SYNTHESIS AND BIOSYNTHESIS OF PYRROLIZIDINE ALKALOIDS AND ANALOGUES

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

by

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To my mother and father

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ABBREVIATIONS

Ac	-	acetyl
AIBN	-	azobisisobutyronitrile
br	-	broad
d	-	doublet
DBU	-	1,8-diazabicyclo[5.4.0]undec-7-ene
DEPT	-	distortionless enhancement by polarisation transfer
DIBAL	-	di-isobutylaluminium hydride
DME	-	1,2-dimethoxyethane
DMF	-	N,N-dimethylformamide
DMSO	-	dimethylsulphoxide
Et	-	ethyl
HMPA	-	hexylmethylphosphoramide
i.r.	-	infra red
LDA	-	lithium di-isopropylamine
m	-	multiplet
Me	-	methyl
MEM	-	methoxyethoxy methyl
n.m.r.	-	nuclear magnetic resonance
NOE	-	Nuclear Overhauser Effect
PTSA	-	p-toluene sulphonic acid
q	-	quartet
s	-	singlet
t	-	triplet
THF	-	tetrahydrofuran

t.l.c. - thin layer chromatography

TMS - tetramethylsilane

u.v. - ultra violet

DEAD - diethylazodicarboxylate

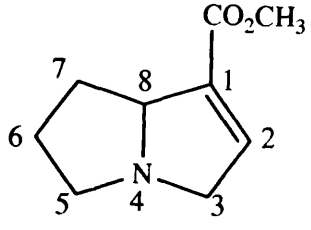
4-DMAP - 4-dimethylaminopyridine

MCPBA - m-chlorobenzoic acid

NIS - N-iodosuccinimide

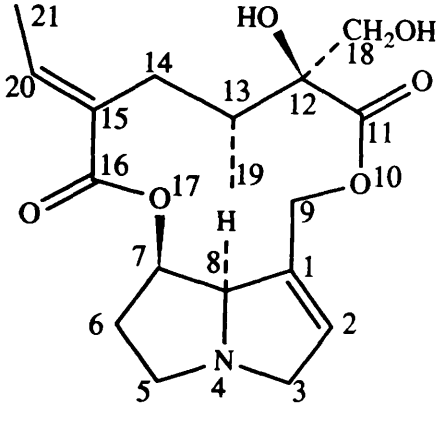
NOTES ON NOMENCLATURE

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

The pyrrolizidine macrocyclic diesters are numbered in accordance with the system proposed by Culvenor et al., (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, 5653). For example, usaramine is shown



SUMMARY

Pyrrolizidine alkaloids are important because they have a widespread occurrence and many of the alkaloids are hepatotoxic. The work presented in this thesis is divided into three sections: (a) investigations into the synthesis of pyrrolizidine bases; (b) feeding of optically active putrescine analogues to plants which produce pyrrolizidine alkaloids; (c) structural studies on seeds which produce pyrrolizidine alkaloids.

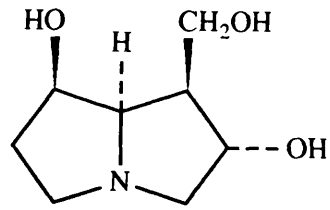
(A) Investigations into the synthesis of pyrrolizidine bases

A route aimed at providing a range of necines, principally in optically active form, from the stereoisomeric forms of malic and tartaric acids was studied. A series of intermediate imides was prepared (e.g. A) from these diacids and development of the pyrrolizidine ring system was achieved (e.g. B) through condensation of the sodium salt of these imides with ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate. However, attempts to convert the products of this cyclisation into the desired products (such as C and D) were unsuccessful. A brief summary of the route pursued is outlined in Scheme I for the synthesis from L-tartaric acid diethyl ester.

A series of problems beset this route, including the failure of well precedented reactions in the latter stages of the reaction sequence. In addition, poor yields were encountered for certain steps together with epimerisation of key intermediates. Attempts to modify the strategy could not overcome these problems.

(B) Feeding of optically active putrescine analogues to plants which produce pyrrolizidine alkaloids

The feeding of putrescine analogues to Senecio pleistocephalus (family Compositae) plants was undertaken. Putrescine is known to be involved in the biosynthesis of rosmarinecine (E), the base portion of the pyrrolizidine macrocyclic diester (rosmarinine) produced by these plants. A route was devised to enable preparation of optically active 2-methoxy-



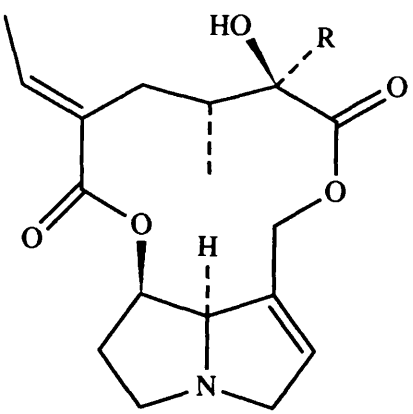
(E)

putrescines from the optically active malic acids. This is illustrated in Scheme II for synthesis of one enantiomer from L-malic acid.

For feeding experiments these substituted putrescines were successfully prepared in radiolabelled form by the use of [^{14}C]methyl iodide in the methylation step on the route from malic acid. Feeding of the [^{14}C-Me]-2-methoxyputrescines indicated that neither of the enantiomeric forms was readily utilised in the biosynthetic pathway to rosmarinecine. No evidence of likely intermediates in the biosynthetic pathway or of the formation of rosmarinecine analogues was obtained. An intermediate trapping experiment demonstrated that the 2-methoxyputrescines do not remain intact within the plant.

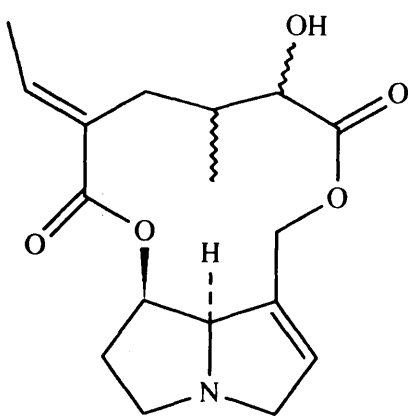
(C) Structural studies on seeds which produce pyrrolizidine alkaloids

Seeds of Crotalaria lanceolata (family Leguminosae) were extracted and the alkaloidal mixture was separated by chromatography. Three alkaloids were present in the seeds in a ratio of 85:10:5 (F:G:H). These were identified by spectroscopic studies as the known pyrrolizidine alkaloids usaramine (F), nilgirine (G), and integerrimine (H).

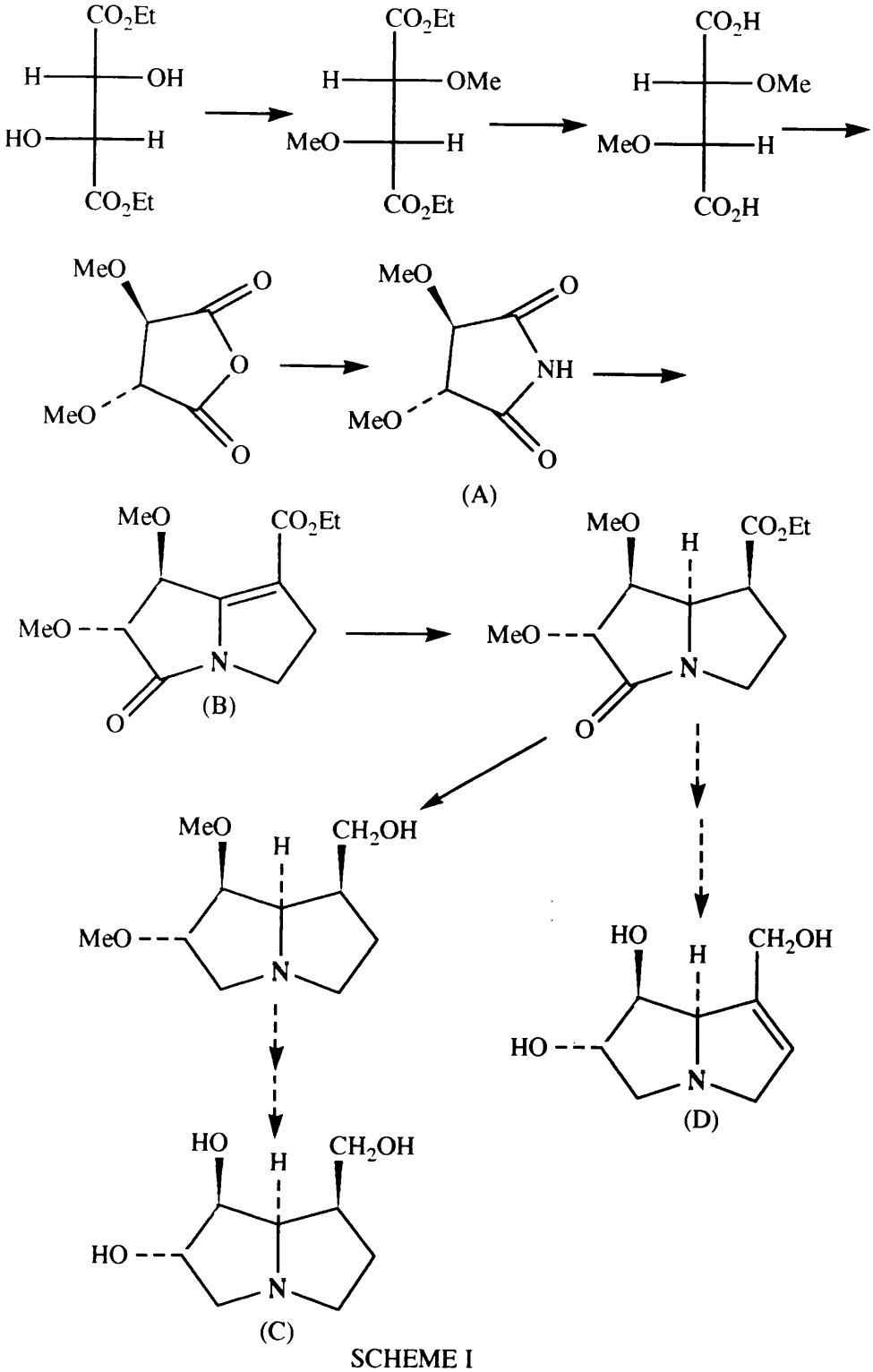


$R = CH_2OH$: (F)

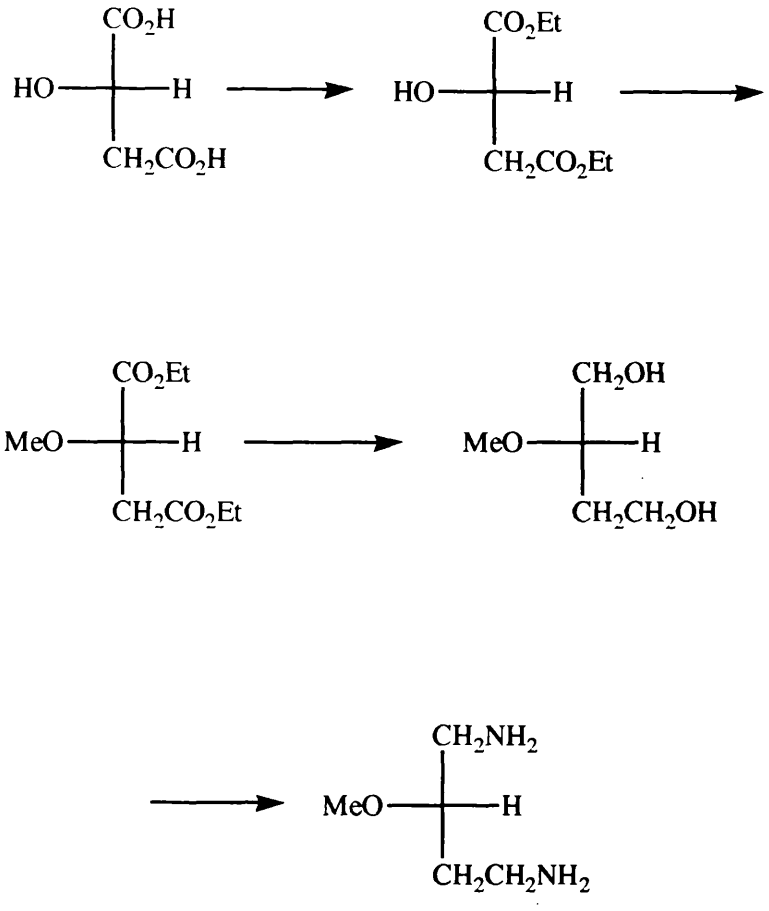
$R = CH_3$: (H)



(G)


CO2Et
H
OH
HO
H
CO2Et
CO2Et
H
OMe
MeO
H
CO2Et
CO2H
H
OMe
MeO
H
CO2H
MeO
O
O
MeO
O
MeO
O
NH
MeO
O
(A)
MeO
CO2Et
MeO
N
O
(B)
MeO
H
CO2Et
MeO
N
O
MeO
H
CH2OH
MeO
N
HO
H
CH2OH
HO
N
(D)
HO
H
CH2OH
HO
N
(C)

SCHEME I


CO2H
HO
H
CH2CO2H
CO2Et
HO
H
CH2CO2Et
CO2Et
MeO
H
CH2CO2Et
CH2OH
MeO
H
CH2CH2OH
CH2NH2
MeO
H
CH2CH2NH2

SCHEME II

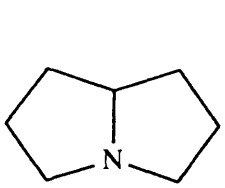
CHAPTER 1

INTRODUCTION

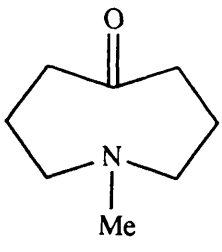
1.1 The Pyrrolizidine Alkaloids

The pyrrolizidine alkaloids comprise a substantial class of natural products.[1] They have aroused much interest by their widespread occurrence and, more significantly, by the diverse range of biological activities exhibited by the group.[2-4] Pyrrolizidine derivatives are found commonly in plants. In excess of 200 such alkaloids have been isolated.[1] Pyrrolizidine alkaloid-containing plants are found on all continents and have a liberal distribution throughout the plant kingdom. The presence of pyrrolizidine alkaloids in over 60 genera, spanning 13 plant families, reflects this botanical diversity.[1] The most commonly studied of the genera are Senecio, Crotalaria, Cynoglossum, and Heliotropium.[2]

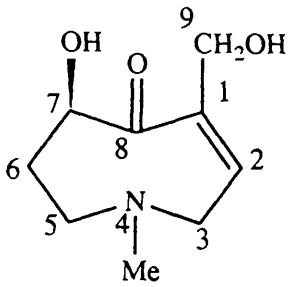
The 1-azabicyclo[3.3.0]octane system (1) is the structural backbone common to most pyrrolizidine alkaloids. A smaller, but sizeable grouping contain a closely related structure (2) as their nucleus, otonecine (3) being an example. Almost all pyrrolizidine bases possess a hydroxymethyl group at C-1. In addition, many are hydroxylated at C-7 and a few are further hydroxylated at C-2 or C-6. The stereochemistry of these substituents, together with the bridgehead proton at C-8, is variable. Pyrrolizidines with a saturated ring system are known, but 1,2-unsaturation predominates. There are also a few examples of the more oxidised pyrroles documented.[5,6] The most common type of pyrrolizidines are the diols either saturated or 1,2-unsaturated. Retronecine (4) is the base portion (necine) encountered most often in pyrrolizidine systems.



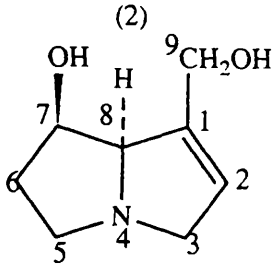
(1)



(2)

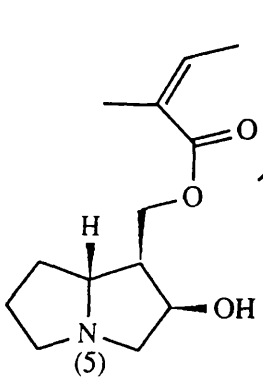


(3)



(4)

Although many of these necines have themselves been isolated, most are found in the form of esters.[1] These can occur as monoesters [like (5)], diesters [such as (6)], or macrocyclic diesters [like (7)], the last being the most common variety. The 1,2-unsaturated macrocycles,



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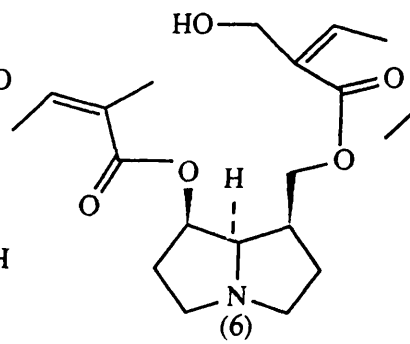
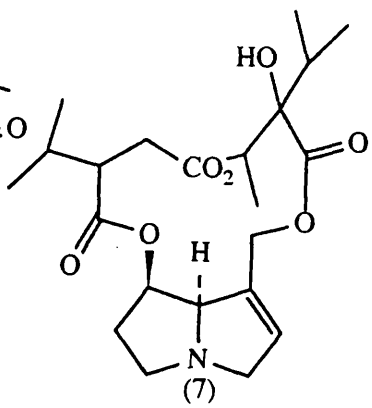


(6)



(7)

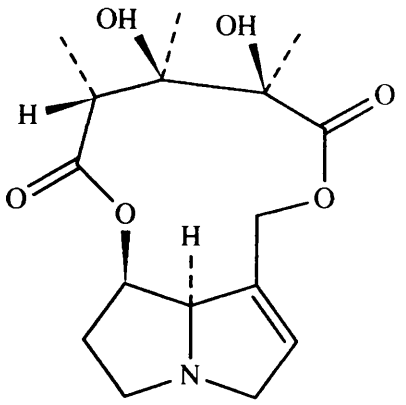
like (7), are recognised as the most toxic members of the pyrrolizidine family.[4,7] Amongst these bislactones the 11- and 12-membered species are the most common, monocrotaline (8) and retrorsine (9) are respective examples. A few 13-membered macrocycles are known,[8,9] all of which contain either a crotanecine (10) or retronecine (4) nucleus. Recently, a smaller number of 14-membered derivatives of retronecine, such as parsonsine (7), have been discovered.[10]

The acid moieties of the pyrrolizidine esters, known as the necic acids, contain five to ten carbon atoms, are often branched, and are frequently hydroxylated. They exist as either mono or diacids, with most being γ- or δ-hydroxyacids.[1] Those which contain ten carbon atoms, such as seneciphyllic acid (11), are by far the most common of the group.

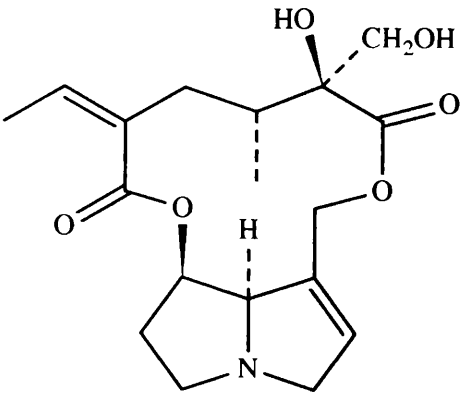
Loline (12) is one of a small collection of pyrrolizidine alkaloids, of novel structure, which have been isolated in non-esterified form.[11] It should also be noted that in their natural environment, pyrrolizidines often occur as a mixture of the tertiary base and the corresponding N-oxide.

1.2 Toxicity of Pyrrolizidine Alkaloids

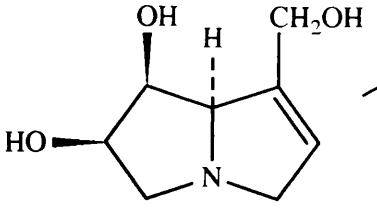
In the past 20 years there has been a marked increase in awareness of the physiological disorders caused by pyrrolizidine alkaloid poisoning of mammalian species.[4] During this period numerous pyrrolizidine alkaloids have been tested and many have been found to induce hepatotoxicity.[12-14] Pyrrolizidine derivatives have also been shown to act as antitumour, hypotensive, local anaesthetic, antispasmodic, anti-inflammatory, and carcinogenic agents.[4] In addition to the liver, damage may also be inflicted on the lungs,[15] heart,[16] and, occasionally, the kidneys.[17]


OH
OH
H
O
O
O
O
H
N

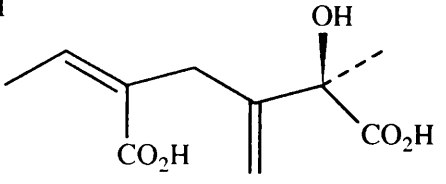
(8)


HO
CH2OH
O
O
O
O
H
N

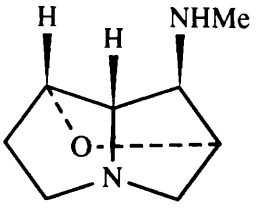
(9)


OH
H
CH2OH
HO
N

(10)


OH
CO2H
CO2H

(11)


H
H
NHMe
O
N

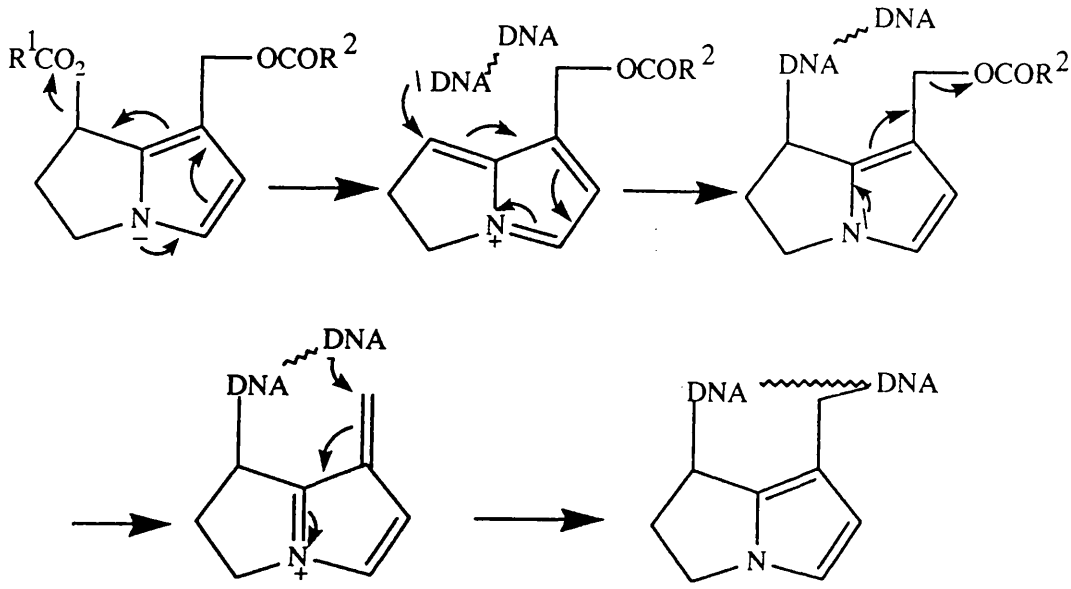
(12)

Ingestion of plants containing pyrrolizidine alkaloids by livestock has long been recognised as a serious environmental hazard.[18-20] Indeed, the first suspected case of pyrrolizidine alkaloid poisoning was recorded as early as 1787, when farmers in Britain suspected Senecio jacobaea of being harmful to livestock. The complexity of the disease and its occurrence in many distant locations caused the condition to be referred to by several alternative names, including Pictou and Winton disease.[2]

In many areas, loss of livestock by pyrrolizidine alkaloid poisoning is of increasing economic concern. Recently in Australia for instance, a number of cattle died and many were afflicted by retarded growth due to consumption of Senecio lautus,[21] while a fatal outbreak of chicken poisoning was detected, this time resulting from contamination of fodder by seeds of Heliotropium europaeum.[22] Ingestion of pyrrolizidine alkaloids by humans is now also recognised as a worldwide health problem. Mortality has resulted in Afghanistan[23] and India[24] as a consequence of contaminated food supplies. An outbreak of veno-occlusive disease developed in Arizona, U.S.A. due to consumption of a herbal tea derived from Senecio longilobus.[25] Other cases of poisoning have been attributed to herbal teas and traditional remedies which incorporate pyrrolizidine alkaloids.[26]

Pyrrolizidine alkaloids and their N-oxides are known to be metabolised by mammals to pyrrole derivatives, the latter category via the basic alkaloid.[4] Such metabolism invariably occurs in the liver, by oxidase enzymes, and it is these pyrrolic metabolites which are responsible for the adverse effects associated with pyrrolizidine alkaloids. Although most tissue damage is confined to their site of production - the liver,

migration of these pyrroles can lead to toxic action on other organs. Their toxicity arises from activation of the ester groups through conjugation with the pyrrole (Scheme 1), enabling these metabolites to behave as bifunctional alkylating agents which can initiate cellular disturbance.[27] Nucleophilic sites of a DNA molecule can become covalently bound to these pyrrolizidine metabolites, leading to cross-linking of the DNA. This can prevent cell mitosis which may ultimately lead to the death of the organism.



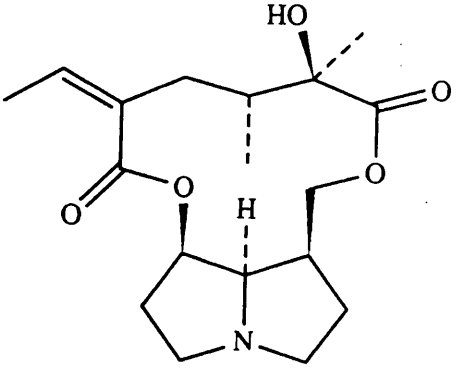
SCHEME 1

Essential structural features required for pyrrolizidine alkaloids to enable formation of these toxic metabolites are 1,2-unsaturation of the basic nucleus and esterification at C-9. Toxicity is further enhanced by esterification at C-7 and substitution in the α-position of the acid moiety.[4] The macrocyclic diesters adopt specific conformations and this may also influence their biological activity.[28]

1.3 Therapeutic Nature of Pyrrolizidine Alkaloids

Despite there being a preponderence of pyrrolizidine alkaloids known to display hepatotoxicity there are some which have demonstrated activity of therapeutic value. These alkaloids, some of which are discussed, either have a saturated pyrrolizidine skeleton or are quaternary amines, particularly N-oxides.

The macrocyclic diester platyphylline (13), has been used



(13)

extensively for several years in the U.S.S.R. in the treatment of hypertension and internal ulcers.[29,30] The saturated ring system is thought to account for its non-hepatotoxic nature. Recently, an ophthalmic drug became available which contains 1% platyphylline tartrate.[31]

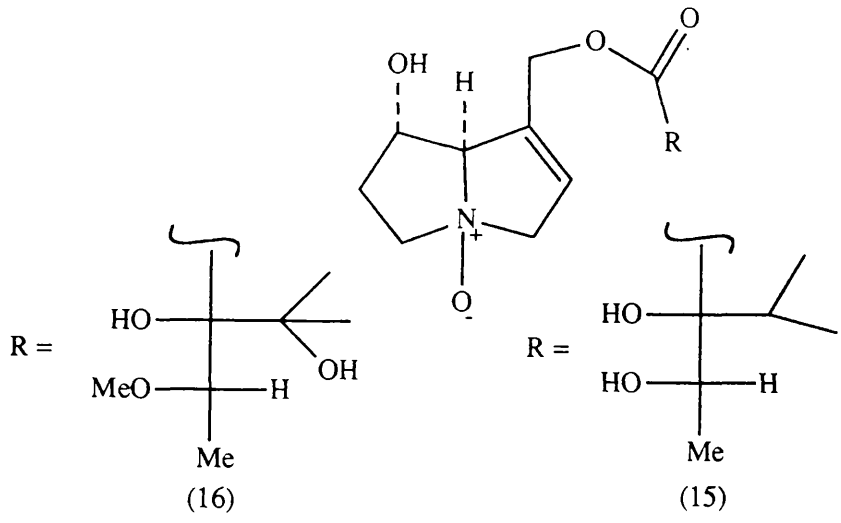
The N-oxides of indicine (14), echinatine (15), and europine (16) display anti-tumour activity in experimental tumour systems.[32] Indicine N-oxide is the only pyrrolizidine alkaloid to have been used in a clinical trial. It was found to be effective against advanced cancers, although side effects, principally myleosuppression, were observed.[33] However, no acute liver damage developed after it was administered to patients. The semi-synthetic pyrrolizidine (17) prepared by Zalkow et al.,[34] has proved to be a more potent anti-tumour agent than indicine N-oxide when tested on similar experimental systems.

The semi-synthetic pyrrolizidine (18) is one of a series of similar compounds which have displayed ganglion and neuromuscular blocking properties in rats.[35] The quaternary 1,2-unsaturated pyrrolizidine (19) was the most potent of a collection of pyrrolizidine thiols which inhibited nerve muscle transmissions in mice.[36]

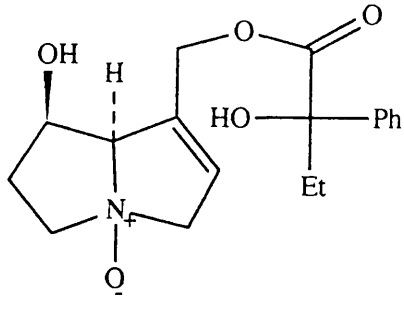
1.4 Aims of Project

One of the greatest challenges confronting the Organic Chemist is in the development of synthetic routes to natural product systems. The increasing availability of optically active reagents as potential synthons, coupled to the considerable advances in synthetic organic chemistry, have enabled natural products to be synthesised increasingly in optically active form. This trend has been reflected in the pyrrolizidine alkaloids, with a number of syntheses of optically active necines recently reported. This work is reviewed in Chapter 2.

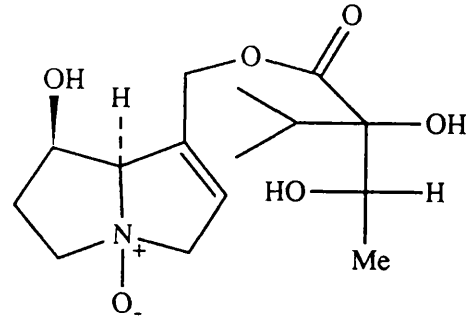
Most of the synthetic routes to the optically active necines are lengthy. In addition, most are restricted to the production of a small number of necines. Consequently, it was considered imperative to try to



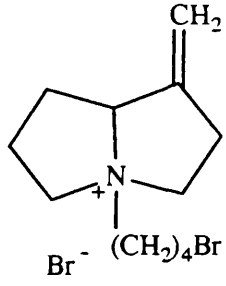
(16) (15)



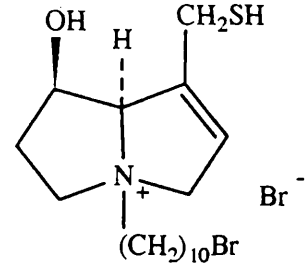
(17)



(14)



(18)



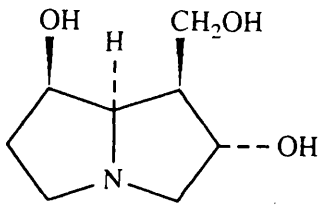
(19)

develop a concise, efficient route, capable of yielding a range of necines in optically active form. Accordingly, the emphasis of this project centred on an investigation into the feasibility of a synthetic route from the stereoisomeric forms of malic and tartaric acids. This work is described in Chapter 4.

The biosynthesis of natural products is of fundamental interest to the Chemist and Biochemist alike. Although the origin of many natural products remains unclear, considerable progress has been achieved in our understanding of the salient features of pyrrolizidine alkaloid biosynthesis. The work reported in this area is summarised in Chapter 3.

Putrescine (20) has been shown to be an efficient precursor in the biosynthesis of the pyrrolizidine bases, retronecine (4) and rosmarinecine (21). However, no work has yet been reported on the feeding of substituted putrescines to plants known to produce pyrrolizidine alkaloids. This may produce analogues of pyrrolizidine alkaloids which can be assessed for useful biological activity, or, if there is interference with the biosynthetic pathway, analogues of biosynthetic intermediates may be produced which will provide information about the biosynthetic pathway. Thus, optically active methoxyputrescines were prepared, in radiolabelled form, from the enantiomers of malic acid and administered, as their dihydrochlorides, to Senecio pleistocephalus plants. This work is discussed in Chapter 5.

(20)



(21)

The distribution of pyrrolizidine alkaloids within plants is of increasing importance. This arises as knowledge of the chemical constituents of plants is becoming increasingly influential in the classification of plant species.

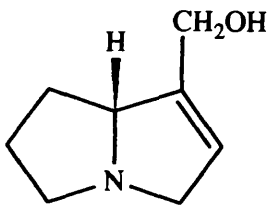
Furthermore, the structural relationship of alkaloids occurring together may be of value in helping to determine the biogenesis of these compounds. Therefore, an investigation of the alkaloidal content of seed of Crotalaria lanceolata was undertaken; this is outlined in Chapter 6.

CHAPTER 2

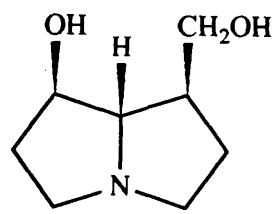
SYNTHESIS OF OPTICALLY ACTIVE PYRROLIZIDINE BASES

The wide range of biological activities associated with pyrrolizidine alkaloids (especially those exhibiting biological activity of therapeutic value) has served to underline the importance of developing practical synthetic routes to these compounds, preferably in optically active form.

The first pyrrolizidine bases to be synthesised in optically active form were the 1-hydroxymethylpyrrolizidines, such as (+)-supinidine (22). Soon afterwards, synthetic routes to optically active diols, such as (-)-hastanecine (23) and (+)-retronecine (4) were reported. (+)-Rosmarinecine (21) is one of three saturated necine triols that has been synthesised recently in optically active form, while two elaborate routes to the 1,2-unsaturated triol, (+)-crotanecine (10), have been published.



(22)



(23)

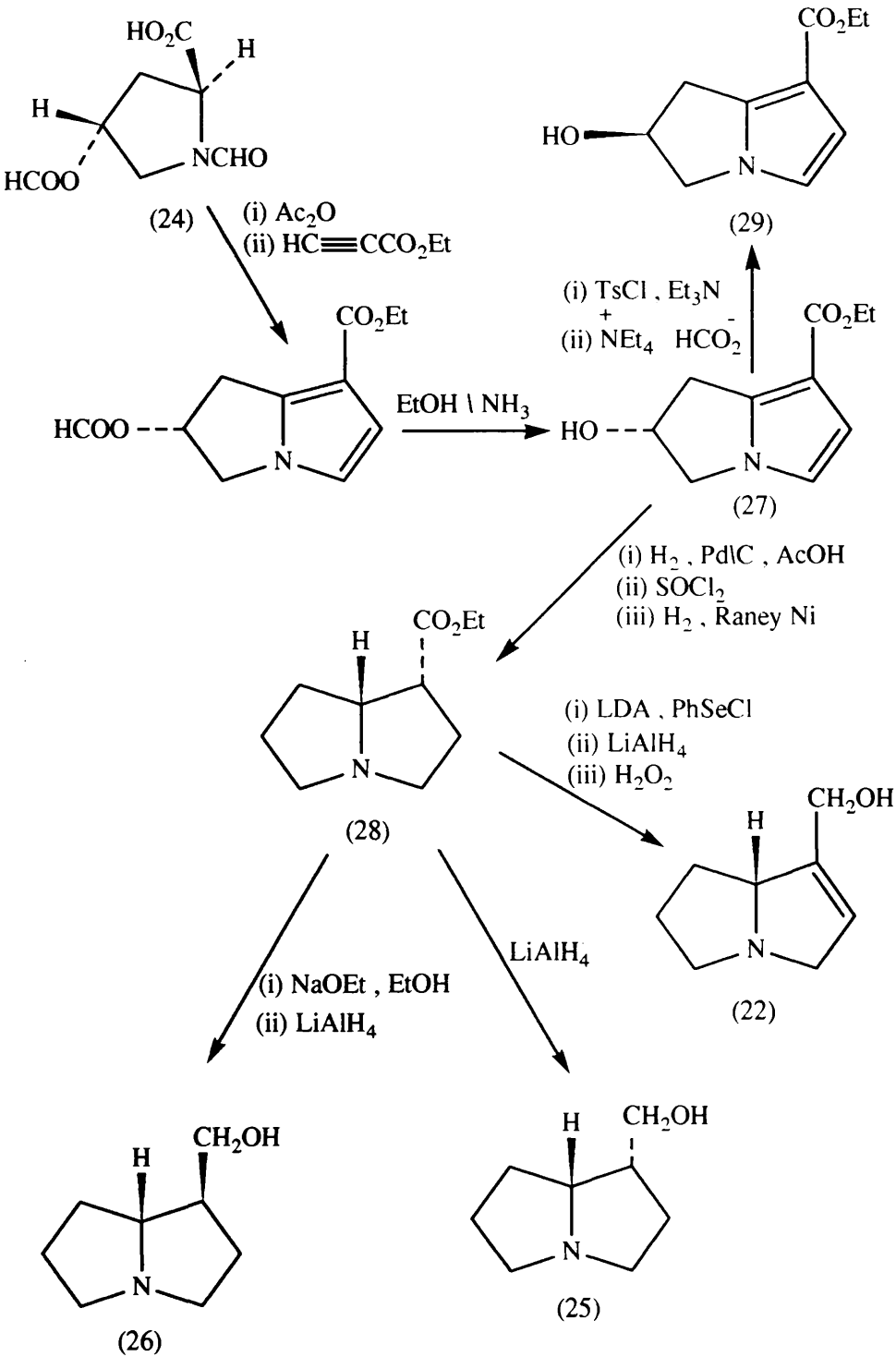
The first reported synthesis of a pyrrolizidine base was in 1931 by Cleo and Ramage.[37] Since this initial breakthrough, extensive effort has been expended in the development of synthetic routes to the necine bases. Although many routes have been successfully employed during this period, the formation of optically active pyrrolizidines has only been

witnessed in recent years. Prior to the last decade, production of optically active forms was achieved only by implementation of classical resolution techniques.[38,39]

The first synthesis of optically active pyrrolizidine alkaloids was reported in 1979 by Robins and Sakdarat.[40,41] Employing the (-)-4-hydroxy-L-proline derivative (24) as their chiral source, they generated (+)-isoretronecanol (25), (+)-trachelanthamidine (26), and (+)-supinidine (22), all with optical purities in excess of 80% (Scheme 2). The key feature is the use of the α-hydroxy substituent of the intermediate pyrrole ester (27) to control the stereochemistry of the subsequent hydrogenation step.

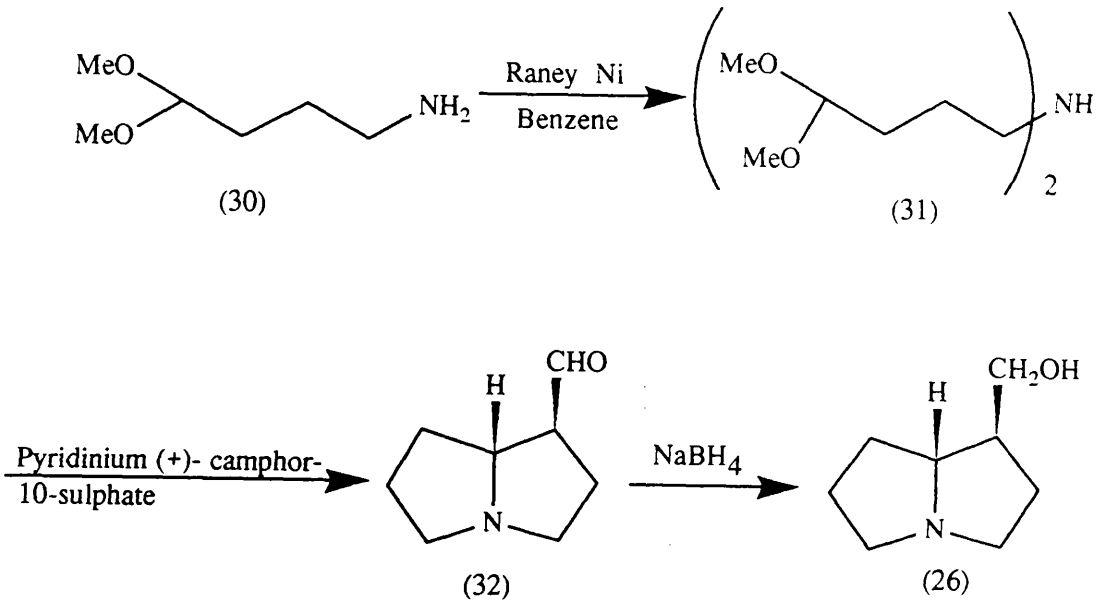
The pyrrole ester (27) was prepared by regiospecific 1,3-cycloaddition of ethyl propiolate with the NO-diformyl derivative of (-)-4-hydroxy-L-proline (24),[42] followed by deformylation. Addition of hydrogen to the less hindered β-face of (27), replacement of the 6α-hydroxy group, and reduction of the saturated ester (28) yielded (+)-isoretronecanol (25). Epimerisation of endo-ester (28), at C-1, gave the thermodynamically more stable exo-ester, which afforded (+)-trachelanthamidine (26) on reduction. The saturated ester (28) was also converted into (+)-supinidine (22) by a sequence of selenenylation α to the ester, followed by reduction of the isomeric selenide mixture, oxidation, and finally thermal elimination of the selenoxide. The same workers later reported the synthesis of the enantiomeric forms of these alkaloids by an analogous sequence of reactions from ester (29), produced from its enantiomer (27).[41]

Soon afterwards, Tanako et al. prepared (+)-trachelanthamidine


HO2C
H
H
NCHO
HCOO
(24)
(i) Ac2O
(ii) HC≡CCO2Et
CO2Et
HCOO
N
EtOH \ NH3
HO
(27)
(i) TsCl , Et3N
(ii) NEt4 HCO2
(29)
(i) H2 , Pd\C , AcOH
(ii) SOCl2
(iii) H2 , Raney Ni
(i) LDA , PhSeCl
(ii) LiAlH4
(iii) H2O2
CH2OH
(22)
(28)
LiAlH4
(i) NaOEt , EtOH
(ii) LiAlH4
(26)
(25)

SCHEME 2

(26) of 23% optical purity from an achiral precursor[43] (Scheme 3). Deaminative dimerisation of acetal (30) gave the symmetrical amine (31) which underwent asymmetric cyclisation to afford selectively the exo-aldehyde (32) on treatment with a solution containing pyridinium (+)-camphor-10-sulphonate. Reduction of this aldehyde with sodium borohydride yielded optically active trachelanthamidine (26).



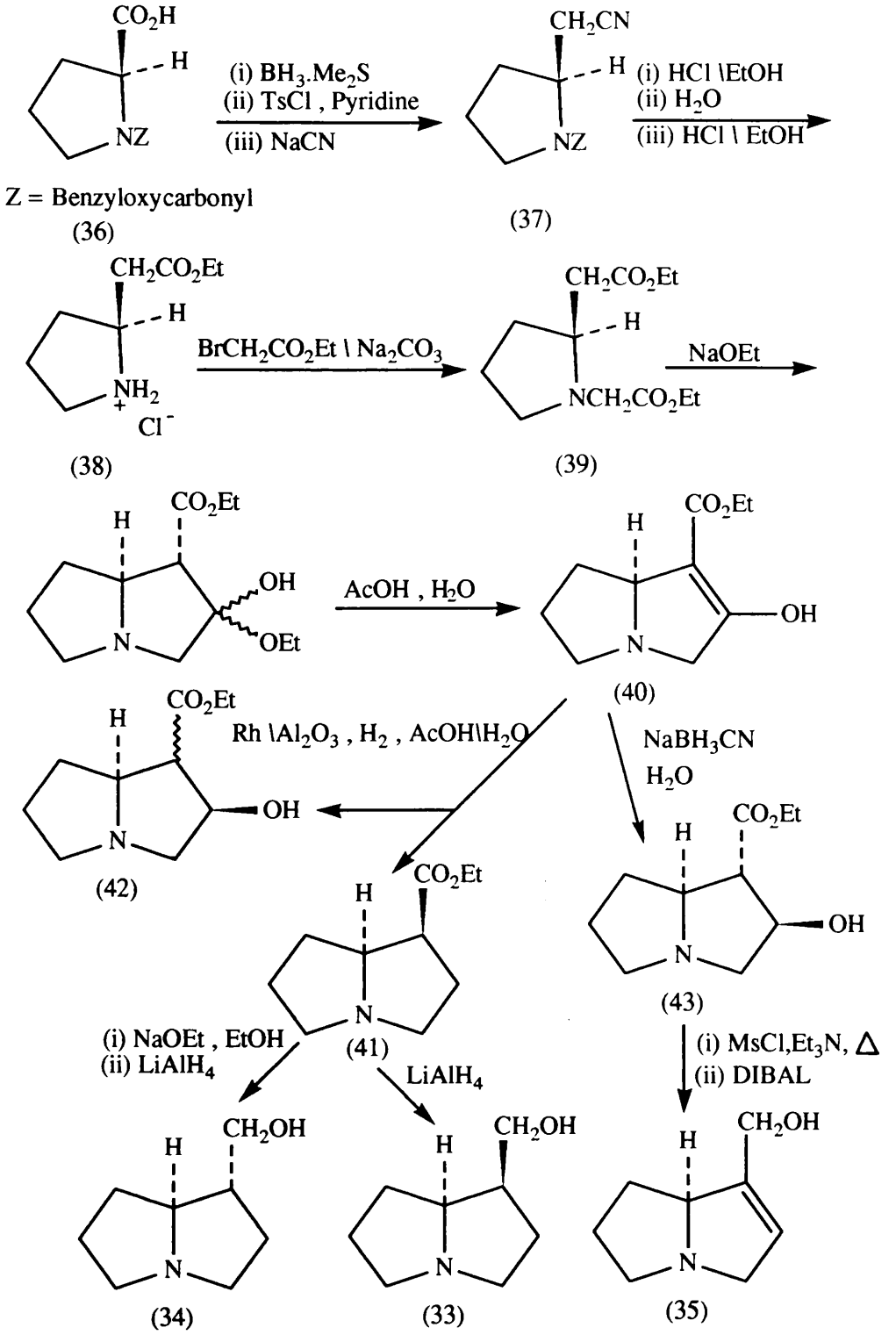
SCHEME 3

In 1982, Rüeger and Benn, like Robins and Sakdarat reported using a L-proline derivative to generate optically active necines - namely, (-)-isoretronecanol (33), (-)-trachelanthamidine (34), and (-)-supinidine

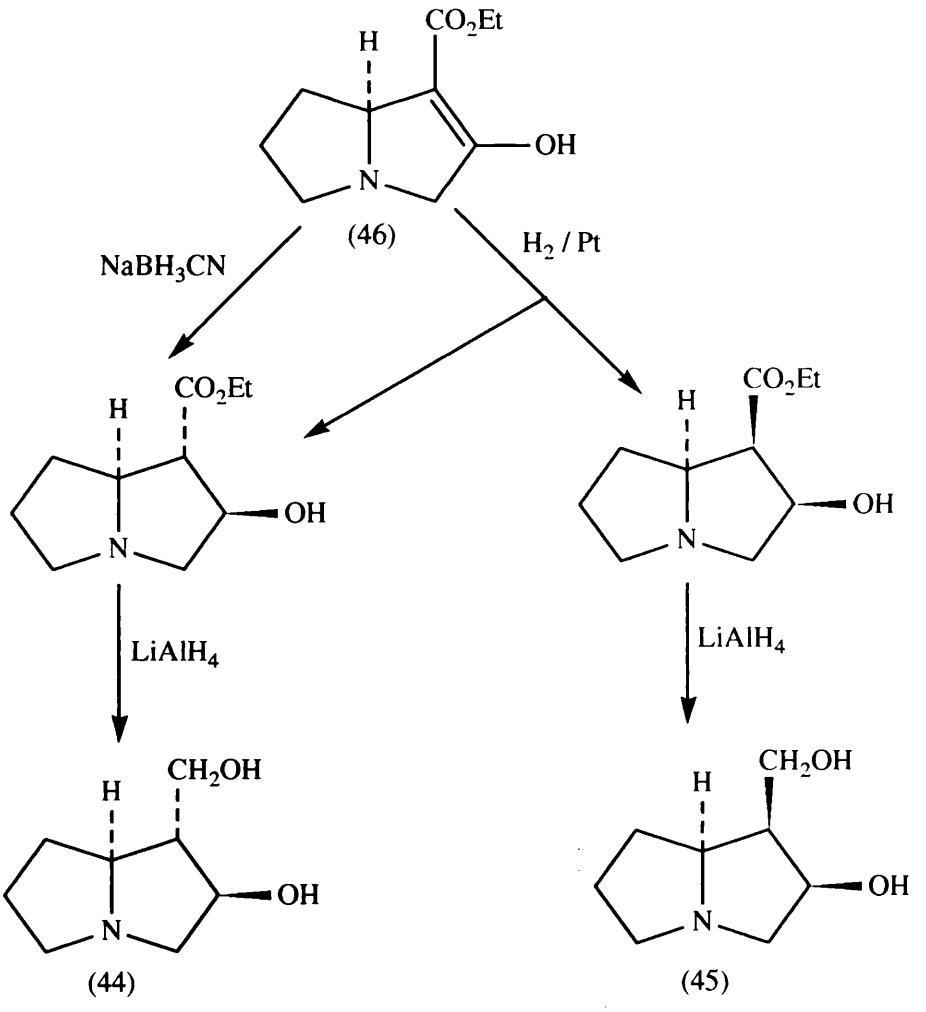
(35)[44] (Scheme 4). This route employed a Dieckmann cyclisation to create the pyrrolizidine nucleus. Borane reduction of the N-protected proline (36), then tosylate formation, followed by nucleophilic displacement of the tosylate by cyanide, yielded the nitrile (37). Exposure of (37) to acidic ethanolysis gave the ester (38) which readily underwent N-alkylation, affording (39). Dieckmann cyclisation of (39), followed by elimination of ethanol, produced the key intermediate enol ester (40). Catalytic hydrogenation of (40) gave a mixture, separable by column chromatography, of the desired ester (41) and diastereomeric hydroxy esters (42). Conversion of (41) into (-)-isoretronecanol (33) and (-)-trachelanthamidine (34) was accomplished by the sequence described by Robins and Sakdarat. Cyanoborohydride reduction of (40) gave (43) which was converted into (-)-supinidine (35) by a sequential mesylation-elimination-reduction scheme.

The same research team later reported the first synthesis of petasinecine (44) and its C-1 epimer (45) in optically active from the intermediate enol ester (46),[45] illustrated in Scheme 5.

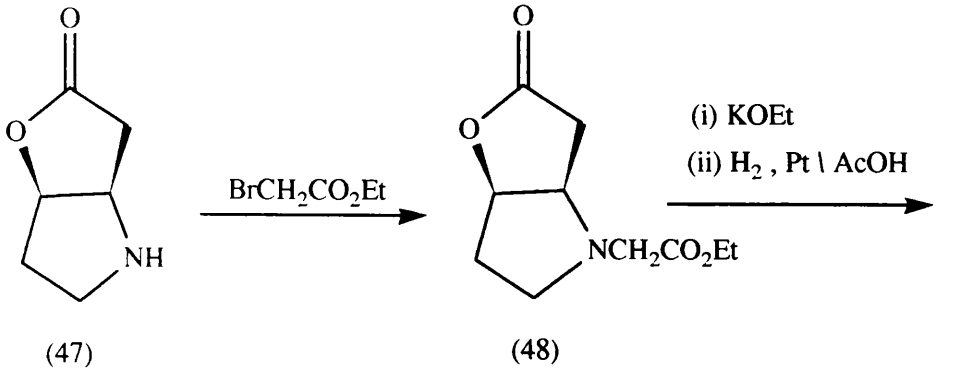
One of the most significant contributions to pyrrolizidine alkaloid synthesis was the route devised by Geissman and Waiss to (±)-retronecine (4).[38] They used β-alanine as their starting material and the key feature of the synthesis was the production, in racemic form, of the bicyclic lactone (47). This reaction sequence employed for the subsequent conversion of this lactone (known thereafter as the Geissman-Waiss lactone) into retronecine is outlined in Scheme 6. A more efficient method of generating retronecine from (48) has recently been reported by Narasaka et al.[46]


CO2H
H
NZ
(i) BH3.Me2S
(ii) TsCl , Pyridine
(iii) NaCN
CH2CN
H
NZ
(i) HCl \EtOH
(ii) H2O
(iii) HCl \ EtOH
Z = Benzyloxycarbonyl
(36)
(37)
CH2CO2Et
H
NH2
+
Cl-
BrCH2CO2Et \ Na2CO3
CH2CO2Et
H
NCH2CO2Et
NaOEt
(38)
(39)
H
CO2Et
OH
OEt
N
AcOH , H2O
OH
(40)
Rh \Al2O3 , H2 , AcOH\H2O
NaBH3CN
H2O
(42)
(41)
(43)
(i) NaOEt , EtOH
(ii) LiAlH4
LiAlH4
(i) MsCl,Et3N, Δ
(ii) DIBAL
CH2OH
(34)
(33)
(35)

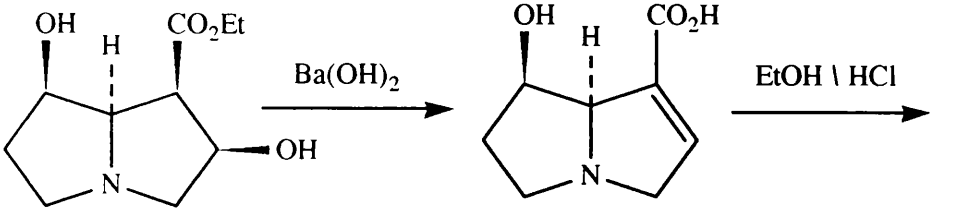
SCHEME 4

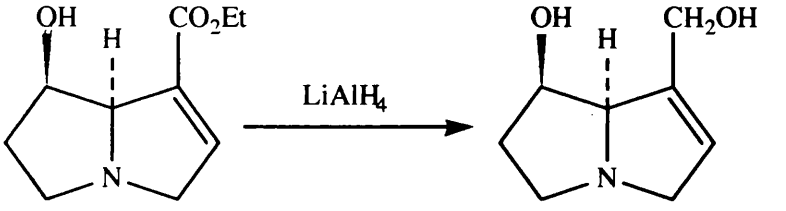

CO2Et
H
OH
N
(46)
NaBH3CN
H2 / Pt
CO2Et
H
OH
N
CO2Et
H
OH
N
LiAlH4
LiAlH4
CH2OH
H
OH
N
CH2OH
H
OH
N
(44)
(45)

SCHEME 5



(47) (48)





(4)

SCHEME 6

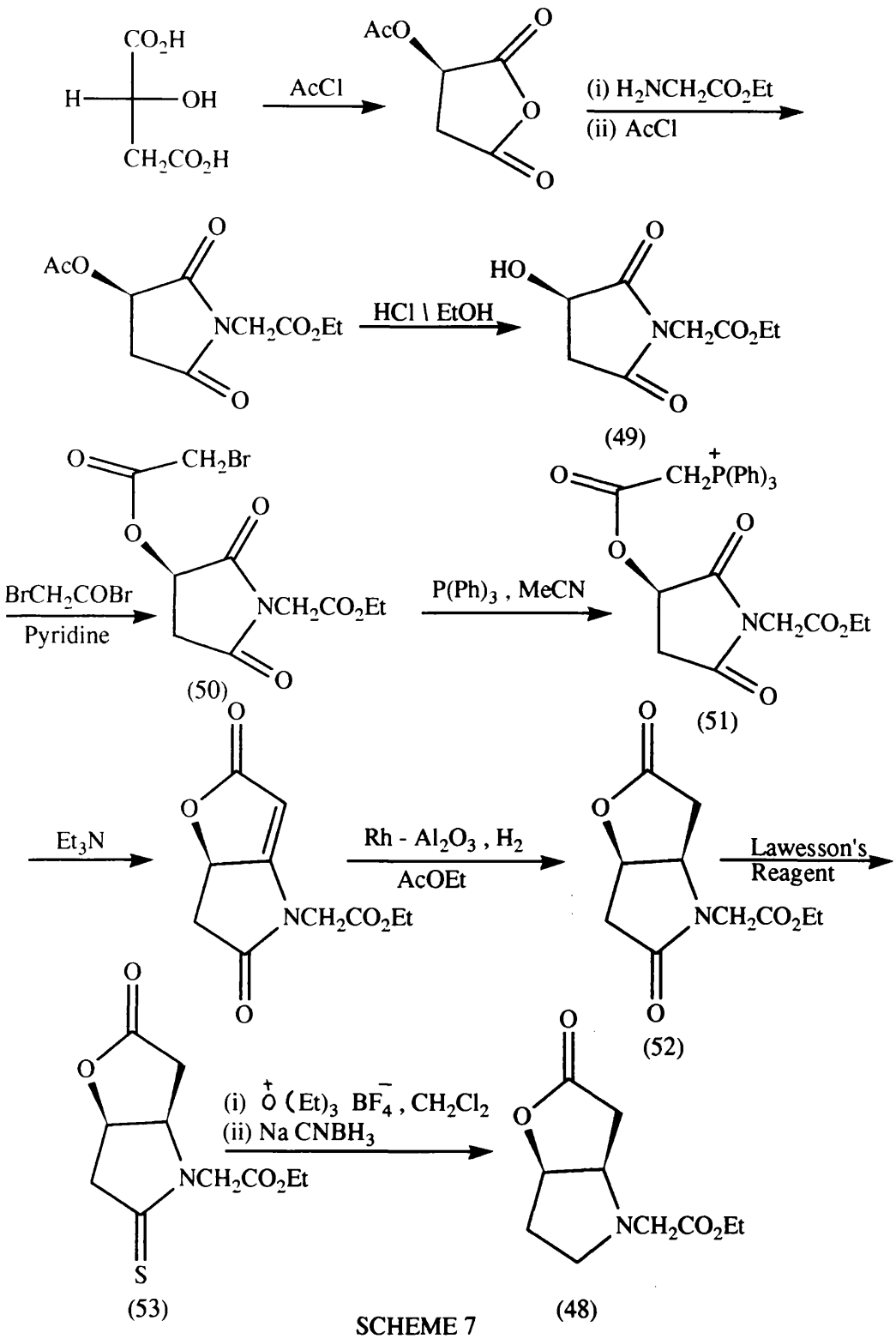
The Geissman-Waiss lactone has proved to be a valuable synthon for optically active necines[47] and the strategy developed by Geissman and Waiss has clearly influenced other enantioselective syntheses.[44,45,48-50]

Recently, several groups[50,51] have reported synthesis of the Geissman-Waiss lactone in optically active form, thus formally establishing a synthesis of (+)-retronecine (4). The most practical method, however, was developed by Niwa et al.,[52] involving the synthesis of the N-(ethoxycarbonyl)methyl derivative (48), employed by Geissman and Waiss in their route to retronecine (Scheme 7).

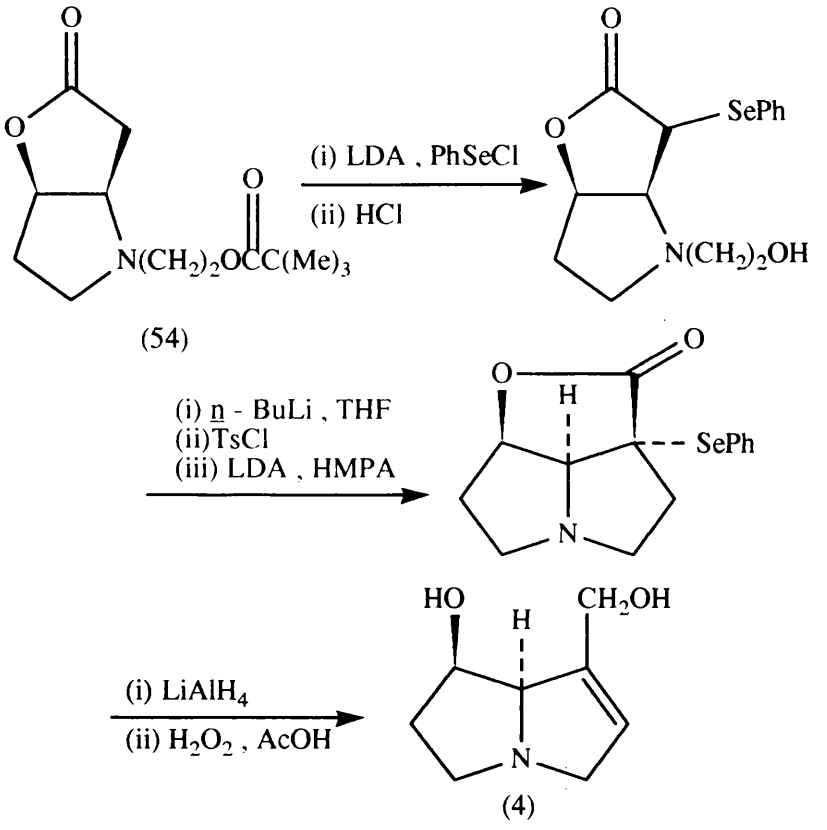
Starting from D-malic acid, Niwa et al. engineered a short sequence to the N-alkylated imide (49). Treatment with $BrCH_2COBr$ in pyridine, transformed (49) into the bromoacetoxy imide (50) which reacted with triphenylphosphine in acetonitrile to afford the phosphonium salt (51). Intramolecular Wittig cyclisation of (51), followed by catalytic reduction, yielded the bicyclic lactam (52). Conversion into (48) was accomplished by selective reduction of the thiolactam (53), produced by the action of Lawesson's Reagent on (52).

Niwa et al. had previously used the same strategy to generate (54), the key intermediate in their total synthesis of (+)-retronecine (4) from D-malic acid. The lactone (54) was converted into retronecine by the simple reaction sequence shown in Scheme 8.

Rüeger and Benn employed the Geissman-Waiss lactone, in optically active form, as the synthon for the first enantioselective synthesis of croalbinecine (55), platynecine (56), and retronecine (4).[47] The sequence illustrated in Scheme 9, also afforded the optically active triol (57), the C-1 epimer of croalbinecine. The enolisable ester (58)


CO2H
H
OH
CH2CO2H
AcCl
AcO
O
O
O
(i) H2NCH2CO2Et
(ii) AcCl
AcO
NCH2CO2Et
HCl \ EtOH
HO
NCH2CO2Et
(49)
CH2Br
BrCH2COBr
Pyridine
NCH2CO2Et
(50)
P(Ph)3 , MeCN
CH2P(Ph)3
NCH2CO2Et
(51)
Et3N
Rh - Al2O3 , H2
AcOEt
Lawesson's
Reagent
NCH2CO2Et
(52)
(i) O (Et)3 BF4 , CH2Cl2
(ii) Na CNBH3
NCH2CO2Et
S
(53)
NCH2CO2Et
(48)

SCHEME 7


O
O
O
N(CH2)2OCC(Me)3
(i) LDA , PhSeCl
(ii) HCl
O
O
SePh
N(CH2)2OH
(54)
(i) n - BuLi , THF
(ii)TsCl
(iii) LDA , HMPA
O
O
H
SePh
N
HO
H
CH2OH
(i) LiAlH4
(ii) H2O2 , AcOH
N
(4)

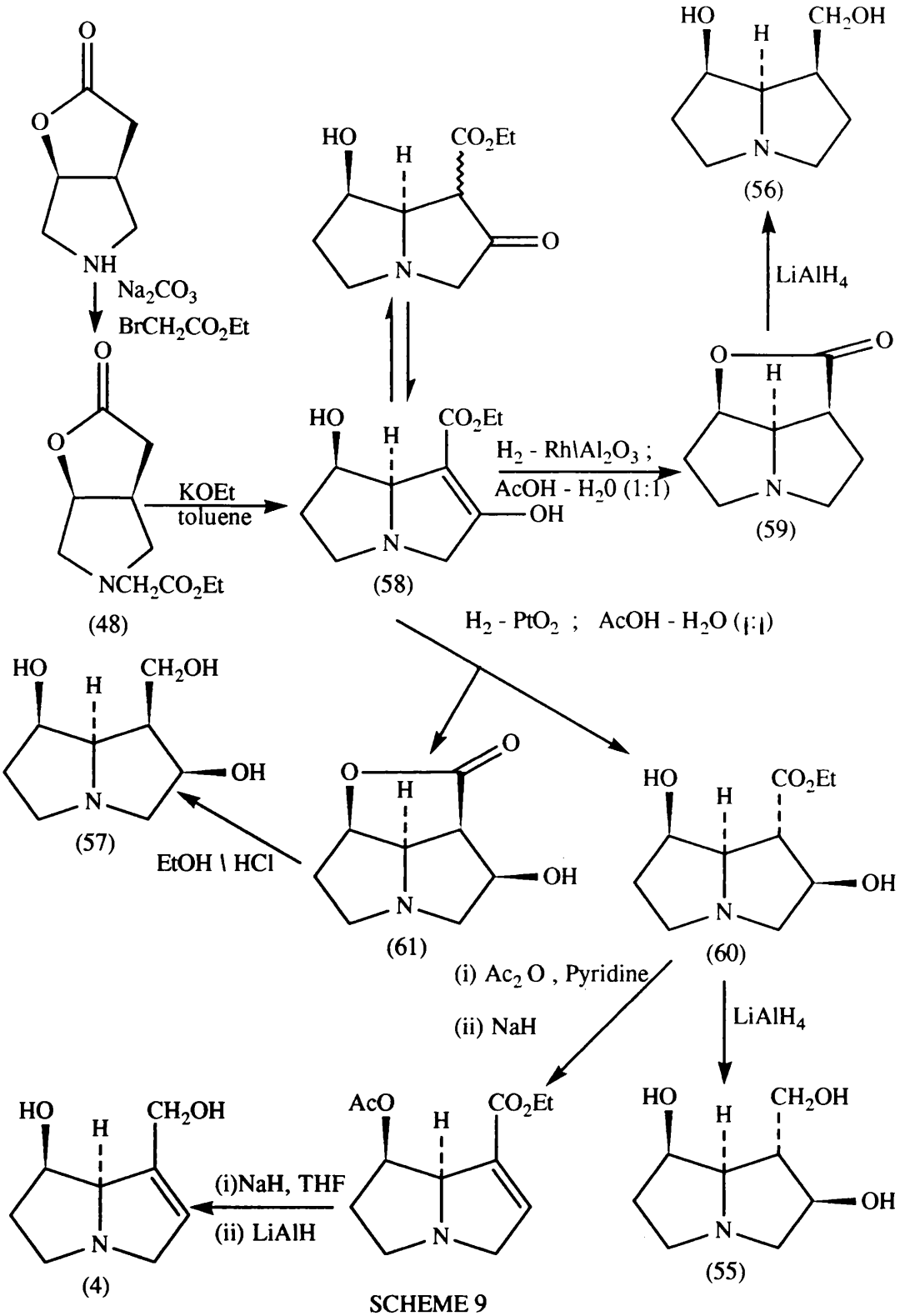
SCHEME 8

was the key intermediate of this route, and was produced via Dieckmann cyclisation of the Geissman-Waiss lactone (48).

Hydrogenation of this enolisable keto ester (58), using 5% rhodium on alumina in acetic acid/water (1:1), gave the lactone (59) which was converted into (-)-platynecine (56) by reduction with lithium aluminium hydride. However, when the hydrogenation step was carried out using Adams' catalyst (PtO_2) and the same solvent mixture, formation of the exo-ester (60) together with a small quantity of lactone (61) was observed. These were separated and subsequent reduction afforded (-)-croalbinecine (55) and its C-1 epimer (57), respectively. (+)-Retronecine (4) was produced from (60) by modification of an established pathway.

At a later date, the same workers, together with Yadav, reported the first synthesis of a pyrrolizidine 1,2-unsaturated triol.[49] They synthesised (+)-crotanecine (10) by an elaborate 15-step sequence, illustrated in Scheme 10. As before, the starting material was the protected L-proline and once again an intermediate bicyclic lactone was employed to help develop the pyrrolizidine skeleton.

Diborane reduction of (62), followed by conversion into the ditosylate, then successive nucleophilic displacements, produced the selenide (63). Oxidation of (63) and thermal elimination of the selenoxide which was produced, gave the anticipated pyrroline (64). This was treated with N-iodosuccinimide in acetic acid, and again reacted in a highly regioselective manner, generating the iodoacetate (65). Solvolysis in acetic acid containing silver acetate gave a complex product mixture which, on acidification, followed by N-alkylation, yielded the desired lactone (66)

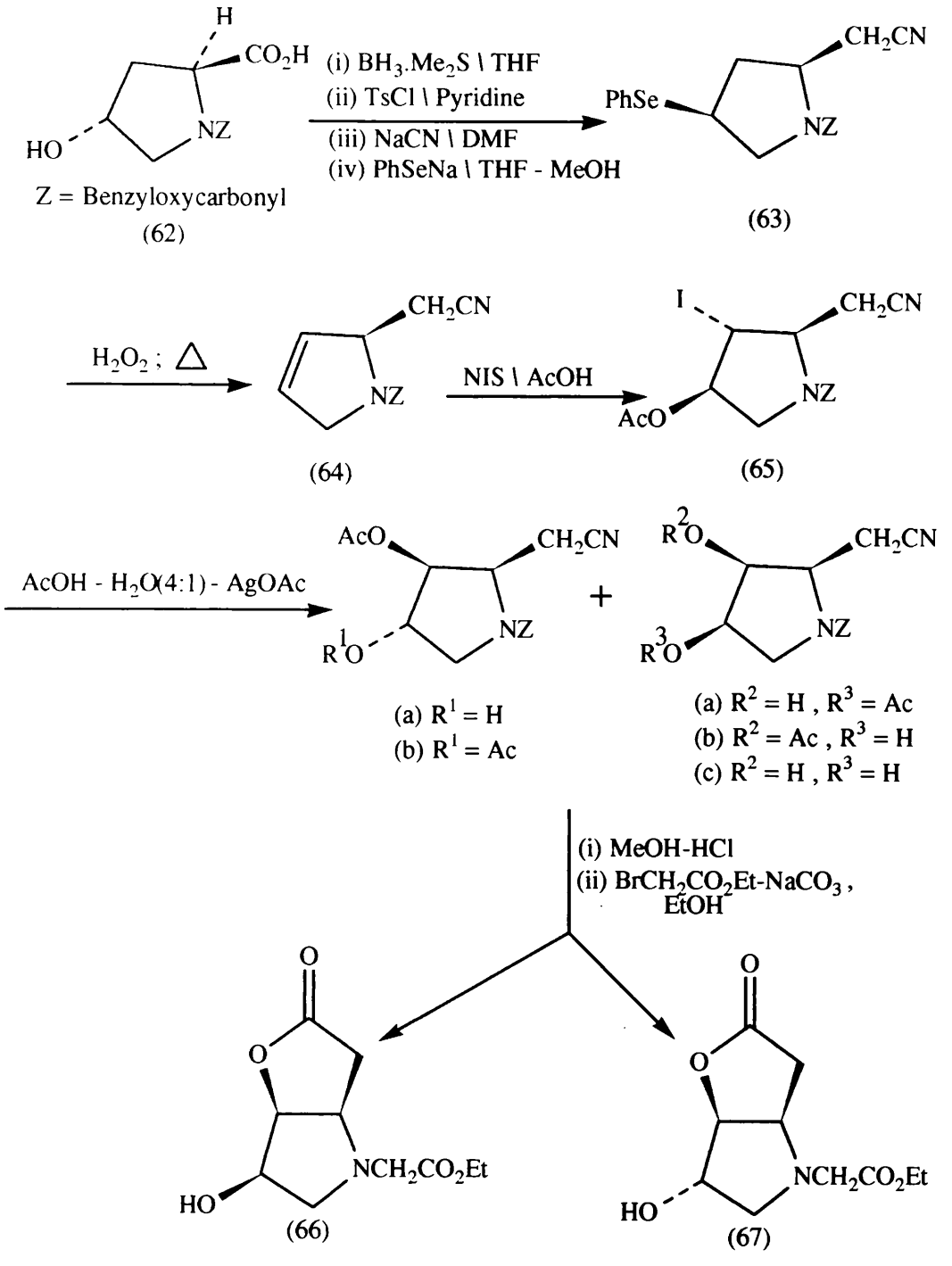

Na2CO3
BrCH2CO2Et
KOEt
toluene
NCH2CO2Et
(48)
(58)
H2 - Rh\Al2O3 ;
AcOH - H20 (1:1)
(59)
LiAlH4
(56)
H2 - PtO2 ; AcOH - H2O (1:1)
(57)
EtOH \ HCl
(61)
(60)
(i) Ac2 O , Pyridine
(ii) NaH
LiAlH4
(i)NaH, THF
(ii) LiAlH
(4)
(55)

SCHEME 9

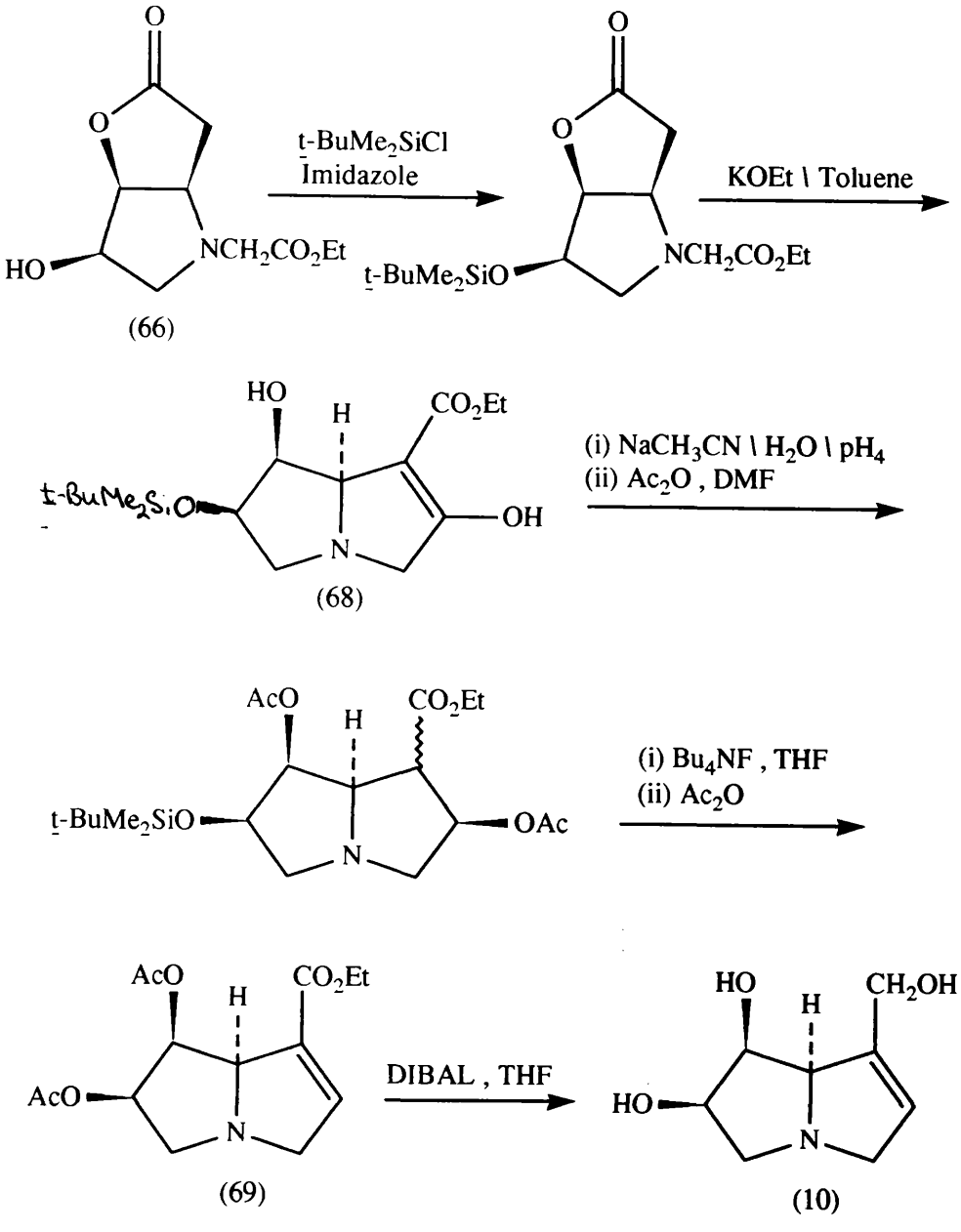
and a small quantity of the epimeric alcohol (67). After chromatographic removal of the unwanted alcohol, the lactone (66) was silylated to aid Dieckmann cyclisation to give the pyrrolizidine system. Cyanoborohydride reduction of enol ester (68), followed by acylation and then removal of the silyl protecting group, gave the conjugated ester (69). Removal of the acetate groups and reduction of the ester was achieved by treatment with DIBAL, thus completing the total synthesis of (+)-crotanecine (10).

Hart and his co-workers have displayed great ingenuity in their synthetic approach to optically active necines. Hart and Yang reported an elegant enantioselective synthesis of both hastanecine (23) and heliotridine (70) from D-malic acid.[53,54] The strategy, outlined in Scheme 11, was dependent upon the production of an N-acyliminium ion capable of inducing a controlled aza-Cope rearrangement to develop the pyrrolizidine skeleton.

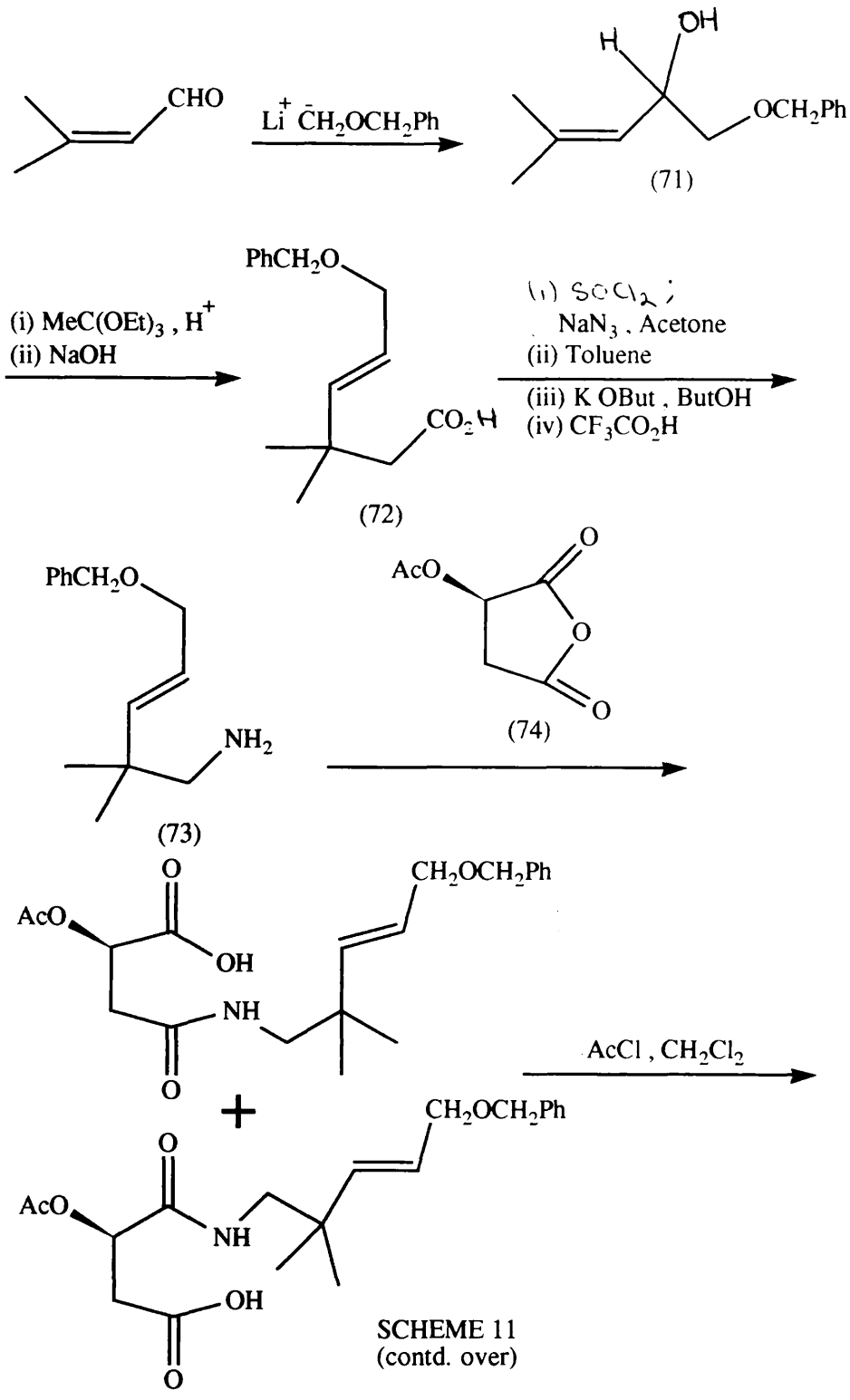
Initially (benzyloxy)methyllithium was reacted with 3,3-dimethylacrolein to yield the vinyl alcohol (71). Rearrangement of (71) afforded the acid (72), which was subsequently transformed into the primary amine (73) by a simple reaction sequence. Addition of (73) to the acylated anhydride of D-malic acid (74), gave a mixture of amic acids which cyclised to the acetoxyimide (75) on treatment with acetyl chloride. Selective reduction of (75) followed by treatment with formic acid, gave a mixture of the alcohol (76) and the corresponding formate (77). The authors accounted for the product stereochemistry by proposing that the initial iminium ion rearrangement occurred via a transition state (78) in which the steric interaction between the (benzyloxy)methyl and acetoxy groups is minimised. Subsequent cyclisation of (78) was consistent with



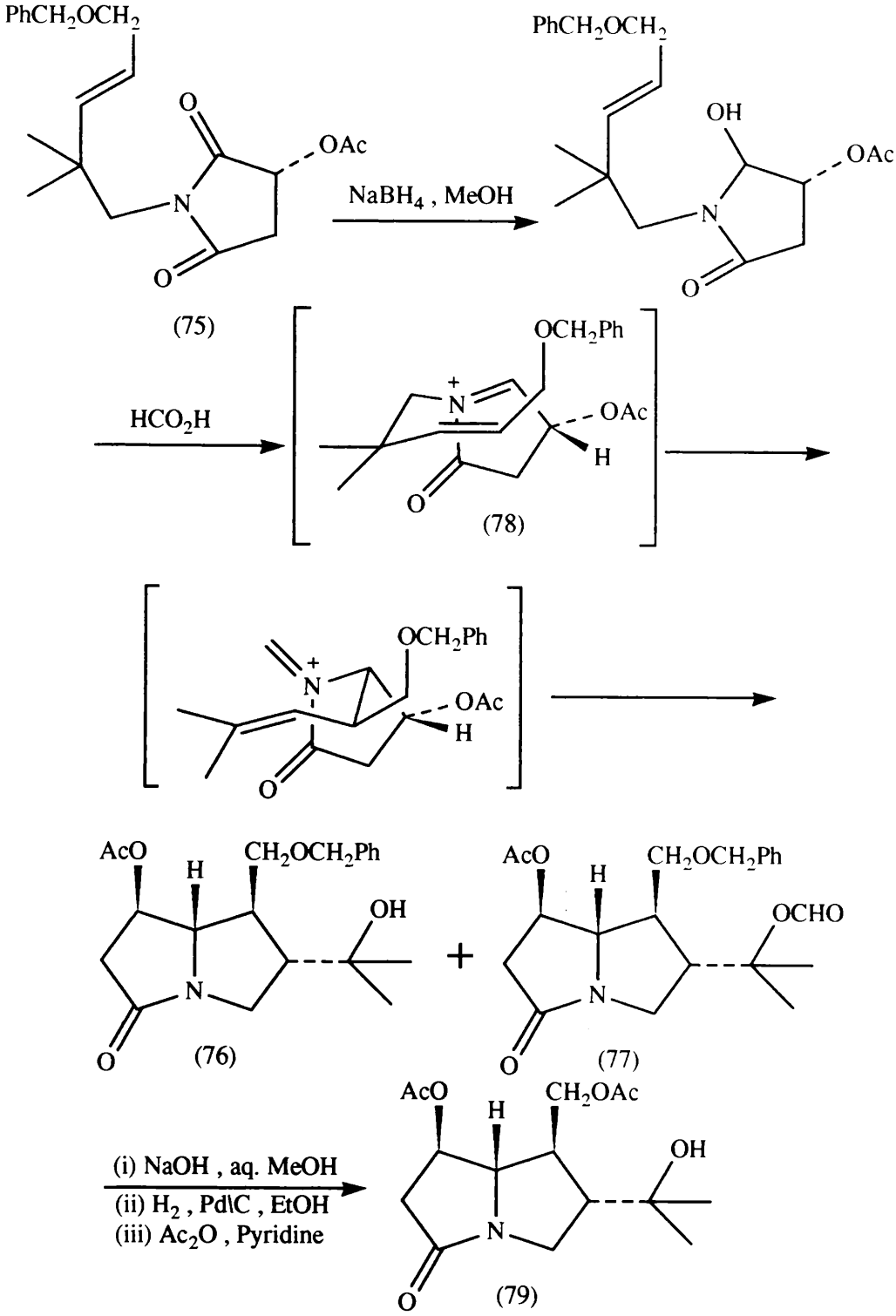
SCHEME 10
(contd. over)


t-BuMe2SiCl
Imidazole
KOEt \ Toluene
HO
NCH2CO2Et
t-BuMe2SiO
(66)
HO
H
CO2Et
OH
N
(68)
(i) NaCH3CN \ H2O \ pH4
(ii) Ac2O , DMF
AcO
(i) Bu4NF , THF
(ii) Ac2O
OAc
DIBAL , THF
CH2OH
(69)
(10)

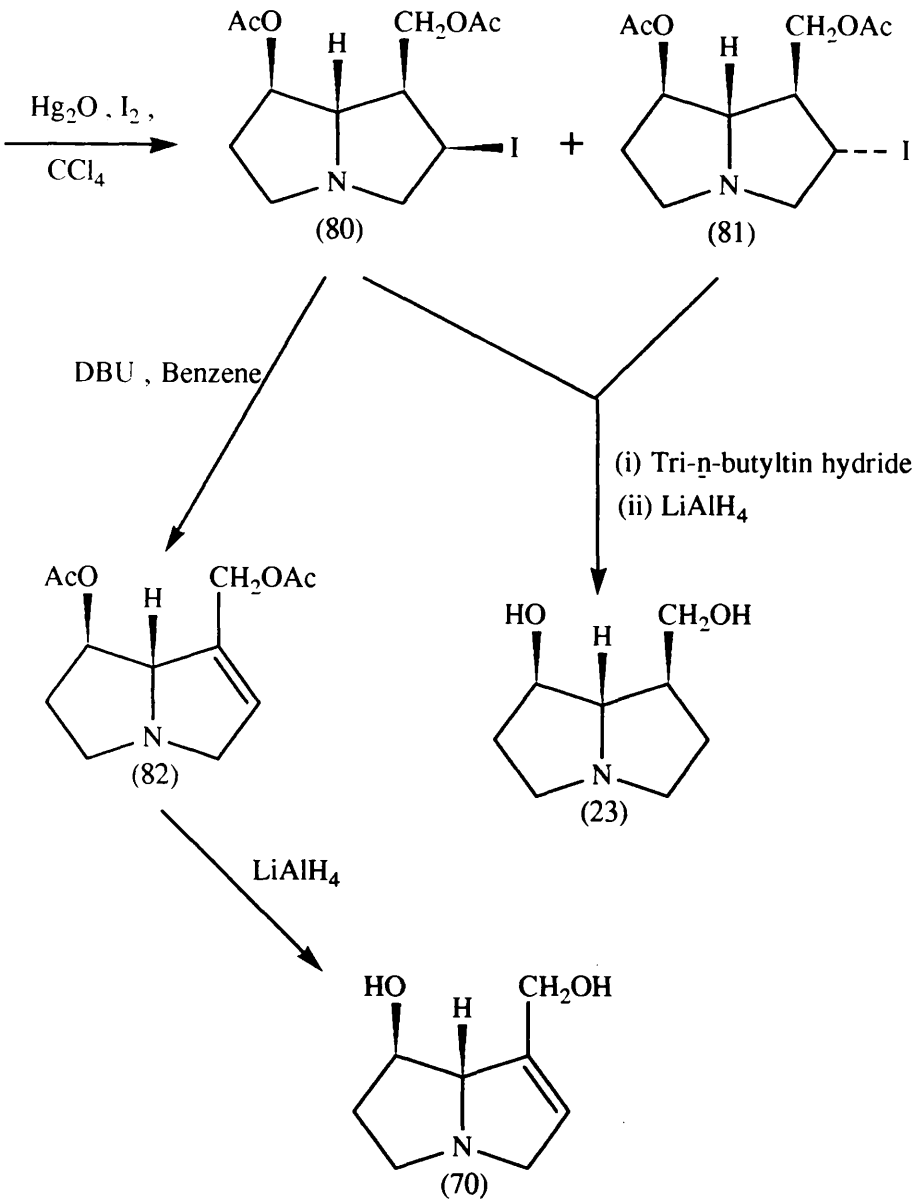
SCHEME 10



SCHEME 11
(contd. over)



SCHEME 11
(contd. over)


AcO
H
CH2OAc
I
N
(80)
+
(81)
Hg2O , I2 ,
CCl4
DBU , Benzene
(i) Tri-n-butyltin hydride
(ii) LiAlH4
HO
CH2OH
(82)
(23)
LiAlH4
(70)

SCHEME 11

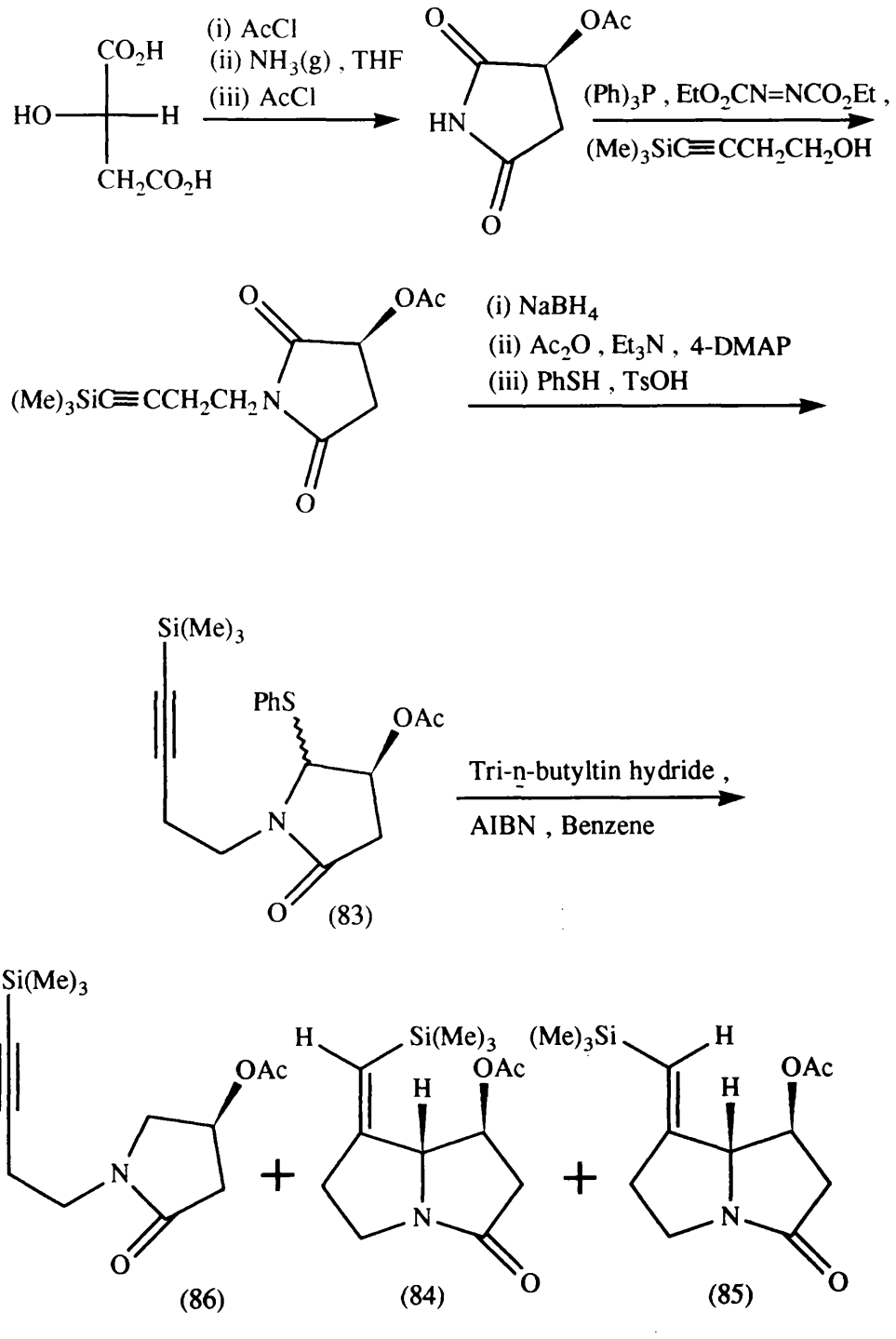
the stereochemistry observed.

Conversion of the product mixture into the diacetate (79), then reduction with mercuric oxide and iodine in carbon tetrachloride, gave a mixture of the separable diastereomeric iodides (80) and (81). Reaction of the β-iodide (80) with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in toluene afforded the lactam (82) which was converted into (-)-heliotridine (70) on treatment with lithium aluminium hydride.

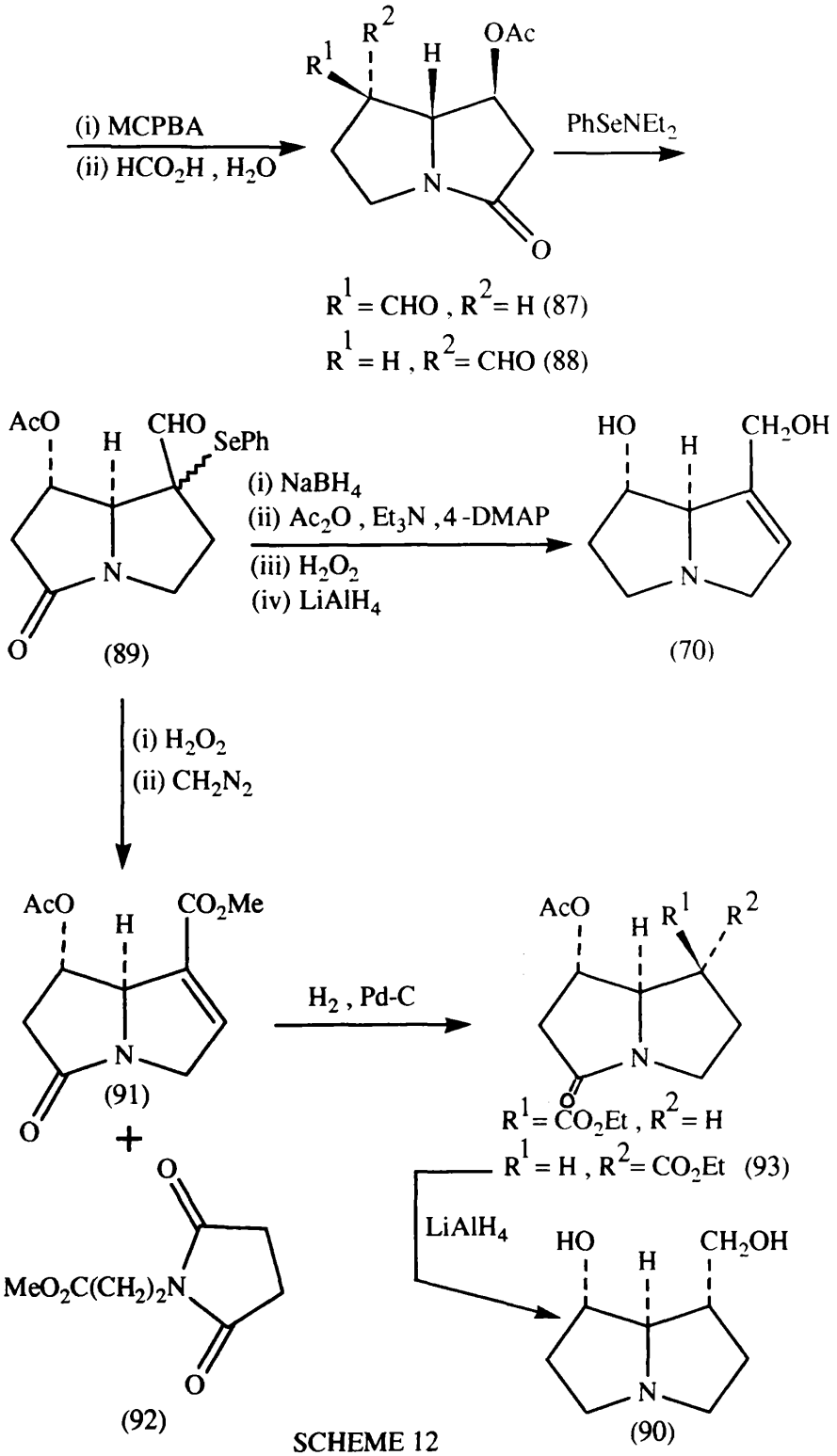
A synthesis of (-)-hastanecine (23) was engineered by the treatment of the iodide mixture (80) and (81) with tri-n-butyltin hydride with azobisisobutyronitrile (AIBN) followed by reaction with aluminium hydride. The enantiomers of hastanecine and heliotridine were also synthesised by substituting L-malic acid into the sequence.

Hart and Choi reported a further enantioselective synthesis of both hastanecine and heliotridine from L-malic acid,[55] illustrated in Scheme 12. On this occasion construction of the pyrrolizidine nucleus was achieved, in highly diastereoselective fashion, by an intramolecular addition of an α-acylamino radical to an alkyne. The N-alkylated lactam (83) was employed as the radical precursor and was synthesised by a strategy related to that used by Hart and Yang.

Treatment of (83) with tri-n-butyltin hydride and AIBN in benzene, generated the desired α-acylamino radical. This radical underwent intramolecular cyclisation to afford a mixture of the geometric isomers (84) and (85), together with a small quantity of the reduction product (86) which was subsequently removed by chromatographic means. Oxidation of the residual silane mixture, followed by treatment with formic acid, gave a 4:1 mixture of the diastereomeric aldehydes (87) and (88). This mixture was readily selenenylated and then converted into



SCHEME 12
(contd. over)


(i) MCPBA
(ii) HCO2H , H2O
PhSeNEt2
R1 = CHO , R2 = H (87)
R1 = H , R2 = CHO (88)
AcO
CHO
SePh
(i) NaBH4
(ii) Ac2O , Et3N , 4-DMAP
(iii) H2O2
(iv) LiAlH4
HO
CH2OH
(89)
(70)
(i) H2O2
(ii) CH2N2
CO2Me
H2 , Pd-C
(91)
MeO2C(CH2)2N
(92)
R1 = CO2Et , R2 = H
R1 = H , R2 = CO2Et (93)
LiAlH4
(90)

SCHEME 12

(+)-heliotridine (70) by established methods.

The selenide (89) was also used to effect a synthesis of (+)-hastanecine (90). Peroxide treatment of (89) followed by reaction with diazomethane yielded the conjugated ester (91) as the major product. A small quantity of the imide (92) was also produced, but removed by chromatographic means. Hydrogenation of (91) gave a 3:1 mixture of the epimeric esters from which (93), the more abundant epimer, was selectively crystallised. Conversion of (93) into (+)-hastanecine (90) was achieved by aluminium hydride reduction.

Chamberlin and Chung have developed a most accomplished and practical route to optically active necines by employing an N-acyliminium ion to effect a highly stereoselective intramolecular cyclisation to the pyrrolizidine ring system. They initially reported an enantioselective synthesis of (+)-heliotridine (70)[56] and later extended the sequence to produce several other pyrrolizidine diols in optically active form.[57] The route employed is illustrated in Scheme 13.

Using L-malic acid as their starting material, they formed the key intermediate ketenethioacetal (94) by a strategy similar to that of Hart and Young. Selective borohydride reduction of (94) was followed, under basic conditions, by an acyliminium induced stereoselective cyclisation to afford the lactam (95). The stereochemistry of the lactam formed is a consequence of the acetoxy group effectively blocking the α-face of the acyliminium ion, resulting in a diastereoselective cyclisation step.

Removal of the acetate protection from (95), followed by thioacetal-assisted regioselective double bond migration, gave the thiane (96). This underwent acetal cleavage, then reduction, to afford (+)-heliotridine

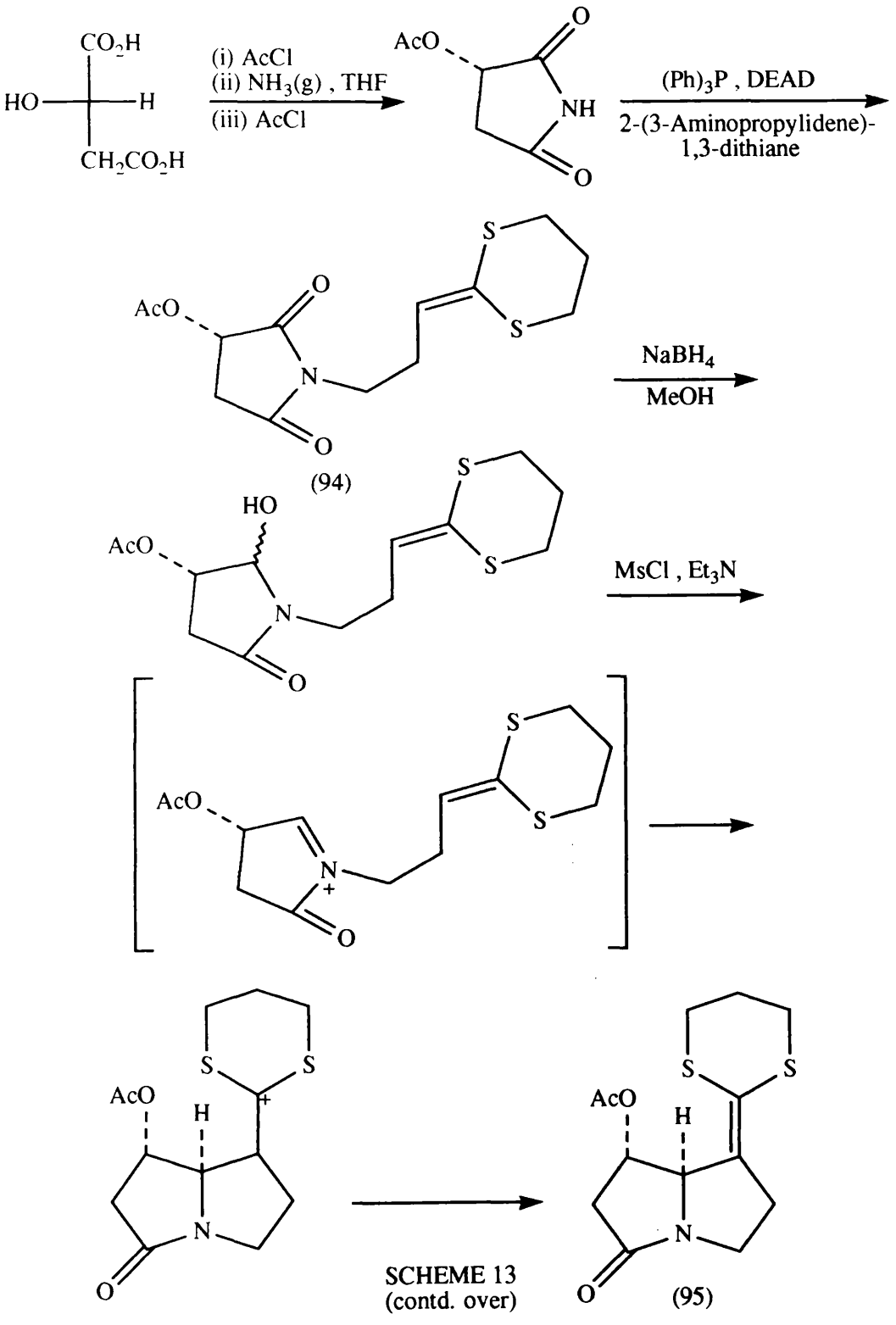
(70). Raney nickel treatment of allylic alcohol (70) yielded the saturated necine (-)-dihydroheliotridine (97).

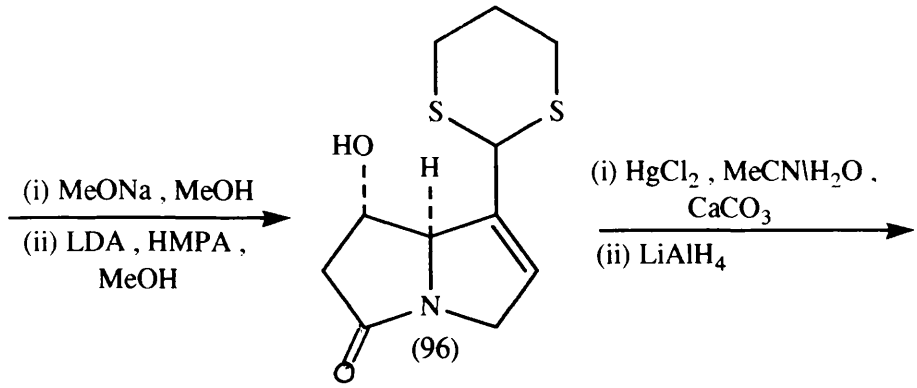
The thioacetate (95) was also used to prepare a further two pyrrolizidine bases, hastanecine (90) and dehydroheliotridine (98) in optically active form. Treatment of (95) with mercuric chloride in acidified methanol, followed by lithium aluminium hydride reduction, yielded (+)-hastanecine (90). The pyrrolizidine alcohol (99), prepared by a sequential hydride reduction and double bond migration, was converted into (+)-dehydroheliotridine (98), by thioacetal cleavage, then sodium borohydride reduction.

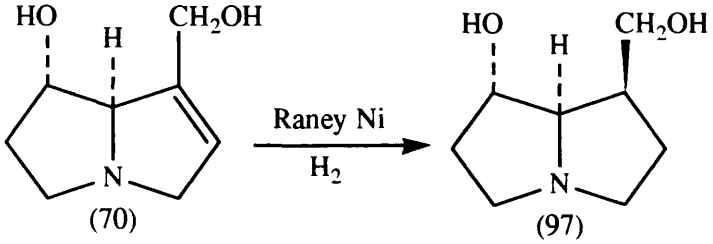
The 7β-hydroxy necines, (+)-retronecine (4), (-)-platynecine (56), and (+)-turneforcidine (100), were also synthesised from (95), via the methyl ester (101). Swern oxidation of alcohol (101), followed by catalytic hydrogenation generated the desired 7β-hydroxypyrrolizidine (102). Hydride reduction of (102) afforded (+)-turneforcidine (100), while treatment with ethoxide anion produced the lactone (103). This lactone was converted into (-)-platynecine (56) and (+)-retronecine (4) by established methods.

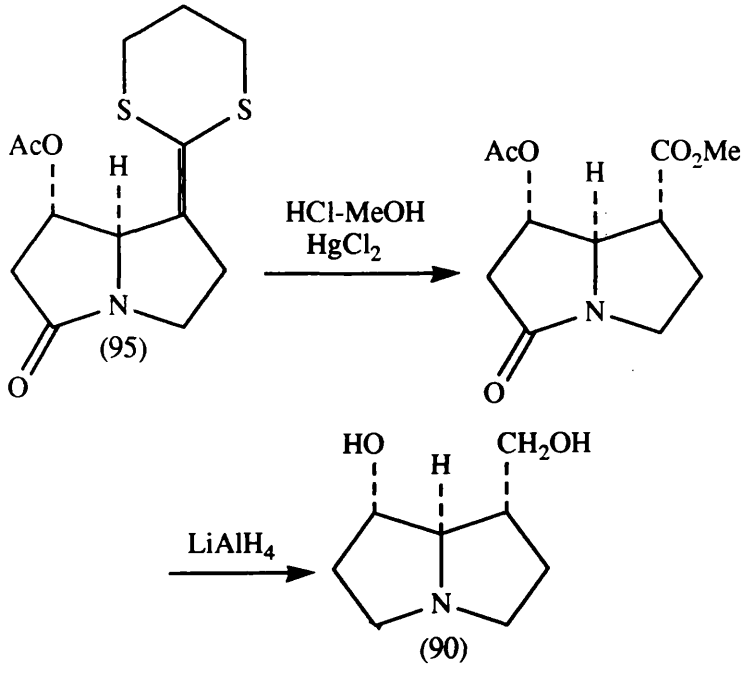
The natural sugars have long been recognised as an important source of stereochemically defined material useful for enantioselective synthesis.[58] Three groups, Tatsuta et al.,[59] Nishimura et al.,[60] and Buchanan et al.[50] have recently reported elaborate routes to optically active necines from such precursors.

Tatsuta and his co-workers produced (-)-rosmarinecine (21) and (-)-isoretronecanol (33) from the furanose (104) illustrated in Scheme 14. Silylation of the furanose (104)[61] was followed by a stereo-

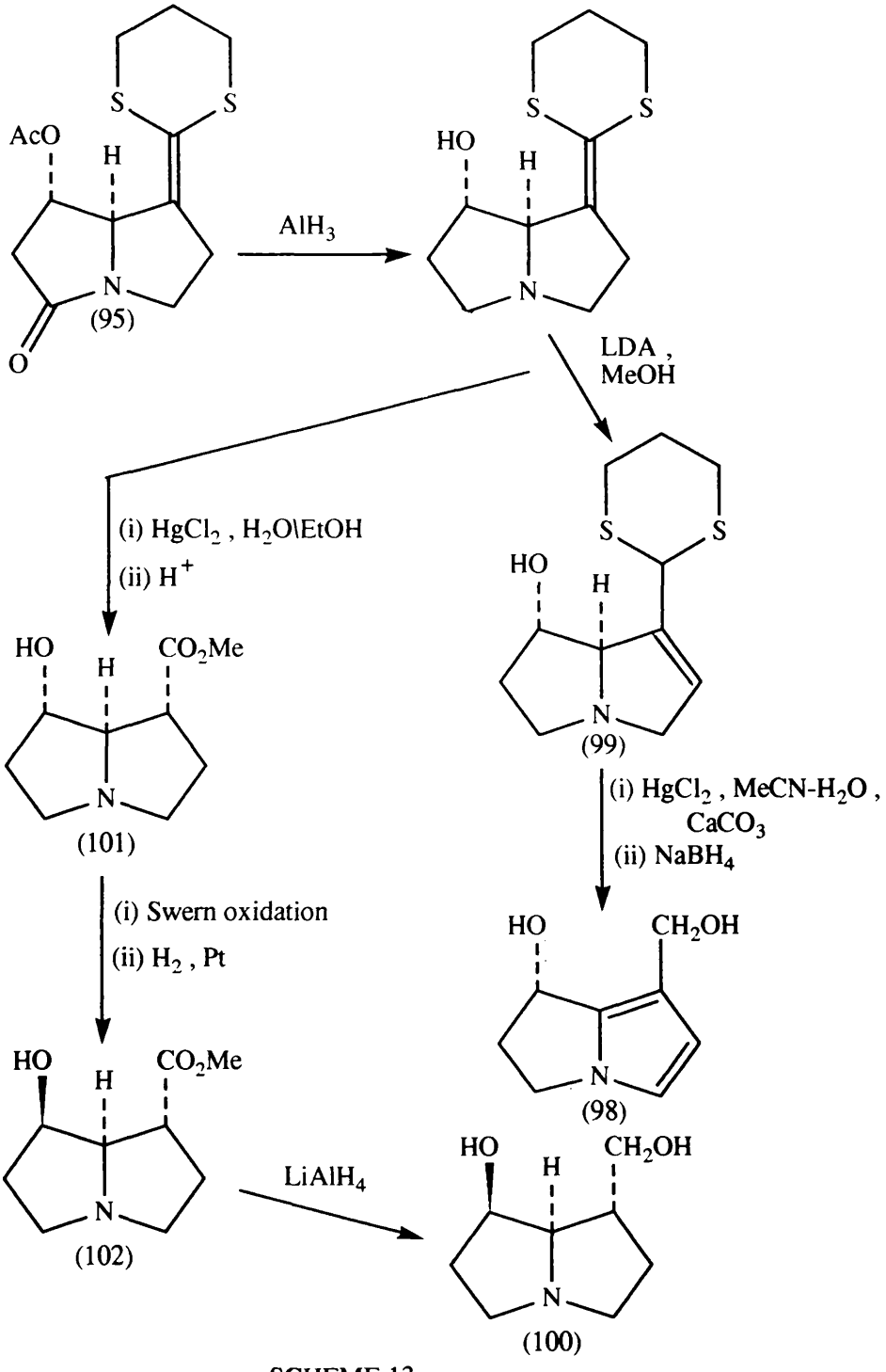

CO2H
HO
H
CH2CO2H
(i) AcCl
(ii) NH3(g) , THF
(iii) AcCl
AcO
O
NH
O
(Ph)3P , DEAD
2-(3-Aminopropylidene)-
1,3-dithiane
S
S
N
NaBH4
MeOH
(94)
HO
MsCl , Et3N
+
SCHEME 13
(contd. over)
(95)



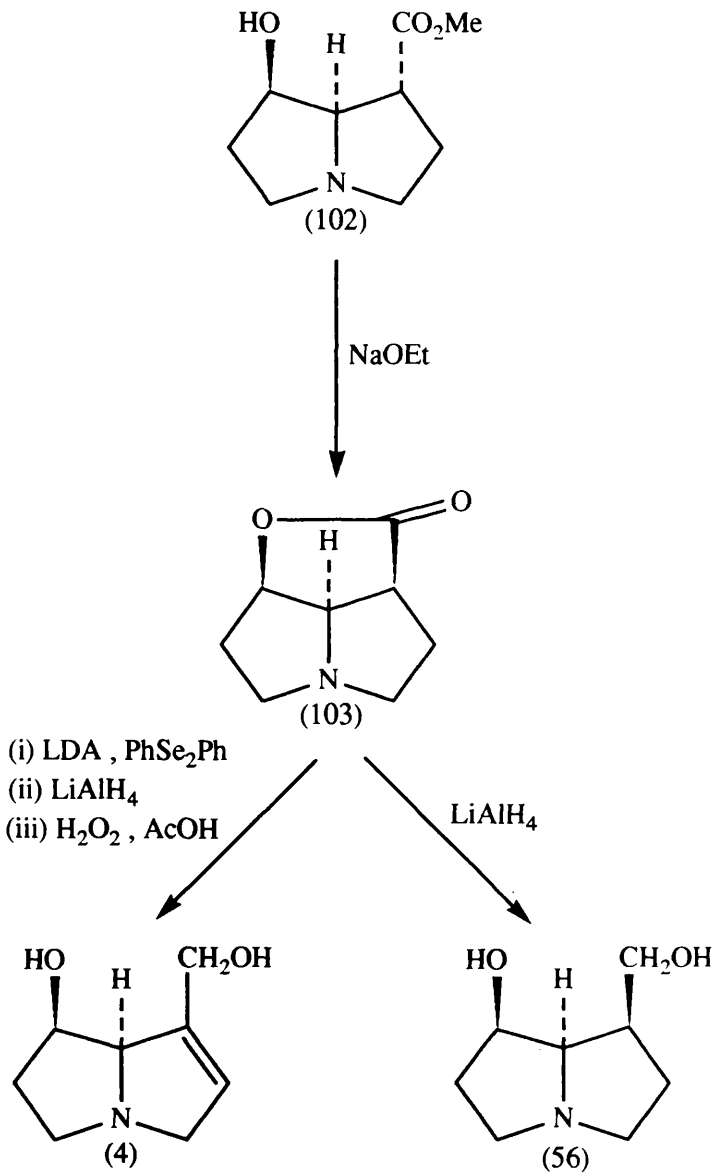




SCHEME 13
(contd. over)



SCHEME 13
(contd. over)


HO
H
CO2Me
N
(102)
NaOEt
O
O
H
N
(103)
(i) LDA , PhSe2Ph
(ii) LiAlH4
(iii) H2O2 , AcOH
LiAlH4
HO
H
CH2OH
N
(4)
HO
H
CH2OH
N
(56)

SCHEME 13

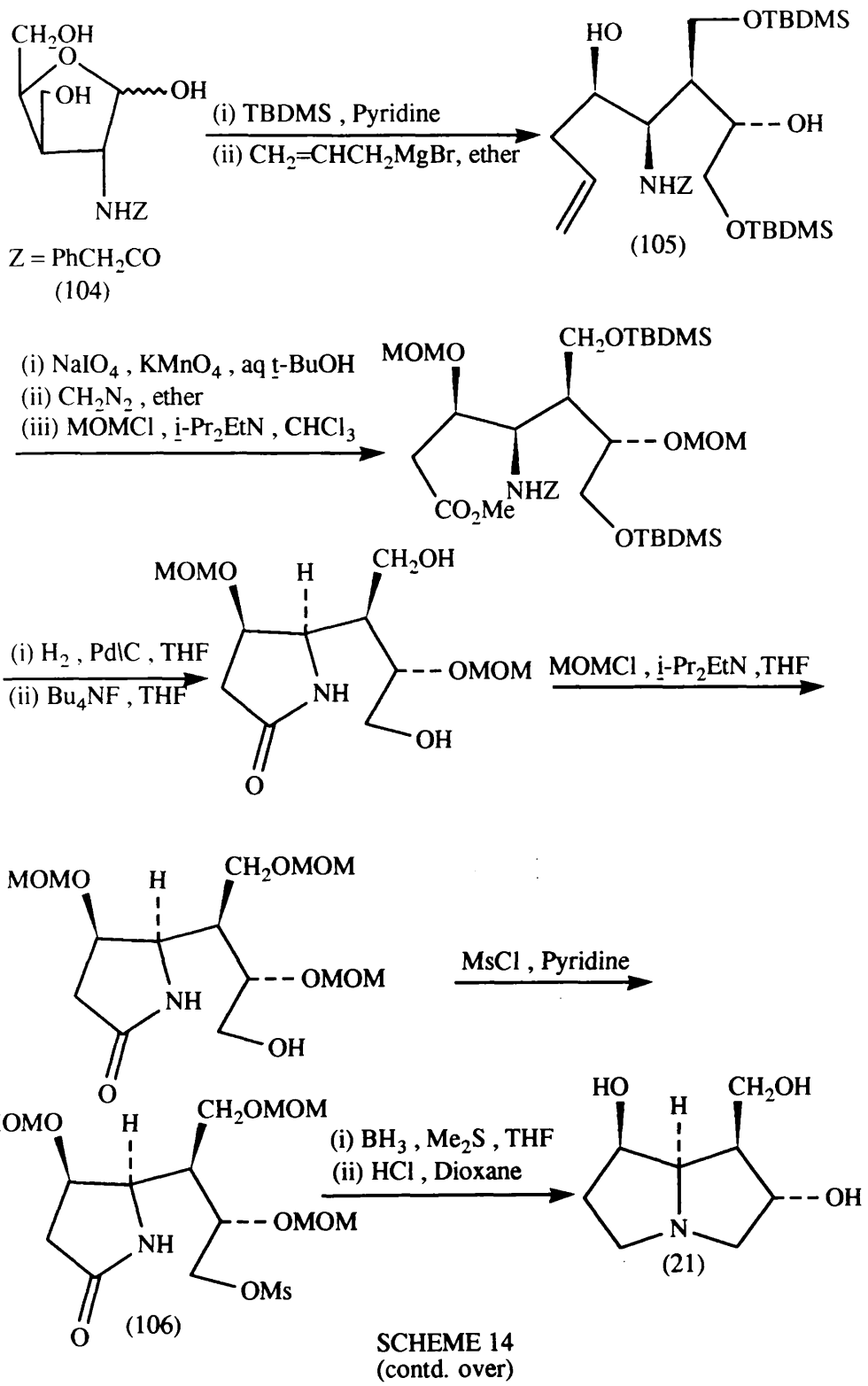
selective Grignard reaction with allylmagnesium bromide to afford (105). This material was subsequently transformed into the lactam (106) by a lengthy series of reactions. Reduction of lactam (106) enabled formation of the pyrrolizidine skeleton, via intramolecular cyclisation, and a subsequent deprotection step afforded (-)-rosmarinecine (21).

A modified sequence was used to generate (-)-isoretronecanol (33) from the furanose (107). Wittig reaction of this dityrityl derivative with (methoxycarbonyl)methylenetriphenylphosphorane gave the α,β-unsaturated ester (108), which was converted into (-)-isoretronecanol (33) by employing a similar extended sequence.

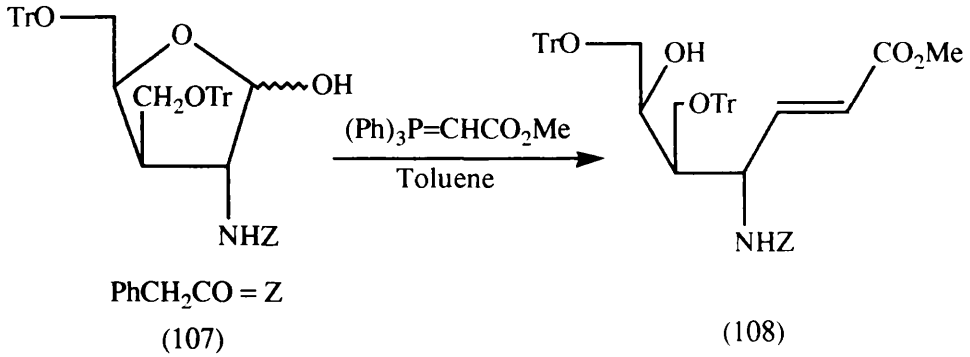
Nishimura et al. later reported synthesis of (+)-retronecine (4) and its enantiomer (109), both in optically pure form, from the azide (110). This azide was produced from D-glucose[62] (Scheme 15). Treatment of azide (110) with Raney nickel produced a primary amine which readily afforded the furanose (111) by intramolecular displacement. A series of established manipulations produced the glycoside (112) which, after Wittig reaction and an ensuing hydroboration-oxidation sequence, afforded the protected triol (113). Nitrogen deprotection of (113) effected cyclisation to the pyrrolizidine (114), which was thenmanipulated to enable the production of both optical forms of retronecine.

Selective deprotection of C-1, by catalytic hydrogenolysis, afforded the 1β-hydroxypyrrolizidine (115). This was then converted into (+)-retronecine (4) by standard methods.

On the other hand, selective removal of the methoxymethyl protecting group at C-7 of (114) produced the 7β-hydroxypyrrolizidine (116). A similar reaction sequence was then implemented to yield (-)-retronecine (109).



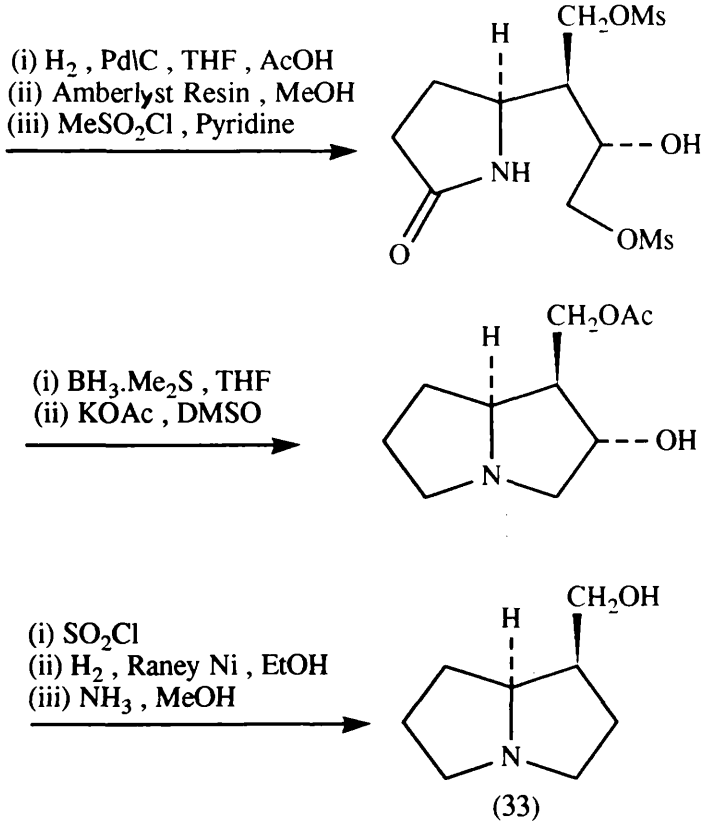
SCHEME 14
(contd. over)



$PhCH_2CO = Z$

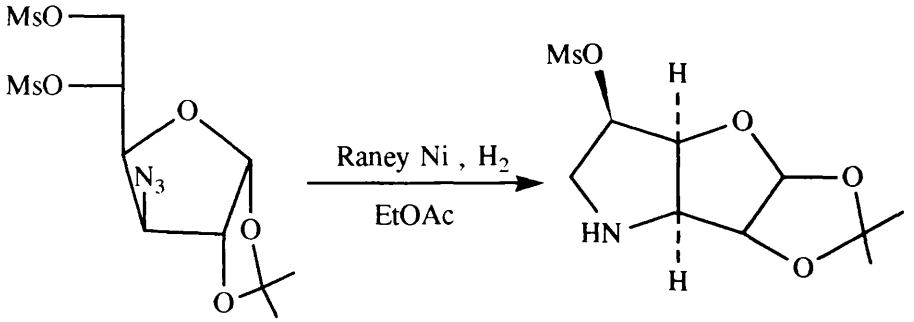
(107)

(108)

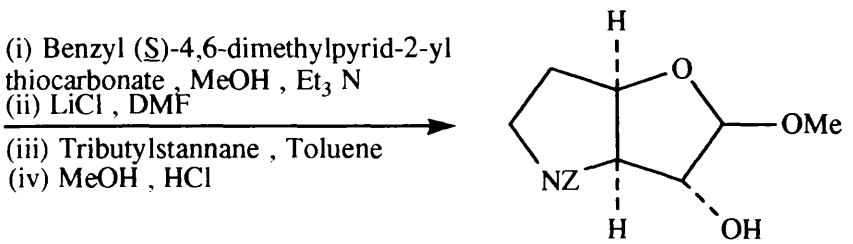


(33)

SCHEME 14

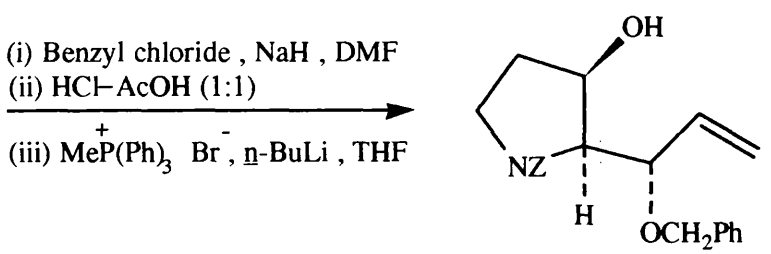


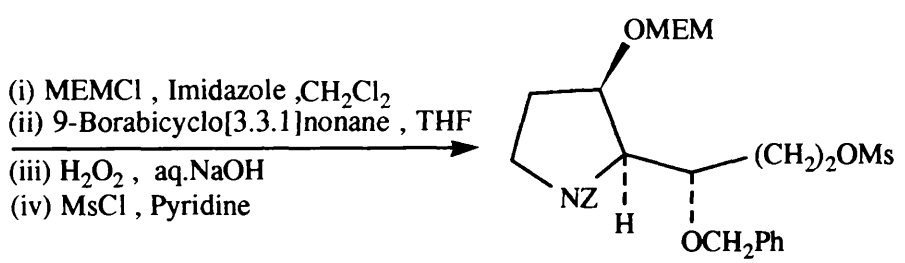
(110) (111)



$PhCH_2CO = Z$

(112)

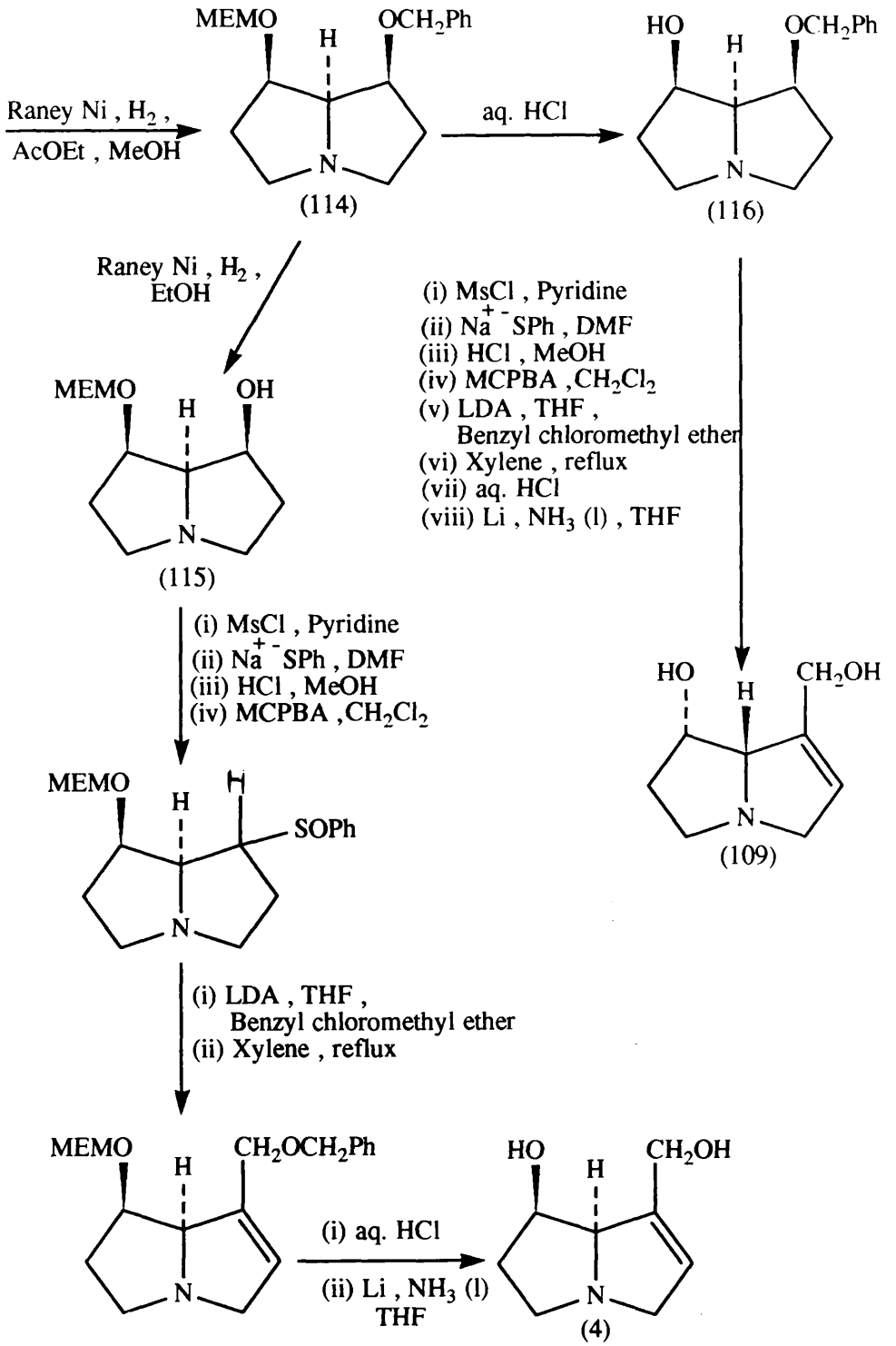




(113)

SCHEME 15

(contd. over)

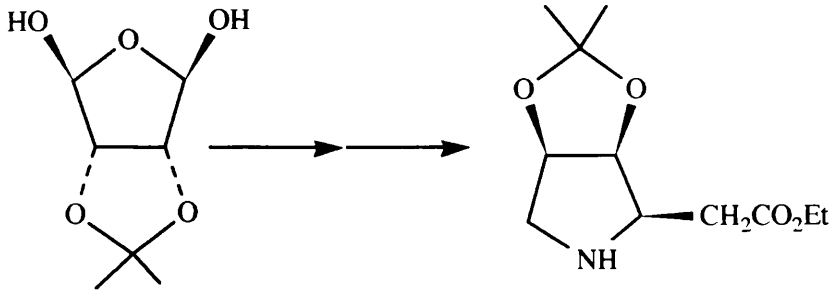

MEMO
H
OCH2Ph
Raney Ni , H2 ,
AcOEt , MeOH
N
(114)
aq. HCl
HO
H
OCH2Ph
N
(116)
Raney Ni , H2 ,
EtOH
MEMO
H
OH
N
(115)
(i) MsCl , Pyridine
(ii) Na+ -SPh , DMF
(iii) HCl , MeOH
(iv) MCPBA ,CH2Cl2
(v) LDA , THF ,
Benzyl chloromethyl ether
(vi) Xylene , reflux
(vii) aq. HCl
(viii) Li , NH3 (l) , THF
HO
H
CH2OH
N
(109)
(i) MsCl , Pyridine
(ii) Na+ -SPh , DMF
(iii) HCl , MeOH
(iv) MCPBA ,CH2Cl2
MEMO
H
H
SOPh
N
(i) LDA , THF ,
Benzyl chloromethyl ether
(ii) Xylene , reflux
MEMO
H
CH2OCH2Ph
N
(i) aq. HCl
(ii) Li , NH3 (l)
THF
HO
H
CH2OH
N
(4)

SCHEME 15

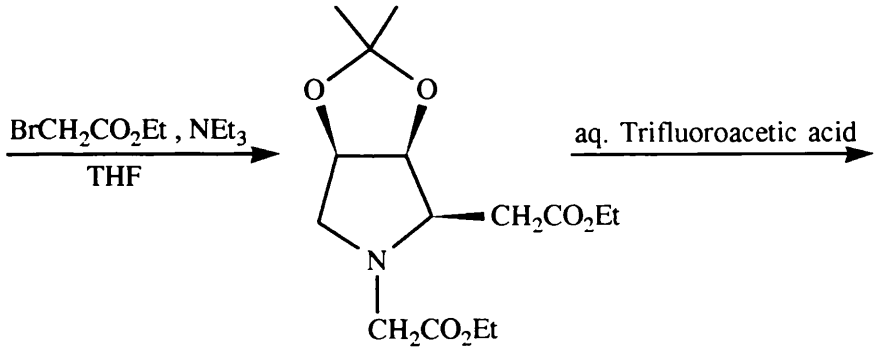
Buchanan et al. also employed a natural sugar derivative as starting material for synthesis of the Geissman-Weiss lactone (47) in optically active form. Starting from the 2,3-O-isopropylidene derivative of ribose (117), Buchanan et al. engineered an intricate scheme to (4), which incorporated the amino ester (118) as a key intermediate. In addition it was noted that (118) could be used to produce crotanecine in optically active form. This strategy, illustrated in Scheme 16, again involved the use of a Dieckmann cyclisation in the construction of the pyrrolizidine ring system.

Initially the amino ester (118) was alkylated, affording the diester (119). Acid hydrolysis of (119) gave the hydroxylactone. This was then silylated prior to Dieckmann cyclisation. Cyclisation was followed by borohydride reduction of the intermediate keto ester (120), and the product was then acylated to give a mixture of diastereomeric diacetates (121) in 40% overall yield from (118). Elimination of acetic acid from (121) generated the α,β-unsaturated ester (122). A reduction-deprotection sequence, employing di-isobutylaluminium hydride as reductant and fluoride anion as desilylating agent, was employed in an effort to convert (122) into (+)-crotanecine (10). However, isolation of crotanecine in good yield proved to be difficult, but Benn and Rüeger had employed (120), (121), and (123) in their synthetic route to (+)-crotanecine from the proline (62). Thus Buchanan et al. had provided an alternative route to (+)-crotanecine by using a natural sugar.

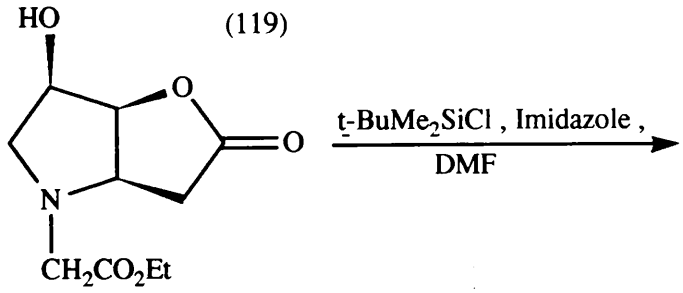
Glinski and Zalkow have reported an efficient route to (+)-heliotridine (70) from its C-7 epimer (+)-retronecine (4)[63] (Scheme 17). A regioselective coupling of (+)-retronecine with benzoic acid

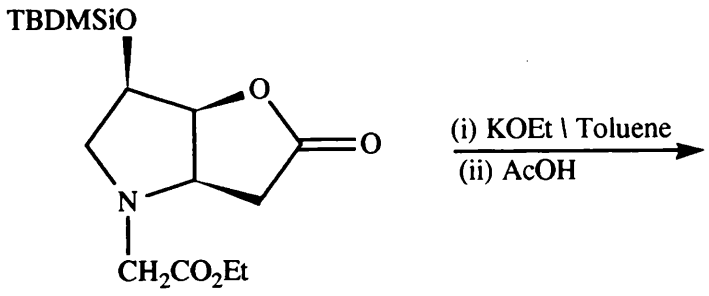


(117) (118)



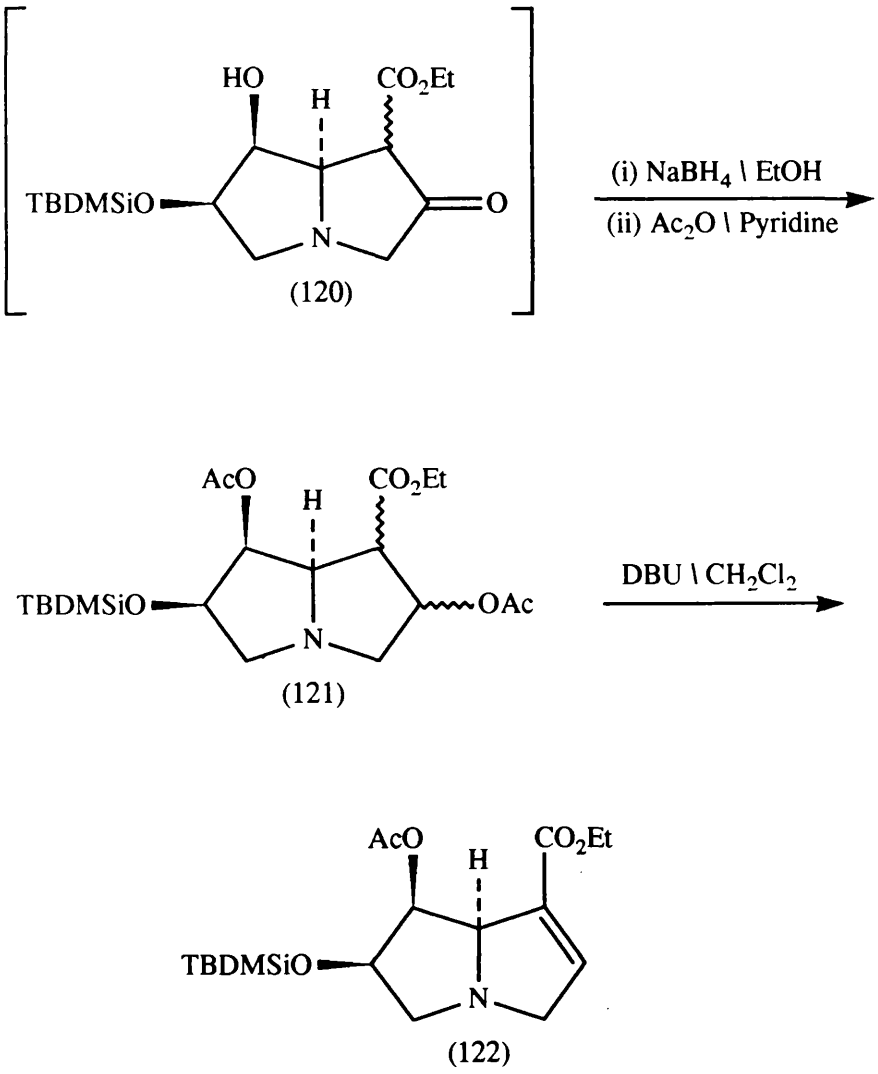
(119)



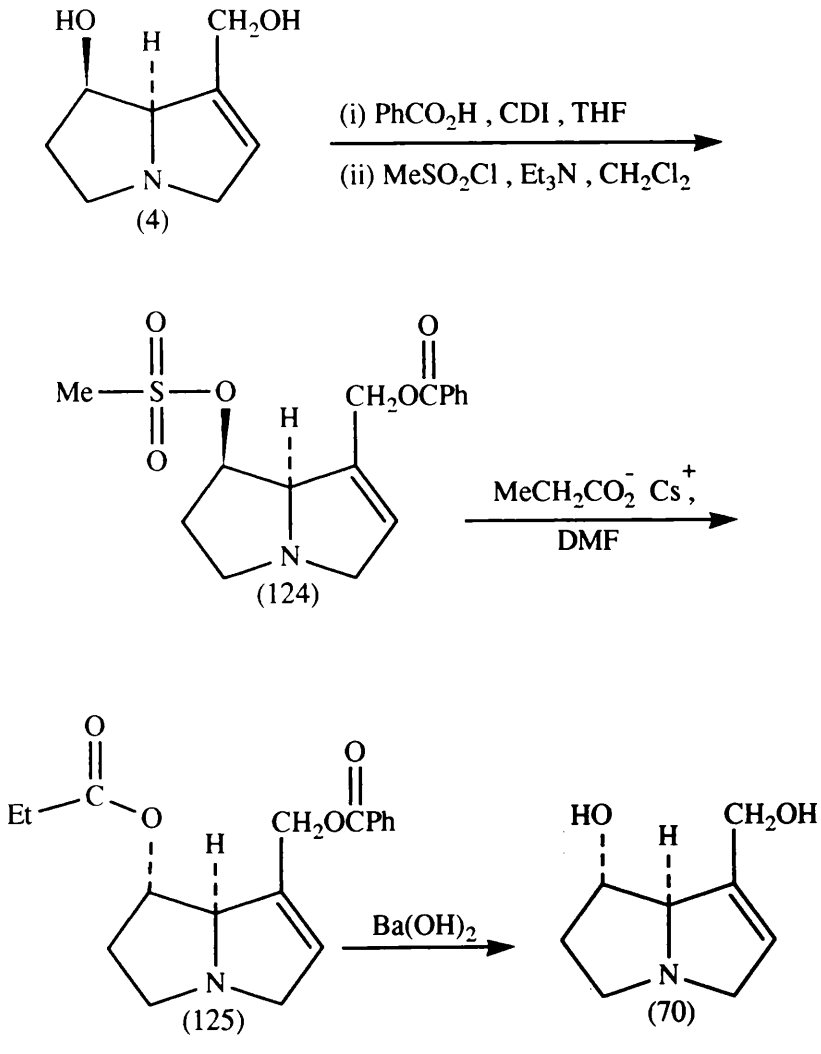


(123)

SCHEME 16
(contd. over)


HO
H
CO2Et
TBDMSiO
O
N
(120)
(i) NaBH4 \ EtOH
(ii) Ac2O \ Pyridine
AcO
H
CO2Et
TBDMSiO
OAc
N
(121)
DBU \ CH2Cl2
AcO
H
CO2Et
TBDMSiO
N
(122)

SCHEME 16

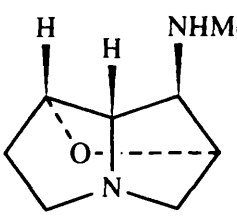

HO
H
CH2OH
N
(4)
(i) PhCO2H , CDI , THF
(ii) MeSO2Cl , Et3N , CH2Cl2
O
Me—S—O
O
O
CH2OCPh
H
N
(124)
MeCH2CO2- Cs+,
DMF
O
Et—C—O
O
CH2OCPh
H
N
(125)
Ba(OH)2
HO
H
CH2OH
N
(70)

SCHEME 17

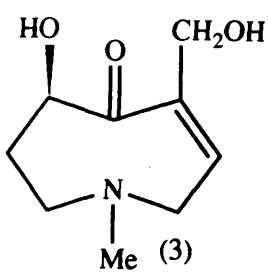
gave the C-9 benzyl alcohol which was then esterified to generate the key intermediate mesylate (124). The desired stereochemical inversion was achieved through nucleophilic displacement of the mesylate using caesium propionate producing (125). Base hydrolysis of (125) yielded (+)-heliotridine (70).

Synthesis of the pyrrolizidine bases has advanced significantly over the past decade with the emergence of routes to several necines, in optically active form, being of particular encouragement. However most of the routes leading to optically active necines are simply too lengthy and often too inflexible to be adopted as practical schemes to generate a range of necines in optically active form. There is still an obvious requirement for improved, shorter routes to the more complex necines.

In addition otonecine (3), a base portion common to several pyrrolizidine macrocyclic diesters, has yet to be produced in optically active form. Another synthetic challenge of the necines is the production of loline (12) and the related necines in optically active form.



(12)



(3)

CHAPTER 3

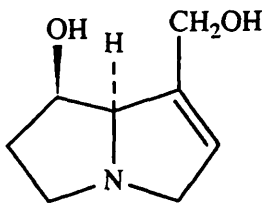
THE BIOSYNTHESIS OF PYRROLIZIDINE ALKALOIDS

3.1 Introduction

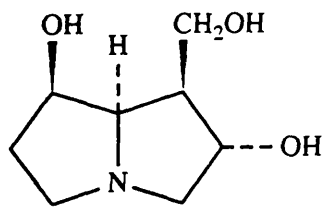
Considerable resources and much effort has been devoted to the study of natural products, including the pyrrolizidine alkaloids. These alkaloids are usually comprised of two units - a necic acid and a pyrrolizidine base. These moieties are derived from different biosynthetic precursors by completely independent biosynthetic pathways. Despite significant contributions which have enhanced our understanding of the biosynthesis of secondary metabolites, particularly over the last decade, the pyrrolizidine alkaloids, in common with most groups of natural products have many aspects of their biosynthesis still to be fully unravelled.

3.2 The Biosynthesis of Pyrrolizidine Bases

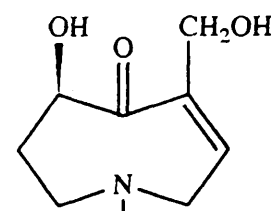
Nearly all the work in this area[64] has been concerned with retronecine (4), the most common base portion of the pyrrolizidine alkaloids. Recently two other pyrrolizidine bases have undergone biosynthetic investigation namely, rosmarinecine (21) and otonecine (3).



(4)



(21)

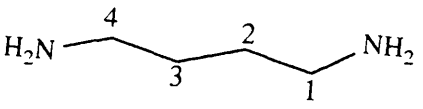


(3)

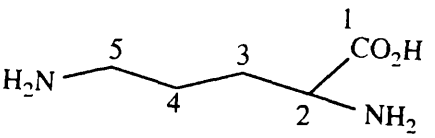
Early experiments involved the use of radiolablled compounds as tracers to establish biosynthetic precursors of retronecine. Initially ^{14}C-labelling was employed but later studies utilised ^{3}H-labelling, often in conjunction with a ^{14}C-label. ^{13}C N.m.r. spectroscopy proved important in confirming the biosynthetic pathway, while stereochemical aspects of the process have recently been elucidated by the use of ^{2}H n.m.r. spectroscopy.

In 1955 Sir Robert Robinson proposed that the biosynthesis of pyrrolizidine alkaloids involved two C_4 units related to ornithine (125).[65] This postulate was supported, in 1962, when Nowacki and Byerrum[66] reported an experiment involving the feeding of [2-^{14}C]ornithine to Crotalaria spectabilis, a plant known to produce monocrotaline (8). After isolation of the alkaloids, radiolabelled monocrotaline was obtained which yielded retronecine (4) and monocrotalic acid (126) on base hydrolysis. Nowacki and Byerrum observed that almost all the activity was associated with the necine portion. Similar experiments involving the feeding of [1-^{14}C]acetate and [1-^{14}C]propionate resulted in most of the label appearing in the acid moiety, monocrotalic acid (126). This demonstrated that neither acetate nor propionate are specific precursors for retronecine.

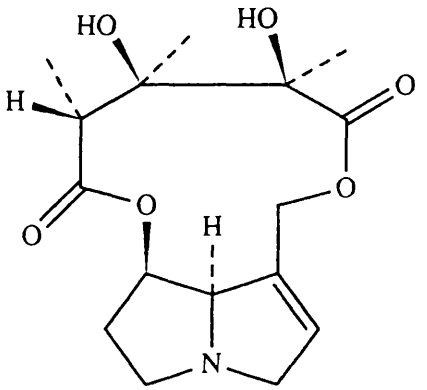
Soon afterwards Bottomley and Geissman[67] confirmed ornithine as a precursor as well as demonstrating that putrescine (20) is also a specific precursor for retronecine. Feeding of [2-^{14}C]- and [5-^{14}C]-ornithine together with [1,4-^{14}C]putrescine to Senecio douglasii, a plant producing several pyrrolizidine alkaloids all with retronecine base portions, again resulted in almost all the activity being confined to the necine portion (94-98%).



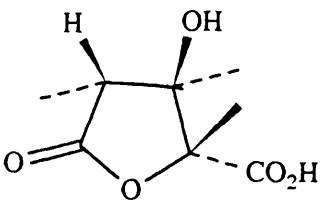
(20)



(125)



(8)

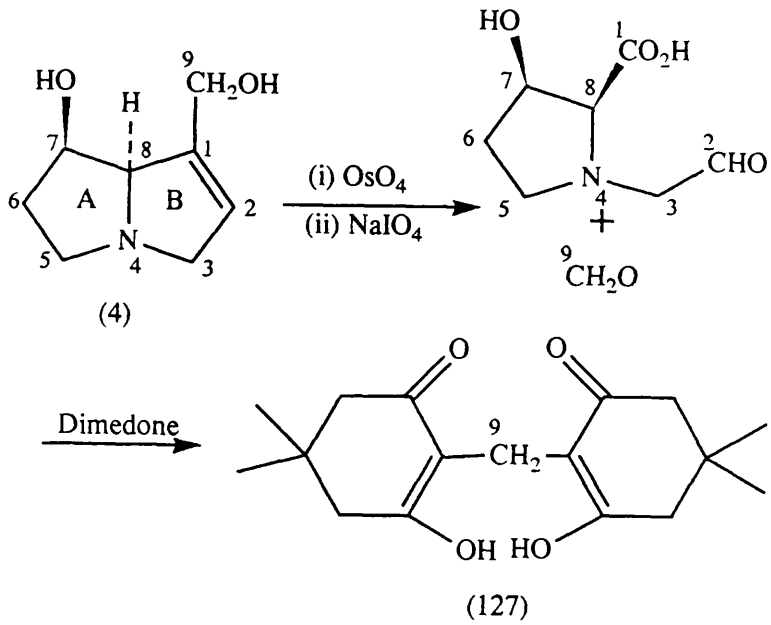


(126)

Bottomley and Geissman extended this investigation by treating the radiolabelled retronecine with osmium tetroxide and sodium periodate to liberate the C-9 atom of retronecine (4) as formaldehyde. The formaldehyde produced was then trapped as the dimedone derivative (127) (Scheme 18).

It was found that retronecine derived from all three experiments had approximately one quarter of the activity associated with C-9. This suggested that during the biosynthetic process C-2 and C-5 of ornithine became equivalent, at least during the formation of ring B of retronecine.

Bale and Crout later employed $^{14}C \rightarrow {}^{3}H$ double labelling to demonstrate that arginine (128) is also a specific precursor of retronecine.[68] These experiments with Senecio magnificus plants also



SCHEME 18

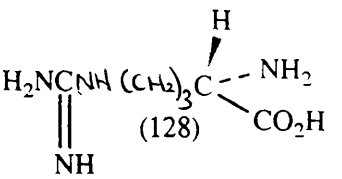
revealed that ornithine is a slightly more efficient precursor for retronecine than arginine.

Similar double labelling experiments were reported by Robins and Sweeney in investigations involving Senecio isatideus.[69] The C_4 portions of both spermine (129) and spermidine (130) were shown to be specific precursors of retronecine whereas L-glutamic acid (131), 4-amino-butanoic acid (132), L-proline (133), and the C_3 component of spermidine were incorporated into both the acid and base components of retrorsine (9), namely retronecine (4) and isatinecic acid (134) [Table 1].

Table 1: Summary of Feeding Experiments carried out by Robins and Sweeney[69] with S. isatideus

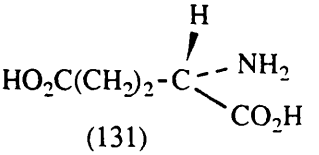
Experiment	Precursor	% Incorporation	% ^{14}C Radioactivity in (4)	% ^{14}C Radioactivity in (134)
1	L-[U-^{14}C]Proline	0.04	68	32
2	L-[U-^{14}C]Glutamic acid	0.12	48	58
3	L-[U-^{14}C]Arginine	0.46	99	6
4	DL-[2-^{14}C]Ornithine	0.29	96	3
5	DL-[5-^{14}C]Ornithine	0.25	97	5
6	[1,4-^{14}C]Putrescine	1.6	94	1
7	[2,3-^{14}C]Putrescine	0.7	95	2
8	NN'-Bis(3-aminopropyl)-1,4-diamino-[1,4-^{14}C]butane(spermidine)	5.2	103	1
9	N-(3-Aminopropyl)-1,4-diamino-[1,4-^{14}C]butane(spermine)	2.0	95	1
10	N-(3-Amino-[3-^{14}C]propyl)-1,4-diaminobutane	0.02	41	57

(129)

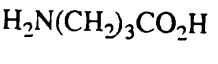


(128)

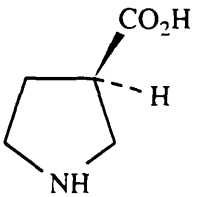
(130)



(131)



(132)



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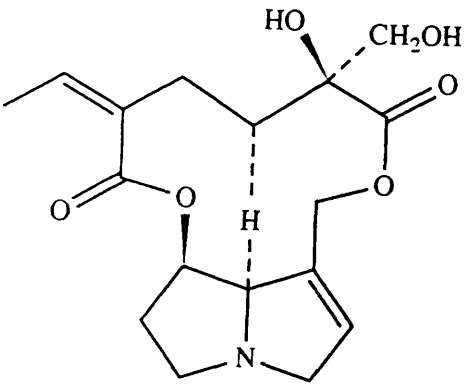
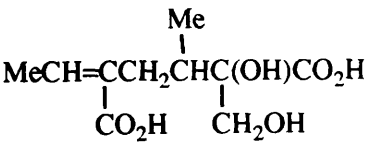


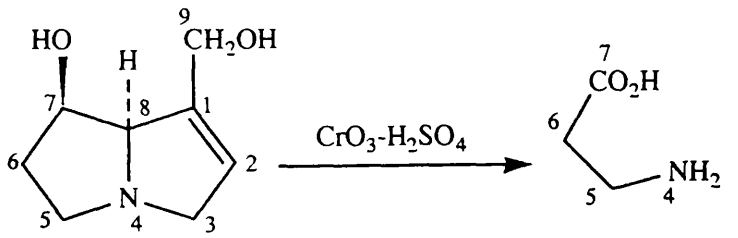
(9)



(134)

Ornithine was found to be the most efficient precursor, a result in accordance with the theory of Geissman and Crout who suggested that putrescine followed ornithine in the biosynthetic pathway.[70]

Robins and Sweeney also successfully employed a modified Kuhn-Roth oxidation[71] to locate partially labelling in ring A of retronecine (Scheme 19). This procedure yielded C(5+6+7) as β-alanine (135), isolated as its 2,4-dinitrophenyl derivative. When this degradation was applied to experiments 4-6 and 8-10 it was shown that approximately one quarter of the activity was associated with the C(5+6+7) fragment. When [2,3-^{14}C]putrescine (expt.7) was administered as precursor, the

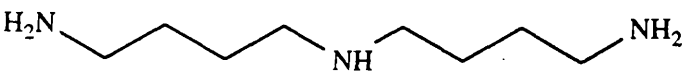


(4) (135)

SCHEME 19

biosynthetically produced retronecine contained 47% of the necine activity in the C(5+6+7) segment of retronecine.

These experiments suggested that the activity was evenly distributed between the two 'rings' of the pyrrolizidine system. Robins and Sweeney accounted for this by proposing that a later symmetrical intermediate, such as homospermidine (136), was involved in retronecine biosynthesis.



(136)

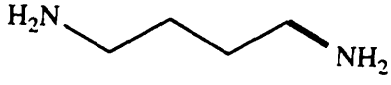
Further evidence for the existence for a symmetrical intermediate was provided by the feeding of ^{13}C-labelled putrescines to S. isatideus.[72] Khan and Robins prepared [1,4-^{13}C]- and [2,3-^{13}C]putrescine [(137) and (138)] which were administered as their dihydrochlorides. Base hydrolysis of the retrorsine isolated afforded ^{13}C-labelled retronecine which, in the case of the [1,4-^{13}C]putrescine feed, gave four equally

enhanced signals in the $^{13}C-\{^{1}H\}$ n.m.r. spectrum corresponding to C-3, C-5, C-8, and C-9 of retronecine (Scheme 20a). The $^{13}C-\{^{1}H\}$ n.m.r. spectrum for the [2,3-^{13}C]putrescine feed exhibited a complementary labelling pattern (Scheme 20b). Two pairs of doublets were observed, corresponding to C-1/C-2 and C-6/C-7, with the four enriched sites displaying nearly equal enhancement factors.

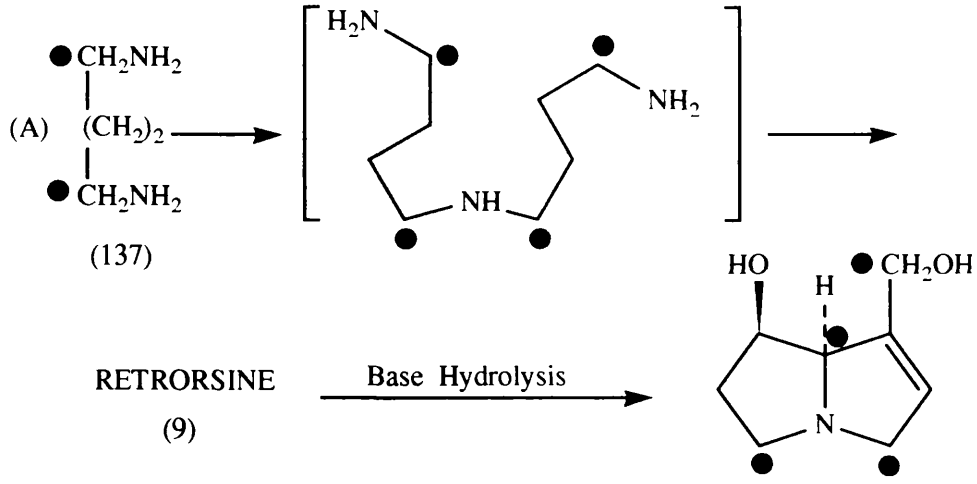
Robins[73] later reported feeding [1,2-$^{13}C_2$]putrescine (139) dihydrochloride to S. isatideus which gave rise to the expected $^{13}C-\{^{1}H\}$ n.m.r. pattern for the biosynthetically produced retronecine (Scheme 20c). The spectrum consisted of eight peaks, each flanked by a doublet, and four sets of $^{13}C-^{13}C$ couplings.

These experiments were again indicative of the existence of a later symmetrical intermediate in the biosynthetic pathway.

The first experimental evidence for a symmetrical C_4-N-C_4 intermediate was provided independently by Grue-Sørensen and Spenser,[74] and by Khan and Robins.[75] This was achieved[74] through the feeding of [1-amino-^{15}N, 1-^{13}C]putrescine (140) dihydrochloride to S. vulgaris, a plant known to produce several pyrrolizidine alkaloids all with a retronecine backbone.

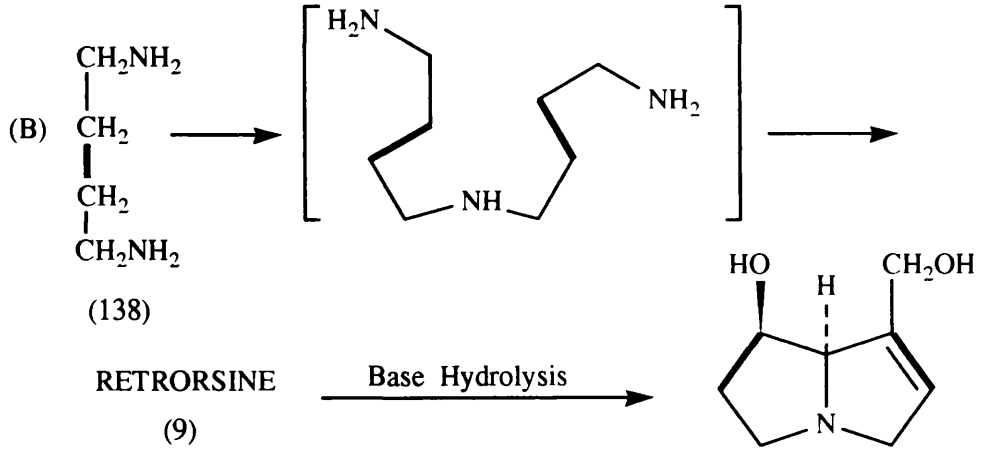


(140)



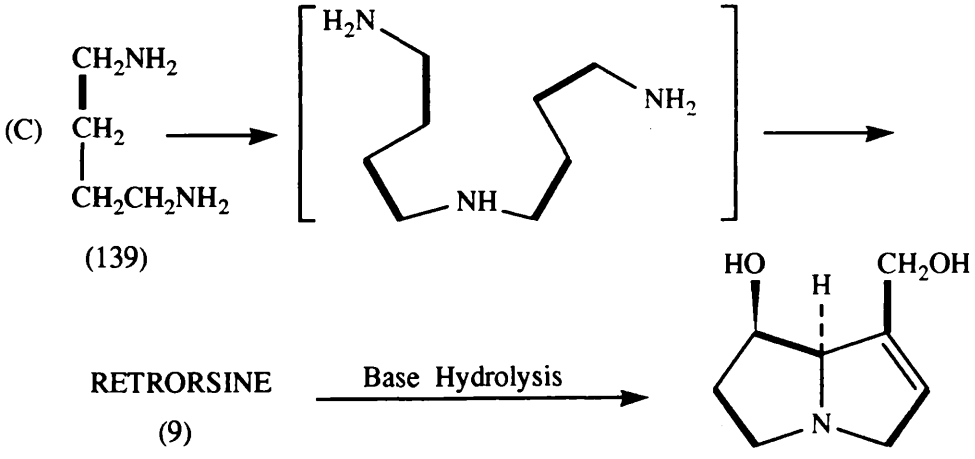
(137)

RETRORSINE (9) — Base Hydrolysis



(138)

RETRORSINE (9) — Base Hydrolysis



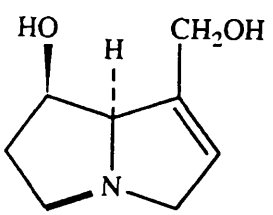
(139)

RETRORSINE (9) — Base Hydrolysis

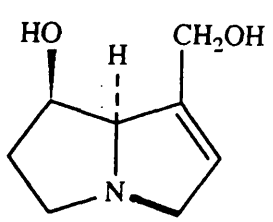
SCHEME 20

Grue-Sørensen and Spenser had noted that the labelling patterns obtained in retronecine from the feeding of [1-^{14}C]putrescine and [5-^{14}C]-ornithine could be explained by two conflicting pathways[76] (Scheme 21). By employing [1-amino-^{15}N, 1-^{13}C]putrescine as a precursor Grue-Sørensen and Spenser believed that this ambiguity could be eradicated as ^{13}C n.m.r. spectroscopy would be able to distinguish which was the relevant pathway in retronecine biosynthesis.

If retronecine was produced via a non-symmetrical intermediate (route B) then feeding of [1-amino-^{15}N, 1-^{13}C]putrescine would be expected to produce a single species of intramolecularly ^{13}C, ^{15}N-labelled retronecine (141). However, if retronecine biosynthesis proceeded via a symmetrical intermediate (route A) then an equimolar mixture of ^{13}C, ^{15}N-labelled products, (141) and (142) would be expected.

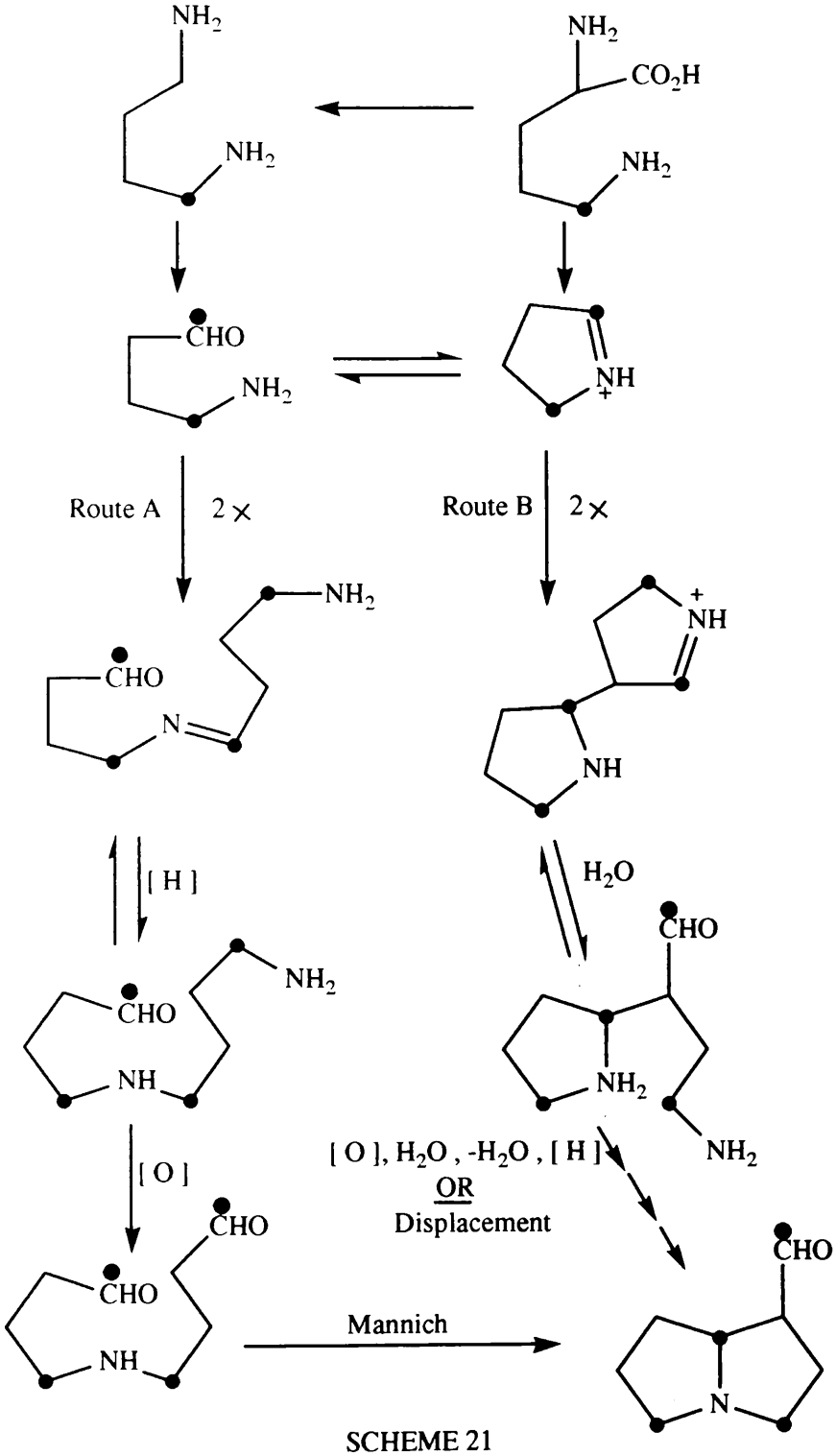


(141)



(142)

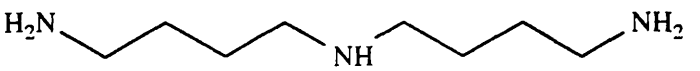
Analysis of the ^{13}C-{^{1}H} n.m.r. spectrum of the labelled retronecine revealed, as expected, four enhanced signals corresponding to C-3, C-5, C-8, and C-9. Careful examination of the difference spectrum (^{13}C-labelled - natural abundance), indicated that the signals at C-3 and C-5 were comprised of a doublet superimposed on a singlet. The doublets, which were of equal intensity, are characteristic of ^{13}C-^{15}N species and the singlets arise from ^{13}C-^{14}N species. Thus the observed


NH2
CO2H
NH2
NH2
CHO
NH2
NH
Route A
2 ×
Route B
2 ×
NH2
CHO
N
NH
NH
[H]
H2O
NH2
CHO
NH
CHO
NH2
NH2
[O], H2O , -H2O , [H]
OR
Displacement
[O]
CHO
CHO
NH
Mannich
CHO
N

SCHEME 21

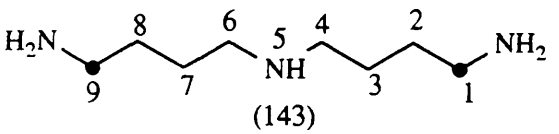
spectrum indicated the existence of a later symmetrical intermediate in retronecine biosynthesis.

Khan and Robins reported analogous findings to Grue-Sørensen and Spenser through feeding [1-amino-^{15}N, 1-^{13}C]putrescine (140) dihydrochloride to S. isatideus.[75] Of greater significance was their identification of the symmetrical C_4-N-C_4 intermediate as 1,6,11-triazundecane (homospermidine) (136). Feeding of [1,9-^{14}C]homospermidine (143) trihydrochloride generated specifically labelled retrorsine (9). Alkaline hydrolysis yielded ^{14}C-labelled retronecine which when degraded was found to have 44% of the radiolabel associated with C-9 of retronecine and only 2% pertaining to the C(5+6+7) fragment, again isolated as the 2,4-dinitrophenyl derivative of β-alanine. These labelling patterns were once again consistent with the intact incorporation of homospermidine into retronecine.

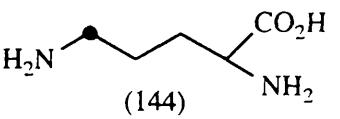


(136)

In addition homospermidine was shown to be produced by S. isatideus by means of an intermediate trapping experiment. DL-[5-^{14}C]-ornithine (144) was administered to S. isatideus and after one day the basic material was extracted into aqueous trichloroacetic acid containing unlabelled homospermidine trihydrochloride. Subsequent formation of the N-phenylthiourea derivative of homospermidine, then purification, yielded the desired derivative in radioactive form. This indicated that homospermidine is probably an intermediate in retronecine biosynthesis.



(143)

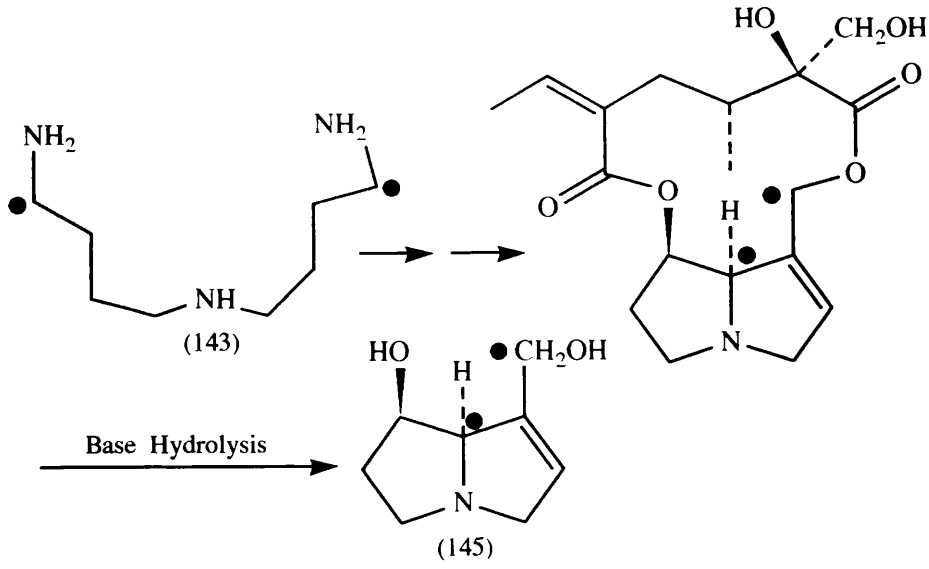


(144)

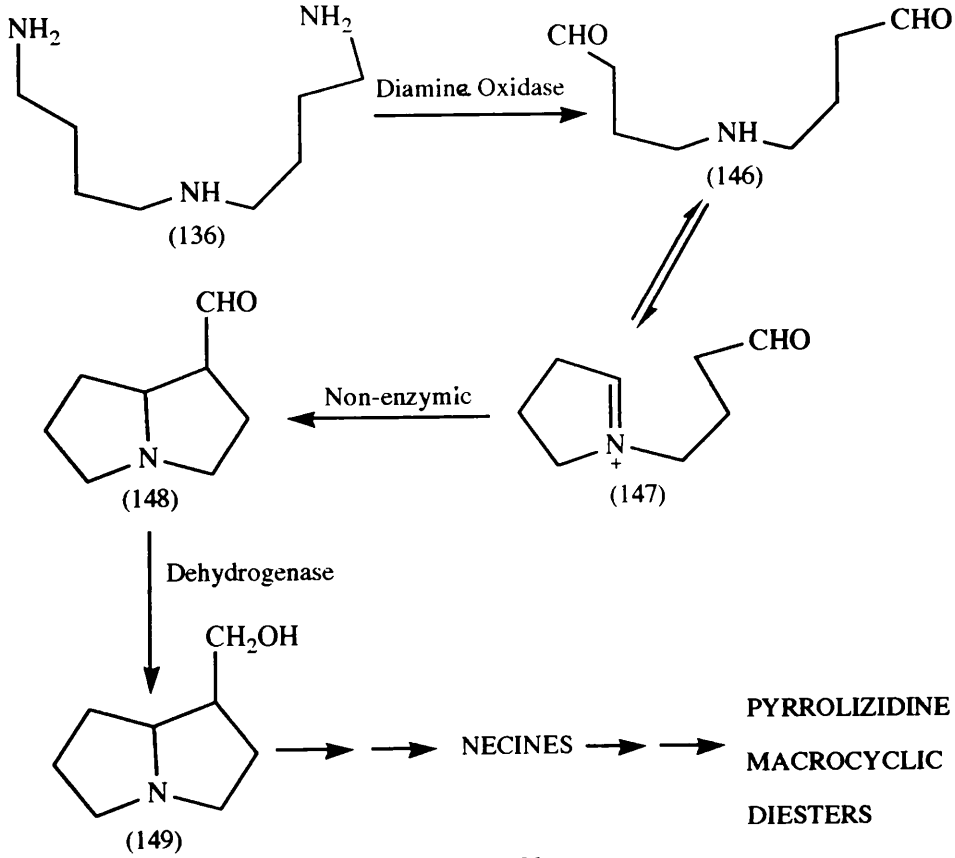
Further evidence emerged when Rana and Robins fed [1,9-^{13}C]-homospermidine (143) trihydrochloride to S. isatideus.[77] The ^{13}C-{^{1}H} n.m.r. spectrum of the retronecine generated (145) was found to possess two enhanced signals corresponding to C-8 and C-9 (Scheme 22). Moreover a geminal coupling between C-8 and C-9 was observed (6 Hz), providing powerful evidence for the intact conversion of homospermidine into retronecine.

With strong evidence linking homospermidine as a key intermediate in pyrrolizidine alkaloid biosynthesis, emphasis now shifted to detailing how homospermidine could be converted into the pyrrolizidine nucleus (Scheme 23). It was possible that homospermidine could be oxidised by diamine oxidase enzymes to afford the dialdehyde (146), thought by Geissman and Crout to be involved in retronecine biosynthesis. The dialdehyde exists in equilibrium with its iminium salt (147), which could undergo cyclisation to afford the pyrrolizidine ring system. Reduction of the 1-formylpyrrolizidine generated (148) could provide 1-hydroxymethylpyrrolizidine (149) which could, in turn, be transformed to yield the necine bases.

The proposed pathway gained more credence when Robins demonstrated that homospermidine could be converted into (±)-trachelanthamidine (26) by the action of diamine oxidase then dehydrogenase



SCHEME 22



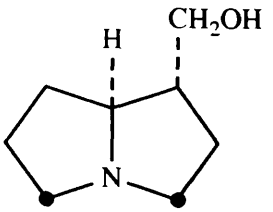
SCHEME 23

enzymes under physiological conditions (Scheme 23).[78]

Further support materialised when Rana and Robins[79] fed (±)-[3,5-^{14}C]trachelanthamidine (150) and (±)-[5-^{3}H]isoretronecanol (151) to Senecio riddelli, a plant known to produce riddelline (152). Degradation studies revealed that both trachelanthamidine and isoretronecanol are incorporated specifically into the retronecine portion of riddelline, with the former being the more efficient precursor.

Robins and Kunec[80] carried out a similar investigation feeding (±)-[5-^{3}H]trachelanthamidine and (±)-[5-^{3}H]isoretronecanol to S. isatideus and S. pleistocephalus [the latter is known to produce rosmarinine (153)]. This study revealed that isoretronecanol is incorporated considerably more efficiently into rosmarinecine (21), the base portion of rosmarinine. Indeed, isoretronecanol was incorporated approximately five times more efficiently than putrescine into rosmarinecine. Feeding of these radiolabelled precursors to S. isatideus provided the opposite result, with trachelanthamidine being more readily incorporated into the base portion of retrorsine, retronecine. These results suggested that 1-hydroxymethylpyrrolizidines are later intermediates in necine biosynthesis, and shows that the pathways to retronecine and rosmarinecine diverge, probably during cyclisation to form the pyrrolizidine system.

Further evidence for these pathways was recently provided when Kelly and Robins[81] demonstrated that the iminium ion (154) is involved in necine biosynthesis. The iminium salt was prepared in radiolabelled form (^{14}C) and administered to both S. isatideus and S. pleistocephalus and found to be a more efficient precursor than putrescine for the respective base portions, retronecine and rosmarinecine. In addition an intermediate trapping experiment verified the presence of the iminium ion in S. pleistocephalus.



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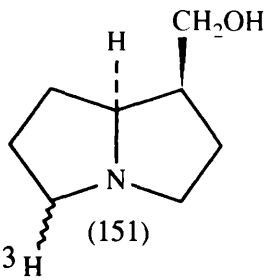

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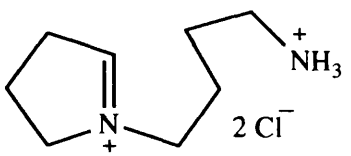

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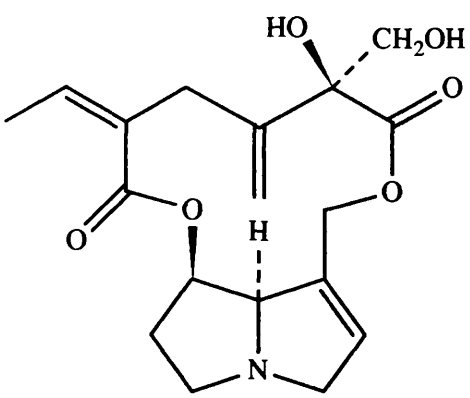

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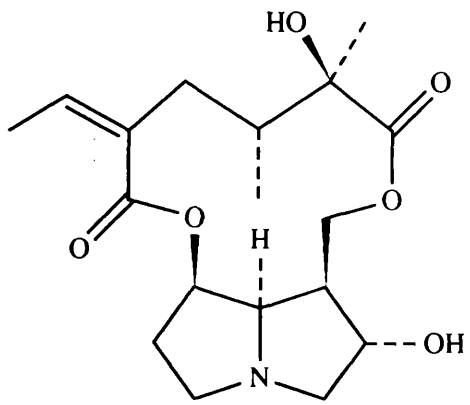
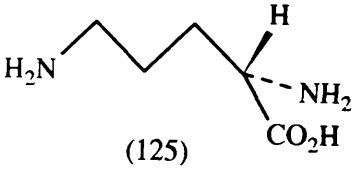


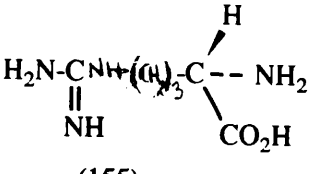
(153)

The efficient incorporation of this iminium ion also suggested that the oxidation of the amino groups of homospermidine proceeds in two discrete steps. A summary of the biosynthetic pathways to retronecine and rosmarinecine is illustrated in Scheme 24.

Stereochemical aspects of retronecine biosynthesis were first reported in 1983 by Robins and Sweeney.[82] A detailed study employing $^{14}C/^{3}H$ labelling established that retronecine is derived from the L-enantiomers of arginine (155) or ornithine (125), with little contribution from the opposite enantiomer forms.



(125)



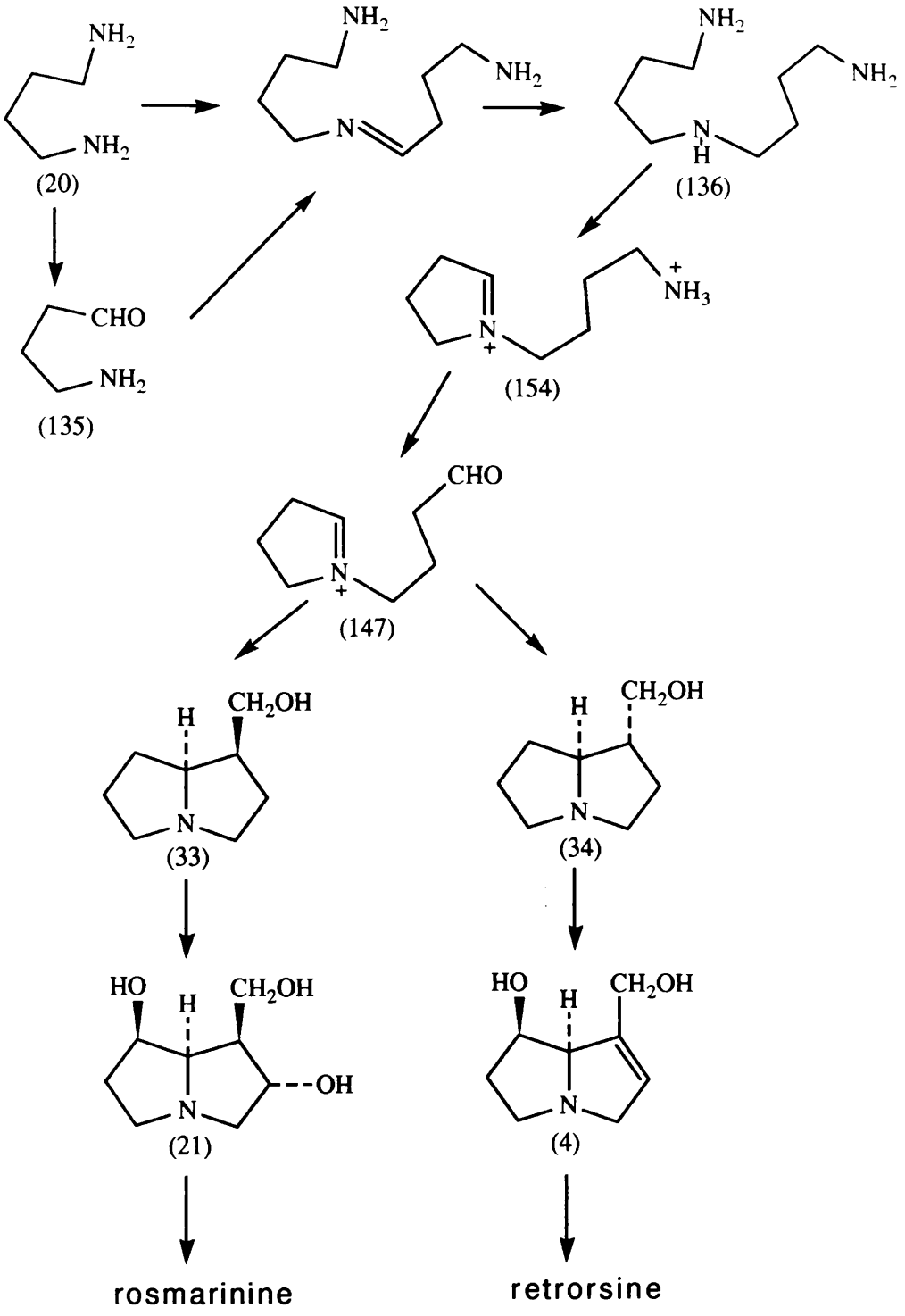
(155)

Rana and Robins[83] amassed considerable stereochemical information about retronecine biosynthesis through the feeding of ^{2}H-labelled putrescines in optically active form. (R)-[1-^{2}H]- and (S)-[1-^{2}H]-putrescines [(156) and (157)] were prepared by an established procedure and fed as their dihydrochlorides to S. isatideus. Analysis of the biosynthetically produced retrorsine, from the (R)-enantiomer feed, by ^{2}H-{^{1}H} n.m.r. spectroscopy, established that deuterium labels were located at C-3β, C-5α, C-8α, and C-9 pro-S of retrorsine (158). This labelling pattern was interpreted in the following manner (Scheme 25).

Initially enzymic oxidation of putrescine to 4-aminobutanal (159) occurs. Diamine oxidases are known to remove specifically the pro-S hydrogen atom of a methylene group adjacent to an amine group undergoing oxidation.[84] Thus for the (R)-enantiomer the deuterium label is retained. Condensation of 4-aminobutanal with a further putrescine molecule generates an imine (160) which undergoes a stereospecific reduction, with hydride being delivered from the C-si face of the imine. This produces homospermidine (161). Further amine oxidation takes place with retention of the pro-R hydrogen and loss of the pro-S to yield the labelled aldehyde (162). This aldehyde is converted into the iminium ion (163) which then undergoes intramolecular addition on the C-re face to generate the 8α-formylpyrrolizidine (164). Finally, a stereospecific reduction of the aldehyde by delivery of the hydride donor on the C-re face of the carbonyl group accounts for the labelling pattern witnessed in retrorsine (158).

Feeding of the opposite enantiomer, (S)-[1-^{2}H]putrescine dihydrochloride, gave a ^{2}H-{^{1}H} n.m.r. spectrum of retrorsine displaying deuterium signals at C-3α and C-5β positions (Scheme 26). Two fewer labels are present because of the removal of the pro-S hydrogen atoms during the oxidation of the primary amino groups.

^{2}H-{^{1}H} N.m.r. spectroscopy was again used to good effect in further stereochemical studies of retronecine biosynthesis.[85] Kunec and Robins fed (R)-[2-^{2}H]- and (S)-[2-^{2}H]putrescine [(166) and (167)] dihydrochlorides to S. isatideus and established that oxygenation of the pyrrolizidine system at C-7 does not proceed via a keto or enol intermediate because the pro-R hydrogen is retained. This also shows that the hydroxylation proceeds with retention of configuration. The 1,2-unsaturation of retronecine arises from the removal of the pro-S hydrogen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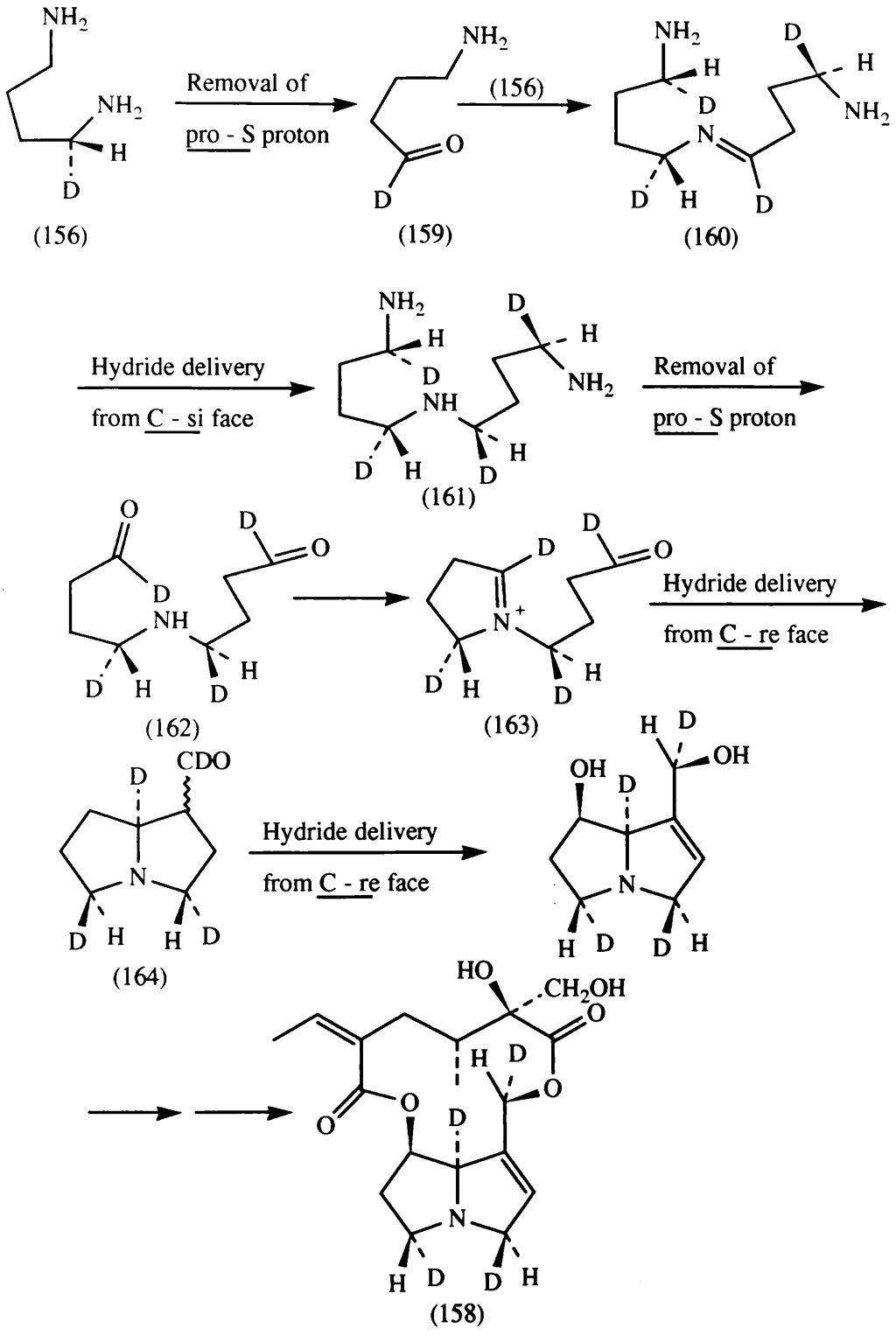
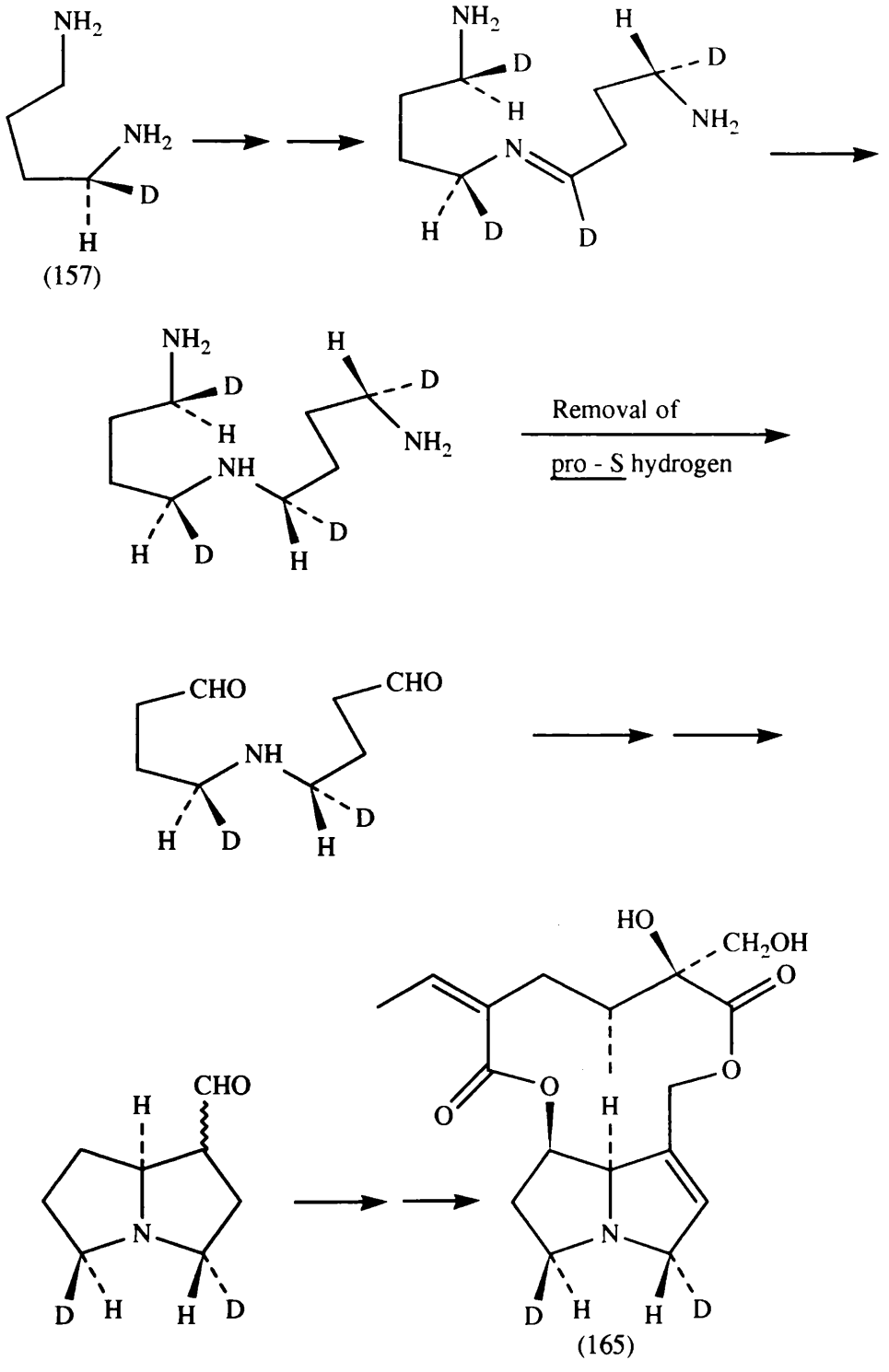
NH2
(20)
NH2
N
NH2
NH2
N
H
(136)
CHO
NH2
(135)
+
NH3
N+
(154)
CHO
N+
(147)
H
CH2OH
N
(33)
H
CH2OH
N
(34)
HO
H
CH2OH
OH
N
(21)
HO
H
CH2OH
N
(4)
rosmarinine
retrorsine

SCHEME 24


Removal of
pro - S proton
(156)
(159)
(160)
Hydride delivery
from C - si face
(161)
Removal of
pro - S proton
(162)
(163)
Hydride delivery
from C - re face
Hydride delivery
from C - re face
(164)
(158)

SCHEME 25


NH2
NH2
D
H
(157)
NH2
D
H
H
D
N
H
D
D
H
D
NH2
NH2
D
H
NH
H
D
H
D
H
D
NH2
Removal of
pro - S hydrogen
CHO
CHO
NH
H
D
D
H
HO
CH2OH
O
O
O
O
H
CHO
H
N
D
H
H
D
N
D
H
H
D
(165)

SCHEME 26

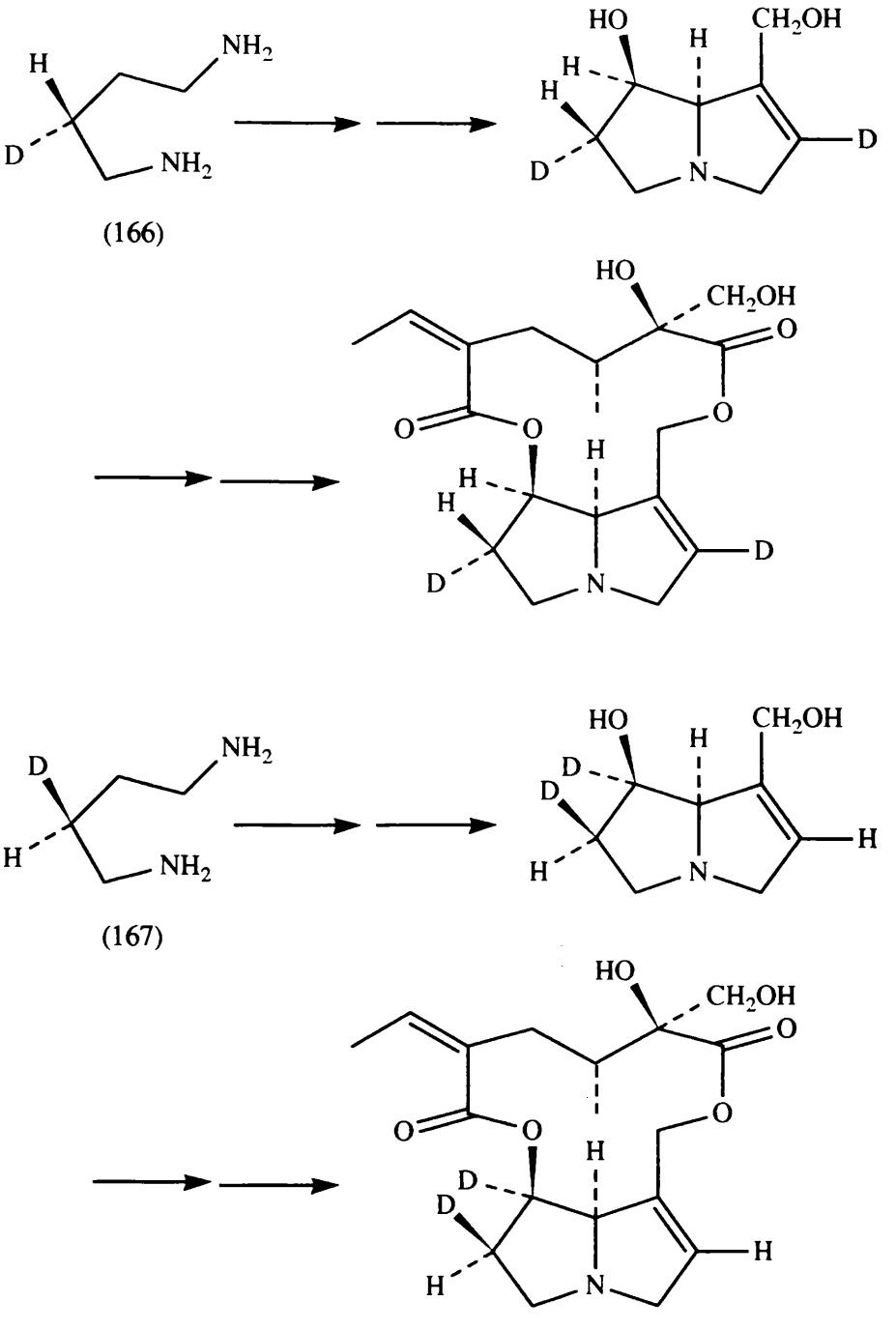
atom at the carbon which becomes C-2 of retronecine. The pro-R hydrogen at this centre is retained during this process (Scheme 27).

Recently Kelly and Robins have provided additional depth to our understanding of necine biosynthesis by implementing a comprehensive study into the biosynthesis of rosmarinecine through the feeding of labelled precursors to Senecio pleistocephalus.

Earlier experiments by Kunec and Robins had demonstrated that (±)-isoretronecanol (25) is a much better precursor for rosmarinecine (21) than its C-1 epimer (±)-trachelanthamidine (26), whereas the converse was identified for retronecine (4) biosynthesis. Consequently Kelly and Robins centred their initial investigations on the early stages of rosmarinecine biosynthesis to ascertain if there is a parallel with the pathway involved in the biosynthesis of retronecine.[86]

Feeding of ^{13}C-labelled putrescine dihydrochlorides to S. pleistocephalus, and subsequent analysis of the biosynthetically produced rosmarinecine by ^{13}C n.m.r. spectroscopy, established that rosmarinecine, like retronecine, is derived biosynthetically from two molecules of putrescine. The observed labelling patterns were similar to those encountered in analogous experiments involving retronecine biosynthesis, although incorporations were much higher. This indicated that a symmetrical C_4-N-C_4 intermediate might also be involved in rosmarinecine biosynthesis.

Administration of [1-amino-^{15}N, 1-^{13}C]putrescine (140) dihydrochloride to S. pleistocephalus yielded a labelling pattern in agreement with the conversion of two molecules of putrescine into a symmetrical C_4-N-C_4 intermediate, such as homospermidine (136). Confirmation of the involve-



(166)

(167)

SCHEME 27

ment of homospermidine in the biosynthesis of rosmarinecine was provided through the feeding of [1,9-^{13}C]homospermidine (143) trihydrochloride to S. pleistocephalus. The ^{13}C-{^{1}H} n.m.r. spectrum of the biosynthetically produced rosmarinine contained two enriched signals corresponding to C-8 and C-9 of rosmarinine. This demonstrated the intact conversion of homospermidine into rosmarinecine and confirmed that the early steps of rosmarinecine biosynthesis are similar to those documented for retronecine biosynthesis.

Having demonstrated the uniformity of the early stages of retronecine and rosmarinecine biosynthesis, Kelly and Robins then changed the emphasis of their investigation in an effort to establish the stereochemical features of rosmarinecine biosynthesis.[87] This was prompted by the divergence of the respective biosynthetic pathways at the 1-hydroxymethylpyrrolizidine stage (Scheme 24) and by the absence of a 1,2-double bond in rosmarinecine. The lack of this unsaturation would enable the stereochemistry of the hydroxylation at C-2 of rosmarinecine to be investigated as well as providing a valuable insight into the stereochemistry of pyrrolizidine ring formation by addressing the fate of the protons destined to become C-1 of rosmarinecine. These stereochemical aspects were explored through the feeding of ^{2}H-labelled precursors to S. pleistocephalus plants.

Kelly and Robins commenced work in this area by feeding [1,1,4,4-^{2}H]putrescine (168) dihydrochloride to S. pleistocephalus. The ^{2}H-{^{1}H} n.m.r. spectrum of the isolated rosmarinecine contained three main signals corresponding to deuterium labelling at C-3β, C-3α, and C-9 pro-S of rosmarinecine. A similar experiment, employing [1,1-^{2}H]-putrescine (169) dihydrochloride as precursor gave rise to rosmarinine

again labelled at C-3β, C-3α, and C-9 pro-S, but with further deuterium labelling present at C-5α, C-5β, and C-8 of rosmarinine.

Rana and Robins had previously encountered an analogous labelling pattern in retrorsine, produced from administering these ^{2}H-labelled putrescines to S. isatideus.[84] They explained this distribution by invoking the involvement of deuterium isotope effects during the biosynthesis of retronecine. This explanation is outlined for [1,1,4,4-^{2}H]-putrescine, and is illustrated in Scheme 28.

Conversion of [1,1,4,4-^{2}H]putrescine (168) into [1,4,4-^{2}H]-4-aminobutanal (170), catalysed by diamine oxidase enzymes, is known to be subject to an intermediate deuterium isotope effect of 1.26.[88] If a similar appreciable deuterium isotope effect operates with diamine oxidases in S. isatideus plants then the oxidation of (168) is most likely to produce the ^{2}H-labelled homospermidine (171). If this intermediate is also subject to a similar deuterium isotope effect during the next oxidation step then this ^{2}H-labelled homospermidine would be converted into the aldehyde (172), which in turn would be transformed into the iminium ion (173). A further oxidation, involving the primary amine side chain, followed by intramolecular cyclisation, would generate retrorsine (174) with a preponderence of deuterium labelling in ring B of the necine.

Kelly and Robins explained the ^{2}H-labelling pattern found in rosmarinine by envisaging the presence of similar deuterium isotope effects in rosmarinecine biosynthesis.

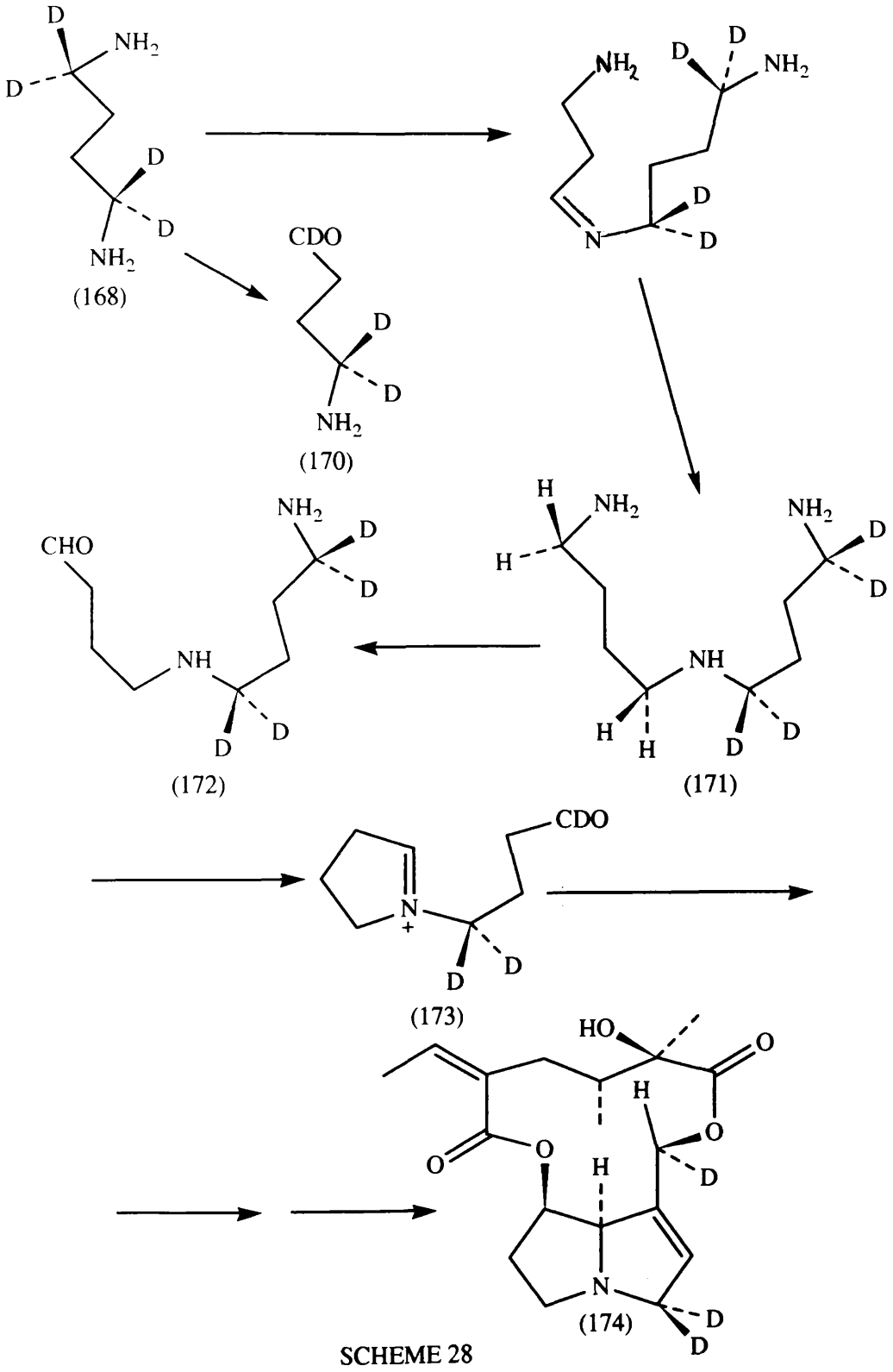
Kelly and Robins then considered the stereochemistry of the enzymic processes which govern the hydrogen atoms initially located at C-1 and C-4 of putrescine. Therefore, (R)-[1-^{2}H]- and (S)-[1-^{2}H]-

putrescine [(156) and (157)] dihydrochlorides were prepared and fed to S. pleistocephalus plants. ^{2}H-{^{1}H} N.m.r. spectroscopy established that the (R)-isomer gave rise to deuterium labels at C-3β, C-5α, C-8α, and C-9 pro-S in rosmarinine (Scheme 29), while feeding of the (S)-isomer produced rosmarinine labelled with deuterium at only two sites, namely C-5β and C-3α of rosmarinine (Scheme 30).

These labelling patterns were again consistent with those witnessed in retrorsine when the same precursors were fed to S. isatideus. This demonstrates that both retronecine and rosmarinecine biosynthesis involve the stereospecific retention of the pro-R and removal of the pro-S protons during the conversion of putrescine into 4-aminobutanal and the oxidation of the terminal carbon atoms of homospermidine. In addition, both pathways feature the formation of homospermidine via hydride (or equivalent) attack on the C-si face of the intermediate iminium ion, and generation of the pyrrolizidine nucleus occurs by hydride attack on the C-re face of the iminium ion (154).

The feeding of (R)- and (S)-[1-^{2}H]putrescine dihydrochlorides to S. pleistocephalus also established that isoretronecanol (25) is produced via hydride attack on the C-re face of 8α-pyrrolizidine aldehyde (175). An analogous stereospecific reduction of the 8β-aldehyde (148) occurs in retronecine biosynthesis affording trachelanthamidine (26).

Kelly and Robins discerned the remaining stereochemical aspects of rosmarinecine biosynthesis through the feeding of (R)-[2-^{2}H]- and (S)-[2-^{2}H]putrescine [(166) and (167)] dihydrochlorides to S. pleistocephalus plants. ^{2}H-{^{1}H} N.m.r. spectroscopy established that feeding of the (R)-isomer gave rise to rosmarinine labelled with deuterium at C-2β and C-6α (Scheme 31), whereas administering the


D
NH2
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D
NH2
(168)
CDO
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NH2
(170)
NH2
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D
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H
NH2
H
NH
H
H
D
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(171)
CHO
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D
NH
D
D
(172)
CDO
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(173)
HO
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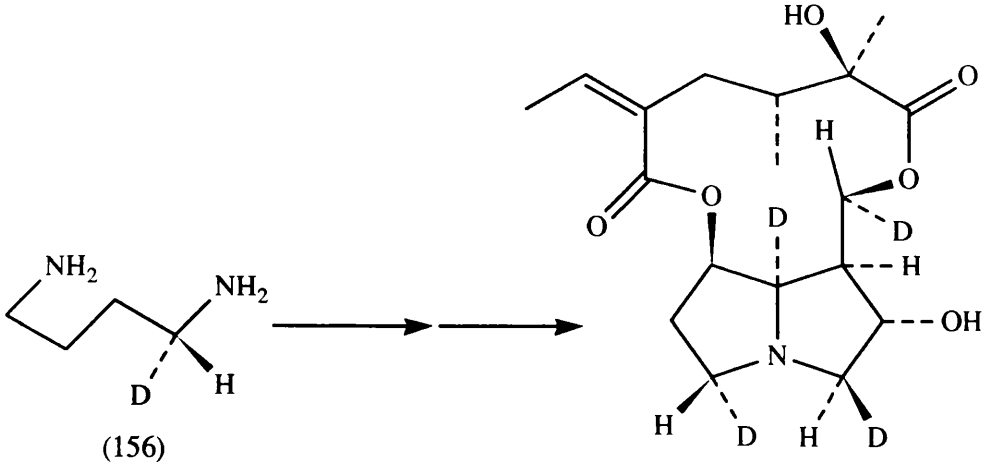
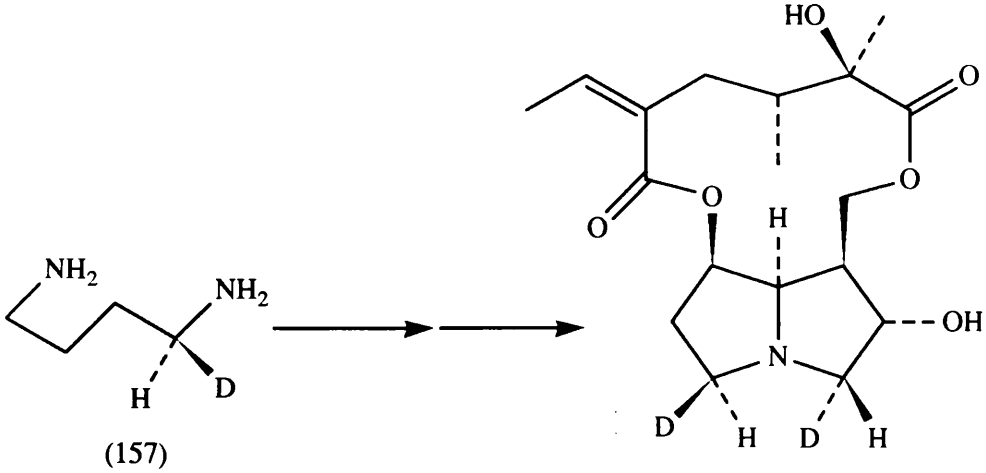
O
O
O
H
D
N
D
D
(174)

SCHEME 28



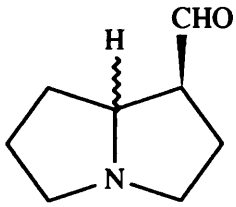
(156)

SCHEME 29



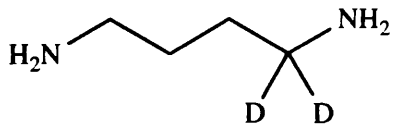
(157)

SCHEME 30

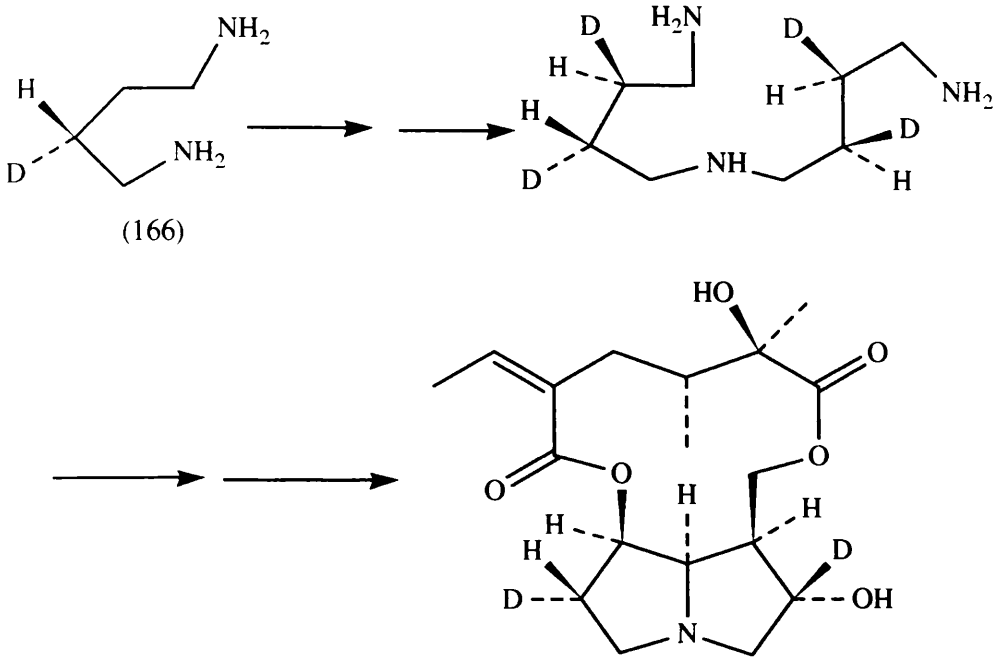


8α - H (175)

8β - H (148)

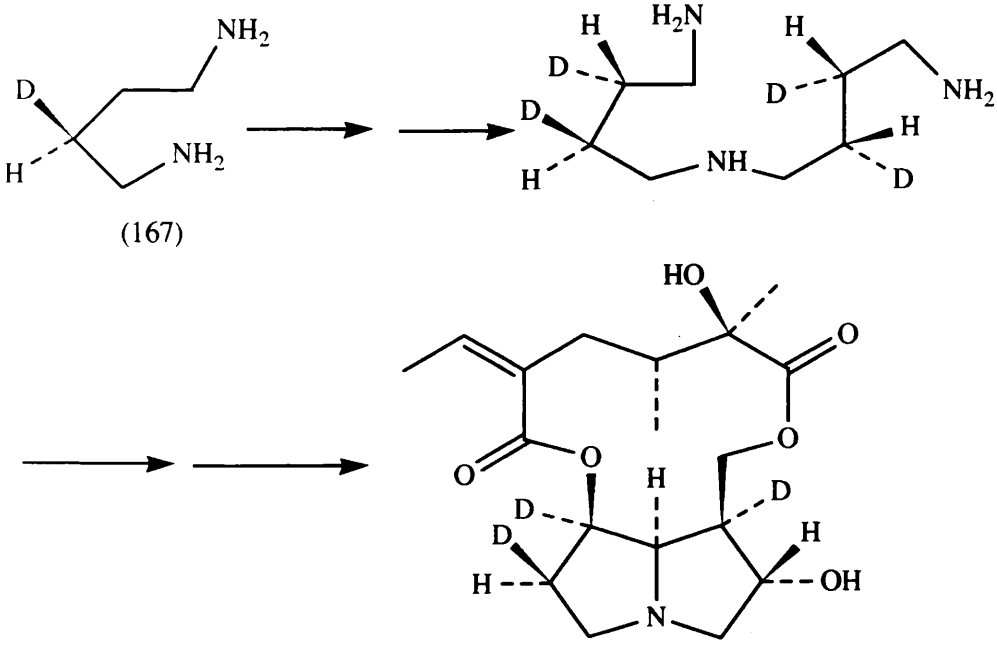


(169)



(166)

SCHEME 31



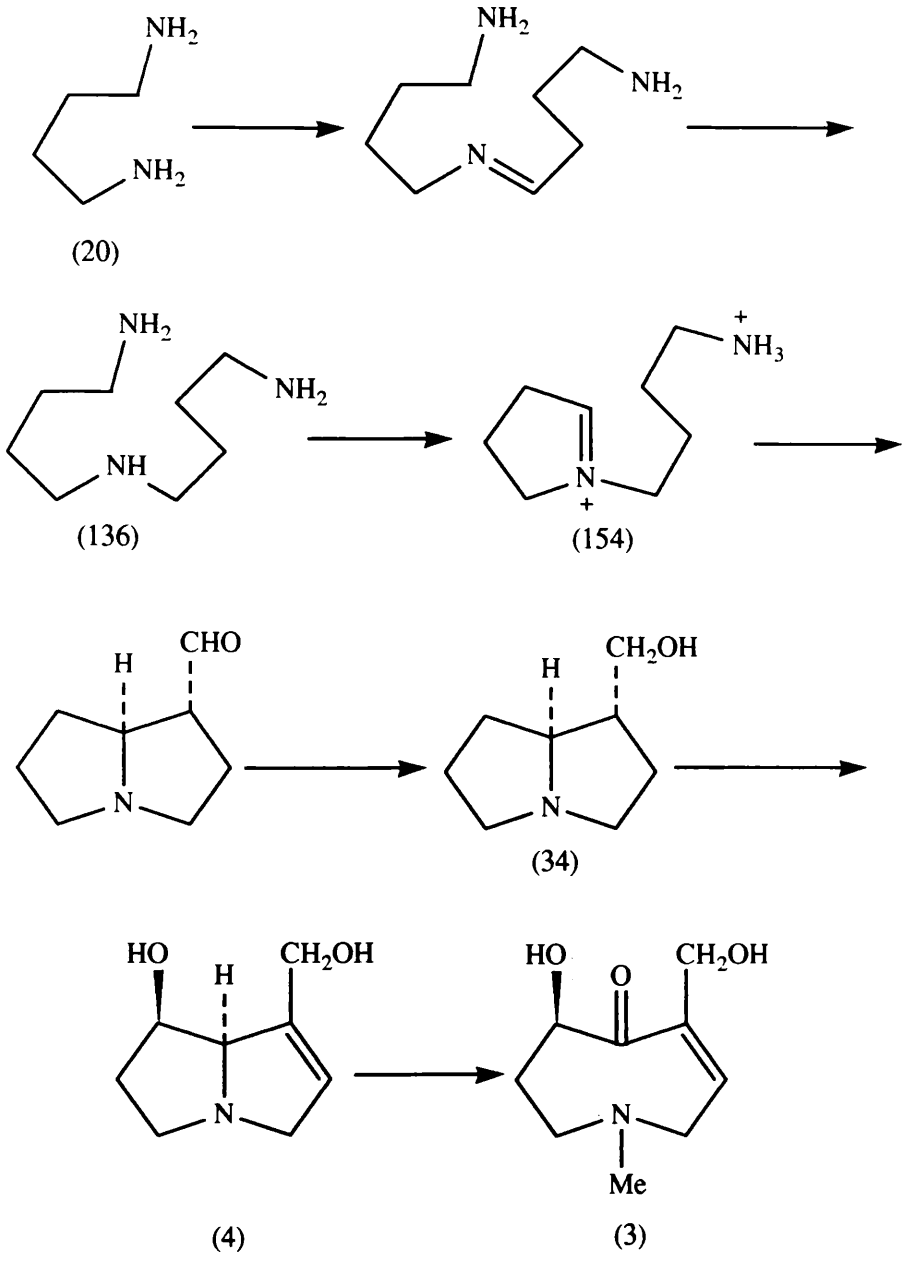
(167)

SCHEME 32

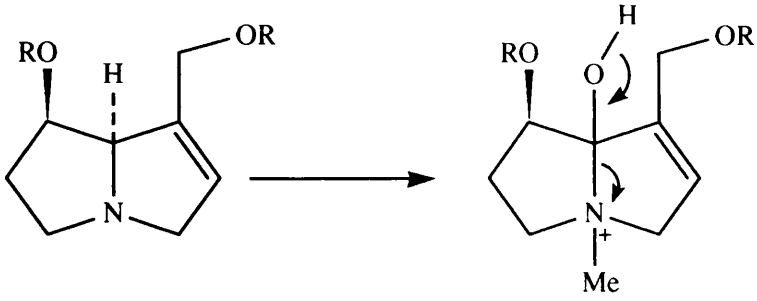
(S)-isomer yielded rosmarinine labelled with deuterium incorporated at C-1α, C-6β, and C-7α (Scheme 32).

These experiments indicated that formation of the 8α-pyrrolizidine aldehyde (175) involves the stereospecific removal of the pro-R hydrogen and retention of the pro-S hydrogen at the carbon atom earmarked to become C-1 of rosmarinine. Furthermore, these experiments established that the hydroxylation processes at C-2 and C-7 in rosmarinecine biosynthesis occur with complete retention of configuration.

Robins and his co-workers recently reported work on the biosynthesis of otonecine (3).[89] This involved the feeding of precursors known to be involved in retronecine biosynthesis in radiolabelled form, to Emilia flammea (family Compositae), a plant known to produce the 12-membered macrocyclic diester emiline (176). As for retronecine biosynthesis, it was established that putrescine (20), homospermidine (136), iminium ion (154), and trachelanthamidine (34) are all good precursors for otonecine (Scheme 33). A later experiment involving the administration of ^{3}H-labelled retronecine to Emilia flammea confirmed retronecine as an efficient precursor for otonecine. This also served to demonstrate that the N(4)-C(8) bond in the pyrrolizidines (34) and (4) is cleaved at a late stage in biosynthetic pathway to otonecine. It is thought that formation of otonecine could arise by hydroxylation of C-8 and N-methylation of retronecine (or an ester derivative), affording (177) (Scheme 34). Subsequent ketone formation and ring cleavage could afford otonecine (3).

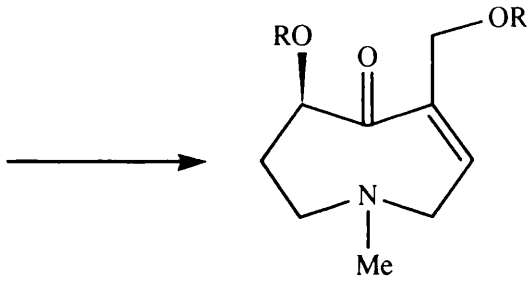

NH2
NH2
NH2
N
(20)
NH2
NH2
NH
(136)
+
NH3
N
+
(154)
H
CHO
N
H
CH2OH
N
(34)
HO
H
CH2OH
N
HO
O
CH2OH
N
Me
(4)
(3)

SCHEME 33



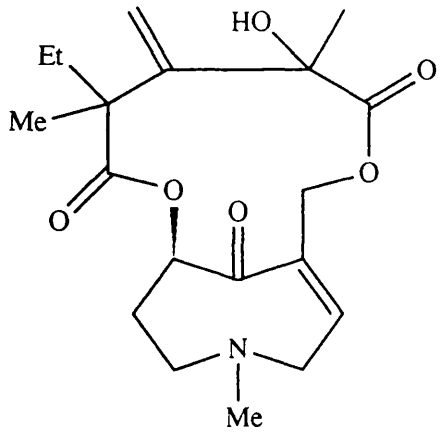
R = H (4)

(177)



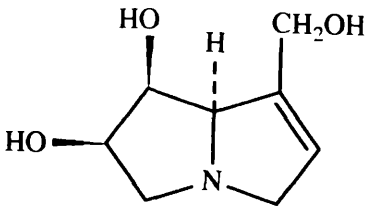
R = H (3)

SCHEME 34

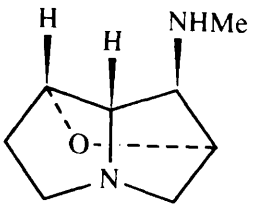


(176)

The biosynthetic pathways to rosmarinecine and retronecine have been established and the many similarities involved in the processes have been highlighted. The divergence in the two pathways has been established. A number of intermediates involved in the biosynthesis of these bases have been identified and almost all the stereochemical aspects of the respective pathways elucidated. The considerable knowledge and expertise gained during these studies should, in time, help to provide a more detailed understanding of necine biosynthesis. Although the recent investigation of otonecine biosynthesis is encouraging, more pyrrolizidine bases require to be investigated before a definitive understanding of necines biosynthesis can emerge. Further studies may address other necines present in pyrrolizidine macrocyclic diesters, such as crotanecine (10). There is also a need to investigate the biosynthesis of necines of different structure, such as loline (12).



(10)

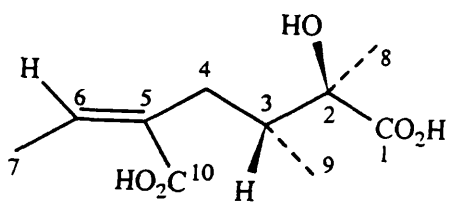


(12)

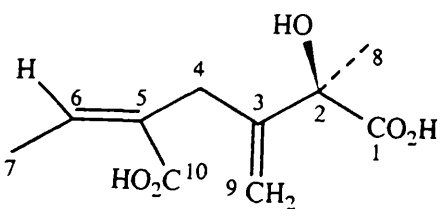
3.3 The Biosynthesis of Necic Acids

Studies relating to the biosynthesis of the necic acids have stimulated much interest. Although this thesis involves no formal investigation into the biosynthesis of these compounds, it is nevertheless appropriate to detail the significant accomplishments in this area.

Until now, the information accumulated on the biosynthesis of necic acids has been gained through the feeding of radiolabelled precursors (^{3}H, ^{14}C) to plants producing pyrrolizidine alkaloids. Most attention has been focussed on the abundant C_{10} diacids, particularly senecic (178) and seniciphyllic acids (11), produced mainly by Senecio species (family Compositae).



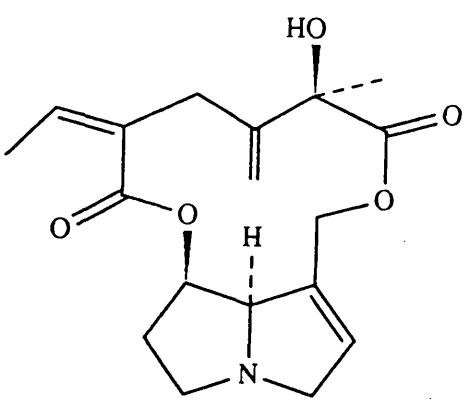
(178)



(11)

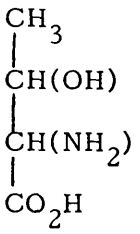
Most necic acids are C_{10} units and it was initially thought that these compounds could originate from the coupling of two C_5 units, related to isoprene, even although the carbon skeletons and oxygenation patterns of the necic acids are distinct from those of terpenoid compounds.[90]

However, work performed by Crout et al.[91] indicated that neither acetate nor mevalonate are specific precursors of the C_{10} necic acids. Feeding of ^{14}C-labelled acetate and mevalonate to Senecio douglasii resulted in randomisation of the ^{14}C activity in both the acid and base portions of seneciphylline (179), the main pyrrolizidine alkaloid produced by these plants.

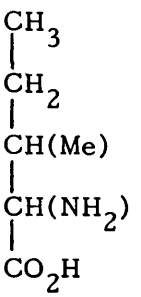


(179)

Attempts to establish the biosynthetic pathway to the necic acids included the screening of various amino acids as potential precursors. Crout et al. found that isoleucine (180) and its biological precursor threonine (181) are both efficient and specific precursors of seniciphyllic acid in Senecio douglasii.[92]



(181)



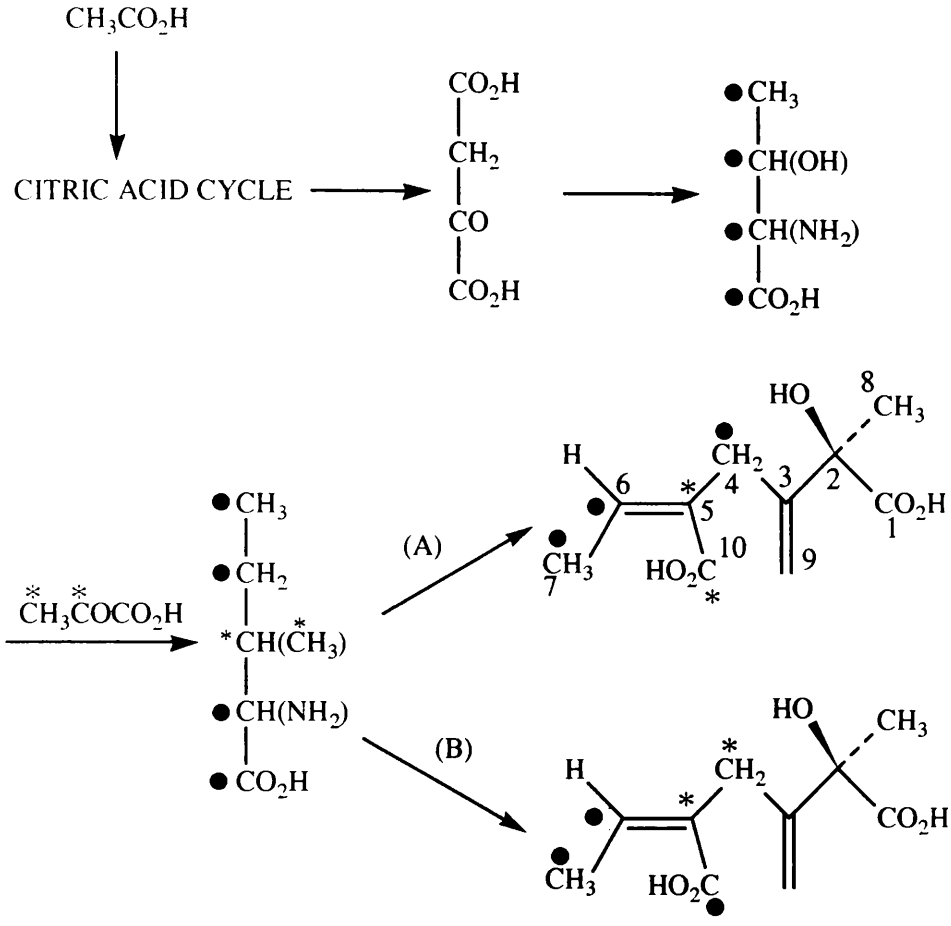
(180)

Feeding of [U-^{14}C]-L-isoleucine resulted in a five carbon unit, encompassing C-4, -5, -6, -7, and -10, of seneciphyllic acid, being labelled most prominently. In addition, when [1-^{14}C]-L-isoleucine was employed as a precursor, it was incorporated into the necic acid with less than one tenth of the efficiency of [U-^{14}C]-L-isoleucine. This indicated that C-1 of L-isoleucine was not incorporated into seneciphyllic acid.

These results enabled Crout et al. to propose that the 'left hand' C_5 unit of seneciphyllic acid is derived from isoleucine by the route outlined in Scheme 35(a).

An alternative route, Scheme 35(b), was refuted on the basis of the labelling pattern obtained after administering [U-^{14}C]-L-threonine. If route 35(b) was adhered to in the biosynthetic process, then equal labelling at C-6, C-7, and C-10 of seneciphyllic acid should arise. However, degradation of the necic acid revealed that the C-6, C-7 unit contained more than four times the activity associated with C-10, hence route 35(a) was deemed the more plausible.

Evidence became available which suggested that this assessment might require refinement. Enzyme systems are known which could induce inequality in the labelling of carbon atoms derived from [U-^{14}C]-L-


CH3CO2H
CITRIC ACID CYCLE
CO2H
CH2
CO
CO2H
CH3
CH(OH)
CH(NH2)
CO2H
(A)
(B)
HO
8
CH3
H
6
CH2
4
3
2
CO2H
1
5
10
9
7
HO2C
CH3COCO2H
CH(CH3)
CH(NH2)
CO2H

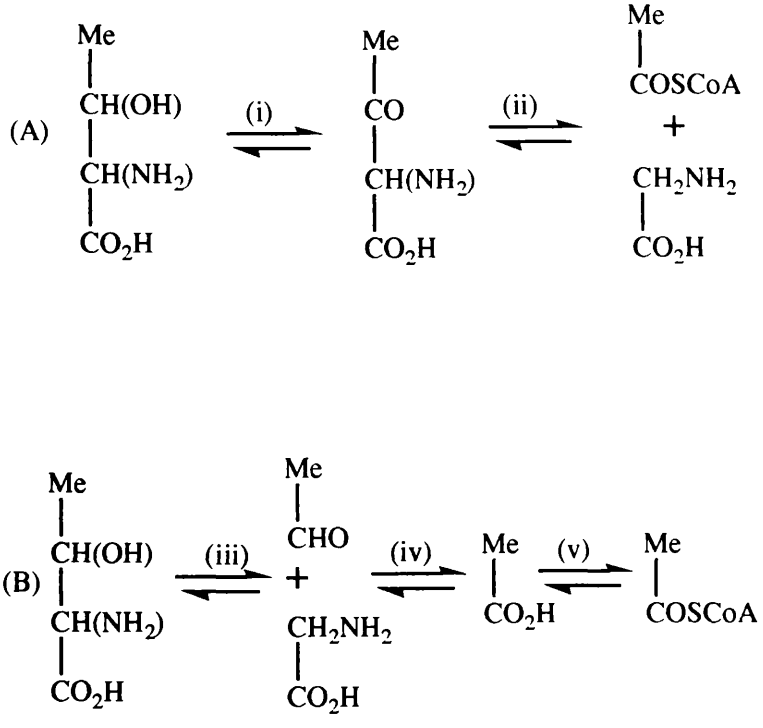
SCHEME 35

threonine in metabolites.

Threonine dehydrogenase and α-amino-oxobutyrate-CoA ligase can operate on threonine to yield an equimolar mixture of acetyl CoA and glycine (Scheme 36(a)).[93,94] A similar effect is observed by the sequential action of threonine aldolase, acetaldehyde dehydrogenase, and acetyl CoA synthetase (Scheme 36(b)).[95] If either, or both, of these systems is reversible in vivo then a distortion in the distribution of activity in [U-^{14}C]threonine could arise after dilution of the labelled glycine and acetyl CoA with unlabelled material. This could be reflected in deficient amounts of the radiolabel at (C-1 + C-2) and (C-3 + C-4) positions of threonine. Evidently from the information then available, it could not be established which was the correct incorporation mode of L-threonine into seneciphyllic acid.

Crout et al. did, however, establish that the route depicted in Scheme 35(b) was indeed the correct mode of incorporation of L-isoleucine into the 'left hand' component of C_{10} necic acids.[92] This was achieved through feeding experiments involving Senecio magnificus plants, a species known to display a small, but nonetheless significant amount of threonine aldolase activity. The presence of the enzyme increased the possibility of incorporation via route 35(b).

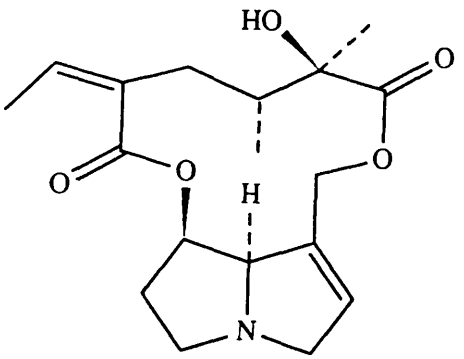
Administration of [2-^{14}C]- [5-^{14}C]-, and [6-^{14}C]-isoleucine revealed a labelling pattern in senecic acid consistent with only route 35(b). Further investigation of the senecic acid, obtained by hydrolysis of the parent alkaloid senecionine (182), revealed that the 'right hand' C_5 unit of the necic acid, comprising C-1, -2, -3, -8, and -9 is also derived from L-threonine. The proposed pathway of incorporation is



Reagents : (i) , Threonine dehydrogenase ; (ii) , α -amino-oxobutyrate-CoA ligase ;
(iii) , threonine aldolase ; (iv) , acetaldehyde dehydrogenase ;
(v) , acetyl CoA synthetase

SCHEME 36

illustrated in Scheme 37

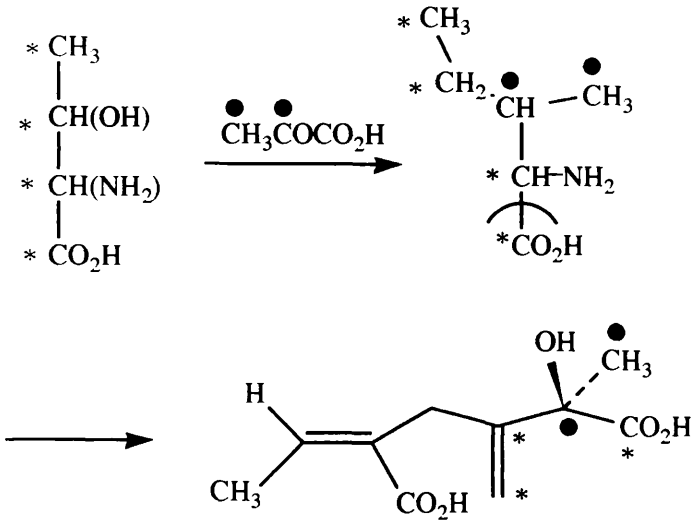


(I82)

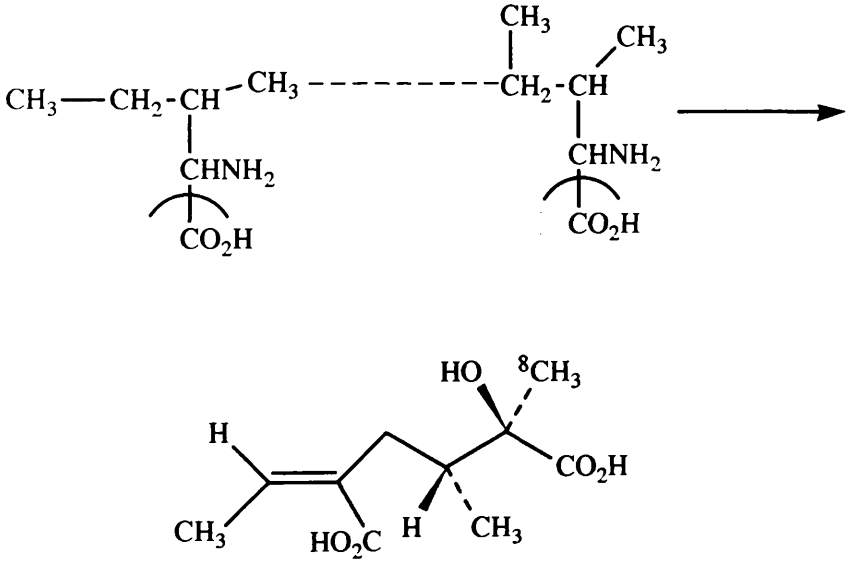
Again C-1 of L-isoleucine was not incorporated into the necic acid. Further degradation experiments also revealed that C-2 of senecic acid was inactive after administering [U-^{14}C]-L-threonine, which was consistent with the proposed scheme. Thus it had been established that C_{10} necic acids are biosynthesised from two C_5 units derived from isoleucine (Scheme 38).

Crout et al. had established, in experiments involving the biosynthesis of seneciphyllic acid, that [Me-^{14}C]methionine is a relatively efficient precursor of C-8 of the necic acid. This was explained by assuming that the methyl group of methionine (183) is converted by Senecio species into the methyl group of pyruvate (184) via serine (185). This supposition was supported by the conversion of serine into pyruvate in vivo (Scheme 39).[96]

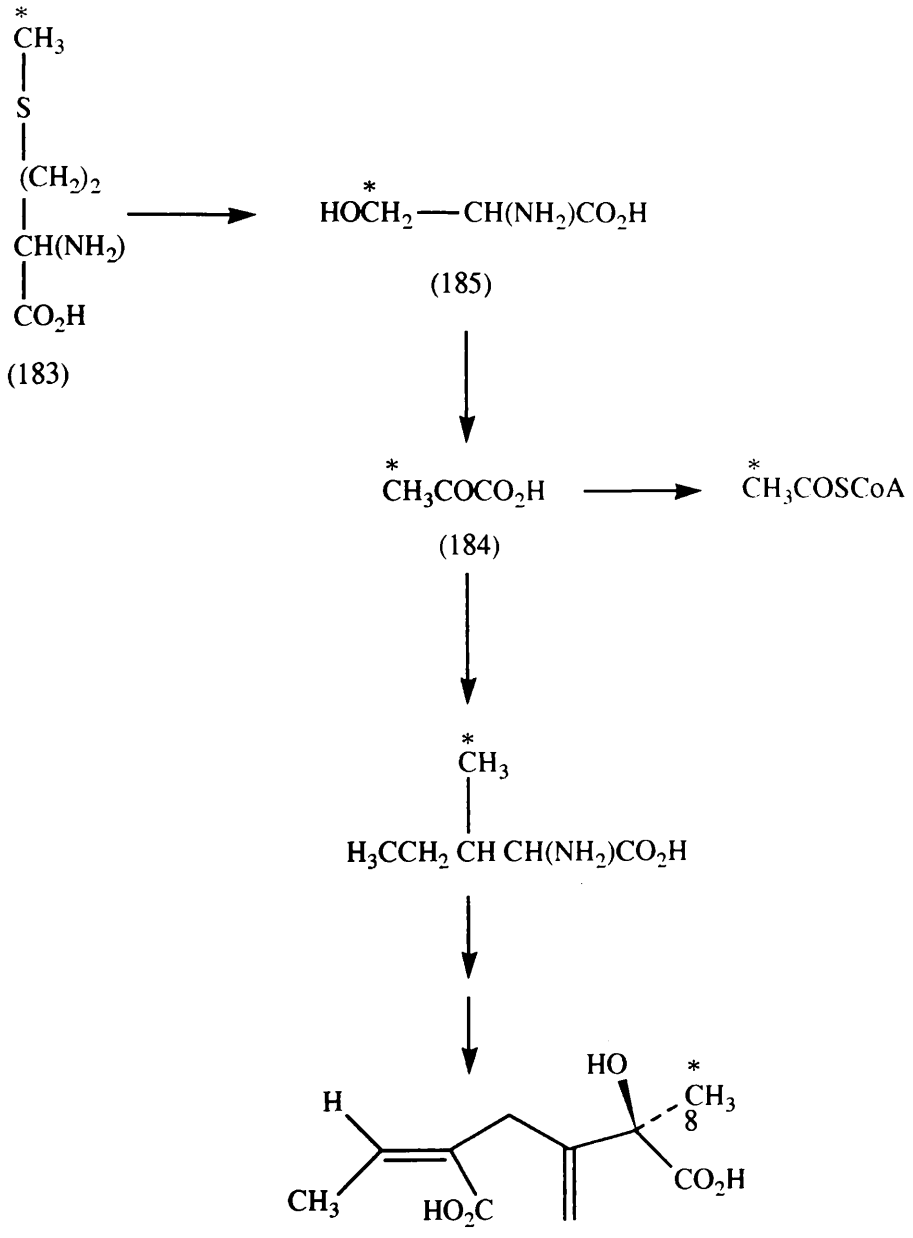
Tangible evidence emerged to support this theory when [Me-^{14}C]-L-methionine was fed to pea seedlings. Degradation of the isolated serine revealed that the radiolabel was confined to the hydroxymethyl group of serine.



SCHEME 37



SCHEME 38

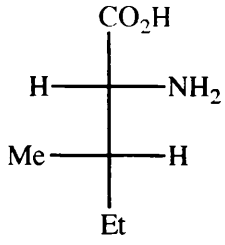

*CH3
S
(CH2)2
CH(NH2)
CO2H
(183)
*HOCH2—CH(NH2)CO2H
(185)
*CH3COCO2H
(184)
*CH3COSCoA
*CH3
H3CCH2 CH CH(NH2)CO2H
HO
*CH3
8
H
CH3
HO2C
CO2H

SCHEME 39

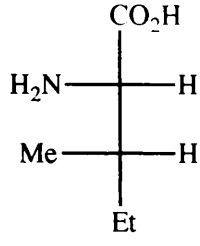
Certain microbial species are known to utilise all four stereoisomers of isoleucine for growth.[97,98] Davies and Crout demonstrated, by use of ^{3}H- and ^{14}C-labelled isoleucine (180) and alloisoleucine (186), that only L-isoleucine (187) is an efficient and specific precursor of senecic acid in Senecio magnificus.[99]

Having established the amino acid basis for necic acid biosynthesis, Crout and co-workers endeavoured to elucidate the pathway by which isoleucine is converted into necic acids. Investigations initially centred on possible C_5 intermediates of isoleucine metabolism, namely 2-methylbutanoic acid (188), angelic acid (189), and 2-methyl-3-oxobutanoic acid (190).[100] On administering radiolabelled forms of these compounds to Senecio magnificus it was evident that none is a specific precursor of seneciphyllic acid.

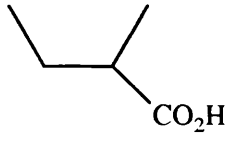
Attention then switched to address the fate of the hydrogen atoms located at C-4 and C-6 of isoleucine, the sites involved in coupling to afford the necic acids. Accordingly, [6-^{3}H, 6-^{14}C]isoleucine was prepared and administered to Senecio magnificus. The senecic acid isolated retained approximately 5/6 of the tritium label. This indicated that at least one of the hydrogen atoms of C-6 of isoleucine is preserved during its conversion into the methylene group at C-4 of senecic acid. In a parallel experiment, [4,4-^{3}H]isoleucine was fed to S. isatideus. In this instance half the tritium label was retained in the necic acid, isatinecic acid (191). These labels were believed to be at H-3 and H-6 of the diacid (191). Together, these results demonstrated that a carbonyl function at the sites which become C-3 or C-4 of the necic acid is not involved in the coupling to generate the necic acid. Moreover it



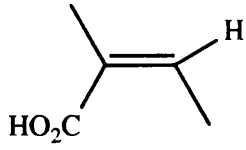
(186)



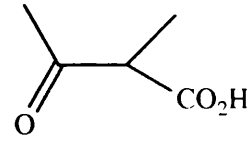
(187)



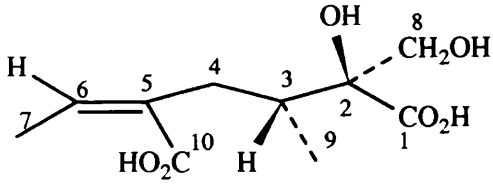
(188)



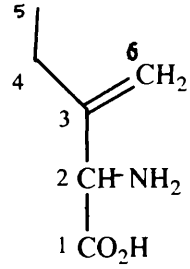
(189)



(190)



(191)

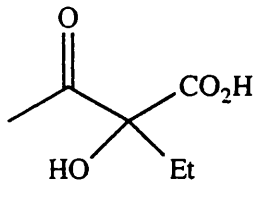


(192)

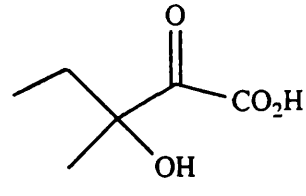
suggested that the functionality introduced at the C-3, C-6 side chain of isoleucine prior to coupling might be CH_2OH or $C{=}CH_2$. The latter was regarded as the more appealing since the corresponding α-amino acid, β-methylenenorvaline (2-amino-3-methylenepentanoic acid) (192), was known to occur in nature.[101] In addition it was envisaged that the $C{=}CH_2$ functionality is a more appropriate unit to instigate the desired linkage.

A study was then embarked upon to develop a viable synthesis of β-methylenenorvaline. Once established, this route was used to prepare the amino acid in radiolabelled form. DL-β-[^{3}H]Methylenenorvaline was administered to S. magnificus and found to be a specific precursor of senecic acid. However, it was not possible to determine if β-methylenenorvaline was incorporated into one or both halves of the necic acid.

A key aspect of L-isoleucine biosynthesis is the tertiary ketol rearrangement of 2-ethyl-2-hydroxy-3-oxobutanoic acid (193) to 3-hydroxy-3-methyl-2-oxopentanoic acid (194).[102]



(193)



(194)

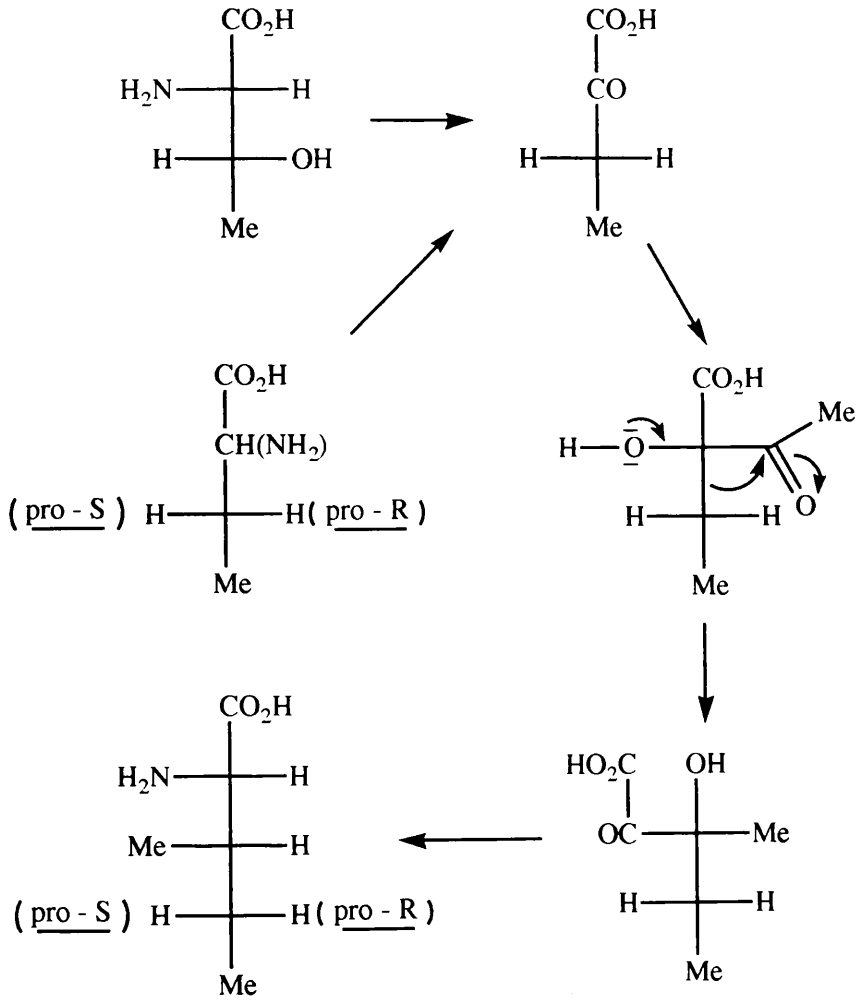
The stereochemistry of this process was ingeniously determined by Cahill et al. by the subtle use of radiolabelled 2-aminobutanoic acid precursors.[103] These precursors were stereospecifically labelled with tritium at C-3 and administered to plants of S. isatideus and S. magnificus. It was assumed that the appropriate enzymes, such as amino acid transferases or oxidases, would operate on 2-aminobutanoic acid to afford 2-oxobutanoate in situ (Scheme 40). Such a conversion would then provide an appropriate means of investigating the tertiary ketol rearrangement.

The precursors fed were (2RS,3S)-[3-^{3}H]-, (2RS)-[3-^{3}H]-, (2S)-[3-^{3}H]-, and (2R)-[3-^{3}H]-2-aminobutanoic acid, in conjunction with [3-^{14}C]-2-aminobutanoic acid.

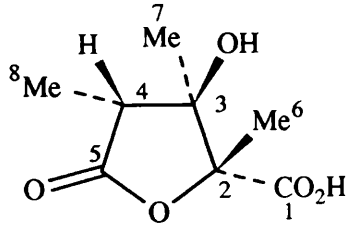
These experiments revealed that both (2S)- and (2R)-2-aminobutanoic acid are incorporated specifically into necic acids. Of much greater importance however, was that during the generation of both C_5 portions of the necic acid, the C-3 pro-S hydrogen of 2-aminobutanoic acid was lost. In contrast, the C-3 pro-R hydrogen atom of both C_5 portions was retained during the process.

To assess the stereochemical changes occurring at C-4 of L-isoleucine, a similar series of experiments were pursued involving the feeding of (2RS,4RS)-[3,4-^{3}H]-, (2S,4S)-[3,4-^{3}H]-, (2S)-[4-^{3}H], and (2S,4R)-[4-^{3}H]isoleucine, together with L-[U-^{14}C]isoleucine.

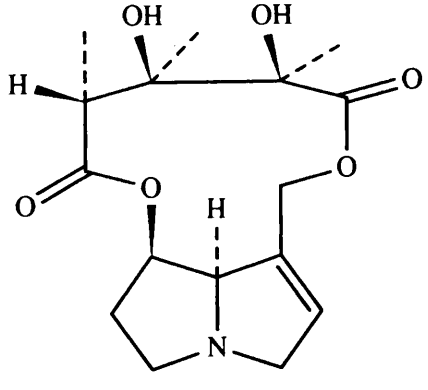
These experiments disclosed that isoleucine is incorporated into both halves of the necic acid with loss of the C-4 pro-S proton and retention of the C-4 pro-R hydrogen, thereby revealing that the hydrogen atoms at C-13 and C-20 of retrorsine (9) and C-20 of senecio-



SCHEME 40

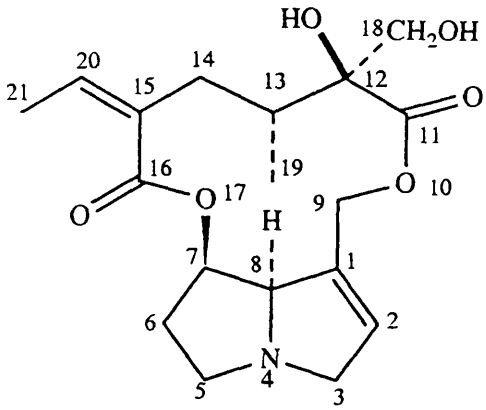


(126)



(8)

nine (182) are derived from the C-4 pro-R hydrogen atom of L-isoleucine.



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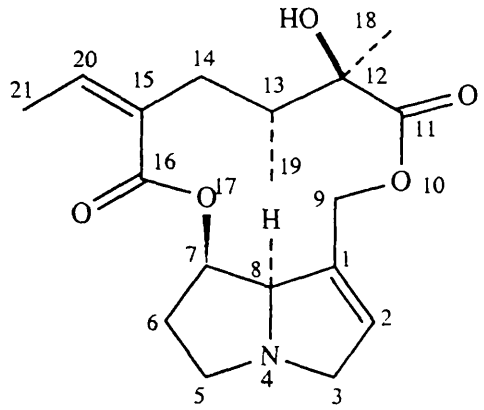


(182)

These results are consistent with the incorporation of 2-amino-butanoic acid via isoleucine and established that the ethyl migration step proceeds with retention of configuration.[104]

Studies on the biosynthesis of monocrotalic acid (126), the C_8 esterifying portion of monocrotaline (8), established that both L-threonine and L-isoleucine are specific precursors of this necic acid.[105] Feeding of [U-^{14}C]-L-isoleucine and [U-^{14}C]-L-threonine to Crotalaria species (family Leguminosae) showed that the C_5 unit of monocrotalic acid, comprising C-1, -2, -3, -6, and -7, is derived from isoleucine. The remaining three carbon atoms may originate from propionate.[106]

Valine (195) and leucine (196) are other amino acids which have been identified as specific precursors of necic acids.[107]

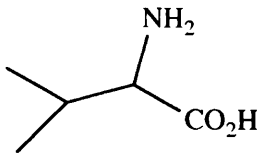
Echimidinic acid (197), the 'right hand' esterifying component of heliosupine (198), was found to be derived from valine through feeding

experiments with Cynoglossum oficinale (family Boraginaceae). The angelic acid moiety of heliosupine, was found to originate from L-isoleucine.

Devlin and Robins studied the biosynthesis of trichodesmic acid (199) in Crotalaria globifera, a plant known to produce trichodesmine (200) as the main pyrrolizidine alkaloid.[108] Labelling experiments indicated that the 'right hand' C_5 unit of this branched lactone is derived from threonine or isoleucine, while the 'left hand' C_5 unit originates from valine or leucine (Scheme 41).

Many important aspects of necic acid biosynthesis have been established, particularly for senecic and seneciphyllic acids. However, our knowledge of the biosynthesis of these acids is incomplete. Up until now the number of necic acids investigated has been comparatively few. Further investigations, including studies on more necic acids, are necessary before a more detailed understanding of the biosynthesis of necic acids can emerge.

Experiments to date have relied upon radiolabelled precursors to extract information about the biosynthetic pathways. Stable isotopes (^{13}C, ^{2}H) have been used to good effect to enhance our understanding of necine biosynthesis. It is conceivable that these isotopes could be used with equal success in the elucidation of necic acid biosynthetic pathways. One of the areas most likely to be investigated by this means is the mode of coupling of the two C_5 units generating the C_{10} diacids.



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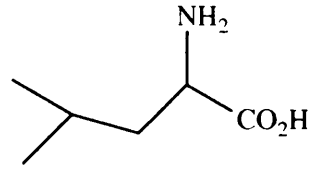

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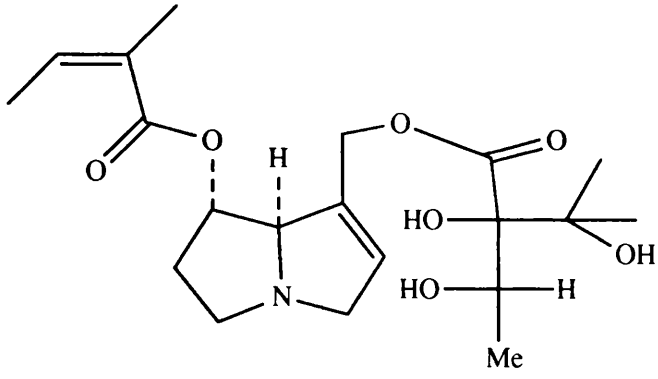

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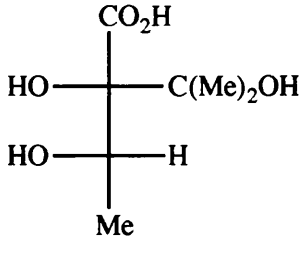

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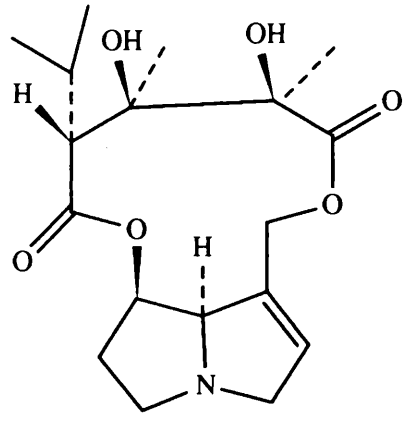

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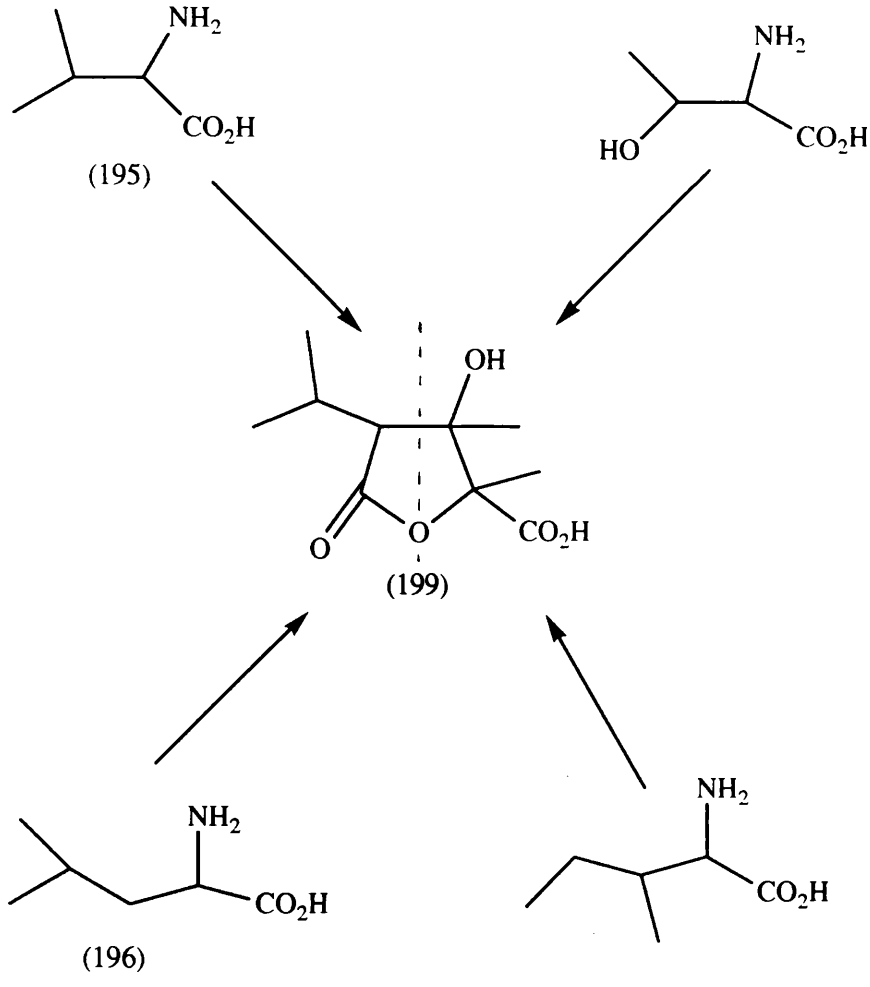


SCHEME 41

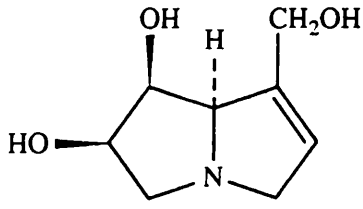
CHAPTER 4

INVESTIGATIONS INTO THE SYNTHESIS OF PYRROLIZIDINE BASES

4.1 Introduction

Many synthetic routes to pyrrolizidine bases have been reported, including the recent upsurge in the synthesis of necines in optically active form. However, as highlighted in Chapter 2, there is still a pressing need for the provision of routes which enable the production of a range of necines in optically active form.

Accordingly, the major aim of this research project was in trying to develop a concise, flexible route capable of generating several necines in optically active form. In addition, it was important that such a scheme should be capable of yielding the more highly oxygenated pyrrolizidine bases, such as crotanecine (10). Indeed at the advent of this research study only a few synthetic routes to necine triols were available.[47,49,59]



(10)

The pyrrolizidine bases have proved to be demanding synthetic targets for the Organic Chemist. The considerable problems associated with their synthesis arise largely from three basic structural characteristics.

Firstly, difficulty can be encountered in the construction of the pyrrolizidine skeleton itself. This is often compounded by the need to develop a suitable substitution pattern, prior to the development of the pyrrolizidine system, to enable production of the desired necine. For necine synthesis this is most commonly expressed by the introduction of protection for hydroxyl groups prior to formation of the pyrrolizidine nucleus. Intramolecular cyclisation is the most frequently encountered means of generating the pyrrolizidine ring system. Secondly, control of the stereochemistry of the bridgehead proton of the pyrrolizidine system, at C-8, may have to be exercised. Such a consideration is often crucial to the success of a synthetic strategy and is frequently accomplished by the incorporation of a stereoselective hydrogenation step.

A final source of difficulty may be presented by the need to develop 1,2-unsaturation of the pyrrolizidine ring system - a feature common to many necines. Although this unsaturation is invariably introduced after the development of the pyrrolizidine nucleus any successful synthesis has to incorporate an appropriate means to facilitate the generation of this unsaturation - often early in a synthetic sequence. Pyrrolizidine systems bearing an ester substituent at C-1, like (28), have been used to good effect to bring about the desired unsaturation. This is accomplished by selenenylation α to the ester (at C-1) followed by a short reduction-oxidation-elimination sequence (Scheme 2).

Obviously any potential synthetic route to the necines must address these problems satisfactorily. Moreover, any scheme aimed at providing products in optically active form has the further complication in both identifying an appropriate starting material and then maintaining the

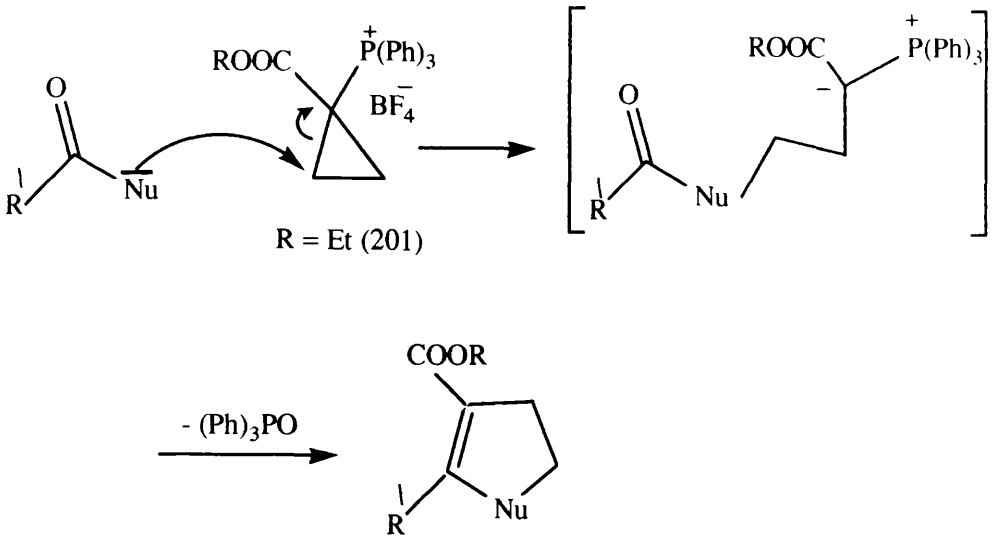
desired stereochemistry throughout the duration of a synthetic sequence.

One of the best ways of constructing the pyrrolizidine ring system was reported by Muchowski and Nelson.[109] This work was based on earlier studies by Fuchs[110] who demonstrated the versatility of ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate (201) in the formation of five-, six-, and seven membered rings (Scheme 42). Fuchs reacted the phosphonium salt with nucleophiles, such as anions of carboxylates or β-diketones, to induce ring opening of the cyclopropane ring. An intramolecular Wittig reaction then occurred, effecting cyclisation.

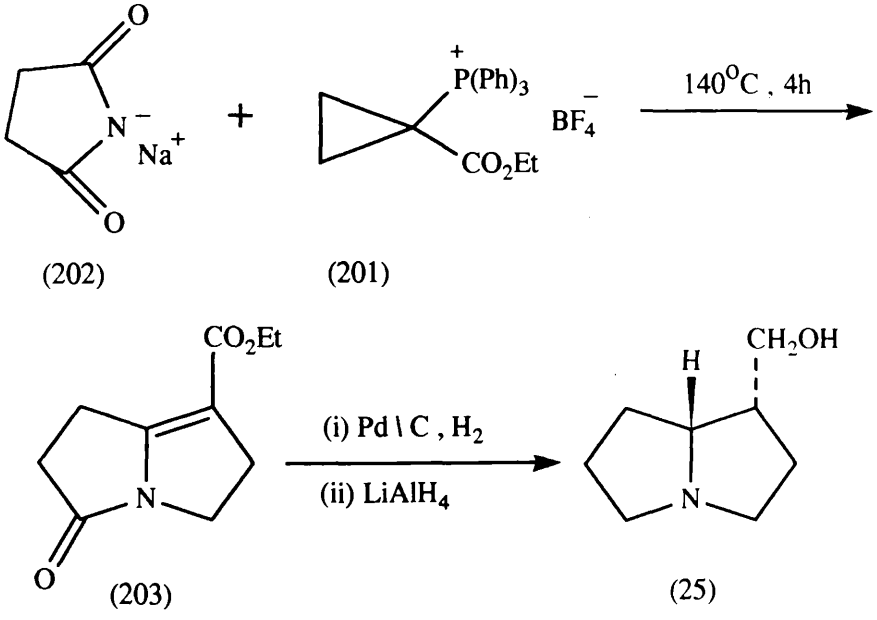
Muchowski and Nelson broadened the scope of this reaction by generating the pyrrolizidine nucleus from imide salts. Reaction of sodium succinimide (202) with cyclopropane salt (201), under melt conditions, generated the expected product of cyclisation, the conjugated ester (203), in 50% yield (Scheme 43).[109] The ester was subsequently transformed into (±)-isoretronecanol (25) by a simple hydrogenation-reduction sequence (Scheme 43).[109]

Flitsch and Wernsman later modified this procedure to boost the yield of the condensation of imide salt (202) with (201) to 84%. This improvement was achieved by employing xylene as solvent and maintaining the reaction under reflux conditions for 2½ hours.[111,112]

Rather surprisingly this novel construction of the pyrrolizidine nucleus appears to have received only limited attention. Indeed no other synthetic route to date has employed this method, though Pinnick and Chang used a related strategy in their conversion of imide (204) into isoretronecanol (25) (Scheme 44).[113]

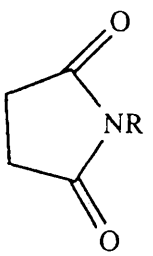


SCHEME 42



SCHEME 43

The lack of applications of this method is somewhat curious since several syntheses have used imides as the building block for 'ring A' of the pyrrolizidine skeleton. However most of these routes involve only the production of the simple necines isoretronecanol (25) and trachelanthamidine (26) from either succinimide (205) or an N-substituted succinimide (206).[12,13]



R = H (205)

R = Protecting Group (206)

In addition three research teams, namely Niwa et al.,[48] Chamberlain and Chung,[56] and Hart and co-workers[53-55] demonstrated the value of the malic acids in providing synthesis of C-7 hydroxylated necines via imides. Necines produced included heliotridine (70) and retronecine (4), in optically active form. In these studies the pyrrolizidine ring system was developed by intramolecular cyclisation.

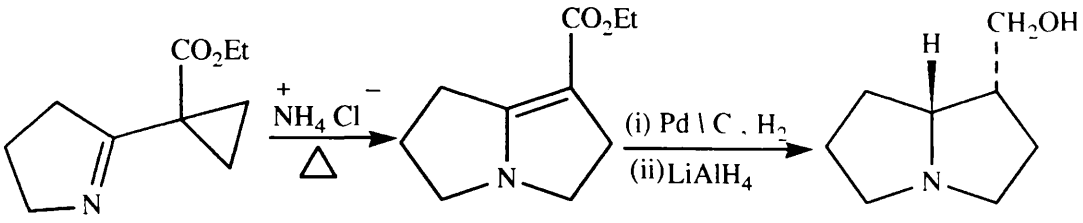
Up until now these routes represent the only reported examples of imides being used in the synthesis of necine diols. However the scope for extension of the routes to produce other necines is limited and none of the routes have been able to provide synthesis of any necine triol.

In common with these studies it was believed that imides, prepared from the L- and D-malic acids, could provide a suitable means of generating necine diols, such as retronecine (4), in optically active form. As before the hydroxyl group of the malic acid could be used to

generate the appropriate oxygenation pattern in 'ring A' in the formation of these necines (Scheme 45(a)). Of greater significance though was the consideration that the tartaric acids could be used to generate a complementary series of intermediate imides which could act as suitable synthons for the more demanding synthetic targets of the necine triols, such as crotanecine (10) [Scheme 45(b)].

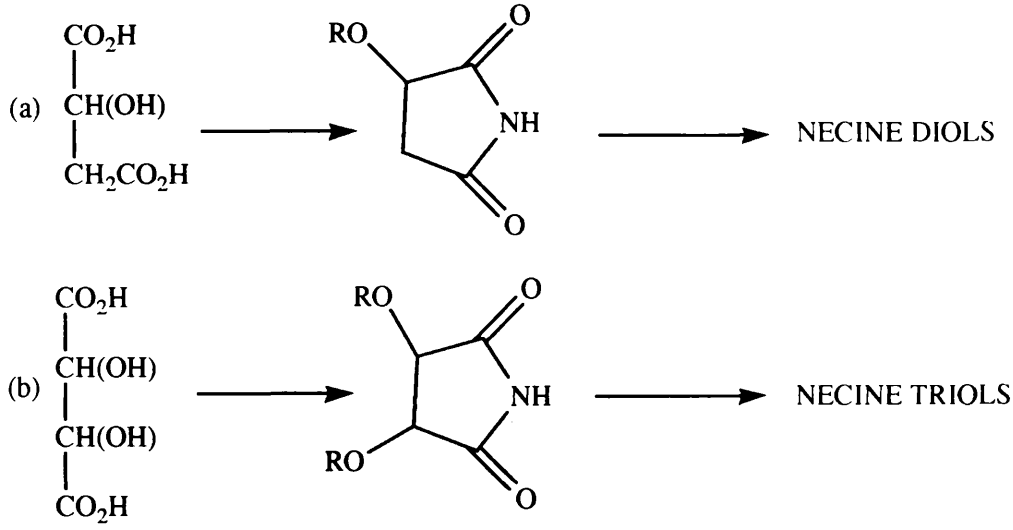
It was anticipated that these intermediate imides, bearing appropriate protection on the hydroxyl group(s), could be reacted with ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate (201) to generate the pyrrolizidine skeleton. The products of this condensation could then be manipulated to the desired necine diol or triol by standard synthetic methods. The stereochemistry of the bridgehead proton, at C-8 introduced by hydrogenation, should be controlled by the substituents present on ring A of the cyclised products.

An important consideration was for the production of the key intermediate imides in a concise a manner as possible. The major challenge faced in their synthesis was in developing the desired imide free of N-protection but bearing appropriate protection on the hydroxyl groups. The most practical means of achieving this aim appeared to be initial development of an imide complete with a N-protecting species which could be removed readily. Having secured a tertiary nitrogen, the hydroxyl protecting species could then be introduced in a subsequent step. Selective removal of the N-protecting species would afford the desired intermediate imide. This strategy is illustrated in Scheme 46 for the conversion of D-malic acid (207) into the hydroxyl-protected imide (208).

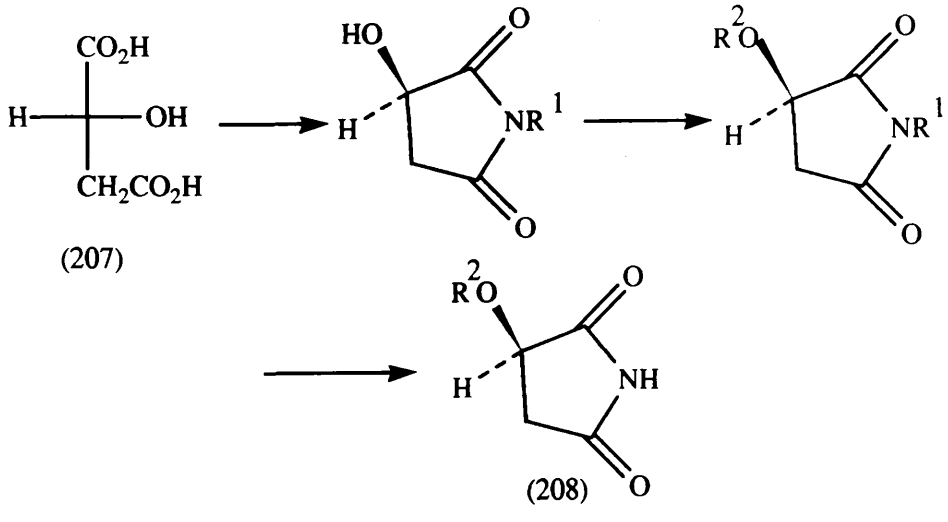


(204) (25)

SCHEME 44



SCHEME 45



(207) (208)

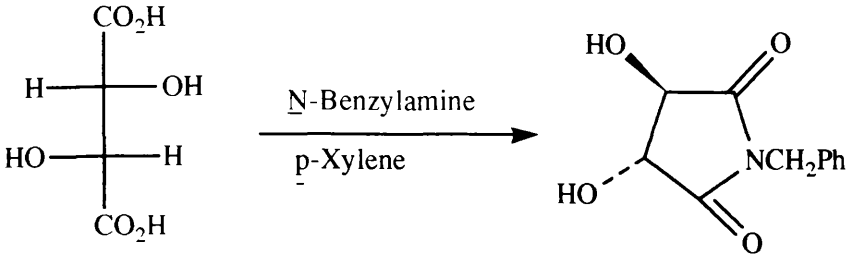
SCHEME 46

Wong et al.[114] had reported treating L-tartaric acid (209) with N-benzylamine to produce tartrimide (210) in their route to diacetyl-anisomycin (211) (Scheme 47). It was felt that this reaction could serve as a suitable means of providing imides with a N-protecting species which could be removed readily. The N-benzyl protection could be removed by hydrogenolysis as Wong et al. employed in their route to (211) from N-benzyl protected amine (212).

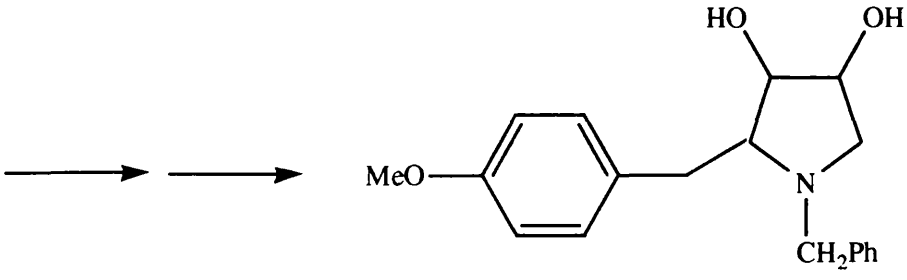
Having introduced the N-benzyl protecting group protection of the hydroxyl groups could then be undertaken. The most appropriate form of protection for the hydroxyl groups of such imides appeared to be as methyl ethers. This was particularly attractive because of the number of methylation procedures documented and the relative stability of the ether linkage. In addition, Yamada and co-workers had reported an efficient method for liberating free alcohols from methyl ethers under mild conditions.[115]

The achiral imide (213) which could be generated from meso-tartaric acid (214) possesses hydroxyl groups with a relative cis-stereochemistry. In this instance a more suitable form of protection might be as an acetal (Scheme 48). Although this linkage is more labile than the methyl ether, it was anticipated that it should still be maintained intact throughout the course of the planned synthesis. If this protection was found to be unsuitable then methylation of the hydroxyl groups of (213) could be used as an alternative. Again it was anticipated that the hydroxyl protecting groups would remain intact during removal of the N-benzyl protection by hydrogenolysis.

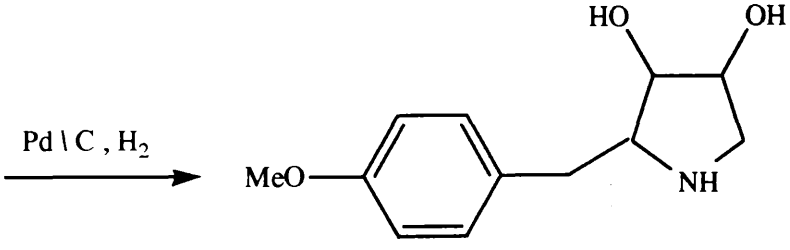
At the advent of this research no synthesis of crotanecine (10)



(2O9) (210)

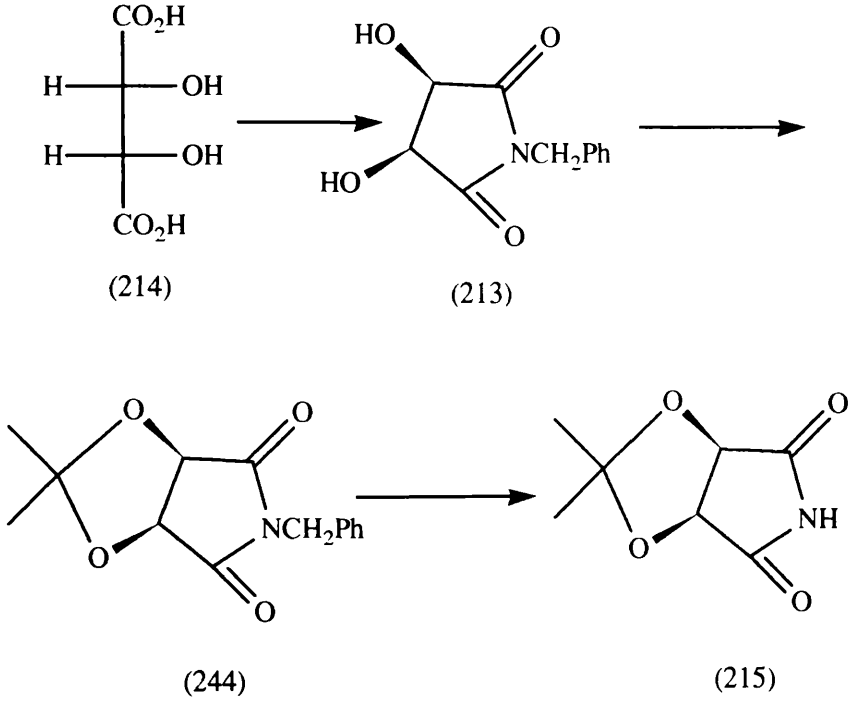


(212)



(211)

SCHEME 47



(214) (213)

(244) (215)

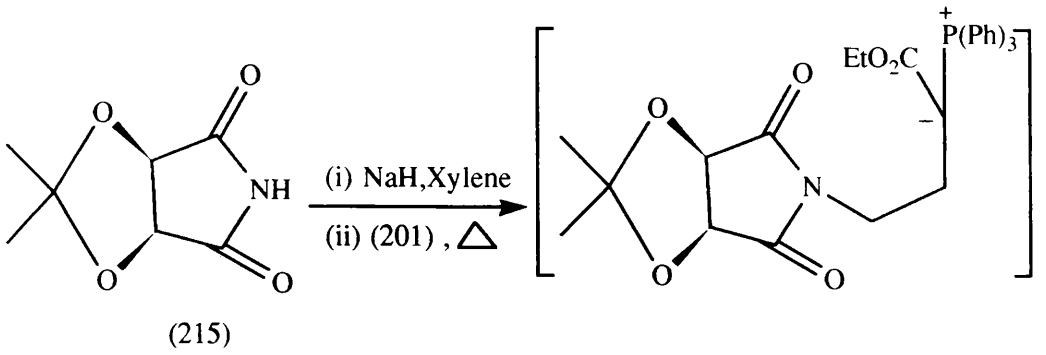
SCHEME 48

had yet been reported. However it was thought that this could be rectified by employing a brief synthetic route from intermediate imide (215) (Scheme 49).

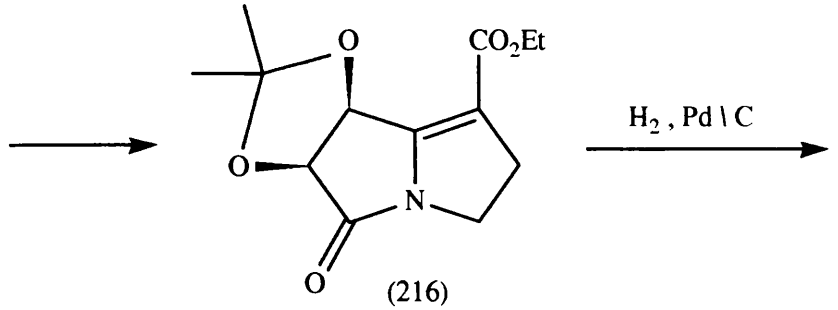
It was anticipated that the condensation of the sodium salt of imide (215) with ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate (201)[110] would generate the conjugated pyrrolizidine lactam (216). Catalytic hydrogenation of (216), influenced by the acetal grouping on ring A, would be expected to yield the racemic 8α-pyrrolizidine (217).[12] The desired 1,2-unsaturation would then be introduced by established methods - namely a selenenylation/reduction/thermal oxidative elimination sequence producing the C-9 alcohol (218). Acidic hydrolysis would then liberate (±)-crotanecine (10) from (218). Modification of this strategy would also enable synthesis of the saturated necine triol (219) from (217), again in racemic form.

In addition to the acetal imide (215), imides derived from the optically active malic and tartaric acids could employ this route to generate necines. This should enable a series of necines to be produced in optically active form, by the same general synthetic strategy.

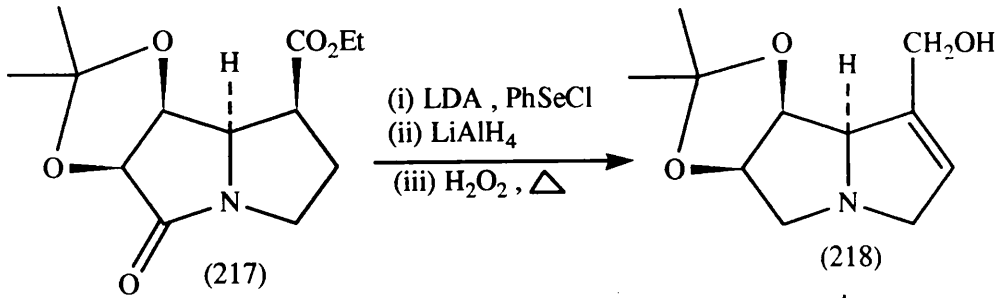
During this study Pestchanker et al.[116] reported isolation of a 13-membered pyrrolizidine macrocyclic diester, uspallatine (220), from plants of Senecio uspallatensis, growing in Argentina. Base hydrolysis of (220) gave the unsaturated necine (221), subsequently known as uspallatinecine.[116] This necine, possessing a 6,7-trans-hydroxylation pattern, could be generated in optically active form from imide (222), derived from L-tartaric acid (209) (Scheme 50).



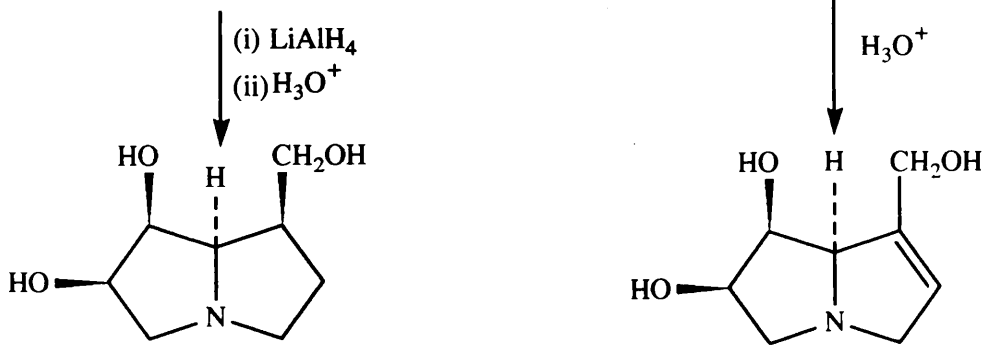
(215)



(216)

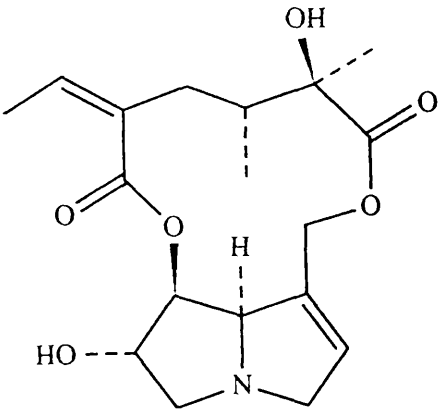


(217) (218)

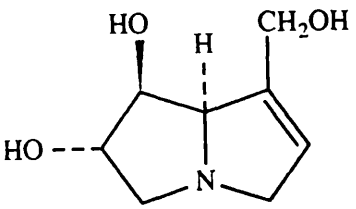


(±)- (219) (±) - (10)

SCHEME 49



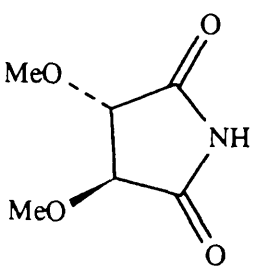
(220)



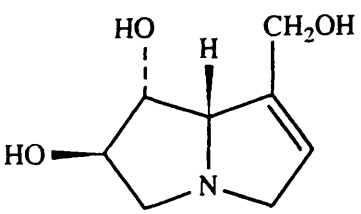
(221)

The methoxyl substituent at C-7 of (223) was expected to have the greater influence on the catalytic hydrogenation step leading to the formation of 1-endo-ester (224). Deprotection of pyrrolizidine (225), affording (+)-uspallatinecine (221), would be accomplished by treatment with BBr_3/15-crown-5 in dichloromethane.[115] The saturated necine (226) should be produced by a reduction-deprotection sequence from (224).

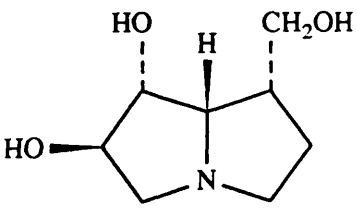
Imide (227), derived from D-tartaric acid should generate the enantiomeric forms of (221) and (226), (228) and (229) respectively, by this route.



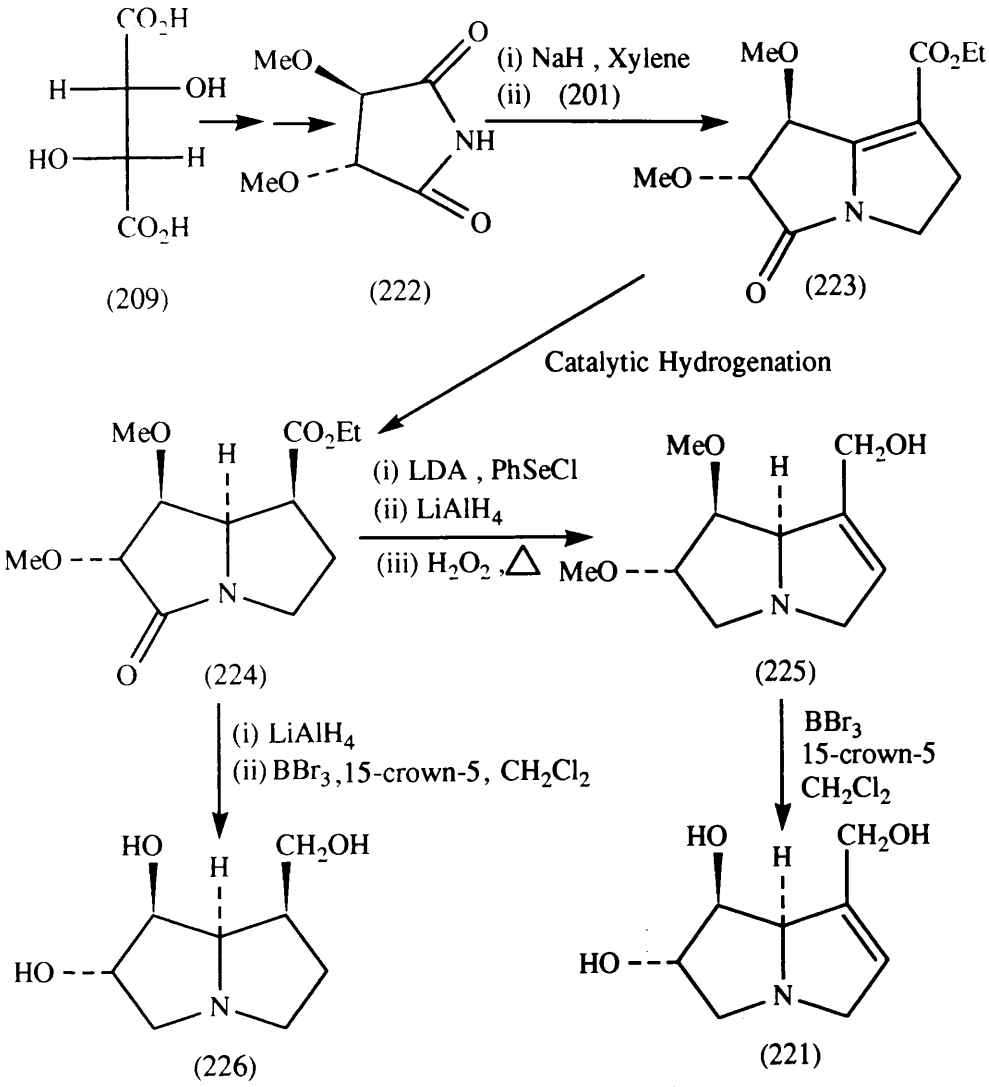
(227)



(228)



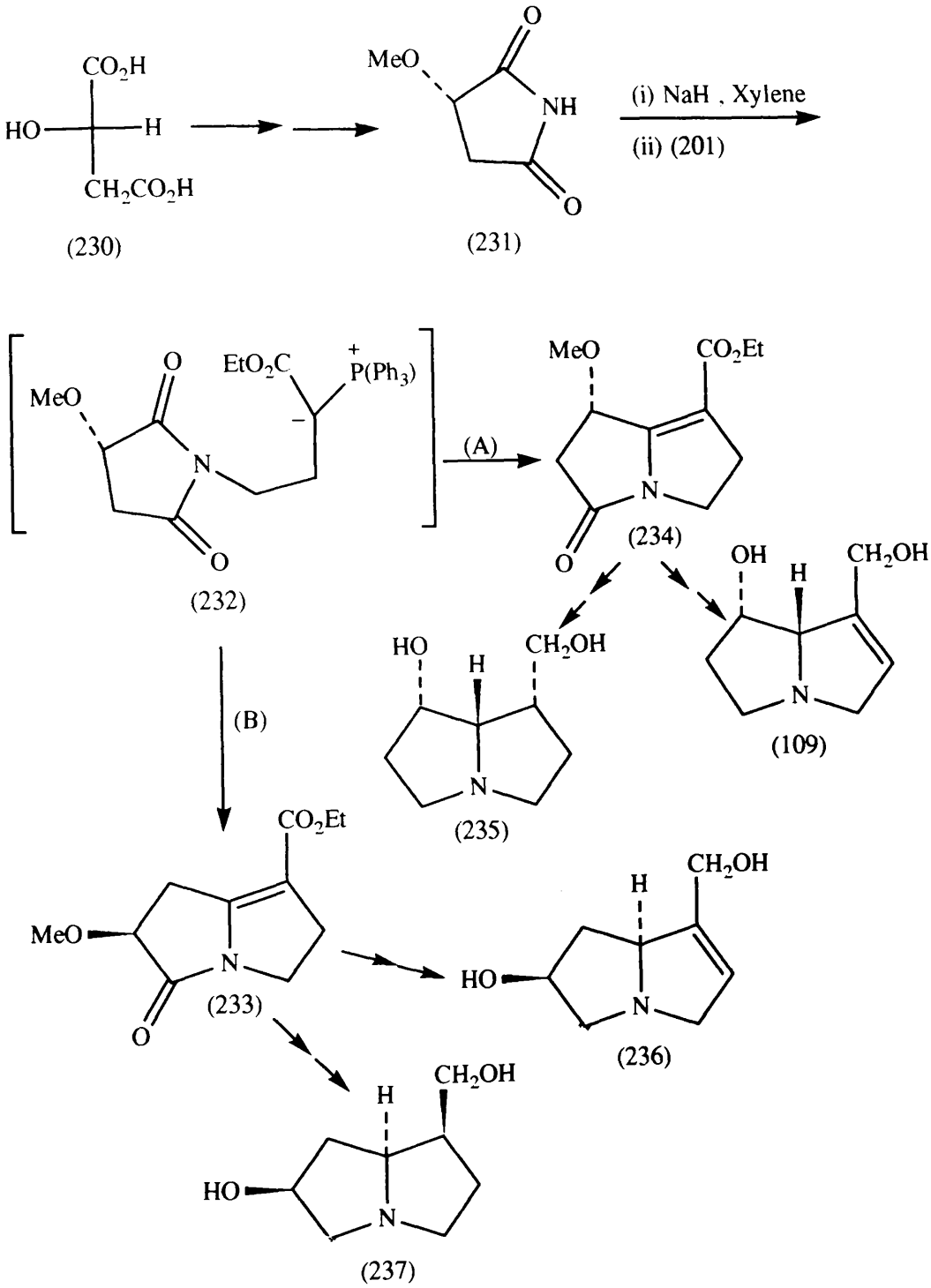
(229)


CO2H
H
OH
HO
H
CO2H
(209)
MeO
O
NH
MeO
O
(222)
(i) NaH , Xylene
(ii) (201)
MeO
CO2Et
MeO
N
O
(223)
Catalytic Hydrogenation
MeO
H
CO2Et
MeO
N
O
(224)
(i) LDA , PhSeCl
(ii) LiAlH4
(iii) H2O2 ,△
MeO
H
CH2OH
MeO
N
(225)
(i) LiAlH4
(ii) BBr3 ,15-crown-5, CH2Cl2
BBr3
15-crown-5
CH2Cl2
HO
H
CH2OH
HO
N
(226)
HO
H
CH2OH
HO
N
(221)

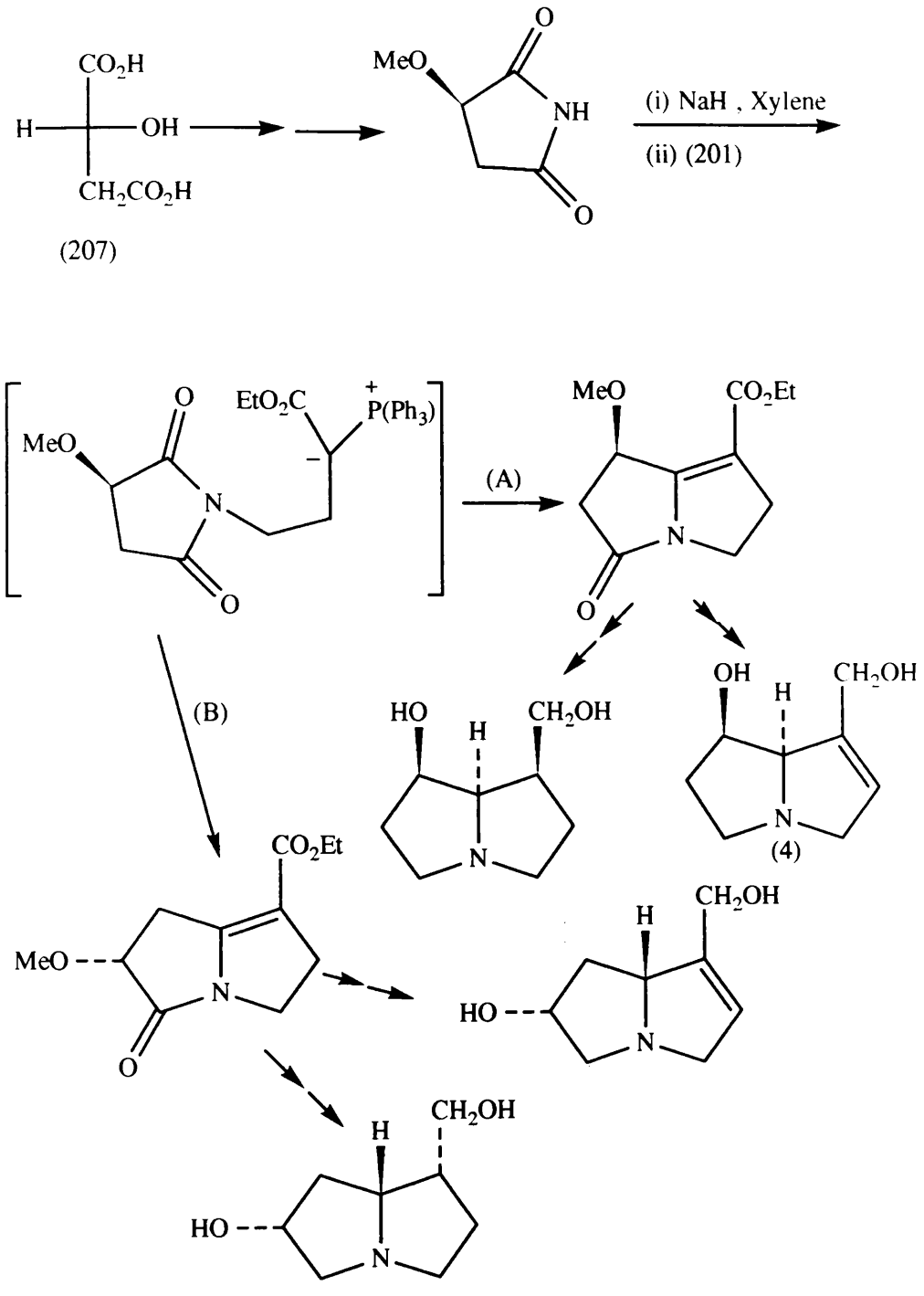
SCHEME 50

Imides, derived from the malic acids, could be used to generate necine diols, in optically active form, by adopting the same synthetic strategy. However, unlike the tartaric acid analogues, the intermediate imides could react with ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate (201) to give two possible products on cyclisation (Scheme 51). The products eventually produced would reflect the outcome of this cyclisation, illustrated in Scheme 51 for synthesis from L-malic acid (230).

Two competing factors are evident on the condensation of the sodium salt of imide (231) with phosphonium salt (201). Firstly the steric effects induced by a nearby methoxyl substituent may help favour cyclisation at the more accessible carbonyl centre of intermediate imide (232) resulting in the preferential formation of the 6β-methoxypyrrolizidine (233).[109] However, the presence of the methoxyl substituent on (232) may favour the opposite mode of cyclisation due to increased electrostatic interaction between the oxygen of the methoxyl group and the phosphorus substituent of (232). This would generate the 7α-methoxypyrrolizidine (234) as the major product.[117] If the conjugated ester (234) was produced on cyclisation then (-)-retronecine (109) and (+)-platynecine (235) could be generated [Scheme 51(a)]. However condensation governed by steric considerations [Scheme 51(b)] would enable synthesis of both (236) and (237) in optically active form. If, as expected, a mixture of products was obtained on cyclisation then it was hoped that separation of the components would be effected by conventional chromatographic techniques. It was again anticipated that stereoselective hydrogenation of the conjugated ester [(233) or (234)] would be dictated


CO2H
HO
H
CH2CO2H
(230)
MeO
O
NH
O
(231)
(i) NaH , Xylene
(ii) (201)
MeO
O
EtO2C
P(Ph3)
N
O
(232)
(A)
MeO
CO2Et
N
O
(234)
OH
H
CH2OH
N
(109)
HO
H
CH2OH
N
(235)
(B)
CO2Et
MeO
N
O
(233)
HO
H
CH2OH
N
(236)
HO
H
CH2OH
N
(237)

SCHEME 51


CO2H
H
OH
CH2CO2H
(207)
MeO
O
NH
O
(i) NaH , Xylene
(ii) (201)
EtO2C
P(Ph3)
(A)
(B)
CO2Et
HO
CH2OH
OH
(4)

SCHEME 52

by the methoxyl group present on ring A.

The complementary sequence is illustrated in Scheme 52 for the possible products from D-malic acid (207) - the enantiomeric form of the anticipated products from Scheme 51.

4.2 Preparation of Imides for Necine Synthesis

The corresponding N-benzylimides were successfully produced by reacting the malic and tartaric acids with N-benzylamine, as outlined by Wong et al.[114] The optically active imides generated from L- and D-malic acid (238) and (239) respectively, together with the dihydroxy-imides (210) and (240), derived from L- and D-tartaric acid were produced in yields comparable to that reported for (210) by Wong et al.[14] In contrast the achiral imide (213), generated from meso-tartaric acid, was synthesised in a disappointing yield (27.8%). The reduced figure, less than half the yield observed for synthesis from the other diacids, might be accounted for by the additional water present in the reaction mixture. This arises because the diacid is provided as a monohydrate.

Attempts to protect the hydroxyl groups of the N-benzylimides presented much greater difficulty. Three methylation procedures, each involving methyl iodide as the methylating agent, were tried but none enabled synthesis of the anticipated product.

Johnstone and Rose[118] reported a facile method suitable for the alkylation of alcohols. The reaction was performed in THF using MeI and KOH to induce methylation. However, attempts to methylate (238), (210), and (213) by this method did not result in the generation of the desired products [(241), (242), and (243)]. Even the introduction

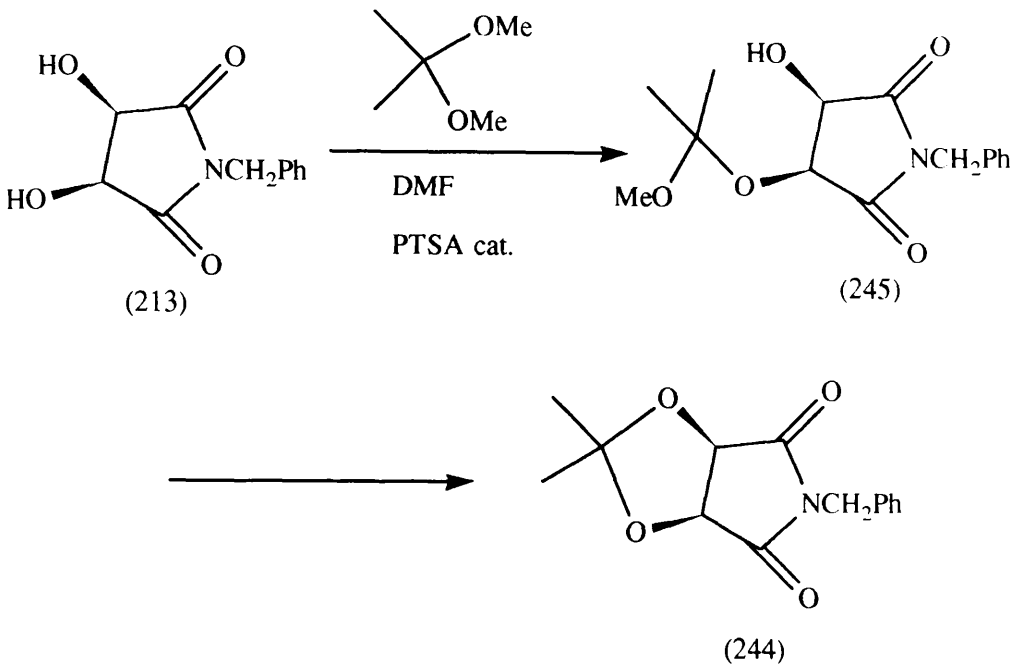
of elevated temperatures and prolonged reaction times failed to generate the desired product. Indeed a considerable quantity of starting material was recovered in all instances.

A second method, reported by Diner et al.,[119] employed methyl iodide, sodium hydride as base and DMSO as solvent. Again this method proved unsuitable for methylation of the N-protected imides. This method is reported to function at room temperature but application of these conditions did not generate the desired product, although no starting material was evident at the end of the reaction. The result was repeated when the reaction was carried out at reduced temperatures with shorter reaction times.

A third methylation procedure involved the mild Ag_2O/methyl iodide method utilised by Purdie and Irvine[120] in the methylation of esters of the tartaric acids. However, this method again failed to provide a suitable means of synthesising the desired compounds.

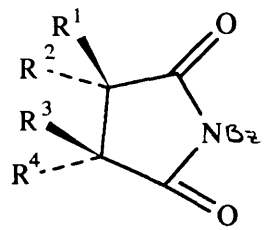
Several methods were pursued, with contrasting results, in an effort to generate acetonide (244) from dihydroxyimide (213). The most productive was undoubtedly through alcohol exchange with excess 2,2-dimethoxypropane in DMF in the presence of a PTSA catalyst (Scheme 53). The reaction proceeded in 62% yield, with the presence of DMF shown to be crucial. Employing only 2,2-dimethoxypropane as its own solvent[121] for this reaction gave little product, with almost all starting material remaining unreacted, even after protracted reaction times. A second product was evident by t.l.c., thought to be the intermediate alkylation product (245) of this reaction.

Other methods tried were acetone in petroleum-ether with PTSA



(213) (245) (244)

SCHEME 53



$R^2 = OH, R^1 = R^3 = R^4 = H$ (238)

$R^1 = OH, R^2 = R^3 = R^4 = H$ (239)

$R^1 = R^4 = OH, R^2 = R^3 = H$ (210)

$R^2 = R^3 = OH, R^1 = R^4 = H$ (240)

$R^1 = R^3 = OH, R^2 = R^4 = H$ (213)

$R^2 = OMe, R^1 = R^3 = R^4 = H$ (241)

$R^1 = R^4 = OMe, R^2 = R^3 = H$ (242)

$R^1 = R^3 = OMe, R^2 = R^4 = H$ (243)

catalyst; acetone in benzene employing PTSA catalyst;[122] and acetone in acetic anhydride using H_2SO_4 catalyst.[123] However, all of these methods were found to be unsuitable for efficient synthesis of (244). Indeed only the second of these methods (acetone/benzene/PTSA cat.) gave any evidence for the formation of acetal (244), although almost all material recovered was starting material (213).

Having encountered difficulty in the protection of the hydroxyl groups of the N-benzylimides the proposed route became less attractive when it became apparent that the hydrogenolysis step to remove the N-benzyl protection from the imides did not proceed smoothly, even under forcing conditions. Attempts to remove the N-benzyl protection from acetal (244), and optically active imides (238) and (210) were unsuccessful. The catalysts employed for these reactions were PtO_2 and Pd/C.

Having encountered considerable problems in the synthesis of the intermediate imides, via N-benzyl protected imides, it was decided to seek an alternative route for their synthesis.

Attention was focussed on the literature preparation of dimethoxyimide (222), in optically active form, from diethyl L-tartrate (246). This strategy is outlined in Scheme 54. Purdie and Irvine[120] reported the synthesis of diethyl dimethoxysuccinate (247) by methylation of (246), employing methyl iodide and Ag_2O. The step proceeded in high yield (91%) and, in common with the other steps of the route, the stereochemistry was maintained. The same report described the generation of diacid (248) by base hydrolysis of diester (247). At a later date Purdie and Young[124] generated anhydride (249) by treatment of diacid (248) with excess acetyl chloride. Young later reported the

conversion of anhydride (249) into imide (222).[125] This proceeds via the amic acid/ammonium salt (250). However, conversion of (250) into dimethoxyimide (222) was reported to proceed in a yield of less than 30%.

Although this route is more lengthy than ideally sought, it did provide a suitable means of generating the key intermediate imides from the malic and tartaric acids. The use of optically active starting materials would enable the key intermediate imides for necine synthesis to be prepared in optically active form.

Three imides were prepared by this route. These were dimethoxyimide (222); methoxyimide (231) from L-malic acid (230) as starting material; and the achiral dimethoxyimide (251) from meso-tartaric acid (214).

L-Malic acid (230) and meso-tartaric acid (214) were converted into the corresponding diesters [(252) and (253)] by the esterification procedure reported by Locquin and Elghozy[126] (Scheme 55). The starting material for synthesis of imide (222) was diethyl L-tartrate (246).

Although the three methoxyimides were successfully prepared by this route, certain problems were apparent in their synthesis.

Some epimerisation was noted in the synthesis of anhydride (254) and imide (251) from diacid (255) and amic acid/ammonium salt (256) respectively. This resulted in the generation of (±)-anhydride (249) and (±)-imide (222).

Epimerisation was particularly severe in the synthesis of imide (251), with an approximately equal quantity of (±)-(222) being produced. A negligible quantity of meso-imide (251) was formed in the synthesis of imide (222) from anhydride (249). However, the detection of meso-imide

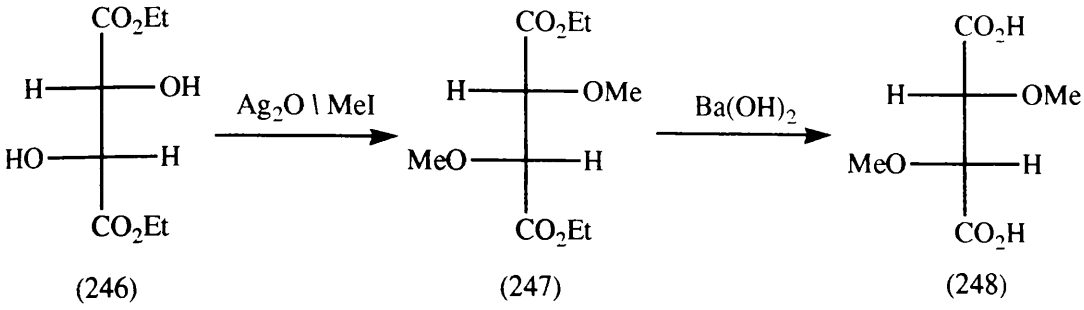
(251) was only achieved by t.l.c., and recrystallisation of the crude product of the reaction, in benzene, yielded only pure (222). There was no evidence for the formation of anhydride (254) during the synthesis of (249) from diacid (248). The yield witnessed for the formation of methoxyimide (222) from anhydride (249), 64%, was a considerable improvement upon the literature value.

The synthesis of the optically active imide (231) was characterised by a very poor yielding final stage [8.3% conversion of anhydride (257) into imide (231)]. The reaction generated many unidentified by-products and the desired imide was isolated only after extensive chromatography.

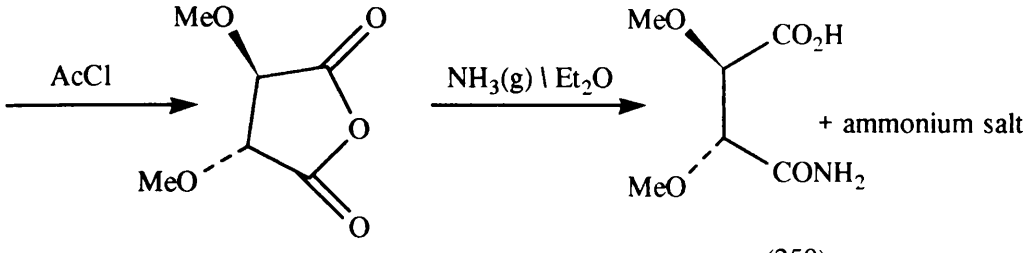
The intermediate anhydrides [(254), (249), and (257)] were all prone to conversion into the corresponding diacids on exposure to moisture. Purdie and Irvine had encountered a similar problem during synthesis of anhydride (249).[120]

Another problem associated with the synthetic route was the expense of employing Ag_2O as a reagent early in the synthetic sequence. In doing so this limited the quantity of intermediate imides which could be produced. Two other methylation procedures were investigated in an effort to overcome this problem but neither proved to be a suitable alternative to the Ag_2O/MeI method.

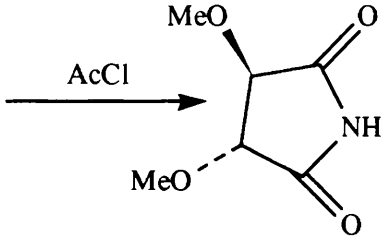
Attempts to produce methylated diester (247) from (246), employing NaH/MeI as the methylating reagent,[127] resulted in epimerisation of the product (247) as well as incomplete methylation of (246). The use of K_2CO_3/dimethyl sulphate proved to be unsuitable with incomplete methylation of starting material evident, even with protracted reaction times.[128]



(246) (247) (248)

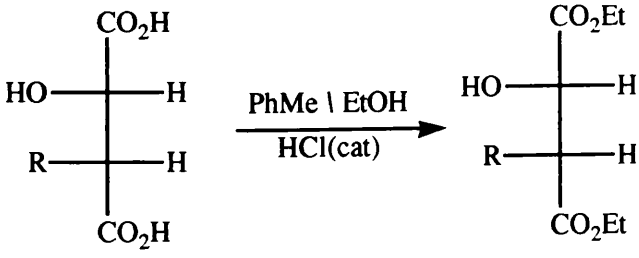


(249) (250)



(222)

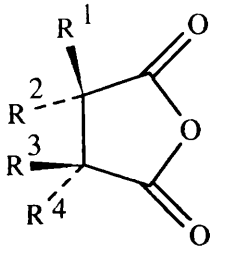
SCHEME 54



R = H (230)
R = OH (214)

R = H (252)
R = OH (253)

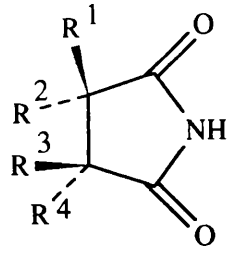
SCHEME 55



$R^1 = R^4 = MeO$; $R^2 = R^3 = H$ (249)

$R^1 = R^3 = MeO$; $R^2 = R^4 = H$ (254)

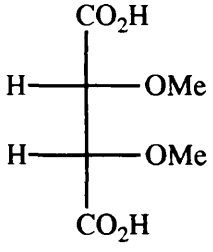
$R^1 = R^3 = R^4 = H$; $R^2 = MeO$ (257)



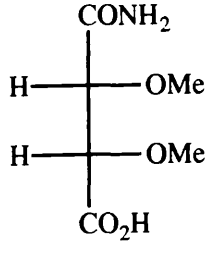
$R^1 = R^4 = MeO$; $R^2 = R^3 = H$ (222)

$R^1 = R^3 = MeO$; $R^2 = R^4 = H$ (251)

$R^1 = R^3 = R^4 = H$; $R^2 = MeO$ (231)



(255)



+ ammonium salt

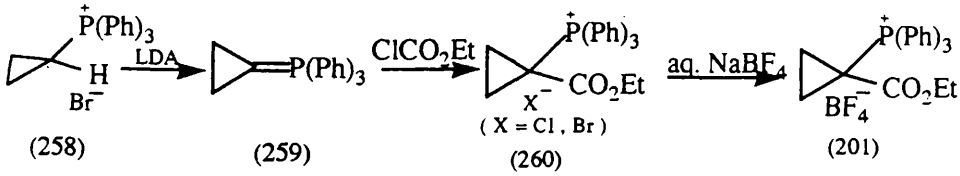
(256)

The yields obtained for the methylation of the hydroxydiesters using Ag_2O/MeI were slightly better than the value reported for synthesis of (247). However, the use of Et_2O as solvent for this reaction, or employing Ag_2O prepared from $AgNO_3$ indicated no improvement on the literature yield for this reaction.

4.3 Preparation of Ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate (201)

Fuchs reported the synthesis of (201) from cyclopropyltriphenylphosphonium bromide (258)[110] (Scheme 56).

This procedure involves initial treatment of (258) with LDA (lithium diisopropylamine) to generate the ylide (259). Addition of ethyl chloroformate to the reaction mixture affords the cyclopropane ester (260) which undergoes anion exchange using excess aq. $NaBF_4$ to complete the synthesis of (201).



(258) (259) (260) (201)

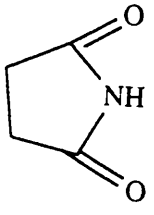
SCHEME 56

Initial attempts to produce phosphonium salt (201) by this method in sufficient purity for subsequent use proved unsuccessful, even

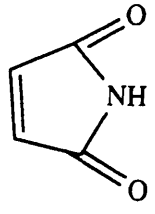
when a more thorough experimental procedure provided by the author was implemented.[129] Although ^{1}H n.m.r. spectroscopy indicated the formation of (201) problems were encountered on trying to purify the product by recrystallisation. However, these difficulties were overcome by the insertion of a filtration step into the experimental procedure prior to anion exchange with excess aq. $NaBF_4$. The filtrate obtained was dried and then anion exchange undertaken. This enabled phosphonium salt (201), of improved quality, to be produced in a 58.1% yield from (258) after recrystallisation.

4.4 Development of the Pyrrolizidine Ring System from the Condensation of Imides with Ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate (201)

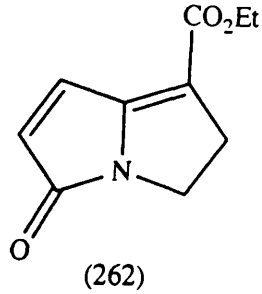
Before reaction of the methoxyimides produced from the malic and tartaric acids with phosphonium salt (201) was carried out, a study was undertaken in an effort to gain a better understanding of the influence which the parent imide, solvent, and base employed exert on the condensation with (201) to generate the pyrrolizidine ring system. For this purpose two imides were used, namely succinimide (205) and malemide (261).



(205)



(261)



(262)

Reaction of malemide (261) with phosphonium salt (201) gave the expected product, the conjugated ester (262), but in poor yield. High resolution mass spectrometry established that the product of this cyclisation corresponded to a molecular formula of $C_{10}H_{11}NO_3$. An i.r. spectrum gave maxima at 1735, 1690 and 1650 cm^{-1}. These peaks correspond to an α,β-unsaturated ester, conjugated amide, and an olefinic peak. A ^1H n.m.r. spectrum was also consistent with the formation of (262), including olefinic signals centred at δ6.38 and 6.44 ppm.

The results of the experiments for the formation of (262) are displayed in Table 2.

Table 2. Production of (262) from condensation of malemide (261) with (201)

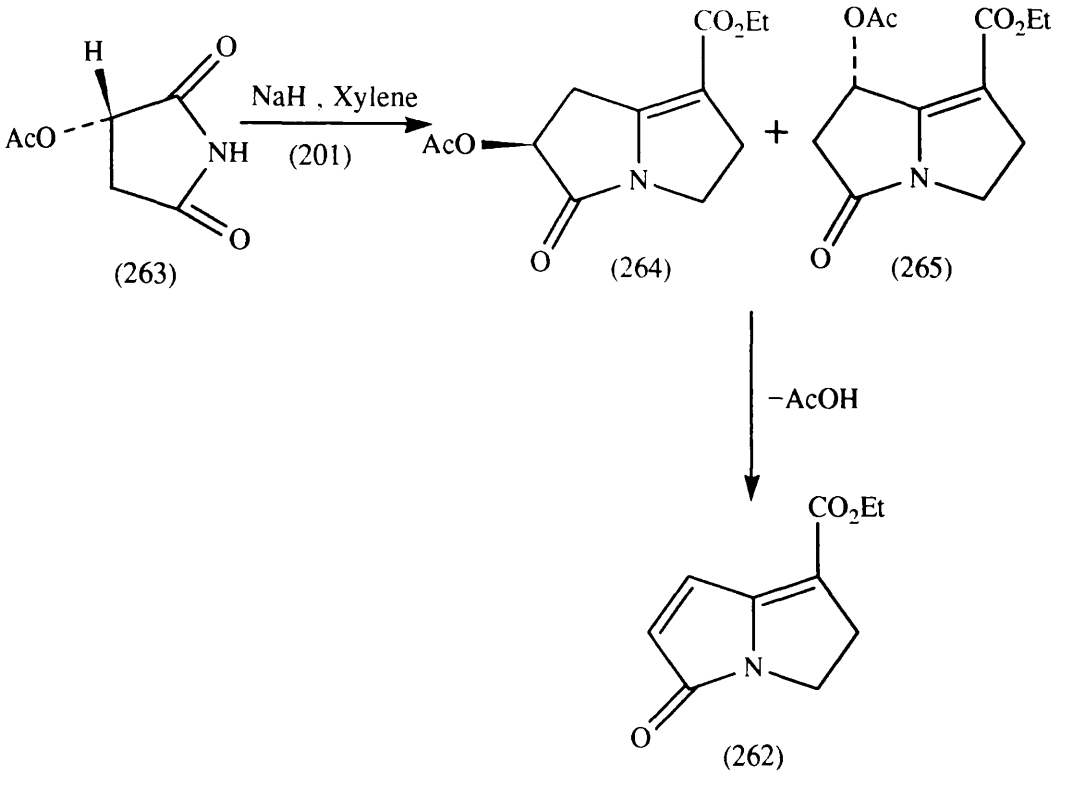
Base (no. of equiv.)	No. of Equiv. of (201)	Solvent	Reflux Time	Yield of (262) obtained
n-BuLi	1.0	Toluene	2½ h	5.5%
n-BuLi	1.0	Benzene	2½ h	10.1%
n-BuLi	1.0	Xylene	2½ h	7.0%
NaH	1.0	Xylene	2½ h	2.3%

These results suggested that benzene might be the most appropriate solvent for carrying out this particular reaction. It is possible that the lower reaction temperature ensuing from employing benzene as solvent enhances the stability of (262), though (262) was found only to be stable for a couple of days at low temperatures, enabling spectroscopic data to be obtained.

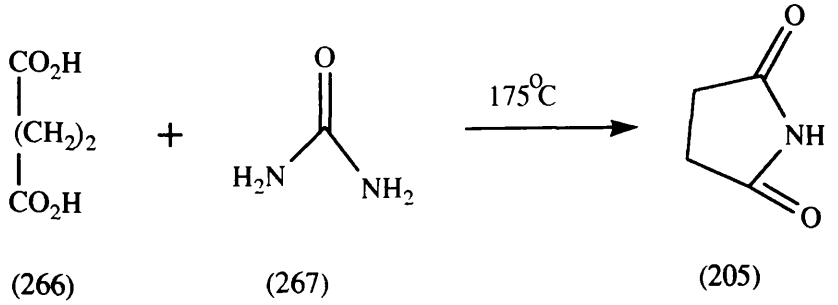
At a later date it was learned that Flitsch and his co-workers had reacted (S)-2-acetoxysuccinimide (263) with phosphonium salt (201) to generate a mixture of acetoxyesters (264) and (265) in a combined yield of 2%.[130] It was also noted that both these esters readily eliminated the elements of acetic acid to form (262), which was itself reported to be unstable (Scheme 57).

Most of the preliminary work on this cyclisation, however, used succinimide (205) as the imide source. Initially experiments were carried out using succinimide (205) generated by the procedure reported by Sheng-Ma and Sah (Scheme 58).[131] This generates succinimide (205) by reaction of succinic acid (266) and urea (267) under melt conditions. The crude succinimide obtained from this reaction was then purified by recrystallisation from ethanol. Although this method proved successful for the synthesis of succinimide (205) it could not be adapted for the generation of methoxyimides for necine synthesis from the malic and tartaric acids.

A series of experiments assessed the influence which three bases have on the condensation of succinimide (205), prepared by the method of Sheng-Ma and Sah,[131] with phosphonium salt (201). The bases were sodium methoxide, sodium hydride, and n-butyllithium. In addition the fusion of the sodium salt of succinimide (202) with (201) was investigated, while succinimide was sublimed before use in some later experiments when it became apparent that the presence of water in the reaction mixture was influencing the outcome. These experiments are documented in Table 3.



SCHEME 57



SCHEME 58

Table 3. Condensation of succinimide (205), prepared by the method of Sheng-Ma and Sah,[131] with phosphonium salt (201)

Expt. no.	Form of reactant imide	Base used (no. of equiv.)	Solvent used (reflux time)	% Yield of unknown (268)	% Yield of ester (269)	No. of equiv. of (201)	Comments
1	Succinimide	NaOMe (1.05)	Xylene (5h)	11	26	1.04	
2	Succinimide	NaOMe (1.11)	Xylene (15½h)	16	18	1.15	
3	Succinimide	NaOMe (1.11)	Xylene (40h)	14	19	1.09	
4	Succinimide	n-BuLi (1.10)	Xylene (43h)	42	18	1.09	
5	Succinimide	n-BuLi (1.02)	Xylene (48h)	34	20	1.09	
6	Succinimide	NaH (1.25)	Xylene (2½h)	32	21	1.01	
7	Sodium Succinimide	-	Xylene (22h)	12	19	1.05	Salt prepared beforehand
8	Sodium Succinimide	-	Xylene (4½h)	11	18	1.00	Salt prepared beforehand
9	Sodium Succinimide	-	Xylene (3½h)	10	22	1.08	Salt prepared beforehand
10	Sodium Succinimide	-	Xylene (20h)	14	16	1.10	Salt prepared beforehand
11	Sodium Succinimide	-	140°C / 4h	12	11	1.00	Melt Conditions[109]
12	Succinimide (sublimed)	n-BuLi (1.00)	Xylene (2½h)	11	16	1.00	
13	Succinimide (sublimed)	NaH (1.25)	Xylene (2½h)	31	22	1.00	
14	Succinimide (sublimed)	NaH (1.29)	Xylene (2½h)	23	27	1.00	
15	Succinimide (sublimed)	NaH (1.25)	Xylene (4h)	22	24	1.10	
16	Succinimide	NaH (1.30)	Xylene (2h)	19	20	1.00	
17	Succinimide	NaH (1.25)	Xylene (2½h)	15	4	1.00	
18	Succinimide	NaH (1.25)	Xylene (2½h)	17	19	1.00	

A further series of experiments was later carried out with succinimide obtained from a chemical reagent supplier (Aldrich). The results obtained from this experimental programme are illustrated in Table 4.

Table 4. Reaction of succinimide (205) with phosphonium salt (201)

Expt. no.	Base (no. of equiv.)	No. of equiv. of (201)	Solvent	Reflux Time	% Yield of (203)
1	NaH (1.20)	1	Xylene	2½h	36
2	NaH (1.22)	1	Xylene	2½h	41
3	NaH (1.25)	1	Xylene	2½h	47
4	NaH (1.78)	1	Xylene	2h	33
5	NaH (1.25)	1	Toluene	2h	18
6	NaH (1.25)	1	Benzene	3h	16
7	n-BuLi (1.0)	1	Xylene	2½h	18
8	n-BuLi (1.1)	1	Xylene	2h	12
‡9	NaH (1.25)	1	Xylene	2½h	21

‡ H_2O added to reaction

Intriguingly it was found that different products were produced on reaction of the two succinimide sources with phosphonium salt (201).

Reaction of succinimide produced by the method of Sheng-Ma and Sah invariably gave rise to a mixture of two succinimide derived products [unknown (268) and ester (269)] despite significant variation in the experimental conditions. In contrast, only one succinimide-derived product was evident from reaction of succinimide (205) supplied by Aldrich

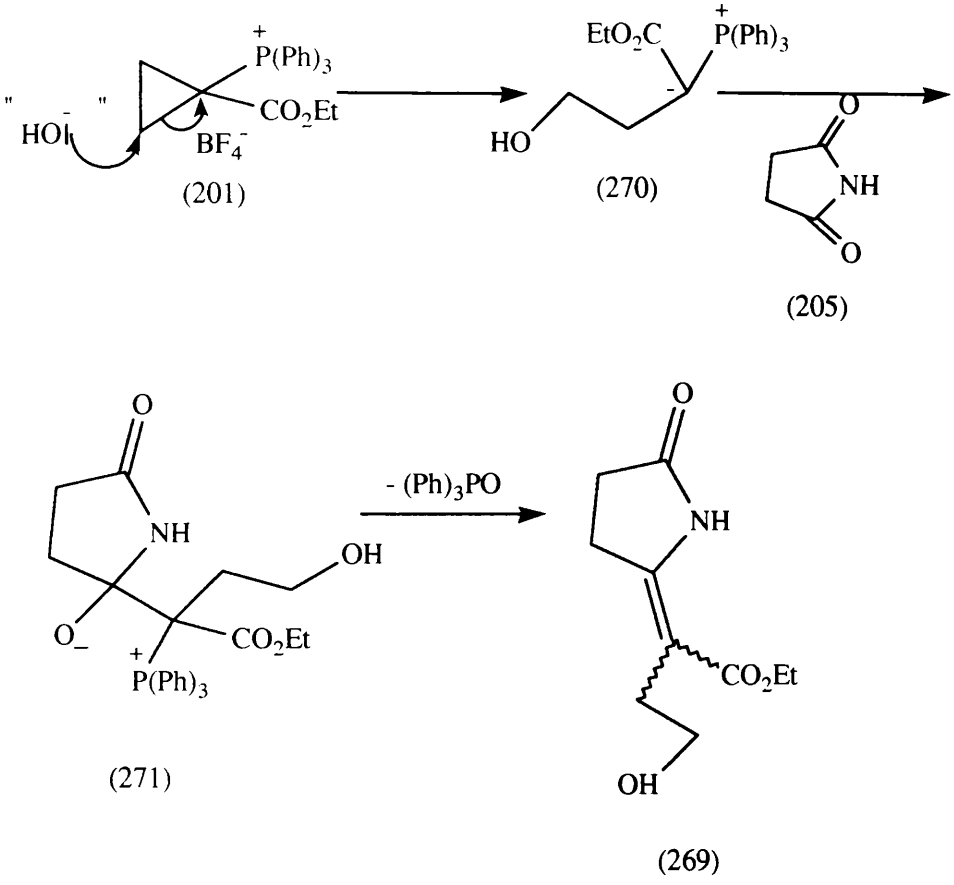
in this reaction. However this was the expected product, conjugated ester (203).

The production of conjugated esters (269) is thought to arise from the involvement of water in the reaction process (Scheme 59). The presence of water, under basic conditions, can be regarded as a potential source of hydroxide anion. Reaction of 'hydroxide' with phosphonium salt (201) can afford the aliphatic ylide (270). Condensation of (270) with succinimide (205) would generate the intermediate Zwitterion (271), from which rapid elimination of $Ph_3P(O)$ could ensue to produce a mixture of isomeric esters (269).

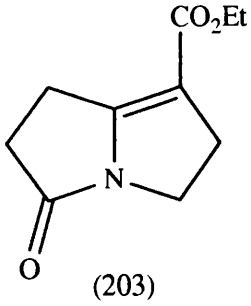
The principal source of water in this reaction is thought to originate from the recrystallisation of succinimide, as a monohydrate, from ethanol. However, reaction of crude succinimide produced by the method of Sheng-Ma and Sah (before recrystallisation) again generated a mixture of unknown (268) and (269), but with the relative proportion of the latter significantly reduced (Table 3, Experiment 17). This may indicate the presence of a reduced quantity of water in the crude succinimide before recrystallisation.

Addition of water to the reaction involving the use of 'pure' succinimide generated neither (268) or (269) as product, although the yield of ester (203) obtained was reduced (Table 4, Experiment 9). This suggests that a further impurity is responsible for the generation of (268) and (269).

The results displayed in Table 3 indicate that succinimide prepared by the method of Sheng-Ma and Sah yields a greater quantity of unknown (268) when n-BuLi, the strongest base tested, was used. By


P(Ph)3
CO2Et
HO
BF4-
(201)
EtO2C
P(Ph)3
HO
(270)
O
NH
O
(205)
O
NH
OH
O
CO2Et
P(Ph)3
(271)
- (Ph)3PO
O
NH
CO2Et
OH
(269)

SCHEME 59


CO2Et
N
O
(203)

contrast, hydroxyester (268) was the dominant product of the mixture when sodium methoxide was employed as base in this reaction.

The experiments using commercially available succinimide for reaction with phosphonium salt (201), confirmed xylene as the most effective solvent of those employed and verified the optimum level of 1.25 equivalents of NaH reported for this reaction.

The unidentified product of the cyclisation, (268), was initially thought to be ester (203). In common with (203) the unidentified product (268) demonstrated a parent ion of m/z 195 in high resolution mass spectrometry - corresponding to a molecular formula of $C_{10}H_{13}NO_3$. In addition, an i.r. spectrum of (268) revealed maxima at 1645, 1690, and 1730 cm^{-1}. The conjugated ester (203) first prepared by Muchowski and Nelson,[109] demonstrates maxima in an i.r. spectrum at 1650 (C=C), 1685 (amide), and 1730 cm^{-1} (α,β-unsaturated ester).

Assignment was made more difficult by the reported ^1H n.m.r. spectral assignments for ester (203). Flitsch and co-workers had assigned a spectrum of (203) with respect to 14 protons[111] and later for 15 protons[112] instead of the intended 13. The ^1H n.m.r. spectrum of (268) indicated a mixture of compounds - by the duplication of the ester proton signals (Figure 1). In comparison to a ^1H n.m.r. spectrum of (203) [Figure 2], (268) displayed two protons shifted downfield to δ4.40 from around 3.75 ppm. In addition, two protons were evident upfield at δ2.70-3.05 compared to a signal at δ3.0-3.1 ppm for (203). The u.v. traces for (203) and (268) were also similar with maxima at 280 nm (203) and 290 nm (268).

However, significant differences were apparent between the two

compounds. Whereas ester (203) was readily hydrogenated, unknown (268) proved resistant to reaction under these conditions. T.l.c. also indicated that (268) was a considerably less polar compound than ester (203). The respective R_f values were 0.36 for (203) and 0.69 for (268) [run in $CH_2C\ell_2$/AcOEt (1:1)]. In addition (268) was a pale yellow oil at ambient temperature while ester (203) was a white solid of m.p. 60-61°C. Ester (203) was a comparatively stable compound whereas unknown (268) underwent decomposition at ambient temperature within a few days of synthesis. It was also observed that (268) was resistant to treatment with D.B.U. (1,8-diazabicyclo[5.4.0]undec-7-ene).

^1H N.m.r. and i.r. spectroscopic data indicated that (268) did not possess either an amine or hydroxyl proton. In addition, ^1H n.m.r. spectroscopy served to highlight the absence of any olefinic protons from (268).

Having carried out preliminary investigation of the cyclisation with malemide and succinimide, attention was then directed to reaction of the methoxyimides, prepared from the malic and tartaric acids, with (201).

Condensation of dimethoxyimide (222) with phosphonium salt (201) proceeded smoothly when NaH was employed as base to afford the anticipated conjugated ester (223) in optically active form (Scheme 60). The cyclised product gave a parent ion corresponding to a molecular formula of $C_{12}H_{17}NO_5$. In addition an i.r. spectrum demonstrated maxima at 1735, 1695, and 1650 cm^{-1}. These corresponded to α,β-unsaturated ester, amide, and olefinic peaks respectively. In addition, ^{13}C n.m.r. and ^1H n.m.r. (Figure 3) spectra were in agreement with (223) being the structure of the cyclised product. No olefinic proton signal was evident

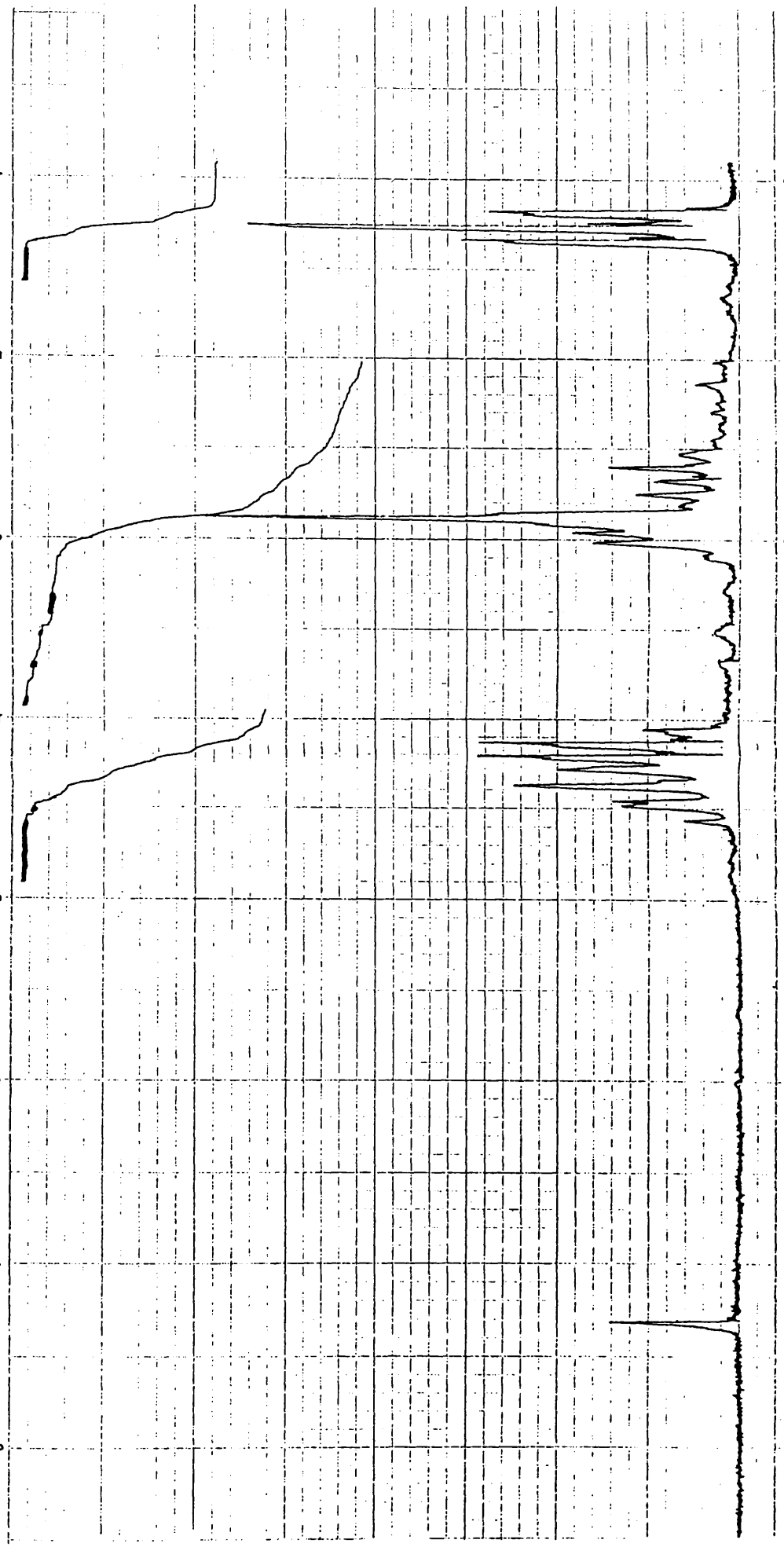


Figure 1. ^{1}H N.m.r. spectrum for unknown (268).

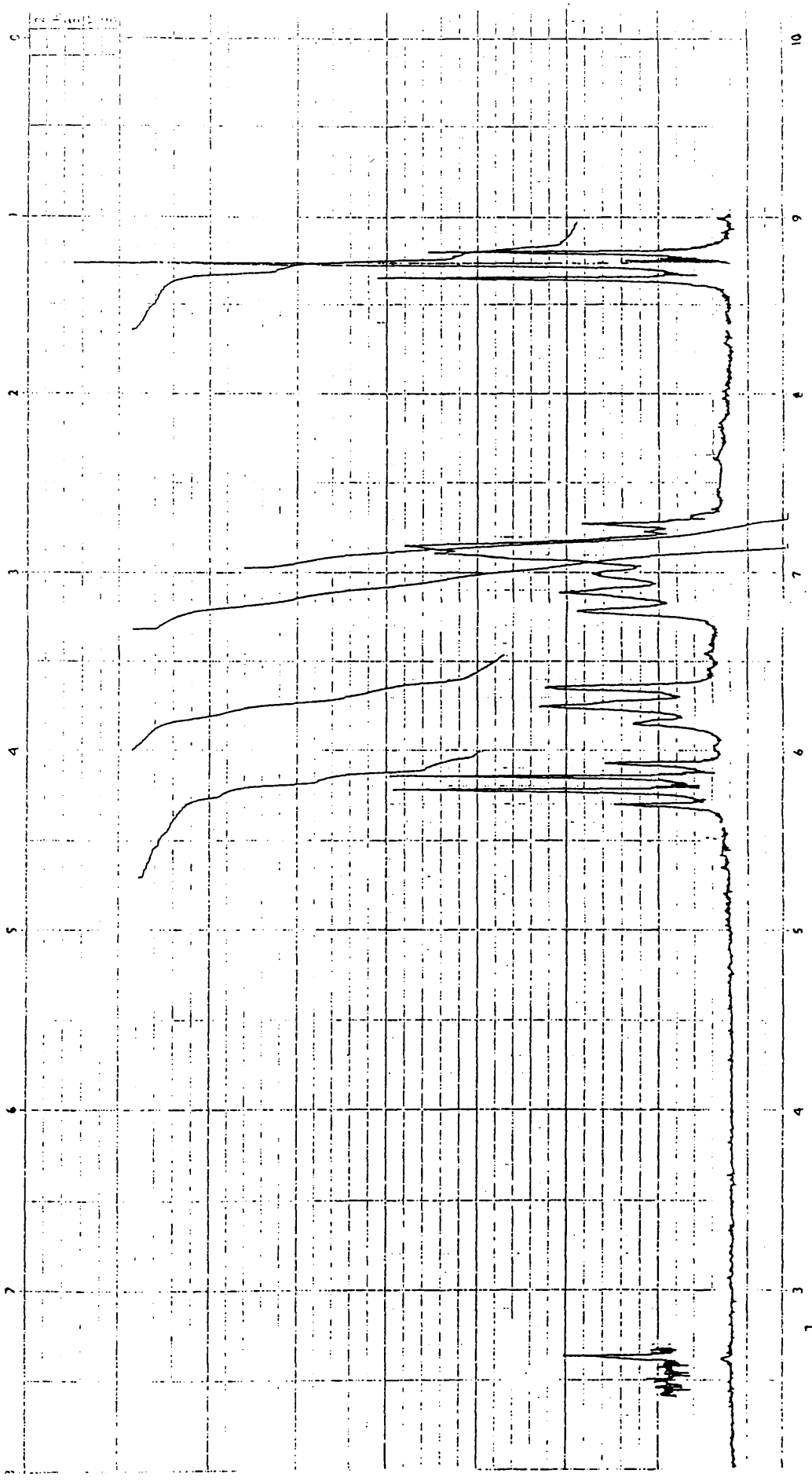


Figure 2. ^{1}H N.m.r. spectrum for pyrrolizidine ester (203).

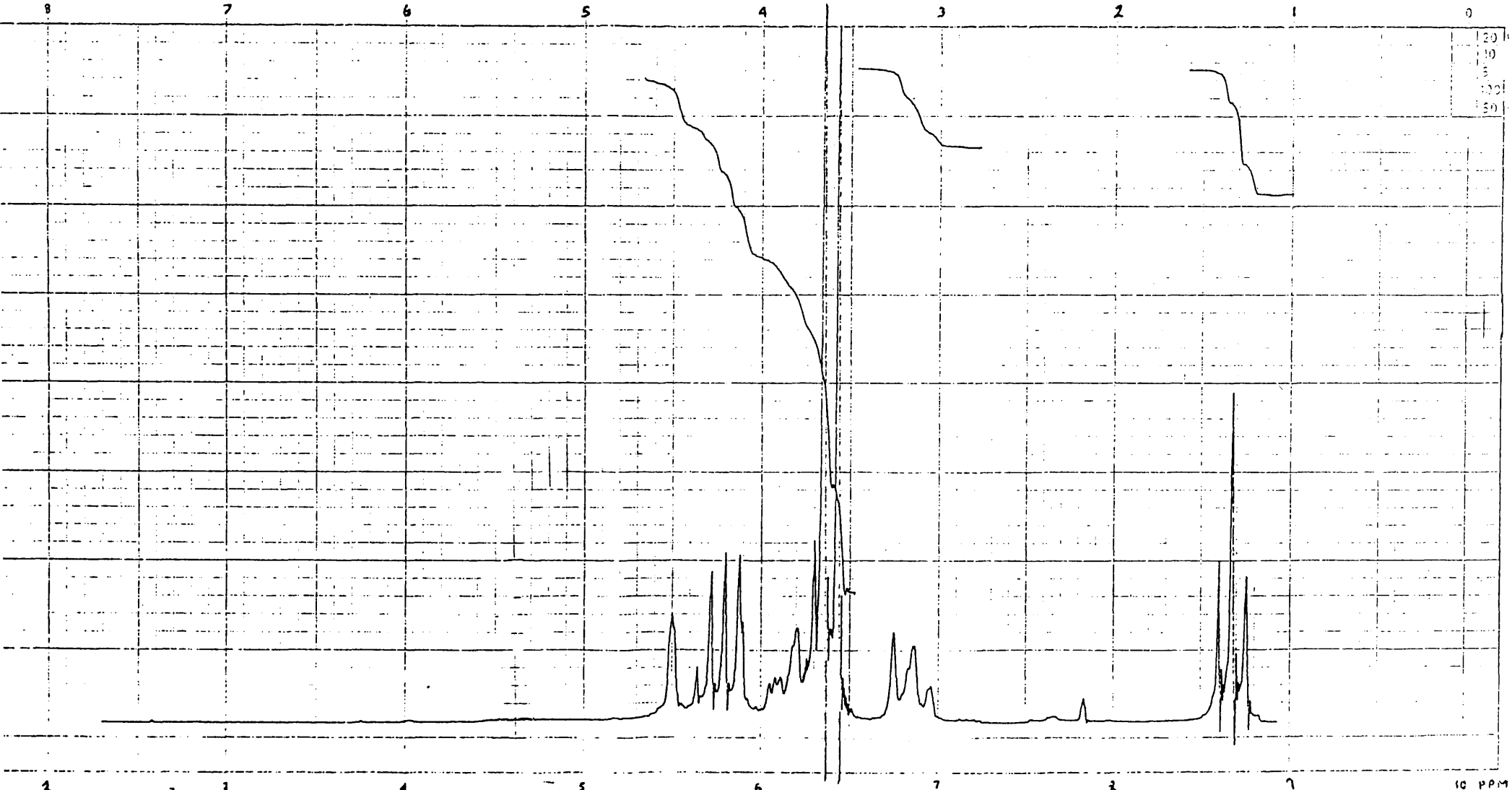


Figure 3. ^{1}H N.m.r. spectrum for dimethoxypyrrolizidine ester (223).

while methoxyl proton signals were at δ 3.40 and 3.46 ppm in the ^1H n.m.r. spectrum.

The result of this cyclisation was encouraging with yields as high as 70% being recorded (Table 5). It was also noted that there was no evidence of epimerisation during the reaction even when excess NaH was used.

However, replacement of NaH with n-butyllithium as base for this reaction did not generate the desired ester (223). Moreover no starting material, imide (222), was recovered from this reaction.

Table 5. Yield of ester (223) obtained from condensation of imide (222) with phosphonium salt (201)

No. of equiv. of NaH	% Yield of (223) obtained
1.20	40
1.23	55
1.27	70
1.35	63
1.97	60

Reaction of the achiral dimethoxyimide (251) with phosphonium salt (201) proceeded in good yield. However, two condensation products were produced in this reaction and these were found to be present in an approximate ratio of 2:1 (Scheme 61) - estimated by ^1H n.m.r. spectroscopy - and separated by column chromatography.

The major product was the anticipated meso-ester (272), but this cyclised product could not be separated from the by-product of the

cyclisation, triphenylphosphonium oxide.

A ^{1}H n.m.r. spectrum of the mixture of (272) and triphenylphosphonium oxide gave methoxyl proton signals at δ 3.45 and 3.57 ppm. In addition a doublet (J 6 Hz) was observed at δ 4.76 corresponding to the C-6 proton being coupled to the C-7 proton. The signal for the C-6 proton is found at δ 4.29 in a ^{1}H n.m.r. spectrum of (223).

The minor product of this reaction was (±)-(223) produced, presumably, by epimerisation of either (251) or (272). The combined yield of (272) and (223) from this reaction was in the order of 60%.

Reaction of imide (231) with phosphonium salt (201) gave a mixture by t.l.c. of products presumed to be the 6β-methoxy- and 7α-methoxypyrrolizidine esters [(233) and (234)] (Scheme 62). The combined yield of product from this reaction was only 14% and ^{1}H n.m.r. spectroscopy suggested that the components were present in a relative ratio of 7:2. This estimate was based on the relative strength of the two sharp methoxyl proton signals in the ^{1}H n.m.r. spectrum of the mixture. The methoxy proton signals were present at δ 3.79 and 3.52 ppm respectively with the upfield signal being the more intense. This suggested that the 7α-pyrrolizidine ester (234) was the major product of the reaction. An i.r. spectrum of the mixture gave the anticipated peaks at 1730, 1695, and 1650 cm^{-1}.

The product mixture witnessed was in accord with the findings of Kayser and Breau[132,133] who recently reported highly regioselective condensations between alkoxy-substituted maleic/phthalic anhydrides and stabilised phosphoranes. This is thought to arise from the oxygen atom of the alkoxyl group acting as a Lewis base towards the electron deficient

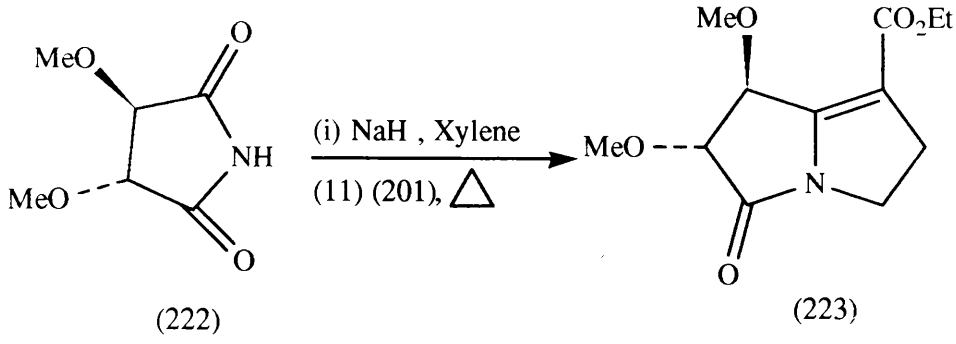
phosphorus of the ylide (Scheme 63). Complexation between the oxygen atom of the substituent and the phosphorus atom of the ylide leads to a lower energy transition state intermediate and consequently promotes preferential reaction at the carbonyl group adjacent to the alkoxy substituent.

Hydrogenation of ester (203) proceeded smoothly, affording the 1-endo-pyrrolizidine ester (273) by employing either Pd/C or PtO_2 as catalyst (Scheme 64). All spectroscopic and physical data for (273) were in agreement with reported values.[112]

However, when similar hydrogenation conditions were applied to the pyrrolizidine ester derived from the malic and tartaric acids, contrasting results were obtained.

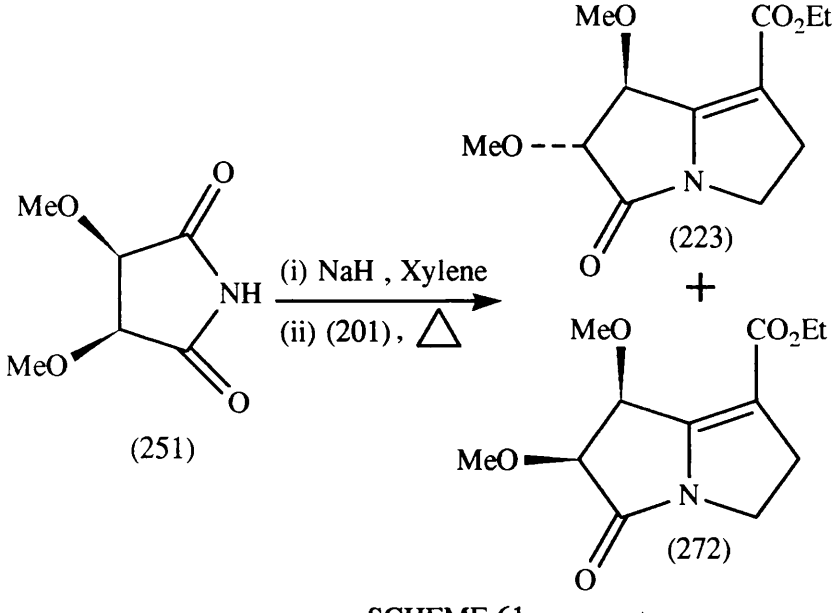
Hydrogenation of (223) using PtO_2 in AcOH, for 48h, gave a mixture of two products, separable by column chromatography, in a 3:1 ratio (Scheme 65). The major product was assigned as the 1β-ester (224) arising from cis-hydrogenation to the less hindered α-face. This was separated from the minor component - thought to be ester (274) produced by epimerisation of ester (224) under acidic conditions - by column chromatography. The hydrogen uptake for this reaction indicated the reaction was complete after 6h.

High resolution mass spectrometry indicated a parent ion, for both (224) and (274), corresponding to molecular formula $C_{12}H_{19}NO_5$. I.r. spectroscopy gave the anticipated ester and amide peaks for these compounds, and confirmed that hydrogenation had taken place by the absence of an olefinic signal. ^1H and ^{13}C N.m.r. spectra [^{13}C n.m.r. for (274) only] were in agreement with the proposed structure. Attempts



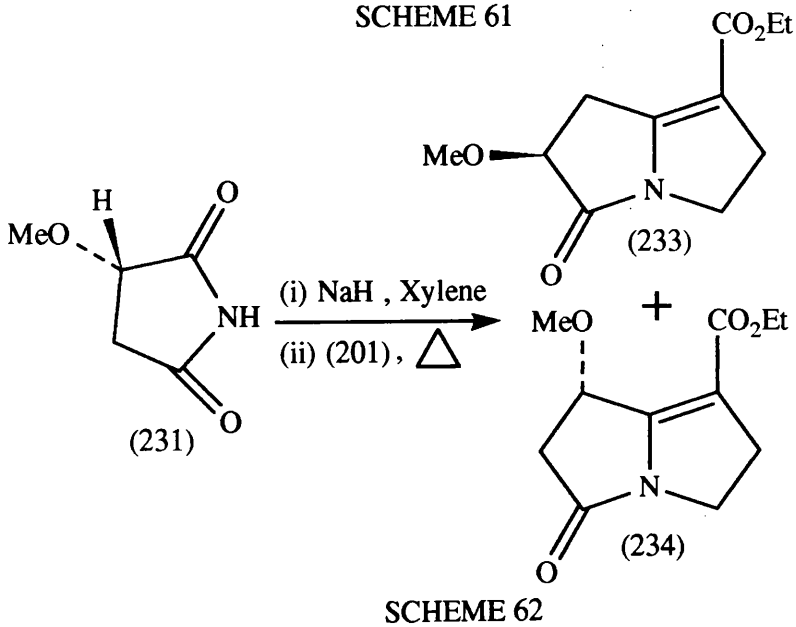
(222) (223)

SCHEME 60



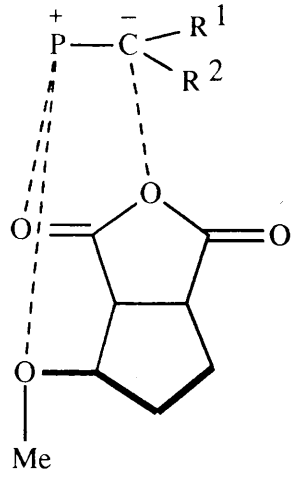
(251) (223) (272)

SCHEME 61

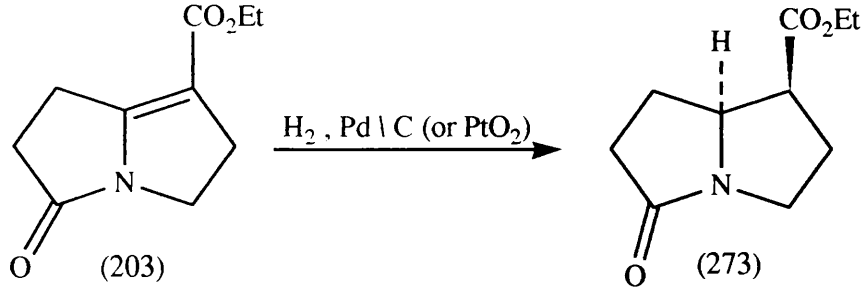


(231) (233) (234)

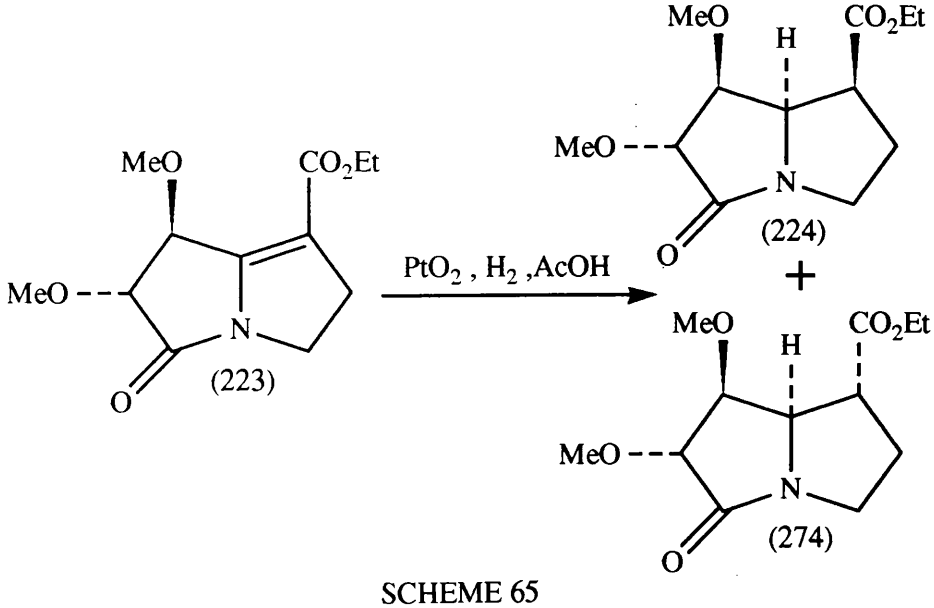
SCHEME 62



SCHEME 63



SCHEME 64



SCHEME 65

to confirm these structures using a N.O.E. experiment were unsuccessful due to product instability.

The use of Pd/C in acidified ethanol for hydrogenation of (223), again generated the same two pyrrolizidine esters, but the composition of ester (274) in the mixture was reduced to around 1%. As before the products were separated by column chromatography.

Ester (224) was readily reduced to the C-9 pyrrolizidine alcohol (275) by treatment with $LiAlH_4$ (Scheme 66). High resolution mass spectrometry gave a parent ion corresponding to molecular formula $C_{10}H_{19}NO_3$, while an i.r. spectrum gave a hydroxyl peak at 3470 cm^{-1} with amide and ester signals absent. The ^1H n.m.r. spectrum obtained was also consistent for the formation of (275).

Alcohol (275) was found to degrade slowly, even at low temperatures.

Hydrogenation of ester (272) did not proceed under either of the catalytic hydrogenation procedures used in the reduction of (223). This was thought to be a consequence of poisoning of the metal catalyst by triphenylphosphine oxide which could not be separated entirely from (272) despite repeated attempts.

The poor yield of product encountered on condensation of imide (231) with phosphonium salt (201), together with the difficulty in separation of the methoxyesters [(233) and (234)], dictated that hydrogenation was carried out on the mixture of methoxyesters produced by this condensation.

However, it appeared that each component of the mixture gave rise to two products on hydrogenation - using either PtO_2 or Pd/C as

catalyst. A ^{1}H n.m.r. spectrum of the product mixture from hydrogenation indicated two pairs of methoxy proton signal peaks. These were at δ3.54 and 3.62 ppm and δ3.81 and 3.88 ppm, respectively. The relative chemical shifts, together with the intensity of the signals, suggested that the upfield pair of methoxy proton signals might be associated with C-7 methoxy-substituted amides [(276) and (277)] while the downfield signal pair might arise from C-6 methoxy-substituted products [(278) and (279)]. It is thought that the thermodynamically less stable endo-ester is the major component of each pair [(276) and (278)] corresponding to methoxy signals at δ3.62 and 3.81 ppm, respectively. The minor component of each pair could be the exo-esters [(277) and (279)] if analogy is made with the work of Flitsch and Wernsmann.[111] The estimated relative intensity of these peaks, in ascending chemical shift, was 2:9:2:1 for both use of PtO_2 or Pd/C. An i.r. spectrum of the mixture indicated an ester peak at 1730 cm^{-1} and an amide signal at 1685 cm^{-1}, with no evidence for a signal representing an olefinic peak. In addition, high resolution mass spectrometry on the mixture gave a parent ion in accordance with hydrogenated products. A chromatogram of the extract indicated four compounds all of similar R_f value (0.23-0.24) - run in $CH_2C\ell_2$/AcOe (1:1).

Owing to the poor yields obtained in both the preparation of imide (231) and the subsequent condensation with phosphonium salt (201), together with the non-specific nature of the later hydrogenation step, it was decided not to pursue the route to the necine diols from malic acid any further. In addition, the contamination of achiral dimethoxypyrrolizidine (272) with triphenylphosphine oxide precluded further investigation of synthesis of crotanecine (10) from (272).

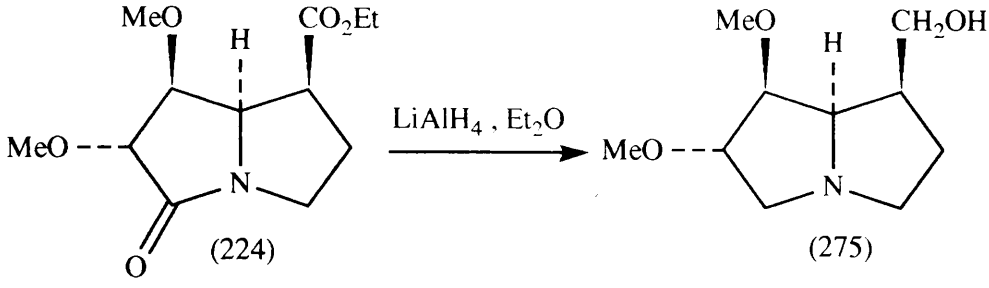
4.5 Attempts to Produce Uspallatinecine (221) from 1β-pyrrolizidine ester (224)

Attempts to develop the desired C-9 alcohol functionality and the 1,2-unsaturation found in uspallatinecine (221) centred on the selenenylation-reduction-thermal oxidative elimination sequence employed to good effect by Robins and Sakdarat in their synthesis of (+)-supinidine (22) (Scheme 2). The first stage of this process involved attempts to generate selenide (280) from the 1β-pyrrolizidine ester (224) (Scheme 67). This involved the abstraction of the acidic proton α to the ester substituent at C-1 of (224), which generated the intermediate anion (281). Addition of PhSeCℓ or Ph_2Se_2 to (281) should then produce selenide (280).

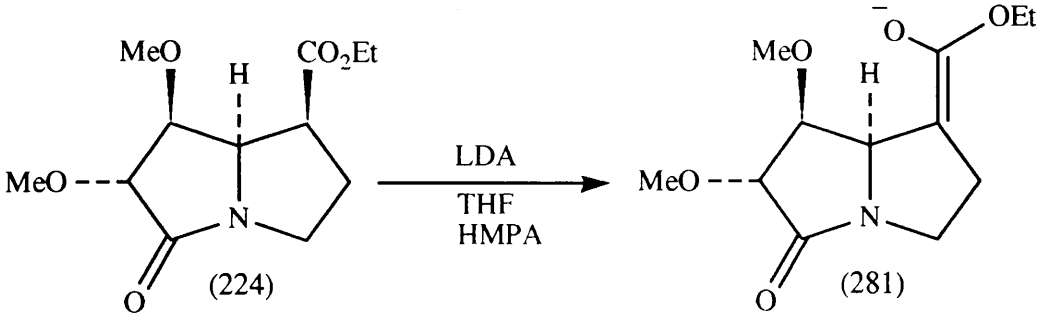
Although an immediate colour change in the reaction mixture was noted on addition of LDA to a solution containing (224) - suggesting anion formation - the desired product, (280), could not be identified in the product mixture obtained. A small quantity of starting material, ester (224), was recovered from this reaction.

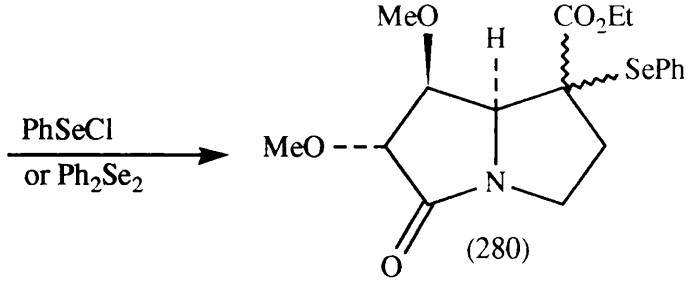
In addition, treatment of the product mixture of this reaction with $LiAlH_4$ gave no indication for the formation of the pyrrolizidine alcohol (282), although a small quantity of the unsaturated 1-hydroxymethyl-pyrrolizidine (275) was recovered from the reduction step. The introduction of a small quantity of HMPA to the selenenylation reaction, in an effort to promote anion formation, gave similar results.

Attention was then directed towards the synthesis of uspallatinecine (221) by a modified strategy (Scheme 68). It was felt that removal of the amide group from (224) might be beneficial to the selenenylation process. Accordingly,[134,135] two routes were investigated which

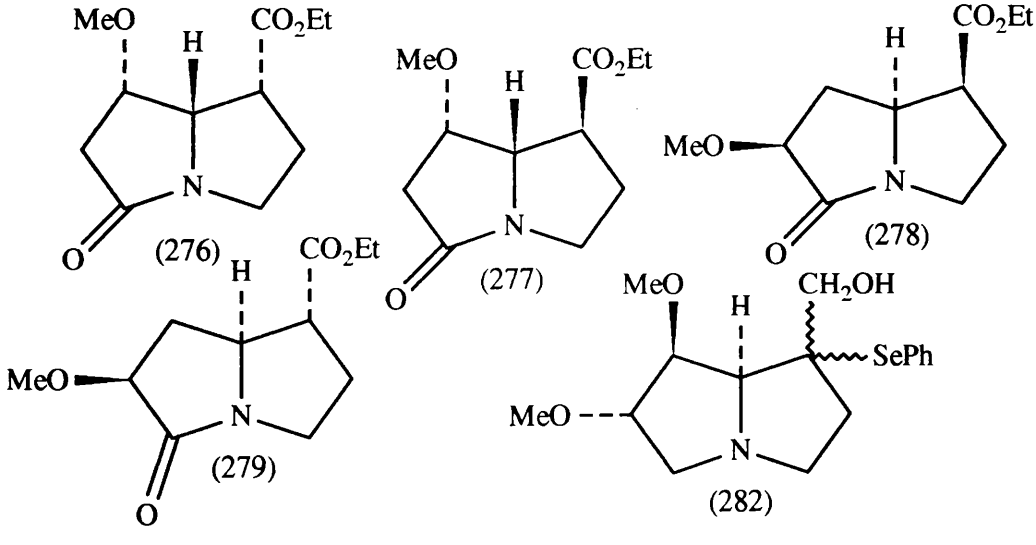


SCHEME 66





SCHEME 67



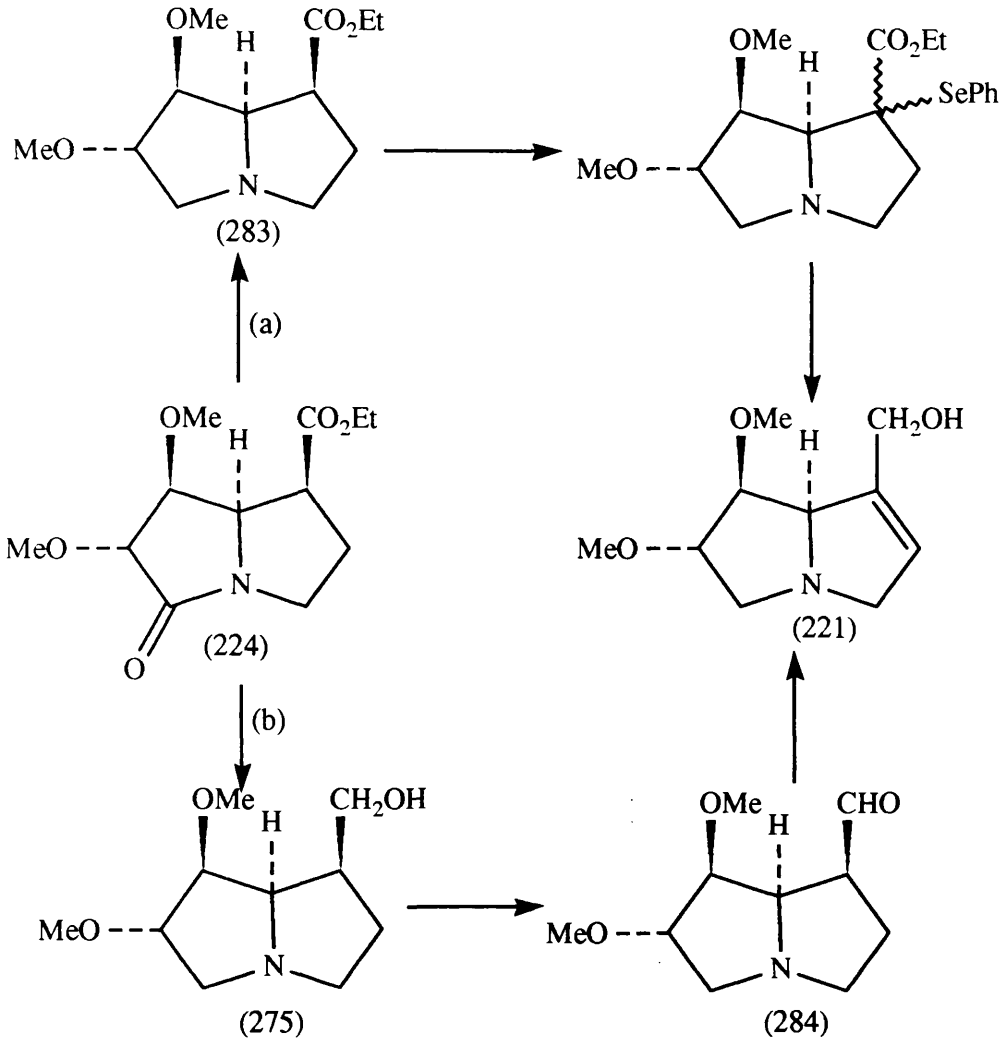
would introduce the desired selenide functionality after reduction of the amide had been effected.

Scheme 68(a) proposed the selective reduction of the amide functionality of (224), generating the endo-ester (283) which could then be transformed into uspallatinecine. The alternative route, Scheme 68(b), would proceed via that pyrrolizidine alcohol (275). A Swern oxidation[136,137] performed on (275) could generate the C-9 aldehyde (284) which could then be used to develop the desired 1,2-unsaturation for uspallatinecine (221), again via a selenide intermediate.

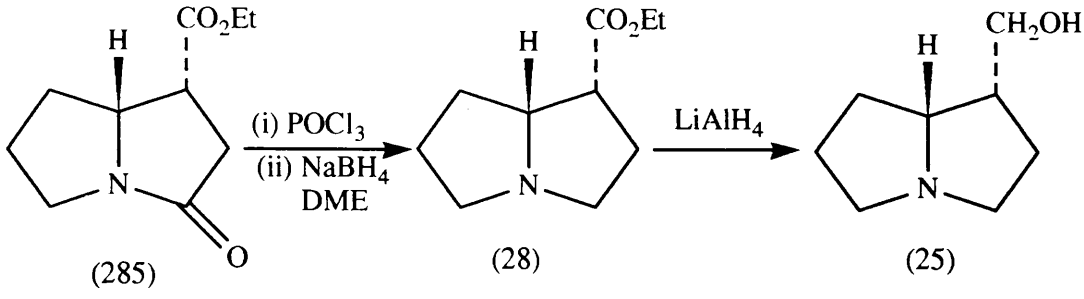
Brown et al.[134] reported the use of diborane for the selective reduction of amides to amines. However, the use of this method proved unproductive, with no evidence for product formation and a poor recovery of starting material (224) was encountered. It had been noted that recovery of products from such a reaction could be made difficult by the formation of a diborane-nitrogen complex.[138]

The other method investigated in an effort to produce (283) was based on the strategy employed by Pinnick and Chang[135] for the reduction of lactam (285) to pyrrolizidine ester (28) in their synthesis of (±)-isoretronecanol (25) [Scheme 69]. However, this method, featuring reduction using $POC\ell_3$ and $NaBH_4$, again proved to be unsuitable for generation of (283).

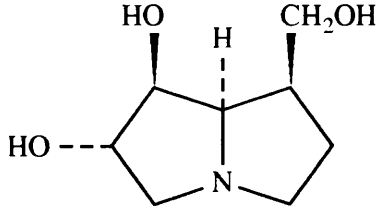
Attempts to produce uspallatinecine by the alternative strategy [Scheme 68(b)] were again unsuccessful. A Swern oxidation[136] performed on pyrrolizidine alcohol (275) did not yield the desired aldehyde (284).


OMe
H
CO2Et
MeO
N
(283)
(a)
OMe
H
CO2Et
SePh
MeO
N
OMe
H
CO2Et
MeO
N
O
(224)
(b)
OMe
H
CH2OH
MeO
N
(275)
OMe
H
CH2OH
MeO
N
(221)
OMe
H
CHO
MeO
N
(284)

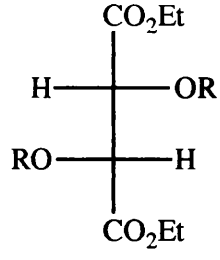
SCHEME 68



SCHEME 69



(226)



R = H (246)
R = Me (247)

The lack of available materials together with the instability of (275) precluded carrying out the deprotection to the saturated necine triol (226).

However, deprotection of diethyl (2S,3S)-dimethoxysuccinate (247), affording hydroxyester (246), was carried out successfully by employing the method of Yamada and co-workers.[115] This involves deprotection using BBr_3 in the presence of 15-crown-5-ether.

Summary

Despite initial problems intermediate imides were prepared from the stereoisomeric malic and tartaric acids. Condensation of these imides with phosphonium salt (201), generated the desired pyrrolizidine ring system, though attempts to produce targeted necines were unsuccessful. Severe problems were associated with this work, most notably application of established reactions to more heavily substituted pyrrolizidines; product stability; poor yields; epimerisation of key intermediates; and the restrictions imposed by eventually adopting a lengthy synthetic strategy exacerbated by the use of a prohibitively expensive reagent (Ag_2O) early in the sequence.

CHAPTER 5

FEEDING OF OPTICALLY ACTIVE 2-METHOXYPUTRESCINES TO SENECIO PLEISTOCEPHALUS PLANTS

5.1 Introduction

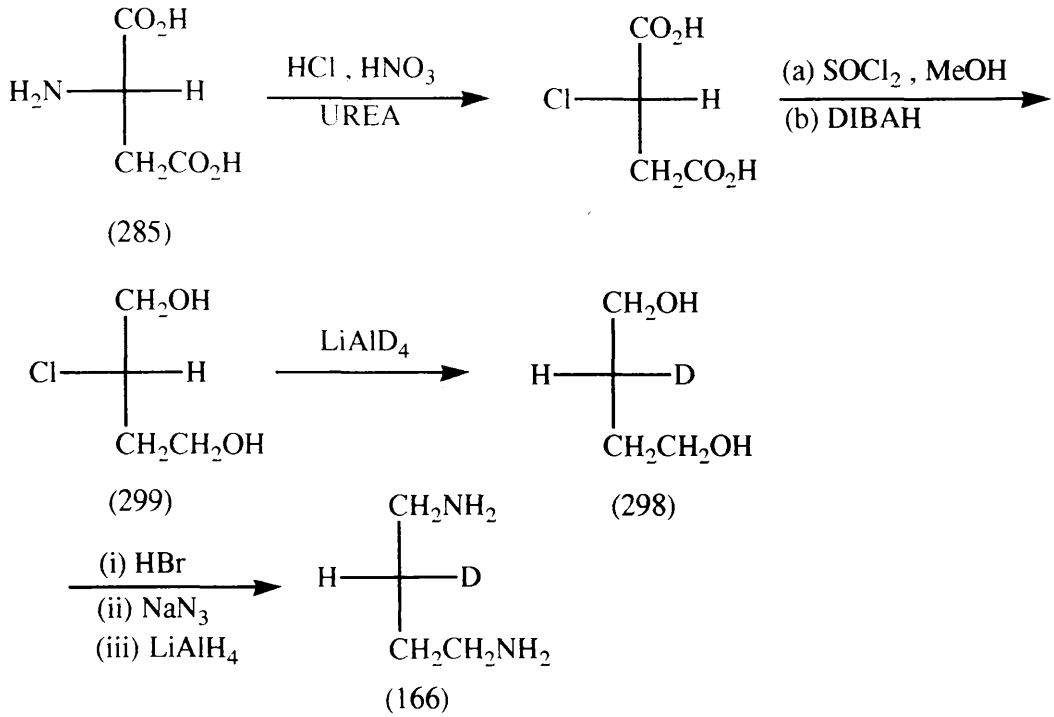
As outlined in Chapter 3.2 the biosynthesis of the pyrrolizidine bases retronecine (4) and rosmarinecine (21) is now well established. However, as yet, there has been no report concerning the feeding of analogues of compounds known to be involved in necine biosynthesis to plants containing pyrrolizidine alkaloids. Experiments involving the feeding of such derivatives might give some indication of the enzyme selectivity associated with pyrrolizidine alkaloid biosynthesis through partial incorporation of unnatural substrates into the biosynthetic pathway. However, if the enzymic systems involved in pyrrolizidine alkaloid biosynthesis display sufficient flexibility in their acceptance of abnormal substrates, then feeding of such analogues might lead to the production of novel pyrrolizidine alkaloid analogues with useful biological activity. Evidently it was of considerable importance for work to be carried out in this area.

Arigoni and Eliel[139] summarised a simple chemical route to (R)- and (S)-[2-^{2}H]succinic acids from the enantiomeric aspartic acids. Recently, Kunec and Robins[85] modified this strategy to provide synthesis of both (R)- and (S)-[2-^{2}H]putrescine from the same starting materials. The route adopted by Kunec and Robins[85] is illustrated in Scheme 70 for the conversion of (S)-aspartic acid (285) into (R)-[2-^{2}H]-

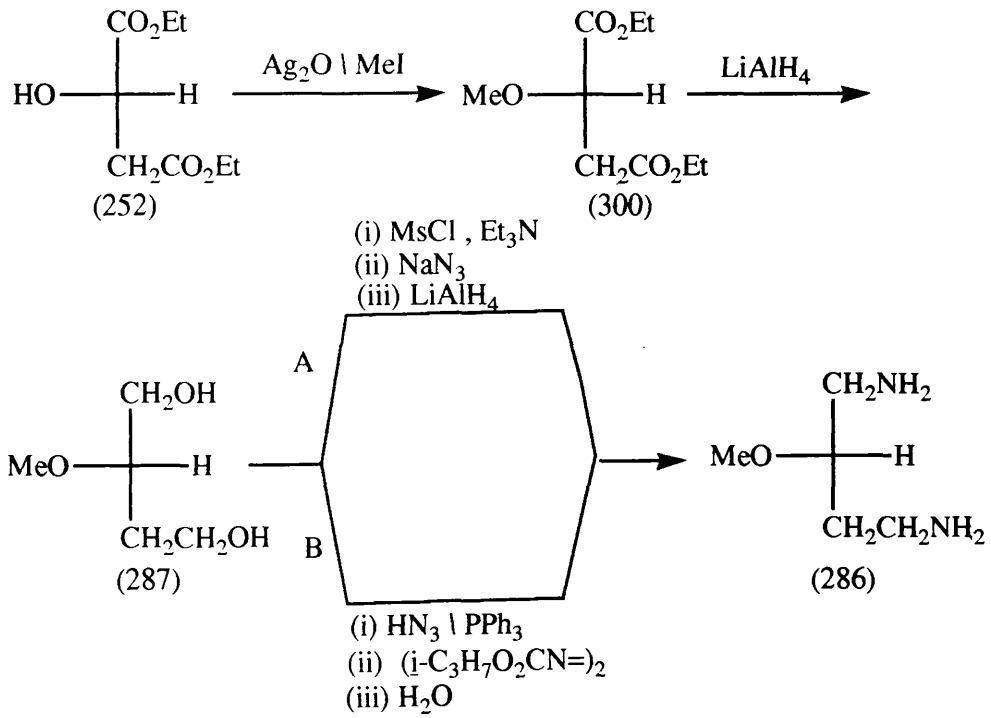
putrescine (166). It was felt that a similar synthetic strategy, complemented by knowledge gained in investigations of necine synthesis (Chapter 4), could provide a suitable basis for the production of (R)- and (S)-2-methoxyputrescine from the enantiomeric malic acids.

Two possible routes to substituted putrescines from the malic acids were considered. The proposed sequences, illustrated in Scheme 71 for the conversion of diethyl (S)-2-hydroxysuccinate (252) into (S)-2-methoxyputrescine (286) differ only in the conversion of the intermediate diol (287) into the desired putrescine (286). Scheme 71(a), like the work of Kunec and Robins, proceeds via a diazide intermediate, whereas Scheme 71(b) involves a one-pot conversion of diol (287) into putrescine (286) by making use of a recent report by Golding and co-workers[140] detailing the conversion of primary or secondary alcohols into the corresponding amines (Scheme 72). The latter method involves a subtle combination of known reactions, namely Mitsunobu[141] reaction to convert diol (288) into diazide (289), followed by Staudinger[142] reaction of (289) with triphenylphosphine to produce an iminophosphorane intermediate (290). Hydrolysis of (290) liberates the free amine (291).

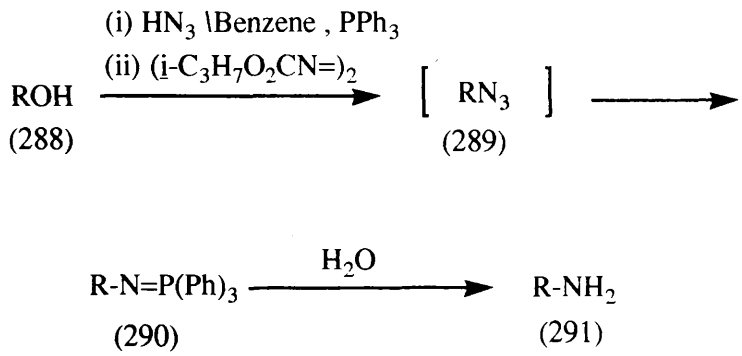
To aid the identification of the fate of the 2-methoxyputrescines to be fed, it was important to introduce a distinctive label. This would be accomplished by using ^{14}C-labelled methyl iodide in the methylation of hydroxyester (252). In using optically active starting materials, the malic acids, this would enable production of the desired methoxyputrescines in optically active form. The provision of optically active putrescine analogues would be beneficial in identifying any divergence in behaviour between the enantiomeric forms during their time in the plants.



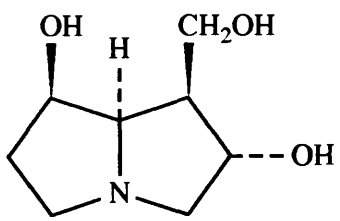
SCHEME 70



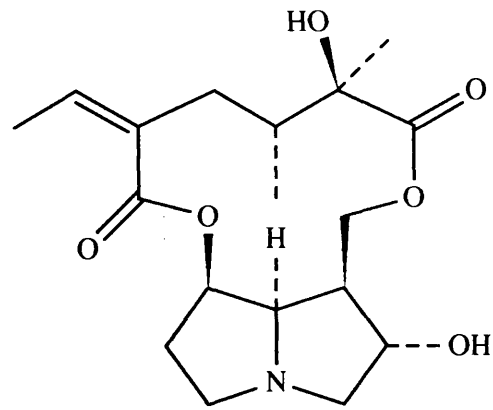
SCHEME 71



SCHEME 72



(21)



(153)

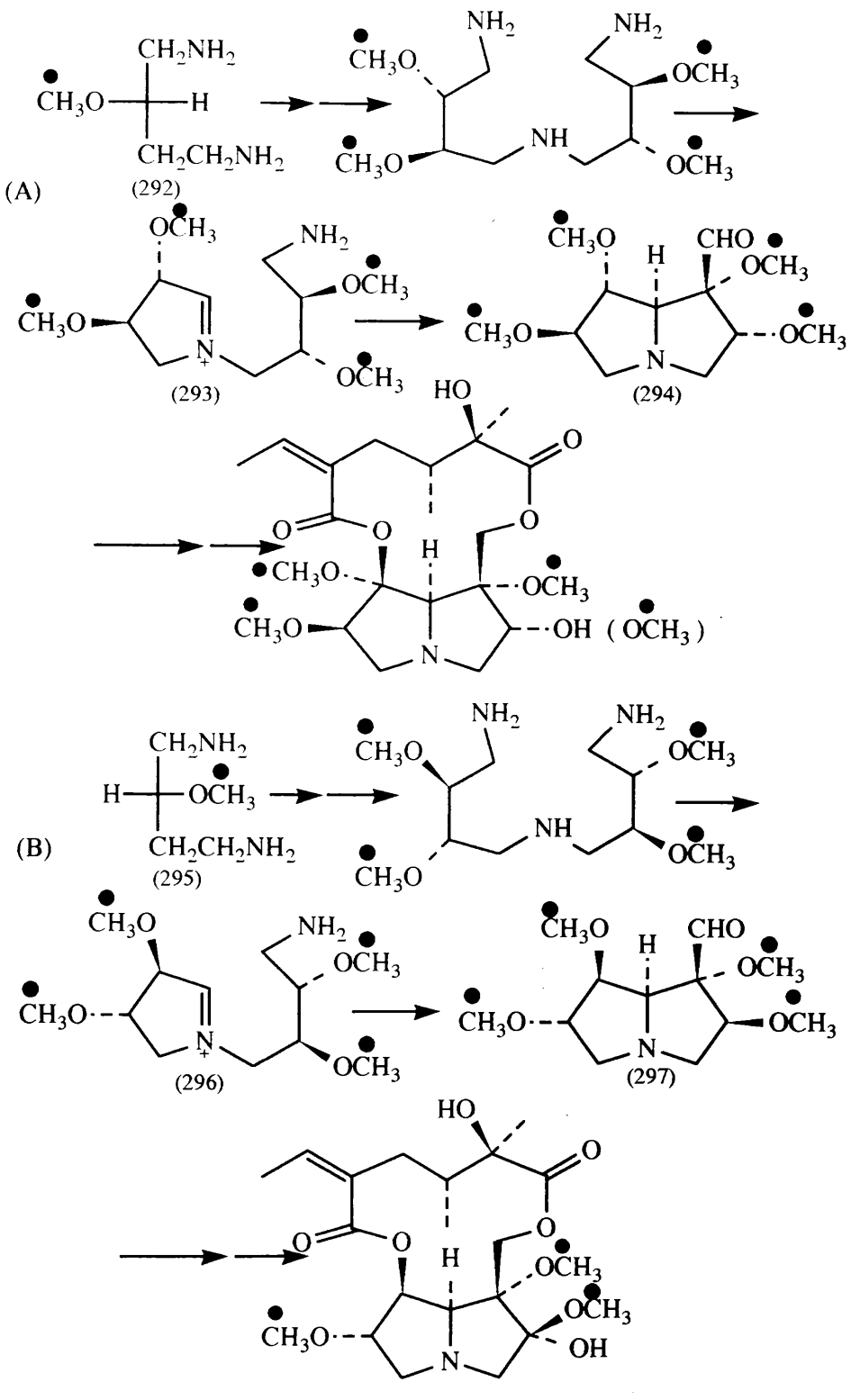
The choice of 2-methoxyputrescines as analogues presented three distinct advantages. Firstly, methoxy groups should be readily identified by ^{1}H n.m.r. spectroscopy and the presence of a ^{14}C-label on this grouping could also be of benefit in detection of analogues and proof of location of labels by degradation. Secondly, the proposed scheme would incorporate the ^{14}C-label at a high yielding stage, namely the methylation of (252). It was anticipated that this would help to maximise the specific activity of the putrescines to be administered. If too great a quantity of precursor was fed then this might interfere with the experiment through poisoning of the plants. Thirdly, if the feeding of the ^{14}C-labelled methoxyputrescines gave rise to encouraging results then a complementary series of experiments could be introduced involving the feeding of ^{3}H-labelled methoxyputrescines. This could be achieved by simply substituting ^{3}H-labelled methyl iodide into the outlined synthetic sequence and ^{3}H-labelled methoxyputrescines could be produced with higher specific activities (Scheme 71). Further scope was provided by the possibility of producing radiolabelled dimethoxyputrescine from the tartaric acids. This might serve to produce analogues of the more highly oxygenated pyrrolizidine alkaloids.

It was intended to feed the optically active radiolabelled methoxyputrescines to plants of S. pleistocephalus. Robins and co-workers had administered labelled putrescines to these plants to help in the elucidation of the biosynthetic pathway to rosmarinine (153) (Chapter 3). These experiments were characterised by a high incorporation of the labelled putrescines into the biosynthetically produced rosmarinine (153). Moreover, these plants are known to produce only

one pyrrolizidine alkaloid, rosmarinine (153). This would be important in helping to simplify the interpretation of spectra and purification of any novel compounds which might be produced from the feeding of the methoxyputrescines.

If the 2-methoxyputrescines were found to be readily incorporated into the earlier stages of the biosynthetic pathway to rosmarinecine (21) then four possible sites displaying methoxyl labelling could be expected in the intermediate iminium ion (154). Scheme 73(a) outlines where methoxy labelling would be expected to be found from the feeding of [^{14}C-Me]-(S)-2-methoxyputrescine (292) to S. pleistocephalus. Feeding of this precursor could provide valuable information concerning the latter stages of the biosynthetic pathway to rosmarinecine (21). Firstly, cyclisation of the iminium ion (293) to the pyrrolizidine aldehyde (294) involves the removal of the pro-R proton from the carbon atom destined to become C-1 of the aldehyde (294). It is possible that the steric congestion created by the nearby methoxyl group may interfere with the enzymic process. Secondly, the feeding of this precursor could be used to probe the stereospecific hydroxylation at C-2 of the necine. This process involves the removal of the pro-S proton at C-2 of (294), a site which could be occupied by a methoxyl substituent. Finally, steric effects created by adjacent methoxyl groups could influence the enzymic processes governing the stereospecific hydroxylation at C-7 of the pyrrolizidine system together with the subsequent esterification at C-7 and C-9.

An experiment involving feeding of the enantiomer, [^{14}C-Me]-(R)-2-methoxyputrescine (295) might also give rise to a labelling pattern



SCHEME 73

Note : these diagrams represent composite labelling patterns

which would provide valuable information about the biosynthetic process [Scheme 73(b)]. Incorporation of (295) into the earlier stages of the biosynthetic process might give rise to iminium ion (296) labelled at four possible sites. Cyclisation of (296) to generate the pyrrolizidine aldehyde (297) requires removal of the pro-R proton from the carbon atom designated to become C-1 of (297). However, this could be impeded by the presence of a methoxyl substituent at this site. Secondly, if (297) was formed then the presence of the C-7β methoxyl group could effect the subsequent stereospecific hydroxylation at this centre. This process involves removal of the pro-S proton at C-7 - a site which could be occupied by a methoxyl substituent. In addition the presence of a methoxyl substituent in close proximity might have a profound effect on the stereospecific hydroxylation at C-2 and esterifications at C-7 and C-9 of rosmarinecine.

5.2 Evaluation of Routes to Optically Active 2-Methoxyputrescines

Work on the synthesis of necines (Chapter 4) had established that malic acid could be readily converted into the corresponding diethyl ester (252). In addition, methylation of (252) had been confirmed as a high yielding process with retention of stereochemistry. Further investigation ascertained that incomplete methylation of (252) was encountered if less than three equivalents of methyl iodide were used relative to (252). Therefore, in order to maximise the specific activity of the methoxyputrescines to be fed, it was imperative that the radio-labelled methyl iodide should, initially at least, be reacted with diethyl ester (252) in a quantity of methyl iodide amounting to no more than one

equivalent relative to (252). The ^{14}C methyl iodide source to be used was a 100 μCi sample of specific activity, 59.7 mCi $mmol^{-1}$. The small volume of the radiolabelled source (< 0.1 μL) dictated that the radiolabelled material would have to be transferred from the storage ampoule under vacuum and distilled into a sample of inactive methyl iodide (amounting to less than one equivalent). On completion of this transfer, using the vacuum line apparatus illustrated in Figure 4, the now diluted radiolabelled methyl iodide source would be added to the reaction mixture. After an appropriate delay, further methyl iodide would be added to ensure that the methylation was driven to completion.

Kunec and Robins, in their route to the optically active ^{2}H-putrescines, had employed four equivalents of $LiAlD_4$ to produce the deuteriated diol (298) via displacement of chloride from (299) (Scheme 70). However, a reduced quantity of $LiAlH_4$ was sufficient to enable effective reduction of diester (300) into diol (287). Replacing Et_2O with THF as solvent for this reduction step appeared to encourage dissolution of lithium salt residue which made subsequent purification more difficult. Therefore it was decided that Et_2O was a more suitable solvent for this step.

Conversion of diol (287) into the corresponding mesylate (301) was accomplished in good yield by treatment with mesyl chloride/Et_3N in dichloromethane. However, attempts to convert (301) into the desired putrescine (286), via diazide (302), proved to be unsuccessful.

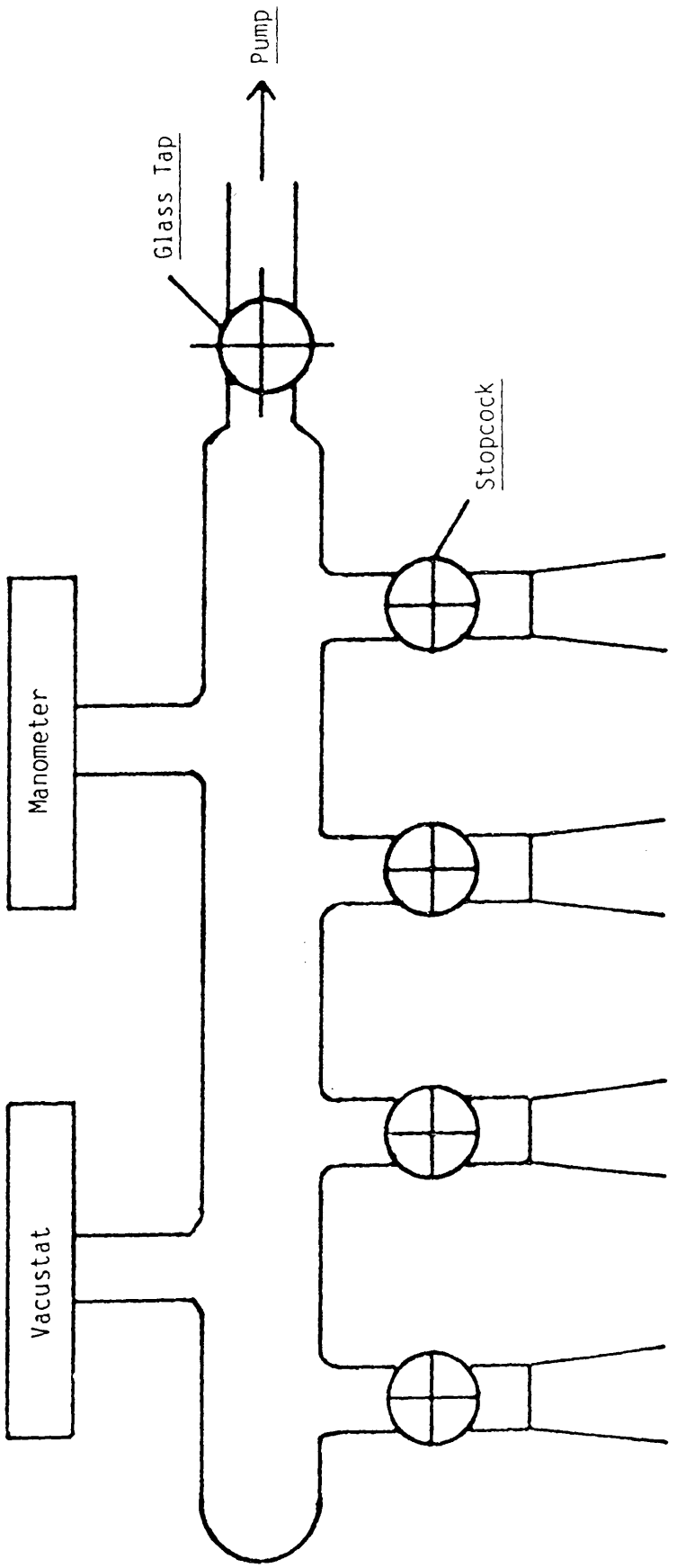
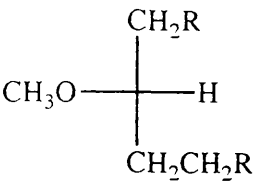


Figure 4. Vacuum apparatus employed for transfer of ^{14}C-labelled methyl iodide.



R = OH (287)

R = OMs (301)

R = N_3 (302)

R = NH_2 (286)

$R'O_2CN{=}NCO_2R'$

$R' = C_2H_5$ (303)

$R' = i\text{-}C_3H_7$ (304)

Having encountered difficulties in the later stages of Scheme 71(a), attention now switched to the possibility of converting diol (287) into the desired diamine (286) dihydrochloride by the method of Golding and co-workers[140] [Scheme 71(b)]. Two dicarboxylates, diethyl azodicarboxylate (303) and diisopropyl azodicarboxylate (304), were tried in the reaction and both generated the desired product - (S)-2-methoxyputrescine (286) dihydrochloride - in acceptable yield. However, the 2-methoxyputrescine produced from the use of diethyl azodicarboxylate (303), was characterised by an intense purple colouration, even after repeated recrystallisations. Indeed optical activity measurements could not be made due to the discolouration of this material. However (286) produced by the use of the alternative reagent, diisopropyl azodicarboxylate (304), was not subject to nearly such a severe discolouration problem, enabling optical rotation values to be determined. Consequently, diisopropyl azodicarboxylate was used in the preparation of the radio-labelled methoxyputrescines.

5.3 Initial Attempt to Prepare [^{14}C-Me]-(S)-2-Methoxyputrescine (292) dihydrochloride

The hydroxy diester (252) was prepared from L-malic acid (230) by the method described in Chapter 4. Diethyl [^{14}C-Me]-(S)-methoxysuccinate (305) was prepared by the reaction of (252) with Ag_2O and [^{14}C-Me]methyl iodide. The radiolabelled methyl iodide was present at less than one equivalent for four hours under reflux conditions before a subsequent methyl iodide addition was made. A third and final addition of methyl iodide was made eight hours into the 16 hour reflux used for this reaction.

The radiolabelled diester (305) was isolated and purified by distillation to yield material of specific activity 1.86 μCi mmol^{-1}. This represented a 25.2% incorporation of the radiolabel into the product.

The diester (305) was then treated with $LiAlH_4$ (3 equivalents) to generate radiolabelled diol (306). However, attempts to convert (306) into the desired putrescine (292) dihydrochloride by the method of Golding and co-workers[140] were unsuccessful. ^{1}H N.m.r. spectroscopy and t.l.c. data on the residue from this reaction gave no evidence for the formation of (292).

After this unsuccessful attempt to generate radiolabelled methoxyputrescines a further evaluation of the route employed was undertaken, using unlabelled material, in an effort to improve the conversion of diester (252) into putrescine (286).

It was observed that reduction of diester (300) to diol (287) could be accomplished in under two hours and required only 1.3 equivalents of $LiAlH_4$. In addition, incorporation of a continuous extraction of

the lithium salt residue from this reaction into the process gave a modest improvement in yield of (287) obtained (69%). Distillation of (287) appeared to encourage the formation of a by-product (307), particularly when carried out at higher temperatures or over a protracted time span. T.l.c. indicated (307) to be a much less polar component than diol (287). The respective R_f values were 0.09 for diol (287) and 0.92 for by-product (307) - where the stationary phase was silica and eluant CH_2Cl_2/AcOEt (1:1). ^1H and ^{13}C N.m.r. spectroscopy failed to discriminate between the two compounds with spectroscopic data obtained from the mixture being indistinguishable from that of pure (287). However, it should be noted that the hydroxyl signals observed in the ^1H n.m.r. spectrum of (287) were shown to be both solvent and concentration dependent. Identification was further complicated by the absence of a parent ion peak for (287) in high resolution mass spectrometry. The highest value ion peak observed for (287) and in a mixture with by-product (307) was m/z 102, corresponding to a molecular formula of $C_5H_{10}O_2$, i.e. the removal of the elements of water from (287). The less polar nature of (307) together with the apparent similarities in spectroscopic data with (287), suggested (S)-2-methoxyfuran (307) as a possible structure for this by-product. This could arise from the intramolecular cyclisation of (287), eliminating water in the process.

Preparation of diol (287) using THF as solvent appeared to be more susceptible to formation of (307) on distillation, possibly because of the small quantity of lithium salt residue which dissolves in THF.

Therefore, it was decided to reduce the quantity of $LiAlH_4$ used in the reduction of diester (300) to diol (287). In addition, a continuous

extraction of lithium salt residue from this reaction would be introduced to boost the yield. A careful distillation of (287) could be undertaken and meticulously monitored for the presence of furan (307) in the distillate.

The quantity of radiolabel successfully incorporated into the process was also of concern. It was hoped to improve incorporation by allowing a longer reaction period with only one equivalent of methyl iodide present during formation of (300). In addition, it was anticipated that the introduction of a small quantity of ether into the reaction would improve mixing without having too adverse an effect on the yield of product obtained.

5.4 Preparation of [^{14}C-Me]-(R)-2-Methoxyputrescine (295) dihydrochloride

The diester (308) was prepared from D-malic acid (207) by the method reported in Chapter 4.[126] In an effort to maximise the specific activity of the methoxyputrescine to be fed, the procedure reported in 5.3 for the preparation of diethyl [^{14}C-Me]-(S)-2-methoxysuccinate (309) was modified.

After the initial transfer of the radiolabelled methyl iodide to the reaction flask, a small quantity of Et_2O was added. The reflux period for the reaction was extended to 30 hours with further addition of methyl iodide taking place after 8, 12, and 22 hours reflux. Each of these subsequent methyl iodide additions was accompanied by addition of an equal volume of Et_2O.

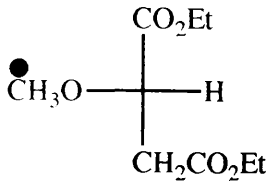
These alterations in procedure generated (309) in a yield of 94.9% and increased the specific activity of the product to 2.84 μCi mmol^{-1}.

This represented a 59% incorporation of the ^{14}C-label into the product (309).

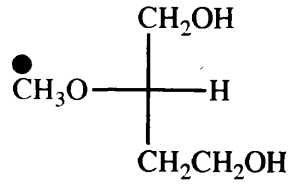
Conversion of (309) into [^{14}C-Me]-(R)-2-methoxybutan-1,4-diol (310) was carried out using the amendments in procedure outlined in 5.3. A continuous extraction helped to increase the yield of (310) obtained to 64.8%, after distillation.

Distillation of (310) again gave rise to the formation of by-product - furan (311). This was particularly noticeable during the latter stages of distillation. T.l.c. again confirmed by-product (311) to be considerably less polar than diol (310). A radioscan of the distillate from the later stages of the distillation demonstrated two active peaks corresponding to (310) and (311). As before a ^{1}H n.m.r. spectrum of the product mixture resembled that of diol (310).

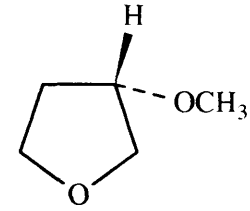
Diol (310) was converted successfully into [^{14}C-Me]-(R)-2-methoxyputrescine (295) dihydrochloride by the method of Golding and co-workers.[140] Two separate preparations of (295) from diol (310) were carried out, giving yields of 23 and 6.6% of product, respectively. The higher figure was obtained using diol (310) freshly distilled while the reduced yield was encountered on using diol (310) several weeks after distillation. T.l.c. indicated that some of diol (310) appeared to be converted into (311).



(305)



(306)



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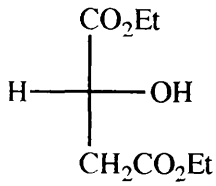

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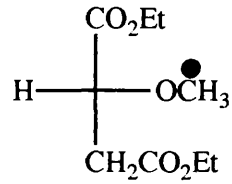

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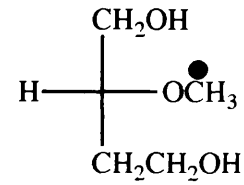

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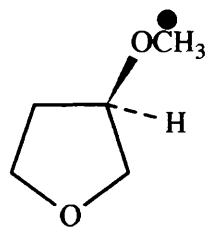
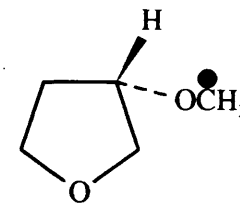


(311)



(312)

5.5 Preparation of [^{14}C-Me]-(S)-2-Methoxyputrescine (292) dihydrochloride

The procedure adopted for the methylation of (252) (see 5.3, 5.4) underwent further refinement in an effort to achieve a higher incorporation of the ^{14}C-label into the product. This involved the use of a greater quantity of Et_2O to aid mixing, and extension of the reflux associated with this reaction to 70 hours. In addition, the first subsequent addition of methyl iodide was deferred until 24 hours reflux had elapsed. Further addition of methyl iodide was made after 47 and 60 hours reflux to ensure methylation was complete. These modifications resulted in a 64.7% incorporation of the ^{14}C-radiolabel into (305), producing material of specific activity 3.28 μCi mmol^{-1}.

The radiolabelled diester (305) was then converted into diol (306). As in the production of the enantiomer the latter stages of the distillation of (306) were accompanied by generation of by-product, assumed to be (312). A radioscan of a chromatogram of material from the latter stages of the distillation again confirmed (312) to be radiolabelled.

The diol (306) was converted into the desired 2-methoxy-putrescine (292) dihydrochloride by the method of Golding and co-workers.[140] Initially [^{14}C-Me]-(S)-2-methoxyputrescine (292) dihydrochloride was produced from diol (306) in a disappointing 16.6% yield. A subsequent conversion of diol (306) into (292) increased the yield to 36.6% on using 2.6 equivalents of HN_3[143] in this reaction. Diol (306) used in this reaction had been distilled several weeks beforehand and by-product (312) was in evidence from t.l.c.. However, use of additional HN_3 appears to be of no benefit to the yield of putrescine obtained when freshly distilled diol was used.

5.6 Feeding of (R)-and (S)-2-Methoxyputrescine dihydrochloride to S. pleistocephalus

5.6.1 Introduction

To assess the relative incorporation of the 2-methoxyputrescines into the biosynthetic pathway to rosmarinine (153) each was fed in conjunction with a tritiated precursor. The precursor used was [1,4-^{3}H]-putrescine dihydrochloride. For each experiment a mixture of ^{3}H- and ^{14}C-labelled putrescines was fed to S. pleistocephalus plants. Measurement of the ^{3}H/^{14}C ratios before and after feeding provided additional reference data. The wick procedure for feeding was adopted in preference to the xylem pricking technique. The wick method entails the threading of a needle and cotton through the stem of the plant. The ends of the length of cotton are immersed in an aqueous solution of the compounds to be adminstered to enable uptake by the plant.

The radiolabelled precursors were adminstered over a five-day period and seven days after completion of the feed the alkaloid content of the plants was extracted by established methods. Attempts were also made to isolate any methoxyputrescine still intact within the plant. This was carried out by an intermediate trapping experiment as the N-phenylamino (thiocarbonyl) derivative.[144]

5.6.2 Feeding of [^{14}C-Me]-(R)-2-Methoxyputrescine dihydrochloride to S. pleistocephalus

[^{14}C-Me](R)-2-Methoxyputrescine (295) dihydrochloride and [1,4-^{3}H]putrescine dihydrochloride were mixed to give an initial ^{3}H/^{14}C feed ratio of 14.7. The alkaloidal material extracted from S. pleistocephalus

plants on completion of this experiment was found to contain total incorporations of 4.94% of the ^{3}H and 0.58% of the ^{14}C activities fed. The alkaloidal material isolated accounted for 0.16% of the plant weight prior to harvesting and exhibited a $^{3}H/^{14}C$ ratio of 124.

A ^{1}H n.m.r. spectrum of the alkaloid extract was consistent with that of rosmarinine (153). The absence of any methoxyl proton peaks from the spectrum, coupled with the low incorporation of the ^{14}C-radiolabel, suggested that (R)-2-methoxyputrescine is not readily utilised in the biosynthetic pathway to rosmarinine (153).

A chromatogram of the extract gave one major spot. This resulted in a positive test when sprayed with o-chloranil solution and corresponded to rosmarinine. A radioscan of a chromatogram of the extract gave rise to only one significant peak. This again corresponded to rosmarinine. Preparative t.l.c. established that most of the radioactivity of the extract was associated with rosmarinine (86.8% of the ^{3}H and 45.4% of the ^{14}C). The melting point of the crude extract compared favourably to the value documented for rosmarinine.

The experiment was repeated with the radiolabelled precursors fed to S. pleistocephalus plants which had been previously pruned in an effort to reduce the amount of endogenous rosmarinine and to stimulate alkaloid production. The radiolabelled precursors were fed in a $^{3}H/^{14}C$ ratio of 18.5. Analysis of the alkaloid extract obtained from this experiment indicated an incorporation of 4.0% of the ^{3}H and 0.31% of the ^{14}C activities fed. The $^{3}H/^{14}C$ ratio of the extract was 239. ^{1}H N.m.r. spectroscopy and t.l.c. again indicated rosmarinine (153) as the sole component of the extract. Attempts were made to detect any (R)-2-

methoxyputrescine still intact within the plant by means of an intermediate trapping experiment. Unlabelled (R)-2-methoxyputrescine was added to the extract and the phenylamino(thiocarbonyl) derivative was isolated.[144] However, this derivative was inactive, suggesting that (R)-2-methoxyputrescine does not remain intact within the plant.

5.6.3 Feeding of [^{14}C-Me]-(S)-2-Methoxyputrescine (292) dihydrochloride to S. pleistocephalus

[^{14}C-Me]-(S)-2-Methoxyputrescine (292) and [1,4-^{3}H]putrescine were fed, as their dihydrochlorides, to S. pleistocephalus plants. The $^{3}H/^{14}C$ ratio of the putrescines fed was 27.9. The alkaloidal material extracted from the plants on completion of the experiment accounted for 0.2% of the plant weight before masceration. The $^{3}H/^{14}C$ ratio of the extract was 248 and accounted for 5.87% of the ^{3}H activity and 0.67% of the ^{14}C activity administered to the plants.

In keeping with the results obtained from the feeding of the enantiomer, ^{1}H n.m.r. spectroscopy and a chromatogram of the extract indicated rosmarinine (153) as the sole component. Once again there was no evidence for analogues of rosmarinine being produced from utilisation of 2-methoxyputrescine in the biosynthetic pathway to rosmarinine. A radioscan of a chromatogram of the extract again gave only one significant peak - corresponding to rosmarinine. Preparative chromatography of the extract again demonstrated that most of the activity of the extract was associated with rosmarinine (87.7% of ^{3}H and 60.7% of ^{14}C activities). The melting point of the crude alkaloid extract compared favourably with the reported value for rosmarinine.

The experiment was repeated with the radiolabelled putrescines being administered to pruned S. pleistocephalus plants. The $^{3}H/^{14}C$ ratio for this experiment was 14.7. The alkaloidal extract from this experiment displayed a $^{3}H/^{14}C$ activity ratio of 37.4. This represented incorporation of 2.02% of the ^{3}H and 0.79% of the ^{14}C activities fed. As in the initial experiment only rosmarinine appeared to be present, with no evidence for the formation of analogues of rosmarinine. An intermediate trapping experiment[144] indicated that (S)-2-methoxyputrescine does not remain intact within the plant after feeding. The very small apparent incorporation of ^{14}C label into rosmarinine suggests that the ^{14}C-labelled 2-methoxyputrescine is broken down and may then be incorporated into rosmarinine.

Summary

^{14}C-Labelled samples of (R)- and (S)-2-methoxyputrescine were successfully prepared. Feeding of these optically active 2-methoxyputrescines to S. pleistocephalus plants indicated that these substituted putrescines are not utilised in the biosynthetic pathway to rosmarinine (153). Degradation of the 2-methoxyputrescines appears to occur. A small amount of the ^{14}C activity may then be incorporated into rosmarinine.

CHAPTER 6

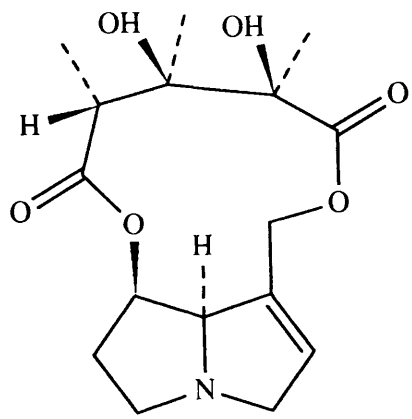
PYRROLIZIDINE ALKALOIDS FROM SEEDS OF CROTALARIA LANCEOLATA

6.1 INTRODUCTION

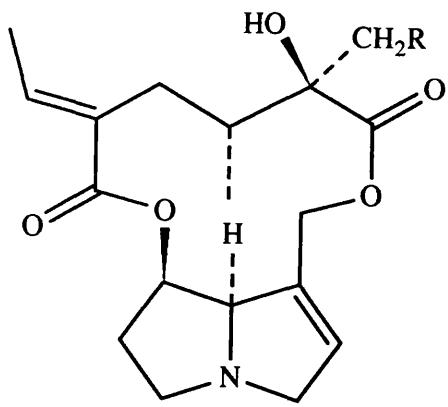
An investigation was undertaken to determine the pyrrolizidine alkaloid content of seeds of Crotalaria lanceolata. Crotalaria together with Andenocarpus and Cytisus, represent the genera of the Leguminosae plant family which have been shown to contain pyrrolizidine alkaloids. However, up until now the literature documents comparatively few such examples from Andenocarpus[145] and Cytisus[146] species. Indeed, with in excess of fifty Crotalaria species already identified as containing pyrrolizidine alkaloids, only the genus Senecio, (family Compositae) contains more species which have been shown to contain pyrrolizidine alkaloids.

Crotalaria species, indigenous to tropical or subtropical climates, have been shown to produce a collection of pyrrolizidine alkaloids displaying great structural variety. Nevertheless, most of the pyrrolizidine alkaloids found within the species are either 11- or 12-membered macrocyclic diesters or simple unesterified pyrrolizidines. The pyrrolizidine alkaloid most common to Crotalaria species is monocrotaline (8). Other macrocyclic diesters frequently found are integerrimine (313) and usaramine (314). The necine portion of pyrrolizidine alkaloids found in Crotalaria species can be simple hydroxymethyl pyrrolizidines or the more elaborate necine diols and triols. Retronecine (4) is the

most frequently encountered base portion. Many Crotalaria species have been shown to contain at least two pyrrolizidine alkaloids, invariably with the same base portion. Seeds of certain Crotalaria species are renowned for an unusually high pyrrolizidine alkaloid content. For example, one strain of seeds of C. retusa was reported to contain in excess of 9% of monocrotaline (8).[147]



(8)



R = H (313)
R = OH (314)

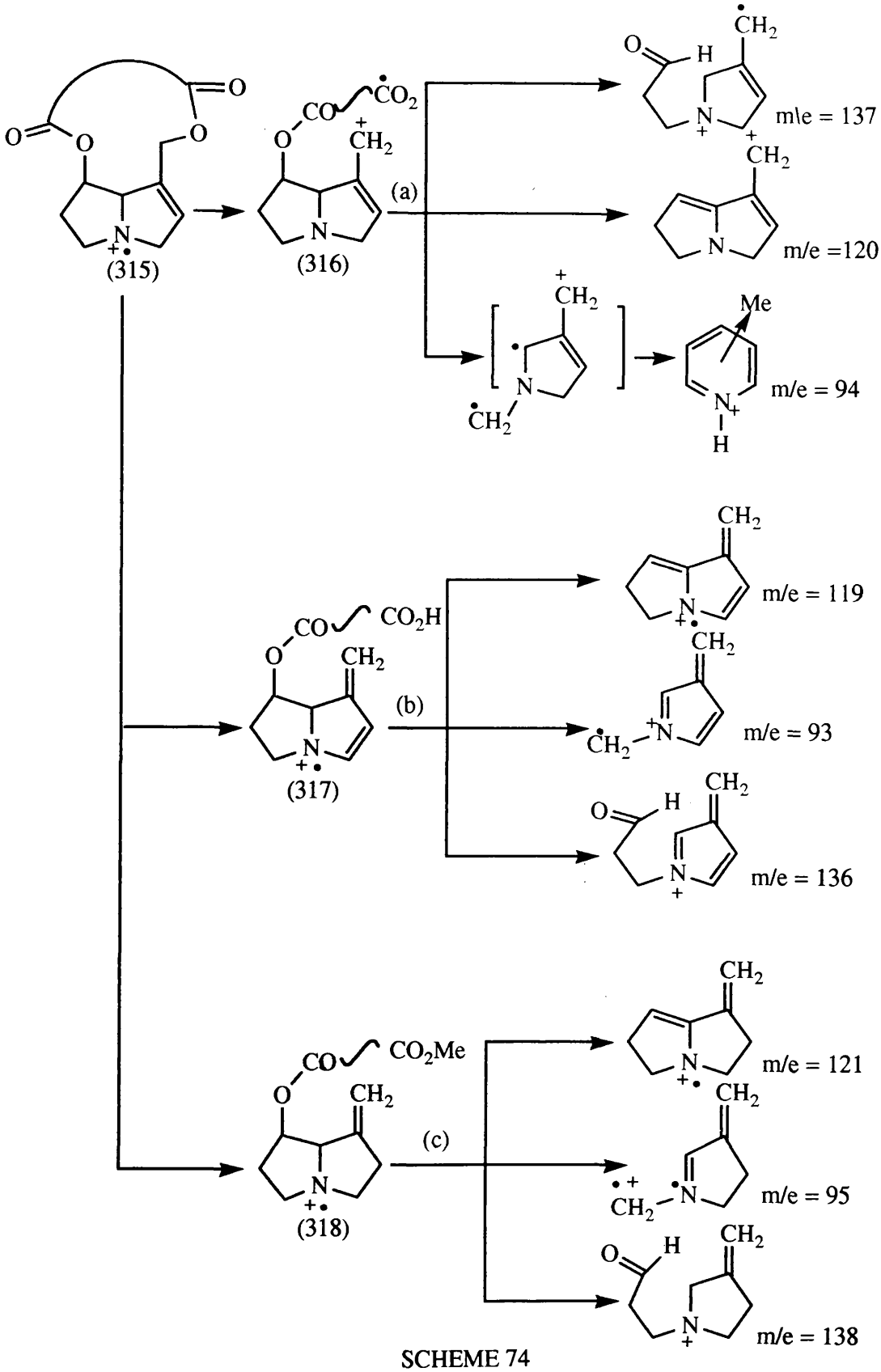
6.2 Extraction of Seeds of Crotalaria lanceolata

Seeds of Crotalaria lanceolata were obtained from Natal Province, Republic of South Africa, by courtesy of Professor D.A.H. Taylor. Initially the seed was immersed in petroleum ether to remove any fats. After removal of the fats, further organic material was extracted from the seeds by blending them in methanol. The resulting extract, after concentration, was taken up in dilute sulphuric acid and washed with methylene chloride to remove non-basic organic material. Thereafter, the acidic solution was stirred with zinc powder to reduce any N-oxides to the corresponding amines. Following removal of the zinc residue by filtration, the solution was basified to pH9 by addition of

conc. ammonia. The alkaline solution was then extracted with methylene chloride to yield, on removal of solvent, three alkaloids of combined content 0.2% based on the initial weight of dry seeds.

6.3 Analysis of Alkaloid Mixture from Seeds of Crotalaria lanceolata

A chromatogram of the extract (developed in $CHCl_3$/MeOH/NH_3; 85:14:1) gave rise to three distinct purple spots when sprayed with o-chloranil followed by Ehrlich's reagent.[148] This positive test indicated the presence of three pyrrolizidine alkaloids all with 1,2-unsaturation of the necine portion. The most intense of these spots was the most polar component (R_f = 0.33). The remaining spots displayed R_f values of 0.38 and 0.40, respectively, with the latter appearing to be the least intense of the alkaloid extract. High resolution mass spectrometry of the extract displayed a fragmentation pattern consistent with the breakdown of macrocyclic diesters possessing a retronecine base portion[149] (Scheme 74). Allylic pyrrolizidine macrocyclic diesters preferentially undergo fusion at the C-9(O) bond in three distinct ways. This results in the generation of three separate series of ions differing only by one mass unit. Simple allylic fusion of (315) generates the intermediate ion (316) which can undergo degradation affording a further three ion peaks (Scheme 74(a)). Rearrangement from (315) gives rise to intermediate ions (317) and (318) which in turn degrade to the ionic components illustrated in Scheme 74(b) and (c), respectively. A rearrangement of (315) involving the loss of a hydrogen atom from the pyrrolizidine ring affords (317), while rearrangement featuring the gain of a hydrogen atom generates (318). The indicative secondary ion peaks of these processes were evident from high


(315)
(316)
(a)
m\e = 137
m/e =120
m/e = 94
(317)
(b)
m/e = 119
m/e = 93
m/e = 136
(318)
(c)
m/e = 121
m/e = 95
m/e = 138

SCHEME 74

resolution mass spectrometry analysis of the alkaloid mixture.

Further scrutiny of the mass spectrum indicated probable parent ion peaks corresponding to $C_{18}H_{25}NO_6$, $C_{18}H_{25}NO_5$, and $C_{17}H_{23}NO_5$. An i.r. spectrum of the mixture displayed peaks at 1710 and 1735 cm^{-1}. The higher frequency corresponds to the carbonyl stretching mode for an αβ-unsaturated ester system. Also evident was an olefinic peak at 1650 cm^{-1}. Analysis of a ^1H n.m.r. spectrum of the mixture revealed the familiar AB system associated with the diastereotopic C-9 protons of a 1,2-unsaturated pyrrolizidine macrocyclic diester.

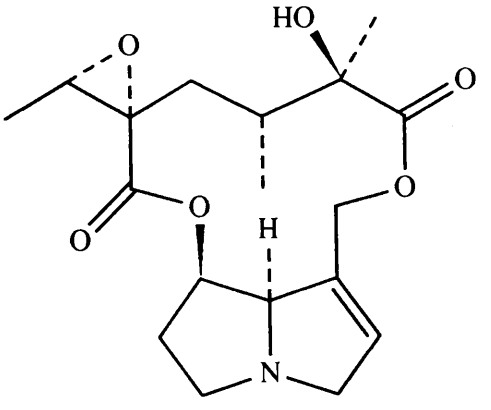
6.4 Separation of the Alkaloid Mixture

To identify the components of the alkaloid mixture the extract was subjected to column chromatography, employing basic alumina as the stationary phase and CH_2Cl_2/MeOH (99:1) as eluant. The principal component of the mixture (R_f = 0.33) was readily separated and accounted for 85% of the alkaloid extract. However, further column chromatography was required to enable even a partial separation of the remaining components. It was established, in conjunction with H.P.L.C., that these components were present in a relative ratio of 2:1.

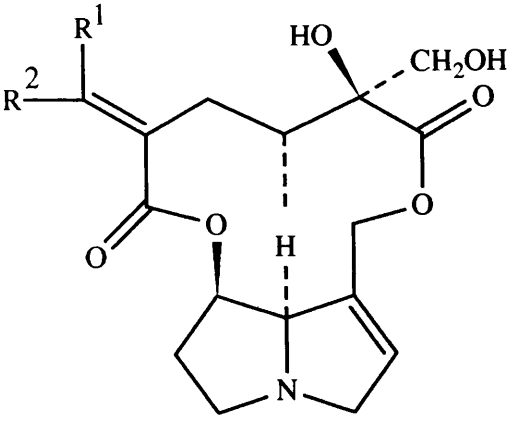
6.5 Identification of Usaramine (314)

High resolution mass spectrometry established that the main component of the alkaloid extract (R_f = 0.33) corresponded to molecular formula $C_{18}H_{25}NO_6$. In addition, the fragmentation pattern observed was indicative of a retronecine-based macrocyclic diester. Four retronecine-based macrocyclic diesters of molecular formula $C_{18}H_{25}NO_6$

have been reported.[1] These are grantaline (319), jacobine (320), retrorsine (9), and usaramine (314).

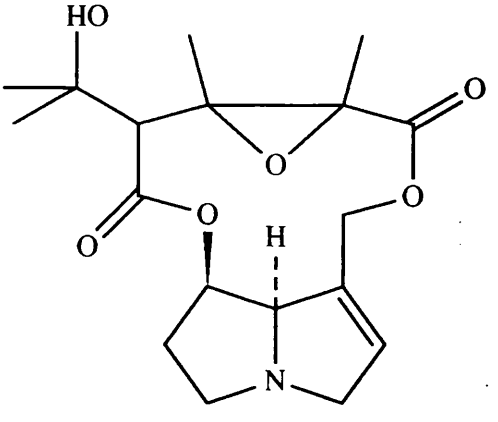


(320)



$R^1 = H$, $R^2 = Me$ (9)

$R^1 = Me$, $R^2 = H$ (314)



(319)

However, consideration of the ^1H and ^{13}C n.m.r. spectroscopic data indicated usaramine (314)[150] as the likely structure of the alkaloid.

A ^1H n.m.r. spectrum of the alkaloid indicated the presence of two olefinic protons at δ6.52 and 6.20, respectively. The former of these signals appears as a quartet (J 7 Hz) and corresponds to the C-20 vinyl proton, with coupling to C-21 protons evident. The relative downfield shift on this olefinic proton is indicative of a trans-arrangement around

this double bond - protons in such a system are normally found in the range of δ6.5-7.0. In contrast a cis-arrangement around this double bond, as in retrorsine (9), tends to result in the ensuing quartet being centred in the range δ6.0-6.2. The multiplet at δ6.20 is for the C-2 proton. The C-9d proton is evident as a doublet (of an AB system) at δ5.41 (J 12 Hz). The C-7 proton is a complicated signal at δ5.01. A complex multiplet, encompassing the two hydroxyl protons, the C-8 proton, and the C-9u proton is located at δ3.95-4.40. The C-3d proton appears as a multiplet at δ3.90. The C-18 protons appear as a doublet at δ3.67 through coupling to the C-13 protons (J 3 Hz). The C-3u proton signal is centred on δ3.46, while the C-5d signal appears at δ3.26. A complex signal δ1.90-2.70 incorporates the C-5u, C-13, C-14, and C-6 protons. The methyl protons at C-21 appear as a doublet (J 7 Hz) at δ1.71 through coupling to the C-20 olefinic proton. The C-19 protons appear as a doublet (J 7 Hz) centred on δ0.83 from coupling to the C-13 proton.

The ^{13}C n.m.r. spectrum of the alkaloid gave a signal pattern consistent with usaramine (314) as structure. In addition, optical activity and melting point measurements were in good agreement with reported values for usaramine (314).[150]

6.6 Identification of Nilgirine (321)

High resolution mass spectrometry established the molecular formula of the second component of the alkaloid extract (R_f = 0.38) to be $C_{17}H_{23}NO_5$. The observed fragmentation pattern was consistent with a retronecine-based macrocyclic diester. Only two such diesters have been reported to be of molecular formula $C_{17}H_{23}NO_5$.[1] These are doronecine (322) and nilgirine (321).

However, consideration of the ^{1}H and ^{13}C n.m.r. spectroscopic data for the alkaloid indicated nilgirine[151] (321) as the likely structure.

A ^{1}H n.m.r. spectrum of the alkaloid (Figure 5), run in deuteriochloroform, displayed the C-19 vinyl proton as a quartet at δ6.53 (J 7 Hz) through coupling with the C-20 protons. The downfield shift of this proton signal is indicative of trans-stereochemistry around this double bond. The complex signal at δ6.15 is for the olefinic proton at C-2. An AB system arising from the coupling of the diastereotopic protons at C-9 appears at δ4.11 and 5.34 (J 12 Hz). Coupling of the C-7 proton to the C-8 proton, together with the non-equivalent C-6 protons give rise to a doublet of doublet of doublets centred at δ5.15. The bridgehead proton, at C-8, appears as a multiplet δ4.24-4.34. Coupling of the C-12 proton with the proton at C-13 gives rise to a doublet at δ4.04. The non-equivalence of the C-3 protons results in the signal for the C-3d proton appearing as a doublet at δ3.92 through coupling to the C-3u proton (J 16 Hz). The C-3u proton is coupled additionally to the C-2 and C-8 protons giving rise to a doublet of doublet of doublets at δ3.39. Similar signal patterns to that of the C-3u proton are observed for the C-5 protons. The C-5d proton signal is centred at δ3.25 with mutual coupling between the diastereotopic C-5 protons evident, together with coupling to the non-equivalent C-6 protons. The C-5u proton is observed at δ2.54 with coupling to the C-6 protons again observed. The broad singlet δ2.75-2.95 is for the hydroxyl proton at C-12. The elaborate signal centred at δ2.30 is for the C-13 proton. The C-14 protons appear as doublets at δ2.16. The complex signal centred on δ2.08 is for the C-6 protons. The methyl protons at C-20 appear as a doublet at δ1.74 through coupling to the C-19 vinyl proton. The C-18 protons appear as a doublet at δ1.03

through coupling to the C-13 protons.

The ^{13}C n.m.r. spectrum of the alkaloid was assigned on the basis of a D.E.P.T. experiment (Figure 6). This gave rise to a pattern consistent with nilgirine (321) as the structure for the alkaloids. In addition, optical activity and melting point measurements were in good agreement with reported values for nilgirine.[151]

The stereochemistry at the C-12 and C-13 centres of nilgirine has not been established. Attempts to elucidate the stereochemistry of these sites by X-ray crystal structure analysis were unsuccessful due to imperfect crystal quality.

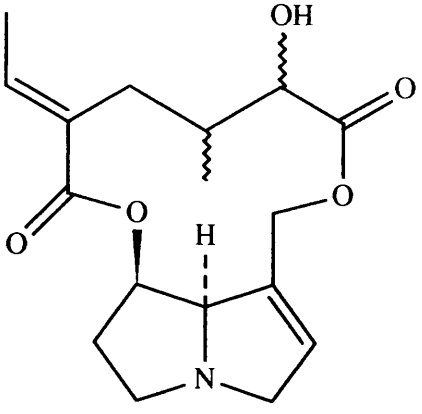
This is only the third reported occurrence of nilgirine.[151,152]

6.7 Identification of Integerrimine (313)

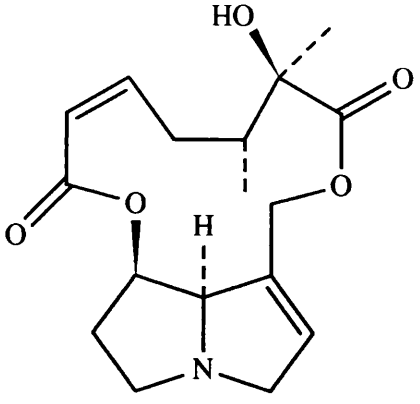
The least abundant component of the alkaloid extract (R_f = 0.40) was shown to have a molecular formula of $C_{18}H_{25}NO_5$ by high resolution mass spectrometry. The fragmentation pattern observed was again consistent with that of a retronecine-based macrocyclic diester. The literature documents four such pyrrolizidine alkaloids of molecular formula $C_{18}H_{25}NO_5$. These are integerrimine (313), its C-20 geometric isomer senecionine (323), senecivernine (324), and retroisosenine (325). However, consideration of the observed ^1H n.m.r. spectroscopic data enabled integerrimine (313) to be proposed as the likely structure for the alkaloid. ^1H N.m.r. spectroscopy (Figure 7) indicates the presence of two olefinic protons, one of which is coupled to an adjacent methyl group. The stereochemistry around the double bond bearing this proton is likely to be trans, determined by the downfield shift of this quartet at δ6.51.

This corresponds to the C-20 proton of integerrimine. The C-2 vinyl proton appears as a multiplet at δ6.21. The AB system generated by the mutual coupling of the diastereotopic C-9 protons is evident at δ4.11 and 5.41 (J 12 Hz). The C-7 proton appears as a doublet of doublet of doublets through coupling to the bridgehead proton at C-8 and the non-equivalent C-6 protons. This signal is centred at δ5.01. The broad signal δ4.31-4.66 is for the bridgehead proton at C-8. The C-3d proton appears as a doublet at δ3.95. The large coupling constant arises from coupling between the non-equivalent C-3 protons (J 15 Hz). The signal observed for the C-3u proton is more complicated. Further coupling between the C-3 proton and the non-equivalent C-5 protons is witnessed. This gives rise to a doublet of doublet of doublets centred at δ3.39. The complex signals at δ2.46 and 3.25 are for the C-5d and C-5u protons, respectively. The C-13 proton signal is a multiplet at δ2.20-2.30 while the C-14 proton signal is evident at δ2.05-2.18. The C-6 protons appear as a complex pattern δ1.95-2.05, with individual couplings not deciphered. The methyl protons at C-21 appear as a doublet at δ1.74 (J 7 Hz) from coupling with the olefinic proton of C-20. The C-19 protons appear as a doublet at δ0.91 through coupling to the C-13 proton. A further doublet at δ1.29 arises for the C-18 methyl protons.

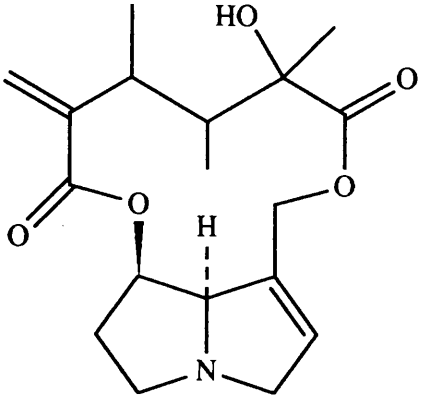
Melting point and optical rotation measurements for the alkaloid were in good agreement with literature values for integerrimine.[153-155] However, it should be noted that some disparity has been shown in values of optical activity for integerrimine. Values have ranged from +4.3°[153] to -22.1°[154] (both measurements in $CHCl_3$).



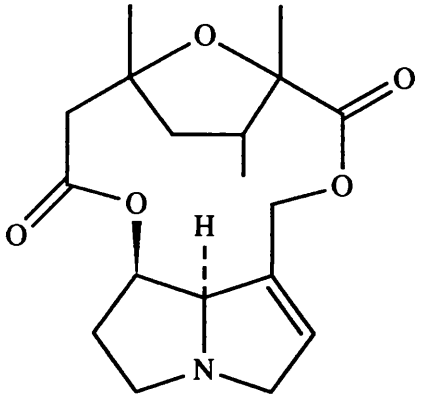
(321)



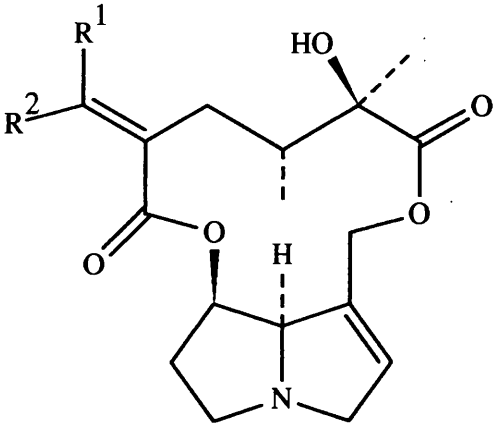
(322)



(324)



(325)



$R^1 = Me$, $R^2 = H$ (313)

$R^1 = H$, $R^2 = Me$ (323)

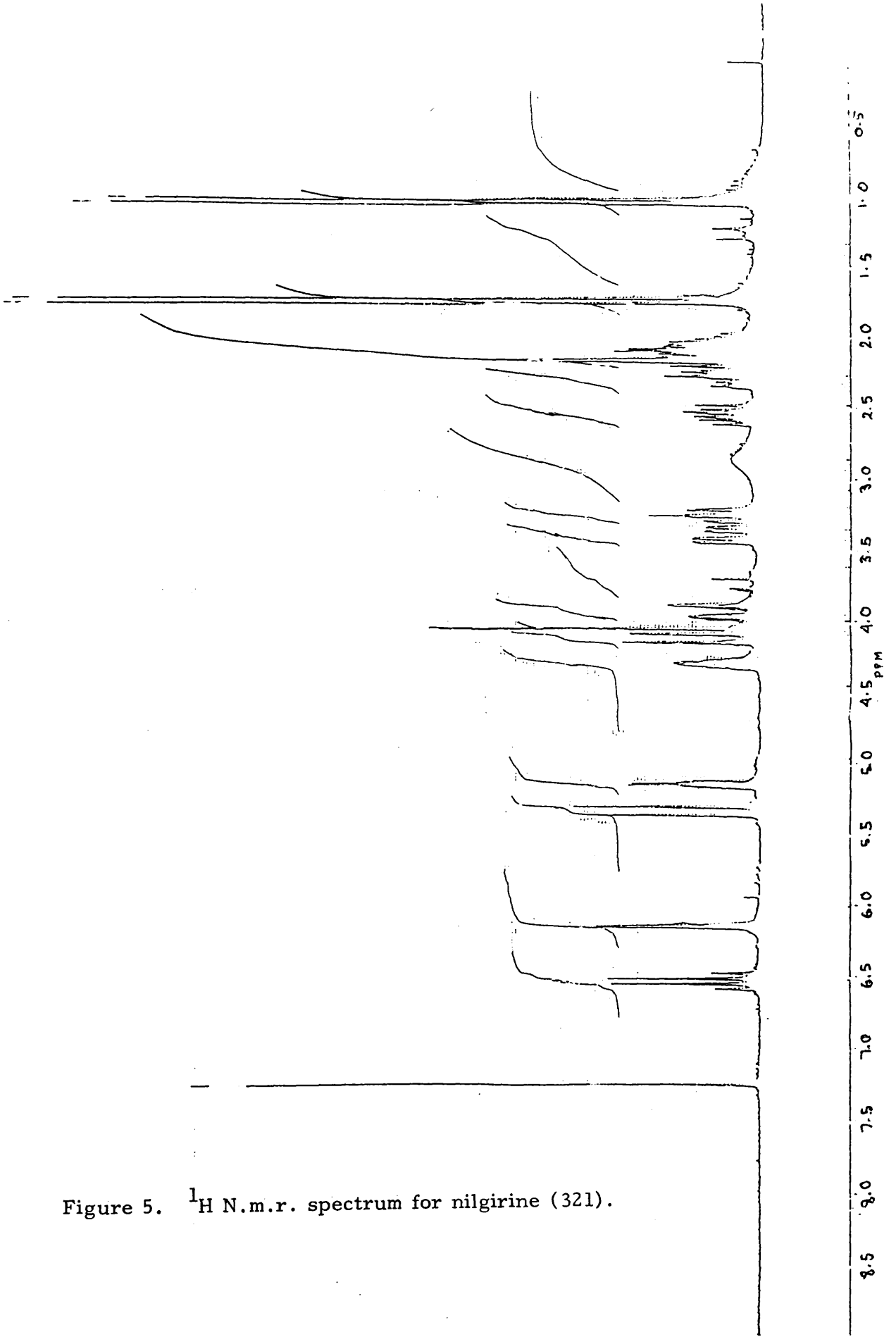


Figure 5. ^{1}H N.m.r. spectrum for nilgirine (321).

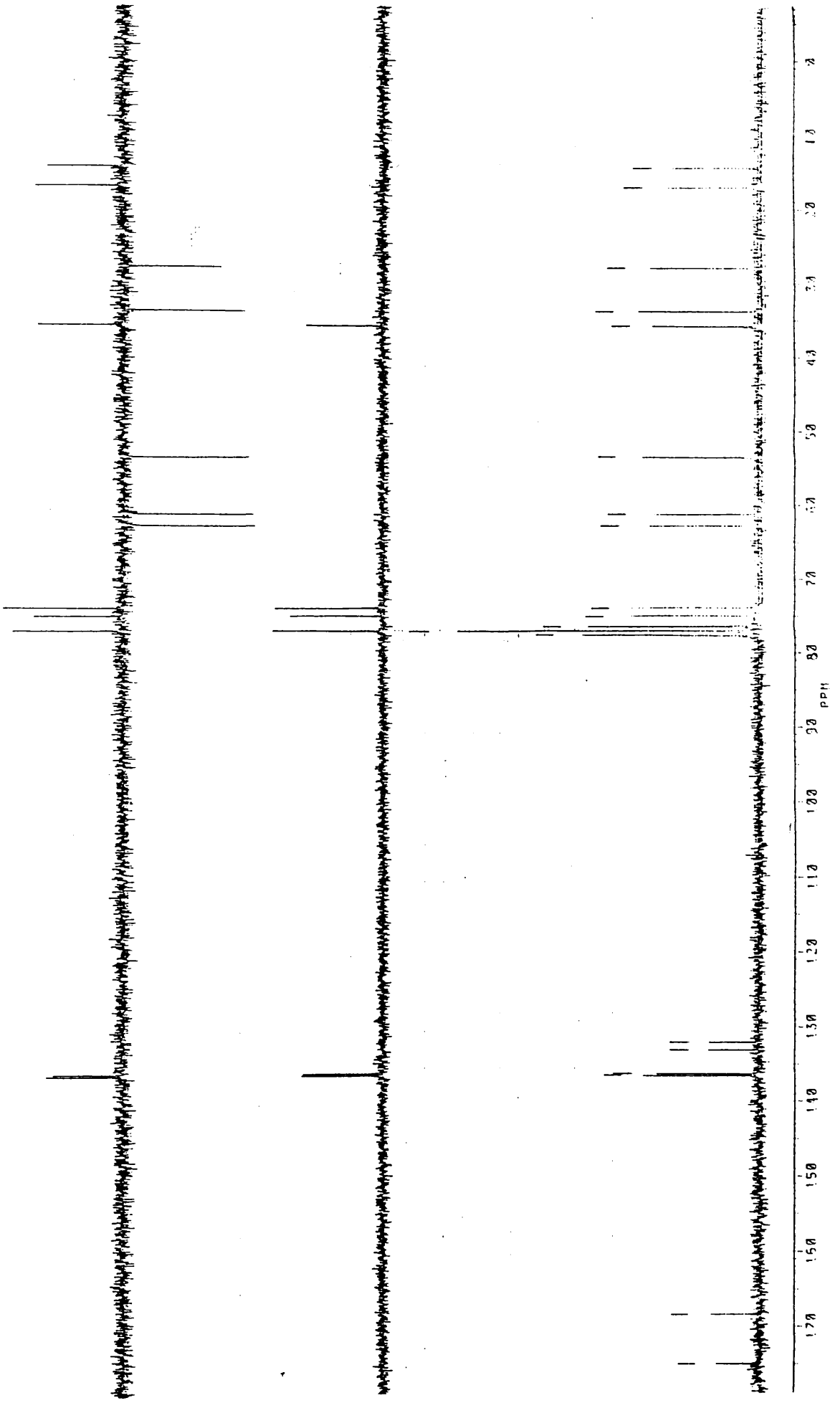


Figure 6. D.E.P.T. spectrum for nilgirine (321).

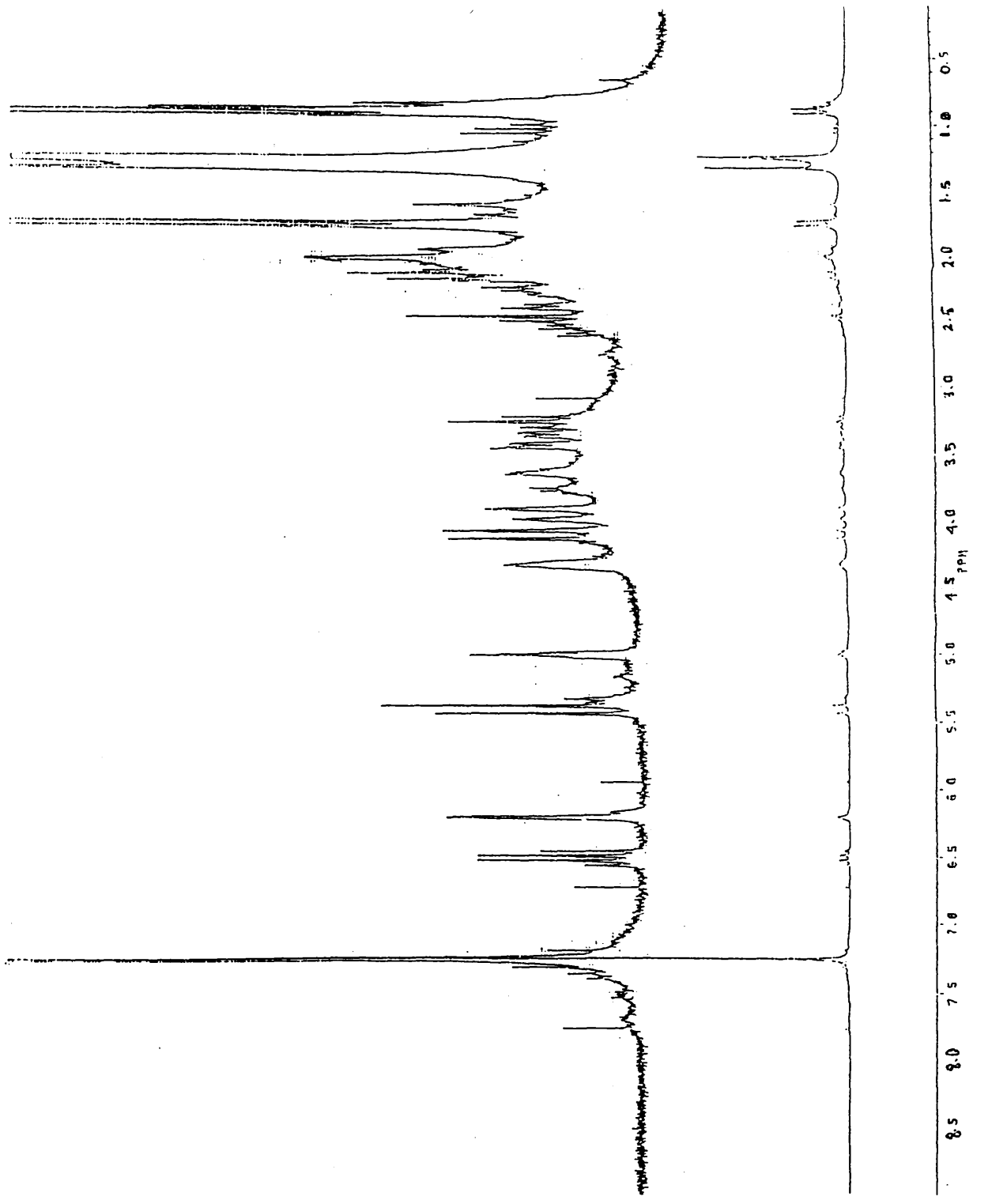


Figure 7. ^{1}H N.m.r. spectrum for integerrimine (313).

Summary

The pyrrolizidine alkaloid content of Crotalaria lanceolata is around 0.2%. Three macrocyclic diesters containing retronecine as base portion were found in the seeds, namely usaramine (314), nilgirine (321), and integerrimine (313). These were present in a relative ratio of 85:10:5 [(314):(321):(313)]. The work has been accepted for publication.[155]

CHAPTER 7

EXPERIMENTAL

7.1 General

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 recorded on a Perkin Elmer 580 spectrophotometer. Nuclear magnetic resonance spectra were obtained with a Perkin Elmer R32 spectrometer operating at 90 MHz (δ_H), a Varian XL-100 spectrometer operating at 25 MHz (δ_C), and a Bruker WP200-SY spectrometer operating at 200 MHz (δ_H) and 50 MHz (δ_C). Spectra were run for solutions in deuteriochloroform unless otherwise stated with tetramethylsilane acting as internal standard.

T.l.c. was carried out on Kieselgel G plates 0.25 mm thickness. Alkaloids were detected by the modified Dragendorff reagent,[156] or by oxidation with o-chloranil followed by treatment with Erhlich's reagent.[148]

Radiochemicals were purchased from Amersham International p.l.c., with radioactivity measurement quantified with a Philips PW4700 Liquid Scintillation Counter using toluene-methanol solutions. Accumulation of sufficient counts was undertaken to ensure a standard error of less than 1% for each determination. Radioactive samples prepared for feeding purposes were distilled or recrystallised to constant specific radioactivity and 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 then sodium benzophenone. Diethyl ether (Et_2O) was dried by distillation from lithium aluminium hydride while N,N-dimethylformamide (DMF) was purified by distillation from calcium hydride and stored over 3Å molecular sieves.[157] Dichloromethane and chloroform were pre-dried with calcium chloride then distilled from phosphorus pentoxide. Acetone was dried utilising 3Å molecular sieves and diisopropylamine was distilled from sodium hydroxide and stored over 4Å molecular sieves.[158]

Organic solutions were dried over either anhydrous magnesium or sodium sulphate and solvents were removed by evaporation under reduced pressure, below 50°C.

7.2 Experimental to Chapter 4

Preparation of N-Benzyl protected imides from stereoisomeric malic and tartaric acid

This method was based on the preparation by Wong et al.[114]

L-Tartaric acid (209) (10.0g, 66.7 mmol) was added to p-xylene (325 ml) in a flask equipped with a Dean and Stark apparatus. The mixture was heated to reflux and benzylamine (8.5 ml, 81.0 mmol) was added over a 30 min period. The resulting reaction mixture was maintained at reflux for a further 3h. The solution was then cooled to 0°C and the crystalline product was separated by filtration. Recrystallisation from water afforded (2R,3R)-N-benzyl-dihydroxysuccinimide (210) (8.68g, 58.9%) (lit.,[114] 60.7%) m.p. 195-197°C (lit.,[114] 196-198°C); $[\alpha]_D^{21}$ + 137.9° (C 4.6, MeOH) (lit.,[114] $[\alpha]_D^{25}$ + 126°); ν_{max} (KBr disc)

3300, 1710, 1665, 1525, and 1460 cm^{-1}; δ_H (90 MHz) [$CO(CD_3)_2$] 4.50 (2H, s), 4.62 (2H, s), 5.30-5.60 (2H, br s, 20H) and 7.20-7.40 ppm (5H, m); δ_C (25 MHz) [$CO(CD_3)_2$] 43.9, 74.5, 128.2, 128.5, 129.5, 139.8, and 179.8 ppm; m/z 221 (M^+, 20.3%), 203, 165, 164, 133, 132, 106, 105, 104, 92, 91 (100%), 79, 78, and 71 (Found: M^+, 221.0689. $C_{11}H_{11}NO_4$ requires M, 221.0688). (Found: C, 59.77; H, 4.80; N, 6.26. $C_{11}H_{11}NO_4$ requires C, 59.73; H, 5.01; N, 6.33%).

The enantiomer (2S,3S)-N-benzyl dihydroxysuccinimide (240) was prepared in the same way[114] from D-tartaric acid, 58.3%, $[\alpha]_D^{16}$ - 136.9° (C 5.8, MeOH).

(S)-N-Benzyl-2-hydroxysuccinimide (238) was prepared from L-malic acid (230) by the procedure of Wong et al.[114] in 56.3% yield, m.p. 179-180°C, $[\alpha]_D^{18}$ - 7.9° (C 7, MeOH); ν_{max} (KBr disc) 3300, 2980, 1695, 1640, 1545, and 1455 cm^{-1}; δ_H (90 MHz) [$CO(CD_3)_2$] 2.58, 2.85 (2H, ABX system, J_{AB} 15 Hz, J_{AX+BX} 9 Hz), 4.37 (1H, J_{AX+BX} 9 Hz), 4.35 (2H, s), 5.40-5.60 (1H, br s, OH) and 7.10-7.45 ppm (5H, m); δ_C (25 MHz) [$CO(CD_3)_2$] 41.7, 43.8, 70.3, 128.2, 128.5, 129.5, 139.9, 172.3, and 176.2 ppm; m/z 205 (M^+ 6.1%) 178, 106, 92, 91 (100%), 89 and 65. (Found: M^+, 205.0745. $C_{11}H_{11}NO_3$ requires M, 205.0739). (Found: C, 64.49; H, 5.35; N, 6.94. $C_{11}H_{11}NO_3$ requires C, 64.38; H, 5.40; N, 6.83%).

The enantiomer (R)-N-benzyl-2-hydroxysuccinimide (239) was prepared from D-malic acid by the method reported by Wong et al.,[114] in 55.9% yield; $[\alpha]_D^{17}$ + 8.1° (C 9.4, MeOH).

N-Benzyl-meso-2,3-dihydroxysuccinimide (213) was prepared from meso-tartaric acid (214) by the method reported by Wong et al.,[114] in 28.6% yield, m.p. 167-169°C; ν_{max} (Nujol) 3280, 1710, 1640, and 1460 cm^{-1}; δ_H (90 MHz) [$CO(CD_3)_2$] 4.42 (2H, s), 4.65 (2H, s), 4.65-4.85 (2H, br s, OH), and 7.25-7.45 ppm (5H, m); δ_C (25 MHz) [$CO(CD_3)_2$] 43.0, 76.2, 128.9, 129.2, 129.6, 137.1, and 175.8 ppm; m/z 221 (M^+, 35.7%), 164, 106, 105, 104, 92, 91 (100%), 79, 78, and 77. (Found: M^+, 221.0690. $C_{11}H_{11}NO_4$ requires M, 221.0688). (Found: C, 59.89; H, 5.01; N, 6.26. $C_{11}H_{11}NO_4$ requires C, 59.73; H, 5.01; N, 6.33%).

Preparation of meso-2,3-diacetoxy-N-benzylsuccinimide (244)

To a solution containing DMF (25 ml) and 2,2-dimethoxypropane (25 ml) was added diol (213) (458 mg, 2.07 mmol) followed by PTSA (11 mg, 0.06 mmol). The resultant mixture was stirred at room temperature for 6h and then neutralised by the addition of calcium carbonate. The reaction mixture was then diluted by addition of chloroform (100 ml) and washed in turn with 5% potassium carbonate solution (100 ml) followed by water (2 x 50 ml). The resultant organic extract was dried and then solvent was removed under reduced pressure. Recrystallisation of the crude product obtained from acetone, gave a white crystalline solid (336 mg, 62%), m.p. 175°C; ν_{max} (KBr disc) 2900, 1770, 1710, 1500, and 1395 cm^{-1}; δ_H (90 MHz) (CD_3OD) 1.45 (6H, s), 4.43 (2H, s), 4.60 (2H, s), and 7.20-7.40 ppm (5H, m); m/z 261 (M^+, 0.5%), 246, 221, 164, 146, 106, 105, 92, 91 (100%), 77 and 71. (Found: M^+ 261.1004. $C_{14}H_{15}NO_4$ requires M, 261.1001).

Attempted synthesis of (2R,3R)-dimethoxy-N-benzylsuccinimide (242)

Synthesis of (242) was attempted using the methylation procedure reported by Diner et al.[119]

To a solution of diol (210) (1.32g, 5.97 mmol) in DME (50 ml) was added methyl iodide (0.9 ml, 14.5 mmol) followed by careful addition of sodium hydride (314 mg, 13.7 mmol). Ten minutes after the completion of the sodium hydride addition a further quantity of methyl iodide (0.2 ml, 3.21 mmol) was added and the reaction was stirred at room temperature for 2h. Concentration of the reaction mixture under reduced pressure was followed by addition of ether (50 ml). The mixture was filtered and the inorganic residue was washed with ether (3 x 20 ml). The washings and filtrate were dried and then concentrated under reduced pressure to give a dark brown oil. ^{1}H N.m.r. spectroscopy and t.l.c. indicated that this oil contained several unidentified products.

Employing DMF as solvent for this reaction, using reduced temperatures and shorter reaction periods, gave similar findings.

Attempts to use this method to protect the hydroxyl groups of imides (238) and (213) gave similar results.

Attempted synthesis of (2R,3R)-dimethoxy-N-benzylsuccinimide (242)

Synthesis of (242) was attempted based on the procedure reported by Johnstone and Rose.[118]

To a solution containing potassium hydroxide (0.621g, 11.1 mmol) in DMSO (20 ml) at 60°C was added diol (210) (1.20g, 5.43 mmol) immediately followed by methyl iodide (1 ml, 16 mmol). Stirring was continued for 65 min at 60°C then the reaction mixture was cooled to room

temperature. The reaction mixture was poured into water (20 ml) and extracted with dichloromethane (3 x 30 ml). The organic extracts were combined, washed with water (2 x 50 ml), filtered through cotton wool, then dried. Concentration of the filtrate under reduced pressure gave only starting material, with no indication for the formation of (242). This procedure carried out over longer reaction periods gave a similar result. Attempts to protect the hydroxyl groups of N-protected imides (238) and (213) by this method again gave only starting material.

Attempted synthesis of (2R,3R)-dimethoxy-N-benzylsuccinimide (242)

Synthesis of (242) was attempted using the procedure of Purdie and Irvine.[120] [See p.196 for the preparation of diethyl (2R,3R)-dimethoxysuccinate (247)]. However, ^{1}H n.m.r. spectroscopy in conjunction with t.l.c. evidence indicated several unidentified products from reaction of imide (210) with Ag_2O/methyl iodide.

Attempted synthesis of (2R,3R)-2,3-diacetoxysuccinimide (215)

To a solution of acetal (244) (114 mg, 0.44 mmol) in ethanol (10 ml) was added a few drops of concentrated hydrochloric acid followed by 5% Pd/C (20 mg). The resultant slurry was maintained under a hydrogen atmosphere (1 atm. pressure) for 16h at room temperature then filtered through Celite. The Celite pad was washed with ethanol (2 x 10 ml) and the filtrate and washings were combined. Removal of solvent under reduced pressure gave only starting material (244).

A similar result was obtained for this reaction when PtO_2 (in AcOH) was employed. Attempts to remove the N-benzyl protection from imides (210) and (238) by these catalytic procedures again gave

only starting material. Increased temperatures (up to 55°C) and pressures (up to 9 atm.) in these reactions again gave only starting material.

Preparation of diethyl L-malate (252)

This preparation was based on the procedure reported by Locquin and Elghozy.[126]

To a solution of ethanol (24 ml) and toluene (12 ml) was added L-malic acid (230) (7.98g, 41 mmol) followed by a few drops of conc. hydrochloric acid. The reaction mixture was heated to ensure the water/toluene/ethanol azeotrope (b.p. 74.4°C) was freely distilling. Once distillation of this azeotrope had stopped similar quantities of ethanol (24 ml) and toluene (12 ml) were added to the reaction mixture and the azeotropic distillation was recommenced. Once the azeotrope stopped distilling the reaction mixture was cooled and solvents were removed under reduced pressure to afford, after distillation, a colourless oil (9.74g, 86.1%), b.p. 120°C/5mm (lit.,[159] 253°C/760mm); $[\alpha]_D^{16}$ - 9.8° (C 6.8, $CHCl_3$) (lit.,[159] $[\alpha]_D^{18}$ - 10.1°); ν_{max} (neat) 3470, 2980, and 1735 cm^{-1}; δ_H (90 MHz) (CD_3OD) 1.21 (3H, t, J 7 Hz), 1.24 (3H, t, J 7 Hz), 2.76 (2H, d, J 6 Hz), 3.65-3.80 (1H, br s, OH), 4.15 (2H, q, J 7 Hz), 4.17 2H, q, J 7 Hz) and 4.52 ppm (1H, t, J 6 Hz); δ_C (25 MHz) (CD_3OD) 18.6, 18.8, 44.2, 65.4, 66.0, 72.7, 175.5, and 178.1 ppm; m/z 145 (M^+ - 45, 8.5%), 127, 117, 99, 89, 75, 71 (100%), 47, 45, and 43. (Found: M^+ -45, 145.0517. $C_6H_9O_4$ requires 145.0502) (Found: C, 50.69; H, 7.37. $C_8H_{14}O_5$ requires C, 50.52; H, 7.42%).

The enantiomer diethyl D-malate was prepared by this method

from D-malic acid (207); $[\alpha]_D^{17}$ + 9.7° (C 6.6, $CHCl_3$).

Preparation of meso-tartaric acid diethyl ester (253)

Preparation of the title compound, from meso-tartaric acid (214), was based on the procedure reported by Locquin and Elghozy.[126] Diester (253) was produced in 81.3% yield, m.p. 65°C; ν_{max} (neat) 3380, 2980, and 1740 cm^{-1}; δ_H (90 MHz) [$CO(CD_3)_2$] 1.25 (6H, t, J 7.5 Hz), 4.21 (4H, q, J 7.5 Hz), 4.54 (2H, s), and 4.90-5.30 ppm (2H, br s, OH); δ_C (25 MHz) [$CO(CD_3)_2$] 14.1, 62.2, 73.1, and 171.2 ppm; m/z 188 (M^+ - 18, 8.2%), 161, 133, 105, 104 (100%), 103, 99, 76, 75, 61, 60 and 31. (Found: M^+ - 18, 188.0677. $C_8H_{12}O_5$ requires M, 188.0674).

Preparation of diethyl (2R,3R)-dimethoxysuccinate (247)

The procedure used was modified from that reported by Purdie and Irvine.[120]

Under a nitrogen atmosphere Ag_2O (7.66g, 33 mmol) was added to a solution of diethyl L-tartrate (252) (2.27g, 11 mmol) in methyl iodide (3 ml, 48 mmol). The vigorous reaction which ensued was moderated by cooling at 0°C for 15 min before a further methyl iodide addition (1.1 ml, 18 mmol) was made to the reaction mixture. The resultant slurry was then heated to reflux and maintained under these conditions for 16h. The reaction mixture was cooled and ether (80 ml) was added. The silver residue was removed by filtration and washed with ether (2 x 40 ml). The ethereal extracts were combined, dried, and concentrated under reduced pressure to afford, on distillation, a clear oil (2.50g, 96.9%), b.p. 115°C/2mm (lit.,[120] 155°C/25mm); $[\alpha]_D^{17}$ + 92.8° (C 36, $CHCl_3$) (lit.,[120] $[\alpha]_D^{20}$ + 98.7°); ν_{max} ($CHCl_3$) 2930 and 1730 cm^{-1}; δ_H (90 MHz)

[$CO(CD_3)_2$] 1.24 (6H, t, J 7.5 Hz), 3.36 (6H, s), and 4.05-4.35 ppm (6H, m); δ_C (25 MHz) 14.3, 59.6, 73.4, 81.3, and 169.2 ppm; m/z 234 (M^+, 7.4%), 202, 173, 161, 133, 132, 131, 117 (100%), 105, 104, 103, 89 and 74. (Found: M^+, 234.1114. $C_{10}H_{18}O_6$ requires M, 234.1103).

Preparation of diethyl meso-dimethoxysuccinate

The title compound was prepared from meso-tartaric acid diethyl ester (253), by a procedure based on that reported by Purdie and Irvine[120] in 94% yield, b.p. 115°C/2mm; ν_{max} (neat) 2980, 1735, and 1195 cm^{-1}; δ_H (90 MHz) 1.22 (6H, t, J 7.5 Hz), 3.45 (6H, s), 3.73 (2H, s), and 4.11 ppm (4H, q, J 7.5 Hz); m/z 234 (M^+, 2.3%), 173, 161, 147, 145, 133, 131, 119, 118, 117 (100%), 105, 104, 103, 89, 77 and 74. (Found: M^+, 234.1109. $C_{10}H_{18}O_6$ requires M, 234.1103).

Preparation of diethyl (S)-methoxysuccinate (300)

The title compound was prepared from diester (252) by a procedure based on the method reported by Purdie and Irvine,[120] in 94.5% yield, b.p. 105°C/1.5mm (lit.,[160] 126°C/17mm); $[\alpha]_D^{15}$ - 48.4° (C 5.3, $CHCl_3$) (lit.,[160] $[\alpha]_D^{18}$ - 50.1°); ν_{max} (KBr disc) 2980 and 1735 cm^{-1}; δ_H (90 MHz) 1.22 (3H, t, J 7 Hz), 1.25 (3H, t, J 7 Hz), 2.65-2.90 (2H, m), 3.44 (3H, s), and 4.05-4.40 ppm (5H, m); δ_C (25 MHz) 13.6, 14.2, 37.9, 58.7, 61.0, 61.3, 76.9, 170.2, and 171.3 ppm; m/z 204 (M^+, 0.8%), 174, 131, 117, 103, 89 (100%), 71, 61, and 43. (Found: M^+, 204.0989. $C_9H_{16}O_5$ requires M, 204.0981).

The enantiomer, diethyl (R)-methoxysuccinate, was prepared from D-malic acid diethyl ester; $[\alpha]_D^{13}$ + 47.9° (C 6.9, $CHCl_3$).

Attempts to prepare diethyl (2R,3R)-dimethoxysuccinate (247)

To a suspension of K_2CO_3 (2.12g, 15.4 mmol) in diethyl L-tartrate (252) (0.54g, 0.26mmol) at 0°C was added acetone (60 ml) followed by dimethyl sulphate (0.55 ml, 5.36 mmol). The reaction mixture was then carefully heated to reflux and maintained under these conditions for 18h. The reaction mixture was cooled and excess K_2CO_3 was removed by filtration. The resultant solution was then concentrated under reduced pressure and ether (100 ml) was added. The ethereal solution was then washed with ammonia solution (2 x 100 ml), dried, and concentrated. However, ^1H n.m.r. spectroscopy and t.l.c. data of the product indicated incomplete methylation of (246), together with the formation of several unidentified compounds.

Attempts to prepare diethyl (2R,3R)-dimethoxysuccinate (247)

Synthesis of the title compound was attempted by a procedure based on the method of Brown and Barton.[127]

Under a nitrogen atmosphere, a solution containing diethyl L-tartrate (246) (4.87g, 24 mmol), methyl iodide (4.5 ml, 72 mmol) and THF (20 ml) was carefully added to a solution containing sodium hydride (1.42g, 59 mmol) in THF (40 ml) at 0°C. The resultant reaction mixture was then heated to reflux and maintained under these conditions for 70 min. The reaction mixture was then cooled and wet THF was carefully added (30 ml). The reaction mixture was then concentrated under reduced pressure and partitioned between ether (150 ml) and water (80 ml). The aqueous layer was isolated and washed with ether (2 x 30 ml) and the ethereal extracts were combined and washed with brine (2 x 50 ml). The

ethereal solution was then dried and concentrated under reduced pressure. Separation of the product mixture by column chromatography afforded (247) (3.39g, 61.3% on distillation (120°C/3mm) (lit.,[120] 155°C/25mm); $[\alpha]_D^{18}$ + 25.2° (C 25, $CHCl_3$) (lit.,[120] $[\alpha]_D^{20}$ + 98.7°). The significant decrease in optical activity from the earlier preparation of (247) indicated that epimerisation had occurred.

Preparation of (2R,3R)-dimethoxysuccinic acid (248)

To a warm (70°C) aqueous barium hydroxide solution (9.4g, 5 mmol in 275 ml water) was added diester (247) (5.19g, 22 mmol) and the reaction mixture was maintained at this temperature for 90 min. The reaction mixture was then cooled, acidified to pH2 by addition of conc. hydrochloric acid and water was removed under reduced pressure. n-Butanol (200 ml) was added to the residue and the inorganic component of this residue was removed by filtration through Celite. Removal of solvent under vacuum afforded a white crystalline solid (3.10g, 78.4%), m.p. 153°C (lit.,[120] 151°C); $[\alpha]_D^{19}$ + 95.4° (C 3.6, acetone) (lit.,[120] + 95.8°); ν_{max} (KBr disc) 3430, 1730, and 1630 cm^{-1}; δ_H (90 MHz) (D_2O) 3.40 (6H, s) and 4.24 ppm (2H, s); δ_C (25 MHz) (D_2O) 60.1, 72.1, and 175.0 ppm; m/z 178 (M^+, 1.8%), 146, 133, 104, 90, 89, 88, 74, 73 (100%), and 72. (Found: M^+, 178.0475. $C_6H_{10}O_6$ required M, 178.0478).

Preparation of meso-2,3-dimethoxysuccinic acid (255)

The title compound was prepared from diethyl meso-dimethoxysuccinate by the method used in the preparation of (248); 72.9% yield, m.p. 160°C; ν_{max} (KBr disc) 3420, 1725, and 1640 cm^{-1}; δ_H (90 MHz)

(D_2O) 3.25 (6H, s), and 4.25 ppm (2H, s); m/z 178 (M^+, 0.8%), 146, 133, 102, 89, 74, 73 (100%), 72, 61, and 45. (Found: M^+, 178.0470. $C_6H_{10}O_6$ requires M, 178.0478).

Preparation of (S)-2-methoxysuccinic acid

The title compound was prepared from diethyl (S)-methoxysuccinate (300) by the method used in the preparation of (248) in 70.1% yield, m.p. 89-90°C (lit.,[160] 89°C); $[\alpha]_D^{16}$ - 32.1° (C 11.4, H_2O) (lit.,[160] $[\alpha]_D^{11}$ - 32.9°); ν_{max} (KBr disc) 3420, 1725, and 1635 cm^{-1}; δ_H (90 MHz) (D_2O) 2.45, 2.78 (2H, ABX system, J_{AB} 12 Hz, J_{AX+BX} 9 Hz), 3.39 (3H, s) and 4.12 (1H, J_{AX+BX} 9 Hz); m/z 148 (M^+, 1.3%), 133, 119, 104, 103, 90, 89 (100%), 61, 59, 58, 55, and 45. (Found: M^+, 148.0366. $C_5H_8O_5$ requires M, 148.0372).

Preparation of (2R,3R)-2,3-dimethoxysuccinic anhydride (249)

The title compound was prepared by a modified procedure to that reported by Purdie and Young,[121]

Under a nitrogen atmosphere excess acetyl chloride (6 ml, 84 mmol) was added to diacid (248) (1.46g, 8.2 mmol) and the resultant mixture was heated to reflux and maintained under these conditions for 3h. Excess acetyl chloride was removed under reduced pressure and recrystallisation of the residue from ether afforded a white crystalline solid (249) (1.09g, 83%), m.p. 80-82°C (lit.,[121] 80-82°C); $[\alpha]_D^{13}$ + 148.3° (C 3.4, acetone) (lit.,[121] $[\alpha]_D^{20}$ + 148.9°); ν_{max} ($CHCl_3$) 3030, 1760, and 1735 cm^{-1}; δ_H (90 MHz) [$CO(CD_3)_2$] 3.44 (6H, s), and 4.28 ppm (2H, s); δ_C (25 MHz) [$CO(CD_3)_2$] 59.6, 81.8, and 170.1 ppm; m/z 160 (M^+, 0.3%), 146, 133, 104, 102, 89 (100%), 85, 74, 73, 72, 61, and 45.

(Found: $\underline{M}^+$, 160.0368. $C_6H_8O_5$ requires $\underline{M}$, 160.0372).

Preparation of meso-2,3-dimethoxysuccinic anhydride (254)

Preparation of the title compound was carried out from meso-2,3-dimethoxysuccinic acid, by a procedure related to that reported by Purdie and Young,[121] in 66% yield, m.p. 71-72°C; ν_{max} ($CHCl_3$) 3010, 1755, and 1730 cm^{-1}; δ_H (90 MHz) 3.41 (6H, s), and 4.27 ppm (2H, s); $\underline{m}/\underline{z}$ 160 ($\underline{M}^+$, 1.1%), 133, 119, 104, 103, 102, 90, 89 (100%), 85, 73, 72, 61 and 45. (Found: $\underline{M}^+$ 160.0363. $C_6H_8O_5$ requires $\underline{M}$, 160.0372).

A small quantity of anhydride (249) [in racemic form] was isolated by column chromatography and amounted to around 10% of the reaction product.

Preparation of (S)-2-methoxysuccinic anhydride (257)

Preparation of the title compound was from (S)-2-methoxy-succinic acid by employing the procedure reported by Purdie and Young.[121] The title compound was produced in 49% yield, b.p. 145°C/1.3mm (lit.,[121] 180°C/17mm); $[\alpha]_D^{14}$ - 84.3° (C 9.8, acetone) (lit.,[121] $[\alpha]_D^{20}$ - 83.9°); ν_{max} ($CHCl_3$) 3010, 1750, and 1725 cm^{-1}; δ_H (90 MHz) 2.57, 2.84 (2H, ABX system, J_{AB} 12 Hz, J_{AX+BX} 9 Hz), 3.45 (3H, s), and 4.21 ppm (1H, J_{AX+BX} 8 Hz); $\underline{m}/\underline{z}$ 130 ($\underline{M}^+$, 0.9%), 115, 103, 97, 85, 84, 73 (100%), 72, 59, and 45. (Found: $\underline{M}^+$, 130.0274. $C_5H_6O_4$ requires 130.0266).

Preparation of (2R,3R)-2,3-dimethoxysuccinimide (222)

The title compound was prepared by a modified form of the procedure reported by Young.[125]

To a solution of dimethoxysuccinic anhydride (249) (4.28g, 24

mmol) in dry ether (200 ml) was passed dry ammonia gas. Instantly, a bulky, crystalline deposit was evident, corresponding to a mixture of the amic acid (250) and its ammonium salt. (For the mixture of amic acid (250) and ammonium salt; ν_{max} (KBr disc) 3435, 3115, 2830, 1675, and 1375 cm^{-1}). The mixture was filtered and the crude product was dried and then reacted, under a nitrogen atmosphere, with excess acetyl chloride (45 ml, 633 mmol) under reflux conditions for 3h. The reaction mixture was cooled and excess acetyl chloride was removed under reduced pressure. Dichloromethane (100 ml) was added to the residue and the inorganic salt was removed by filtration through a florosil pad. Removal of solvent under vacuum gave an impure white solid which on recrystallisation from benzene afforded (2R,3R)-dimethoxysuccinimide (222) (2.73g, 64.2%) m.p. 108-110°C (lit.,[125] 108-110°C); $[\alpha]_D^{19}$ + 240.1° (C 0.9, acetone) (lit.,[125] $[\alpha]_D^{20}$ + 235.5°); δ_H (90 MHz) 3.67 (6H, s), 4.22 (3H, s), and 8.80-9.25 ppm (1H, br s, NH); δ_C (25 MHz) 59.7, 81.9 and 172.5 ppm; m/z 159 (M^+, 11.0%), 157. 145, 144, 142, 130, 129, 127, 116, 115, 88 (100%), 74, 73, and 72. (Found: M^+, 159.0540. $C_6H_9NO_4$ requires M, 159.0534). (Found: C, 45.11; H, 5.62; N, 8.48. $C_6H_9NO_4$ requires C, 45.28; H, 5.70; N, 8.84%).

Preparation of meso-2,3-dimethoxysuccinimide (251)

The title compound was prepared from meso-dimethoxyanhydride (254), by a modified procedure to that reported by Young,[125] in 27% yield, m.p. 95-96°C; ν_{max} (KBr disc) 3220, 1785, and 1720 cm^{-1}; δ_H (90 MHz) 3.55 (6H, s), 4.20 (2H, s) and 8.60-8.90 (1H, br s, NH); m/z 159 (M^+, 11.5%), 144, 130, 129 (100%), 101, 88, 85, 73, and 72. (Found: M^+,

159.0528. $C_6H_9NO_4$ requires M, 159.0534).

This reaction also generated (±)-2,3-dimethoxysuccinimide (222) - separated by column chromatography - in an approximately equal portion to (251).

Preparation of (S)-2-methoxysuccinimide (231)

The title compound was prepared from (S)-methoxyanhydride (257), by a procedure based on the method of Young,[125] in a yield of 8.3%, m.p. 88-89°C; $[\alpha]_D^{16}$ - 53.8° (C 17, $CHCl_3$); ν_{max} (KBr disc) 3250, 1770, and 1720 cm^{-1}; δ_H (90 MHz) 2.64, 3.01 (2H, ABX system, J_{AB} 15 Hz, J_{AX+BX} 13 Hz), 3.42 (3H, s), 4.30 (1H, J_{AX+BX} 13 Hz) and 7.40-7.80 ppm (1H, br s, NH); m/z 129 (M^+, 1.8%), 115, 114, 99, 86, 85, 74, 72, 58, 55, 44, and 43 (100%). (Found: M^+, 129.0444. $C_5H_7NO_3$ requires 129.0428).

Preparation of ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate (201)

The title compound was prepared by a method adapted from that reported by Fuchs.[110]

Under a nitrogen atmosphere, diisopropylamine (8.4 ml, 60 mmol) was added to dry THF (300 ml) at -60°C. At the same temperature n-butyllithium (38.2 ml, 55 mmol, 1.44M in hexane) was added. The solution was then slowly warmed to -15°C and stirring maintained for 25 min. Cyclopropyltriphenylphosphonium bromide (258) (19.9g, 52 mmol) was then added to the reaction mixture, affording a bright orange solution immediately on addition. The reaction mixture was then cooled to -78°C and ethyl chloroformate (7.2 ml, 75 mmol) in dry THF (5 ml) was added over a 10 min period. Stirring was maintained for a further 3h

at -78°C before the reaction was warmed to room temperature and stirred for a further 16h. The reaction mixture was filtered and the precipitate obtained was dissolved in chloroform (500 ml) and washed with water (2 x 150 ml). The organic extract was dried and then concentrated under vacuum to afford an amorphous solid (20.25g). The amorphous solid was dissolved in methanol (20 ml) and added to an aqueous $NaBF_4$ solution (152g of $NaBF_4$ in 500 ml water) and stirred for 4h at room temperature. The aqueous solution was then extracted with chloroform (3 x 200 ml) and the organic washings were combined, dried and then concentrated under reduced pressure to afford crude (201), which on recrystallisation from $CHCl_3$-Et_2O gave a pale yellow crystalline solid (13.9g, 58.1%), m.p. 178-181°C (lit.,[110] 179-181°C); ν_{max} (KBr disc) 2980, 1740, 1715, and 1440 cm^{-1}; δ_H (90 MHz) 0.91 (3H, t, $\underline{J}$ 7 Hz), 1.15-1.60 (2H, m), 2.10-2.35 (2H, m), 4.04 (2H, q, $\underline{J}$ 7 Hz), and 7.60-7.90 ppm (15H, m); δ_C (25 MHz) 13.2, 15.0, 16.8 ($\underline{J}_{PC}$ 90 Hz), 63.3, 117.5 ($\underline{J}_{PC}$ 91 Hz), 130.5 ($\underline{J}_{PC}$ 15.7 Hz), 133.9 ($\underline{J}_{PC}$ 9.5 Hz), 135.3, and 167.6 ppm ($\underline{J}_{PC}$ 7.2 Hz); $\underline{m/z}$ 361, 278, 277 (100%), 262, 201, 199, 185, 183, 152, 108, 107, 97, and 51. (Found: C, 62.10; H, 5.15; F, 16.11; P, 6.91. $C_{24}H_{24}O_2PBF_4$ requires C, 62.36; H, 5.23; F, 16.91; P, 6.70%).

Preparation of succinimide (205)

Synthesis of the title compound was based on the procedure reported by Sheng-Ma and Sah.[131]

Succinic acid (26.3g, 0.22 mol) and urea (6.61g, 0.11 mol) were mixed and heated at 175°C for 15 min. The resultant slurry on cooling to room temperature afforded a white crystalline solid. Recrystallisation from ethanol afforded succinimide monohydrate (18.1g, 69.3%), m.p.

126-128°C (lit.,[131] 126-128°C); ν_{max} (KBr disc) 3300, 1775, and 1710 cm^{-1}; δ_H (90 MHz) 2.70 (4H, s), and 11.0 ppm (1H, br s); m/z 99 (M^+, 22.9%), 74 (100%), 73, 56, 55, and 47. (Found: M^+, 99.0310. $C_4H_5NO_2$ requires M, 99.0320).

Preparation of ethyl 1,8-didehydro-6α,7β-dimethoxy-5-oxopyrrolizidine-1-carboxylate (223)

The title compound was prepared from (2R,3R)-dimethoxy-succinimide (222) by a procedure based on the method reported by Flitsch and Russkamp[112] (see Table 5).

Under a nitrogen atmosphere, sequential additions of imide (222) (129 mg, 0.81 mmol) and NaH (25 mg, 1.03 mmol) were made to a flame-dried flask containing xylene (8.25 ml, sodium dried). After stirring for 1h at room temperature, ethoxycarbonylcyclopropyltriphenyl-phosphonium tetrafluoroborate (201) (381 mg, 0.81 mmol) was added and the reaction mixture heated to reflux and maintained under these conditions for 2½h. Wet xylene (2 ml) was carefully added to the cooled reaction mixture and inorganic residue was removed by filtration and washed with toluene (2 x 5 ml). The organic extracts were combined, dried, and concentrated under reduced pressure. Separation of the components of the residue afforded (223) (145 mg, 70.1%), R_f 0.41 (CH_2Cl_2/AcOEt); m.p. 70-72°C; $[\alpha]_D^{20}$ + 31.1° (C 36, $CHCl_3$); ν_{max} ($CHCl_3$) 1735, 1695, and 1650 cm^{-1}; δ_H (90 MHz) 1.12 (3H, t, J 7 Hz), 2.94 (2H, t, J 10 Hz), 3.40 (3H, s), 3.46 (3H, s), 3.50 (2H, t, J 10 Hz), 3.91 (1H, d, J 3 Hz), 4.04 (2H, q, J 7 Hz), and 4.29 ppm (1H, d, J 3 Hz); δ_C (25 MHz) 14.4, 32.0, 41.0, 60.2, 60.3, 65.5, 78.4, 86.9, 108.1, 153.0, 167.2, and 171.3 ppm; m/z 255 (M^+, 19.2%), 253, 212, 210,

209, 184, 182, 124, 122, 94, 85, and 82 (100%). (Found: $\underline{M}^+$, 255.1114. $C_{12}H_{17}NO_5$ requires $\underline{M}$, 255.1107).

Preparation of ethyl (±)-1,8-didehydro-6β,7β-dimethoxy-5-oxopyrrolizidine-1-carboxylate (272)

The title compound was prepared from meso-dimethoxysuccinimide (251) by the method reported by Flitsch and Russkamp;[112] ν_{max} ($CHCl_3$) 1730, 1690, and 1650 cm^{-1}; δ_H (90 MHz) [$CO(CD_3)_2$] 1.22 (3H, t, $\underline{J}$ 7 Hz), 3.02 (2H, t, $\underline{J}$ 10 Hz), 3.45 (3H, s), 3.57 (3H, s), 3.68 (2H, t, $\underline{J}$ 10 Hz), 4.00-4.25 (3H, m) and 4.76 ppm (1H, d, $\underline{J}$ 6 Hz). Pyrrolizidine (223), in racemic form, was produced in this reaction as the minor reaction product. The information quoted for (272) is for a mixture with triphenylphosphine oxide which could not be entirely separated. The ratio of (272):(223) produced by this reaction was approximately 2:1, with a combined yield of between 50-60%.

Preparation of cis/trans-5'-carbethoxy-5'-hydroxyethylpyrolid-5-ene-2-one (269) and unidentified compound (268)

A mixture of products, (268) and (269), was prepared by reaction of succinimide prepared by the method of Sheng-Ma and Sah[131] with ethoxycarbonylcyclopropyltriphenylphosphonium tetrafluoroborate[112] (201). See Table 3 for details of yields from this reaction under modified conditions:- (269); m.p. 101-103°C; ν_{max} (KBr disc) 3380, 3185, 1730, 1695, 1680, and 1635 cm^{-1}; uv (ethanol) λ_{max} (ε) 285 nm (22,500); δ_H (90 MHz) 1.27 (3H, t, $\underline{J}$ 7 Hz), 2.49 (2H, t, $\underline{J}$ 7 Hz), 2.87 (2H, t, $\underline{J}$ 10 Hz), 2.97 (2H, t, $\underline{J}$ 7 Hz), 4.19 (2H, q, $\underline{J}$ 7 Hz), 4.33 (2H, t, $\underline{J}$ 10 Hz), and 5.80-6.55 ppm (2H, br s, N$\underline{H}$ and O$\underline{H}$); δ_C (25 MHz) 14.5, 23.7, 29.9,

32.8, 59.8, 70.7, 128.3, 131.8, 166.3 and 172.6 ppm; m/z 213 (M^+, 34.3%), 196, 169, 167, 139, 125, 123 (100%), 122, 97, 96, and 95. (Found: M^+, 213.0996. $C_{10}H_{15}NO_4$ requires 213.1001).

:- unknown (268), ν_{max} ($CHCl_3$) 2975, 1730, 1690, and 1645 cm^{-1}; uv (ethanol) λ_{max} (ε) 290 nm (20,100); δ_H (90 MHz) 1.21 (3H, t, J 7 Hz), 2.60 (2H, t, J 7 Hz), 2.70-3.05 (4H, m), 4.13 (2H, q, J 7 Hz), and 4.30-4.50 (2H, m); m/z 195 (M^+, 2.3%), 168, 125, 115, 101, 56 (100%), and 55. (Found: M^+, 195.0892. $C_{10}H_{13}NO_3$ requires M, 195.0895).

Preparation of ethyl 1,8-6,7-tetradehydro-5-oxopyrrolizidine-1-carboxylate (262)

The above compound was prepared from malemide (261) by a procedure based on that reported by Flitsch and Russkamp[112] (Table 2); (262), m.p. 104-105°C; ν_{max} ($CHCl_3$) 2870, 1735, 1690, and 1650 cm^{-1}; δ_H (90 MHz) 1.31 (3H, t, J 7 Hz), 3.27 (2H, t, J 6 Hz), 3.79 (2H, t, J 6 Hz), 4.26 (2H, q, J 7 Hz), 6.38 (1H, d, J 9 Hz), and 6.44 ppm (1H, d, J 9 Hz); m/z 193 (0.1%), 189, 155, 153, 100, 99 (100%), and 71; (Found: M^+, 103.0721. $C_{10}H_{11}NO_3$ requires M, 193. 0739).

Preparation of ethyl 1,8-didehydro-5-oxopyrrolizidine-1-carboxylate (203)

The title compound was prepared from succinimide (205) by a procedure based on the method reported by Flitsch and Russkamp[112] (see Table 4 for details of yield); m.p. 60-61°C (lit.,[112] 62°C); ν_{max} ($CHCl_3$) 1730, 1685, and 1650 cm^{-1}; uv (ethanol) λ_{max} (ε) 280 nm (21,000); δ_H (90 MHz) 1.27 (3H, t, J 7 Hz), 2.70-3.30 (6H, m), 3.76 (2H, t, J 9 Hz), and 4.19 ppm (2H, q, J 7 Hz); m/z 195 (M^+, 18%), 167, 154, 150, 123, 122, 105, 94, and 55 (100%). (Found: M^+, 195.0888. $C_{10}H_{13}NO_3$ requires M, 195.0895).

Preparation of ethyl 1,8-didehydro-6β-methoxy-5-oxopyrrolizidine-1-carboxylate (233) and ethyl 1,8-didehydro-7α-methoxy-5-oxopyrrolizidine-1-carboxylate (234)

The title compounds were prepared from (S)-2-methoxysuccinimide (257) by a procedure based on the method reported by Flitsch and Russkamp.[112] The product mixture, in a combined yield of 14%, was estimated to be a 7:2 mixture of (234):(233) on the basis of methoxy proton signal in the ^{1}H n.m.r. spectrum of the product mixture, δ_H (90 MHz) OCH_3 at δ3.52 and 3.79 ppm; ν_{max} ($CHCl_3$) 1730, 1695, and 1650 cm^{-1}; $[\alpha]_D^{14}$ (for mixture) - 9.8% (C 3.1 $CHCl_3$).

Preparation of ethyl 6α,7β-dimethoxy-5-oxo-8α-pyrrolizidine-1β-carboxylate (224) and ethyl 6α,7β-dimethoxy-5-oxo-8α-pyrrolizidine-1α-carboxylate (274)

A slurry containing PtO_2 (20 mg) and ester (223) (132 mg, 0.52 mmol) in acetic acid (10 ml) was reacted under a hydrogen atmosphere (1 atm.) for 48h with stirring. The slurry was filtered through Celite and the filtrate was washed with acetic acid (2 x 10 ml). Combination of the organic extracts, then concentration, followed by column chromatography afforded ester (224) (81g, 60%), $[\alpha]_D^{18}$ + 67.4° (C 27.6, $CHCl_3$); ν_{max} ($CHCl_3$) 2980, 1725, and 1670 cm^{-1}; δ_H (90 MHz) 1.28 (3H, t, J 7.5 Hz), 2.10-2.45 (2H, m), 2.95-3.35 (2H, m), 3.45 (3H, s), 3.65 (3H, s), 3.60-3.95 (3H, m), 4.17 (1H, m), and 4.20 ppm (2H, q, J 7.5 Hz); δ_C (25 MHz) 14.2, 29.8, 41.0, 45.0, 58.1, 58.8, 61.1, 64.0, 83.9, 86.1, 170.2, and 171.8 ppm; m/z 257 (M^+, 32.5%), 227, 212, 198, 196, 186, 180, 154, 142, 126, 105, 88 (100%), and 85. (Found: M^+, 257.1251. $C_{12}H_{19}NO_5$ requires 257.1263).

Ester (274) (26 mg, 19.3%); $[\alpha]_D^{14}$ + 10.5° (C 5, $CHCl_3$); ν_{max} ($CHCl_3$) 2990, 1725, and 1705 cm^{-1}; δ_H (90 MHz) 1.25 (3H, t, J 7.5 Hz), 1.95-2.20 (2H, m), 2.65-3.10 (2H, m), 3.36 (3H, s), 3.54 (3H, s), 3.50-3.90 (3H, m), 4.25 (2H, q, J 7.5 Hz) and 4.30-4.45 ppm (1H, m); m/z 257 (M^+, 17.8%), 228, 227, 226, 225, 212, 198, 196, 180, 142, 126, 88 (100%), and 85. (Found: M^+, 257.1269. $C_{12}H_{19}NO_5$ requires M, 257.1263).

The use of Pd/C catalyst, employing EtOH as solvent, generated the same mixture of products, but with the contribution from (274) amounting to less than 1% of the combined yield. The components were again separated by column chromatography, which afforded (224) in 85% yield.

Attempted synthesis of ethyl (±)-6β,7β-dimethoxy-5-oxo-8α-pyrrolizidine-1β-carboxylate

Attempts to produce the title compound from ester (272) by the hydrogenation procedures described for reaction of ester (223) were unsuccessful, although starting material was recovered. It is thought that the presence of triphenylphosphine oxide in the sample of (272) employed for reaction may have hindered the hydrogenation process by poisoning of the catalyst. Introduction of an increased quantity of catalyst gave a similar result.

Preparation of ethyl 7α-methoxy-5-oxo-8β-pyrrolizidine-1α- and 1β-carboxylate [(276) and (277)] together with ethyl-6β-methoxy-5-oxo-8α-pyrrolizidine-1α- and 1β-carboxylate [(279) and (278)]

Reaction of an estimated 7:2 mixture of esters (233) and (234) (the latter being the major component) in either of the hydrogenation procedures described for reaction of (223) to produce (224) gave rise to a product mixture of four components by t.l.c., thought to be (276), (277), (278), and (279). Estimation of the relative abundance of the components was made on the basis of the relative intensity of the four methoxyl proton signals in the ^{1}H n.m.r. spectrum of the mixture. The two downfield methoxyl peaks were assumed to belong to the 6β-methoxy esters while the two upfield signals were assumed to be indicative of 7α-methoxy esters. The estimated ratio of components by this basis was 9:2:2:1 [(276):(277):(278):(279)]. The combined yield was 80-85%.

For the mixture: R_f (four spots) 0.23-0.24 [CH_2Cl_2/AcOEt (1:1)]; $[\alpha]_D^{13}$ - 9.6° (C 1.7, $CHCl_3$); ν_{max} ($CHCl_3$) 2990, 1730, and 1685 cm^{-1}; δ_H (90 MHz) OCH_3 peaks at 3.54, 3.62, 3.81 and 3.88 ppm [ratio of peaks is 2:9:2:1 in ascending chemical shift; m/z 227 (M^+, 1.3%)].

Preparation of 6α,7β-dimethoxy-1β-hydroxymethyl-8α-pyrrolizidine (275)

Under a nitrogen atmosphere a solution of ester (224) (31 mg, 0.12 mmol) in dry ether (2 ml) was added to a slurry containing $LiAlH_4$ (15.5 mg, 0.41 mmol) in dry ether (8 ml). The resultant mixture was maintained at room temperature, with stirring, for 3h. Wet ether (5 ml) was then carefully added to the mixture followed by addition of a few drops of 1M NaOH solution. The lithium salt residue was removed by

filtration and washed with lukewarm chloroform (20 ml). The organic extracts were combined, dried, and concentrated under reduced pressure to afford a pale yellow oil (21 mg, 86%), R_f 0.11 (EtOAc); $[\alpha]_D^{18}$ + 22.8° (C 2.6, $CHCl_3$); ν_{max} ($CHCl_3$) 3470, 3020, 1120, and 1100 cm^{-1}; δ_H (90 MHz) 2.50-3.00 (2H, m), 3.05-3.30 (3H, m), 3.33 (3H, s), 3.46 (3H, s), 3.30-3.60 (5H, m), and 3.70-4.20 ppm (3H, m); m/z 201 (M^+, 12.3%), 199, 186, 170, 168, 138, 136, 124, 113, 112, 111, 101, 82 (100%) and 71. (Found: M^+, 201.1364. $C_{10}H_{19}NO_3$ requires M, 201.1365).

Attempted synthesis of ethyl 6α,7β-dimethoxy-1-phenylselenyl-8α-pyrrolizidine-1-carboxylate (280)

Under a nitrogen atmosphere a solution of ester (224) (141 mg, 0.55 mmol) in dry THF (5 ml), followed by HMPA (0.5 ml), were carefully added to a solution containing lithium diisopropylamine (58 mg, 0.55 mmol) in THF (3 ml) at -78°C. The resultant reaction mixture was allowed to stir for 15 min then a solution of phenylselenium chloride (130 mg, 0.68 mmol) in THF (4 ml) was added and the reaction was warmed to -35°C and maintained at this temperature for a further 3h. The reaction mixture was then warmed to 0°C and poured into water (30 ml). The resultant solution was extracted with dichloromethane (3 x 15 ml) and the organic extracts were combined, dried, and concentrated under reduced pressure. The residue obtained was dissolved in benzene (25 ml) and washed with saturated lithium chloride solution (3 x 20 ml). The organic extract was dried, and concentrated under vacuum to afford a product mixture, which after column chromatography, was shown not to contain selenide (280). A small quantity of starting material (224) was recovered from this reaction.

The same result was obtained when phenylselenium chloride was replaced by diphenyldiselenide in this reaction or when HMPA was not incorporated into the experimental procedure or in variation of the quantities of LDA and selenide reagent employed.

Attempted synthesis of 6α,7β-dimethoxy-1β-formyl-8α-pyrrolizidine (284)

Synthesis of the title compound was attempted by a method based on the procedure reported by Swern and co-workers.[137]

To a solution of oxalyl chloride (0.022 ml, 0.25 mmol) in dichloromethane (5 ml), cooled to -60°C, was added DMSO (0.04 ml, 0.54 mmol) in dichloromethane (2 ml). After stirring for 5 min a solution of alcohol (274) (45 mg, 0.22 mmol) in dichloromethane (2 ml) was added and the reaction mixture was maintained at -60°C for a further 20 min. Triethylamine (0.16 ml, 1.12 mmol) was then added and the reaction mixture was allowed to warm to room temperature. Water (15 ml) was added to the mixture and the organic phase was isolated. The aqueous extract was washed with dichloromethane (3 x 10 ml) and the organic extracts were combined and in turn washed with brine (10 ml), saturated sodium carbonate solution (10 ml), and, finally water (10 ml). The resulting organic layer was dried, and then concentrated under reduced pressure to afford a dark brown oil. Analysis of this oil indicated that aldehyde (284) had not been formed. T.l.c. indicated a mixture of several unidentified products.

Attempted synthesis of ethyl 6α,7β-dimethoxy-8α-pyrrolizidine-1β-carboxylate (283)

This was based on the method reported by Pinnick and Chang.[135]

To flame dried apparatus, under a nitrogen atmosphere, was added pyrrolizidine ester (224) (125 mg, 0.49 mmol) followed by phosphoryl chloride (0.5 ml, 5.45 mmol). The mixture was maintained at room temperature for 40 min then excess phosphoryl chloride was removed under vacuum. The resulting residue was dissolved in DME (1.5 ml) and cooled to 0°C. Sodium borohydride (38 mg, 0.98 mmol) in ethanol (10 ml) was added to the reaction mixture over a 15 min period. The reaction was warmed to room temperature and stirred for 30 min. The reaction mixture was acidified to pH2 by addition of 5M HCl. Ethanol was removed under reduced pressure and the reaction mixture was stirred for 30 min, then water (10 ml) was added. The aqueous solution was extracted with ether (4 x 5 ml), cooled to 0°C, then basified (pH8) with powdered K_2CO_3. The resulting aqueous solution was washed with ether (4 x 5 ml) and the ethereal washings combined and in turn washed with water (10 ml). The resulting ethereal solution was dried and concentrated under reduced pressure to afford a dark brown oil. However, analysis of this oil indicated that ester (283) had not been formed. Several unidentified products were evident by t.l.c.

Attempted synthesis of ethyl 6α,7β-dimethoxy-8α-pyrrolizidine-1-carboxylate (283)

Attempts to produce the title compound from ester (224) were made using a procedure based on the method reported by Brown et al.[134]

Under a nitrogen atmosphere 1M borane solution in THF (0.3 ml, 0.30 mmol) was carefully added to a refluxing solution containing ester (224) (69 mg, 0.27 mmol) in THF (3 ml). After the addition was complete the reaction mixture was maintained at reflux for a further 15 min before being allowed to cool to room temperature, whereupon methanolic hydrogen chloride was slowly added (0.4M, 0.75 ml, 0.3 mmol). The reaction mixture was then heated to 60°C and THF and methyl borate were removed by distillation. However, spectroscopic analysis of the crude product obtained indicated that (283) had not been formed. T.l.c. indicated the presence of several unidentified compounds in the product mixture.

Preparation of diethyl L-tartrate (246)

The title compound was prepared by the procedure reported by Yamada and co-workers.[115]

Under a nitrogen atmosphere 15-crown-5-ether (4.8 ml, 24.2 mmol) in dichloromethane (80 ml, saturated with sodium iodide) was added to a solution of ester (247) (380 mg, 1.63 mmol) in dichloromethane (3 ml) at -30°C, followed by addition of BBr_3 (9.75 ml, 9.75 mmol, 1.0M solution in dichloromethane). The reaction mixture was stirred for 3h at this temperature then wet dichloromethane (20 ml) was carefully added to the reaction mixture. The mixture was extracted with saturated brine solution (3 x 50 ml) followed by water (2 x 50 ml). The organic extract was dried and concentrated under reduced pressure to afford a pale yellow oil shown by t.l.c. to be comprised of several compounds. Separation of the components of the product mixture by column chromatography, followed by distillation gave a clear oil (246), (63 mg, 18.9%),

b.p. 125°C/3 mm; $[\alpha]_D^{15}$ + 5.6° (C7, $CHCl_3$); ν_{max} ($CHCl_3$) 3520 and 1730 cm^{-1}; δ_H (90 MHz) 1.24 (6H, t, J 7 Hz), 3.37-3.57 (2H, br s, OH), 4.21 (4H, q, J 7 Hz) and 4.44 ppm (2H, s); δ_C (25 MHz) 14.1, 62.2, 73.1, and 171.2 ppm; m/z 161 (M^+ - 45, 0.2%), 133, 104 (100%), 77, 76, 59, and 47. (Found: M^+ - 45, 161.0440. $C_6H_9O_5$ requires M - 45, 161.0450). (Found: C, 46.43; H, 6.82. $C_8H_{14}O_6$ requires C, 46.60; H, 6.80%).

7.3 Experimental to Chapter 5

Preparation of (S)-2-methoxybutan-1,4-diol (287)

A solution of diethyl-(S)-2-methoxysuccinate (300) (903 mg, 4.43 mmol) in dry ether (15 ml) was added dropwise to a suspension of lithium aluminium hydride (222 mg, 5.84 mmol) in dry ether (50 ml). After addition of the ester was complete the mixture was stirred at room temperature for 1½h. Excess lithium aluminium hydride was destroyed by the slow addition of wet ether (10 ml) followed by 0.1M NaOH solution (1 ml). The resulting suspension was filtered and the lithium salt residue was washed with lukewarm chloroform (2 x 20 ml) and then subjected to a 12h continuous extraction with chloroform. The organic solutions were combined, dried, and then concentrated under reduced pressure to afford, after distillation, a colourless oil (287) (368 mg, 69.3%), b.p. 105°C/1.2 mm Hg; $[\alpha]_D^{17}$ - 14.6° (C 11.3, $CHCl_3$); ν_{max} ($CHCl_3$) 3420, 2960, and 1090 cm^{-1}; δ_H (90 MHz) 1.70-1.93 (2H, m), 3.45 (3H, s), 3.50-3.80 (5H, m), and 4.15-4.45 ppm (2H, br, OH)‡;

‡ Disappeared on addition of D_2O. Signal found to be both concentration and solvent dependent.

δ_C (25 MHz) 33.7, 57.1, 59.1, 63.3, and 79.9 ppm; m/z 102 (M^+ - 18, 0.7%), 89, 75, 71, 59 (100%), 45 and 41. (Found: M^+ - 18, 102.0670. $C_5H_{10}O_2$ requires M - 18, 102.0681).

Preparation of (R)-2-methoxybutan-1,4-diol

This was prepared by the method described for the enantiomer. All properties were identical except $[\alpha]_D^{13}$ + 14.8° (C 10.1, $CHCl_3$).

Preparation of (S)-2-methoxybutan-1,4-diol dimesylate (301)

Under a nitrogen atmosphere, mesyl chloride (853 mg, 5.90 mmol) was added to a solution containing (S)-2-methoxybutan-1,4-diol (287) (321 mg, 2.68 mmol) and dry CH_2Cl_2 (5 ml). The reaction mixture was then cooled to -78°C and triethylamine (600 mg, 5.90 mmol) was added via syringe. The resulting mixture was then maintained at -78°C for a further 30 min and then allowed to warm up to ambient temperature. The mixture was then poured into water (30 ml) and extracted with chloroform (3 x 40 ml). The organic extracts were combined and washed with brine (2 x 30 ml), dried, and concentrated under reduced pressure to afford crude (301), which on distillation gave a pale yellow oil (301) (561 mg, 76%); b.p. 160°C/1.3 mm Hg; $[\alpha]_D^{15}$ - 30.8° (C 6.1, CH_2Cl_2); ν_{max} ($CHCl_3$) 3030, 1360, 1180, and 975 cm^{-1}; δ_H (90 MHz) 1.70-1.95 (2H, m), 2.89 (3H, s), 2.93 (3H, s), 3.30 (3H, s), and 3.95-4.35 ppm (5H, m); m/z 261 (M^+ - 15, 6.6%), 260, 167, 155, 78, 72, and 71 (100%). (Found: M^+ - 15, 261.0119. $C_6H_{13}O_7S_2$ requires M - 15, 261.0103).

Attempts to prepare (S)-2-methoxyputrescine (286) dihydrochloride from dimesylate (301)

Dimesylate (301) (291 mg, 1.06 mmol) was dissolved in DMSO (2 ml) and added to a solution of sodium azide (245 mg, 3.77 mmol) dissolved in DMSO (5.5 ml). The resultant mixture was allowed to stir at room temperature for 18h, then poured into water (50 ml) and extracted with ether (3 x 15 ml), dried, and concentrated under reduced pressure to afford a yellow oil (167 mg). The oil was redissolved in dry ether (10 ml) and added dropwise to a suspension of lithium aluminium hydride (102 mg, 2.68 mmol) in ether (30 ml) and the resultant mixture stirred at room temperature for 12h. Excess lithium aluminium hydride was destroyed by addition of wet ether (5 ml) followed by saturated sodium sulphate solution (0.5 ml). The lithium salt residue was removed by filtration and HCl gas bubbled through the etherate extract. Concentration of the extract, under reduced pressure, gave a dark brown oil. However, spectroscopic data indicated that (286) was not formed by this method.

Preparation of (S)-2-methoxyputrescine (286) dihydrochloride

This preparation was based on the procedure reported by Golding and co-workers.[140]

To a solution of diol (287) (938 mg, 7.82 mmol) in dry THF (10 ml) was added a solution of 1.36M hydrazoic acid in benzene (13.8 ml, 18.77 mmol) followed by a solution of diisopropyl azodicarboxylate (304) (3.31g, 16.37 mmol) in dry THF (5 ml). To the resulting solution was added, over a 20 min period, triphenylphosphine (9.038g, 34.46 mmol) in

dry THF (50 ml). After completion of this addition, the reaction mixture was warmed to 50°C and maintained at this temperature for 3h. Water (2 ml) was added to the mixture and the reaction maintained for a further 3h period at 50°C, with stirring. The reaction mixture was then cooled and concentrated under reduced pressure to afford a brown oil. The oil was partitioned between dichloromethane (40 ml) and 1M hydrochloric acid (40 ml). The acidified extract was washed with dichloromethane (3 x 40 ml), and water was removed under reduced pressure. Recrystallisation from methanol-ether afforded a cream solid (286) dihydrochloride (642 mg, 43%), m.p. 216-218°C; $[\alpha]_D^{14}$ - 53.6° (C 1.7, MeOH); ν_{max} (KBr disc) 3000, 2020, 1610, and 1470 cm^{-1}; δ_H (90 MHz) (D_2O) 1.90-2.25 (2H, m), 3.10-3.45 (4H, m), 3.55 (3H, s), and 3.70-4.00 ppm (1H, m); δ_C (25 MHz) (D_2O) 29.0, 36.8, 42.0, 57.7, and 75.8 ppm. (Found: C, 31.48; H, 8.36; N, 14.51; Cl, 37.28. $C_5H_{16}N_2OCl_2$ requires C, 31.4; H, 8.44; N, 14.66; Cl, 37.11%).

Substitution of diisopropyl azodicarboxylate (304) by diethyl azodicarboxylate (303) in this reaction gave a purple solid (286) dihydrochloride in 45% yield. However, optical activity values for this material could not be determined due to the colouration of the product.

Preparation of (R)-2-methoxyputrescine dihydrochloride

The title compound was prepared by the same method as for the enantiomer (286) dihydrochloride. All data recorded were identical to those for (286) dihydrochloride except m.p. 215-218°C; $[\alpha]_D^{13}$ + 54.3° (C 5, MeOH).

Standard Procedure for Extracting Rosmarinine (153) from Senecio pleistocephalus

Fresh leaves and stems (700g) of S. pleistocephalus S. Moore plants (family Compositae), were blended in methanol (12L) in portions. The methanolic extract was concentrated under reduced pressure to afford a green residue (26.3g). The residue was dissolved in dichloromethane (170 ml) and partitioned with 2M hydrochloric acid (150 ml). The organic portion was extracted with a further quantity of 2M H_2SO_4 (150 ml) and the acidic extracts were combined and washed with dichloromethane (6 x 100 ml). Powdered zinc (18g) was added and the mixture was stirred at room temperature for 2h. After filtration through Celite, the filtrate was made basic by addition of concentrated ammonia solution (pH9). The basic solution was then extracted with dichloromethane (3 x 400 ml). The organic extracts were combined, dried, and concentrated under vacuum to afford crude rosmarinine (153), which was recrystallised from dichloromethane/acetone (1:1) to give fine white crystals (1.529g), m.p. 203-204°C (lit.,[161] 202-204°C); $[\alpha]_D^{18}$ - 84.9° (C 2.1 MeOH) (lit.,[161] $[\alpha]_D^{24}$ - 85.3°); (Found: M$^+$, 353.1815. $C_{18}H_{27}NO_6$ requires M, 353.1839).

^1H and ^{13}C n.m.r. spectra were identical with those of an authentic sample.[87]

Preparation of diethyl [^{14}C-Me]-(S)-2-methoxysuccinate (305)

This preparation was based on the procedure reported by Purdie and Irvine.[120]

A vial containing [^{14}C]methyl iodide (100 μCi, 59.7 mCi mmol^{-1}) together with a collecting tube containing unlabelled methyl

iodide (0.7 ml, 11.29 mmol) were attached to the vacuum line apparatus shown in Figure 4. The inactive methyl iodide sample was solidified by cooling with a liquid nitrogen trap (-196°C) and a vacuum applied to the apparatus (0.02 mm Hg). The seal of the vial containing the radiolabelled methyl iodide was broken by the dropping of a magnet. The radiolabelled methyl iodide then distilled along the vacuum line and solidified in the collecting tube containing inactive methyl iodide (at -196°C). The now diluted [^{14}C]methyl iodide sample was detached from the vacuum line apparatus and slowly allowed to melt then transferred to a suspension containing Ag_2O (7.32g, 31.58 mmol), diethyl-(S)-hydroxysuccinate (252) (4.00g, 21.05 mmol), and dry ether (5 ml). The collecting tube was washed with further portions of ether (3 x 10 ml) and these washings were added to the reaction mixture. The reaction mixture was then warmed to 0°C and maintained at this temperature for 30 min before heating to reflux. Reflux was maintained for 70h in total with addition of methyl iodide after 24, 47, and 60h reflux. Each of these later additions of methyl iodide (1 ml, 16.09 mmol) was accompanied by addition of ether (1 ml) to the reaction mixture. After 70h reflux the reaction mixture was cooled and the silver residue was removed by filtration, and washed with ether (4 x 100 ml). The ethereal extracts were combined with the filtrate, dried, and concentrated under vacuum to afford, on distillation, diethyl [^{14}C]-(S)-2-methoxysuccinate (305) (4.02g, 3.28 μCi mmol^{-1}, 93.6%). ^{1}H N.m.r. spectroscopy data and t.l.c. were identical to those of unlabelled material.

Preparation of diethyl [^{14}C-Me]-(R)-2-methoxysuccinate (309)

The title compound was prepared by the method outlined for the enantiomer.[120] However, a shorter reflux time of 30h was adopted for this reaction and a reduced quantity of ether was used for the washing out of the [^{14}C]methyl iodide collecting tube. The diester was generated in a 94.9% yield with a specific activity of 2.84 μCi mmol^{-1}. ^{1}H N.m.r. spectroscopy data and t.l.c. were identical to those of unlabelled material.

Preparation of [^{14}C-Me]-(S)-2-methoxybutan-1,4-diol (306)

The title compound was prepared by the method described in the synthesis of unlabelled material. Diethyl [^{14}C-Me]-(S)-2-methoxysuccinate (305) (3.89g, 1.91 mmol) was reacted with 1.3 equivalents of lithium aluminium hydride to afford (306) (1.46g, 3.09 μCi mmol^{-1}, 63.9%). ^{1}H N.m.r. spectroscopy and t.l.c. data were identical to those of unlabelled material.

Preparation of [^{14}C-Me]-(R)-2-methoxybutan-1,4-diol (310)

The title compound was prepared by the method described in the synthesis of unlabelled material of the enantiomer. Diethyl [^{14}C-Me]-(R)-2-methoxysuccinate (309) (4.22g, 20.7 mmol) was reacted with 1.3 equivalents of lithium aluminium hydride to afford the title compound (1.61g, 2.76 μCi mmol^{-1}, 64.8%).

^{1}H N.m.r. spectroscopy and t.l.c. data were in agreement with those of unlabelled material.

Preparation of [^{14}C-Me]-(S)-2-methoxyputrescine (292) dihydrochloride

The title compound was prepared from [^{14}C-Me]-(S)-2-methoxybutan-1,4-diol (306) by the method of Golding and co-workers.[140] An initial preparation afforded (292) dihydrochloride (123 mg, 3.16 μCi mmol^{-1}, 16.6%). A later preparation, employing 2.6 equivalents of diisopropyl azodicarboxylate (304) in this reaction, afforded (292) dihydrochloride (394 mg, 3.31 μCi mmol^{-1}, 36.6%). All physical and spectroscopic data were in agreement with unlabelled material.

Preparation of [^{14}C-Me]-(R)-2-methoxyputrescine (295) dihydrochloride

The title compound was prepared from [^{14}C-Me]-(R)-2-methoxybutan-1,4-diol (310) by the procedure reported by Golding and co-workers.[140] An initial preparation afforded (295) dihydrochloride (184 mg, 2.87 μCi mmol^{-1}, 23%). A later preparation gave (295) dihydrochloride (114 mg, 2.95 μCi mmol^{-1}, 6.6%). All physical and spectroscopic data were in agreement with unlabelled material.

Feeding Methods.- The Royal Botanic Garden, Edinburgh, identified and supplied Senecio pleistocephalus S. Moore plants which were cultivated from stem cuttings and grown in pots in a standard compost in a greenhouse. Two well-rooted plants were employed for each experiment. A sample of [1,4-^{3}H]putrescine dihydrochloride was added to each [^{14}C-Me]-2-methoxyputrescine to assess the relative incorporation of the methoxyputrescines. For each experiment the mixture of radiolabelled putrescines was divided into equal portions and fed by the wick method on five successive days. Sterile water was used for uptake of the

radiolabelled putrescines by the plants. One week after the feeding of the precursors had been completed, the plants were harvested. The alkaloidal content was isolated by the standard procedure. In each experiment radioscans of the t.l.c. plates showed one radioactive band coincident with authentic rosmarinine [R_f = 0.29, NH_3/MeOH/$CHCl_3$ (1:14:85)]. ^{1}H N.m.r. spectroscopy of the extract for each experiment was in good agreement with that for rosmarinine (153). The melting point of the alkaloidal extract from each experiment was also in good agreement with that for rosmarinine. Analysis of the extract by preparative chromatography established that almost all of the activity was associated with rosmarinine. An intermediate trapping experiment was carried out in an effort to trap any 2-methoxyputrescine as its N,N'-diphenylamino (thiocarbonyl) derivative.[144] Incorporation figures for each experiment are listed in Table 6.

Preparation of N,N'-diphenylamino(thiocarbonyl) derivative of (S)-2-methoxyputrescine for Intermediate Trapping Experiments[144]

A mixture of [^{14}C-Me]-(S)-2-methoxyputrescine (292) dihydrochloride and [1,4-^{3}H]putrescine dihydrochloride were fed to S. pleistocephalus plants over a five-day period. Seven days after feeding was complete the plants were blended in methanol (5.7L) and the extract was then divided into two equal portions. One of the portions was concentrated and used in an intermediate trapping experiment.

Inactive (S)-2-methoxyputrescine (286) dihydrochloride (50 mg, 0.26 mmol) and sodium borohydride (193 mg, 5.1 mmol) were added to the concentrated methanolic extract (150 ml) from the feeding experiment, and the mixture was stirred for 24h. Isothiocyanatobenzene (1 ml, 8.37

Table 6. Incorporation of putrescines fed to S. pleistocephalus plants

Experiment	Radiolabelled 2-Methoxyputrescine fed	Quantity of [1,4-^{3}H]putrescine dihydrochloride solution fed	Initial $^{3}H/^{14}C$ feed ratio	Incorporation of radiolabels in alkaloid extract
1	(S)-2-Methoxyputrescine (292) dihydrochloride (115 mg, 3.16 μCi mmol^{-1})	60 μL	27.9	0.67% for ^{14}C 5.87% for ^{3}H
2	(S)-2-Methoxyputrescine (292) dihydrochloride (202.5 mg, 3.31 μCi mmol^{-1})	60 μL	14.7	0.79% for ^{14}C 2.02% for ^{3}H (plants pruned before putrescines fed)
3	(R)-2-Methoxyputrescine (295) dihydrochloride (160 mg, 2.87 μCi mmol^{-1})	40 μL	14.7	0.58% for ^{14}C 4.94% for ^{3}H
4	(R)-2-Methoxyputrescine (295) dihydrochloride (108.5 mg, 2.95 μCi mmol^{-1})	35 μL	18.5	0.31% for ^{14}C 4.01% for ^{3}H (plants pruned before putrescines fed)

mmol) was added to the reaction mixture which was stirred for a further 70h at room temperature. Brine (100 ml) was added and the resultant solution was extracted with dichloromethane (4 x 100 ml). The organic extracts were combined, dried, and then concentrated under reduced pressure. A radioscan of the product mixture obtained developed in CH_2Cl_2-MeCN (9:1) indicated that the band corresponding to the N,N'-diphenylamino(thiocarbonyl) derivative of (286) was not labelled.

N,N'-Diphenylamino(thiocarbonyl) derivative of (286).- m.p. 165-166°C; $[\alpha]_D^{17}$ - 31.9° (C 8.9, CH_2Cl_2); ν_{max} ($CHCl_3$) 3410, 1740, 1595, 1555, 1495, 1435, and 1230 cm^{-1}; δ_H (90 MHz) 1.60-1.85 (2H, m), 3.12 (3H, s), 3.40-3.85 (5H, m), 6.30-6.55 (1H, br s, NH), 6.65-6.90 (1H, br s, NH), 7.05-7.55 (10H, m), 8.20-8.30 (1H, br s, NH), and 8.35-8.45 ppm (1H, br s, NH).

Similar results were obtained in an intermediate trapping experiment with [^{14}C-Me]-(R)-2-methoxyputrescine (295) dihydrochloride. For the unlabelled N,N'-diphenylamino(thiocarbonyl) derivative[144] with all spectroscopic and physical data were identical to the enantiomer except $[\alpha]_D^{13}$ + 30.4° (C 6, CH_2Cl_2).

7.4 Experimental to Chapter 6

Standard procedure for extracting alkaloid from seeds of C. lanceolata

Seeds (100g) of Crotalaria lanceolata (family Leguminosae) were immersed in petroleum ether (b.p. 40-60°C) (250 ml). The seeds were then separated from the ether washings, dried, and then finely ground using a mortar and pestle. The crushed seeds were then blended,

in portions, with methanol (2.5L), and the methanolic filtrates were concentrated under reduced pressure. The resulting residue (8.18g) was dissolved in dichloromethane (70 ml) and extracted with 2M sulphuric acid (3 x 50 ml). The acidic extracts were combined and, in turn, washed with dichloromethane (6 x 50 ml). Powdered zinc metal (8g) was added to the acidified solution and stirred at ambient temperature for 2½h. The mixture was filtered through Celite and the Celite pad was washed with 2M sulphuric acid (2 x 30 ml). The acidified filtrations were then combined and concentrated ammonia solution was added to increase the pH to 9. The alkaline solution was then extracted with dichloromethane (4 x 100 ml). The organic extracts were combined and washed with water (100 ml). The ensuing dichloromethane extract was then dried, and concentrated under reduced pressure to afford a mixture of three alkaloids (205 mg, 0.2%). The alkaloids were separated by column chromatography. Basic alumina (grade 3) was employed as the stationary phase and $CHCl_3/CH_2Cl_2$ (1:1) as the eluant. Usaramine (314), the major component of the extract, was readily separated but further column chromatography was necessary to achieve a partial separation of the remaining components, nilgirine (321) and integerrimine (313). The alkaloids were present in a relative ratio 85:10:5 [(314):(321):(313)].

Usaramine (314): R_f 0.33 [NH_3/MeOH/$CHCl_3$ (1:14:85)]; m.p. 184-185°C (lit.,[150] 182.5-183.5°C); $[\alpha]_D^{12}$ + 8.8° (C 3.15, EtOH) (lit.,[150] $[\alpha]_D^{20}$ +7.1°): ν_{max} ($CHCl_3$) 3600, 1735, 1710, and 1650 cm^{-1}; δ_H (90 MHz) 0.83 (3H, d, J 7 Hz, 19-H_3), 1.71 (3H, d, J 7 Hz, 21-H_3), 1.90-2.70 (6H, m, 5u-H, 13H, 14-H_2, and 6-H_2), 3.26 (1H, m, 5d-H),

3.46 (1H, m, 3u-H), 3.67 (2H, d, J 3 Hz, 18-H), 3.90 (1H, m, 3d-H), 3.95-4.40 (4H, m, 2OH, 8-H, and 9u-H), 5.01 (1H, m, 7-H), 5.41 (1H, d, J 12 Hz, 9d-H), 6.20 (1H, m, 2-H), and 6.52 ppm (1H, q, J 7 Hz, 20-H); δ_C (25 MHz) 12.3 (C-19), 14.1 (C-21), 29.7 (C-6), 33.7 (C-14), 37.0 (C-13), 53.0 (C-5), 61.1 (C-9), 62.6 (C-3), 67.1 (C-18), 75.6 (C-7), 78.3 (C-8), 81.4 (C-12), 131.7 (C-1), 133.5 (C-15), 135.6 (C-20), 136.6 (C-2), 168.9 (C-16), and 175.6 ppm (C-11); m/z 351 (9.4%), 220, 138, 136, 120, 119 (100%), 95, 94, 93, 80 and 55. (Found M^+, 351.1710. $C_{18}H_{25}NO_6$ requires M, 351.1681).

Niligirine (321): R_f 0.38 [NH_3/MeOH/$CHCl_3$ (1:14:85)]; m.p. 126-128°C (lit.,[151] 127-128°C); $[\alpha]_D^{21}$ + 28.9° (C 1.31, EtOH) (lit.,[151] $[\alpha]_D^{18}$ + 31.5°); δ_H (200 MHz) 1.03 (3H, d, J 7 Hz, 18-H_3), 1.74 (3H, d, J 7 Hz, 20-H_3), 2.08 (2H, m, 6-H_2), 2.16 (2H, dd, J 3.5 Hz and 3.8 Hz, 14-H_2), 2.30 (1H, m, 13-H), 2.54 (1H, ddd, J 9 Hz, 11 Hz, and 6 Hz, 5u-H), 2.72-2.95 (1H, br s, OH), 3.25 (1H, ddd, J 9 Hz, 8 Hz, and 2 Hz, 5d-H), 3.39 (1H, ddd, J 16 Hz, 6 Hz, and 1.75 Hz, 3u-H), 3.92 (1H, d, J 16 Hz, 3d-H), 4.04 (1H, d, J 2 Hz, 12-H), 4.11 (1H, d, J, 12 Hz, 9u-H), 4.24-4.34 (1H, m, 8-H), 5.15 (1H, ddd, J 4 Hz, 4 Hz, and 2.9 Hz, 7-H), 5.34 (1H, d, J 12 Hz, 9d-H), 6.15 (1H, m, 2-H), and 6.53 ppm (1H, q, J 7 Hz, 19-H); δ_C (50 MHz) 14.2 (C-18), 16.9 (C-20), 27.8 (C-6), 33.7 (C-14), 35.7 (C-13), 53.5 (C-5), 61.1 (C-9), 62.7 (C-3), 73.8 (C-8), 74.9 (C-7 or C-12), 77.6 (C-12 or C-7), 132.0 (C-15), 133.0 (C-1), 136.3 (C-2), 136.5 (C-14), 168.3 (C-11), and 175.0 (C-16); m/z 321 (M^+, 16.7%), 220, 184, 138, 136, 121, 120, 119 (100%), 118, 117, 95, 94, 93, 80, and 53. (Found: M^+, 321.1584. $C_{17}H_{23}NO_5$ requires M, 321.1576).

Integerrimine (313): R_f 0.40 [NH_3/MeOH/$CHCl_3$ (1:14:85)]; m.p. 168-169°C (lit., 168-169°C[154], 171-172°C[152]); $[\alpha]_D^{16}$ - 20.1° (C 1.1, $CHCl_3$) (lit., [154] $[\alpha]_D$ - 21.4°); δ_H (200 MHz) 0.91 (3H, d, J 7 Hz, 19-H_3), 1.29 (3H, d, J 18 Hz, 18-H_3), 1.74 (3H, d, J 7 Hz, 21-H_3), 1.95-2.05 (2H, m, 6-H_2), 2.05-2.18 (2H, m, 14-H_2), 2.20-2.30 (1H, m, 13-H), 2.46 (1H, m, 5u-H), 3.25 (1H, m, 5d-H), 3.39 (1H, ddd, J 15 Hz, 1.8 Hz, and 6 Hz, 3u-H), 3.95 (1H, d, J 15 Hz, 3d-H), 4.11 (1H, d, J 12 Hz, 9u-H), 4.31-4.66 (1H, m, 8-H), 5.01 (1H, ddd, J 4 Hz, 4 Hz, and 1.7 Hz, 7-H), 5.41 (1H, d, J 12 Hz, 9d-H), 6.21 (1H, m, 2-H), and 6.51 ppm (1H, q, J 7 Hz, 20-H); m/z 335 (M^+, 7.8%), 291, 248, 220, 153, 149, 139, 138, 137, 136, 122, 121, 120, 119 (100%), 118, 109, 95, 94, 93, 80, and 67. (Found: M^+, 335.1738. $C_{18}H_{25}NO_5$ requires M, 335.1733).

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