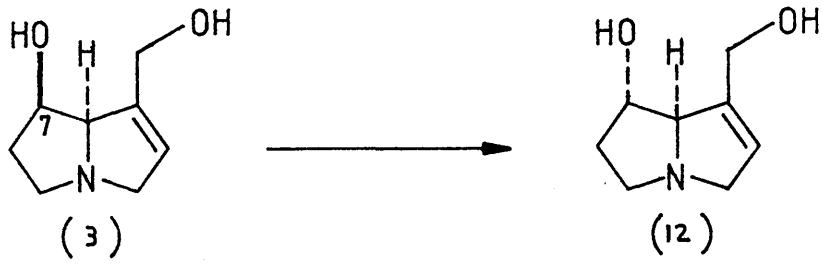


Scheme 19 : Reagents : (i) Ac_2O, pyr, DMAP, (ii) NaH, THF, (iii) EtOH,HCl

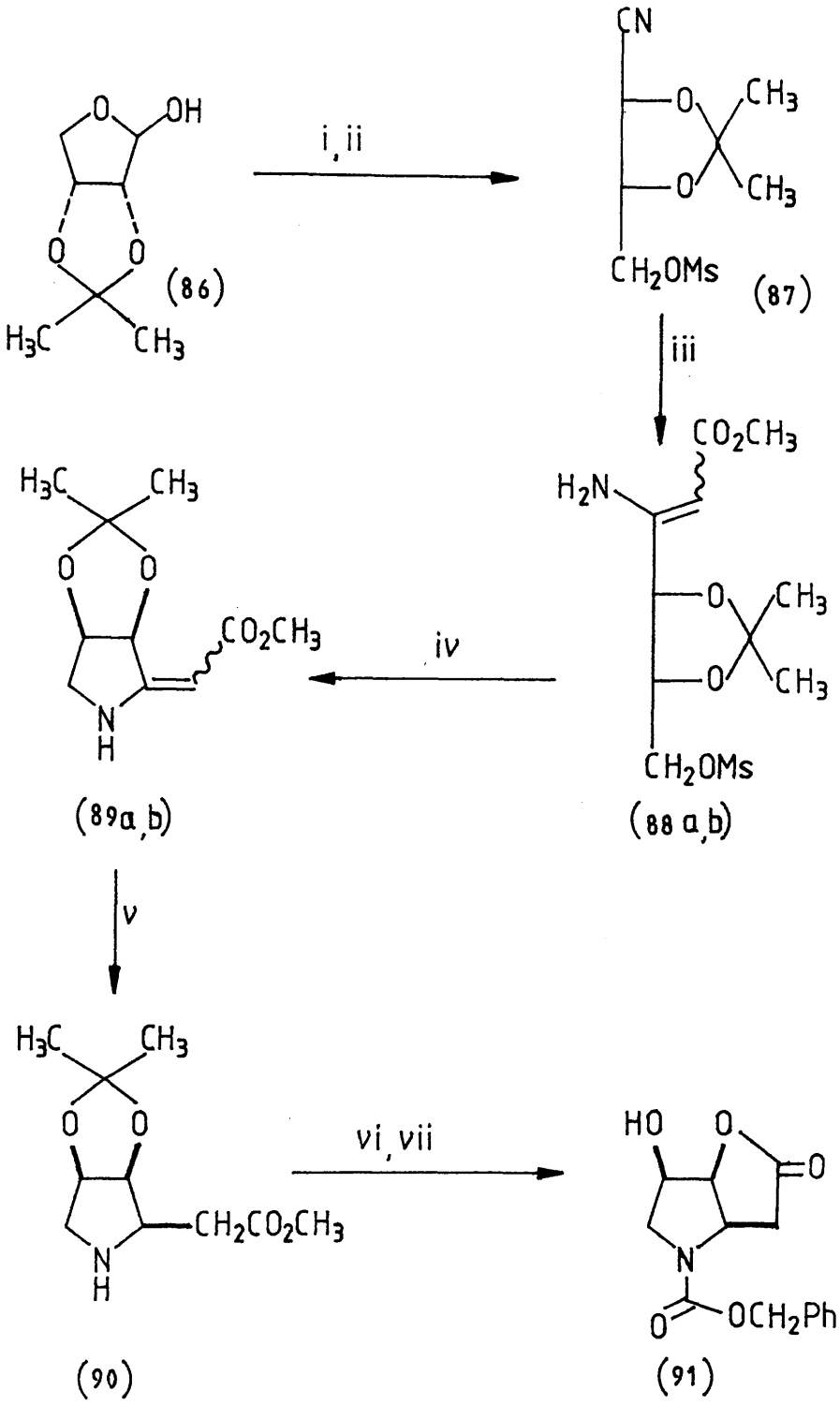
(iv) $LiAlH_4$, (v) NaOEt(cat.), EtOH, (vi) $H_2/Rh/Al_2O_3$, AcOH, H_2O

This was probably due to the steric effects of the acetal group. Hydrolysis of the acetal after N-protection gave the optically active lactone (91) which could be easily converted into lactone (74) or its 4-oxy-derivative (85). Either of these could then be used to produce optically active necine bases.

An interesting feature of the previous syntheses, and indeed of many other synthetic routes, is the interconversion between the various necine alcohols. This was demonstrated, at an elementary level by Glinski and Zalkow, who converted naturally occurring (+)-retronecine (3) into its C-7 epimer (+)-heliotridine (12).[51] This was achieved by a sequence of protection of the primary hydroxyl, mesylation of the secondary hydroxyl, epimerisation at C-7 with caesium propionate and hydrolysis of the esters.



(3) (12)



Scheme 20 : Reagents : (i) $NH_2OH.HCl$, C_5H_5N, (ii) $MeSO_2Cl$, (iii) activated Zn, $BrCH_2CO_2CH_3$, (iv) DBU, (v) $NaBH_3CN$, (v) $PhCH_2OCOCl$, (vii) CF_3CO_2H.

A rather different approach to the synthetic problems is to use the transannular interaction of a 1-aza-5-oxocyclo-octane system. It is known that molecules of this type exist in the 1-aza-[3.3.0]bicyclo-octane form (Fig. 5), when protonated.[52] The infra-red spectrum of (92b) after protonation shows no carbonyl stretching frequency. The ^{1}H n.m.r spectrum shows only a singlet for the benzyl protons, rather than the doublet expected if N-protonation had taken place.

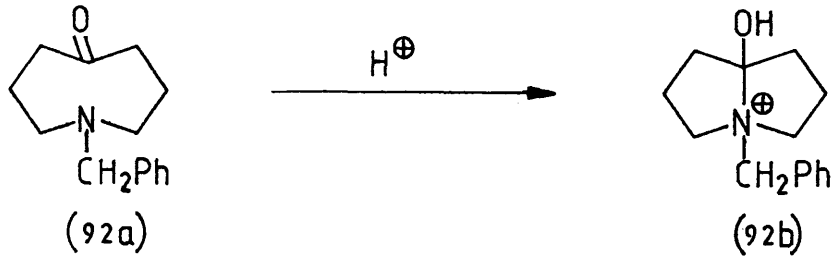
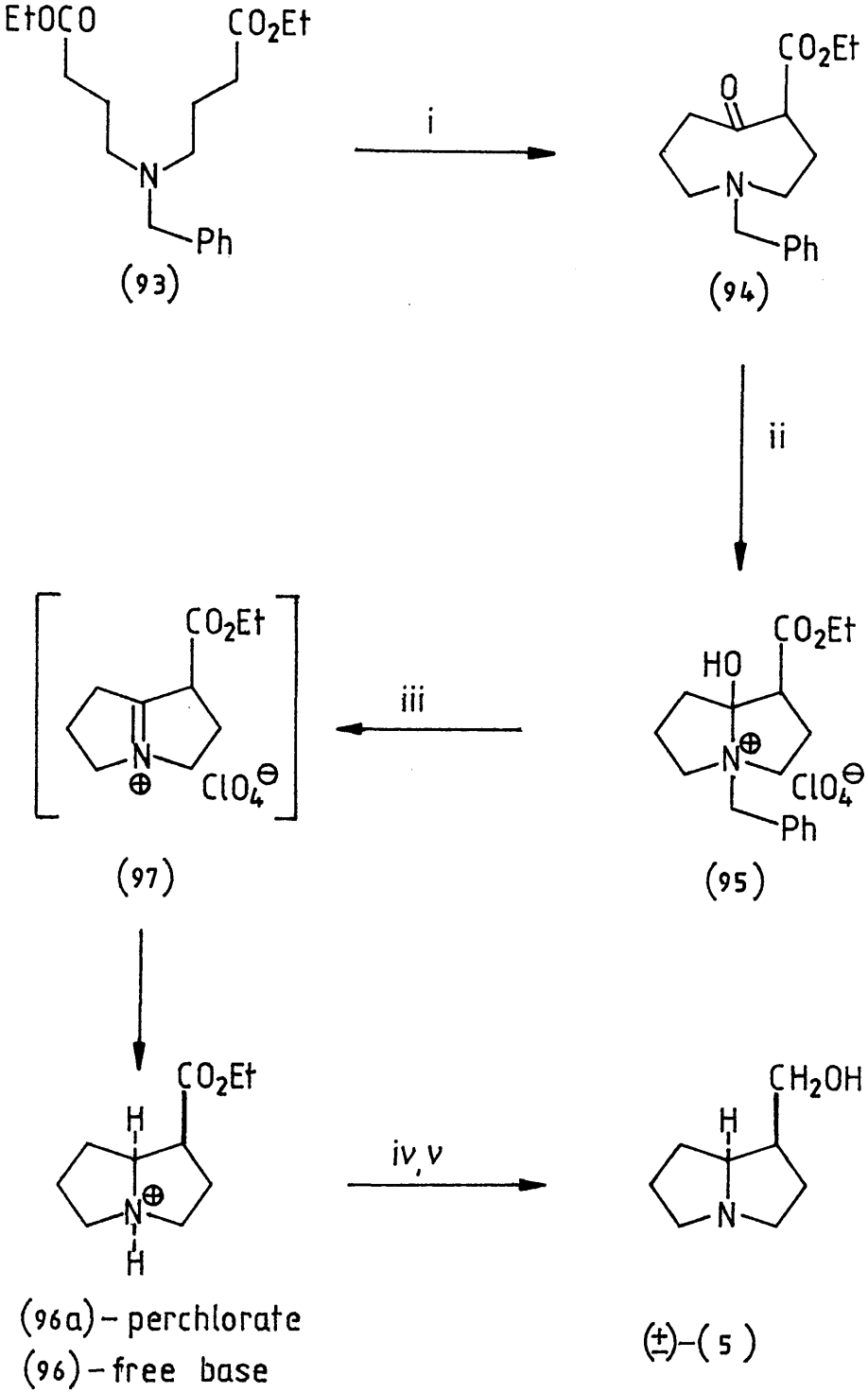


Figure 5.

The ^{1}H n.m.r. spectrum also shows a broad signal corresponding to the hydroxyl proton.

This observation therefore presents an acid-mediated method for the construction of the pyrrolizidine ring system. Leonard and Sato used this type of procedure in their 1969 synthesis of (±)-isoretronecanol (5).[53] Their synthesis, shown in Scheme 21, started from the ester (93) which was subjected to a high dilution Dieckmann cyclisation with potassium t-butoxide. The cyclic product (94) was converted into its perchlorate which was shown by spectral data, to have the transannular structure (95). [The infra-red spectrum showed no band for a ketone and two bands at 3550 and 3370 cm^{-1} for OH.] Catalytic hydrogenation of the perchlorate salt (95) gave a good yield of ethyl (±)-isoretronecanolate perchlorate (96a). This reaction



Scheme 21 : Reagents : (i) $KOBu^t$, (ii) $HClO_4$ (iii) H_2(1-3 atm), Pd/C, (iv) NaOH, (v) $LiAlH_4$.

probably goes through the iminium species (97) which would account for the cis-nature of the ester product (96a). Liberation of the free base (96) followed by reduction with $LiAlH_4$ gave (±)-isoretronecanol (5) which was isolated as its picrate salt.

The same group also tried the direct hydrogenation of the keto-ester (94) under non-acidic conditions. The result was a mixture of ethyl (±)-isoretronecanolate (96) and the αβ-unsaturated ester (98). To complete the hydrogenation of ester (98) required the use of acidic media. The formation of ester (98) is accounted for by dehydration of the β-hydroxy ester (99c) which is in equilibrium with (99a) and (99b) (Figure 6).

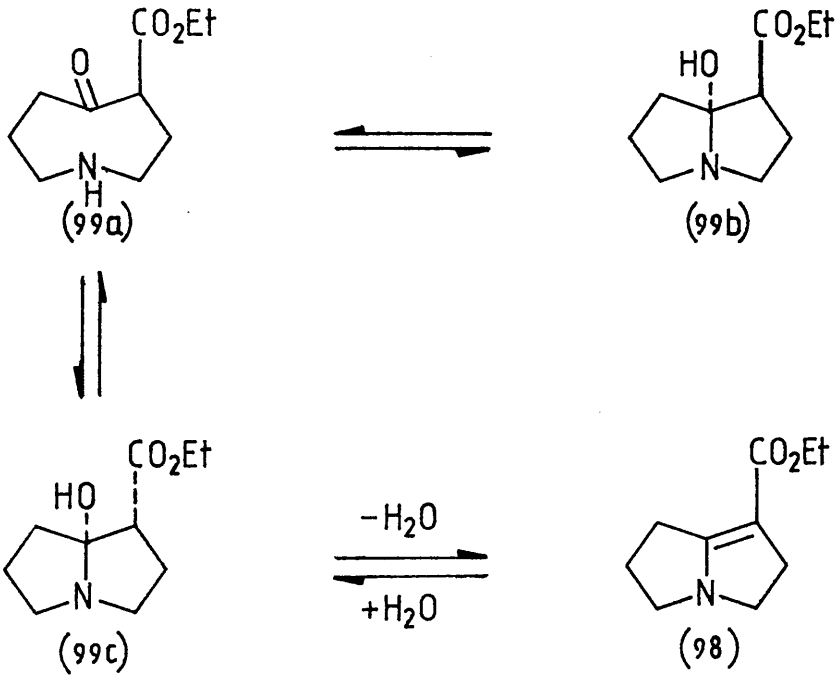
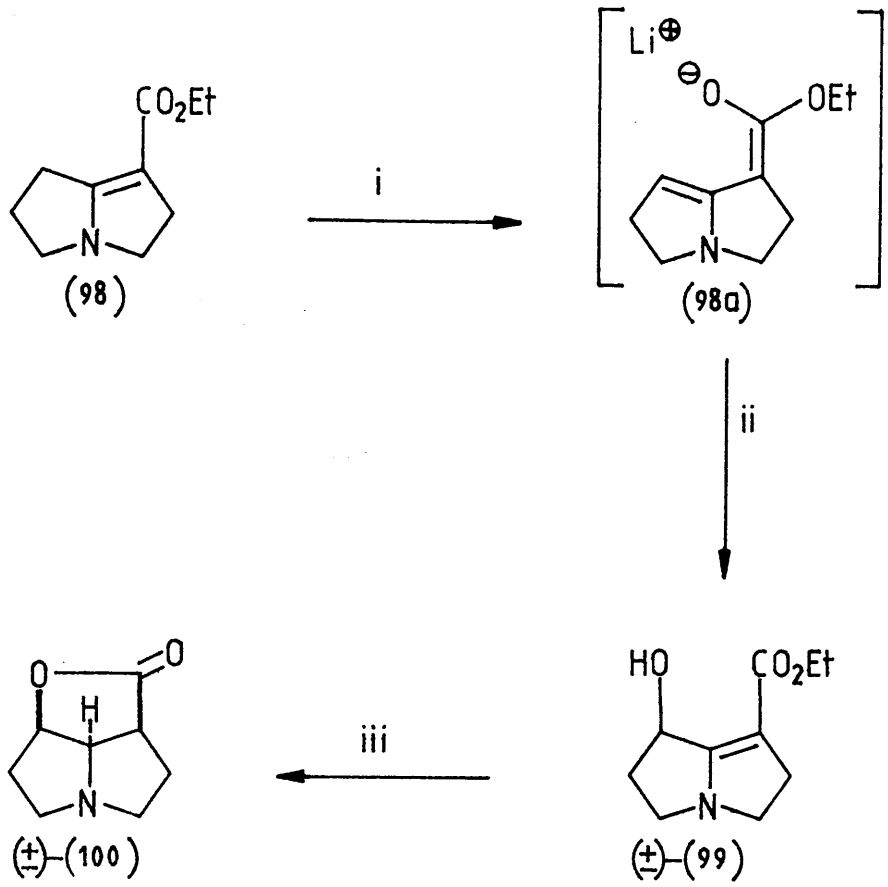


Figure 6.

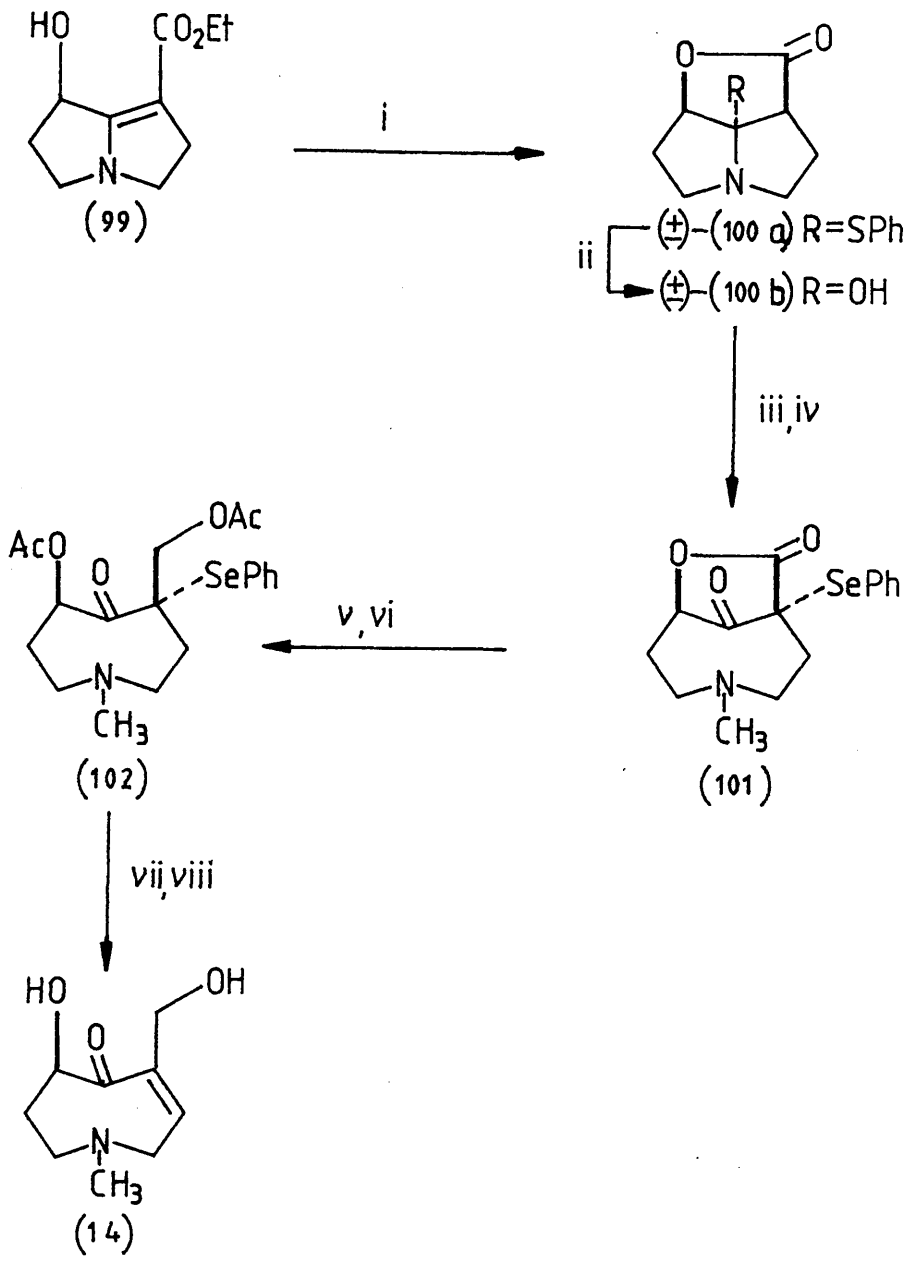
In a later synthesis of (±)-retronecine (3), Niwa et al. used the αβ-unsaturated ester (98) as a key intermediate.[54] They were extending the use of the γ-hydroxylation reaction of lithium enolates



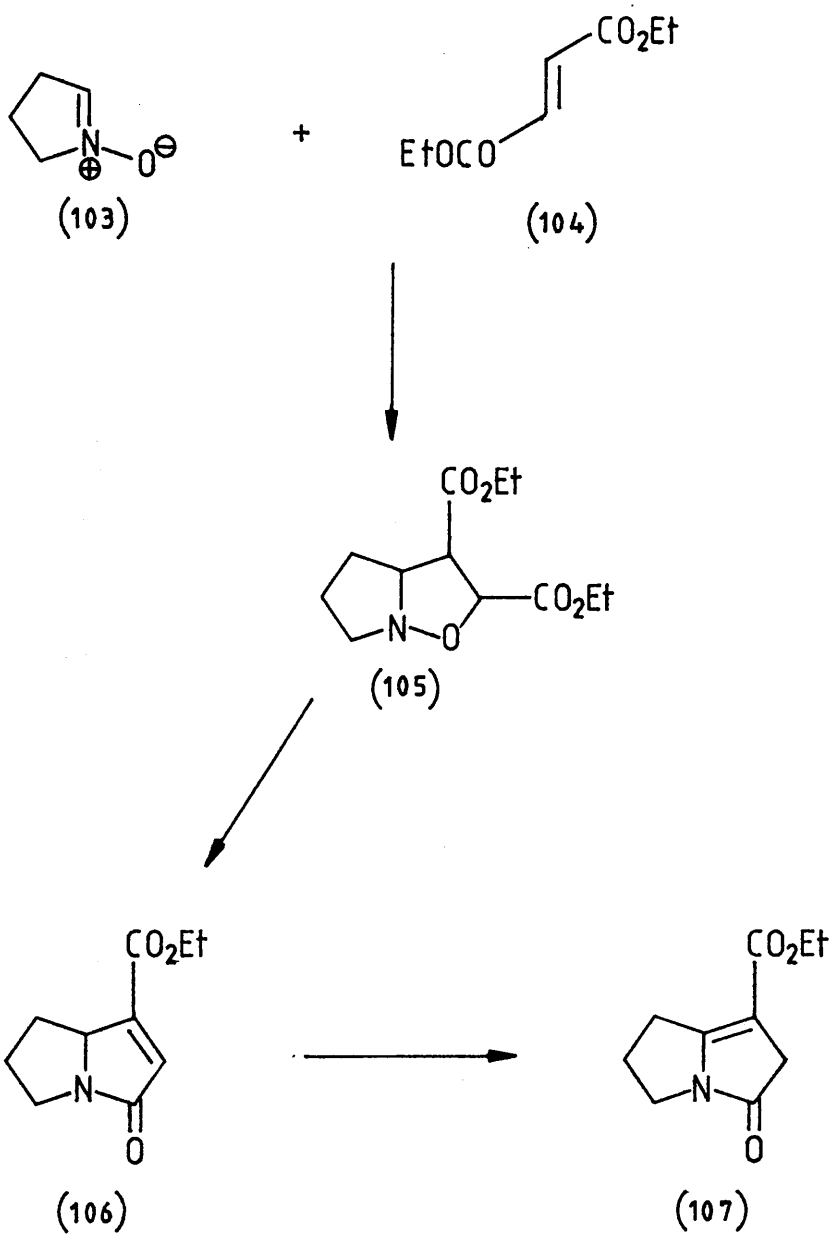
Scheme 22 : Reagents : (i) LDA, HMPA, (ii) MoO_5, (iii) H_2, PtO_2.

Tufariello and Tette,[56] (Scheme 24). They used the nitrone (103) as a 1,3-dipole and, initially, diethyl fumarate (104) as the dipolarophile, giving the adduct (105). However, the product after hydrogenolysis and dehydration was not the Δ^1-compound (106) expected but was the Δ^7-ester (107). It is thought that isomerisation occurs of the cross-conjugated keto-ester (106) to the more stable lactam (107). An alternative to this was to use an unsymmetrical dipolarophile, such as methyl 4-hydroxycrotonate (108). Studies with this dipolarophile had shown that the cycloaddition regiochemistry gave the adduct (109a) and not its isomer (110). Therefore, hydrogenolysis of the mesylate derivative (109b) followed by intramolecular substitution and elimination of water gave the (±)-unsaturated ester (111). This was reduced using the selective hydride reagent lithium aluminium hydride/aluminium chloride to give (±)-supinidine (11) (Scheme 25). Later, Tufariello and Lee extended this synthesis to make (±)-retronecine (3).[57] Using the new nitrone (112) in an exactly analogous manner to that described above, they obtained the ketal ester (113) which was easily transformed into the diol (3) which had spectral and physical properties identical to authentic (±)-retronecine (3) (Scheme 26).

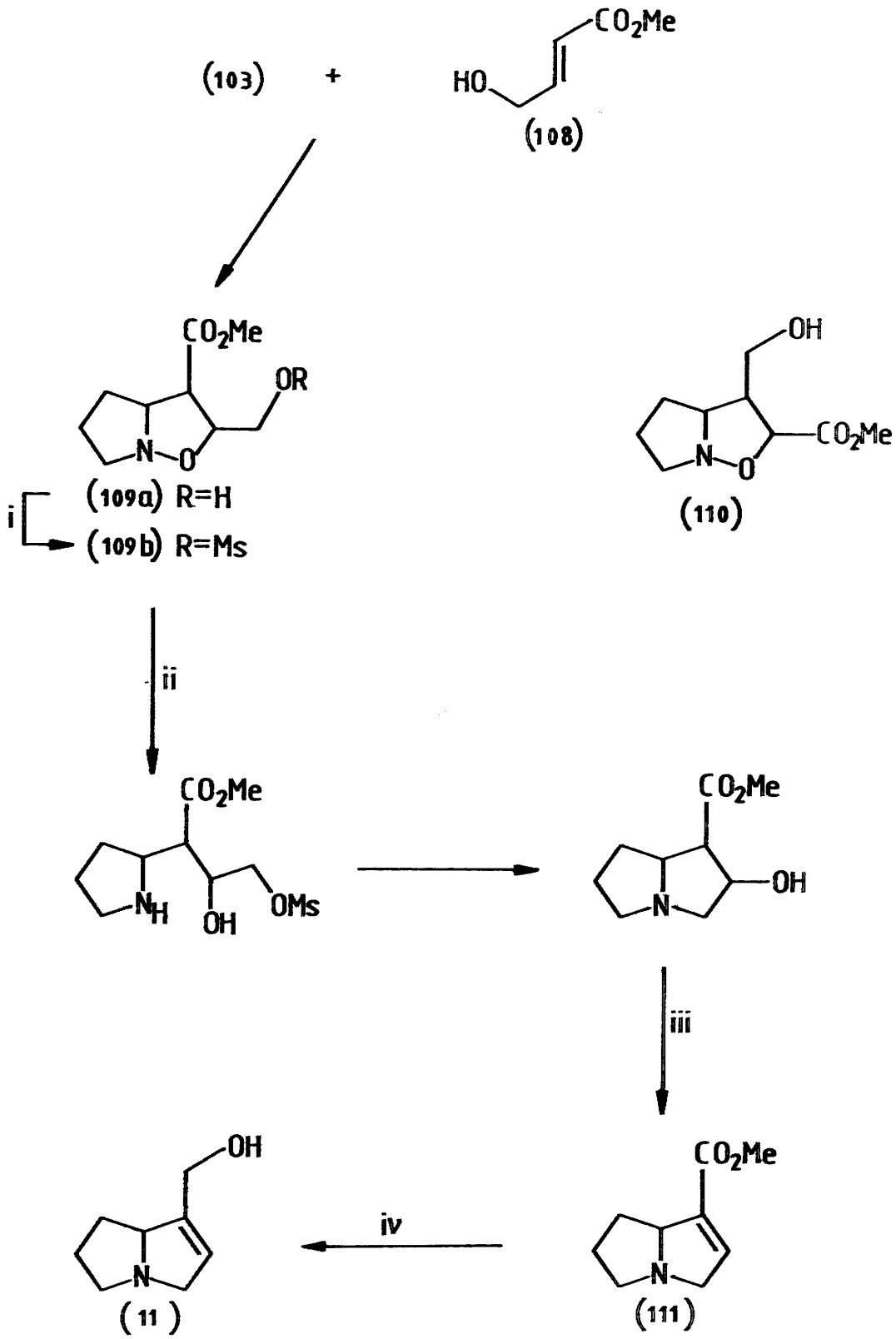
These last two syntheses did not use the full potential of the 1,3-dipolar cycloaddition, in that the ring formed was not kept in the final pyrrolizidine product. A recent synthesis of (±)-retronecine (3) by Vedejs *et al.*[58] used a similar system to Tufariello *et al.*, but the cycloaddition reaction was utilised to form the second ring of the pyrrolizidine nucleus. Instead of using the imine oxides (103) or (112) they used the imidate species (114) which was generated *in situ* from the imidate triflate salt (115) using CsF (Scheme 27). Cycloaddition of the imidate (114) to methyl acrylate gave the adduct (116) which was



Scheme 23 : Reagents : (i) LiSPh, (ii) $Hg(OAc)_2$, (iii) MeI, (iv) NaH, PhSeCl, (v) DIBAL, Et_2AlCl, (vi) Ac_2O, pyr (vii) H_2O_2, (viii) NaOMe, MeOH.



Scheme 24



Scheme 25 : Reagents : (i) MsCl, pyr (ii) H_2Pd/C, (iii) $POCl_3$,pyr

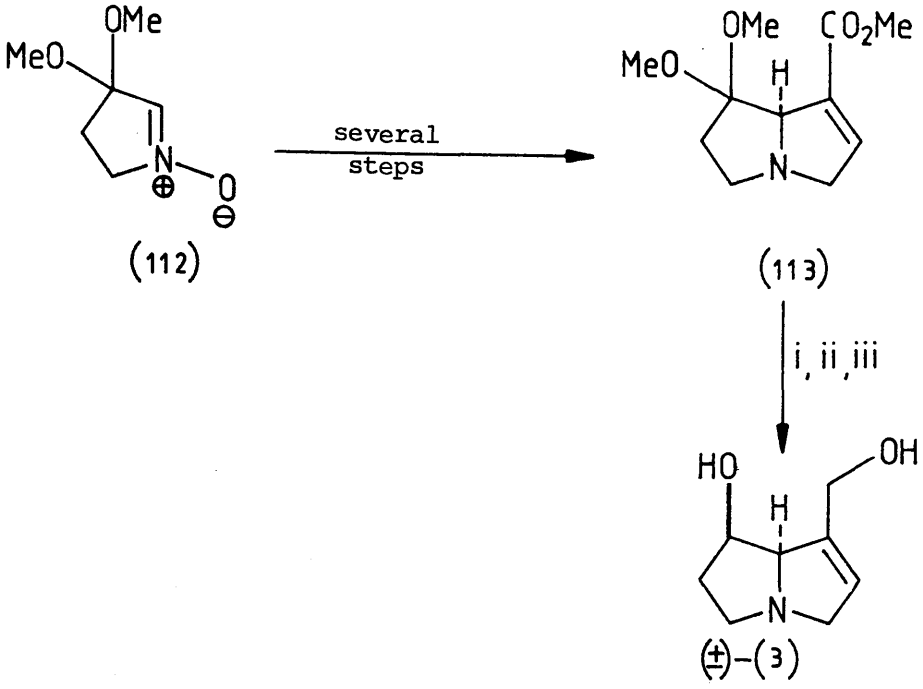
(iv) $LiAlH_4/AlCl_3$

transformed into (±)-retronecine (3) by standard reactions.

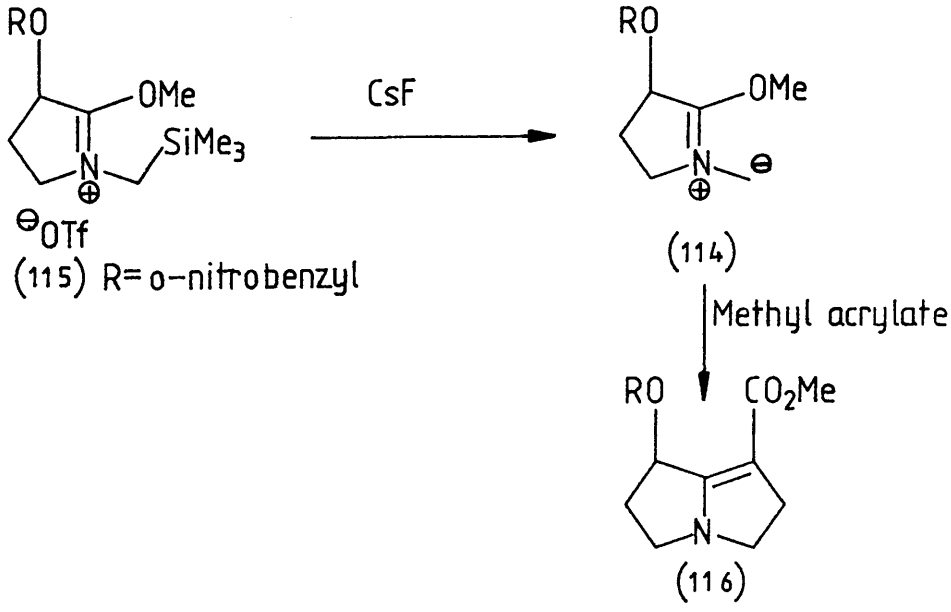
Given the length and relative complexity of the routes above it is not surprising that much shorter routes have been found which make use of the 1,3-dipolar system. The first important report came from Pizzorno and Albonico in their 1974 synthesis of (±)-isoretronecanol (5).[29]

Using N-formyl-L-proline (117) as their starting point they formed the 1,3-dipolar species, probably (118).[59] Cycloaddition with ethyl propiolate gave the adduct (119) which, spontaneously lost CO_2 via a retro-Diels-Alder process. The pyrrole ester (120) produced was then hydrogenated with a Pd/C catalyst and, as previously noted, delivery of the H-atoms occurs from one side only. Therefore the product obtained was exclusively ester (96). Reduction gave a sample of (±)-isoretronecanol (5) which was identical to authentic material. The sequence is shown in Scheme 28.

In 1979, Robins and Sakdarat extended this route to make (±)-supinidine (11).[60] The method used was the phenyl-selenenylation of the ester (96) followed by the reduction/oxidation/elimination steps, as mentioned previously. Shortly after, they came up with an adaptation of this route which allowed them to report the first synthesis, in optically active forms, of the four stereoisomers of 1-hydroxymethyl-pyrrolizidine and two enantiomers of supinidine (11).[61] These workers noticed that the 1,3-dipolar cycloaddition reaction mentioned above could also be carried out on the N,O-diformyl derivative (121) of (-)-(4 R)-4-hydroxy-L-proline (123) which is a readily available starting material. Heating the diformyl compound (121) with Ac_2O in the presence of ethyl propiolate gave the adduct (123a) as a single product in high yield. As shown in Scheme 29, the pyrrole (123a) was deformylated to give the



Scheme 26 : (i) 37% HCl, (ii) $NaBH_4$,MeOH (iii) AlH_3



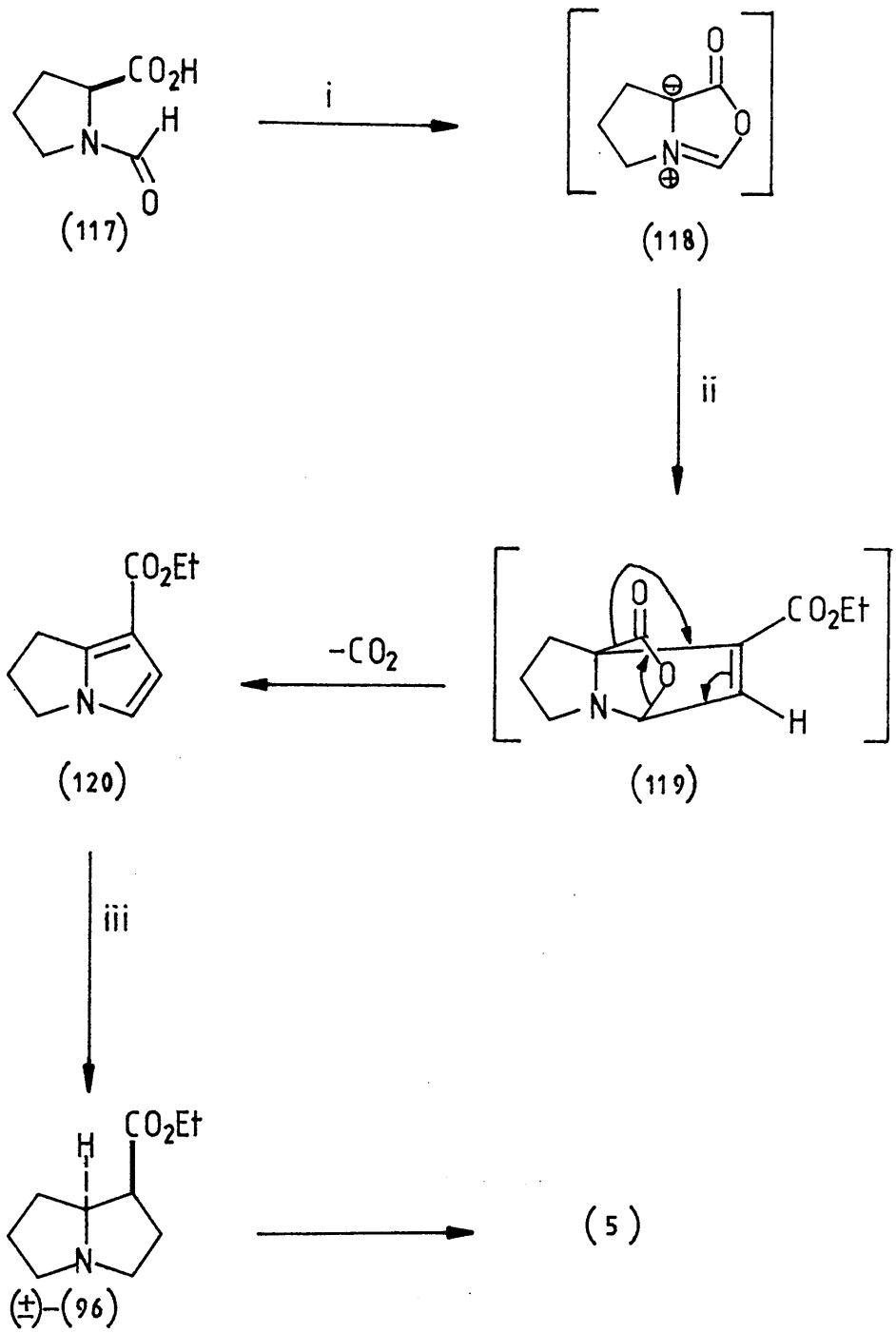
Scheme 27

free alcohol (123b). In the subsequent hydrogenation it is this alcohol which controls the stereochemistry of the new chiral centres at C-1 and C-8. The alcohol is α to the ring, so the cis-H atoms are delivered from the less hindered β-face. Hence, hydrogenation of pyrrole (123b) yielded the saturated ester (124) with 1R, 6R, 8R-stereochemistry. Replacement of the 6α-hydroxy group by chlorine[62] followed by catalytic hydrogenation gave the ester (+)- (96). Hydride reduction of ester (96) afforded optically pure (+)-isoretronecanol (5) which was identical in all respects to the natural material. It was then noted that since ester (96) is the less stable endo isomer, base treatment should induce epimerisation. Using NaOEt in EtOH, according to the method of Bradange and Lundin[63] gave complete conversion to the 1-β isomer (125). Hydride reduction gave the alcohol (+)-trachelanthamidine (6). Ester (96) was also converted into (+)-supinidine (11) using the same conditions as for the (±)-mixture.[60]

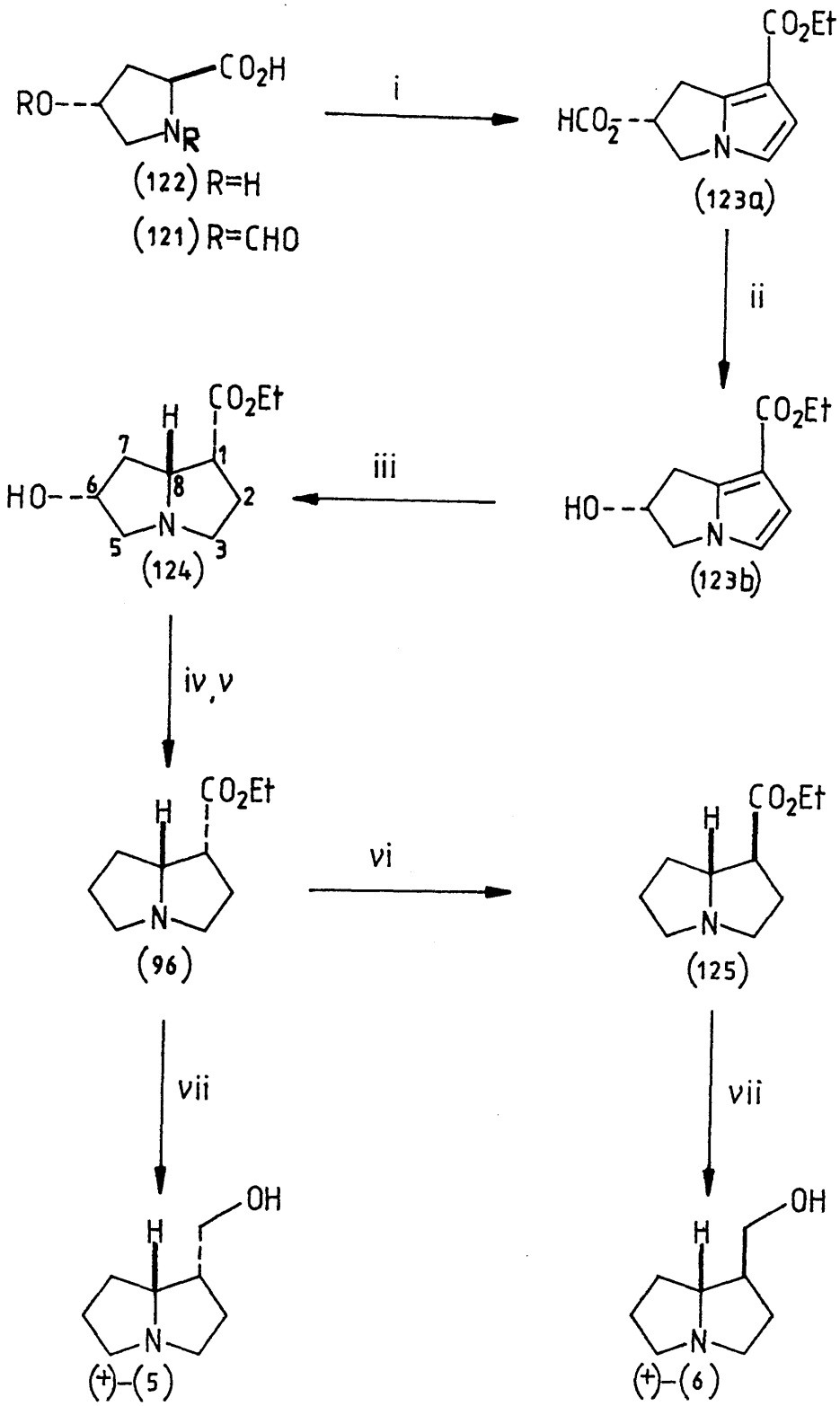
Robins and Sakdarat then made the corresponding 8 α-compounds. They did this by epimerising at C-6 of pyrrole (123) via formate substitution of the C-6 tosylate. This gave the (-)-enantiomer of pyrrole (123), which was used to make (-)-isoretronecanol (5), (-)-trachelanthamidine (6) and (-)-supindine (11) by the same methods as above.

Very recently, Kelly and Robins used the diol derived from ester (124) in their synthesis of a set of macrocyclic pyrrolizidine alkaloid analogues.[64]

The regiochemistry of the addition of ethyl propiolate to the azomethine ylid, as used in the last few syntheses, appears to be completely selective. However the product obtained is not the one



Scheme 28 : Reagents. (i) Ac_2O, (ii) $HC{\equiv}CCO_2Et$, (iii) H_2, Pd/C



Scheme 29 : Reagents. (i) Ac_2O, $HC{\equiv}CCO_2H$, (ii) NH_3/MeOH, (iii) H_2,Pd/C (iv) $SOCl_2$, (v) H_2, Raney Ni, (vi) NaOEt/EtOH, (vii), $LiAlH_4$

which would be predicted mechanistically.

A full Frontier Molecular Orbital (FMO) treatment is not possible due to the lack of data on the energies of the azomethine ylides that are involved. However, it is possible to give a qualitative description of the system, and so justify the resulting regiochemistry. There are two questions for each system to be examined . Firstly, which component supplies the HOMO for the reaction and which the LUMO? Secondly, are the coefficients of the orbitals such that large/large and small/small interactions are maximised, thus controlling the regiochemistry?

For the reaction of N-formyl-L-proline (117) with ethyl propiolate the dipole is the azomethine ylide (118). Calculations have shown that an electron withdrawing group, such as the carbonyloxy group in (118), causes a lowering of the LUMO energy and a smaller lowering of the HOMO energy.[65] The approximate energy values are shown in Figure 7, for the oxazolone as a 1,3-dipole (118).[66] Photoelectron spectroscopy

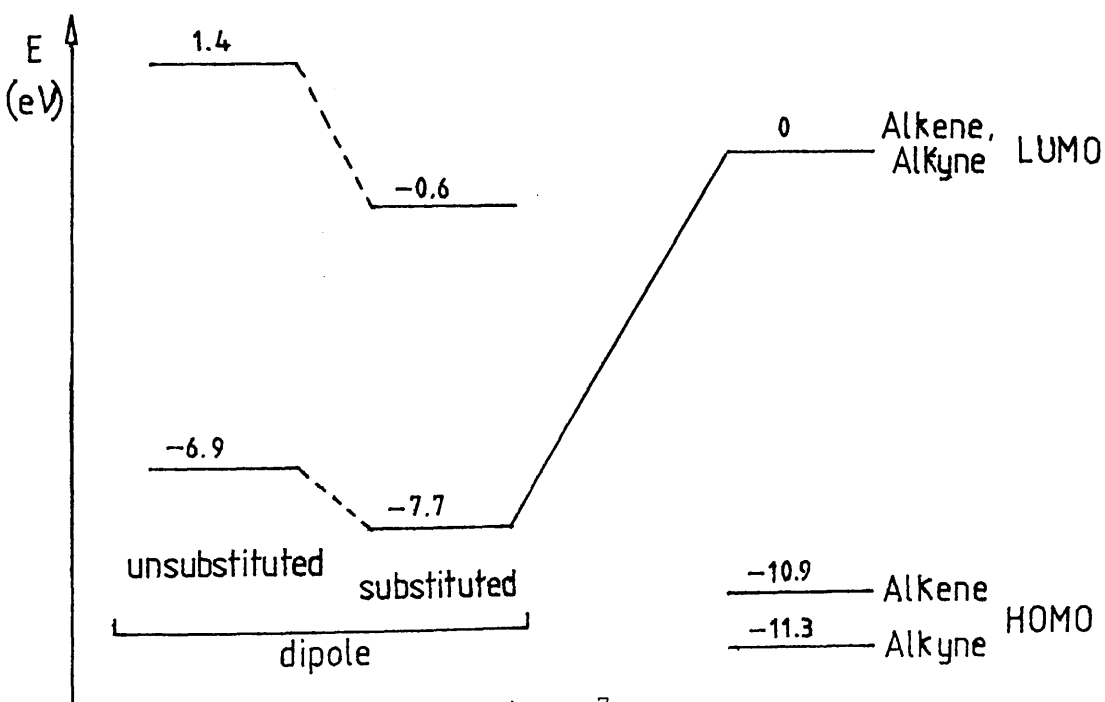


Figure 7.

indicates that the HOMO of an alkyne is some 0.4 to 0.9 eV less than that for the corresponding alkene. The LUMO of the alkyne is virtually identical to the LUMO of the analogous alkene, as can be seen from the approximate energy values for ethyl propiolate (alkyne) and ethyl acrylate as shown in Figure 7.[66]

The HOMO-LUMO combination which best matches the energies of the frontier orbitals is clearly the HOMO of the 1,3-dipole (118) and the LUMO of the dipolarophile, ethyl propiolate. The energy difference is 7.7 eV, indicating a dipole-HOMO controlled reaction.

The regiochemistry is controlled by the overlap of the frontier orbitals which maximises the large/large and small/small orbital coefficient interactions. The HOMO in a 1,3-dipole has the largest coefficient on the anionic carbon. For an azomethine ylide, such as (118), both of the carbon atoms bear substantial negative charges (-0.14 to -0.21 for the "neutral" carbon, -0.21 to -0.40 for the "anionic" carbon and +0.23 to +0.47 for the central nitrogen atom).[65] The HOMO and the LUMO of an alkyne (or alkene) has the larger coefficient at the unsubstituted end when attached to an electron withdrawing group

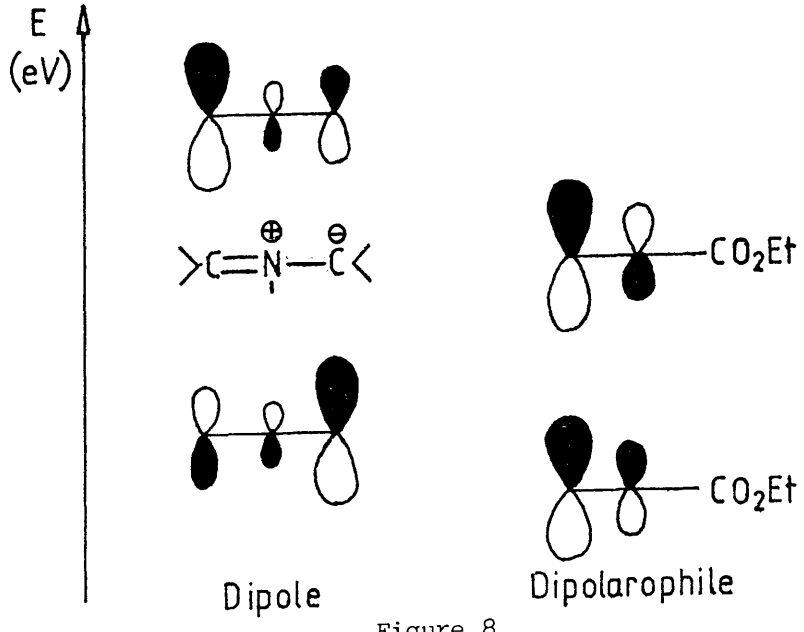


Figure 8

(Figure 8). This reasoning, however, leads to the prediction of the wrong regiochemistry. This inconsistency is most likely due to the effect of having electron-withdrawing and releasing substituents on the ends of the dipolarophile. It may be that the carbonyloxy group reduces the coefficient at the "anionic" end of the dipole and coupled with an increase in the coefficient at the "neutral" end due to the oxy-substituent, this could lead to the regiochemistry observed.

Clearly more data on the coefficients and energy levels are required to explain this phenomenon fully, and to enable predictions to be made.

3.3 Conclusions

It is clear from the previous section that the synthetic methods towards the pyrrolizidine bases range from the short and simple to the long and complex; some are total syntheses while others are modifications of naturally derived materials.

The last technique is one which was used in the route to a radio-labelled biosynthetic intermediate and will be discussed in the second part of Chapter 6. In another approach, the 1,3-dipolar cycloaddition method was chosen in the attempted synthesis of a biologically interesting necine base. This will be discussed in the first part of Chapter 6.

CHAPTER 4.

SYNTHESIS OF MACROCYCLIC PYRROLIZIDINE ALKALOIDS AND ANALOGUES.

4.1 Introduction

The range of biological activities, particularly hepatotoxicity, and the widespread occurrence of the pyrrolizidine alkaloids has focussed attention upon them. The main toxic effect of the alkaloids is thought to be due to the presence of an allylic ester function with the double bond as part of the pyrrolizidine nucleus.[3] The most toxic of this class of compounds are the macrocyclic diesters, formed by the double esterification of a necic diacid with a pyrrolizidine diol or triol. Naturally occurring diesters with ring sizes of 11-14 have been isolated.[1]

To allow the development of structure-activity relationships, alkaloids and related analogues have been developed. These will be reviewed in the next section.

4.2 Macrocyclic Pyrrolizidine Diesters

The few syntheses of macrocyclic diesters, which have been reported, have appeared in the last eight or nine years. The first, by Robins and Sakdarat,[67] was the preparation of an 11-membered diester (126) of (+)-retronecine (3). The important step in this, and in each of the subsequent synthetic routes is the method of activation of the diacid portion towards the necine diol. The particular solution in this case was to use the Corey-Nicolaou "double activation" method.[68] With this technique the carbonyl group is activated by the formation of a pyridine-2-thiol ester, and the hydroxyl is activated by intramolecular proton transfer, as shown in Figure 9.

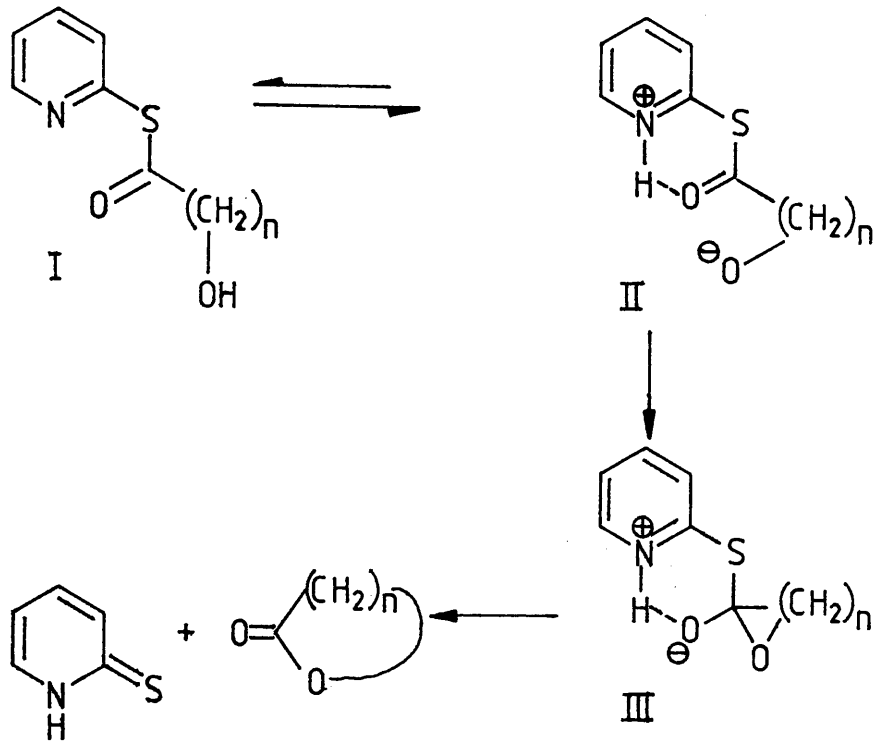


Figure 9.

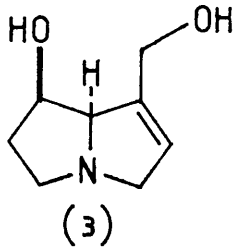
This transfer is promoted by the formation of the H-bonded intermediate II which spontaneously cyclises, under the reaction conditions, to give III. Elimination of 2-pyridithione gives the desired lactone product.

The full report of the synthesis of dilactone (126) and a set of similar compounds (127 - 131) was published later (Scheme 30).[69] Treatment of (+)-retronecine, obtained from natural sources,[70] with 3,3-dimethylglutaric anhydride gave a mixture of the 9- and 7- monoesters (132) and (133) which precipitated from the chloroform solution. There was no evidence of any diester formation, which may have been because the zwitterionic monoester precipitated immediately. To

effect the lactonisation, the 9- and 7- pyridine-2-thiol esters (134) and (135) were formed by the addition of 2,2'-dithiodipyridine (136) and triphenyl phosphine (137). The thioester mixture was then added to refluxing chloroform. After lactonisation at high dilution was complete, the product was purified by preparative thin layer chromatography.

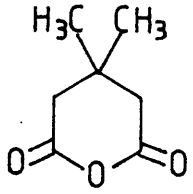
The most notable feature of the ^{1}H n.m.r spectrum of (126) was the AB system (J 12 Hz) observed for the two diastereotopic protons at C-9. The chemical shift difference [$\Delta\delta$(H-9)] between these protons was found to be 1.24 ppm. This feature along with the other spectral data, particularly the i.r. carbonyl frequency at 1738 cm^{-1}, provided convincing evidence that the dilactone had formed. A similar non-equivalence in the protons at C-9 was also seen in analogues (127) (128), (130) and (131) with $\Delta\delta$(H-9) values of 1.23, 0.62, 0.51 and 0.99 p.p.m. respectively.

Very shortly after this, Devlin and Robins reported the first synthesis of a naturally occurring macrocyclic alkaloid,[71] dicrotaline (138), and its C-13 epimer (139). Using the same lactonisation method as above, reaction of (+)-retronecine with 3-O-TMS-3-hydroxy-3-methylglutaric anhydride, gave a mixture of two basic compounds, one of which was coincident on t.l.c. with authentic dicrotaline (138). The two were separated by preparative t.l.c. and identified as dicrotaline (138) and its C-13 isomer (139). Again, both diesters had an AB system in their ^{1}H n.m.r. spectra corresponding to the C-9 protons, with $\Delta\delta$(H-9) 1.24 p.p.m. for dicrotaline (138) and 0.98 p.p.m for its epimer (139). In addition, the stereochemistry at the C-13 position in each was identified by hydrogenolysis of the allylic ester,



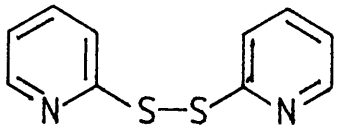
(3)

+

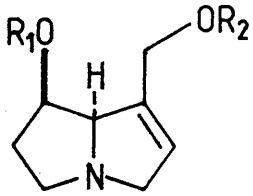


1) 20°C, 12h

2) PPh_3 (137),



(136)



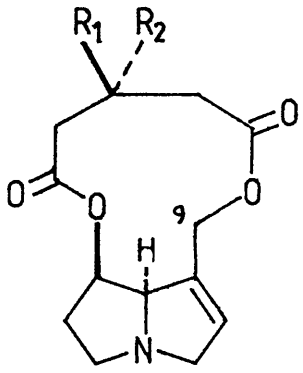
(132) R_1 = H; R_2 = $COCH_2C(CH_3)_2CH_2CO_2H$

(133) R_1 = $COCH_2C(CH_3)_2CH_2CO_2H$; R_2=H

(134) R_1=H ; R_2=$COCH_2C(CH_3)_2CH_2COSPy$

(135) R_1=$COCH_2C(CH_3)_2CH_2COSPy$; R_2=H

$CHCl_3$, reflux 26h



(126) R_1=R_2=CH_3

(127) R_1R_2=$(CH_2)_4$

(128) R_1=R_2=H

(129) R_1=R_2=Ph

(130) R_1=H, R_2=CH_3

(131) R_1=CH_3, R_2=H

(138) R_1=OH, R_2=CH_3

(139) R_1=CH_3, R_2=OH

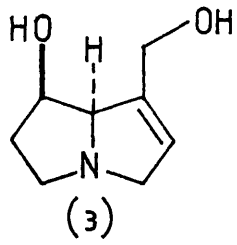
Scheme 30

selective reduction of one of the carbonyl groups and lactonisation to give optically active mevalonolactones.

A similar synthesis by Brown et al.,[72] produced the two diastereomers (12R, 14S)- and (12S, 14R)- 12,14-dimethyl-1,2-didehydrocrotaline (140) and (141), respectively. These were made to investigate what biological effect would be observed by steric hindrance around the ester groups. Hydrolysis of such ester groups is thought to be a key step in the detoxification of pyrrolizidine alkaloids.[3] The two dilactones were made as above, coupling (+)-retronecine (4) with meso-2,4-dimethyl glutaric anhydride. Separation of the dilactones by column chromatography on basic alumina gave (140) and (141) in 33 and 24% yields respectively. The absolute stereochemistry of each analogue was assigned by chemical degradation of each of the macrocyclic diesters into optically active tetrahydro-3,5-dimethyl-2H-pyran-2-ones (142) and (143) (Scheme 31). Comparison with known compounds gave the stereochemical assignments of diesters (140) and (141). Additionally, an X-ray crystal analysis of the (12S, 14R)-isomer (141) confirmed the structure and stereochemistry. It was also seen that the two ester carbonyl groups are syn-parallel and directed below the plane of the ring.

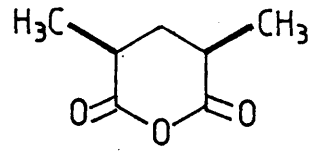
All of the above syntheses have been of 11-membered macrocyclic esters, some of which are found in nature. There are no natural examples of 10-membered macrocyclic esters of (+)-retronecine although succinic acid derivatives are present in plants.

The first synthesis of 10-membered macrocyclic dilactones of (+)-retronecine (3) was reported by Burton and Robins.[73] These were made by the reaction of (+)-retronecine with succinic anhydride, (±)-



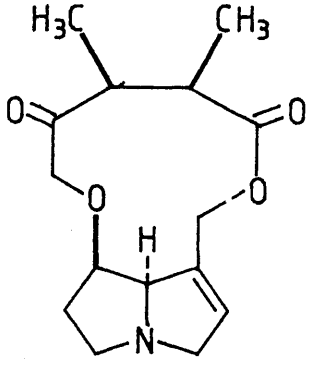
(3)

+

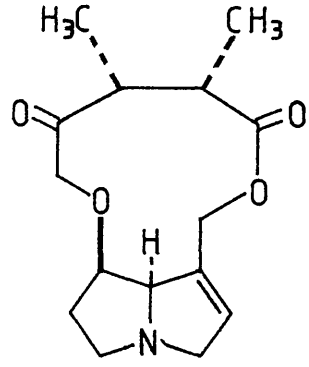


i) 20°C, 16h

ii) Dithiodipyridine (136), Ph_3P (137), Δ



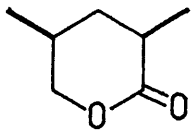
(141)



(140)

1) $H_2/PtO_2/AcOH$

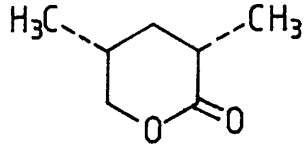
2) $LiBH_4$



(142)

1) $H_2/PtO_2/AcOH$

2) $LiBH_4$



(143)

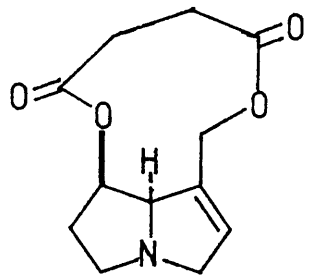
Scheme 31

trans-cyclohexane dicarboxylic anhydride and phthalic anhydride, followed by lactonisation via the pyridine-2-thiol esters. This gave the four compounds (144), (145), (146) and (147). Diesters (145) and (146) were formed as an inseparable diastereomeric pair. An X-ray structure analysis of the succinate diester (144) showed that the two ester carbonyl groups adopt an antiparallel conformation.

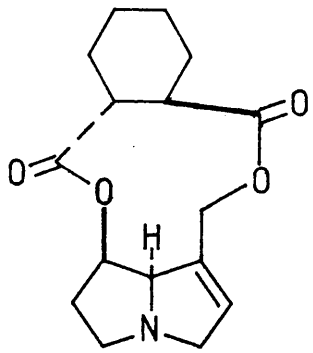
The relatively low yield for dilactone (147) led Burton and Robins to design a route which would give improved yields when the diacid portion contains an unsaturated or aromatic group.[74]

It had been shown previously[75] that the (-)-hydrochloride of (7R, 8R)-1-chloromethyl-1,2-didehydro-7-hydroxy-pyrrolizidine (148)[76] can be used to form C-9 esters of retronecine by treatment with the salts of various acids. The allylic chloride (148) was made by treatment of retronecine with thionyl chloride. Reaction of (148) with phthalic anhydride followed by treatment with base gave the desired macrocyclic diester (147). Various bases were tried, including N,N-diisopropylethylamine (Hunig's base) and potassium hydroxide, but the best yield (57%) of the diester (147) was obtained using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base. Six new ten-membered diesters (149) - (154), one eleven-membered (155) and one twelve-membered (156) macrocyclic dilactones were made by this method.

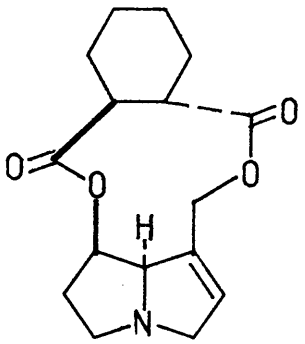
A rather different strategy was adopted by Narasaka et al.[44] in the first synthesis of the 12-membered macrocyclic alkaloid, integerrimine (157). The strategy behind the synthesis was to produce suitably protected retronecine (3) and integerrinecic acid (158), couple the two forming an αβ-unsaturated ester, and then lactonise. In the production of the integerrinecic acid (158) the methylthiomethyl (MTM)



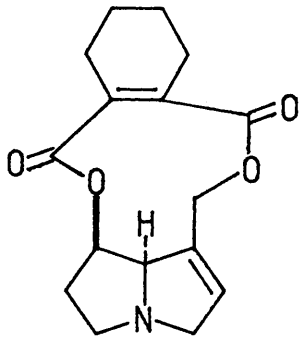
(144)



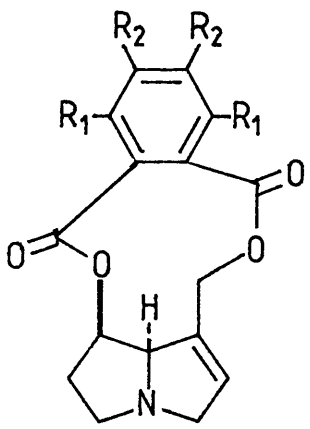
(145)



(146)



(152)

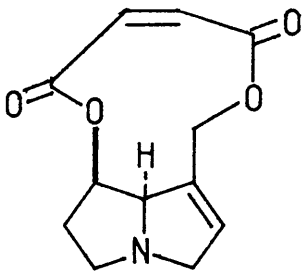


(147) $R_1=R_2=H$

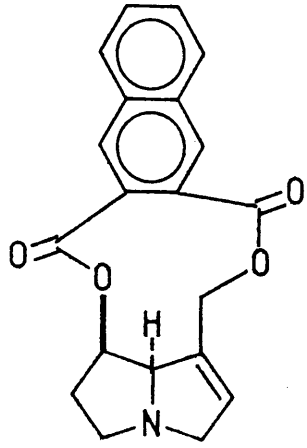
(149) $R_1=H$, $R_2=Cl$

(150) $R_1=R_2=Br$

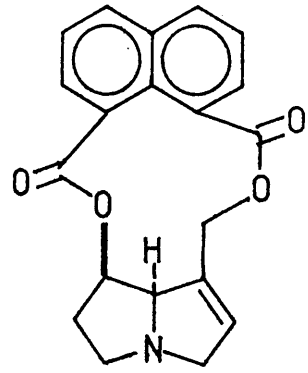
(151) $R_1=R_2=Cl$



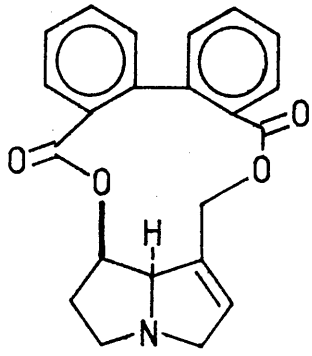
(153)



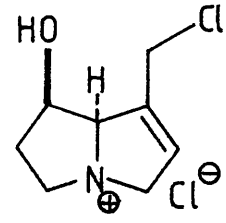
(154)



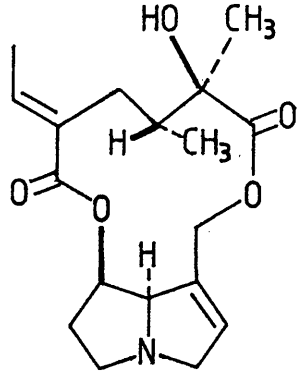
(155)



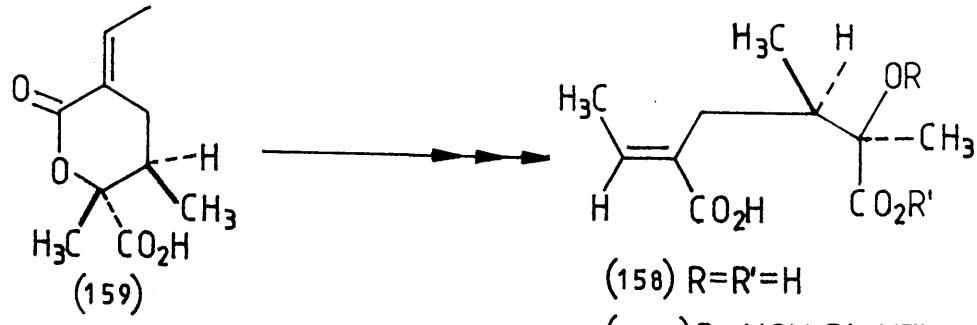
(156)



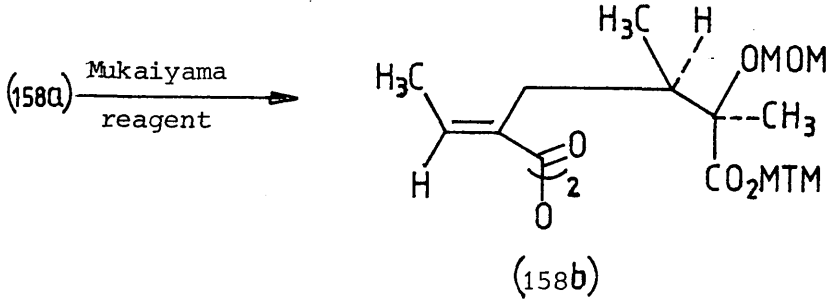
(148)

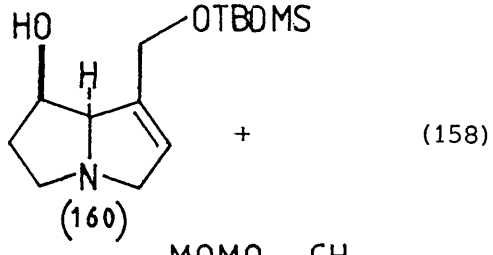


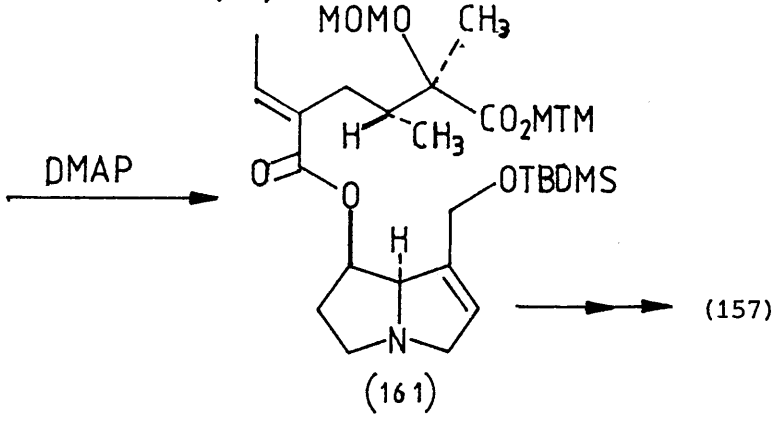
(157)



MOM-methoxymethyl







Scheme 32

group was used to great effect. MTM esters were made by reaction of the carboxylate with MTM chloride, and were removed by oxidation to the sulphone followed by aqueous alkaline hydrolysis. The (±) integerinecic acid lactone (159)[77] was opened and protected as the mono-acid anhydride (158b)

(±)-Retronecine (3) was produced according to the method of Geissman[42] (see Chapter 3) and protected as the 9-O-tert-Butyldimethylsilyl (TBDMS) ether (160). Reaction of this with the acid anhydride (158) gave the ester (161) (Scheme 32). The lactonisation was carried out by a novel method. The workers had noticed that if an alkoxide was used in place of a hydroxide, in the removal of the MTM group, then the product was an ester. Therefore, the TBDMS group was selectively removed, after oxidation of the thiomethyl to the sulphone. Formation of the lithium alkoxide affected cyclisation, in 41% yield, giving the two isomers (162a) and (162b). These were separated by preparative t.l.c. on alumina affording , after acid hydrolysis, (±) integerrimine (157).

Masamune et al.[79] developed a new method for ring closure to give macrocyclic lactones, using tert-butyl thioesters and mercuric trifluoroacetate or trifluoromethanesulphonate (Scheme 33). It is thought that the species in Figure 10 is a reaction intermediate rather than the carboxylic trifluoroacetic acid anhydride. Other thiophilic

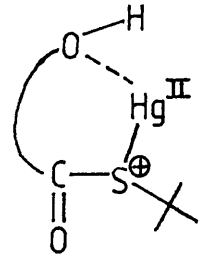
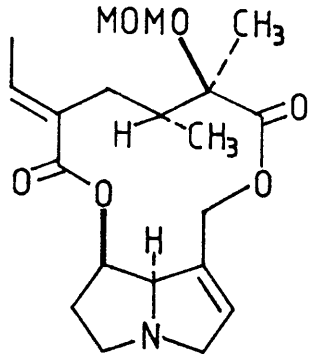
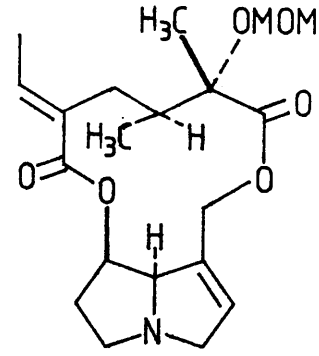


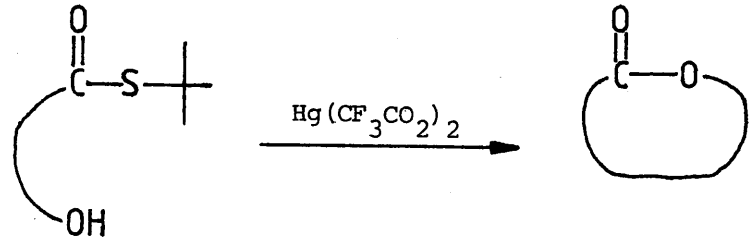
Figure 10



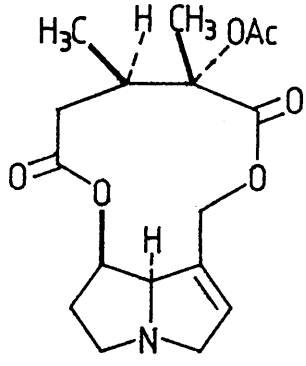
(162a)



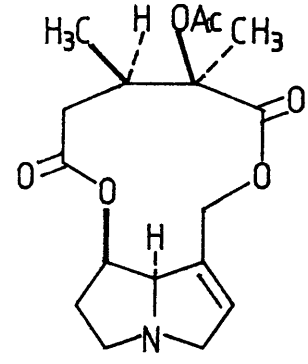
(162b)



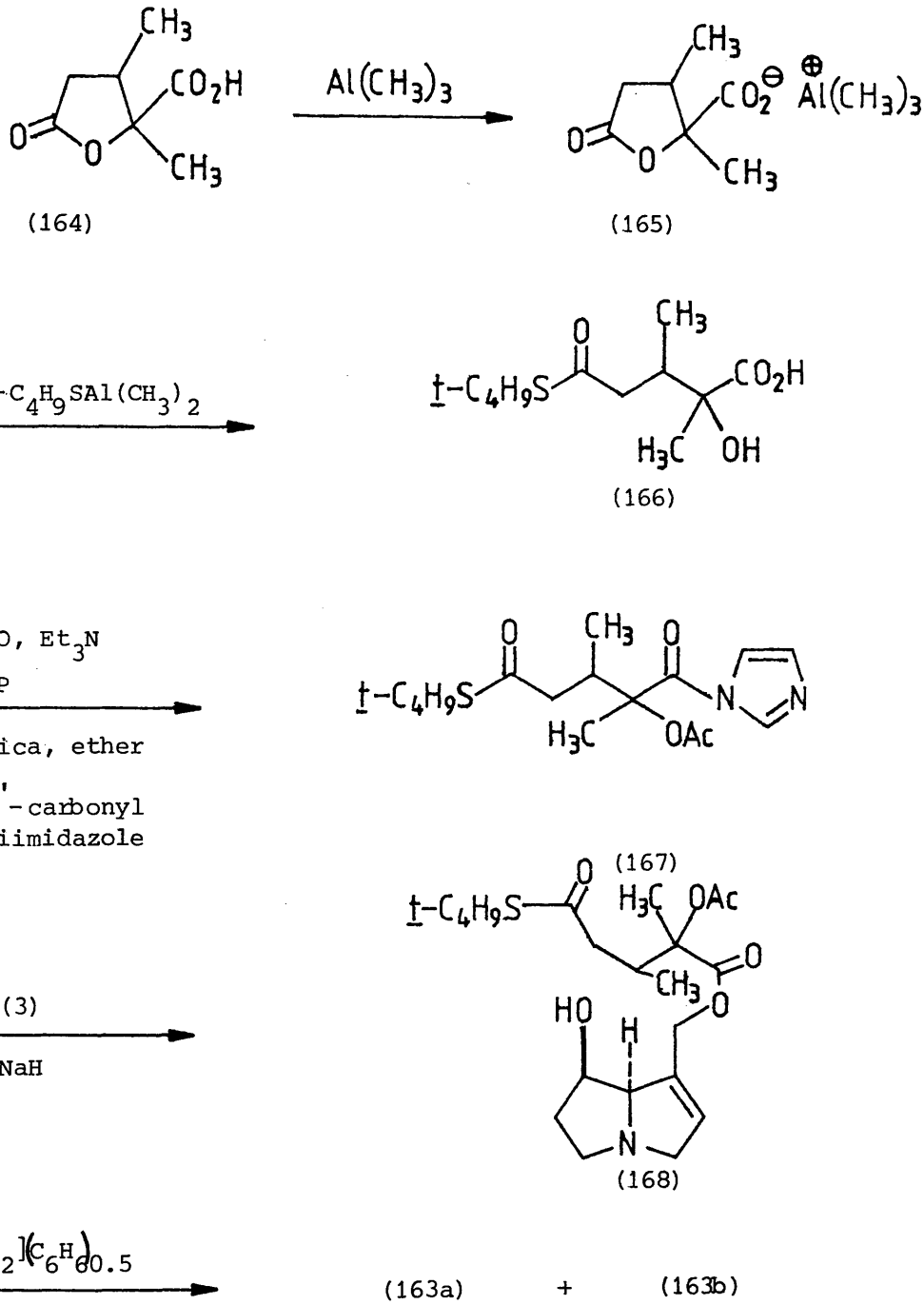
Scheme 33



(163a)



(163b)


CH3
CO2H
O
O
CH3
(164)
Al(CH3)3
CH3
CO2⊖
⊕Al(CH3)3
O
O
CH3
(165)
t-C4H9SAl(CH3)2
O
CH3
t-C4H9S
CO2H
H3C
OH
(166)
1. Ac2O, Et3N
DMAP
2. Silica, ether
3. N,N'-carbonyl
diimidazole
O
CH3
O
t-C4H9S
N
N
H3C
OAc
(167)
O
H3C
OAc
t-C4H9S
O
CH3
(3)
NaH
HO
H
O
N
(168)
Cu[CF3SO2](C6H6)0.5
(163a) + (163b)

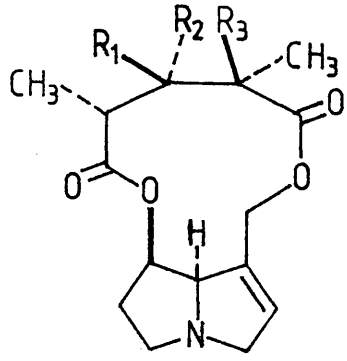
Scheme 34.

cations, such as Ag^{I}, Cu^{I} and Cu^{II} can also be used in place of Hg^{II}.

Using the above strategy in the lactonisation step, Huang and Meinwald devised a synthesis of crobarbatine acetate (163a) and a diastereomer (163b).[80] Having established that a trans-crobarbatic acid lactone (164) corresponded to the diacid portion of the alkaloid, they set about to condense a suitably protected (+)-trans-crobarbatic acid lactone (164) with (+)-retronecine (3). The protection steps were (Scheme 34) the formation of the methylene chloride soluble trimethyl aluminium salt (165) which was easily converted into the tert-butylthio ester (166) and then into the acylated imidazolide (167). Coupling with (+)-retronecine gave monoester (168) which was lactonised using the copper(I) trifluoromethanesulphonate-benzene complex.[79] This gave rise to two diastereomeric components which were separated to give (163a) and (163b).

The configurations of (163a) and (163b) were determined by hydrolysis and comparison of the Cotton curves of the acids obtained with other five-membered lactonic acids.[81] Deacylation of either of the acetates (163a) and (163b) proved to be very difficult giving only the deacylated version of (163b), in low yield. Since the authors could not obtain an authentic sample of crobarbatine or its acetate (163a), they were unable to say which of their products corresponded to the natural product.

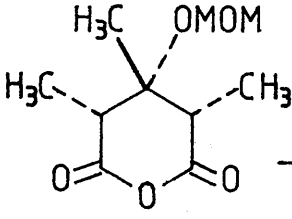
A different lactonisation method was used by Vedejs et al. in their syntheses of monocrotaline[82] (169a), fulvine (169b) and crispatine (169c).[83] Each of these used a fluoride induced cyclisation of a carboxylate with a mesylate (Scheme 35), to give the desired product



(169a) R_1=OH, R_2=CH_3, R_3=OH

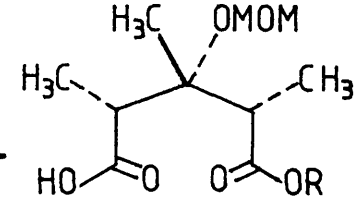
(169b) R_1=OH, R_2=CH_3, R_3=H

(169c) R_1=CH_3, R_2=OH, R_3=H

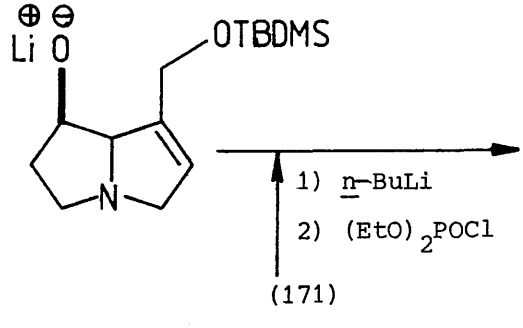


(170)

$(CH_3)_2AlOCH_2CH_2Si(CH_3)$



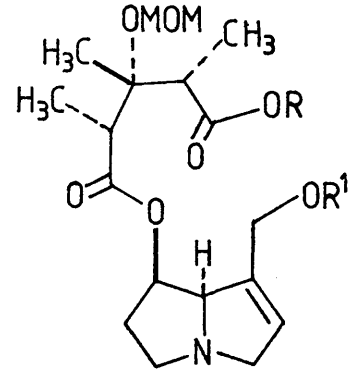
(171) R=$CH_2CH_2Si(CH_3)_3$



1) n-BuLi

2) $(EtO)_2POCl$

(171)



(173a) R=$CH_2CH_2Si(CH_3)_3$

R^1 = TBDMS

(173b) R=$CH_2CH_2Si(CH_3)_3$

R^1=SO_2CH_3

Scheme 35

in a protected form. The diacid portion for each alkaloid was converted into the protected anhydride (170), selectively ring opened to give the less hindered monoacid (171), and then activated as the phosphoric anhydride. This is shown in Scheme 35 for the crispatine synthesis. The anhydride was coupled to the protected retronecine alkoxide (172) giving the ester (173a) which was then selectively desilylated and mesylated to give the ester (173b). Treatment of this ester (173b) with tetra-n-butyl ammonium fluoride released the free carboxylate which spontaneously cyclised to give (±)-crispatine (169c) in its protected form. Deprotection gave material which was identical to the authentic natural product. The syntheses of (±)-fulvine and (±)-monocrotaline were carried out in a similar fashion.

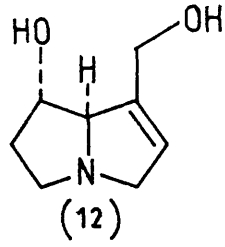
The lactonisation by intramolecular displacement of a mesylate was also used in the synthesis of integerrimine (161) by White and Ohira.[84]

All of the previous syntheses have involved the use of (±)- or (+)-retronecine. However, recently some different diols have been used to make macrocyclic diesters. A diastereoisomer of (+)-retronecine (3), (+)-heliotridine (12) has been incorporated as the base portion of a set of new 11-membered macrocyclic analogues.[85] The necine (12) was obtained by basic hydrolysis of the alkaloids extracted from *Cynoglossum officinale*. The lactonisation step was carried out after the formation of the pyridine-2-thiol-esters, giving the lactones (174a - e). All spectroscopic data were consistent with the formation of 11-membered macrocyclic diesters. An important feature in each of the ^{1}H n.m.r. spectra was the AB system due to the diastereotopic protons at C-9. The $\Delta\delta$(H-9) values were in the range 0.12 - 0.74 p.p.m.

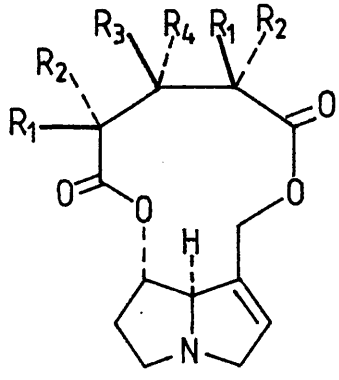
As a result of the lengthy procedures involved in both the synthesis and extraction of the unsaturated necine diols required for the above syntheses, it was decided to look for a more accessible necine or analogue. Mattocks had shown previously that 2,3-bis-(hydroxymethyl)-1-methyl-2,5-dihydropyrrole (175) (synthanecine A) could be made and that its diester derivatives caused similar damage to animal livers as that caused by pyrrolizidine alkaloids.[3]

Barbour and Robins[86] using a modified synthesis of (±)-synthanecine A (175) as shown in Scheme 36, made a series of 10-[87] and 11-membered[86] macrocyclic diesters with various substituted succinic and glutaric anhydrides. Lactonisation was achieved *via* the pyridine-2-thiol esters. Using the synthanecine A derivative, (±)-3-chloromethyl-2-hydroxy-methyl-1-methyl-2,5-dihydropyrrolium chloride (176), they made a set of different 10- and 11-membered macrocyclic diesters of synthanecine A (175).[88] In addition, using a combination of the previous two esterification methods they synthesised a set of macrocyclic diesters of (±)-synthanecine A containing 12- to 16-membered rings.[89]

Very recently, Kelly and Robins[64] have reported the synthesis of a set of macrocyclic diesters using a synthetic, saturated necine base, (+)-(1*R*, 6*R*, 8*R*)-6-hydroxy-1-hydroxymethyl-pyrrolizidine (177). The diol (177) was made by the route given in Scheme 37,[90] and was reacted with various glutaric anhydride derivatives. After lactonisation, via the pyridine-2-thiol esters, 12-membered diesters (178) - (182) were isolated. Reaction with 2,2-dimethylglutaric anhydride gave only one lactonised product but it was not possible to assign its structure (183a) or (183b) unambiguously. An 11-membered



(12)



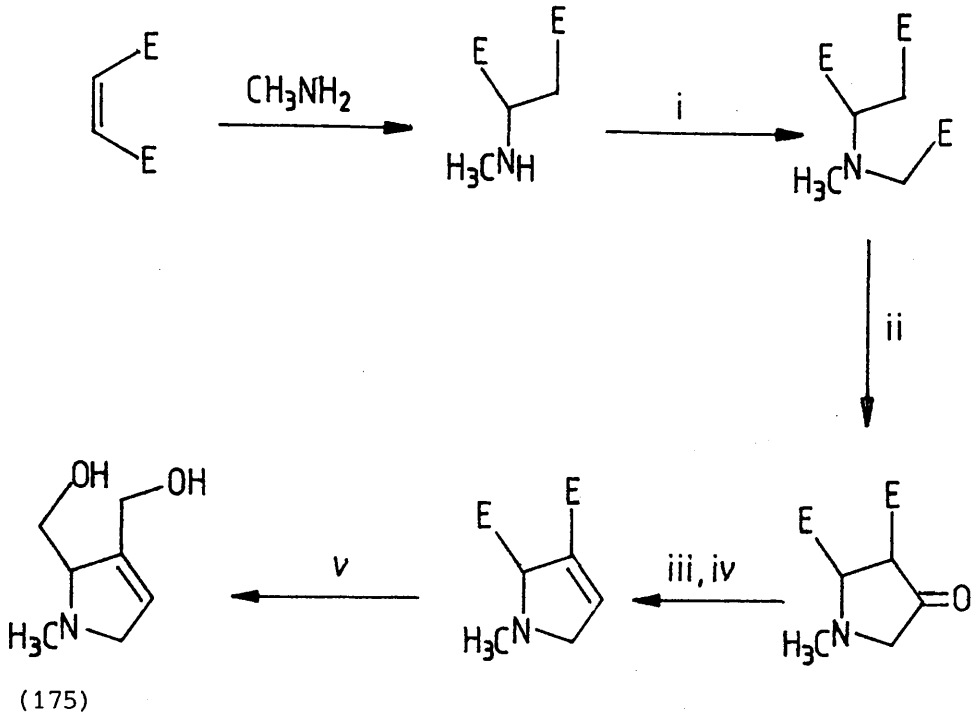
(174a) $R_1=R_2=H$, $R_3=R_4=CH_3$

(174b) $R_1=R_2=H$, $R_3R_4 = -(CH_2)_4$

(174c) $R_1=R_2=H$, $R_3R_4 = -(CH_2)_5$

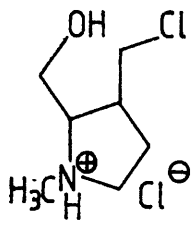
(174d) $R_1=R_2=R_3=R_4=H$

(174e) $R_1 = CH_3$ or H $\quad R_2 = H$ or CH_3 $\quad R_3=R_4=H$

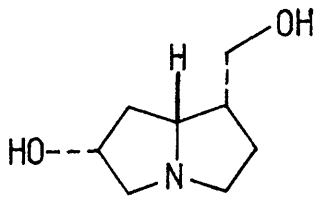


(175)

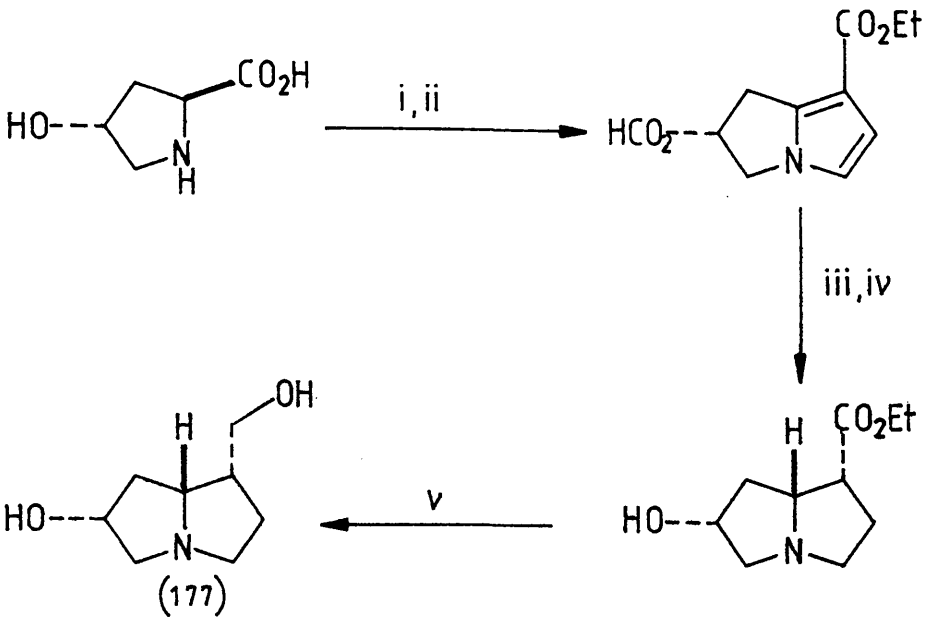
Scheme 36 : Reagents : i) $BrCH_2CO_2Et, K_2CO_3$; ii) NaH, toluene ; iii) $NaBH_4$, 2% NaOH ; iv) $4\text{-}CH_3C_6H_4SO_2Cl$, pyridine ; v) $Bu^i{}_2AlH$.



(176)



(177)



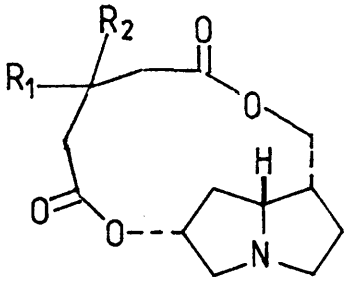
Scheme 37

Reagents: i) Ac_2O, HCO_2H

ii) Ac_2O, $HC{\equiv}CCO_2Et$

iii) NH_3 ; iv) $H_2/Rh/C$

v) $LiAlH_4$

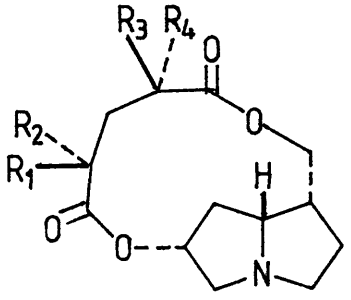


(178) $R_1{=}R_2{=}H$

(179) $R_1{=}R_2{=}CH_3$

(180) $R_1R_2{=}$ $(CH_2)_4$

(181) $R_1R_2 = (CH_2)_5$



(183) a) $R_1{=}R_2{=}CH_3$, $R_3{=}R_4{=}H$

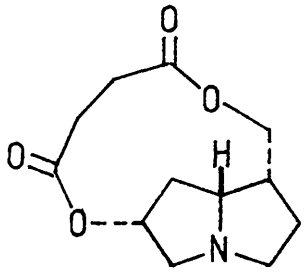
b) $R_1{=}R_2{=}H$, $R_3{=}R_4{=}CH_3$

(182) $R_1{=}R_3{=}$ H or CH_3 , $R_2{=}R_4{=}$ CH_3 or H

lactone (184) was also produced by reaction of the diol (177) with succinic anhydride.

4.3 Conclusions

With the growth in the number of synthetic routes to macrocyclic pyrrolizidine diesters, the way is now clear for both the synthesis of the more complex alkaloids and of biologically interesting analogues. Through such syntheses, a better understanding of the chemistry and biochemistry of the pyrrolizidine alkaloids should result.



(184)

To this end, this volume contains a description of the synthesis of a set of new pyrrolizidine alkaloid analogues. These analogues are discussed in Chapter 8.

CHAPTER 5.

THE BIOSYNTHESIS OF EMILINE

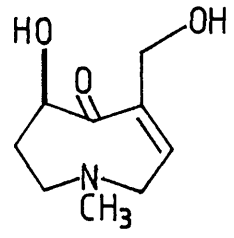
5.1 Introduction

Until recently, retronecine (3) was the only necine to have its biosynthesis investigated in any detail. Some studies have been reported on a few other necines, particularly the saturated necine triol rosmarinecine (10)[28,35,36] but there have been no reports on the biosynthesis of the seco-pyrrolizidine base, otonecine (14). Therefore, it was decided to start a detailed study on the biosynthetic pathway to this unusual base. The alkaloid chosen for this work was the macrocyclic diester, emiline (22).

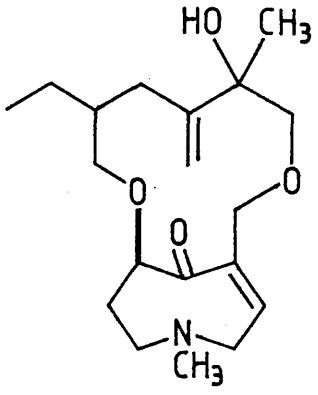
5.2 Emiline.

The diester emiline (22) is the major alkaloid constituent of the plant *Emilia flammea* Cass. (family Compositae),[91,92] and a revised structure was published by Barbour and Robins.[93] In some recent work, Kelly et al.[94] showed that (±)-trachelanthamidine (6) is an efficient precursor for emiline (22), whereas the diastereoisomer (±)-isoretronecanol (5) is not. This observation is the same as that for the biosynthesis of retronecine (3) in *S. isatideus*[28] and *S. riddellii*[95] and is the opposite of that observed for rosmarinine in *S. pleistocephalus*.[28] These results would appear to indicate a link between the biosynthetic pathways to retronecine (3) and otonecine (14), but the pathway to rosmarinecine (10) is divergent from these. However, it was essential to establish first the early steps in the pathway.

To enable a detailed study of the emiline biosynthetic pathway



(14)



(22)

Signal irradiated	% increase in signal integrals									
	2	3a	3b	5a	5b	6a	6b	7	9a	9b
2	-	1.72	2.53	0	0	0	1.36	0	1.04	5.66
3a	1.64	-	8.59	0	1.92	0	0.62	0	0	0
3b	2.85	7.50	-	0	0	0	0.47	0	0	0
5a*	-	-	-	-	-	-	-	-	-	-
5b	0	2.29	0.11	>40	-	7.20	0.50	0.54	0	0
6a	0	0.28	0	1.43	-	-	2.26	3.37	0	0
6b	0	0	0	7.98	1.32	1.94	-	0.72	0	0
7	0	0	0	0	0	2.71	1.70	-	0	0
9a	1.53	0	0	0	0	0	0	0	-	67.6
9b	5.47	0	0	0	0	0	0	0	64.4	-

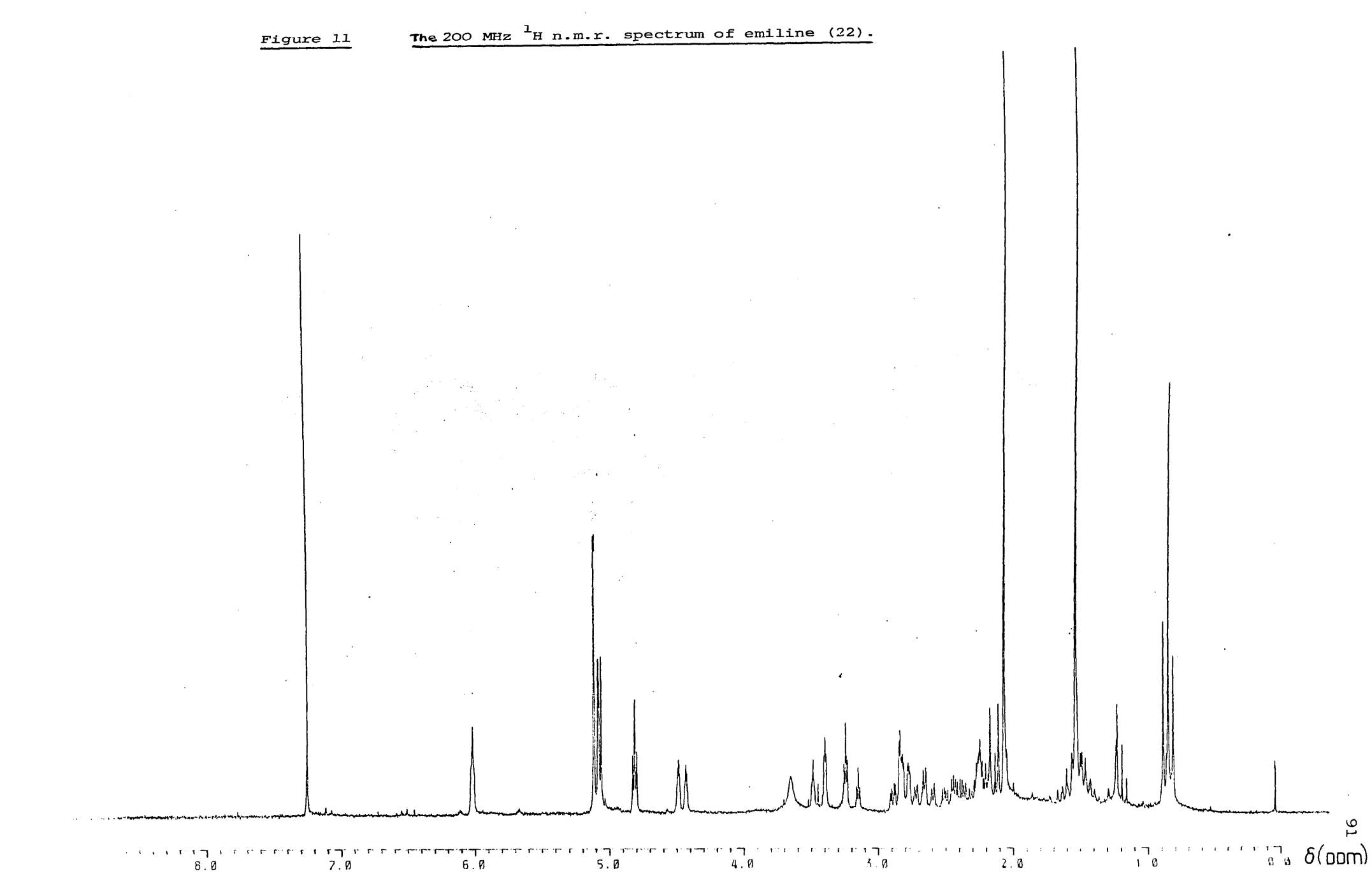
*Signal 5a was not irradiated due to technical difficulties.

Table 3.

to take place, using ^{2}H-labelled precursors, it was essential to assign each of the H-atoms on the otonecine (14) portion of emiline (22) to the correct signal in the ^{1}H n.m.r spectrum. A high field spectrum of the alkaloid (22) is shown in Figure 11. It has been shown from X-ray crystallographic studies,[96] that 12-membered alkaloids which contain otonecine (14) adopt a solid-state conformation which is similar to that of other 12-membered alkaloids which contain retronecine (3), such as retrorsine (17). Also ^{1}H n.m.r spectroscopic data[31] and X-ray crystallographic studies[97] on retrorsine (17) have shown that the pyrrolizidine alkaloid conformation is similar in organic solution and in the solid state. 12-Membered otonecine alkaloids, such as emiline (22) can adopt a similar conformation via a trans-annular interaction of the N-4 and C-8 atoms of the eight-membered ring.

The best method available to assist in the ^{1}H n.m.r spectroscopic assignments was the use of nuclear Overhauser enhancement (n.O.e) difference spectroscopy, using the NEOMULT.AU program.[98] The results of these n.O.e. experiments are shown in Table 3. In addition, Figure 12 shows graphically the important nuclear interactions. The irradiation at 6.02 p.p.m. (H-2) produced an enhancement of 2.53% at 3.20 p.p.m but only 1.72% at 3.44 p.p.m. This observation along with comparison with models and known assignments for retrorsine indicate that the absorption at 3.44 p.p.m is due to the 3β-hydrogen while the absorption at 3.20 p.p.m is due to the 3α-hydrogen. Similarly, irradiation at 3.44 p.p.m (H-3β) produced an enhancement at 2.65 p.p.m. but not at 2.85 p.p.m. This suggests that the signal at 2.65 p.p.m is due to the 5β-hydrogen. In the same way, the 5α-, 6α- and 6β-hydrogens were assigned. Also, the

Figure 11 The 200 MHz ^{1}H n.m.r. spectrum of emiline (22).



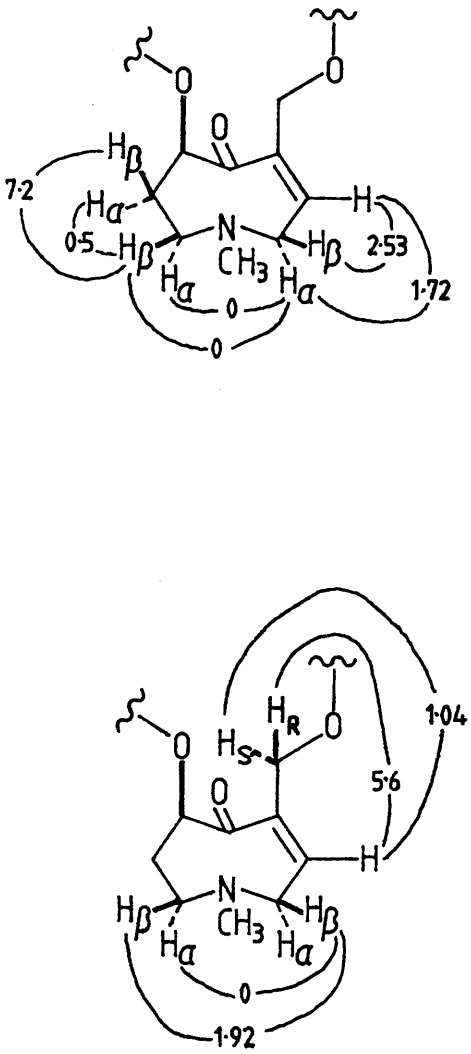


FIGURE 12.

C-9 pro-S and pro-R H atoms were assigned from their interactions with the C-2 proton.

Each of the n.O.e. interactions used in the ^{1}H n.m.r spectral assignments was also observed in a Homonuclear (^{1}H-^{1}H) NOESY experiment, which confirms that the observed n.O.e's were real and not experimental artefacts.

5.3 Incorporation of Radiolabelled Precursors into Emiline (22)

The experiments described in this section were initially carried out on *E. flammea* plants. The first precursor fed was [1,4-^{14}C]-putrescine dihydrochloride, which was administered by the wick method. The stem of the plant was threaded with needle and cotton, and the cotton ends were immersed in a vial containing an aqueous solution of the labelled compound. After feeding, the plant was left for a week, harvested and the alkaloid was extracted. The emiline obtained was recrystallised[93] to constant specific activity. A specific incorporation of ca 0.5%* was observed in the emiline extracted, which was rather disappointing. In addition, the amount of emiline from each plant was very low : ca 100g of fresh plant leaves eventually yielded only ca 5 mg of pure recrystallised alkaloid. While these figures did not make biosynthetic analysis impossible, it was clear that any experiments involving stable isotopes might be difficult in terms of detection by n.m.r spectroscopy.

*Specific radiolabel incorporation per C_4 unit for a putrescine precursor was calculated from [(molar activity of rosmarinine (44) x ½)/(molar activity of precursor)] x 100%.

Shortly after the first few plant experiments had been attempted, a new technique for obtaining the alkaloid emiline (22) became available. Dr. N. Walton at the AFRC Food Research Institute, Norwich provided a sample of hairy root culture[99] derived from *E. flammea*. These cultures can be grown in bulk amounts (1.5 L medium leading to ca 600 g total weight per batch) in a relatively short time (typically three weeks). In a preliminary test feeding with [1,4-^{14}C]-putrescine (27), 25 mg of emiline was obtained from a 600 g batch of hairy root cultures. More importantly, this sample had a specific incorporation of ^{14}C per C_4 unit of 4.4%, which was much better than the incorporations obtained from plants. It was decided, therefore, that the future experiments would involve hairy root cultures instead of plants.

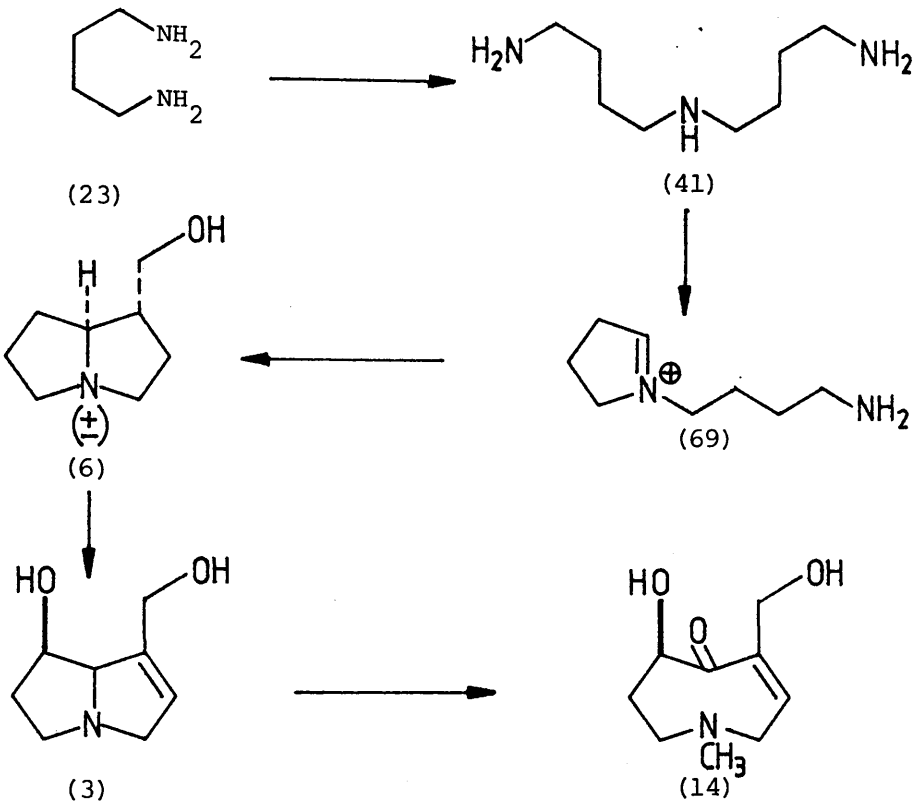
In order to find out the best conditions for feeding, two sets of experiments were carried out. The first of these was to establish how much of a precursor should be used in each feed. Radiolabelled precursors were usually fed as high specific activity materials but for work with precursors labelled with stable isotopes, the amount fed could be critical. Therefore, batches of 10, 30 and 100 mg of unlabelled putrescine were fed to three separate batches of root cultures along with a sample of [1,4-^{14}C]-putrescine (27). Feedings were carried out by dividing the samples among the flasks containing the root cultures. It was found that the specific incorporations of ^{14}C (2.8, 4.0 and 3.2%) were not affected by the amount of material fed. The value of 30 mg was chosen as a suitable amount, as a balance of having enough label present to be incorporated and not overloading the cultures and possibly wasting valuable or scarce precursors.

The second set of experiments was to investigate how important the timing of feeding and extraction would be to the yield of alkaloid and the incorporation of label. So, a sample of [1,4-^{14}C]-putrescine was fed to a batch of root cultures after 7 days of growth. At 11 days growth (that is, 4 days after feeding) part of the culture batch was extracted and the emiline obtained was examined for radiolabel. It was found that there was 4 mg of alkaloid with a specific incorporation* of 5.4% per C_4 unit. At 15 days growth the emiline extracted from a further batch of the culture had a weight of 15 mg, with specific incorporation* 3.5% per C_4 unit. Finally, the remainder of the batch was taken off at 22 days growth. Extraction gave 5 mg of alkaloid with a specific incorporation* of 0.32%. Clearly the ideal timing of the experiment was to extract the cultures at around a week after feeding. This could give rise to reasonably high incorporations while giving a convenient amount of alkaloid. The cultures were fed at seven days old because this was the start of their rapid growth phase.

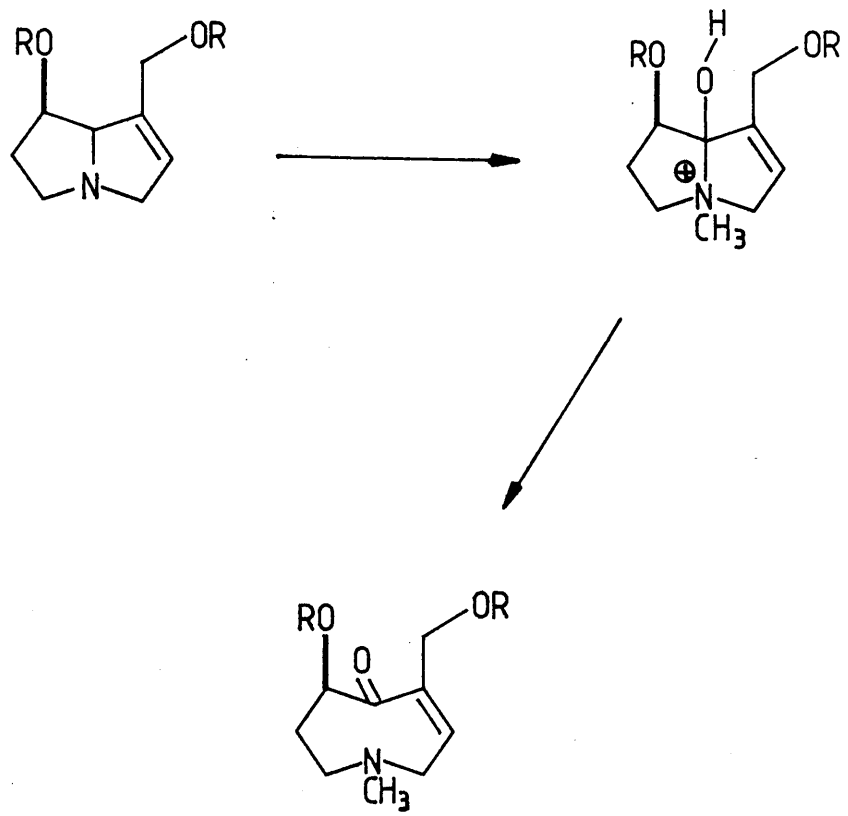
The next important requirement was to measure the relative incorporation of ^{3}H and ^{14}C in putrescine samples. Such a measurement was necessary because later experiments would use ^{3}H- or ^{14}C-labelled putrescines as references in double label feeding experiments. Thus, [1,4-^{3}H]- and [1,4-^{14}C]-putrescine dihydrochloride were mixed to give an initial ^{3}H/^{14}C ratio of 12.0 and the mixture was fed to *E. flammea* hairy root cultures. After 10 days the roots were extracted to give 21 mg of emiline with a ^{14}C specific incorporation* of 1%. The ^{3}H/^{14}C ratio was 10.6 showing that ^{3}H- and ^{14}C-labelled materials were incorporated to about the same extent.

The next objective was to establish the intermediacy of the triamine homospermidine (41) on the biosynthetic pathway. This compound is an intermediate in retronecine (3) biosynthesis. The labelled version of the molecule to be used was [1,9-^{14}C]homospermidine, made by Dr. S.K. Ner using the method of Khan and Robins.[100] So, a sample of [1,9-^{14}C]homospermidine (0.12 μCi/mg) was mixed with [1,4-^{3}H]putrescine (0.31 μCi/mg), giving a ^{3}H/^{14}C ratio of 1.1 and fed to hairy root cultures of *E. flammea* in the usual way. The emiline obtained from this experiment showed a ^{14}C specific incorporation* of 3.6% with a ^{3}H/^{14}C ratio of 0.9. This indicates that homospermidine is incorporated into emiline with similar efficiency to putrescine. In this last feeding the specific activities of the two precursors were adjusted to similar values, in order to provide a more valid comparison of relative incorporations.

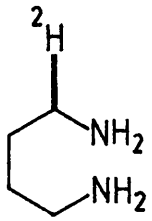
The intermediacy of trachelanthamidine (6) and the iminium ion (69)[94] had already been shown. The last important test was to see if retronecine (3) itself was a specific precursor of emiline (22). A sample of ^{3}H-labelled retronecine was obtained biosynthetically by feeding [1,4-^{3}H]putrescine to *Senecio isatideus* to produce ^{3}H-labelled retrorsine. Hydrolysis afforded ^{3}H-labelled retronecine. This was mixed with a sample of [1,4-^{14}C]putrescine so that a ^{3}H/^{14}C ratio of 9·8 was obtained. Feeding this sample to *E. flammea* root cultures produced a sample of emiline with a ^{3}H specific incorporation* of 2.7 % and a ^{3}H/^{14}C ratio of 10·7 . So retronecine (3) is an efficient precursor of emiline (22) (Scheme 38). This confirms that in the formation of otonecine (14), the (N-4)-(C-8) bond of pyrrolizidines (6) and (3) is cleaved. This may occur by hydroxylation at C-8 and N-methylation of retronecine (3),



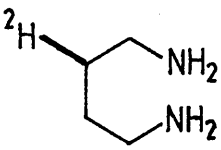
Scheme 38



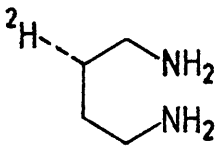
Scheme 39



(185)



(60)



(61)

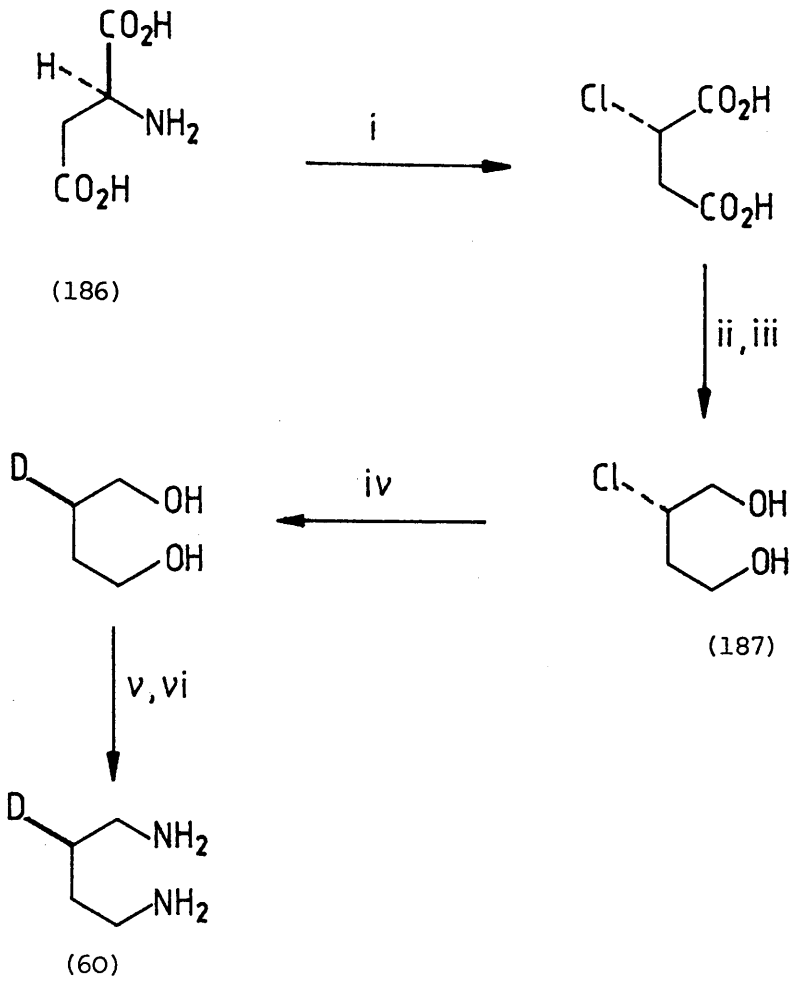
or a derivative, followed by ketone formation with cleavage of the bridging C-N bond (Scheme 39).

In all of the above experiments, the radioactive emiline samples obtained were hydrolysed with acid to give otonecine (14) as the hydrochloride.[101] More than 94% of the ^{3}H and ^{14}C radioactivity was located in otonecine in each case.

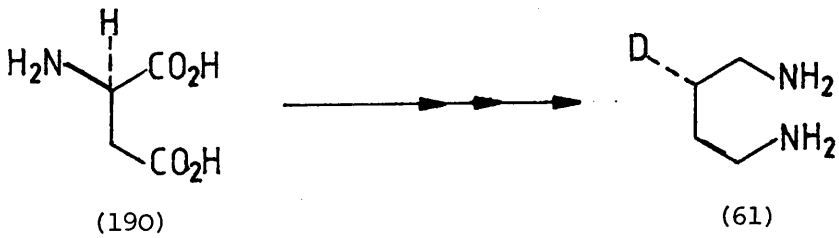
5.4 Incorporation of ^{2}H-Labelled Putrescines into Emiline (22).

Using a combination of ^{2}H-labelled precursors with ^{2}H n.m.r spectroscopy it was possible to examine the stereochemistry of the enzymic processes involved in emiline (22) biosynthesis. The ^{2}H-labelled derivatives of putrescine chosen were the mono-deuteriated compounds (185), (60) and (61).

The (R)-[1-^{2}H]putrescine (185) was made by the enzymatic decarboxylation of L-ornithine (21) in $^{2}H_{2}O$ by L-ornithine decarboxylase.[32] The (2R)-[2-^{2}H]- and (2S)-[2-^{2}H]-putrescines, (60) and (61) respectively, were formed by modifications of the route of Kunec and Robins[34] (Schemes 40 and 41). Starting from (2S)-aspartic acid (186), chlorination, methylation and reduction gave rise to the (2S)-2-chlorobutanediol (187). Reaction with $LiAl^{2}H_{4}$ gave the (2R)-[2-^{2}H]-butane-1,4-diol. In a new strategy, the hydroxyl groups were converted into amino groups using a procedure described by Fabiano and Golding.[102] This involved a Mitsunobu-type[103] reaction to give the azide (188) and an *in situ* Staudinger reaction[104] to give a phospho-imine species such as (189). Aqueous acidic hydrolysis gave the product, (2R)-[2-^{2}H]putrescine (60) as its dihydrochloride,



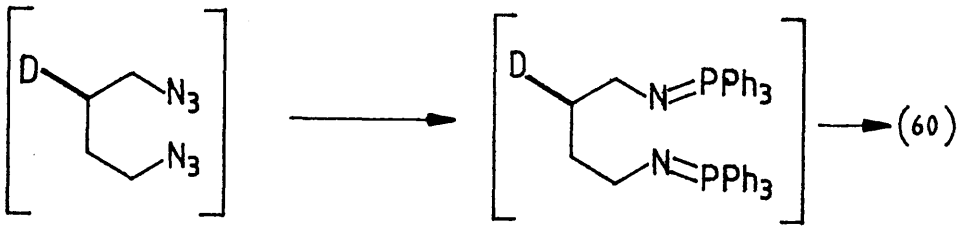
Scheme 40 : i) HCl, HNO_3; ii) MeOH, $SOCl_2$; iii) DIBAL;
iv) $LiAl^2H_4$; v) HN_3, $iPrOCON{=}NCO_2iPr$, Ph_3P; vi) H_2O/HCl



Scheme 41

in a reliable 10.6 % yield from aspartic acid.

The enantiomeric (2S)-[2-^{2}H]putrescine (61) was made in the same way from (2R)-aspartic acid (190) (Scheme 41).



(188) (189)

Feeding the (R)-[1-^{2}H]putrescine (185) to *E. flammea* root cultures gave rise to a sample of emiline (191) containing ^{2}H label. The ^{2}H-{^{1}H} n.m.r spectrum of this sample showed signals at δ_D 3.4 (C-3β), 2.8 (C-5α) and 5.0 (C-9 pro S) (Figure 13a-c). Figures for the specific incorporations of ^{2}H were estimated from ^{2}H-{^{1}H} n.m.r spectrum by comparison of the concentration of sample with the size of the natural abundance peaks for ^{2}H in $CHCl_3$. For each ^{2}H feeding experiment the specific incorporation of ^{2}H was comparable to that of ^{14}C. For the feeding of (R)-[1-^{2}H]putrescine (185) the ^{14}C specific incorporation was 3.8%.

Feeding the (2R)-[2-^{2}H]putrescine (60) gave an emiline sample (192) which had a ^{2}H-{^{1}H} n.m.r spectrum with peaks at δ_D 2.4 (C-6α) and 6.0 (C-2). Feeding the (2S)-enantiomer (61) gave a sample of emiline (193) with ^{2}H-{^{1}H} n.m.r spectral peaks at δ_D 2.2 (C-6β) and 4.81 (C-7α) (Scheme 43). ^{14}C-Specific incorporations for each were 3.4 and 3.5% respectively.

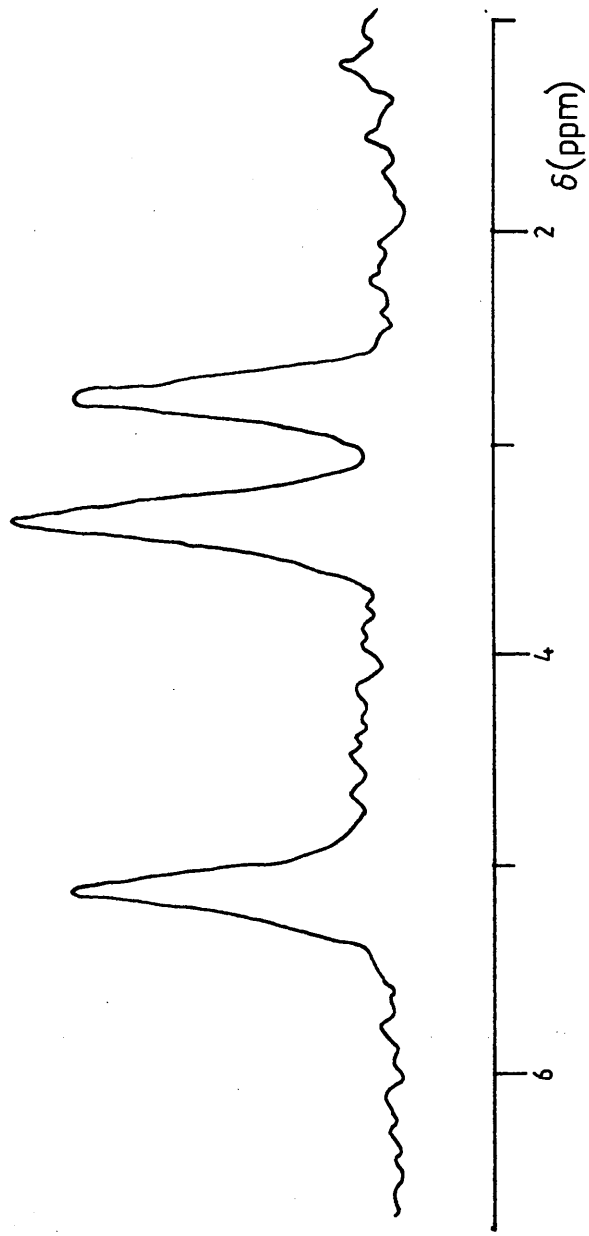


Figure 13a. -30 MHz ^{2}H n.m.r. spectrum of emiline (191) fed with (1R)-[1-^{2}H] putrescine.

Figure 13(b) -30 MHz n.mr. spectrum of emiline (192) fed with (2R)-[2-^{2}H] putrescine.

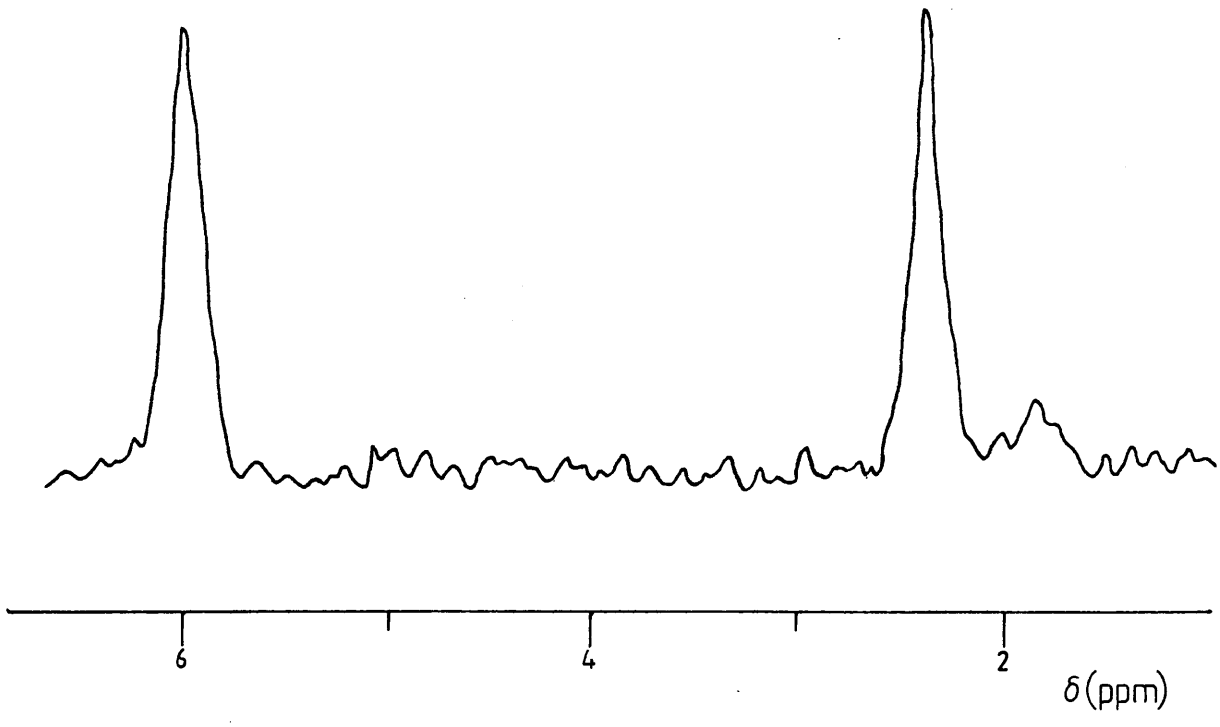
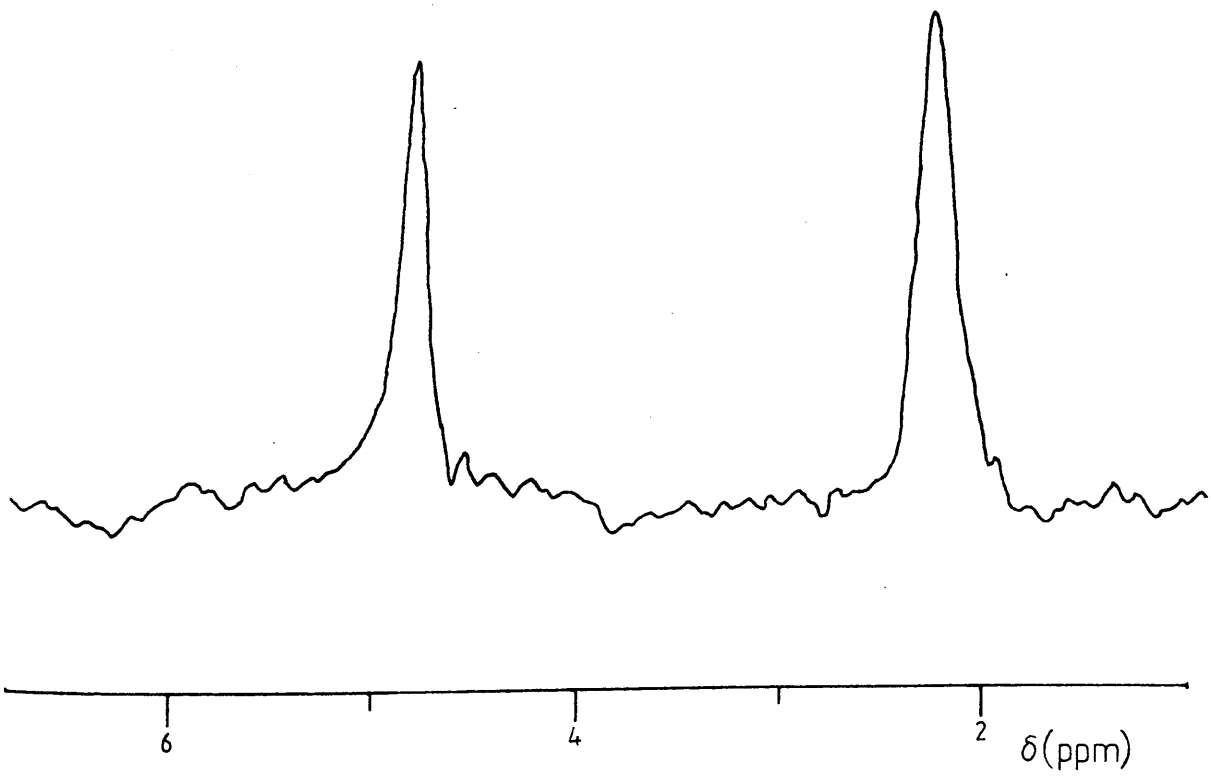
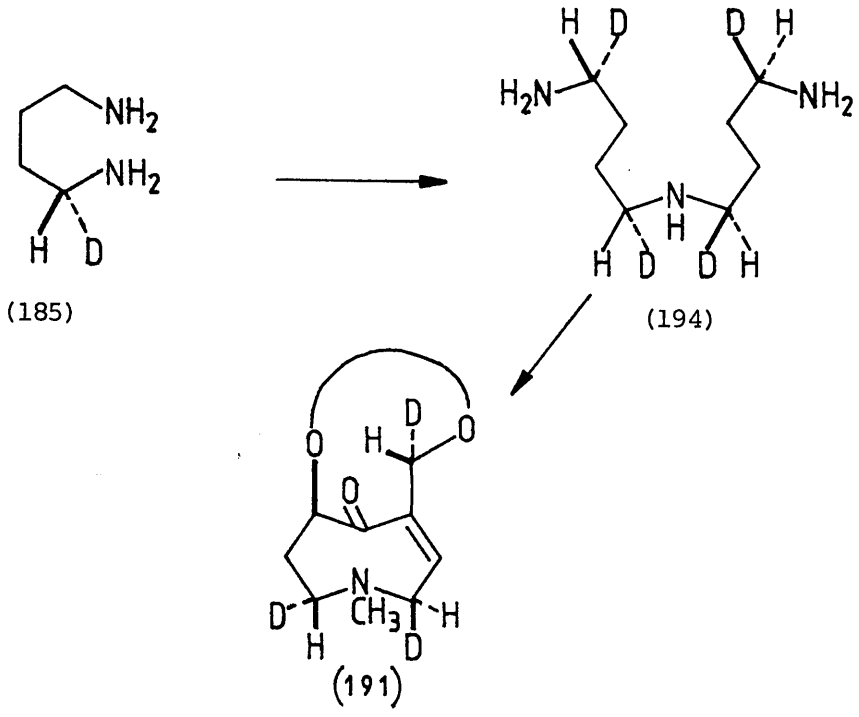
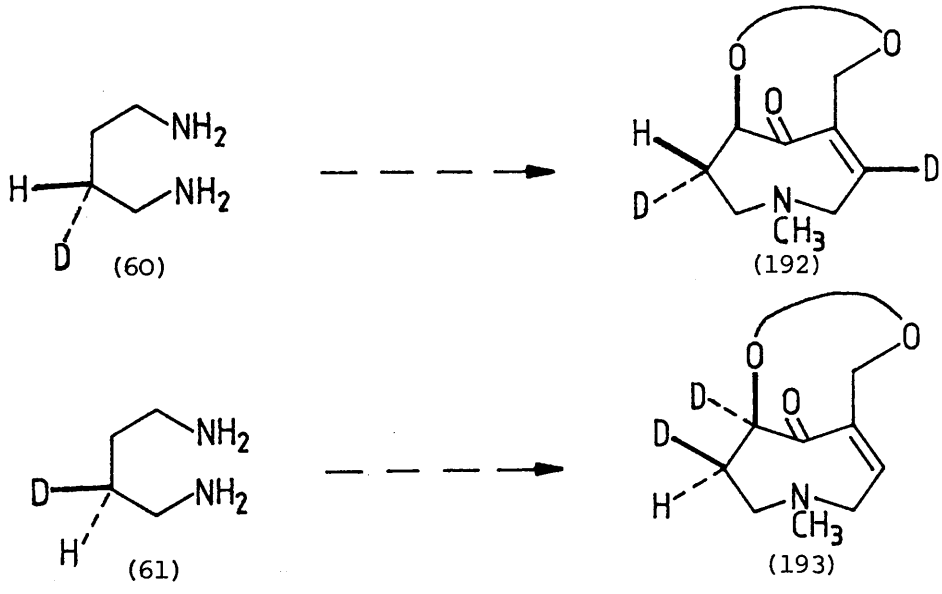


Figure 13(c) -30 MHz n.m.r. spectrum of emiline (193) fed with (2S)-[2-^{2}H] putrescine.





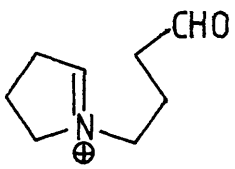
Scheme 42



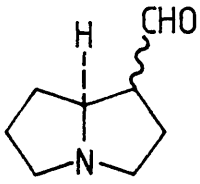
Scheme 43

These observations can be rationalised in the following way.

Putrescine (23) is oxidised to 4-aminobutanal stereospecifically with loss of the 1-pro-S and retention of the 1-pro-R hydrogens. Coupling with a further molecule of putrescine gives an imine which is reduced to give a labelled homospermidine, such as (194). The labelling in the emiline sample (191) from the (R)-isomer (185) indicates that this reduction occurs from the C-si face of the imine. Two more oxidations on homospermidine lead to loss of the pro-S hydrogens at C-1 and C-9 to give the iminium species (47). This cyclises by attack on the C-re face of the double bond to give the 8α-pyrrolizidine aldehyde (195). Reduction of this aldehyde to trachelanthamidine (6) occurs by attack of the hydride equivalent on the C-re face of the carbonyl group. Finally, the hydroxylation at C-7 of emiline proceeds with retention of stereochemistry, while



(47)



(195)

the formation of the 1,2-unsaturation takes place with retention of the 2-pro-R hydrogen of putrescine.

The stereochemistry of the biosynthetic pathway to emiline has been partially elucidated using enantiomerically deuteriated compounds.

This work could be continued in more detail to look at the later steps in the pathway by using ^{2}H-labelled versions of the

later intermediates.

Note:- none of the putrescine precursors (185), (60) or (61) contain more than one ^{2}H atom. The labelling patterns shown in Schemes 5 and 6 are therefore composite representations of all ^{2}H-labelled species present.

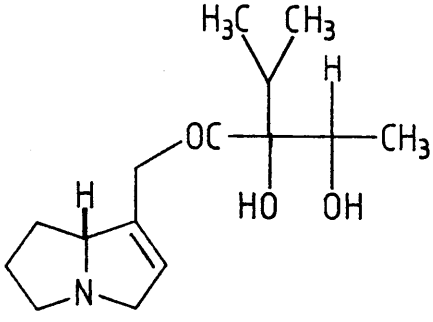
5.5 Biosynthetic Studies on Other Plants.

In order to widen the scope of plants that can be used in the elucidation of the pyrrolizidine biosynthetic pathways, it is necessary to examine the alkaloid content of some plants to see if they are suitable for experimentation. One such plant is *Cynoglossum australe* which is known to contain the alkaloids cynaustine (196) and cynaustraline (197).[105] Previous ^{14}C-labelling experiments with *C. australe* plants gave low incorporations (0 - 0.5%).

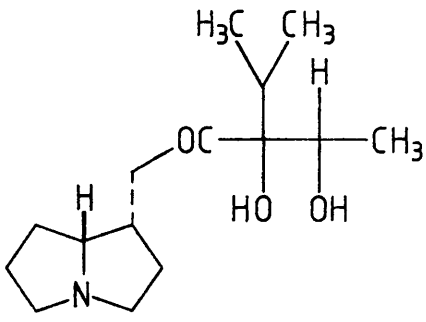
It was hoped that using the hairy root culture method would give better results but it was found that [1,4-^{14}C]putrescine was not incorporated to any great extent (<0.2%).

Wink had reported that in plants producing quinolizidine alkaloids, wounding of the leaves of the plants caused a sharp rise in alkaloid production.[106] An attempt to use a similar technique on *C. australe* failed to increase the incorporation figure.

Clearly, *C. australe* is not an easy plant to use for biosynthetic studies, so an alternative was sought.



(196)

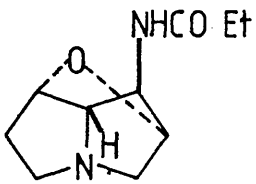


(197)

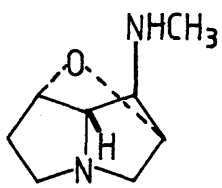
Adenocarpus decorticans Boiss, a member of the family Leguminosae, was reported to contain the alkaloid decorticasine[107] (198). This alkaloid is one of the loline (18) group, with the rather unusual cyclic ether function. There are only a few alkaloids of this type, and no biosynthetic studies have been carried out on them.

It is known also that this species contains the quinolizidine alkaloid, sparteine (199).

Therefore, it was decided to examine the alkaloid content of *A. decorticans* to find out if it would be suitable for use in biosynthetic experimentation.



(198)



(18)

Seeds of A. decorticans were obtained from Kew Botanic Gardens and a supply of plants was grown in open ground and harvested before flowering. The fresh plant material was extracted with methanol and the extract was taken up in dilute acid solution. After washing with chloroform, the solution was stirred with Zn powder to remove any N-oxides, then filtered, basified and extracted with dichloromethane. This procedure gave one alkaloid only in 0.4% yield based on the weight of dry leaves.

A t.l.c of the alkaloid on silica, eluting with $CHCl_3$:MeOH:NH_3 (85:14:1) gave a single spot at R_F 0.11 when developed with

Dragendorff's reagent.[108] The infra-red spectrum showed no carbonyl peaks and one strong peak at 1445 cm^{-1}. A high resolution mass spectrum showed the molecular formula to be $C_{15}H_{26}N_2$. This data itself indicated that the alkaloid was not a pyrrolizidine, and certainly not decorticasine (198). The true identity of the compound became apparent from the ^1H and ^{13}C n.m.r spectra. All of the peaks in the ^1H n.m.r spectrum lie below 2.50 p.p.m and are all fairly broad. An interesting point is that when the ^1H n.m.r spectrum is run in deuteriochloroform the signals are much broader than in the spectrum run in d_6-benzene. This may be due to the small amount of acid present in the deuteriochloroform leading to partial protonation of the nitrogen atoms. The only notable features of the proton resonance spectrum were the doublet splittings at 0.7, 2.03 and 2.22 p.p.m. The 50 MHz ^{13}C n.m.r spectrum, running a DEPT sequence, showed eleven methylene substituted carbons, four methine substituted carbons and no others.

The above spectra were identical to those of a sample of sparteine (199). The optical rotation value of this isolated sample showed that it was (-)-sparteine, an alkaloid more usually found in the *Lupinus* species particularly *Lupinus luteus* and *L. angustifolius*.

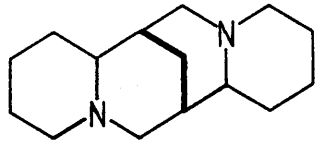
Since there are already other plants available for the biosynthetic study of (-)-sparteine (199), it was decided to stop the work on *A. decorticans* at this point.

It has been suggested that loline alkaloids, such as decorticasine (198) are formed by fungi which infect certain plants.[2]

This may explain our failure to isolate decorticasine (198) from healthy *A. decorticasine*.

5.6 Conclusions.

The results contained within this Chapter have helped to widen the scope of the biosynthetic knowledge on the pyrrolizidine alkaloids. This includes some new work on the previously unexamined necine (14).



(199)

CHAPTER 6

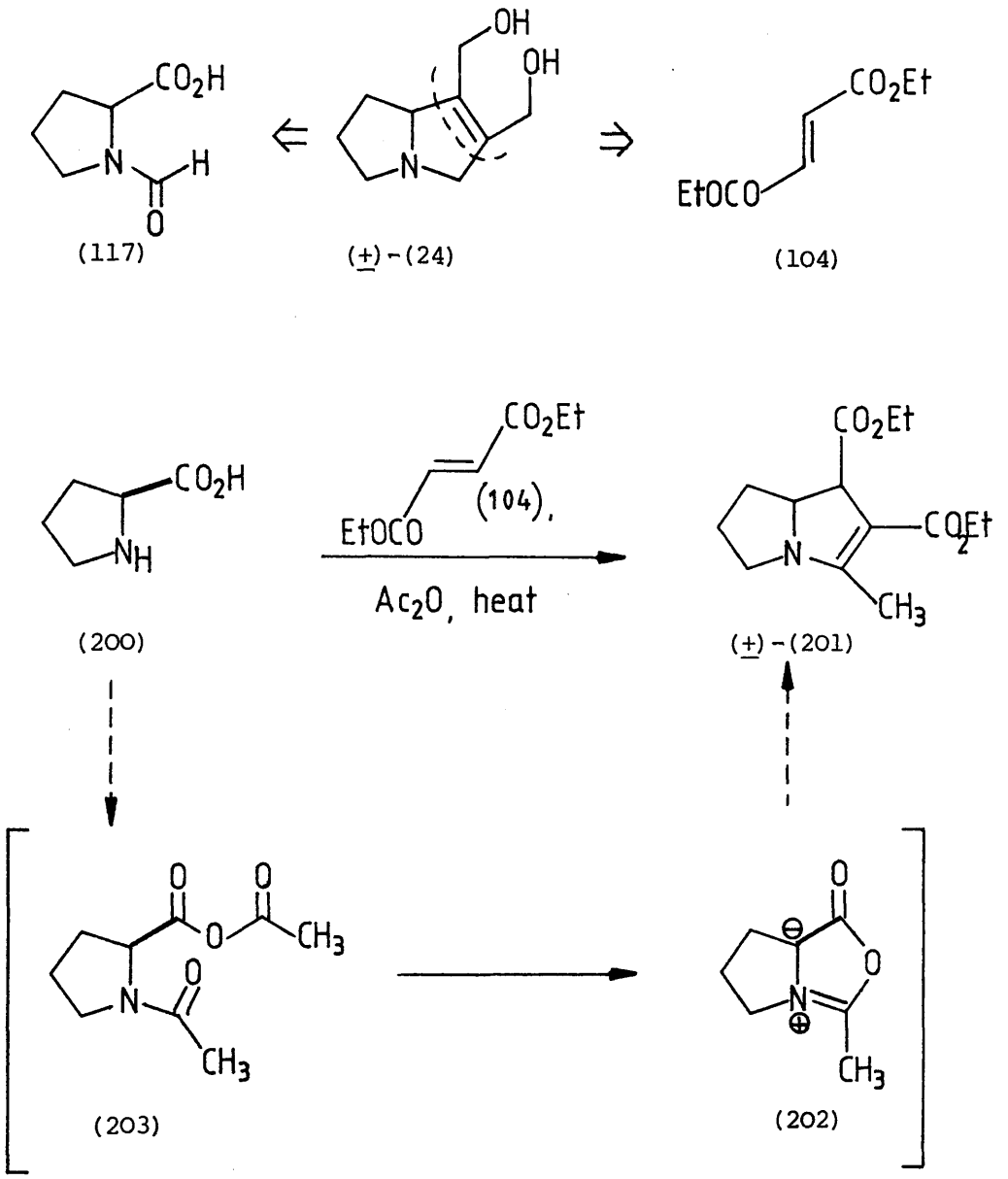
SYNTHESIS OF ANALOGUES OF NECINE BASES

6.1 A New Necine Analogue

As discussed previously the pyrrolizidine alkaloids with the most interesting biological activity are those which carry an allylic ester functionality, such as in retrorsine (17). Most of the pyrrolizidine alkaloid analogues that have been made contain retronecine (3), as the base portion. It was, therefore, envisaged that a useful target from a bio-activity point of view would be the diol (24). Ester derivatives of this diol could be assessed for heptatoxic activity, whereas N-oxides of these ester derivatives might possess anti-tumour activity.

Retrosynthetic analysis of structure (24) suggests that it might be constructed using a 1,3-dipolar cycloaddition reaction between N-formyl-L-proline (117) and a symmetrically substituted alkene. The best dipolarophile is likely to contain ester groups which could subsequently be reduced to the required diol (24). This approach to necines has been discussed in Chapter 3.

Reactions of this type were first reported by Gotthardt and Huisgen in their study of N-substituted oxazolium 5-oxides as 1,3-dipoles.[109] They noticed that by heating L-proline (200) and diethyl fumarate (104) at reflux in acetic anhydride, a bicyclic product (201) was obtained. This was formed by cycloaddition of the oxazolium species (202) with diethyl fumarate (104). The 1,3-dipole (202) was formed after cyclisation of the diacyl L-proline derivative (203) (Scheme 44).


CO2H
N
H
O
OH
OH
CO2Et
EtOCO
(117)
(±)-(24)
(104)
CO2Et
EtOCO
(104),
Ac2O, heat
(200)
(±)-(201)
CH3
(203)
(202)

Scheme 44.

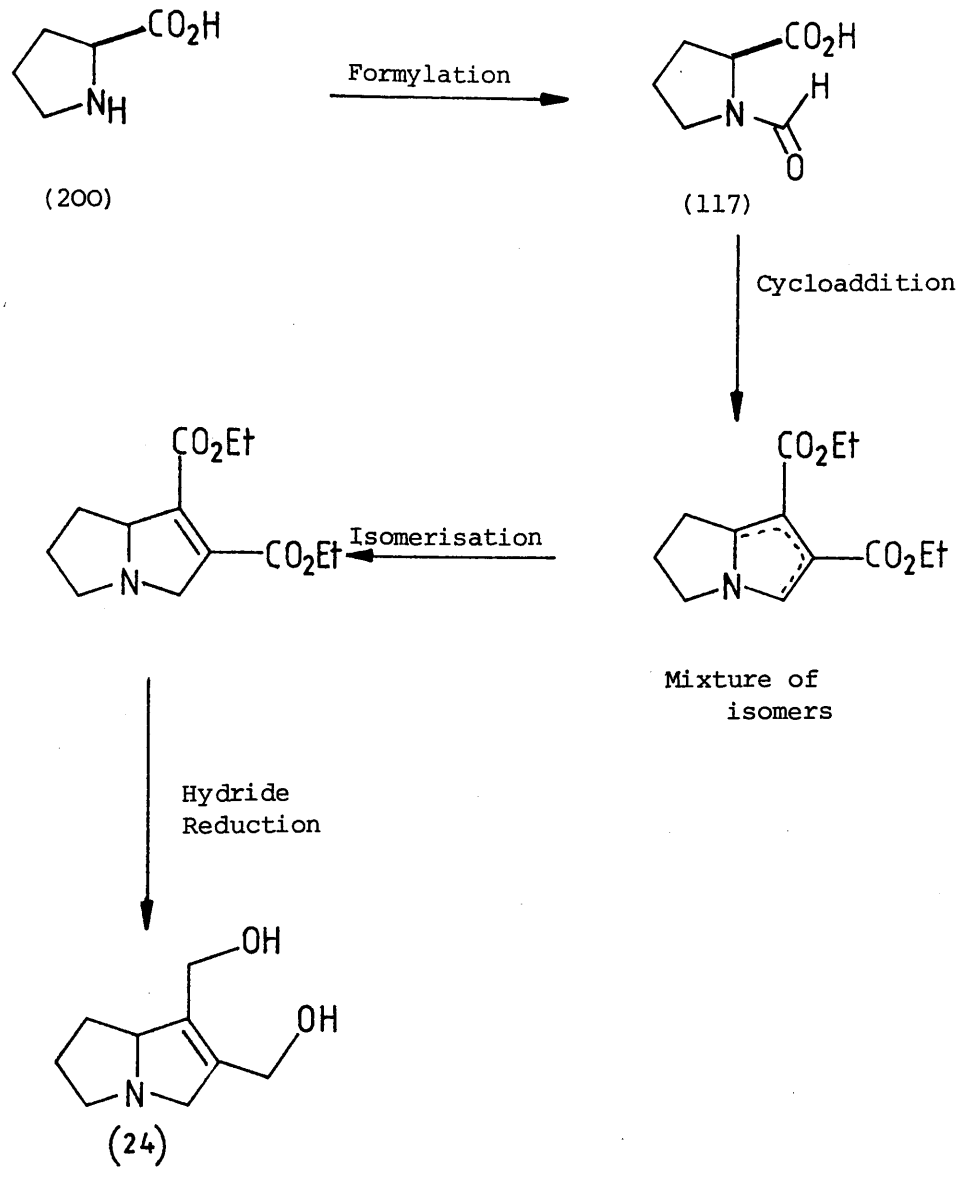
Several years later, Sakdarat in his work on the synthesis of (±)-supinidine noted that the reaction of N-formyl-L-proline (117), with diethyl fumarate (104) gave a mixture of three major compounds.[110] Clearly, Huisgen's observation that only the 2-pyrroline is formed does not apply in this case.

The overall route that was chosen is shown in Scheme 45.

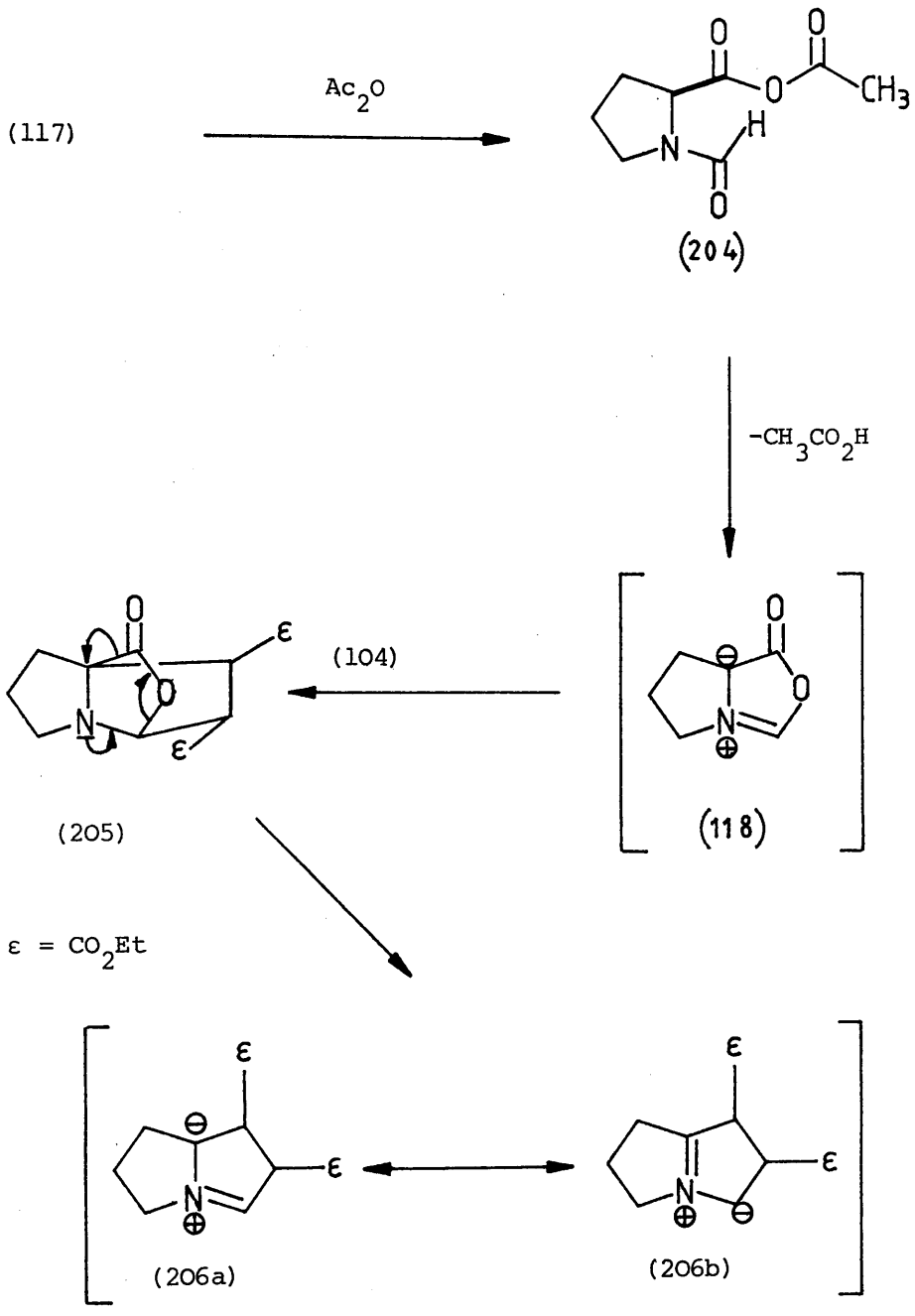
The first step was a simple N-formylation of the readily available L-proline (200) and this proceeded in high yield. There was some difficulty in obtaining a high purity of this material as it was often difficult to remove traces of acetic acid which were invariably present. Multiple crystallisation, at least three times, although expensive in terms of material, gave the best results.

The 1,3-dipolar cycloaddition reaction was carried out by heating at reflux a solution of N-formyl-L-proline (117) and diethyl fumarate (104) for several hours. The probable mechanism for this reaction is shown in Scheme 46. Initially, the N-acyl amino acid forms the mixed anhydride (204) by acylation with acetic anhydride. This diacylated species (204) spontaneously cyclises to afford the azomethine ylide (118) which contains a 1,3-dipole. Cycloaddition with diethyl fumarate (104) gives the initial adduct (205) which, under the reaction conditions, loses CO_2 *via* a *retro*-1,3-dipolar process generating a new azomethine ylide represented by (206a) and (206b).

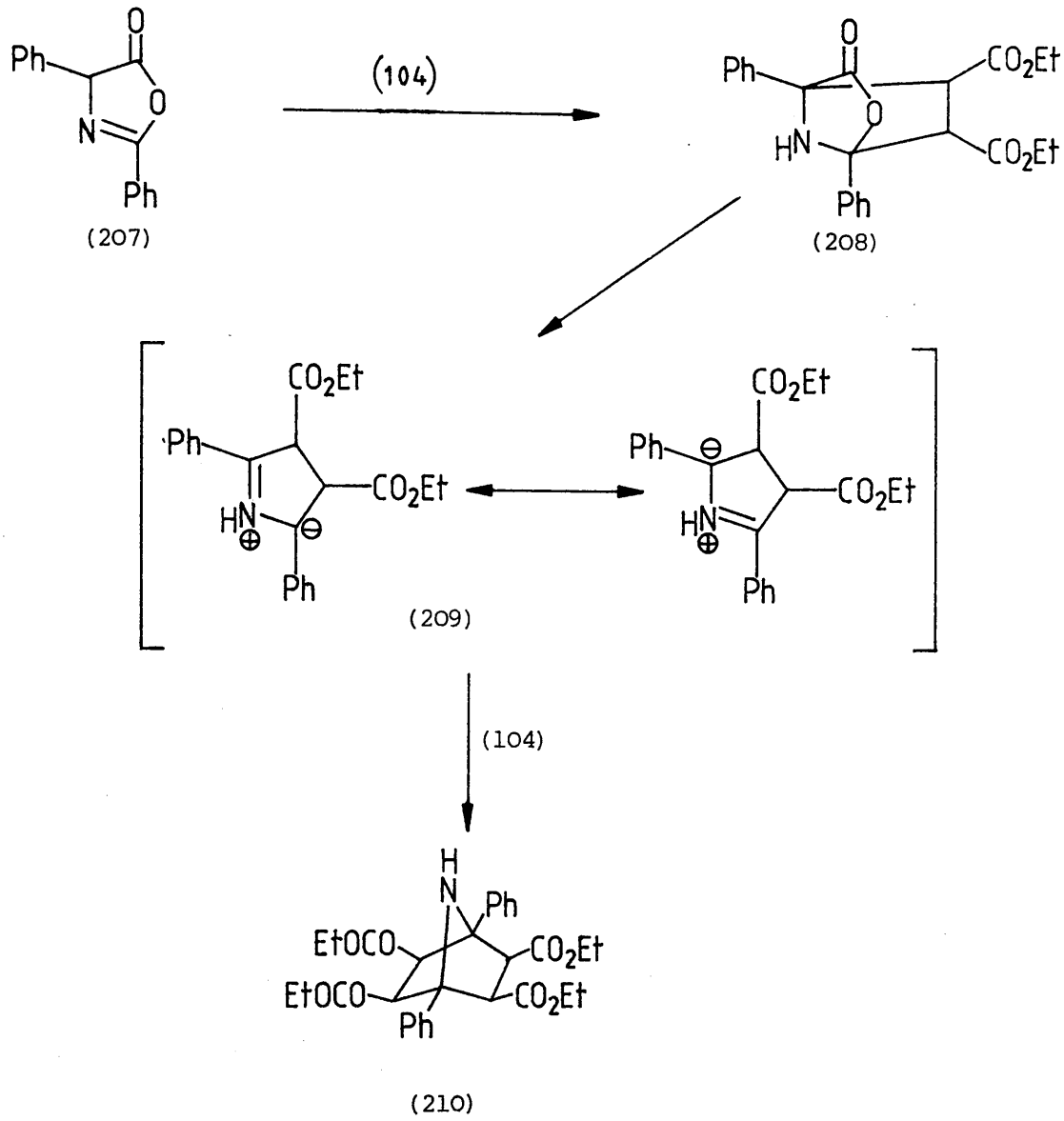
This mechanism is supported by evidence from Huisgen *et al*.[111] They noted that with high concentrations of active dipolarophiles,



Scheme 45. Planned synthesis of a new necine analogue.



Scheme 46


Ph
O
N
O
Ph
(207)
(104)
Ph
O
HN
O
CO2Et
CO2Et
Ph
(208)
CO2Et
Ph
HN
CO2Et
Ph
(209)
CO2Et
Ph
HN
CO2Et
Ph
(104)
H
N
Ph
EtOCO
EtOCO
CO2Et
CO2Et
Ph
(210)

Scheme 47

such as diethyl fumarate, cycloaddition of a second molecule of the dipolarophile can take place. A double addition adduct of this type has been isolated. For instance, the diphenyl azlactone (207) added one molecule of diethyl fumarate (104) to yield the adduct (208). Elimination of CO_2 generated the azomethine ylide (209) which reacted with a further molecule of diethyl fumarate (104) to give the 7-azo-bicyclo[2.2.1] heptane derivative (210), which was an isolable product (Scheme 47).

The usual course of reaction for an azomethine ylide such as (206a) and (206b), in the absence of a dipolarophile, would be to tautomerise to give the 2-pyrrolines which in this case means that the two pyrrolizidines (211) and (212) should be found. We have confirmed Sakdarat's observation, that, in fact, three products are formed.

Several separation methods were used for this mixture, none of which was wholly satisfactory. The various types of column chromatography were not able to separate the components, due to the small difference in their R_F values. In the end preparative t.l.c. was used, although this too brought problems of scale and of product rearrangements.

For the two diesters (211) and (212), and their structural isomer (213), spectral and physical data has been assembled for the assignment of structures (211 - 213).

The mass spectral data for each component showed an $\underline{M}^+$ at 253, corresponding to $C_{13}H_{19}NO_4$, and a large peak at 180 corresponding to loss of CO_2Et. The infra-red spectrum of (211) showed ester

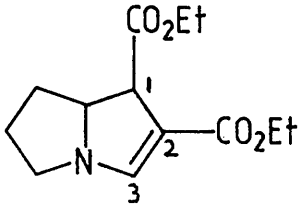
carbonyl frequencies at 1730 and 1695 cm^{-1}. Component (212) had these absorptions at 1735 and 1685 cm^{-1}. However, (213) showed ester carbonyl peaks at 1700 and 1600 cm^{-1}. It appeared that (211) and (212) contained two ester groups of which only one was conjugated to a double bond, whereas (213) contained only conjugated esters.

The U.V. spectra are also indicative of a clear distinction between one of the diesters and the other two. Compound (211) had an absorption at 291 nm (ε 14,900) while (212) had an absorption at 290 nm (ε 14,900). Both of these observations were as expected for the "ene-amine-ester" systems observed in both. The N atom extends the chromophore to a much higher value than would be observed for a simple αβ-unsaturated ester. The auxochromic shift is of the order of 65 nm.

The very different chromophore in (213) had a slightly higher absorption at 300 nm, but a much larger extinction coefficient ε 19,400, indicating a different type of system.

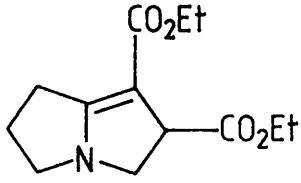
The ^{1}H n.m.r spectra of the three isomers provided the key to their identity. All three spectra were similar with the exception of some important details. For diester (211), a key feature was the singlet at 6.75 p.p.m. (H-3) which did not appear in the spectra of (212) or (213). The spectrum of diester (212) contained a broad triplet at 2.65 p.p.m. (C-7-H_2) which was diagnostic for this molecular structure. The ^{1}H n.m.r spectrum of diester (213) was, as expected, much more complicated. The signals for the C-5, C-6, C-7 and C-8 protons of (213) appeared as a complex band from 2.30 - 3.10 p.p.m. This feature, however, helped to distinguish

(206a)

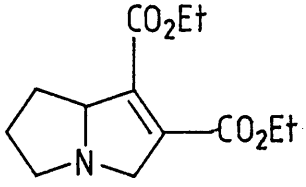


(211)

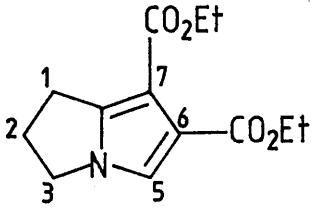
(206b)



(212)



(213)



(214)

the spectrum of (213) from those of (211) and (212).

Having established the structures of the three diesters, the task was then to rationalise the formation of the third isomer (213). This 3-pyrroline derivative was not the expected product of a 1,3-dipolar cycloaddition; its formation had not been observed previously. It appears, that under the conditions of the reaction, the 2-pyrrolines partially isomerised to give the 3-pyrroline. Looking at the structures of the diesters (211) and (212) this is, perhaps, not unexpected. Isomerisation of (211) with its tri-substituted double bond into (213) with its tetrasubstituted double bond would seem to be a feasible step. The double bond in diester (212) may be strained being on the bridgehead of a bicyclo[3.3.0] system. In addition, formation of (213) from (211) or (212) requires the removal of an H atom from the α-position of an ester group. The reverse process is unlikely to occur. Under the conditions, therefore, it is not surprising that isomerisation can take place.

The important question at this point was could the isomerisation be adapted to give only one of the diester products, hopefully diester (213)? In the reaction itself, the isomerisation was obviously not complete so different conditions from AC_2O/AcOH would have to be used.

The most obvious step was to treat the mixture with base and observe, by **t.l.c.**, what happened. Using first of all K_2CO_3, then Et_3N, gave no change to the mixture in either case. Using the non-nucleophilic base DBU, however, did cause a change. The normal **t.l.c.** pattern for the bases (211), (212) and (213) is shown

in Figure 14. From this evidence it appeared that the base treatment caused the amount of isomer (213) to increase. Closer inspection of the system revealed this might not be the whole picture. Normally, the bases were located using the modified

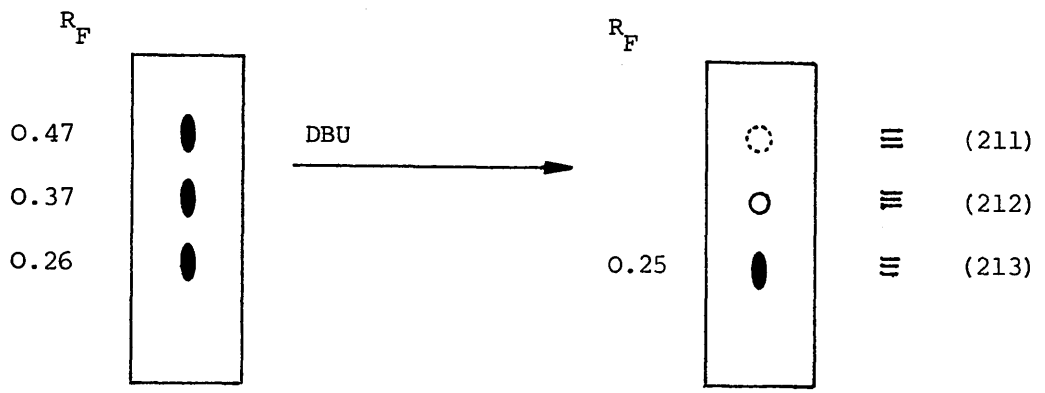


Figure 14.

Dragendorff reagent and the same was true for the base treated mixture.[108] Locating the bases using the Ehrlich reagent[112] gave one bright blue spot at R_F 0.25 which had not been there previously. [The Ehrlich spray is used commonly to detect pyrrolic materials and does not give any reaction for base (213)].

So the process occurring in the presence of DBU is not just isomerisation but some oxidation as well. A detailed mechanism for the reaction is not known, but the product of the oxidation has been isolated. The same product, a viscous brown oil, appeared if the mixture of bases (211), (212) and (213) was allowed to stand open to the air for any length of time. The oxidation product, the pyrrole (214) was isolated (in 17% yield from L-proline) and identified as diethyl 2,3-dihydro-1H-pyrrolizidine-6,7-dicarboxylate (214). Purification by flash silica column chromatography gave

a sample which allowed characterisation. High resolution mass spectrometry showed the molecular formula to be $C_{13}H_{17}NO_4$ with $\underline{M}^+$ at 251. The infra-red spectrum showed two ester carbonyl peaks at 1730 and 1690 cm^{-1} corresponding to the two different ester groups. The ^1H n.m.r spectrum showed a singlet at 6.95 p.p.m. corresponding to the pyrrolic C-5 proton. There were also well defined triplets at 3.00 ($\underline{J}$ 7 Hz) and 3.95 ($\underline{J}$ 8 Hz) corresponding to the methylene groups at C-1 and C-3 respectively.

It was to become clear that this pyrrole (214) would appear repeatedly whenever any work was done on the bases (211), (212) or (213).

Instead of using DBU as a base, DABCO was tried to see if any difference was observed. This time the T.L.C spot at R_F 0.26 corresponding to compound (213) became more intense but so did the pyrrolic compound at R_F 0.25.

Finally, acid isomerisation of the mixture was attempted in the hope that in the presence of acid, the N atom would protonate and thus be removed from the conjugated system in (211) and (212). If this occurred then diester (213) should be the most stable of the three isomers. Unfortunately treatment of the isomeric mixture with either HCl in ethanol or p-toluene sulphonic acid in $CHCl_3$ gave only a black tar as the product. Not unexpectedly, the pyrrole (214) had formed and polymerised.

At this point, it was decided that this route was not going to be successful in terms of producing a new diol and, therefore, the work was stopped. For further work, the next obvious step

would be to change the ester group of the dipolarophile. This might give the cyclised products some extra stability.

6.2 Synthesis Towards a New Diol for use in Biosynthetic Work.

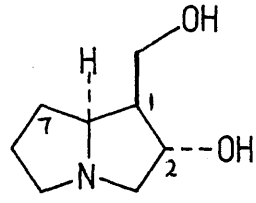
The diol (25) was chosen as a synthetic target because of its possible biosynthetic utility in investigating the pathway to the alkaloid rosmarinine (44). It is known that isoretronecanol (5) is transformed into rosmarinine, but it is not known whether hydroxylation of (5) occurs first at C-2 or C-7. Kelly[113] observed that platynecine (8) (containing a radiolabel) was a precursor for rosmarinine (44), so a comparison with the biological incorporation of diol (25) would be useful.

Any synthetic route would have to allow the introduction of a radiolabel, which further complicates the synthetic problem.

The solution that was chosen used a popular methodology from the literature; that of using a naturally occurring material which could be selectively modified to give the target (25). This diol (25) is closely related to the base portion of rosmarinine (44) (having the same absolute stereochemistry at the 1-, 2- and 8-positions) which is a relatively abundant alkaloid. Thus, rosmarinine (44) was chosen as the starting material for the synthetic route which is shown in Scheme 48. Another advantage of using rosmarinine (44) is that it can be easily obtained in radio-labelled form by feeding the parent plant, *Senecio pleistocephalus*,

with an appropriately radiolabelled precursor.

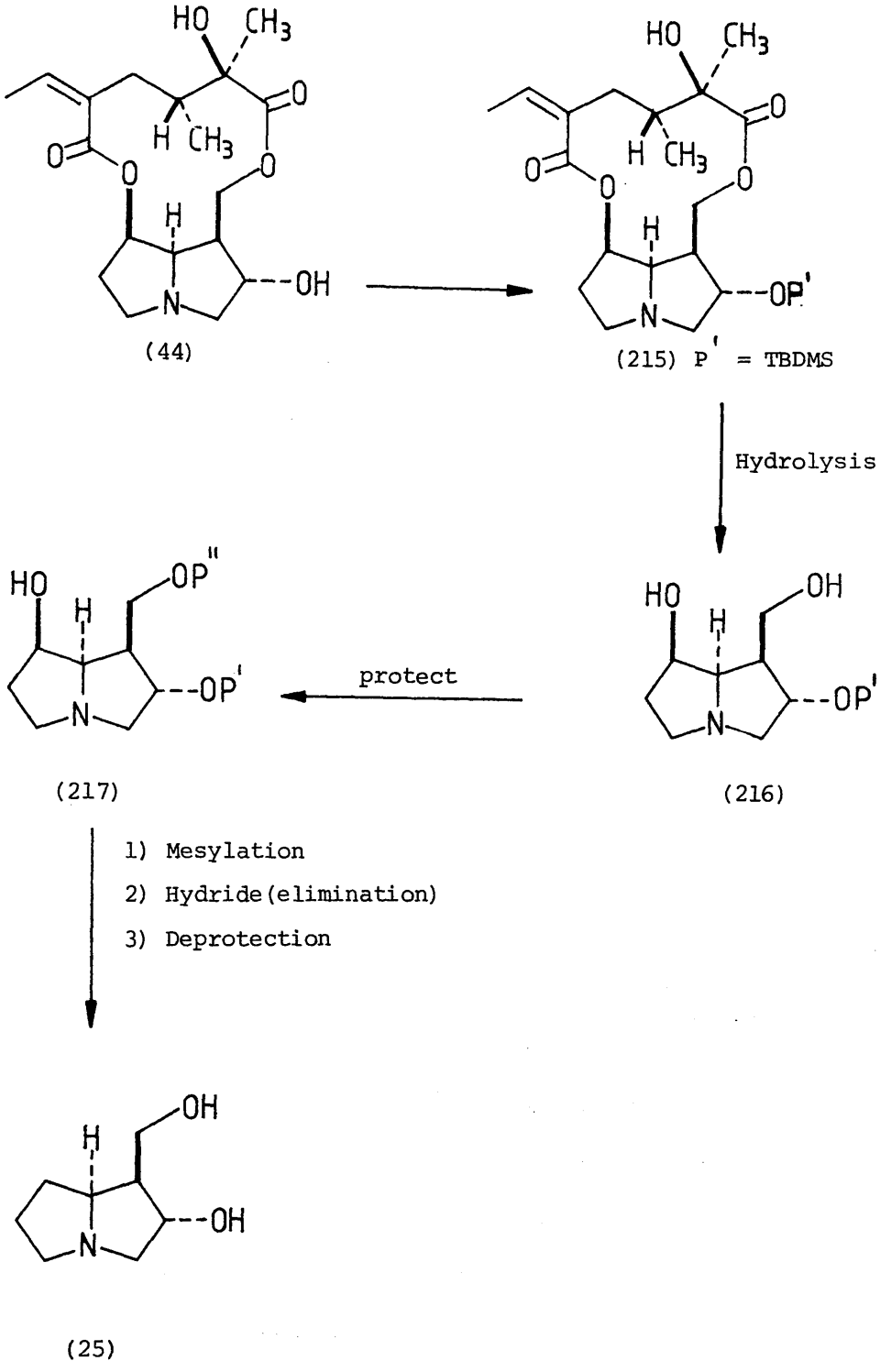
The overall synthetic plan is one which relies on successive and selective protection and deprotection.



(25)

The initial protection of the secondary hydroxyl group was carried out, using TBDMS-Cl in DMF with imidazole, by Kelly.[113] He noted that treatment of rosmarinine (44) with these reagents gave an oil, which contained only one TBDMS group. He proposed that the structure of this adduct was (215), which was confirmed in this work. Using this as the starting point, it was proposed that hydrolysis of some kind would give the monoprotected di-alcohol (216). Further protection of the primary hydroxyl would yield the di-protected mono-ol (217). The secondary hydroxyl would then be removed by reduction of the 7-O-mesylate. Deprotection at the 2-O- and 9-O-positions would then give the diol (25).

Using the method of Kelly[113] gave, as reported, an oil, in high yield. It was clear from the ^{1}H nmr spectrum, however, that the oil still contained an appreciable amount of DMF. This was removed by azeotroping with toluene. Crystallisation of the resulting oil gave a white crystalline solid in high yield. High resolution mass spectrometry showed the presence of only one Si atom, giving a formula of $C_{24}H_{41}NO_6Si$. Microanalytical



Scheme 48. General Route towards (1S, 2S, 8S)-1-hydroxymethyl-2-hydroxypyrrolizidine (25).

data confirmed this to be the correct molecular formula. The infra-red spectrum showed the typical rosmarinine (44) peaks plus an absorption at 1120 cm^{-1} probably due to a Si-O bond vibration.

The question at this point was whether it would be possible to show spectroscopically that the Si-protecting group had become attached to the secondary hydroxyl and not the tertiary hydroxyl. This was a likely proposition but no actual evidence had been quoted to confirm it.

The ^{1}H n.m.r spectrum of the 2-O-TBDMS adduct (215) was almost identical to that of rosmarinine itself, so correct assignment is not possible from this. In general, for TBDMS ethers, the ^{1}H n.m.r spectrum is largely unaffected by the presence of such a functional group.[114] Confirmation of the presence of the protecting groups came from the ^{1}H n.m.r absorptions for the two $Si-CH_3$ groups. In TBDMS-Cl, these appeared as a singlet. In the adduct (215) they appeared as two singlets at 0.02 and 0.07 p.p.m due to their diastereotopic nature. (Throughout this synthesis, the presence of these two signals was a useful indication of the presence of the protected species).

Confirmation of the site of protection came from the ^{13}C n.m.r spectrum. The C-2 signal in this spectrum came at 76.3 p.p.m, compared to 69.1 p.p.m., for rosmarinine itself. The signal due to the C-12 nucleus at 77.4 was largely unchanged from rosmarinine (44). This shift, of 7.2 p.p.m., is of the order expected for such a system.

The next stage of the route was to remove the macrocyclic

diester portion of the molecule (215). The nature of the protecting group ruled out the use of acidic conditions. In addition, TBDMS ethers are unstable to strong aqueous base,[115] so the usual $Ba(OH)_2$ hydrolysis could not be used either. This left the use of hydride reduction of the ester groups. The reagent chosen was DIBAL because it gave a slightly higher yield (61%) than $LiAlH_4$ (54%). The reduction was carried out at 0^oC in CH_2Cl_2 solution and, after work up, the product was purified by column chromatography on basic alumina. The product, a mono-protected triol (216) was obtained as an oil which would not crystallise from a range of solvents. Therefore, analytical data was collected for the oil.

High resolution mass spectrometry showed a mass peak at M 287.1894, corresponding to the formula $C_{14}H_{29}NO_3Si$. The base peak at 212 corresponded to loss of a tert-butyl group and H_2O. The infrared spectrum showed a typical broad band at 3320 cm^{-1}, indicating the presence of hydroxyl groups. There was no sign of any ester carbonyl absorption in the spectrum confirming that reduction had taken place and that an alcohol was the product. There was also an absorption at 1120 cm^{-1} corresponding to the Si-O bond, strong evidence that the TBDMS group had survived the reduction step.

As before, the ^1H n.m.r spectrum did not give a great deal of information in relation to the position of the TBDMS group. There were two singlets, at 0.13 and 0.15 p.p.m. for the diastereotopic silicon methyl protons, and a nine proton singlet at 0.95 p.p.m for the silicon tert-butyl group. An interesting feature of the

spectrum was the AB system for the 9-H protons. In the ^{1}H n.m.r spectrum of rosmarinine (44), these protons appeared as an AB system at 4.08 p.p.m. (J 12.5 Hz and 1.0 Hz) and 4.87 p.p.m. (J 12.6 Hz and 5.2 Hz). For the diol product (216) the AB system at 3.75 p.p.m. had J 12 Hz and 6 Hz. The other half of the system was obscured by the hydroxyl proton absorptions. This was an indication that the 9-O-ester group had been removed. There was also a broad double doublet absorption at 4.50 p.p.m corresponding to the β-proton on the C-2 position. The pattern of the absorption was due to the unequal coupling with the diastereotopic C-3 protons and the C-1 proton.

The ^{13}C n.m.r spectrum was very similar to the spectrum of rosmarinecine (10) except that the C-2 absorption was slightly higher in the protected version (216).

The compound (216) retained its optical activity, with an $[\alpha]_D$ value of -23.9^{o}, compared with the rosmarinecine (10) value of -11.6^{o}.[113]

The combination of physical and spectroscopic data for the diol (216) confirm its structure to be as shown. The presence of the TBDMS group also verifies the previous assertion that the protecting group was located on the oxygen of the pyrrolizidine ring and not on the diacid portion.

The next step was to decide how best to protect selectively the 9-hydroxyl bearing in mind the reactions planned for later in the route. A limiting factor in any choice was the presence of the tertiary nitrogen group. This meant that the reagent chosen had

to protect only the primary hydroxyl without affecting the secondary hydroxyl or the tertiary amine. To attempt to keep the later deprotections more simple, the TBDMS group was again chosen.

Chaudray and Hernandez had reported that their silylating reagent of TBDMS-Cl with DMAP selectively protected primary alcohols in the presence of other alcohols and amines.[116] They noted this during their work on nucleotide synthesis (see Figure 15). Using this method on the diol (216), however, gave no reaction. Increasing the amount of reagent, the reaction time and gently heating failed to produce the required product.

It was deduced from these observations that the primary hydroxyl group was much less reactive than expected, probably due to the presence of the 2-O-TBDMS group. Therefore, it was decided to use the more reactive TBDMS-Cl, DMF and imidazole system, as used in the initial silylation. Once again the reaction did not occur, with only starting material recovered. This time heating the reaction mixture caused decomposition.

It was obvious at this point that both the 9- and the 7-hydroxyl groups were very unreactive and that it might not be possible to silylate one in preference to the other. In an attempt to see if silylation could be effected at all, the highly reactive TBDMS-triflate (218) reagent was used. This is known to silylate a large range of active and non-active alcohols.[114] Stirring the reagent (218) with the diol (216) in the presence of 2,4-lutidine (219) at $0^{\circ}C$ gave no reaction, even after several hours (monitoring by t.l.c). Increasing the temperature also caused decomposition of the starting material.

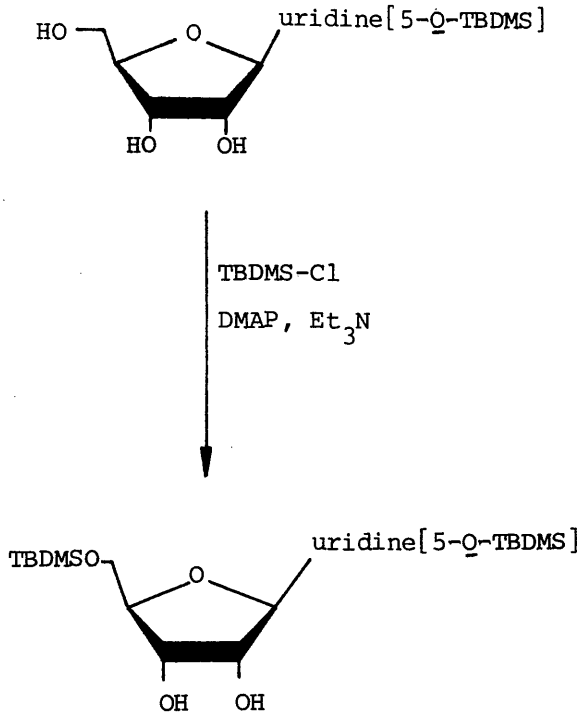
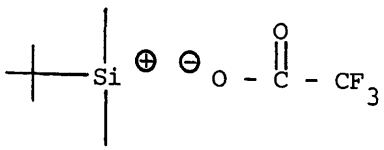
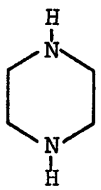

HO
O
uridine[5-O-TBDMS]
HO
OH
TBDMS-Cl
DMAP, Et3N
TBDMSO
O
uridine[5-O-TBDMS]
OH
OH

FIGURE 15



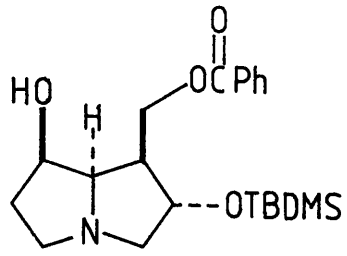
(218)



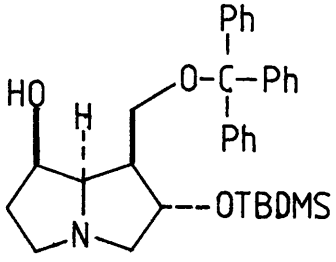
(219)

This last result caused us to look for an alternative protecting group for the 9-OH, one which would be reactive enough to attach to the hydroxyl but not so much as to attach to the nitrogen. The choice for this group was the benzoyl ester which had been used to great effect by Glinski and Zalkow in their synthesis of some heliotridine-type alkaloids.[51] For the diol (216) under study, we firstly used benzoyl chloride in aqueous sodium hydroxide solution. After work up there was no indication, in the ^{1}H n.m.r spectrum, that esterification had occurred to produce ester (220). A similar benzoylation in pyridine gave the same result.

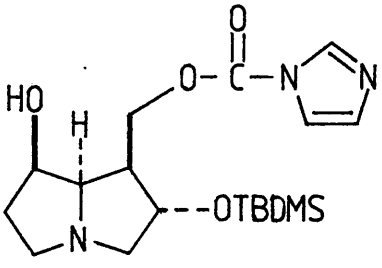
Unfortunately at this point the work had to be stopped due to lack of time.



(220)



(221)



(222)

6.3 Conclusions

A useful route has been set out and established here, and it is a great pity that it was not possible to complete the work. Several options are still open, including the formation of the triphenylmethyl derivative (221) or the 9-carbonyl-imidazole derivative (222). The latter could be used to produce the benzoate ester (220) in a similar manner to that described by Gelbaum *et al.*[117] Once this second protection step is successfully completed the removal of the 7-hydroxyl group should be relatively straightforward.

6.4 The Necine Bases - Conclusions

The synthesis of necine base analogues is an extremely wide field of research employing very many strategies and techniques. The two methods chosen in this Chapter, although not entirely successful, do demonstrate that both could be useful for producing necine base analogues, for whatever purpose. The results dicussed previously add to the wealth of information already available on the synthesis of necines and their analogues.

CHAPTER 7

ANALOGUE SYNTHESIS

7.1 The Synthesis of Putrescine Analogues.

As discussed previously there are several different reasons for making analogues of natural metabolites. Two such reasons are the elucidation of enzyme mechanisms and, linked very closely to this, the design of specific enzyme inhibitors.

There are many opportunities within the pyrrolizidine alkaloid biosynthetic pathway to study the effects of analogues, but it was decided that the best choice would be the conversion of ornithine (21) into putrescine (23). This step was chosen because of the importance of putrescine (23) in general cell metabolism. This diamine (23) is one of a number of ubiquitous polyamines which acts as primary modulators of both normal and pathological cell growth.[118] Putrescine itself is an essential growth factor for many microorganisms (for instance, *H. parainfluenzae* and *A. nidulans*), a follicle stimulating factor releaser in man, and an intermediate in the biosynthesis of the important polyamines spermine (30) and spermidine (29).[119] Clearly any process which reduces the amount of putrescine available within a living cell could have serious consequences on cell growth and function.

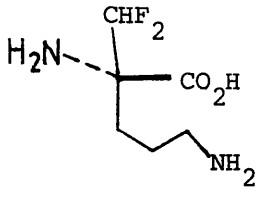
In most cells there are two principal ways of producing putrescine, either from ornithine (21) or from arginine (28),[16,120] so inhibition of one route still leaves an alternative. However, in certain lower microorganisms, such as some fungi, the only biosynthetic source of putrescine is from ornithine. Hence, inhibition of this process effectively stops the growth of the organism. It was therefore our intention to produce some

potential inhibitors of the ornithine to putrescine step. These would be tested for antifungal activity by Dr. D. Walters at the West of Scotland Agricultural College, Auchincruive. In addition, the compounds would be fed to some pyrrolizidine-alkaloid producing plants and hairy root cultures[99] to observe what effect, if any, was obtained.

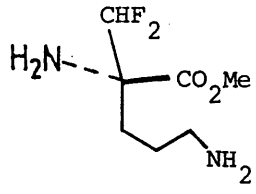
The conversion of ornithine into putrescine is an enzyme mediated process controlled by the enzyme ornithine decarboxylase (O.D.C). This is a relatively well known enzyme and there are several compounds which inhibit this enzyme including the two ornithine analogues, α-difluoromethylornithine (α-DFMO) (223), its methyl ester (224) and putrescine itself. The latter observation gives an indication as to how ODC is regulated; a higher concentration of putrescine results in a lower enzyme reaction rate.

The two ornithine analogues (223) and (224) are the best known inhibitors of ODC.[121] No definite mechanism is known for their action, but it is thought to involve the interaction of the substrate bound to a pyridoxal phosphate cofactor with the enzyme (Figure 16). The decarboxylation process might leave the highly polarised C-F bonds adjacent to some nucleophile within the enzyme active site, and the enzyme could become bound to the substrate. It should be stressed that this is only a postulated mechanism, although it is known that both α-DFMO (223) and its methyl ester (224) are irreversible inhibitors of some kind.[121]

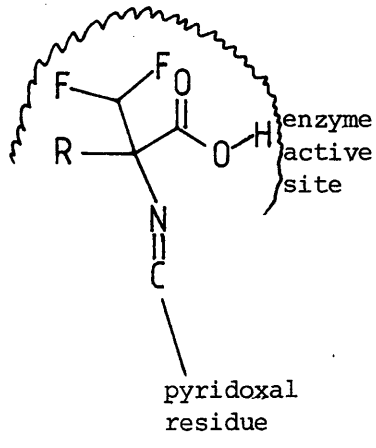
The initial synthetic targets were some simple analogues of putrescine (30) which may become reactive when bound to



(223)



(224)



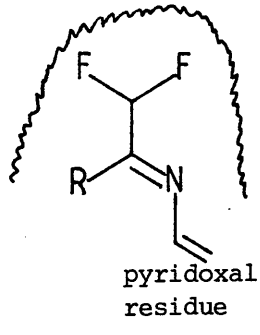
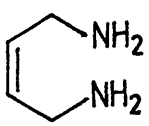
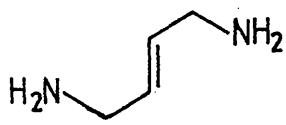


FIGURE 16.

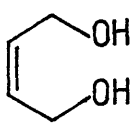
pyridoxal phosphate within O.D.C. The choice was to make the two unsaturated putrescine analogues (225) and (226). These were both known compounds[122] and, in this work, were made by a new method involving a Mitsunobu/Staudinger process as detailed in Chapter 5, from diols (227) and (228). Diol (228) was made by specific reduction of the ester groups of diethyl fumarate (104). The diamines (225) and (226) were tested for anti-fungal activity against *Penicillium canadense I* in Glasgow by Mrs. P. Tait in the Mycology Unit. Zones of inhibition were clearly visible for the trans-isomer (226) at loadings of 10 μg - 1 mg, but not for the cis-diamine (225). More detailed tests are being carried out on rust fungi by Dr. D. Walters at Auchincruive.



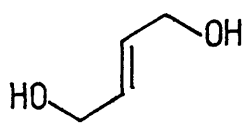
(225)



(226)



(227)



(228)

The next step was to choose targets with similar properties to the diamines (225) and (226) but with greater reactivity or potential reactivity. An important route to designing specific inhibitors is to select a small, inert group that can be modified into a more reactive species by undergoing a transformation.[123] It was felt that a good choice would be a cyclopropyl group, making the targets the new diamines (229) and (230). Other cyclopropane derivatives have been used successfully as enzyme inhibitors, for instance, methanoproline (231) is an inhibitor of proline biosynthesis in plants.[124]

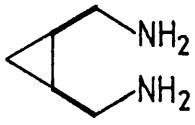
The synthetic route towards these diamines (229) and (230) was originally intended to be an extension of the route for the unsaturated diamines (225) and (226). It appeared that a straightforward carbene-type addition to either the diols (227) and (228) or the diamines (225) and (226) would be the best way to proceed. A report by Conia et al.[125] indicated that using a zinc-silver couple and diiodo methane (232) on an electrophilic double bond, such as in diethyl fumarate (104) would give good results.

A sample of Zn/Ag couple was made using the Conia method and reacted with diiodomethane (232) followed by reaction with redistilled diethyl fumarate (104). Upon work up of the reaction, the only identifiable material was the starting diester, which was returned in 8% yield. The residues left after the work up procedure turned rapidly bright yellow then brown, indicating the presence of some reactive species. It was not possible to say if the diethyl fumarate was reacting in an unexpected way, or if the cyclopropane was initially being formed and then decomposing.

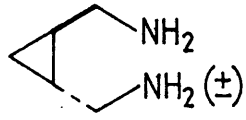
It appeared at this point that either the alkene (104) or the carbenoid species was too reactive to use for this synthesis. An alternative to the Zn/Ag couple was the older Zn/Cu couple,[126] first used by Simmons and Smith. This couple is more difficult to make in reactive form, requiring highly purified starting materials. However it can be made in bulk and stored for some time without loss of activity. In contrast, the Zn/Ag couple has to be made and used in situ.

Using a Zn/Cu couple, prepared by the Simmons and Smith method,[126] along with diiodomethane (232) and diethyl fumarate gave complete recovery of starting materials. To test the activity of the couple, it was decided to form a simple cyclopropane derivative of an alkene. The example chosen was norcarane (233) which was made successfully by Simmons and Smith,[127] and by Le Goff[128] using the Zn/Cu couple. The reaction of cyclohexene with diiodomethane and the Zn/Cu couple produced norcarane (233) in 40% yield. This was less than the quoted figure (56%) but it did show that the Zn/Cu couple prepared was active. Therefore, it was decided to make a different choice of the alkene for the reaction.

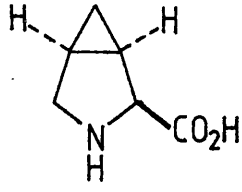
The nature of the carbenoid species in this reaction, and the mechanism of the cyclopropanation are not precisely known. It is believed that the methylene transfer species is as shown in Figure 17, with the copper having no role other than activating the zinc surface.[127] The evidence available supports the view that the reaction mechanism is a one step process. The "quasi-trigonal" [129] methylene group of iodomethylzinc iodide adds to the



(229)



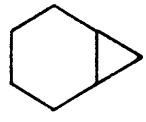
(230)



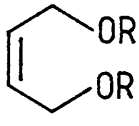
(231)

ICH_2I

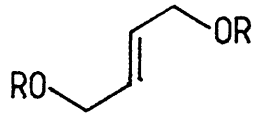
(232)



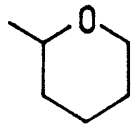
(233)

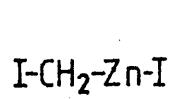


(234)

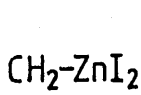


(235)

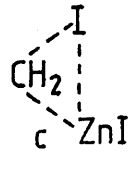
R = 



a



b



c

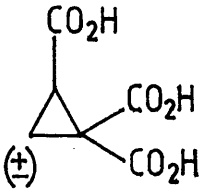
FIGURE 17.

olefinic π-bond such that both new carbon-carbon bonds are formed simultaneously. The main evidence to support this is that cyclopropane formation occurs in stereospecific fashion.[130] Using cis- and trans-alkenes usually gives cis- and trans-cyclopropanes. This reasoning indicated that the cis- and trans-but-2-ene diols (227) and (228) or diamines (225) and (226) might be suitable compounds for the cyclopropanation reactions. In addition, it is known that oxygen functions close to the alkene can coordinate with the zinc reagent, and lead to increased rates and yields.[129] Typically, hydroxyl or ether groups are used for this purpose.

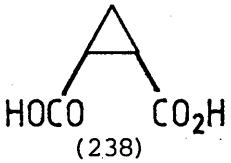
It was decided that a suitable choice of olefinic starting materials would be the diols (227) and (228) and their tetrahydropyranyl (thp) ethers (234) and (235).

The thp ethers were made by a standard method giving satisfactory analytical data. Reaction of these two thp ethers (234) and (235) with a carbenoid species according to the method of Conia or of Simmons and Smith gave no formation of cyclopropane, with complete recovery of starting material. Similar results were obtained with the two but-2-ene diols (227) and (228).

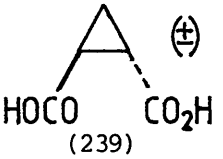
Faced with this lack of success for the carbenoid approach to the synthesis, a new route was devised which avoided the use of such species. In a 1965 report, Abell and Lennon studied the decarboxylation of some 1,1,2-cycloalkane tricarboxylic acids.[131] They noted, in particular, that the products of the thermal decarboxylation of 1,1,2-cyclopropane tricarboxylic acid (236) depended on whether acidic or basic conditions were used. In the presence of



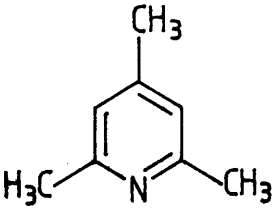
(236)



(238)



(239)

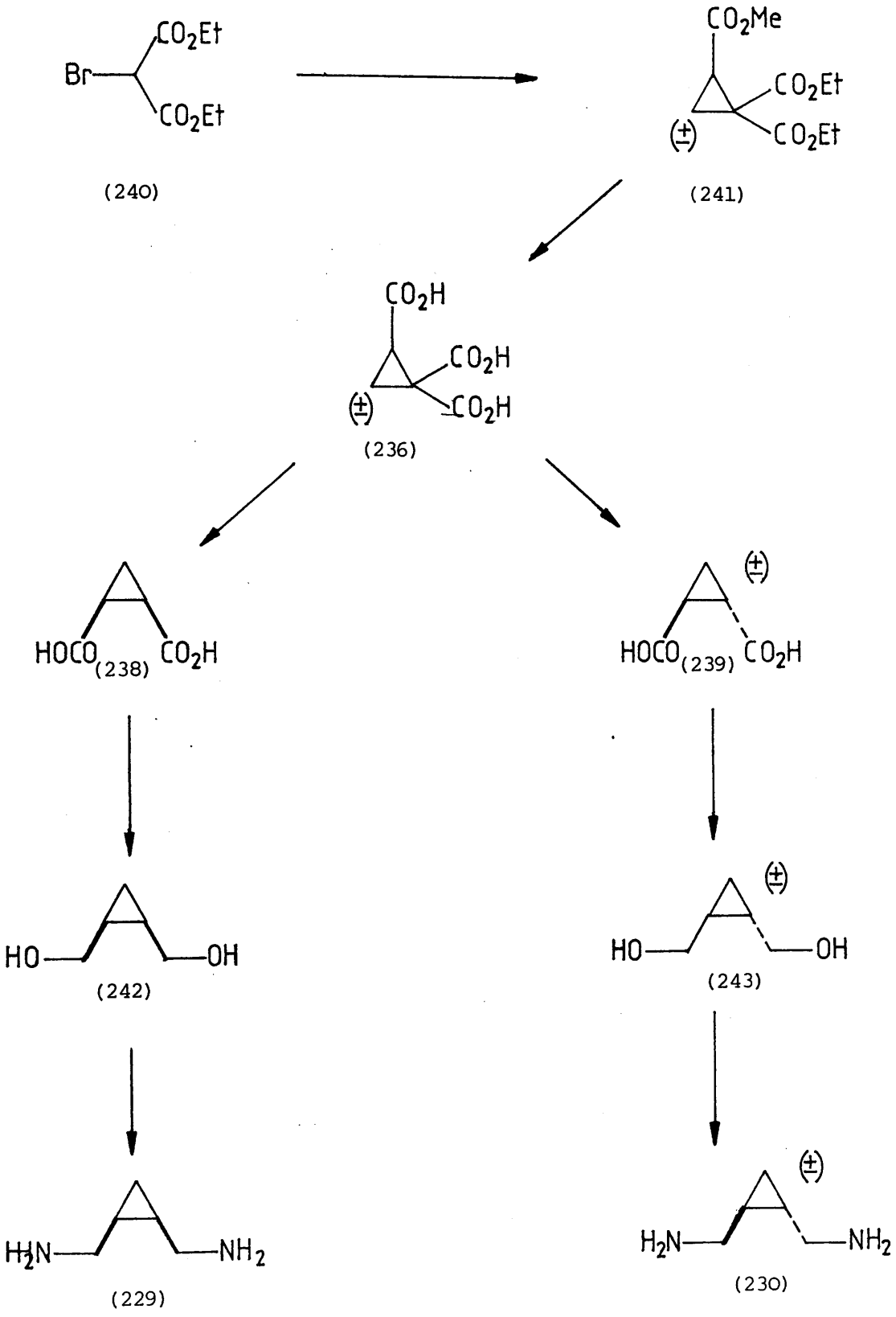


(237)

collidine (2,4,6-trimethylpyridine) (237) the cis-1,2-cyclopropane-dicarboxylic acid (238) was the major product, and the corresponding trans-diacid (239) was a minor constituent. With no solvent present, the proportions of diacids (238) and (239) were approximately equal while in the presence of 5M HCl solution, the trans product (239) was predominant. It was hoped that this observation could be used on a preparative scale in the synthesis of the diamines (229) and (230). A route was devised from readily available starting materials and is shown in Scheme 49.

Diethyl bromomalonate (240) was prepared by a known method[132] and gave the correct analytical data. The formation of the cyclopropane ring was carried out by a modification of the procedure of Bonavent et al.[133] In their method, the bromo-ester such as (240), was reacted with methyl acrylate using sodium ethanoate as the base. An attempt to repeat this appeared to give only a dimerised product. Using sodium hydride in tetrahydrofuran (THF) gave better results, with the cyclised product (241) being obtained in 62% yield, as a clear oil. High resolution mass spectrometry indicated the molecular formula $C_{11}H_{16}O_6$, which corresponded to the desired product. The ^1H n.m.r spectrum showed that the structure was the expected one. Aside from the peaks for the ethyl and methyl esters, there was an ABX system for the three cyclopropyl protons. This was analysed in the usual fashion[134] giving J_{AB} 4.6 Hz, J_{AX} 9.3 Hz and J_{BX} 6.5 Hz.

The triacid derivative (236) was produced by basic hydrolysis of the triester (241), giving a white, crystalline product in reasonable yield (61%). Again, the analytical data for this


Br
CO2Et
CO2Et
(240)
CO2Me
CO2Et
CO2Et
(±)
(241)
CO2H
CO2H
CO2H
(±)
(236)
HOCO
CO2H
(238)
HOCO
CO2H
(239)
(±)
HO
OH
(242)
HO
OH
(±)
(243)
H2N
NH2
(229)
H2N
NH2
(±)
(230)

Scheme 49.

compound were satisfactory. Furthermore, the ^{1}H n.m.r spectrum showed an ABX system, which was very similar to that of triester (241), with J_{AB} 4.8 Hz, J_{AX} 9.4 Hz and J_{BX} 6.5 Hz.

The next stage in the synthesis was the decarboxylation of the triacid (236) to give a mixture of the diacids (238) and (239). In the original paper,[131] the reactions were carried out on an analytical scale, with no comment on whether any experiments using larger amounts of material had been attempted. In any case, it was found that the reported results could not be repeated using apparatus as close as possible to the original equipment. Heating the triacid (236) at 200°C in collidine (237), on a Woods metal bath, gave a crude mixture of decomposed starting material and triacid (236). This was deduced from t.l.c. and ^{1}H n.m.r spectral evidence.

As a modification, the triacid was heated at 200°C, under a nitrogen atmosphere, in a Kugelrohr apparatus in the presence of 6M hydrochloric acid. Again the result was a mixture of starting material and decomposition products.

Finally, the triacid (236) was heated at 200°C in the Kugelrohr apparatus with no solvent. It was suggested that the reaction should proceed better at a low pressure.[135] At a pressure of 0.1 mm Hg, under the above conditions, the solid quickly melted and a gas was evolved. This was presumed to be CO_2 emission. After 10 minutes, the evolution of gas ceased and a white solid appeared in the collector bulbs. Recrystallisation of this solid gave a white crystalline material, which was shown to be cis-1,2-cyclopropane dicarboxylic acid (238). The

remaining brown residue was triturated with diethyl ether leaving a brown gum which was recrystallised to give a white solid. It was reported that the tricarboxylic acid is much more soluble in organic solvents than the corresponding dicarboxylic acids.[131] Hence, the white solid obtained was the (±)-trans-1,2-cyclopropane dicarboxylic acid (239), and the unreacted triacid (236) was removed by the trituration process.

The melting points of each diacid (139 - 142°C for the cis-isomer (238) and 177°C for the trans-isomer (239)) were in agreement with the literature values.[133] The analytical data for the two materials were very similar. High resolution mass spectrometry and microanalytical data for both compounds showed a molecular formula of $C_5H_6O_4$. The infrared spectra were almost identical with bands at around 3000 and 2650 cm^{-1}, characteristic of strongly H-bonded carboxylic acid hydroxyl groups. There were also bands at 1700 cm^{-1} corresponding to the carbonyl groups.

The ^1H and ^{13}C n.m.r spectra of the two isomers were both simple due to their inherent symmetry. The cis-isomer (238) is a meso-compound while the trans-isomer is chiral (although this sample was racemic) with an axis of symmetry. In the ^1H n.m.r spectra, the ring protons of the trans-isomer (239) gave absorptions at slightly higher fields than the cis-isomer (238). The spectra of both were insufficiently resolved to allow extraction of coupling constants. After several runs through the decarboxylation procedure, it was found that the cis-isomer (238) was obtained in 32% and the (±)-trans-isomer (239) in 51% yield.

Having obtained the two dicarboxylic acids in pure form the next stage was the reduction to the corresponding cis- and trans-alcohols (242) and (243). It is known that cyclopropyl groups are stable to hydride reduction agents, such as lithium aluminium hydride (LAH).[136] Therefore, analytical scale reductions using LAH and DIBAL were attempted on the trans-diacid (239). It was found that a better yield of the diol product (243) was obtained using the DIBAL reagent. Therefore, preparative scale reactions were carried out on both diacids (238) and (239), each giving rise to a clear distillable oil.

As for the diacids, the analytical data for the alcohol products, assigned as (242) and (243), were very similar. The dibenzoate derivative of each gave a formula from microanalysis of $C_{19}H_{18}O_4$, indicating that the alcohols had the formula $C_5H_{10}O_2$. The infrared spectra of each were identical, with a characteristic broad hydroxyl peak at 3300 cm^{-1}. The two alcohols (242) and (243) were both known compounds and so could be identified using their boiling points.

The ^1H n.m.r spectra of the isomers (242) and (243) verified the assignments of the structures. The spectrum of the trans-diol (243) showed a doublet of doublets (J 7 Hz and 7 Hz) at 0.35 p.p.m. and a multiplet at 0.90 p.p.m. for the ring protons. This spectrum arises because of the magnetic equivalence of the ring methylene protons. This means that there is cis- and trans- vicinal coupling to the ring methylene protons, but no geminal coupling between these two protons.

In the ^1H n.m.r spectrum of the cis-diol (242), the

magnetic inequivalence of the ring methylene protons results in complex multiplet signals for the ring protons.

The conversion of alcohols (242) and (243) into diamines was carried out by the Mitsunobu/Staudinger procedure detailed in Chapter 5. The yields obtained were satisfactory (47% and 58%) giving two hydrochloride salts which crystallised as needles from aqueous ethanol/acetone. Analysis by mass spectrometry and microanalysis gave the molecular formula for each as $C_5H_{14}N_2Cl_2$. In an analogous fashion to the diols (242) and (243) the ^1H n.m.r spectrum of the trans-diamine (230) was much less complex than the spectrum of the cis-diamine (229). Hence the structural assignments were made.

The products of this route have yet to be tested for anti-fungal activity. It is also our intention to investigate their behaviour with the enzyme diamine oxidase, which is another important enzyme on the pyrrolizidine biosynthetic pathway. It was intended to carry out feeding experiments with these putrescine analogues to study their effect on the pathways to pyrrolizidine alkaloids and to see if analogues of pyrrolizidine alkaloids are produced. For this work, it was considered necessary to have radioisotopically labelled material to measure incorporation of analogues and to aid the location of possible analogues in complex alkaloidal mixtures. There was insufficient time to develop routes to radiolabelled forms of putrescine analogues (225), (226), (229) and (230). This might be achieved in the future by carrying out reduction steps with ^3H-labelled reagents.

7.2 Conclusions

A set of four simple analogues of putrescine have been produced with one showing appreciable anti-fungal activity. This work provides a stimulus to making further analogues which would allow a more detailed study of the structural features required for anti-fungal activity.

CHAPTER 8.

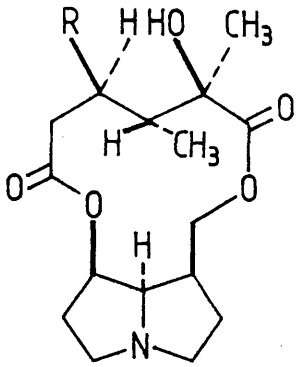
SYNTHESIS OF MACROCYCLIC DIESTERS OF (-)-PLATYNECINE

8.1 Introduction.

The alkaloid platyphylline (244) has been reported to be widely used in the U.S.S.R for the treatment of internal ulcers and hypertension.[1,137] This alkaloid (244) largely lacks the hepatotoxicity usually associated with the pyrrolizidine alkaloids. This is believed to be due to the absence of an allylic ester function such as in retrorsine (17).[3] Therefore, it was decided that it would be desirable to have a set of macrocyclic analogues of platyphylline (244), to allow the study of the pharmacological properties of such compounds. There is only one reported synthesis of macrocyclic diesters of a saturated necine base[64] and no previous synthesis of the dilactones of (-)-platynecine (8), the necine contained in platyphylline (244). In fact, there are only seven known macrocyclic alkaloids which contain (-)-platynecine (8), which have either 12- or 13-membered rings.[2] These are shown in Figure 18.

(-)-Platynecine (8) is difficult to obtain because it occurs in so few alkaloids, but the 1,2-didehydro derivative (+)-retronecine (3) is relatively abundant. A simple catalytic reduction of (+)-retronecine (3) gives (-)-platynecine (8).

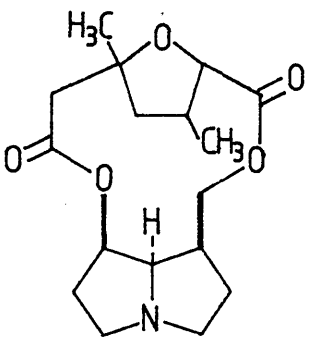
The lactonisation technique used to make the 10- and 11-membered dilactones of base (8) was the Corey-Nicolaou "double activation" method, via the pyridine-2-thiol esters (see Chapter 4).



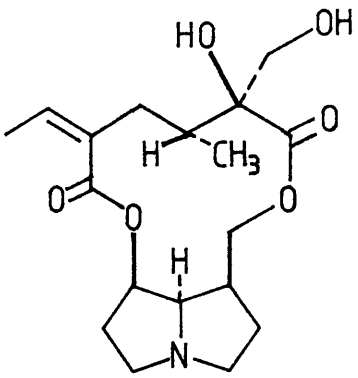
(244) R=H Platyphylline

R=H, geometric isomer
-neoplatyphylline

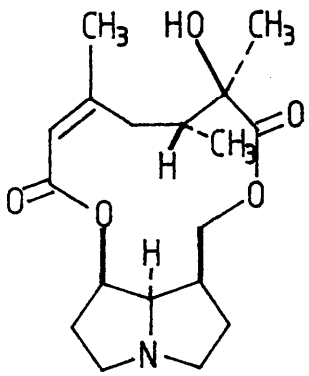
R=OH Hygrophylline



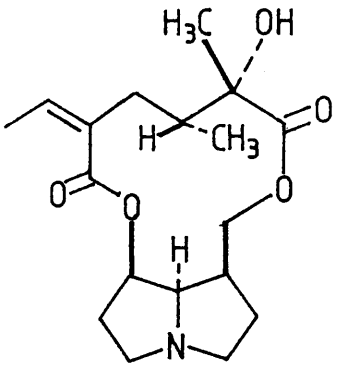
Nemorensine



Dihydroretrorsine



Bulgarsenine



Ligularinine

Figure 18 : Naturally occurring macrocyclic diesters of (-)-platynecine (8).

8.2 Preparation of (-)-Platynecine (8).

The (+)-retronecine (3) used in this synthesis came from hydrolysis of riddelline (52). Dr. Molyneux has isolated huge quantities of riddelline (52) from *Senecio riddellii* for work on toxicity of the alkaloids to livestock. He kindly made available to us the mother liquors from the crystallisation of riddelline (52). The liquors were rich in esters and diesters of (+)-retronecine (3), so hydrolysis yielded the unsaturated base (3) which was extracted and recrystallised from acetone.

Hydrogenation of (+)-retronecine (3) over a Pd/C catalyst gave (-)-platynecine (8), which crystallised from acetone, in high yield. The stereochemistry of the product is rationalised by assuming that attack from the α-face of the molecule is greatly preferred. This is probably due to the β-endo-hydroxyl group at C-7 and the (R)-configuration at C-8. An ^{1}H n.m.r spectrum is shown in Figure 19. An important feature is the AB system at 3.81 and 3.91 p.p.m. due to the diastereotopic H-9 protons.

8.3 Synthesis of 11-Membered Dilactones.

11-Membered dilactones were made using four different glutaric anhydrides with substituents at C-3. All of the anhydrides were symmetrically substituted to avoid the formation of diastereomeric isomers.

Treatment of (-)-platynecine (8) in dry 1,2-dimethoxyethane (DME) with 3,3-dimethylglutaric anhydride (245) gave a quantitative yield of the 7- and 9-monoesters, (246) and (247) respectively.

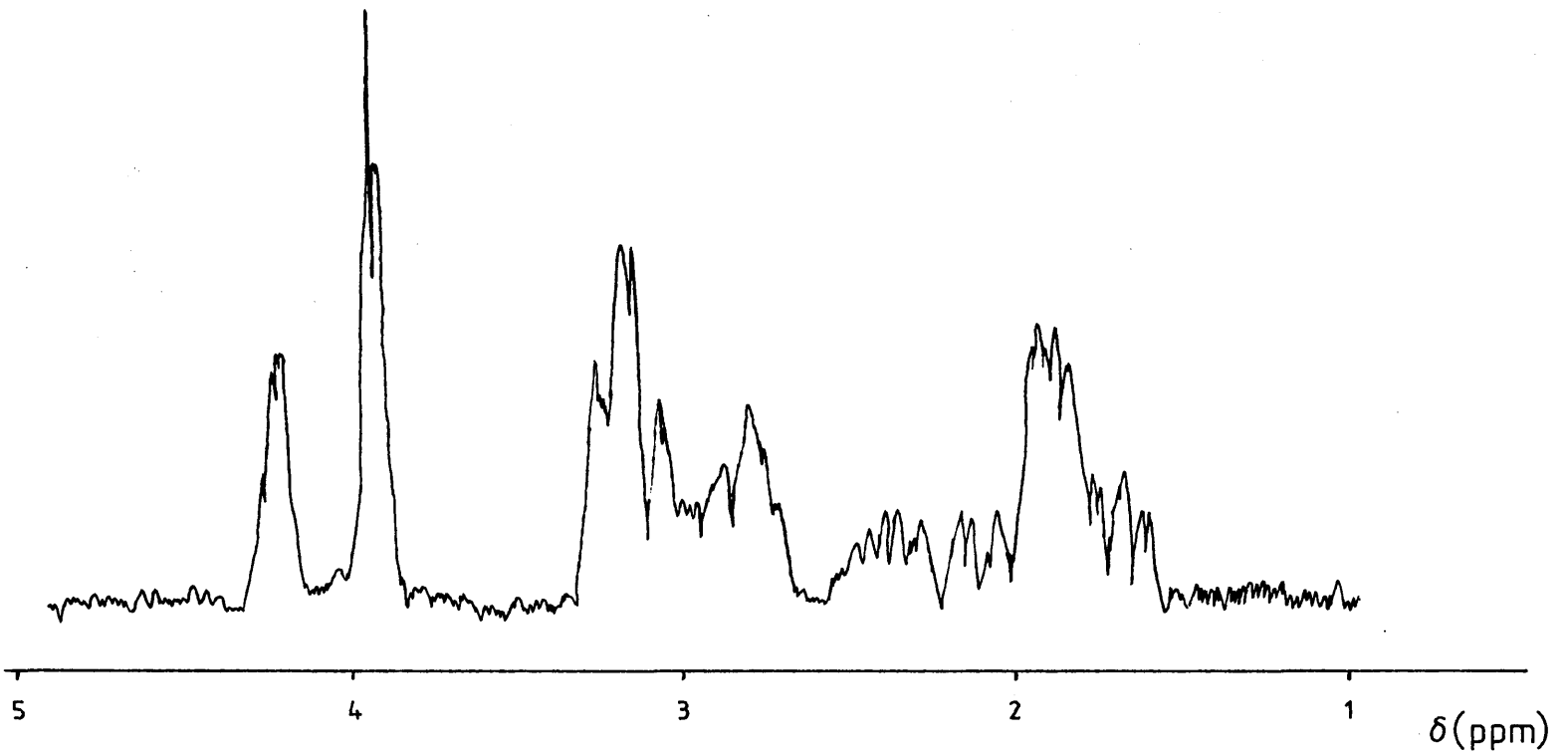


Figure 19: -90 MHz ^{1}H n.m.r. spectrum of (-)-platynecine (8).

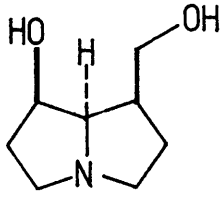
The ^1H n.m.r spectrum of this mixture in deuteriomethanol showed signals at δ 4.2 (1H, m, 7-H) and δ 4.65 and 4.80 (2H, AB part of ABX system, 9-H_2) corresponding to the 9-monoester (247). There were also signals of lower intensity at δ 3.8 and 3.9 (2H, AB part of ABX system, 9-H_2) and 5.41 (1H, m, 7-H) corresponding to the 7-monoester (246). The integrations of these signals indicated a ratio of 6:1 for the 9- : 7- monoesters. The t.l.c. on silica for the mixtures showed a single spot at R_F 0.1.

The Corey-Nicolaou method was chosen for lactonisation because it had been used successfully in a number of related systems (see Chapter 4). The pyridine-2-thiol esters of monoesters (246) and (247) were prepared by the addition of 2,2'-dithiodipyridine (136) and triphenylphosphine (137), using 1.5 equivalents of each. The t.l.c of the thiolesters had R_F 0.6.

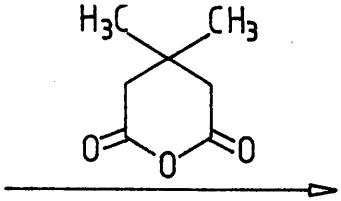
The lactonisation was carried out by adding the thiolester mixture to DME heated at reflux, and continuing the reflux for 5 days. The high dilution conditions involved cut down on any oligomerisation reactions that might have occurred.

Dilactone (248) was partially purified by column chromatography on basic alumina, which removed the 2-thiopyridone (249) and triphenylphosphine oxide (250) by-products. Final purification was achieved by preparative t.l.c on silica. The overall yield from (-)-platynecine (8) was rather disappointing at 11%. Three more macrocyclic diesters (251), (252) and (253) were made in a similar way with yields of 16, 13 and 14%, respectively.

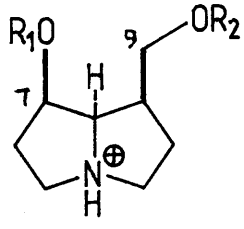
The infra-red spectra of each of the macrocycles showed a peak



(8)



DME

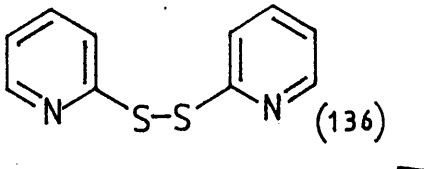


(246) $R_1 = COCH_2C(CH_3)_2CH_2CO_2^{\ominus}$

$R_2 = H$

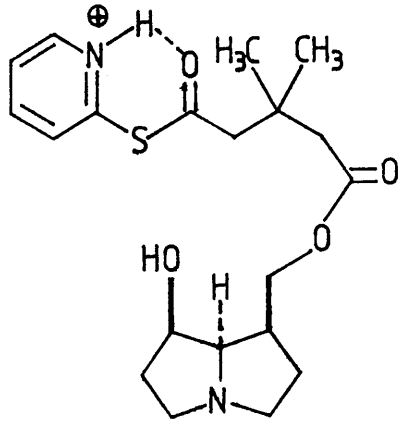
(247) $R_1 = H$,

$R_2 = COCH_2C(CH_3)_2CH_2CO_2^{\ominus}$



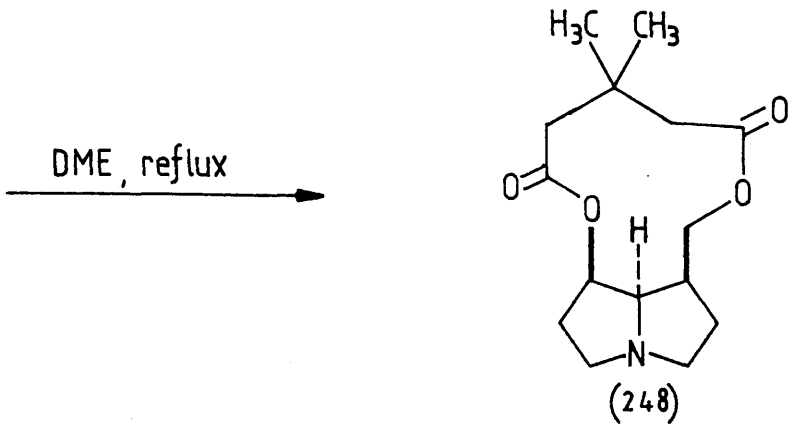
(136)

PPh_3 (137)

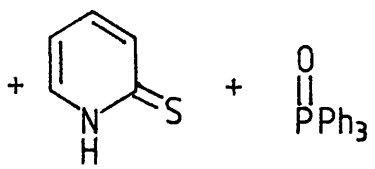


+ 7-thiolester

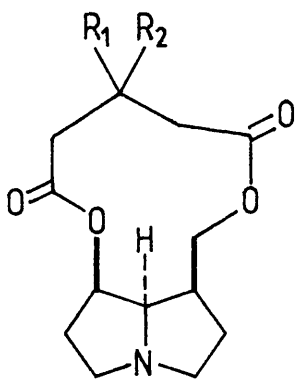
DME, reflux



(248)



(249) (250)



(248) $R_1 = R_2 = CH_3$

(251) $R_1 = R_2 = H$

(252) $R_1 \; R_2 = (CH_2)_4$

(253) $R_1R_2 = (CH_2)_5$

at ca. 1740 cm^{-1} corresponding to the saturated ester carbonyl groups. The dilactones displayed accurate mass measurements giving the appropriate molecular formula in each case. Analysis of the mass spectra showed major fragmentation patterns of $^{m}/_{z}$ $\underline{M}^{+}$, 138, 122, 121, 108, 82, 81, 80. A possible fragmentation pattern is shown in Scheme 50.[138]

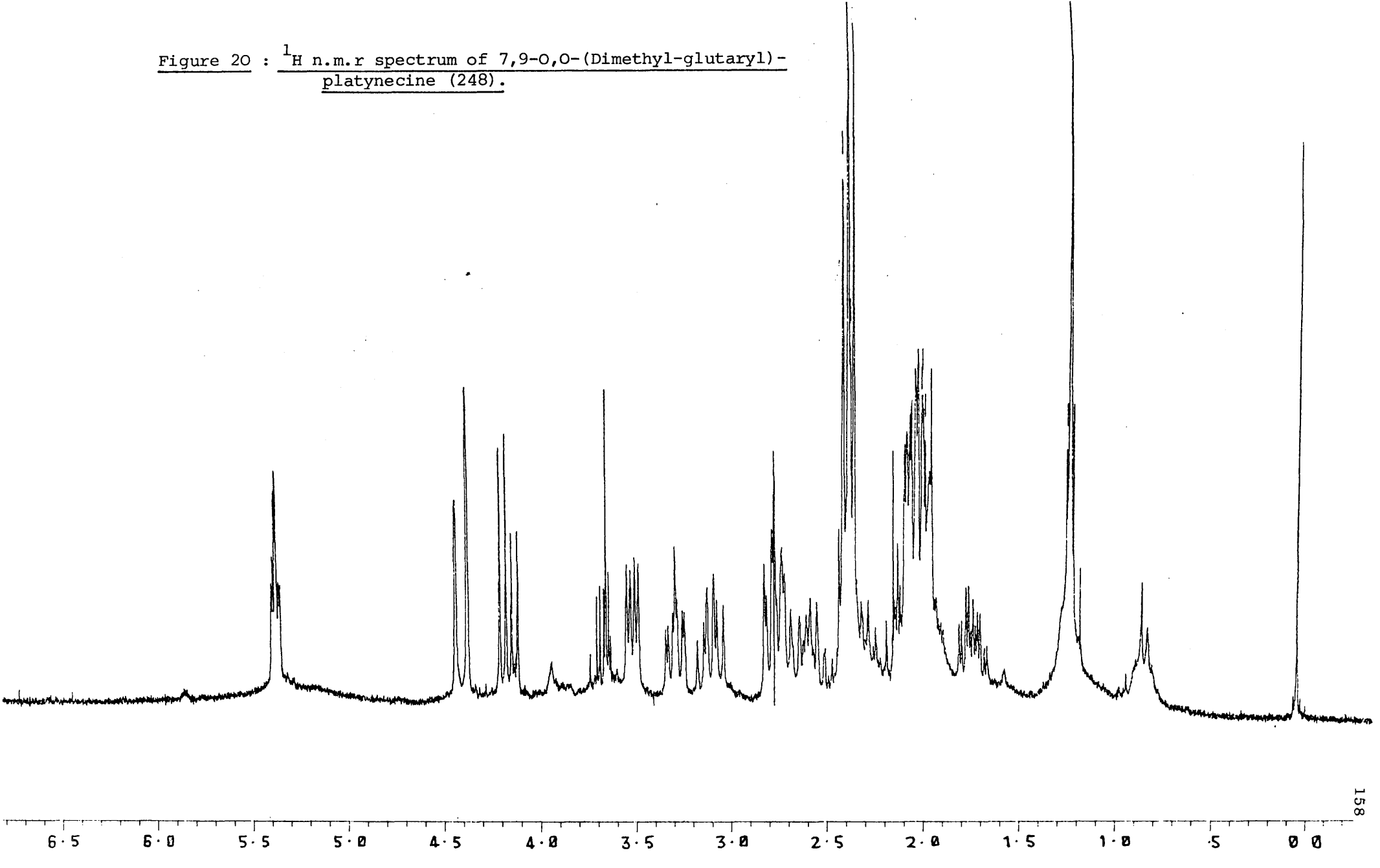
A characteristic feature of each of the ^{1}H n.m.r spectra was the downfield shift of the 7- and 9- protons. In particular, the protons at C-9 showed an AB part of an ABX spectrum. For instance, for analogue (248), the C-9 protons appeared at δ 4.20 and 4.32. Thus the chemical shift difference [Δδ(H-9)] was 0·12 p.p.m. Also, for diesters (248), (252) and (253) AB systems were also present due to the protons at C-12 and C-14. The Δδ(H-9) values for the analogues (251), (252) and (253) were 0.25, 0.08 and 0.25 p.p.m. respectively.

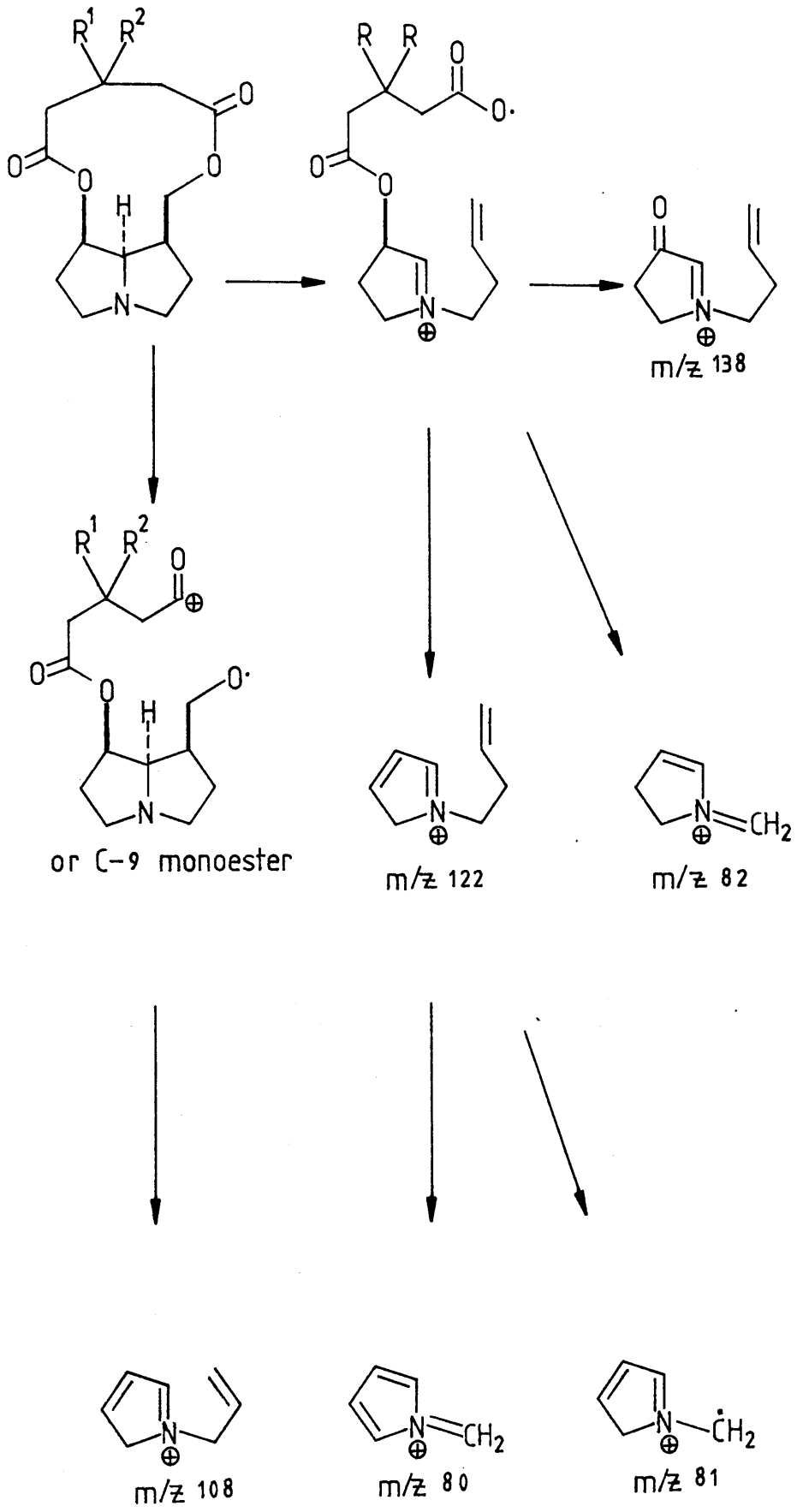
8.4 Synthesis of 10-Membered Dilactones.

The synthesis of these compounds was carried out by reacting (-)-platynecine (8) with derivatives of succinic anhydride then forming the pyridine-2-thiol esters as before. Using succinic anhydride (254) gave, after lactonisation, succinylplatynecine (255) in 12% yield. Accurate mass data showed the molecular formula to be $C_{12}H_{17}NO_4$. The ^{1}H n.m.r. spectrum showed the AB system for the C-9 protons, with a Δδ(H-9) value of 0.30 p.p.m.

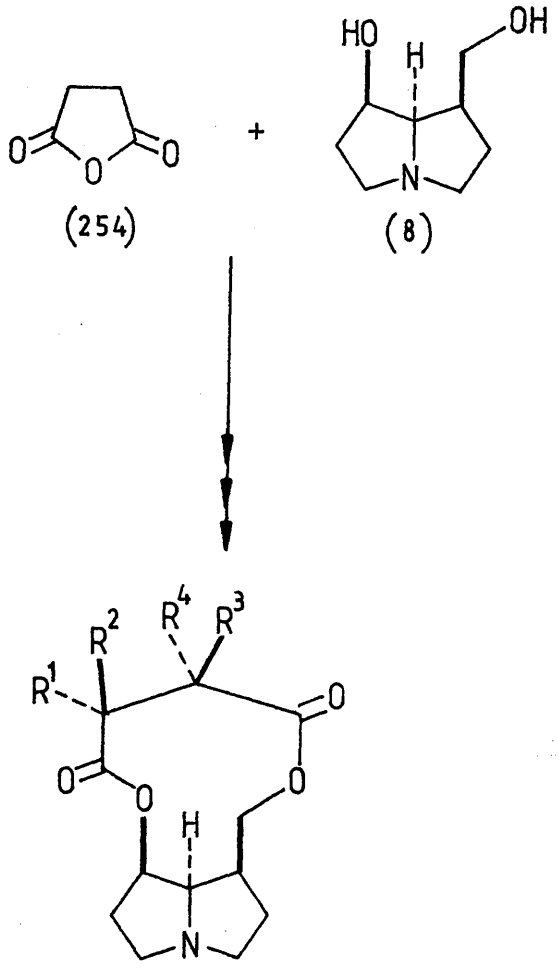
The two other 10-membered analogues (256) and (257) were chosen because they carried substituents at α-positions of the

Figure 20 : ^{1}H n.m.r spectrum of 7,9-O,O-(Dimethyl-glutaryl)-platynecine (248).





Scheme 50.



(254) (8)

(255) $R^1 = R^2 = R^3 = R^4 = H$

(256) $R^1R^3 = (CH_2)_4$, $R^2 = R^4 = H$
$R^1 = R^3 = H$, $R^2R^4 = (CH_2)_4$

(257) $R^1R^4 = (CH_2)_4$, $R^2 = R^3 = H$
$R^1 = R^4 = H$, $R^2R^3 = (CH_2)_4$

diacid portions. The extra steric hindrance around the ester groups is believed to reduce the extent to which they are hydrolysed, and thus detoxified, *in vivo*. Hence, the two should have greater toxicity.[3]

Treatment of both *cis* and *trans*-1,2-cyclohexane dicarboxylic anhydride, separately, gave, after lactonisation, the two mixtures (256) and (257) in 11 and 13% yields respectively. The diastereomeric pairs could not be separated on t.l.c. and so data were collected on the mixtures. The mass spectral, infra-red and ^{1}H n.m.r data for the 10-membered analogues (255) to (257) were consistent with the formation of the macrocyclic system. In particular, the diastereotopic protons at C-9 gave $\Delta\delta$(H-9) values of 0.1 - 0.3 p.p.m. This range is similar to that of the 11-membered analogues with $\Delta\delta$(H-9) values of 0.08 - 0.25 p.p.m.

^{1}H N.m.r spectroscopic data can be used to study the conformations of macrocyclic pyrrolizidine alkaloids in solution, with the value of $\Delta\delta$(H-9) being used as a guide to the conformation adopted. For the analogues described above it would appear that the 10-membered analogues have similar conformations to the 11-membered species. Unfortunately, this could not be confirmed by X-ray crystal structure analysis. This situation contrasts with that of the macrocyclic diesters of (+)-retronecine (3). The $\Delta\delta$(H-9) values are 0 - 0.9 p.p.m. for the 11-membered rings and 1.25 - 1.55 p.p.m. for the 10- or 12-membered dilactones. The 11-membered systems tend to have the ester carbonyls synperiplanar whereas all 10- or 12-membered species which have been subject to X-ray analysis have antiperiplanar conformations. Such

conformational differences may have a bearing on the relative biological activities of the alkaloids and analogues.

8.5 Conclusions.

A series of seven new 10- and 11-membered macrocyclic diesters of (-)-platynecine (8) have been synthesised, using the Corey-Nicolaou lactonisation system. The major problem with the method used was the low yields encountered, even after optimisation of the reaction conditions. This may be due to the relatively unreactive nature of the (-)-platynecine (8) or the instability of the products towards the work-up procedure.

However, the work shown above provides a starting point for the establishment of a study of structure/activity relationships for macrocyclic pyrrolizidine alkaloids containing saturated bases. This work has been accepted for publication.

CHAPTER 9

EXPERIMENTAL

All melting points were measured on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured with an Optical Activity Ltd. AA 10 Polarimeter. Infra red spectra were obtained on a Perkin Elmer 580 spectrophotometer. Nuclear magnetic resonance spectra were recorded with a Perkin Elmer R32 spectrometer operating at 90 MHz (δ_H), a Varian XL-100 spectrometer operating at 25 MHz (δ_C), a Bruker WP200-SY spectrometer operating at 200 MHz (δ_H), 50 MHz (δ_C) and 30.72 MHz (δ_D). Spectra were recorded for solutions in deuteriochloroform unless otherwise stated, with tetramethylsilane as internal standard. Mass spectra were obtained with A.E.I. MS 12 or 902 spectrometers.

T.l.c. was carried out on Kieselgel G plates of 0.25 mm thickness and developed with chloroform-methanol-conc. ammonia (85:14:1) unless otherwise stated. The alkaloids were detected by the modified Dragendorff reagent, or by oxidation with o-chloranil, followed by treatment with Ehrlich's reagent.

Radiochemicals were purchased from Amersham International. Radioactivity was measured with a Philips PW 4700 Liquid Scintillation Counter using toluene-methanol solutions. Sufficient counts were accumulated to give a standard error of less than 1% for each determination. Radioactive samples were recrystallised to constant specific radioactivity and they were counted in duplicate. A Panax thin-layer scanner RTLS-1A was used for radioscanning of t.l.c. plates.

1,2-Dimethoxyethane (DME) and tetrahydrofuran (THF) were dried by distillation from potassium hydroxide and then from sodium-benzophenone under argon prior to use. N,N-Dimethylformamide (DMF) and acetone were dried utilising 3Å molecular sieves. Dichloromethane and chloroform were pre-dried with calcium chloride, and then distilled from phosphorus pentoxide. Organic solutions were dried with anhydrous magnesium sulphate and solvents were evaporated off under reduced pressure below 50°C.

9.1 Experimental to Chapter 5.

(a) *Emilia flammea* Cass. (Compositae) either as fresh plant material (ca. 900 g) or as a hairy root culture[99] (ca. 600 g) was extracted by chopping and blending with methanol. The blended mixture was filtered and the filtrate concentrated in vacuo to leave a green residue. This was extracted with 1M hydrochloric acid (3 x 50 ml) and the combined acidic extracts were washed with chloroform (6 x 100 ml) until the washings were no longer coloured. Zinc powder (2g) was added and the mixture was stirred at room temperature for 2h. The suspension was then filtered through celite, basified (pH 9) with concentrated ammonia solution, and extracted with dichloromethane (5 x 100 ml). The combined organic layers were dried, filtered and concentrated in vacuo to give a crude alkaloid mixture (ca. 100 mg). This was triturated with n-hexane (8 x 2 ml) and upon standing overnight at 0°C, emiline (22) crystallised as a white solid (25 mg), m.p. 105°C (lit.[91,92] 105 - 107°C); R_F 0.34; $[\alpha]_D^{21}$ - 16.5 (c2, $CHCl_3$) (lit.[91] $[\alpha]_D^{20}$ -13.1); ν_{max} ($CHCl_3$) 3020, 2960, 1730 and 1330 cm^{-1}; δ_H[93] 0.85 (3H, t, J 7 Hz, 21-H_3), 1.53 (2H, m, 20-H_2), 1.53 (3H, s, 18-H_3), 2.06 (3H, s, N-CH_3), 2.10 (1H, m, 14-H), 2.20 (1H, m, 6-H_β), 2.25 (1H, m, 15-H), 2.43 (1H, m, 6-H_α), 2.65 (1H, m, 5-H_β), 2.80 (1H, m, 14-H), 2.85 (1H. m, 5-H_α), 3.20 (1H, dt, J 18 Hz and 2.5 Hz, 3-H_α), 3.44 (1H, br d, J 18 Hz, 3-H_β), 3.66 (1H, br, s, O-H), 4.46 (1H, br, d, J 11 Hz, 9-H_α), 4.81 (1H, t, J 3 Hz, 7-H), 5.09 (1H, d, J 11 Hz, 9-H_β), 5.10 (2H, d, J 6 Hz, 19-H_α) and 6.02 (1H, br, s, 2-H); δ_C 12.0 (C-21), 26.5 (C-20), 28.5 (C-18), 36.1 (C-14), 37.5 (C-6), 40.2 (N-Me), 46.9 (C-15), 53.2 (C-5) 58.6 (C-9), 66.6 (C-3), 75.2 (C-12), 77.2 (C-7), 117.9 (C-19), 131.8 (C-2) 135.7 (C-1), 146.5 (C-13), 174.7 and 177.6 (C-11 and C-16) and 191.5 (C-8); m/z 365 (M^+, 7%), 337, 321, 306, 168, 151, 125, 110 and 96

(Found $\underline{M}^+$, 365.1836. $C_{19}H_{27}NO_6$ requires $\underline{M}$, 365.1838).

(1R)-[1-^{2}H]-Putrescine (185) dihydrochloride was prepared according to the method of Richards and Spenser,[32] in a yield of 71% overall m.p. > 300°C (ethanol/acetone); δ_H (D_2O) 1.95 (4H, br m, 2- and 3-H_2) and 3.25 (3H, br, m, 1-H and 4-H_2).

(2R)-[2-^{2}H]-Putrescine (60) Dihydrochloride.

This was prepared according to the work of Kunec and Robins,[28] but some modifications were made and additional spectral data is presented. Therefore, the preparations are given in full.

(2S)-2-Chlorobutanedioic acid. (2S)-Aspartic acid (100 g, 0.752 mol), and urea (10 g) were dissolved in 10M hydrochloric acid (160 ml) and concentrated nitric acid (160 ml). The solution was heated at 70°C for 5h, and vigorous evolution of brown gas was noted. The solution was cooled to room temperature and left for 18h. Crystals of (2S)-2-chlorobutanedioic acid were filtered off, washed with water and dried in vacuo over P_2O_5 (74 g, 65%), m.p. 179 - 180°C (lit.[139] 176°); $[\alpha]_D^{19}$ -21° (c 1, H_2O) (lit.[139] -20.1°); ν_{max} (KBr) 3000 and 1720 cm^{-1} ; δ_H (D_2O) 3.0 (2H, ABX system, J_{AB} 17 Hz, $J_{AX,BX}$ 8 Hz) and 4.2 (1H, t, J 8 Hz); δ_C (D_2O) 41.8 (C-3), 55.0 (C-2), 174.8 and 175.9 (C-1 and C-4). m/z 152 (M^+) (Found : C, 31.28 ; H, 3.30 ; Cl, 23.39. $C_4H_5O_4Cl$ requires C, 31.47; H, 3.28; Cl, 23.28%).

Diethyl (2S)-chlorobutanedioate. (2S)-2-Chlorobutanedioic acid (40 g, 0.26 mmol) was dissolved in dry MeOH (300 ml) and thionyl chloride (64 g) was added at $0^{o}C$ with stirring over 10 m. The solution was stirred for 18 h and excess reagents were removed in vacuo. The residue was partitioned between saturated sodium sulphate solution and diethyl ether. The aqueous layer was extracted with diethyl ether (3 x 20 ml) and the combined organic layers were dried, filtered and concentrated in vacuo. The remaining yellow oil was distilled to give an oil (39.8 g, 84%) b.p. 94-96^{o}C at 10 mm Hg ; $[\alpha]_D^{20}$ - 43^{o} (c 1, $CHCl_3$) (lit.^{140} -42^{o}); ν_{max} 1750 cm^{-1}; δ_H 3.04 (2H, ABX system, J_{AB} 17 Hz, $J_{AX,BX}$ 8 Hz), 3.7 (3H, s), 3.8 (3H,s) and 4.65 (1H,t, J 8 Hz); δ_C 39.0 (C-4), 51.1 (C-3), 51.9 and 53.0 (C-1 and C-6), 168.8 and 169.5 (C-2 and C-5); m/z 180 (M^+) (Found : M^+, 180.0200 ; C, 39.91; H, 4.82; Cl, 19.33. $C_6H_9ClO_4$ requires M, 180.0198; C, 39.89; H, 4.99; Cl, 19.67%).

(2S)-2-Chlorobutane-1,4-diol (187). A solution of dimethyl (2S)-2-chlorobutane dioate (24 g, 0.13 mol) in toluene (350 ml) was cooled to -20^{o}C and di-isobutyl aluminium hydride (1.5M in toluene : 400 ml) was added over 20 m with cooling and stirring. The mixture was stirred for 30 min at -20^{o}C then allowed to come to +20^{o}C over 1h. Ethyl acetate (20 ml) was added and the mixture poured onto a suspension of celite (160 g) in acetone (400 ml). Methanol (50 ml) was added slowly, the mixture was shaken vigorously until gelling occurred then left to stand for 30 min. Water (80 ml) was added to break up the gel and the mixture was filtered and washed with water (2 x 100 ml) and methanol (2 x 100ml). The filtrate was concentrated in vacuo and dried by azeotropic distillation with benzene. The residue

was distilled to give (2S)-2-chlorobutane-1,4-diol (187) as a clear oil (8.8 g, 53%) b.p. 79-83°C at 0.1 mm Hg; $[\alpha]_D^{21}$ -44° (c 2, MeOH) (lit.[28] -45°); ν_{max} 3350 cm^{-1}; δ_H 1.7 - 2.3 (2H, m), 3.6 - 3.9 (4H, m), 4.0 - 4.4 (2H, br m, OH) and 4.4 - 4.7 (1H, m); δ_C 37.0 (C-3), 58.5 (C-4), 60.6 (C-2) and 66.4 (C-1); m/z 124 (M^+) (Found : M^+, 124.0288; C, 38.79; H, 7.12; Cl, 28.91. $C_4H_9ClO_2$ requires M, 124.0318; C, 38.55; H, 7.23; Cl, 28.5%).

(2R)-[2-^{2}H]-Butane-1,4-diol. A solution of (2S)-2-chlorobutane-1,4-diol (4 g, 32 mmol) in dry THF (20 ml) was added to a suspension of lithium aluminium deuteride (98 atom % ^{2}H, 1.6g, 38 mmol) in THF at 0°C with stirring under N_2. The mixture was stirred for 20 h and saturated sodium sulphate solution (8 ml) was added. The mixture was filtered, washed with THF (3 x 5ml) and the combined filtrate and washings were concentrated in vacuo. The residue was distilled to give (2R)-[2-^{2}H]-butane-1,4-diol (1.52 g, 52%) b.p. 83°C at 0.5 mm Hg; ν_{max} 3300 (br) cm^{-1}; δ_H (d_6-acetone) 1.5 - 1.7 (3H, m), 3.5 - 3.8 (4H, m); δ_C (d_6-acetone) 30.1 (C-2 and C-3), 62.6 (C-1 and C-4); m/z 91 (M^+), 71, 57 (Found C, 52.0; H, 10.8. $C_4H_9DO_2$ requires C, 52.7; H, 10.9).

(2R)-[2-^{2}H]-Butanedioic acid. (2R)-[2-^{2}H]-Butane-1,4-diol (100 g, 1.1 mmol) in water (2 ml) was added to a solution of sodium dichromate dihydrate (0.8 g) in dilute sulphuric acid (1M, 5 ml) at 0°C. The solution was stirred and heated at 50°C for 3h, then continuously extracted with diethyl ether for 24 h. The extracts were dried, filtered through celite and concentrated in vacuo. The residue

was recrystallised (acetone) to yield (2R)-[2-^{2}H]-butanedioic acid (112 mg, 86%). m.p. 186-189°C.

(2R)-[2-^{2}H]-Succinic anhydride. A solution of (2R)-[2-^{2}H]-butanedioic acid (100 mg, 0.84 mmol) in acetic anhydride (10 ml) was heated at 60°C for 3h. The solution was cooled and concentrated *in vacuo* to give a solid residue which was recrystallised ($CHCl_3$) to give (2R)-[2-^{2}H]-succinic anhydride (66 mg, 67%) m.p. 119-120°C. Comparison of the mass spectrum of this anhydride with that of unlabelled anhydride showed a ^{2}H content of > 90%.

(2R)-[2-^{2}H]-Putrescine (60) dihydrochloride. A solution of HN_3 in benzene (1.36M, 18 ml)[141] was added to a solution of (2R)-[2-^{2}H]-butane-1,4-diol (0.9 g, 10 mmol) in 10 ml THF. Then a solution of di-isopropyl azodicarboxylate (4.44 g, 22 mmol) in THF (10 ml) was added with stirring. To this mixture was added Ph_3P (11.54 g, 44 mmol) in THF (60 ml) at a rate sufficient to keep the reaction temperature at 40°C. The mixture was allowed to stir for 1h at room temperature and then at 50°C for 3h. Water (2 ml) was added and the solution stirred at 50°C for a further 3h. The solution was cooled and concentrated *in vacuo*, and the residue partitioned between 1M HCl (80 ml) and CH_2Cl_2 (80 ml). The aqueous layer was extracted with CH_2Cl_2 (2 x 80 ml). The aqueous layer was concentrated *in vacuo* leaving a dark red solid, which was recrystallised (aqueous ethanol/acetone) to give (2R)-[2-^{2}H]-putrescine (60) dihydrochloride (500 mg, 31%) m.p. 300°C (dec); ν_{max} (KBr disc) 3020 cm^{-1};

δ_H (D_2O) 1.8 (3H, m) and 3.2 (4H, m); δ_C (D_2O) 24.8 (C-2 and C-3) and 39.9 (C-1 and C-4); $^m/_z$ 89 (M^+) (Found C, 29.1; H, 8.80; N, 17.06. $C_4H_{11}DN_2$ requires C, 29.6; H, 8.69; N, 17.4).

(2S)-[2-^{2}H]-Putrescine (61) Dihydrochloride. This was prepared in an analagous manner from (2R)-aspartic acid. (2R)-2-Chlorobutanedioic acid had m.p. 181°C (lit.[139] 176°C); $[\alpha]_D^{19}$ + 19.8° (c 1, H_2O) (lit.[139] + 20.1); ν_{max} (KBr) 3000 and 1720 cm^{-1}; δ_H (D_2O) 3.0 (2H, ABX system, J_{AB} 17 Hz, $J_{AX,BX}$ 8 Hz) and 4.2 (1H,t, J 8 Hz); δ_C (D_2O) 41.8 (C-3), 54.9 (C-2), 174.9 and 176.0 (C-1 and C-4); $^m/_z$ 152 (M^+) (Found C, 31.27; H, 3.17; Cl, 23.32. $C_4H_5ClO_4$ requires C, 31.48; H, 3.28; Cl, 23.28%). Dimethyl (2R)-2-chlorobutanedioate had b.p. 93-96°C at 10 mm Hg; $[\alpha]_D^{19}$ + 44 (c 1, $CHCl_3$) (lit.[142] + 41.96°); ν_{max} (CCl_4) 1750 cm^{-1}; δ_H 3.00 (2H, ABX system, J_{AB} 17 Hz, $J_{AX,BX}$ 8 Hz), 3.62 (3H, s), 3.71 (3H, s) and 4.60 (1H, t, J 8 Hz); δ_C 38.9 (C-3), 51.1 (C-2), 51.7 and 52.8 (CH_3 x 2), 168.7 and 169.4 (C-1 and C-4); $^m/_z$ 180 (M^+) (Found : M^+, 180.0200; C, 39.98; H, 4.71; Cl, 19.28. $C_6H_9ClO_4$ requires M, 180.0198; C, 39.89; H, 4.99; Cl, 19.67%). (2R)-2-Chlorobutane-1,4-diol had b.p. 79-83°C at 0.1 mm Hg; $[\alpha]_D^{19}$ + 42° (c 1, MeOH) (lit.[28] + 41°); ν_{max} (film) 3350 (br) cm^{-1}; δ_H 1.6 - 2.3 (2H, m), 3.6 - 3.9 (4H, m), 4.0 - 4.3 (2H, br m, OH), 4.35 - 4.55 (1H, m); δ_C 37.0 (C-3). 58.5 (C-4), 60.6 (C-2) and 66.4 (C-1); $^m/_z$ 124 (M^+) (Found M^+, 124.0321; C, 38.58; H, 7.35; Cl, 28.1. $C_4H_9O_2Cl$ requires M, 124.0318; C, 38.55; H, 7.23; Cl, 28.5%). (2S)-[2-^{2}H]-Butane-1,4-diol had b.p. 80-84°C at 0.5 mm Hg; ν_{max} (film) 3300 cm^{-1}; δ_H 1.5 - 1.7 (3H, m), 3.5 - 3.7 (4H, m), 4.6 (2H, br s, OH);

δ_C (d_6-acetone) 29.4 (C-2 and C-3) and 62.3 (C-1 and C-4); $^m/_z$ 91 ($\underline{M}^+$) (Found C, 52.2; H, 10.7. $C_4H_9DO_2$ requires C, 52.7; H, 10.9%).

(2S)-[2-^{2}H]-Butanedioic acid had m.p. 186-189°C. (2S)-[2-^{2}H]-Succinic anhydride had m.p. 119-120°C. Comparison of the mass spectrum of this anhydride with that of unlabelled material indicated a ^{2}H content of >90%. (2S)-[2-^{2}H]-Putrescine (61) dihydrochloride had m.p. > 300° (dec); ν_{max} (KBr disc) 3050, 1450 cm^{-1}; δ_H (D_2O) 1.9 (3H, m) and 3.2 (4H, m); δ_C (D_2O) 24.6 (C-2 and C-3) and 39.7 (C-1 and C-4); $^m/_z$ 89 ($\underline{M}^+$, 8%) (Found C, 29.1; H, 8.80; N, 17.06 $C_4H_{11}DN_2$ requires C, 29.6; H, 8.69; N, 17.4%).

(a) ^{3}H-Labelled retronecine was prepared by feeding [1,4-^{3}H]-putrescine dihydrochloride to *Senecio isatideus*, followed by extraction of the retrorsine (17) and basic hydrolysis. The material had a specific activity of 37 μCi/mmol. This work was done by H.A. Kelly.

[1,9-^{14}C]-Homospermidine (42) was prepared by Dr. S.K. Ner,[100] with a specific activity of 0.12 μCi/mmol.

Feeding methods :

1. *E. flammea* plants were propagated from seed (Suttons Seeds sell them as *Cacalia Coccinea*) in pots in a greenhouse or in an open plot. Various numbers of plants were used for each experiment, typically between 8 and 15. A sample of [1,4-^{14}C]-putrescine (27) dihydrochloride (5 μCi) was added to each feed of ^{2}H- or ^{3}H-labelled precursor. Each feeding sample was divided into equal amounts

Each feeding experiment consisted of a feed of 30 mg of material containing the radiolabel. This was divided among thirty 100 ml flasks containing the root culture and 50 ml of Gamborg B5 medium.

The results of the root culture experiments are given in Table 4.

Compound	Activity fed (μCi)	wt. alkaloid recovered (mg)	Activity recovered (dpm-^{14}C)	specific activity (%)
1. [1,4-^{14}C]putrescine	5	25	38500	4
2. [1,4-^{3}H]putrescine	12	21	32100	1
+[1,4-^{14}C]putrescine	1			
3. [1,9-^{14}C]homospermidine	48	20	30600	3.6
+[1,4-^{3}H]putrescine	5			
4. [5-^{3}H]retronecine	49	23	35300	2.7
+[1,4-^{14}C]putrescine	5			
5.* (1R)-[1-^{2}H]putrescine	5	20	31000	3.8
6.* (2R)-[2-^{2}H]putrescine	5	15	23600	3.4
7.* (2S)-[2-^{2}H]putrescine	5	18	28000	3.5

*Each feed of deuteriated material also contained 5 μCi of [1,4-^{14}C]putrescine.

and fed, via a wick through the plant stem, on alternate days over 6-8 days. After a further 10 days the plants were harvested and extracted. The labelled emiline was subjected to autoradiography in each case, to show one band on the t.l.c. plate coincident with unlabelled emiline at R_F 0.34. Incorporation figures are given in Chapter 5.

2. *E. flammea* hairy root cultures were propagated from cultures provided by Dr. N. Walton at the AFRC Food Research Institute, Norwich and were grown in std medium with a 200 r.p.m. shake rate. At one week old, a batch of typically thirty flasks each containing 50 ml medium was inoculated with the precursor in sterile aqueous solution giving a concentration of 1 mg per 50 ml. A sample of [1,4-$^{14}C_2$] putrescine (5 μCi) was added to each non-radiolabelled precursor. After a further two weeks growth, the cultures were drained and the roots were blended in methanol in the usual manner. Extraction of the alkaloid components gave an emiline sample which showed one band on a t.l.c. plate, by autoradiography, coincident with authentic emiline at R_F 0.34. The various incorporation figures are given in Chapter 5.

Studies on the root cultures of incubation time vs specific incorporation were carried out by taking off a part of each batch at predetermined intervals. The subsequent extractions were done as before to give labelled emiline.

(b) Feedings to the plant *Cynoglossum australe* were carried out in three ways.

1. Complete stem and leaf cuttings were taken and fed by standing the cut ends in a solution containing 5 μCi of [1,4-^{14}C]-putrescine dihydrochloride. Subsequent extraction (by the general method) gave a sample containing a mixture of alkaloids with total incorporations of < 0.1%.

2. The green leaves were cut into strips (10 mm across) and floated on the top of a solution of putrescine (50 mg, 0.3 mmol) containing [1,4-^{14}C]-putrescine (5 μCi) in sterile water (100 ml). Samples of leaves were removed at various time intervals and the % incorporation of ^{14}C into each measured, after the usual extraction procedure.

3. Hairy roots cultures were inoculated with [1,4-^{14}C]-putrescine under the same conditions as for *E. flammea* hairy root cultures. Extraction yielded a small amount of alkaloids (< 5 mg) with no incorporation of ^{14}C, by radioscan. Therefore, the silica from the radioscan plate was cut into bands, extracted and each band counted. No ^{14}C tracer was detected.

(c) The extraction of *Adenocarpus decorticans* (Boiss) was carried out using the same procedure as for *E. flammea* plants (excluding final purification). Freshly collected plants were stripped of leaves (dry wt. 2.1 Kg) which gave a brown oil after the extraction process. Kugelrohr distillation (> 200^{o}C at 0.1 mm Hg) gave a clear oil which was identified as (-)-sparteine (199) (8.3 g, 0.4% from leaves);

R_F 0.11 ; $[\alpha]_D^{21}$ -17° (c 3, $CHCl_3$) (lit.[143] -17.3) ; ν_{max} 2920, 1445 and 1110 cm^{-1}; δ_H (C_6D_6)[144] 0.7 (1H, br d, 8-H), 1.0 (3H, br m, 4- 5- and 13-H), 1.25 (7H, br m, 3- 5- 9-H, 1- and 1-H_2), 1.37 (5H, br m, 3- 4- 6- 13- and 14-H), 1.59 (1H, br s, 7-H), 1.73 (4H, br m, 2- 10- 11- and 15-H), 2.03 (1H, br d, 8-H), 2.22 (1H, br d, 17-H) and 2.40 (3H, br, m, 2- 10- and 15-H); δ_C 24.1 (C-13), 24.3 (C-4), 25.3 (C-14), 25.4 (C-3), 27.0 (C-8), 28.7 (C-5), 32.5 (C-9), 34.1 (C-12), 35.6 (C-7), 52.9 (C-17), 54.8 (C-15), 55.6 (C-2), 61.4 (C-10), 63.7 (C-11) and 65.8 (C-6); m/z 234 (M^+, 14%) (Found M^+, 234.2092. $C_{15}H_{26}N_2$ requires M, 234.2096).

9.2 Experimental to Chapter 6.

A. Synthesis of a Necine Base Analogue.

N-Formyl-L-proline (117):- Formic acid (92 g, 2 mol) was stirred with acetic anhydride (102 g, 1 mol) for 2h at 45°C. A solution of L-proline (11.5 g, 0.1 mol) in formic acid (50 ml) was added and the mixture was stirred for 16 h. Ice water (200 ml) was added and the solution was concentrated in vacuo to give an oil which crystallised from ethyl acetate to give N-formyl-L-proline (6.62 g, 68%) m.p. 84°C $[\alpha]_D$ (c 1, MeOH); ν_{max} 3010, 1725, 1665 (br), 1380 and 1220 cm^{-1}; δ_H ($CDCl_3$) 2.05 (2H, m, 3-H_2), 2.25 (2H, m, 2-H_2), 3.66 (2H, m, 4-H_2), 4.55 (1H, m, 1-H) and 8.32 (1H, br s, CHO). (Found C, 50.31; H, 6.32; N, 9.64. $C_6H_9NO_3$ requires C, 50.35; H, 6.29 N, 9.79).

Cycloaddition of N-formyl-L-proline to diethyl fumarate. Diethyl fumarate (1.72g, 5 mmol) was added to a solution of N-formyl-L-proline (0.71 g, 5 mmol) in acetic anhydride (15 ml) and heated at 130°C with stirring, under N_2 for 4h. Removal of the solvent under reduced pressure gave a brown oil which t.l.c. showed to be made up of three components. Separation of the three by preparative t.l.c., elution with Et_2O/60-80° pet. ether (3:1 v/v) gave the following materials:- Diethyl 5,6,7,8-tetrahydro-1H-pyrrolizine-1,2-dicarboxylate (211) was obtained as an oil (228 mg, 18%); R_F 0.45; ν_{max} 1730, 1695, 1600 and 1200 cm^{-1}; δ_H (CCl_4) 1.25 (6H, m, OCH_2CH_3), 2.0-2.5 (6H, m, 1- and 8-H, 6- and 7-H_2), 3.00 (2H,m, 5-H_2), 4.20 (4H, m, OCH_2) and 6.75 (1H, s, 3-H); m/z 253 (M^+, 12%), 180 (100%), 152, 134, 108, 107 and 106 (Found M^+, 253.1312. $C_{13}H_{19}NO_4$ requires M, 253.1314).

Diethyl-2,5,6,7-tetrahydro-3H-pyrrolizine-1,2-dicarboxylate (212) was obtained as an oil (318 mg, 25%); R_F 0.37; ν_{max} 1730, 1685, 1620 and 1180 cm^{-1}; δ_H (CCl_4) 1.20 (6H, m, OCH_2CH_3), 2.25 (^{2}H, m, 6-H_2), 2.65 (2H, m, 7-H_2), 3.0 (2H, m, 5-H_2), 3.4 (2H, d, J 9Hz 3-H_2), 3.85 (1H, m, 2-H) and 4.05 (4H, m, OCH_2-CH_3); m/z 253 (M^+, 12%), 189, 188, 180 (100%), 152, 108, 107 and 106 (Found M^+, 253.1265. $C_{13}H_{19}NO_4$ requires M, 253.1314).

Diethyl-5,6,7,8-tetrahydro-3H-pyrrolizine-1,2-dicarboxylate (213) was obtained as an oil (144 mg, 12%); R_F 0.27; ν_{max} 1700, 1600 and 1120 cm^{-1}; δ_H (CCl_4) 1.25 (6H, m, OCH_2CH_3), 2.10 - 3.50 (9H, m) and 4.10 (4H, m, OCH_2CH_3); m/z 253 (M^+, 18%), 152, 108, 107 and 106 (Found M^+, 253.1301. $C_{13}H_{19}NO_4$ requires M, 253.1314).

Air oxidation of any of the above three diesters gave rise to a pyrrole (t.l.c. R_F 0.21 {Et_2O/60-80°C pet. ether 3:1}) which was purified by flash silica column chromatography using Et_2O/60-80°C pet. ether as eluant. An oil (40 mg, 6% overall from proline) was obtained and was identified as diethyl 2,3-dihydro-1H-pyrrolizine -6,7-dicarboxylate (214); ν_{max} 1730, 1690 and 1185 cm^{-1}; δ_H (CCl_4) 1.30 (6H, m, OCH_2CH_3), 2.2 - 2.6 (2H, m, 2-H), 3.00 (2H, t, J 7 Hz, 1-H), 3.95 (2H, t, J 8 Hz, 3-H), 4.00 -4.20 (4H, m, OCH_2CH_3) and 6.95(1H, s, 5-H); m/z 251 (M^+, 35%), 206, 205, 178 (100%) and 134 (Found M^+ 251.1160. $C_{13}H_{17}NO_4$ requires M. 251.1158).

B. Synthesis of a Diol Derived from Rosmarinine (44).

2-O-tert-(Butyldimethylsilyl)rosmarinine (215) - Rosmarinine (140 mg, 0.4 mmol) was dissolved in dry DMF (0.75 ml) under an N_2 atmosphere. t-Butyldimethylchlorosilane (90 mg, 0.6 mmol) and imidazole (41 mg, 0.6 mmol) were added and the mixture was stirred at room temperature for 48 h. The mixture was taken up in $CHCl_3$ (10 ml) and washed with saturated brine solution (4 x 10 ml). The combined aqueous layers were extracted with $CHCl_3$ (1 x 40 ml) and the combined organic layers were filtered and concentrated in vacuo. The oil which resulted crystallised from pet. ether (60-80°C) to give 2-O-TBDMS rosmarinine (215) (170 mg, 91%) m.p. 95°C; R_F 0.8; $[\alpha]_D^{20}$ -65° (c 2, $CHCl_3$); ν_{max} 1725, 1250, 1120 and 845 cm^{-1}; δ_H ($CDCl_3$) 0.02 and 0.07 (6H, 2 x s, 2 x CH_3-Si), 0.85 (9H, s, 3 x CH_3-C) and the remaining signals were the same as for rosmarinine; δ_C (d_4-MeOH) - 0.46 and -0.42 (2 x Si-CH_3), 13.5 (C-19), 16.0 (C-21), 19.0 (Si-C), 26.6 (C-18 and 3 x CH_3-C), 35.0 (C-6), 38.8 (C-13), 40.6 (C-14), 50.8 (C-1), 53.5 (C-5), 63.7 (C-3), 64.2 (C-9), 68.3 (C-8), 75.0 (C-7), 76.3 (C-2), 77.4 (C-12), 133.2 (C-15), 136.9 (C-20), 168.9 (C-16), and 178.4 (C-11); m/z 467 (M^+, 20%), 410, 341, 270, 268, 253, 252 (100%), 82 and 75 (Found: M^+, 467.2693; C, 61.57; H, 8.95: N, 3.04: $C_{24}H_{41}NO_6Si$ requires M, 467.2703; C, 61.67; H, 8.78; N, 3.00).

2-O-tert-(Butyldimethylsilyl)rosmarinecine (216). - The TBDMS ether of rosmarinine (215) (170 mg, 0.4 mmol) was dissolved in dry CH_2Cl_2 (10 ml) and cooled to 0°C. A solution of DIBAL in toluene (2.5 ml, 1.0M) was added with stirring under N_2 while

maintaining the temperature at 0°C. After stirring for ½h at 0°C and 1h at room temperature, ethyl acetate (1 ml) was added and the mixture was poured onto a suspension of celite (5 g) in acetone (30 ml). Methanol (2 ml) was added with vigorous shaking until the mixture formed a gel and this was then allowed to stand for ½h. Water (5 ml) was added and the mixture was filtered, washed with water (4 x 10 ml) and MeOH (10 x 10 ml) and concentrated *in vacuo*. The resulting yellow residue was azeotroped with benzene (4 x 1 ml) and purified by column chromatography on basic alumina eluting with 10% $CH_2Cl_2/CHCl_3$. This gave 2-*O*-(tert-Butyldimethylsilyl)rosmarinecine (216) (70 mg, 61%) as a clear oil; R_F 0.3 $[\alpha]_D^{20}$ - 23.9° (*c* 1, CH_2Cl_2); ν_{max} 3320 (br), 1250, 1120 and 840 cm^{-1}; δ_H (d_6 acetone) 0.13 and 0.15 (6H, 2 x s, 2 x Si-CH_3), 0.95 (9H, s, $C(CH_3)_3$), 1.90 (2H, m, 6-H_2), 2.25 (1H, m, 1-H), 2.80 - 3.25 (4H, m, 3- amd 5-H_2), 3.40 (1H, m, 8-H), 3.75 (1H, dd, *J* 12 Hz and 6 Hz, 9-H), 4.15 - 4.35 (4H, br, s, 7-H, 9-H and 2 x OH) and 4.50 (1H, br dd, *J* 9 Hz and 9 Hz, 2-H); δ_C (CD_2Cl_2) - 4.5 and -4.7 (2 x $SiCH_3$), 18.4 (Si-C), 25.9 (-$(CH_3)_3$), 36.9 (C-6), 50.3 (C-1), 54.9)C-5), 57.8 (C-3), 59.0 (C-9), 69.8 (C-8) and 71.8 and 72.0 (C-2 and C-7); *m/z* 287 (M^+, 12%), 243, 230 212 (100%), 99, 86 and 82 (Found M^+, 287.1894. $C_{14}H_{29}NO_3Si$ requires *M*, 287.1916).

9.3 Experimental to Chapter 7.

Zn/Ag couple - Purified Zn dust[145] was used to prepare the Zn/Ag couple according to the method of Conia et al.,[125] giving a black suspension of the metal in diethyl ether. Each couple thus made was used immediately.

Zn/Cu couple - Powdered Zn was used in the method of Smith and Simmons,[126] giving a red/black powder which was stored over P_2O_5 under vacuum. The required amount was used from a bulk stock prepared.

Attempted synthesis of diethyl [2,3-methylene]-fumarate.

1. Diethyl fumarate (1.72 g, 10 mmol) was used in the cyclopropanation method of Conia et al.[125] Upon work up, starting material only was recovered (0.14 g, 8%).

2. Diethyl fumarate (1.72 g, 10 mmol) was used in the method of Smith and Simmons,[126] giving complete recovery of starting material.

1,4-O,O-Bis(tetrahydropyranyl)-(Z)-but-2-ene-1,4-diol (234). (Z)-But-2-ene-1,4-diol (227) (1 g, 11.4 mmol) was dissolved in a stirred solution of 3,4-dihydro-2H-pyran (2.11 g, 25.1 mmol) in dry THF (40 ml). A crystal of p-toluene sulphonic acid was added and the solution was stirred for 18 h at room temperature. Saturated sodium bicarbonate solution (25 ml) was added and the solution was concentrated in vacuo. The residue was partitioned between water (25 ml) and diethyl ether (25 ml). The aqueous

layer was extracted with diethyl ether (5 x 25 ml) and the combined organic layers dried, filtered and concentrated under reduced pressure to give the title compound as a single product (2.43 g, 85%); R_F 0.7; ν_{max} 3450 (br), 3020, 2950 and 1025 cm^{-1}; δ_H ($CDCl_3$) 1.40 - 1.95 (12H, br m, ring-CH_2), 3.55 (2H, m, acetal -CH), 3.80 - 4.00 (4H, m, ring-OCH_2), 4.20 (4H, m, 1- and 4-H_2) and 5.74 (2H, t, J 5Hz, 2- and 3-H); (Found C, 65.7; H, 9.82. $C_{14}H_{24}O_4$ requires C, 65.62; H, 9.38).

1,4-O,O-Bis(tetrahydropyranyl)-(E)-but-2-ene-1,4-diol (235). Using the same method as for the (Z) isomer, (E)-but-2-ene-1,4-diol (228) (1 g, 11.4 mmol) was used to produce the title compound (2.43 g, 85%); R_F 0.7; ν_{max} 3450 (br), 3020, 2950 and 1025 cm^{-1}; δ_H ($CDCl_3$) 1.40 - 1.90 (12H, br m, ring $-CH_2$ 3.60 (2H,m,acetal-H), 3.88 (4H,m,ring $-OCH_2$), 4.18 (4H,m, 1- and 4-H_2) and 5.74 (2H,m, 2- and 3-H) (Found C, 65.1; H, 9.99. $C_{14}H_{24}O_4$ requires C, 65.62; H, 9.38).

Attempted synthesis of cis-1,2-Bis(hydroxymethyl)cyclopropane (242). Following the Method of Conia et al.,[125] or of Smith and Simmons[126] with cis 2-butene-1,4-diol gave no formation of cyclopropane (by n.m.r. data).

Attempted synthesis of trans-1,2-Bis(hydroxymethyl)cyclopropane (243). - The same results were obtained as for the cis isomer.

Attempted synthesis of cis- and trans-1,2-Bis(tetrahydropyranyl-hydroxymethyl)cyclopropane and using either cyclopropanation method,[125,126] gave complete return of starting material.

Diethyl bromomalonate (240). Bromine (16.5 g, 0.1 mmol) was added slowly, with heating for the first few drops to a solution of diethyl malonate (16 g, 0.1 mol) in CCl_4 (15 ml). After addition, the mixture heated at reflux until the colour disappeared and was then allowed to cool. After washing with 2% w/v Na_2CO_3 (5 x 50 ml) the organic layer was dried, filtered, and concentrated in vacuo. The residue was carefully distilled to give diethyl bromomalonate (12.1 g, 51%); b.p. 50-60°C at 0.1 mm Hg (lit.[132] 132 - 136°C at 33 mm Hg); δ_H 1.33 (6H, t, $-CH_3$), 4.31 (4H, q, $-CH_2-$), and 4.86 (1H, s); $\underline{m}/\underline{z}$ 240 and 238 ($\underline{M}^+$) (Found C, 35.28; H, 4.77; Br, 31.17. $C_7H_{11}O_4Br$ requires C, 35.12; H, 4.60; Br, 33.47).

1,1-Bis(carboxyethyl)-2-carboxyethyl cyclopropane (241). To a suspension of NaH (0.24 g dry weight, 0.01 mmol) in dry THF (25 ml) was added methyl acrylate (1.29 g, 0.015 mmol). The solution was cooled to 0°C and diethyl bromomalonate (2.4g, 0.01 mmol) was added with vigorous stirring. After 20 min, water (15 ml) was added and the solvent removed in vacuo. The residue was taken up in HCl (15 ml, 1$\underline{M}$) and extracted with $CHCl_3$ (4 x 20 ml). The combined organic layers were dried and concentrated in vacuo to give an oil which was distilled to give (±)-1,1-bis(carboxyethyl)-2-carboxymethyl cyclopropane (1.52 g, 62%) b.p. 91-92°C at 0.05 mm Hg; ν_{max} 3025, 1725 and 1280 cm^{-1}; δ_H 1.28 (6H, t, CH_2CH_3), 1.65, 1.96 and 2.58 (3H, ABX, $\underline{J}_{AB}$ = 4.6 Hz, $\underline{J}_{AX}$ = 9.3Hz and $\underline{J}_{BX}$ = 6.5Hz),[134] 3.73 (3H, s, OCH_3), and 4.22 (4H, q, $-OCH_2CH_3$); δ_C 13.4 ($-CH_2CH_3$), 18.9 (C-3), 26.9 (C-2), 36.4 (C-1), 51.7 ($-OCH_3$), 61.1 and 61.7 ($-OCH_2-$), 165.2 and 168.0 (C-4 and C-5) and 169.2 (C-6);

m/z 244 (M^+, 4.3%), 199 (100%), 185, 171 and 157; (Found: M^+, 244.0945; C, 53.4; H, 7.26. $C_{11}H_{16}O_6$ requires M, 244.0947 ; C, 54.1; H, 6.56).

(±)-1,1,2-Cyclopropane tricarboxylic acid (236). 1,1-Bis-(Carboxyethyl)-2-carboxymethyl cyclopropane (1 g, 4.1 mmol) as a solution in EtOH (5 ml) was added slowly dropwise to a solution of NaOH (0.7 g) in water (5 ml) with stirring at 80°C. Water (10 ml) was added and the mixture was heated at reflux for 18 h. The solvent was removed under reduced pressure, and the residue was dissolved in HCl (50 ml, 1M), and continuously extracted with diethyl ether for 24 h. Evaporation of the organic solvent under high vacuum gave an off-white solid which was recrystallised from water to give the title compound as a white crystalline solid (0.44 g, 61% yield) m.p. 184-185°C; (lit.[133] 185-186°C); R_F 0.4; ν_{max} 3100, 1690, 1425, 1290 and 1210 cm^{-1}; δ_C (D_2O) 1.6, 1.7 and 2.52 (3H, ABX system, J_{AB} = 4.8 Hz, J_{AX} = 9.4 Hz and J_{BX} = 6.5 Hz); δ_C (D_2O) 20.8 (C-3), 29.5 (C-2), 37.6 (C-1), 171.5, 172.7 and 173.9 (carboxylates); m/z no M^+, 130 ($-CO_2$), 129 ($-CO_2H$) and 128 ($-HCO_2H$) (Found: C, 40.99; H, 3.59; $C_6H_6O_6$ requires C, 41.38; H, 3.45).

Decarboxylation of 1,1,2-cyclopropane tricarboxylate.

Method 1: The triacid (100 mg, 0.6 mmol) was heated at 200°C on a Woods metal bath, under N_2, in the presence of 2,4,6 trimethyl pyridine (4ml). N.m.r. spectroscopy of the crude mixture showed mainly starting material.

Method 2. The triacid (100 mg, 0.6 mmol) was heated at 200°C on a Kugelrohr apparatus, under N_2, in the presence of 6M HCl (4 ml). N.m.r. spectroscopy of the crude mixture showed no identifiable product or starting material.

Method 3. The triacid (100 mg, 0.6 mmol) was heated at 200°C in a pre-heated Kugelrohr oven for 10 min, at a pressure of 0.1 mm Hg. During this time a white solid was deposited on the inside of the receiver bulbs. Recrystallisation from H_2O gave cis-1,2-cyclopropane dicarboxylate (238) (24 mg, 32% yield) m.p. 139 - 142°C (lit.[146] 139°C); R_F 0.1 ($CHCl_3$); ν_{max} 3050, 2950 (br), 2650 (br) and 1700 cm^{-1}; δ_H (D_2O) 1.65 (2H, m, 3-H_2) and 2.35 (2H, m, 1- and 2-H); δ_C (D_2O) 16.56 (C-3), 23.3 (C-1 and C-2) and 177 p.p.m. m/z 130 (M^+, 1.6%), 112, 84 (100%); (Found M^+, 130.0257; C, 46.29; H, 4.61; $C_5H_6O_4$ requires M, 130.0266; C, 46.15; H, 4.61). The brown residue in the reaction vessel was triturated with diethyl ether leaving a brown gum which was recrystallised from water to give (±)-trans-1,2-cyclopropane dicarboxylate (239) (38 mg, 51% yield), m.p. 177-179°C (lit.[146] 175-176°C); R_F 0.1 ($CHCl_3$); ν_{max} 3050 3000 (br), 2650 (br) and 1700 cm^{-1}; δ_H (D_2O) 1.51 (2H, m, 3-H_2) and 2.10 (2H, m, 1- and 2-H); δ_C (D_2O) 15.4 (C-3), 22.2 (C-1 and C-2) and 175.6 p.p.m.; m/z 130 (M^+, 2.1%), 112, 84 (100%); (Found C, 46.45; H, 4.64. $C_5H_6O_4$ requires C, 46.15; H, 4.61).

cis 1,2-Bis(hydroxymethyl)cyclopropane (242). cis-1,2-cyclopropane dicarboxylate (238) (500 mg, 3.85 mmol) was dissolved in dry CH_2Cl_2 (10 ml) and cooled to 0°C. Diisobutylaluminium hydride in toluene (25 ml, 1.0 M, 25 mmol) was added dropwise with stirring,

under N_2. The solution was stirred at 0°C for 30 minutes then at room temperature for 1 h. Ethyl acetate (1 ml) was added and the solution was poured onto a suspension of acetone (50 ml) and celite (10 g). Methanol (5 ml) was added with vigorous shaking until the mixture gelled. After standing for 15 minutes, water (10 ml) was added and the mixture was filtered and washed with water (3 x 20 ml) and methanol (8 x 50 ml). Concentration of the filtrate, *in vacuo*, gave an oil which was distilled to give *cis*-1,2-bis(hydroxymethyl) cyclopropane (242) (257 mg, 65%) b.p. 61°C at 0.2 mm Hg (lit.[147] 107-109°C at 5 mm Hg); ν_{max} 3300 (br), 2950 and 1050 cm^{-1}; δ_H (d_6-acetone) 0.45 (2H, m, 3-H_2), 0.98 (2H, m, 1- and 2-H), 3.40 (4H, br m, O-CH_2) and 4.00 (2H, br s, O-H); δ_C (d_6-acetone) 9.36 (C-3), 23.1 (C-1 and C-2) and 64.2 (C-4 and C-5). The dibenzoate derivative melts at 91-93°C (EtOH) (Found C, 72.98; H, 5.76. $C_{19}H_{18}O_4$ requires C, 73.55; H, 5.81).

trans-1,2-bis(hydroxymethyl)cyclopropane (243). The reduction was carried out on *trans*-1,2-cyclopropane dicarboxylate (239) (500 mg, 3.85 mmol) using the same method as for *cis*-1,2-cyclopropane dicarboxylate. This gave the title compound as an oil (270 mg, 69%), b.p. 150°C (Kugelrohr oven temperature) at 0.1 mm Hg (lit.[147] 95-103°C at 1 mm Hg); $[\alpha]_D^{20}$ 0° (*c* 3 in MeOH); ν_{max} 3300 (br), 2950 and 1055 cm^{-1}; δ_H (d_6-acetone) 0.35 (2H, dd, *J* 7 Hz and 6.7 Hz, 3-H_2), 0.90 (2H, m, 1- and 2-H), 3.26 (2H, dd, *J* 11.1 Hz and 6.9 Hz, 4- and 5-H), 3.46 (2H, dd, *J* 11.3 Hz and 5.7 Hz) and 4.58 (2H, s, OH); δ_C (d_6-acetone) 7.72 (C-3), 19.85 (C-1 and C-2) and 65.7 (C-4 and C-5). The dibenzoate derivative melts at

112-113°C (EtOH), (Found C, 73.14; H, 5.92. $C_{19}H_{18}O_4$ requires C, 73.55; H, 5.81).

cis-1,2-Bis(aminomethyl)-cyclopropane (229) dihydrochloride.

To a solution of cis-1,2-bis(hydroxymethyl)cyclopropane (128 mg, 1.3 mmol) in dry THF (5 ml) was added a solution of HN_3 in benzene (2.34 ml, 1.4M, 3.28 mmol) and a solution of diisopropyl azodicarboxylate (0.58 g, 2.88 mmol) in dry THF (5 ml). Ph_3P (1.50 g, 5.72 mmol) was added dropwise keeping the reaction temperature below 40°C. After addition, the solution was stirred for 30 minutes then heated at 50°C for 2 h. Water (2 ml) was added and heating at 50°C was continued for 2 h. The solvent was removed in vacuo and the residue was partitioned between CH_2Cl_2 (25 ml) and HCl solution (25 ml, 1M). The aqueous layer was washed with CH_2Cl_2 (2 x 25 ml) and concentrated in vacuo. The solid remaining was recrystallised from 90% aqueous ethanol/acetone to give cis-1,2-bis(aminomethyl)cyclopropane (229) dihydrochloride as a white powder (105 mg, 47%) m.p. > 300°C; R_F 0.2 ($MeOH/NH_3$, 9:1); ν_{max} 3040 and 1730 cm^{-1}; δ_H (D_2O) 0.99 (2H, m, 3-H_2), 1.40 (2H, m, 1- and 2-H) and 3.20 (4H, complex, 4- and 5-H_2); δ_C (D_2O) 10.65 (C-3), 15.69 (C-1 and C-2) and 43.70 (C-4 and C-5); (Found C, 34.95; H, 7.76; N, 15.84. $C_5H_{14}N_2Cl_2$ requires C, 34.68; H, 8.23; N, 16.18).

(±)-trans-1,2-Bis(aminomethyl)cyclopropane (230) dihydrochloride. This compound was made in an exactly analogous fashion to cis-1,2-bis(aminomethyl) cyclopropane (229) dihydrochloride to give off-white needles (152 mg, 58%), m.p. > 320°C; R_F 0.2 (MeOH/NH_3, 9:1); ν_{max} 3045 and 1740 cm^{-1}; δ_H (D_2O) 0.91 (2H, br t, 3-H_2), 1.82 (2H, complex, 1- and 2-H), 3.49 (2H, dd, J 13.2 Hz and 7.2 Hz, 4- and 5-H) and 3.69 (2H, dd, J 13.3 Hz and 6.6 Hz, 4- and 5-H); δ_C (D_2O) 9.69 (C-3), 15.00 (C-1 and C-2) and 42.98 (C-4 and C-5); (Found C, 35.03; H, 7.89; N, 16.62. $C_5H_{14}N_2Cl_2$ requires C, 34.68; H, 8.23; N, 16.18).

(E)-But-2-ene-1,4-diol (228). A solution of DIBAL in toluene (39 ml, 1.5M, 0.06 mol) was added dropwise to a solution of diethyl fumarate (2 g, 0.012 mol) in dry toluene (50 ml) at -20°C, under N_2 with stirring. The mixture was stirred at -20°C for 30 minutes then at room temperature for 1 h. Ethyl acetate (2.5 ml) was added and the solution was poured onto a suspension of celite (20 g) in acetone (120 ml). Methanol (20 ml) was added slowly with vigorous shaking until a gel formed. The gel was left to stand for 30 minutes then water was added to break up the gel. The mixture was filtered, washed with water (2 x 100 ml), and methanol (5 x 100 ml) then the combined filtrate and washings were concentrated in vacuo. The residue was distilled to give (E)-But-2-ene-1,4-diol (228) (0.54 g, 51%) b.p. 150°C (Kugelrohr) at 1 mm Hg (lit.[148] 125-127°C at 10 mm Hg); δ_H (d_4-MeOH) 4.13 (4H, d, J 2 Hz) and 5.85 (2H, br s); (Found C, 54.40; H, 9.03. $C_4H_8O_2$ requires C, 54.54; H, 9.09). The dibenzoate derivative was obtained as white crystals from EtOH, m.p. 103-104°C. The dibenzoate derivative of

authentic (Z)-But-2-ene-1,4-diol is m.p. (EtOH) 64-65°C.[148]

(E)-But-2-ene-1,4-diamine (226) dihydrochloride. A solution of HN_3 in benzene (25 ml, 1.86M, 0.05 mol) was added to a solution of (E)-but-2-ene-1,4-diol (228) (0.88 g, 0.01 mol) in THF (10 ml). Diisopropylazodicarboxylate (4.44 g, 0.022 mol) in THF (10 ml) was also added with stirring. A solution of Ph_3P (11.54 g, 0.044 mol) in THF (60 ml) was added dropwise such that the temperature of the reaction was maintained at around 40°C. After addition the solution was stirred for 1 h then heated at 50°C for 3 h. Water (2 ml) was added and the solution was stirred at 50°C for a further 3 h. The solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2 (80 ml) and HCl solution (80 ml, 1M). The aqueous layer was then washed with CH_2Cl_2 (2 x 80 ml) and the water was evaporated in vacuo to leave (E)-but-2-ene-1,4-diamine (226) dihydrochloride (500 mg, 31%) m.p. > 300°C (dec); R_F 0.1 (MeOH/NH_3, 9:1); δ_H (D_2O) 4.0 (4H, m) and 6.15 (2H, m); δ_C (D_2O) 42.8 (C-1 and C-4) and 130.4 (C-2 and C-3); (Found C, 29.96; H, 7.59; N, 17.19. $C_4H_{12}N_2Cl_2$ requires C, 30.19; H, 7.55; N, 17.61).

(Z)-But-2-ene-1,4-diamine (225) dihydrochloride. This was made in an exactly analagous fashion to the (E)-isomer, using (Z)-but-2-ene-1,4-diol (227) (0.44 g, 5 mmol) [supplied by Aldrich]. This gave the title compound (265 mg, 33%), m.p. > 300°C; R_F 0.1 (MeOH/NH_3, 9:1); δ_H (D_2O) 4.1 (4H, m) and 6.2 (2H, m); δ_C (D_2O) 38.6 (C-1 and C-4) and 129.4 (C-2 and C-3). The spectrum also contained two small peaks corresponding to the (E)-isomer at 42.8

and 130.4 p.p.m; (Found C, 30.09; H, 7.77; N, 17.22. $C_4H_{12}N_2Cl_2$ requires C, 30.19; H, 7.55; N, 17.61).

9.4 Experimental to Chapter 8.

(-)-Platynecine (8). (+)-Retronecine (500 mg, 3.2 mmol) was dissolved in absolute ethanol (25 ml) and hydrogenated at 1 atm for 18 h at room temperature over 10% Pd-charcoal (50 mg) as catalyst. The solution was filtered through celite and the solvent was removed in vacuo to give (-)-platynecine (8) as a clear oil, which crystallised from acetone (383 mg, 75%), m.p. 149-151°C (lit.[149] 148-149°C); $[\alpha]_D^{20}$ -58° (c 1, EtOH) (lit.[149] -56.8°); ν_{max} (KBr disc) 3350, 2940, 2880 and 1480 cm^{-1}; δ_H 1.56 - 2.24 (4H, complex, 2 and 6-H_2), 2.41 (1H, m, 1-H), 2.69 - 3.00 (2H, complex, 3- or 5-H_2), 3.04 (1H, m, 8-H), 3.20 (2H, complex, 3- or 5-H_2), 3.81 (1H, dd, J 11.2 Hz and 4.2 Hz, 9-H), 3.91 (1H, dd, J 11.2 Hz and 2.6 Hz, 9-H) and 4.21 (1H, m, 7-H); δ_C (25 MHz) 28.6 (C-2), 36.5 (C-6), 43.9 (C-1), 53.7 and 55.3 (C-3 and C-5), 61.6 (C-9), 71.1 (C-8) and 73.1 (C-7); m/z 157 (M^+, 7%), 113, 83, 82 and 81; (Found: M^+, 157.1108; C, 61.21; H, 9.71; N, 9.02. $C_8H_{15}NO_2$ requires M 157.1103; C, 61.15; H, 9.55; N, 8.92).

General Procedure for Synthesis of Dilactones [(248), (251)-(253), (255)-(257)]. The anhydride (0.76 mmol) was added to a solution of (-)-platynecine (3) (100 mg, 0.64 mmol) in dry DME (20 ml) at room temperature under dry N_2. The solution was stirred for 7 d to form the zwitterionic monoesters (t.l.c., MeOH-NH_3 (9:1), R_F 0.30). Di-2-pyridyl disulphide (220 mg, 1 mmol) and triphenylphosphine (262 mg, 1 mmol) were added and the mixture was stirred at room temperature under N_2 for 48 h until thioester formation was complete (R_F 0.13). The homogeneous yellow solution was added over ½ h by syringe to dry DME (100 ml) heated at reflux under

dry N_2. The mixture was then heated for a further 5 d. The diester was partially purified by application to a basic alumina (activity 1) column and elution with $CHCl_3$. (This removed most of the 2-thiopyridone and triphenyl phosphine oxide). The fractions containing the more polar compounds (R_F 0.0 - 0.4) were concentrated *in vacuo* to give crude dilactones as yellow oils. Final purification was achieved by preparative t.l.c.

(-)-7,9-*O*,*O*-(3,3-Dimethylglutaryl)platynecine (248) was obtained as a non-crystalline solid (11% yield) m.p. 92-94°C (benzene-hexane); R_F 0.15; $[\alpha]_D^{24}$ -24.0° (*c* 2, $CHCl_3$); ν_{max} (CCl_4) 2960, 1740, 1230 and 1180 cm^{-1}; δ_H 1.11 (3H, s, 17-H_3 or 18-H_3), 1.30 (3H, s, 17-H_3 or 18-H_3), 1.94 (2H, m, 2-H_2), 2.08 (2H, m, 6-H_2), 2.17 and 2.30 (2H, AB system, *J* 13.5 Hz, 12-H_2 or 14-H_2), 2.20 and 2.32 (2H, AB system, *J* 13.0 Hz, 12-H_2 or 14-H_2), 2.50-2.70 (1H, m, 1-H), 2.83 (2H, m, 3-H_2 or 5-H_2), 3.15 and 3.35 (2H, m, 3-H_2 or 5-H_2), 3.64 (1H, m, 8-H), 4.20 (1H, dd, *J* 12.4 Hz and 2.3 Hz, 9-H), 4.32 (1H, dd, *J* 12.4 Hz and 6.1 Hz, 9-H), and 5.35 (1H, t, *J* 4.0 Hz, 7-H); δ_C 27.6 (C-17 or C-18), 27.9 (C-2), 31.9 (C-17 or C-18), 33.7 (C-13) 34.6 (C-6), 39.3 (C-1), 45.3 and 45.7 (C-12 and C-14), 52.6 and 53.5 (C-3 and C-5), 60.6 (C-9), 70.8 (C-8), 73.4 (C-7), 170.5 and 171.1 (C-11 and C-15); *m*/*z* 281 (M^+, 12%), 277, 181, 154, 140, 138, 122, 121, 120, 108, 82 (100%), 81, and 80; (Found M^+, 281.1629; C, 63.81; H, 8.45; N, 4.91. $C_{15}H_{23}NO_4$ requires *M*, 281.1627; C, 64.06; H, 8.18; N, 4.98%).

(-)-7,9-*O*,*O*-(Glutaryl)platynecine (251) was obtained as an oil (16% yield); R_F 0.18; $[\alpha]_D^{20}$ -15.0° (*c* 1.4, $CHCl_3$); ν_{max} (CCl_4) 2980, 2930, 2870, 1735 and 1285 cm^{-1}; δ_H 1.22 (2H, m, 13-H_2), 1.90-2.1 (4H, m, 2-H_2 and 6-H_2), 2.35 (4H, m, 12-H_2 and 14-H_2), 2.58 (1H, m, 1-H), 2.77-3.10 (2H, m, 3-H_2 or 5-H_2), 3.39-3.51 (2H, m, 3-H_2 or 5-H_2), 3.65 (1H, m, 8-H), 4.15 (1H, dd, *J* 12.2 Hz and 6.6 Hz, 9-H), 4.40 (1H, br d, *J* 12.2 Hz, 9-H), and 5.37 (1H, m, 7-H); δ_C 20.8 (C-13), 26.4 (C-2), 29.6 (C-6), 34.4 and 34.6 (C-12 and C-14), 39.6 (C-1), 53.6 and 54.4 (C-3 and C-5), 60.4 (C-9), 71.2 (C-8), 74.1 (C-7), 172.0 and 173.4 (C-11 and C-15); *m*/*z* 253 (M^+, 10%) 140, 138, 122, 121, 108, 96, 95, 82 (100%), 81 and 80; (Found *M*, 253.1314. $C_{13}H_{19}NO_4$ requires *M*, 253.1314).

(-)-7,9-*O*,*O*-(3,3-Tetramethyleneglutaryl)platynecine (252) was obtained as an oil (13% yield); R_F 0.13; $[\alpha]_D^{20}$ -30.0° (*c* 1, $CHCl_3$); ν_{max} (CCl_4), 2960, 2930, 1745, 1100, 1030 cm^{-1}; δ_H 1.70 (8H, m, 17-, 18-, 19- and 20-H_2), 1.90-2.30 (4H, complex, 2-H_2 and 6-H_2), 2.24 and 2.39 (2H, AB system, *J* 12.9 Hz, 12-H_2 or 14-H_2), 2.28 and 2.40 (2H, AB system, *J* 14.0 Hz, 12-H_2 or 14-H_2), 2.58 (1H, m, 1-H), 2.85 (2H, m, 3-H_2 or 5-H_2), 3.07 and 3.24 (2H, m, 3-H_2 or 5-H_2), 3.56 (1H, m, 8-H), 4.16 (1H, br d, *J* 4.5 Hz, 9-H), 4.24 (1H, br d, *J* 4.5 Hz, 9-H), and 5.49 (1H, t, *J* 4.0 Hz, 7-H); δ_C 23.0 and 23.7 (C-18 and C-19), 28.1 (C-2), 29.8 (C-6), 34.9 and 36.8 (C-17 and C-20), 39.1 (C-1), 43.6 and 43.8 (C-12 and C-14), 44.5 (C-13), 52.6 and 53.5 (C-3 and C-5), 61.1 (C-9), 71.4 (C-8), 73.3 (C-7), 171.1 and 171.6 (C-11 and C-15); *m*/*z* 307 (M^+, 32%), 148, 181, 140, 139, 138, 122 (100%), 121, 108, 96, 95, 82, 81 and 80; (Found *M*, 307.1790. $C_{17}H_{25}NO_4$ requires *M*, 307.1783).

(-)-7,9-*O*,*O*-(3,3-Pentamethyleneglutaryl)platynecine (253) was obtained as an oil (14%); R_F 0.14; $[\alpha]_D^{20}$ -26.9° (*c* 1, $CHCl_3$); ν_{max} (CCl_4) 2940, 2860, 1740, 1440, 1225, 1160 and 1120 cm^{-1}; δ_H 1.48 (10H, br m, 17-, 18-, 19-, 20- and 21-H_2), 1.90-2.09 (2H, m, 2-H_2), 2.15-2.20 (2H, m, 6-H_2), 2.10 and 2.57 (2H, AB system, *J* 14.0 Hz, 12-H_2 or 14-H_2), 2.26 and 2.38 (2H, AB system, *J* 13.4 Hz, 12-H_2 or 14-H_2), 2.62 (1H, m, 1-H), 2.95 (2H, m, 3-H_2 or 5-H_2), 3.36 and 3.57 (2H, m, 3-H_2 or 5-H_2), 3.79 (1H, m, 8-H), 4.18 (1H, dd, *J* 6.2 Hz and 1.1 Hz, 9-H), 4.43 (1H, dd, *J* 6.2 Hz and 2.8 Hz, 9-H), and 5.40 (1H, t, *J* 3.8 Hz, 7-H); δ_C 21.5 and 21.6 (C-18 and C-20), 25.7 (C-19), 28.2 (C-2), 29.7 (C-6), 34.6 and 35.4 (C-17 and C-21), 36.5 (C-13), 39.1 and 39.6 (C-12 and C-14), 42.4 (C-1), 52.9 and 53.9 (C-3 and C-5), 60.1 (C-9), 71.2 (C-8), 73.3 (C-7), 170.5 and 171.4 (C-11 and C-15); *m*/*z* 321 (M^+, 12%), 181, 138, 122 (100%) 121, 120, 108, 96, 95, 82, 81 and 80; (Found M^+, 321.1923. $C_{18}H_{27}NO_4$ requires *M*, 321.1935).

(-)-7,9-*O*,*O*-(Succinyl)platynecine (255) was prepared as an oil (12% yield); R_F 0.37; $[\alpha]_D^{23}$ -16.0° (*c* 1.5, $CHCl_3$); ν_{max} 2960, 2925, 1760, 1420, 1220 and 1140 cm^{-1}; δ_H 1.23 (5H, complex, 1-H, 2-H_2 and 6-H_2), 2.00 (4H, m, 12-H_2 and 13-H_2), 2.51 (1H, m, 8-H), 2.62 (2H, m, 3-H_2 or 5-H_2), 3.08 (2H, br t, 3-H_2 or 5-H_2), 3.65 (1H, d, *J* 7 Hz, 9-H), 3.95 (1H, br s, 9-H) and 4.49 (1H, br s, 7-H); δ_C 26.6 (C-2), 29.7 (C-12 and C-13), 35.4 (C-6), 41.6 (C-1), 53.8 and 54.6 (C-3 and C-5), 58.8 (C-9), 71.3 and 72.7 (C-7 and C-8), 173.0 and 173.5 (C-11 and C-14); *m*/*z* 239 (M^+, 2%), 138, 122, 121, 108, 82 (100%), 81 and 80; (Found M^+, 239.1163. $C_{12}H_{17}NO_4$ requires *M*, 239.1158).

(-)-7,9-O,O-(cis-Cyclohexane-1,2-dicarbonyl)platynecine (256) was obtained as an inseparable mixture of two diastereomers (11% yield) as an oil; R_F 0.43; $[\alpha]_D^{20}$ -33.5 (c 2, $CHCl_3$); ν_{max} ($CHCl_3$) 2960, 2930, 1730, 1420, 1225 and 1120 cm^{-1}; δ_H 1.18 (4H, br m 2- and 6-H_2), 1.5-2.1 (8H, complex, 16-, 17-, 18- and 19-H_2), 2.69 (3H, m, 1-, 12- and 13-H), 3.13 (1H, m, 3- or 5-H), 3.37 (1H, m, 3- or 5-H), 3.61 (1H, m, 8-H), 3.88 (2H, m, 3- or 5-H_2), 4.93 (1H, dd, J 12.1 and 3.0 Hz, 9-H), 5.03 (1H, d, J 12.4 Hz, 9-H), and 5.36 (1H, br t, 7-H); m/z 293 (M^+, 31%), 156, 138, 122, 121, 108, 96, 95, 82 (100%), 81 and 80; (Found M^+, 293.1632. $C_{16}H_{23}NO_4$ requires M, 293.1686).

(-)-7,9-O,O-(trans-Cyclohexane-1,2-dicarbonyl)platynecine (257) was obtained as a mixture of two diastereoisomers which could not be separated (13% yield); R_F 0.32; $[\alpha]_D^{20}$ - 24.5° (c 1.1, $CHCl_3$); ν_{max} ($CHCl_3$) 2960, 2930, 2855, 1730, 1450, 1420, 1225 and 1120 cm^{-1}; δ_H 1.23 (4H, br m, 2-H_2 and 6-H_2), 1.80 - 2.3 (8H, complex, 16-, 17-, 18- and 19-H_2), 2.40 (2H, m, 12- and 13-H), 2.65 (1H, m, 1-H), 2.78 (2H, m, 3- or 5-H), 3.30 (1H, m, 3- or 5-H), 3.51 (1H, m, 3- or 5-H), 3.79 (1H, m, 8-H), 4.17 (1H, d, J 12.6 Hz, 9-H), 4.42 (1H, br d, J 12.6 Hz, 9-H) and 5.50 (1H, br s, 7-H); m/z 293 (M^+, 8%), 156, 155, 138, 122, 121, 111, 110, 108, 96, 95, 83, 82 (100%), 81 and 80; (Found: M^+, 293.1620. $C_{16}H_{23}NO_4$ requires M, 293.1642).

REFERENCES

1. D.J. Robins, Fortschr. Chem. Org. Naturst., 1982, 41, 115

2. D.J. Robins, Nat. Prod. Rep., 1984, 1, 235; 1985, 2, 213; 1986, 3, 297; 1987, 4, 577.

3. A.R. Mattocks, "Chemistry and Toxicology of Pyrrolizidine Alkaloids", Academic Press, London, 1986.

4. H.D. Tandon, B.M. Tandon and A.R. Mattocks, Amer. J. Gastroenterol, 1978, 70, 607.

5. I. Hirono, H. Mori and M. Haga, J. Natl. Cancer Inst., 1978, 61, 865.

6. M. Boppré, R.L. Petty, D. Schneider and J. Meinwald, J. Comp Physiol., 1978, 126A, 97; G.J. Goss, Environ Entomol., 1979, 8, 487.

7. L.B. Bull, C.C.J. Culvenor and A.T. Dick, "The Pyrrolizidine Alkaloids", North Holland, Amsterdam, 1968.

8. C.C.J. Culvenor, A.T. Dann and A.T. Dick, Nature, 1962, 195, 570.

9. A.R. Mattocks, Nature, 1968, 217, 723: J. Chem. Soc. (C), 1969, 1155.

10. J.S. Kovach, M.M. Ames, G. Powis, C.G. Moertel, R.G. Hahn, and E.T. Creatgan, Cancer Res., 1979, 39, 4540.

11. R. Schoental and A.R. Mattocks, Nature, 1960, 185, 842.

12. R. Schoental, Israel. J. Med. Sci., 1968, 4, 1133.

13. A.R. Mattocks, Toxicol. Lett., 1982, 14, 111.

14. R. Robinson, "The Structural Relations of Natural Products", Clarendon Press, Oxford, 1955.

15. E. Nowacki and R.U. Byerrum, Life Sci., 1962, 1, 157.

16. W. Bottomley and T.A. Greissman, Phytochemistry, 1964, 3, 357.

17. D.J. Robins and J.R. Sweeney, J. Chem. Soc., Perkin Trans 1 , 1981, 3083.

18. T.A. Geissman and D.H.G. Crout, "Organic Chemistry of Secondary Plant Metabolism", Freeman, Cooper and Co., San Francisco, 1969.

19. Y.K. Ho, R.N. Gupta, D.B. MacLean and I.D. Spenser, Can. J. Chem., 1971, 49, 3352.

20. D.J. Robins, J. Chem. Res (S)., 1983, 326.

21. G. Grue-Sørensen and I.D. Spenser, Can. J. Chem., 1982, 60, 643.

22. H.A. Khan and D.J. Robins, J. Chem. Soc., Chem. Commun., 1981, 554; J. Chem. Soc., Perkin Trans 1, 1985, 191.

23. R. Kuttan, A.N. Radhakrishnan, T. Spande and B. Witkop, Biochemistry, 1971, 10, 361; R. Kuttan and A.N. Radhakrishnan, Biochem. J., 1972, 127, 161.

24. J. Rana and D.J. Robins, J. Chem. Res (S), 1983, 146.

25. H.A. Kelly and D.J. Robins, J. Chem. Soc., Perkin Trans. 1, 1987, 177.

26. D.J. Robins, J. Chem. Soc., Chem. Commun., 1982, 1289.

27. J. Rana and E. Leete, J. Chem. Soc. Chem. Commun., 1982, 1821.

28. E.K. Kunec and D.J. Robins, J. Chem. Soc., Chem. Commun., 1986, 250; J. Chem. Soc., Perkin Trans.1, 1989, 1437.

29. M.T. Pizzorno and S.M. Albonico, J. Org. Chem., 1974, 39, 731.

30. D.J. Robins and J.R. Sweeney, Phytochemistry, 1983, 22, 457.

31. J. Rana and D.J. Robins, J. Chem. Soc., Chem. Commun., 1984, 517; J. Chem. Soc., Perkin Trans 1, 1986, 983.

32. J.C. Richards and I.D. Spenser, Can. J. Chem., 1982, 60, 2810.

33. A.R. Battersby, J. Stauton and M.C. Summers, J. Chem. Soc., Perkin Trans. 1, 1976, 1052.

34. E.K. Kunec and D.J. Robins, J. Chem. Soc., Chem. Commun., 1985, 1450; J. Chem. Soc., Perkin Trans. 1, 1987, 1089.

35. H.A. Kelly and D.J. Robins, J. Chem. Soc., Perkin Trans 1, 1987, 2195.

36. H.A. Kelly and D.J. Robins, J. Chem. Soc., Chem. Commun., 1988, 329.

37. D.H.G. Crout, M.H. Benn, H. Imaseki, and T.A. Geissman, Phytochem., 1966, 5, 1.

38. D.H.G. Crout, J. Chem. Soc. (C)., 1966, 1968; N.M. Davies and D.H.G. Crout, J. Chem. Soc., Perkin Trans. 1, 1974, 2079; D.J. Robins, N.M. Bale and D.H.G. Crout, J. Chem. Soc., Perkin Trans. 1, 1974, 2082.

39. N.M. Bale, R. Cahill, N.M. Davies, M.B. Mitchell, E.H. Smith and D.H.G. Crout, J. Chem. Soc., Perkin Trans. 1, 1978, 101; D.H.G. Crout, Chimia, 1976, 30, 270; R. Cahill, D.H.G. Crout, M.B. Mitchell and U.S. Muller, J. Chem. Soc., Chem. Commun., 1980, 419.

40. R.E. Marquis, "Handbook of Experimental Pharmacology", Springer-Verlag, Berlin, 1970.

41. D.J. Robins, Adv. Heterocyclic Chemistry, 1979, 24, 247; and references cited therein.

42. T.A. Geissman and A.C. Waiss, J. Org. Chem., 1962, 27, 139.

43. J.W. Clark-Lewis and P.I. Mortimer, J. Chem. Soc., 1960, 189.

44. K. Narasaka, T. Sakakura, T. Uchimaru, K. Morimoto and T. Mukaiyama, Chem. Lett., 1982, 455.

45. H. Rueger and M. Benn, Heterocycles, 1982, 19, 23.

46. H. Rueger and M. Benn, Heterocycles, 1983, 20, 1331.

47. V.K. Yadav, H. Rueger and M. Benn, Heterocycles, 1984, 22, 2735.

48. J.G. Buchanan, G. Singh and R.H. Wightman, J. Chem. Soc., Chem. Commun., 1984, 1299.

49. S.M. Hannick and Y. Kishi, J. Org. Chem., 1983, 48, 3833.

50. J.G. Buchanan, V.B. Jigajinni, G. Singh and R.W. Wightman J. Chem. Soc., Perkin Trans. I, 1987, 2377.

51. J.A. Glinski and L.H. Zalkow, Tetrahedron. Lett., 1985, 26, 2857.

52. N.J. Leonard and J.A. Klainer, J. Am. Chem. Soc., 1968, 33, 4269.

53. N.J. Leonard and T. Sato, J. Org. Chem., 1969, 34, 1066.

54. H. Niwa, A. Kuroda and K. Yamada, Chem. Lett., 1983, 125.

55. H. Niwa, Y. Uosaki and K. Yamada, Tetrahedron. Lett., 1983, 24, 5731.

56. J.J. Tufariello and J.P. Tette, J. Org. Chem., 1975, 40, 3866.

57. J.J. Tufariello and G.E. Lee, J. Am. Chem. Soc., 1980, 102, 373.

58. E. Vedejs, S. Larsen and F.G. West, J. Org. Chem., 1985, 50, 2170.

59. H.O. Bayer, H. Gotthardt and R. Huisgen, Chem. Ber., 1970, 103, 2356.

60. D.J. Robins and S. Sakdarat, J. Chem. Soc., Perkin Trans 1, 1979, 1734.

61. D.J. Robins and S. Sakdarat, J. Chem. Soc.,Chem. Commun., 1979, 1181.

62. R.S. Sawhney, C.K. Atal, C.C.J. Culvenor and L.W. Smith, Austral. J. Chem., 1974, 27, 1805.

63. S. Brandage and C. Lundin, Acta. Chem. Scand., 1971, 25, 2447.

64. H.A. Kelly and D.J. Robins, J. Chem. Soc., Perkin Trans. 1, 1989,

65. K.N. Houk, J. Sims, R.E. Duke, Jnr., R.W. Strozier and J.K. George, J. Am. Chem. Soc., 1973, 95, 7287.

66. K.N. Houk, J. Sims, C.R. Watts and L.J. Luskus, J. Am. Chem. Soc., 1973, 95, 7301.

67. D.J. Robins and S. Sakdarat, J. Chem. Soc., Chem. Commun., 1980, 282.

68. E.J. Corey and K.C. Nicolaou, J. Am. Chem. Soc., 1974, 96, 5614.

69. J.A. Devlin, D.J. Robins and S. Sakdarat, J. Chem. Soc., Perkin Trans I, 1982, 1117.

70. (+)-Retronecine was obtained by hydrolysis of retrorsine from *Senecio isatideus*. See D.J. Robins and J.R. Sweeney J. Chem. Soc., Perkin Trans. I, 1981, 3083.

71. J.A. Devlin and D.J. Robins, J. Chem. Soc., Chem. Commun., 1981, 1272.

72. K. Brown, M. Burton, D.J. Robins and G.A. Sim., J. Chem. Soc., Perkin Trans I, 1986, 1261.

73. M. Burton and D.J. Robins, J. Chem. Soc., Perkin Trans. 1, 1985, 611.

74. M. Burton and D.J. Robins, J. Chem. Soc., Perkin Trans. 1, 1986, 585.

75. C.C.J. Culvenor, A.T. Dann and L.W. Smith, Chem. Ind. (London), 1959, 20; C.C.J. Culvenor and L.W. Smith, Aust. J. Chem., 1966, 19, 1955; C.C.J. Culvenor, S.R. Johns J.A. Lamberton and L.W. Smith, ibid., 1970, 23, 1279.

76. R. Adams and B.L. Van Duuren, J. Am. Chem. Soc., 1954, 76, 6379.

77. K. Narasaka and T. Uchimaru, Chem. Lett., 1982, 57.

78. T.A. Geissman and A.C. Waiss, J. Org. Chem., 1962, 27, 139.

79. S. Masamune, S. Kamata and W. Schilling, J. Am. Chem. Soc., 97, 3515 (1975).

80. J. Huang and J. Meinwald, J. Am. Chem. Soc., 1981, 103, 861.

81. O. Cervinka, L. Hub, A. Klasek and F. Santavy, Chem. Commun., 1968, 261.

82. E. Vedejs, S. Ahmad, S.D. Larsen and S. Westwood, J. Org. Chem., 1987, 52, 3937.

83. E. Vedejs and S.D. Larsen, J. Am. Chem. Soc., 1984, 106, 3030.

84. J.D. White and S. Ohira, J. Org. Chem., 1986, 51, 5492.

85. D.B. Hagan and D.J. Robins, J. Chem. Soc., Perkin Trans. 1, 1988, 1165.

86. R.H. Barbour and D.J. Robins, J. Chem. Soc., Perkin Trans. I, 1985, 2475.

87. R.H. Barbour, D.J. Robins and A.A. Freer, J. Chem. Soc., Perkin Trans. I, 1987, 2069.

88. R.H. Barbour and D.J. Robins, J. Chem. Soc., Perkin Trans. I, 1988, 1169.

89. R.H. Barbour and D.J. Robins, J. Chem. Soc., Perkin Trans. I, 1988, 1923.

90. D.J. Robins and S. Sakdarat, J. Chem. Soc., Perkin Trans. I, 1981, 909.

91. S. Kohlmuenzer, H. Tomczyk and A. Saint-Firmin, Diss. Pharm. Pharmacol, 1971, 23, 419.

92. H. Tomczyk and S. Kohlmunzer, Herba. Pol., 1971, 17, 226.

93. R.H. Barbour and D.J. Robins, Phytochemistry, 1987, 26, 2430.

94. H.A. Kelly, E.K. Kunec, M. Rodgers and D.J. Robins, J. Chem. Res., 358.

95. E. Leete and J. Rana, J. Nat. Prod., 1986, 49, 838.

96. G. Birnbaum, J. Am. Chem. Soc., 1974, 96, 6165; K.B. Birnbaum, Acta Cryst., 1972, 28, B, 2825.

97. H. Stoeckli-Evans, Acta Crystallogr., Sect. B, 1979, 35, 2798.

98. M. Kinns and J.K.M. Sanders, J. Mag. Reson., 1984, 56, 518.

99. M.Jaziri et al, Phytochemistry, 1988, 27, 419.

100. H.A. Khan and D.J. Robins, J. Chem. Soc., Perkin Trans. 1, 1985, 819.

101. A.V. Danilova, N.I. Koretskaya and L.M. Utkin, J. Gen. Chem., U.S.S.R., 1961, 31, 3560.

102. E. Fabiano and B.T. Golding, Synthesis, 1987, 190.

103. O. Mitsunobu, Synthesis, 1981, 1.

104. H. Golobolov, Tetrahedron, 1981, 37, 437.

105. C.C.J. Culvenor and L.W. Smith, Aust. J. Chem., 1967, 20, 2499.

106. M. Wink, Z. Naturforsch, 1983, 38c, 905.

107. J.P. Soto, Acta. Cient. Compostelana, 1969, 6, 37; I. Ribas and J.J. Barreiro, Anales. Assoc. Quim. Arg., 1953, 41, 27.

108. R. Munier and M. Macheboef, Bull. Soc. Chim. Biol., 1951, 33, 846.

109. H. Gotthardt and R. Huisgen, Chem. Ber., 1970, 103, 2625.

110. S. Sakdarat, Ph.D. Thesis, 1979, University of Glasgow.

111. R. Huisgen, H. Gotthardt and H.O. Bayer, Chem. Ber., 1970, 103, 2368.

112. H.J. Huizing, F. DeBoer and T.M. Malingre, J. Chromatogr., 1980, 195, 407; R.J. Molyneux and J.N. Roitman, ibid., 412.

113. H.A. Kelly, Ph.D. Thesis, 1987, University of Glasgow.

114. E.W. Colvin, "Silicon in Organic Synthesis", Butterworths. 1979 (1st Edn.)

115. T. Greene, "Protecting Groups in Organic Synthesis" 1984.

116. S.K. Chaudhary and O. Hernandez, Tetrahedron Lett., 1979, 99.

117. T. Gelbaum, M.M. Gordon, M. Miles and L.H. Zalkow, J. Org. Chem., 1982, 47, 2501.

118. D. R. Morris and L.J. Marton, Eds., "Polyamines in Biology and Medicine", Marcel Dekker, New York, 1981.

119. H. Tabor and C.W. Tabor, Pharmacol. Rev., 1964, 16, 245.

120. N.M. Bale and D.H.G. Crout, Phytochemistry, 1975, 14, 2617.

121. P. Bey, F. Gerhart, V.Van Dorsselaer and C. Danzin, J. Med. Chem., 1983, 26, 1551.

122. L.H. Amundsen, R.H. Mayer, L.S. Pitts and L.A. Malentacchi J. Am. Chem. Soc., 1951, 73, 2118.

123. C.J. Suckling, Angew. Chem. Int. Ed. Engl., 1988, 27, 537.

124. See Chem. Br., 1986, 22, 395.

125. J.M. Denis, C. Girard and J.M. Conia, Synthesis, 1972, 549.

126. R.D. Smith and H.E. Simmons, Org. Synth., 1961, 41, 72.

127. H.E. Simmons and R.D. Smith, J. Am. Chem. Soc., 1959, 81, 4256.

128. E. LeGoff, J. Org. Chem., 1964, 29, 2048.

129. H.E. Simmons, T.L. Cairns, S.A. Vladuchick and C.M. Hoiness, Org. React., 1973, 20, 1.

130. H.E. Simmons and R.D. Smith, J. Am. Chem. Soc., 1958, 80, 5323.

131. P.J. Abell and D.J.J. Lennon, J. Am. Chem. Soc., 1965, 30, 1206.

132. C.S. Palmer and P.W. MacWhirter, Org. Synth., Coll. Vol. 1, 1941, 245.

133. G. Bonavent , Bull. Soc. Chim. Fr., 1964, 10, 2462.

134. R.J. Abraham, "Analysis of High Resolution n.m.r. Spectra", Elsevier, 1971.

135. S.M. Reid, personal communication.

136. S. Sarel and M.S. Newman, J. Am. Chem. Soc., 1956, 78, 5416.

137. C.C.J. Culvenor, Chem-Biol. Interact., 1976, 12, 299.

138. A.J. Aasen, C.C.J. Culvenor and L.W. Smith, J. Org. Chem., 1969, 34, 4137.

139. P. Karrer, H. Reschofsky and W. Kasse, Helv. Chim. Acta., 1947, 30, 271.

140. "Dictionary of Organic Compounds", Chapman and Hall, Vol. 1, p1070.

141. W. Schmidt, Org. React., 1947, 3, 327.

142. Gaffield et al, Tetrahedron, 1971, 27, 915.

143. S. Okuda and K. Tsuda, Chem. Ind., 1961, 1115.

144. W. Golebiewski, Org. Mag. Reson., 1972, 4, 837; ibid., 1986, 24, 105.

145. "Reagents for Organic Synthesis", Fieser and Fieser, 1967, 1276.

146. C.K. Ingold, J. Chem. Soc., 1921, 119, 305.

147. A.T. Blomquist, J. Am. Chem. Soc., 1959, 81, 2012.

148. Dictionary of Organic Compounds (5th Edn.), 1, 909.

149. R. Adams and E.F. Rogers, J. Am. Chem. Soc., 1941, 63, 537.