STRUCTURAL AND SYNTHETIC STUDIES IN THE PYRROLIZIDINE ALKALOID SERIES

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

by

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Finally, I would like to thank the University of Glasgow for the award of a studentship.

TABLE OF CONTENTS

SUMMARY

This work on pyrrolizidine alkaloids is divided into three parts: (a) Synthesis of pyrrolizidine bases, (b) Synthesis of a macrocyclic diester alkaloid, and (c) Structural studies.

(a) Synthesis of Pyrrolizidine Bases

A novel method for the synthesis of 1,2-didehydro-pyrrolizidine bases from saturated pyrrolizidine esters has been achieved. The saturated pyrrolizidine ester, ethyl (±)-pyrrolizidine-1-*endo*-carboxylate was readily prepared by regio-specific 1,3-dipolar cycloaddition of ethyl propiolate to N-formyl-L-proline followed by stereospecific catalytic hydrogenation. The 1,2-unsaturation was introduced by phenylselenenylation α to the ester function, followed by *syn*-elimination of the derived phenylselenoxide. Using this technique, (±)-supinidine was synthesised.

The use of natural (-)-4-hydroxy-L-proline as a model compound in the 1,3-dipolar cycloaddition reaction with ethyl propiolate followed by catalytic hydrogenation led to the formation of the optically active ethyl (+)-6α-hydroxy-8β-pyrrolizidine-1α-carboxylate. Conversion of this saturated ester into its 1,2-unsaturated analogue was also achieved by the same steps of phenylselenenylation α to the ester function, followed by phenylselenoxide fragmentation to give the new optically active

necine base, (-)-6α-hydroxy-1-hydroxymethyl-5,6,7,8β-tetrahydro-3H-pyrrolizine isolated as its diacetate. Reduction of ethyl (+)-6α-hydroxy-8β-pyrrolizidine-1α-carboxylate yielded a new optically active necine base (+)-6α-hydroxy-1α-hydroxymethyl-8β-pyrrolizidine. Proof of the absolute stereochemistry of these two novel bases was achieved by removal of one chiral centre, the 6α-hydroxy group of the saturated ester, ethyl (+)-6α-hydroxy-8β-pyrrolizidine-1α-carboxylate, followed by conversion to the known naturally occurring 8β-pyrrolizidine bases, (+)-lindelofidine, (+)-laburnine and (+)-supinidine. A procedure has been described for the preparation of the corresponding 8α-pyrrolizidines, (-)-trachelanthamidine, (-)-isoretronecanol, and (-)-supinidine.

A synthesis of (±)-retronecine was attempted. The 1,3-dipolar cycloaddition of ethyl propiolate to N,O-diformyl derivatives of cis-and trans-3-hydroxyproline failed, possibly due to elimination of the formyloxy group. An alternative approach to (±)-retronecine, attempting to introduce oxygen substituents at the 1-position of ethyl 2,3-dihydro-1H-pyrrolizidine-7-carboxylate, was also unsuccessful.

(b) Synthesis of a Macrocyclic Diester Alkaloid

A mixture of the 7- and 9-monoesters of (+)-retronecine was formed from (+)-retronecine and dicrotalic anhydride. Attempts to cyclise this mixture by the Corey-Nicolaou method

to give the macrocyclic alkaloid dicrotaline resulted in dehydration and decarboxylation leading to a mixture of 7- and 9-senecioylretronecine. The use of 3,3-dimethylglutaric anhydride in this procedure led to the formation of an analogue of dicrotaline, 13,13-dimethyl-1,2-didehydrocrotalanine. This is the first synthesis of an 11-membered macrocyclic pyrrolizidine diester.

(c) Structural Studies

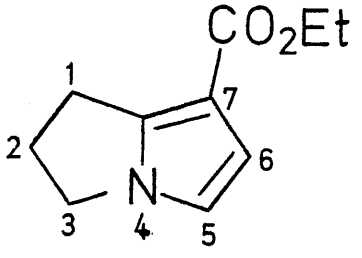
The succulent plant, Senecio odorus was shown to contain three pyrrolizidine alkaloids. Separation by preparative layer chromatography gave two bands. G.l.c. analysis and spectroscopic studies showed that the less polar band was a mixture of integerrimine and senecionine in the ratio 4:1. The more polar band was proved to be senkirkine by spectroscopic studies and comparison with an authentic sample.

PUBLICATION

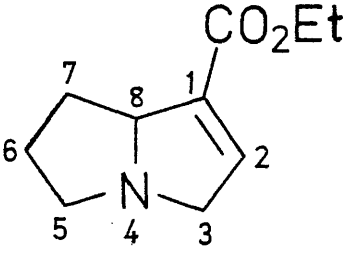
Some of the work described in Chapter 3 has been published: "Synthesis of the Pyrrolizidine Base, (±)-Supinidine, D.J.Robins and S.Sakdarat, J.C.S. Perkin I, 1979, 1734.

NOTE ON NOMENCLATURE

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



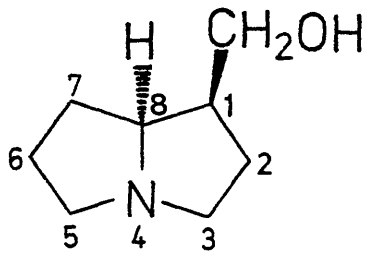
Ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate



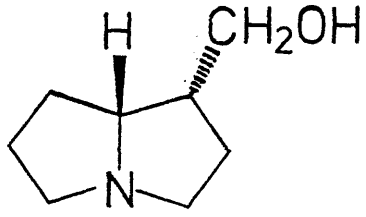
Ethyl 5,6,7,8-tetrahydro-3H-pyrrolizine-1-carboxylate

Fully saturated compounds are named as pyrrolizidine derivatives. Stereochemistry of substituents is indicated by the α and β nomenclature to conform with usual practice in this field. To avoid confusion, (+)-, (-)-, and ($\pm$)-bases

are drawn separately and given individual numbers in the text.

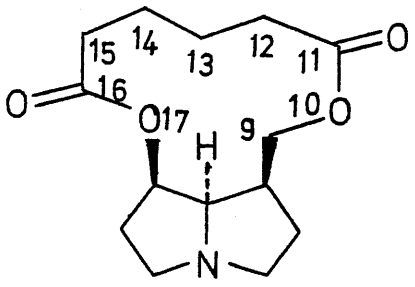


1β-hydroxymethyl-8α-pyrrolizidine [(-)-isoretronecanol]

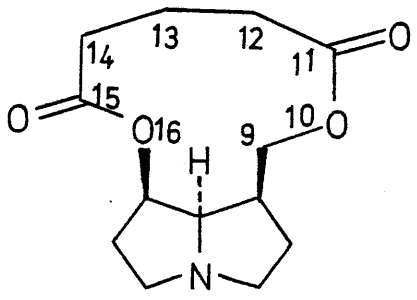


1α-hydroxymethyl-8β-pyrrolizidine [(+)-isoretronecanol]

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



Senecanine



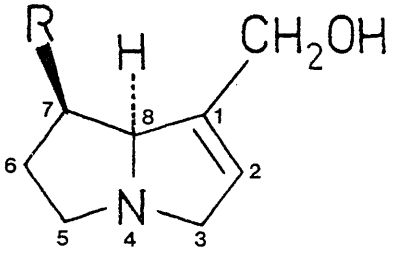
Crotalanine

CHAPTER 1 INTRODUCTION

1.1 Pyrrolizidine Alkaloids

Heavy losses of livestock in several countries over many years, and the recognition that these losses were caused by the consumption of certain species of plants, eventually led to the isolation of a previously unknown group of alkaloids from Senecio latifolius by Watt in 1909.[1] Chemical examination of these toxic alkaloids over a period of several decades revealed the presence of a pyrrolizidine (1-azabicyclo[3.3.0] octane) nucleus. Since then, more than one hundred and fifty naturally occurring pyrrolizidine alkaloids have been isolated and characterised.[2-5] These alkaloids occur in taxonomically widely separated plant families, chiefly the Boraginaceae (Cynoglossum and Heliotropium species), the Compositae (Senecio species), and the Leguminosae (Crotalaria species).

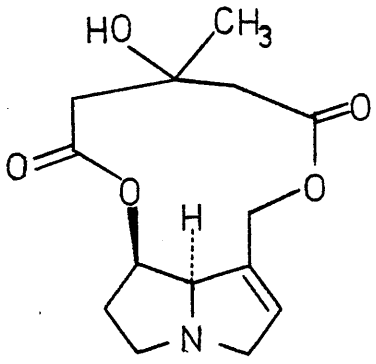
The pyrrolizidine alkaloids contain a base portion, called 'necine', and hydroxyl groups are common substituents of the necine. A double bond is frequently observed in the 1,2-position as in supinidine (1) and retronecine (2). The hydroxyl



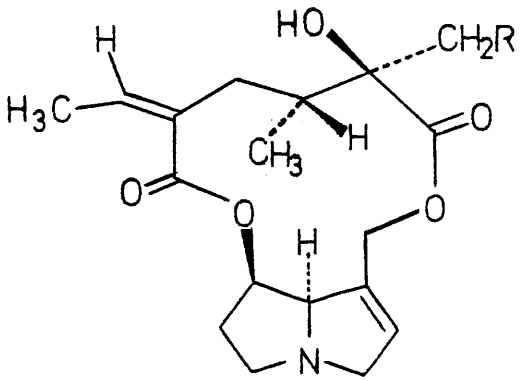
(1) R = H Supinidine

(2) R = OH Retronecine

groups of the necine are usually esterified with carboxylic acids termed 'necic acids'. Most of these acids are unusual, highly-branched, oxygenated compounds containing five to ten carbon atoms. In many alkaloids, both hydroxyl groups on the necine are esterified with a dicarboxylic acid to give either an eleven-membered ring, as in dicrotaline (3) or a twelve-membered ring as in retrorsine (4) and senecionine (5). Pyrrolizidine alkaloids have frequently been found to occur as N-oxides.



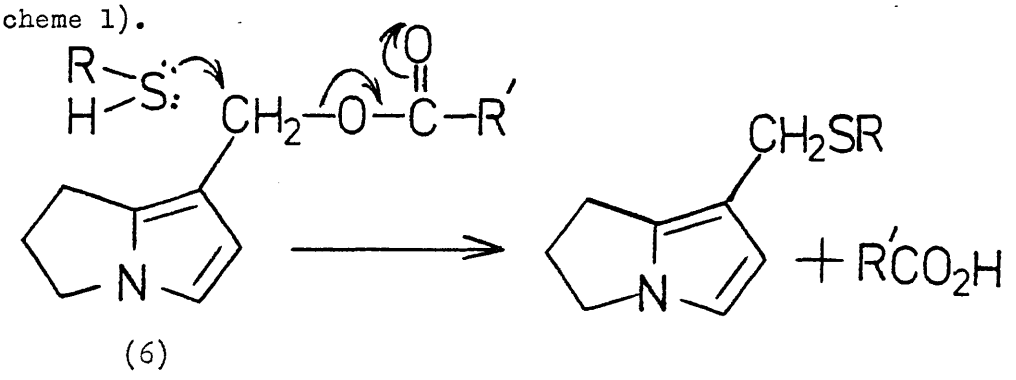
(3) Dicrotaline



(4) R = OH Retrorsine
(5) R = H Senecionine

Much of the current interest in the study of pyrrolizidine alkaloids is based on the long established fact that many of these compounds exhibit specific and irreversible hepatotoxicity towards animals and probably also towards humans.[2,3] Some of the alkaloids are also carcinogenic. The outstanding histological change in the liver is the gradual enlargement of the hepatocytes and their nuclei in a process that is termed megalocytosis. The structural features in the alkaloid which

appear to be necessary for toxicity to be observed are (a) a primary allylic ester grouping, and (b) a high degree of chain branching in the necic acid attached to C-9. It is believed that pyrrole derivatives [such as (6)], produced by the hepatic microsomal enzymes, are the actual toxic agents.[3] These metabolites are thought to become covalently bound to cell nuclei (possibly by alkylation of coenzyme sulphydryl groups[6]) by a mechanism involving alkyl-oxygen fission (Scheme 1).

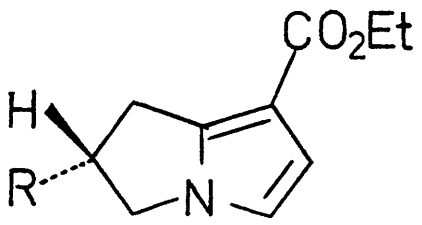


(6)

Scheme 1

1.2 Synthetic Studies on Pyrrolizidine Alkaloids

One of the outstanding developments in pyrrolizidine chemistry over the past few years has been in the increased array of synthetic methods available for the construction of the pyrrolizidine ring system.[3,7] In particular, the 1,3-dipolar cycloaddition of unsaturated esters to N-formyl-L-proline, to produce dehydropyrrolizidine esters [such as (7)], is an extremely facile and efficient one-pot method for producing the pyrrolizidine ring system. This method is discussed in detail in Chapter 2, as it forms the basis for most of the synthetic



(7) R = H

(8) R = OH

work described in this thesis.

Most of the published syntheses have been directed towards fully saturated 1-hydroxymethylpyrrolizidines.[7] It is considerably more difficult to construct the 1,2-didehydropyrrolizidine system present in supinidine (1) and retronecine (2). This area is reviewed in Chapter 2. Because of this lack of good synthetic routes to these 1,2-unsaturated necines (which are the bases present in the toxic alkaloids), it was decided in this work to concentrate on synthetic routes to these compounds. The initial target was (±)-supinidine (1). Various approaches to this compound, and the eventual successful synthesis, are described in Chapter 3.

The next target for synthesis was (±)-retronecine (2), which is the necine most commonly found in macrocyclic diester alkaloids. In order to extend the above strategy to the synthesis of (±)-retronecine, it was necessary to use N-formyl-3-hydroxyproline in the cycloaddition reaction. Initial work with readily available natural 4-hydroxy-L-proline led to the formation of several novel chiral necines. Furthermore, removal of the hydroxyl group after catalytic hydrogenation of the dihydropyrrolizine (8), provided a means of synthesizing

optically active naturally occurring necine bases. This work is discussed in Chapter 4.

Since the use of 4-hydroxy-L-proline had shown that the hydroxyl group in that position did not affect the cycloaddition reaction, it was decided to prepare and use 3-hydroxyproline in an attempt to synthesize retronecine (2). This approach is descibed in Chapter 5.

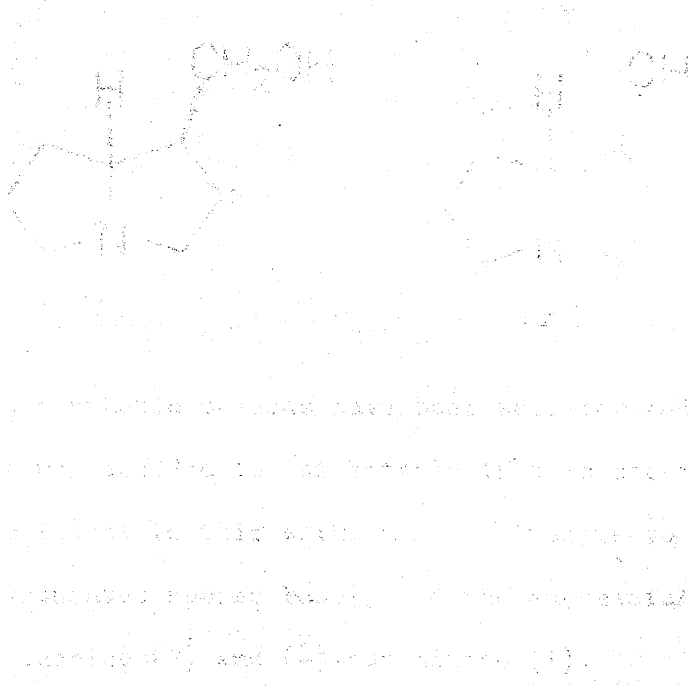
Synthesis of the monoester and diester pyrrolizidine alkaloids has received limited attention. This area is reviewed in Chapter 2**3**. However, no macrocyclic diester alkaloids have yet been synthesized. The simplest example known is dicrotaline (3), and attempts to synthesize this alkaloid are described in Chapter 6. The first successful synthesis of an 11-membered macrocyclic pyrrolizidine diester is also described.

1.3 Structural Studies on Pyrrolizidine Alkaloids

There is much interest in the occurrence of pyrrolizidine alkaloids from several points of view. Their pharmacology, and the relationship of pharmacological activity to structure is important. Greater understanding of the occurrence and distribution of alkaloids is useful from the chemotaxonomic standpoint. Knowledge of the chemical constituents of plants is being increasingly used to aid in the classification of plant species. Finally, the structural relationships between alkaloids occurring together in the same species may help

in determining the biogenesis of these compounds.

It was noticed that very few of the succulent species from the very extensive Senecio genus had been studied for their content of pyrrolizidine alkaloids. Therefore, the alkaloid constituents of Senecio odorus were investigated. This work is described in Chapter 7.

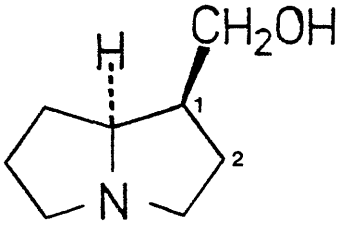


CHAPTER 2 SYNTHESIS OF PYRROLIZIDINE ALKALOIDS

2.1 Synthesis of Pyrrolizidine Bases

(a) Introduction

Many synthetic methods leading to the pyrrolizidine nucleus have been reported in the past few decades. Comprehensive reviews of this large and expanding area are available,[7] and current developments are reviewed annually.[3] Most of the published syntheses are of the fully saturated necine bases, particularly the 1-hydroxymethylpyrrolizidines, (±)-isoretronecanol (9) and (±)-trachelanthamidine (10).



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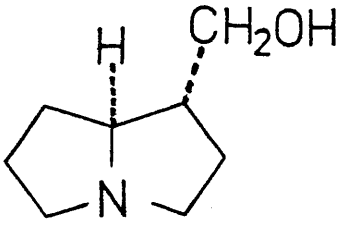
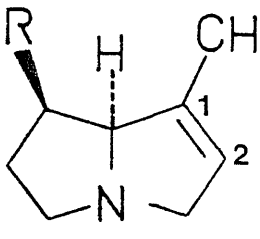


(10)

Since these synthetic methods have been well-covered in the reviews, it was decided to concentrate in this section on syntheses relevant to this work, namely attempts to produce the 1,2-unsaturated necine bases, and the successful syntheses of (±)-retronecine (2) and (±)-supinidine (1).



(1) R = H

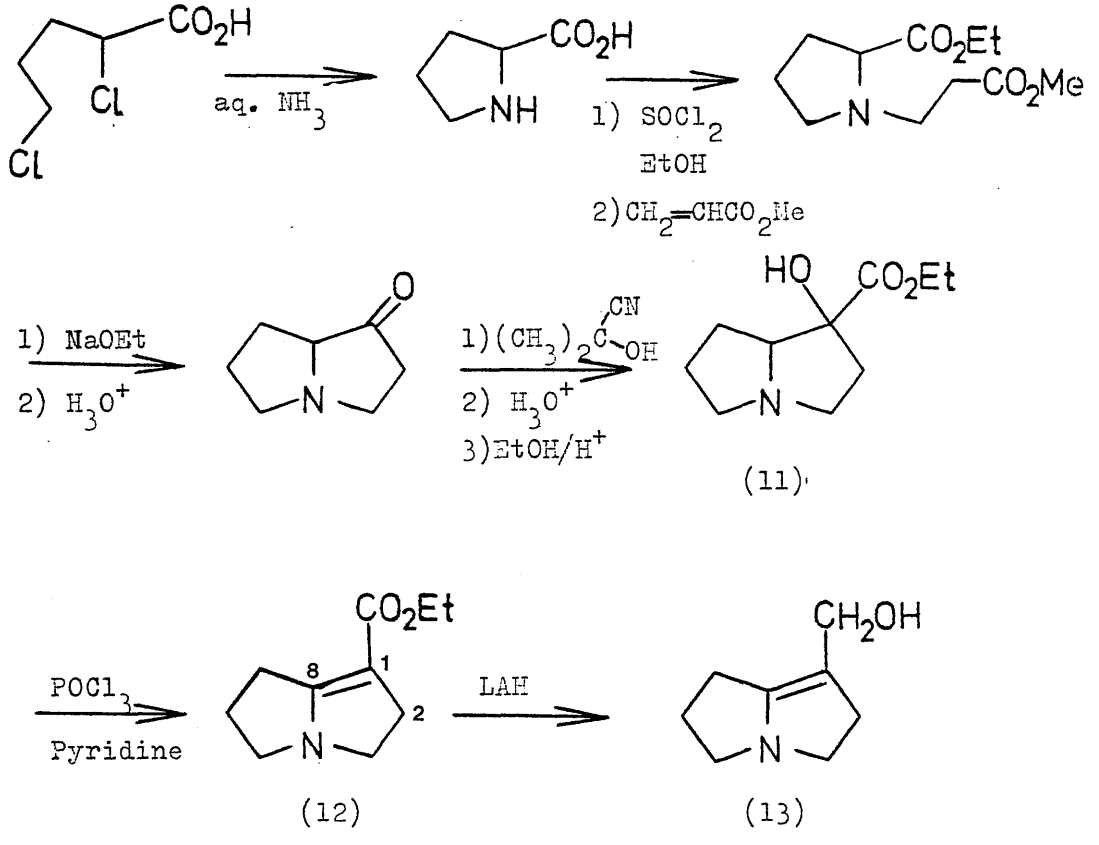
(2) R = OH

(b) Attempted Syntheses of 1,2-Didehydropyrrolizidines

Three groups of workers have attempted to synthesise the simplest 1,2-unsaturated necine, supinidine (1), by introduction of the double bond in this position in elimination processes from suitably substituted pyrrolizidine derivatives.

The first attempt to synthesise (±)-supinidine (1) by Kochetkov et al.[8] is outlined in Scheme 2. These authors

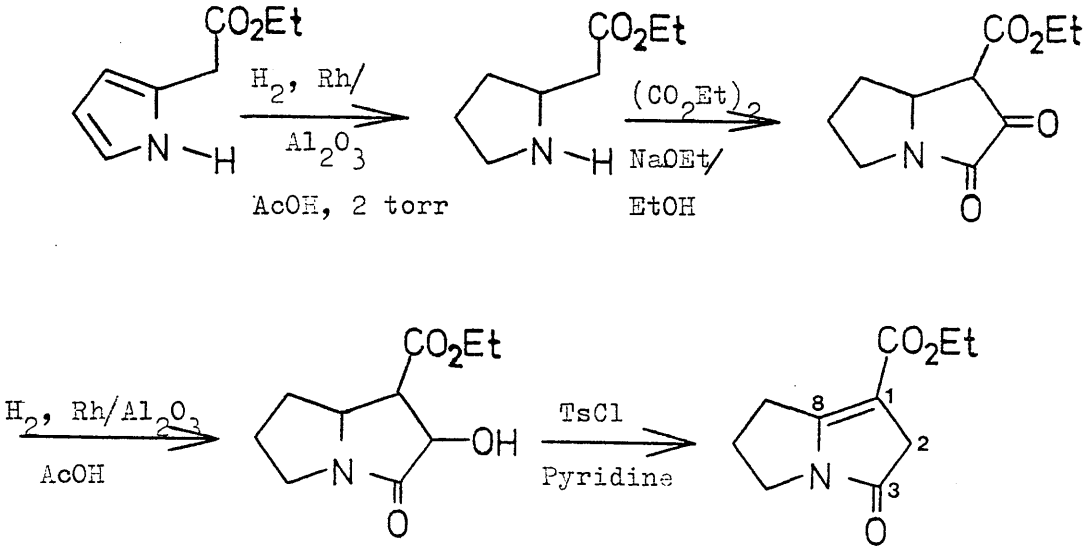
Scheme 2



hoped that in the key step, dehydration of the hydroxy-pyrrolizine ester (11) would afford the 1,2-unsaturated ester. In fact, only the 1,8-unsaturated ester (12) was produced. This was proved by reduction of (12) to the alcohol (13). This unsaturated alcohol behaved like an enamine, and exhibited properties quite different from those of the naturally occurring 1,2-unsaturated alcohol, supinidine (1).[9]

In their attempt to synthesise (±)-supinidine, Adams and co-workers[10] chose what appeared to be a better intermediate, ethyl 2-hydroxy-3-oxopyrrolizidine-1-carboxylate (14). This was prepared as shown in Scheme 3. It was reasonable to assume that dehydration of (14) as its tosylate derivative would yield the desired 1,2-unsaturation, and this was erroneously claimed by the authors.

Scheme 3

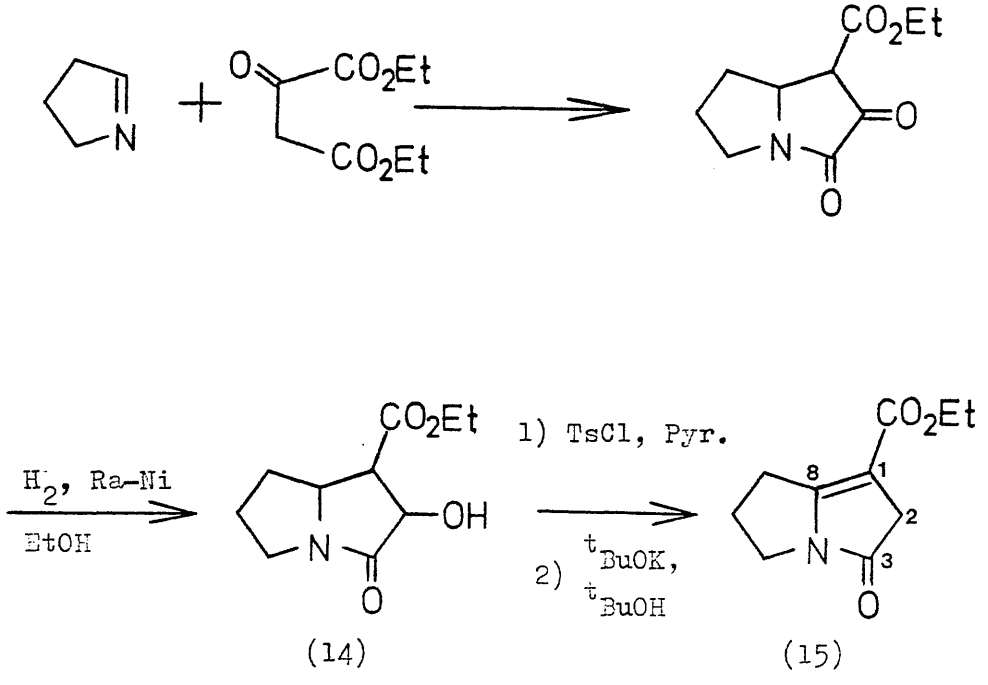


(14) (15)

Goldschmidt[11] demonstrated later that the actual product was the 1,8-unsaturated ester (15). This explains the failure of Adams and his co-workers to reduce this unsaturated ester to supinidine (1), either with lithium aluminium hydride or by stepwise reduction under a variety of conditions.

The third attempt to synthesise (±)-supinidine by Goldschmidt[11] also utilised the key intermediate (14), produced by an improved two step procedure from 1-pyrroline and ethyl oxaloacetate (Scheme 4). Treatment of this intermediate (14) with phosphorus oxychloride in pyridine gave an oil, which was probably the 2-chloro-derivative. Attepts to carry out pyrolytic or acid or iodine catalysed dehydration on (14) all yielded starting material. Conversion of (14) to its

Scheme 4



(14) (15)

tosylate derivative, followed by dehydration under basic conditions gave the 1,8-unsaturated ester (15) as observed earlier by Adams and co-workers.[10]

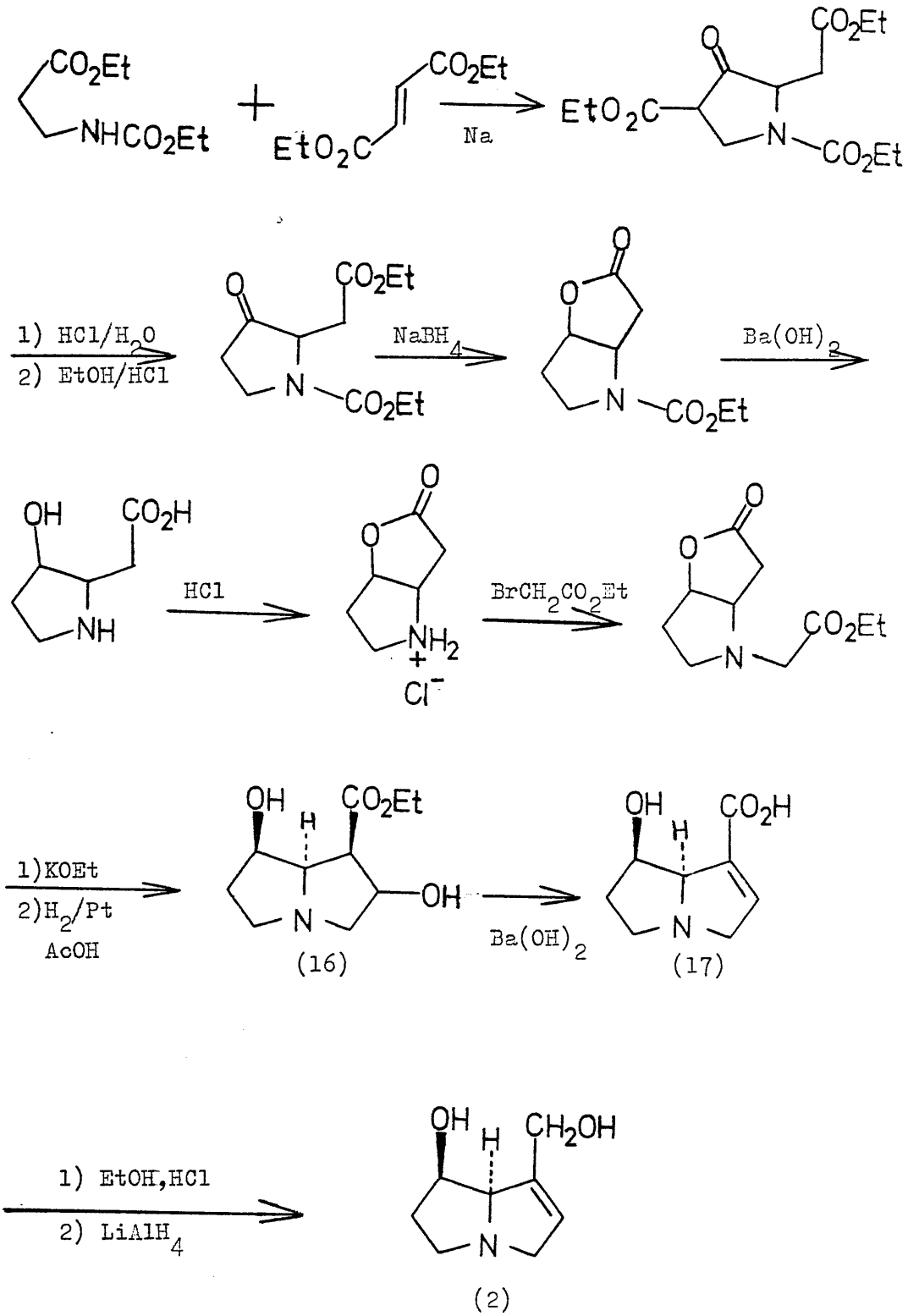
(c) Synthesis of (±)-Retronecine

The total synthesis of (±)-retronecine by Geissman and Waiss[12] is probably the most outstanding achievement of synthetic pyrrolizidine chemistry, although only a very low yield (< 1%) was obtained by the lengthy procedure outlined in Scheme 5. One of the key intermediates in this sequence is the dihydroxypyrrolizidine ester (16). Treatment of this ester with barium hydroxide resulted in hydrolysis of the ester function, together with dehydration to yield the 1,2-unsaturated acid (17). Apparently, none of the isomeric 1,8-unsaturated acid was formed. This was in direct contrast to the results of Adams and co-workers,[10] and Goldschmidt[11] who observed only 1,8-unsaturation on dehydration of their 2-hydroxy-pyrrolizidine esters, although it should be noted that their compounds also contained a 3-oxo group.

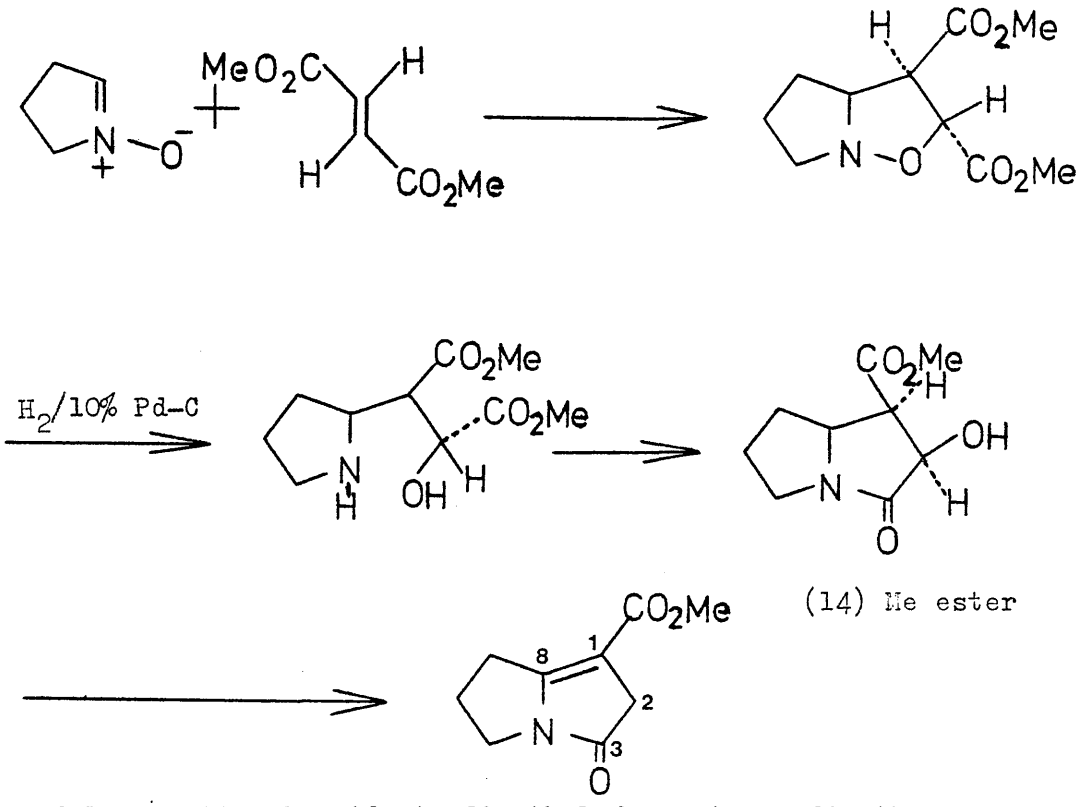
(d) Synthesis of (±)-Supinidine (1)

The total synthesis of (±)-supinidine was accomplished by Tufariello and Tette.[13] Initially, these workers prepared the previously used intermediate (14) (but as a methyl ester) by a different route involving the 1,3-dipolar cycloaddition

Scheme 5

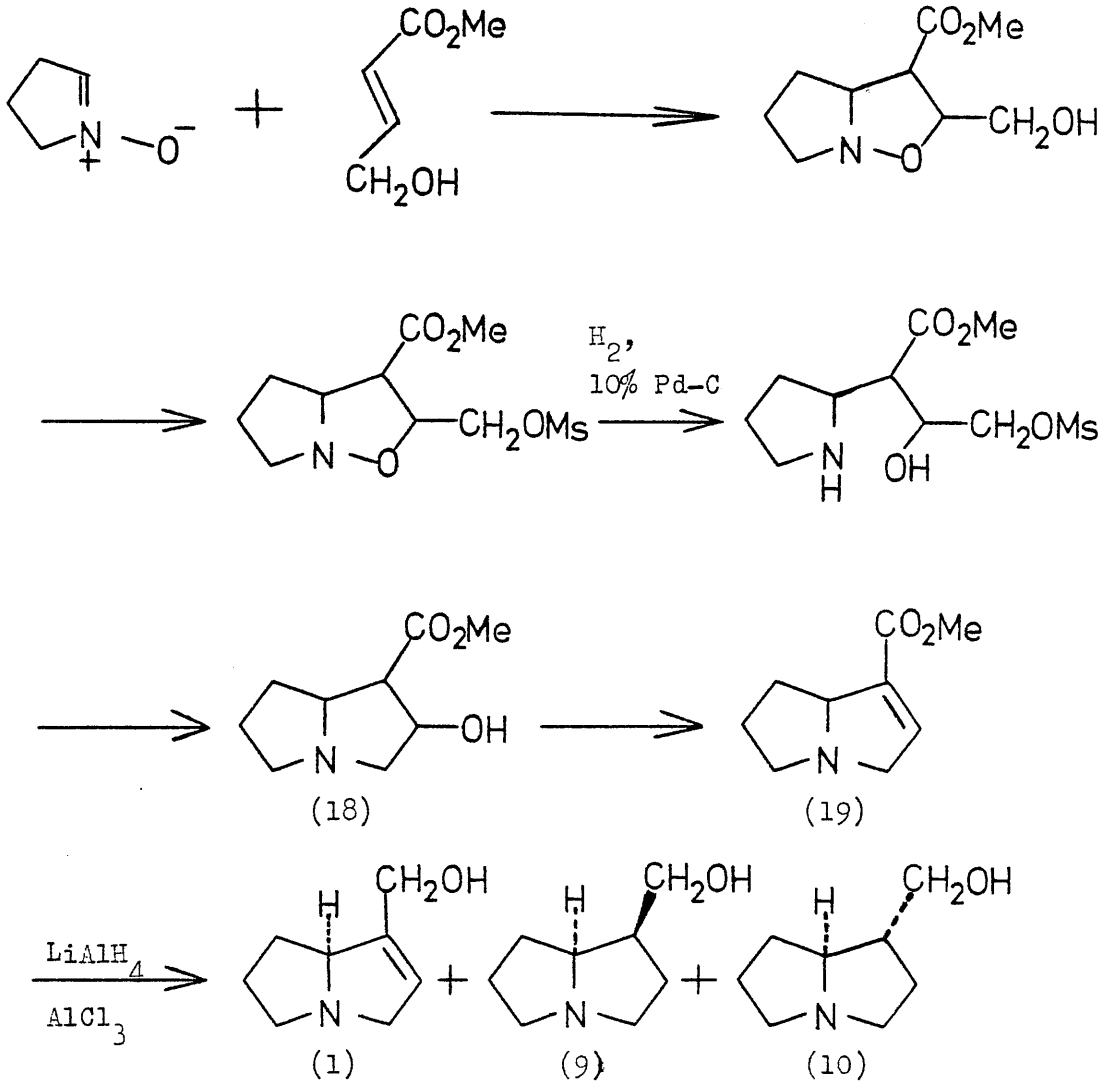

CO2Et
NHCO2Et
EtO2C
Na
1) HCl/H2O
2) EtOH/HCl
NaBH4
Ba(OH)2
OH
CO2H
NH
HCl
NH2
Cl−
BrCH2CO2Et
1)KOEt
2)H2/Pt
AcOH
H
(16)
(17)
1) EtOH,HCl
2) LiAlH4
CH2OH
(2)

Scheme 6



of 1-pyrroline-1-oxide to dimethyl fumarate or dimethyl maleate followed by catalytic hydrogenation (Scheme 6). Dehydration of this ester intermediate via the tosylate derivative gave only 1,8-unsaturated ester as previously noted by Adams and co-workers[10] and Goldschmidt.[11] Again, the presence of the 3-oxo function appears to play a crucial role leading to the formation of the undesired 1,8-unsaturation. Accordingly, Tufariello and Tette decided to synthesise an intermediate (18) without this 3-oxo group, as shown in Scheme 7. Dehydration of this hydroxypyrrolizidine ester (18) proceeded smoothly with phosphorus oxychloride at 0 °C to give the 1,2-unsaturated ester (19). Reduction of this ester (19) with a mixed hydride reagent prepared from lithium aluminium

Scheme 7



hydride and aluminium chloride gave a mixture of (±)-supinidine (1), (±)-trachelanthamidine (10), and (±)-isoretronecanol (9), in a ratio of 6:3:2. Separation of this mixture was effected by preparative g.l.c. The overall yield of (±)-supinidine was ca. 3% from 1-pyrroline-1-oxide.

Conclusion

Attempts to dehydrate 1-hydroxypyrrolizidine esters or 2-hydroxy-3-oxopyrrolizidine esters led to the formation of 1,8-unsaturated ester. The only two successful syntheses of 1,2-unsaturated necines utilized the dehydration of 2-hydroxy-pyrrolizidine esters.

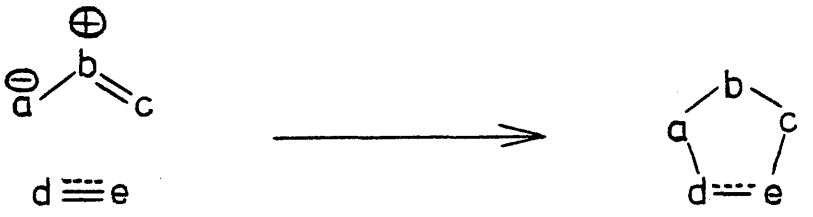
2.2 The 1,3-Dipolar Cycloaddition Reaction and its Application to the Synthesis of the Pyrrolizidine Nucleus

(a) General Description

The 1,3-dipolar cycloaddition reaction is an extremely versatile and important method for the synthesis of many types of heterocyclic compounds. Huisgen and his collaborators began research in this area in 1961[14] by introducing 1,3-dipolar cycloaddition as a general method for the synthesis of five-membered heterocyclic compounds. Since then, it has turned out to be an extremely valuable synthetic method, as the increasing number of applications in heterocyclic chemistry will testify.

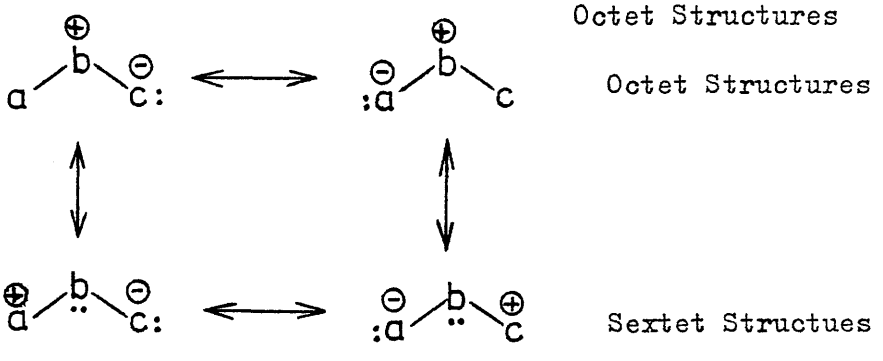
The 1,3-dipole is defined as a species which undergoes 1,3-cycloadditions to a multiple bond system, known as the dipolarophile as shown in Scheme 8.[15] The 'heteroallyl anion' system of 1,3-dipoles can be represented by zwitterionic

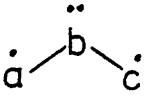
Scheme 8



resonance structures. 1,3-Dipoles can be both nucleophilic and electrophilic as the resonance structures with a terminal sextet suggest (Scheme 9). Note that the spin-paired diradical (20) may also contribute towards the ground state of the 1,3-dipole. The three atoms a, b, and c can be a wide variety of combinations of C, O, and N. The dipolarophile can be virtually any double or triple bond.

Scheme 9



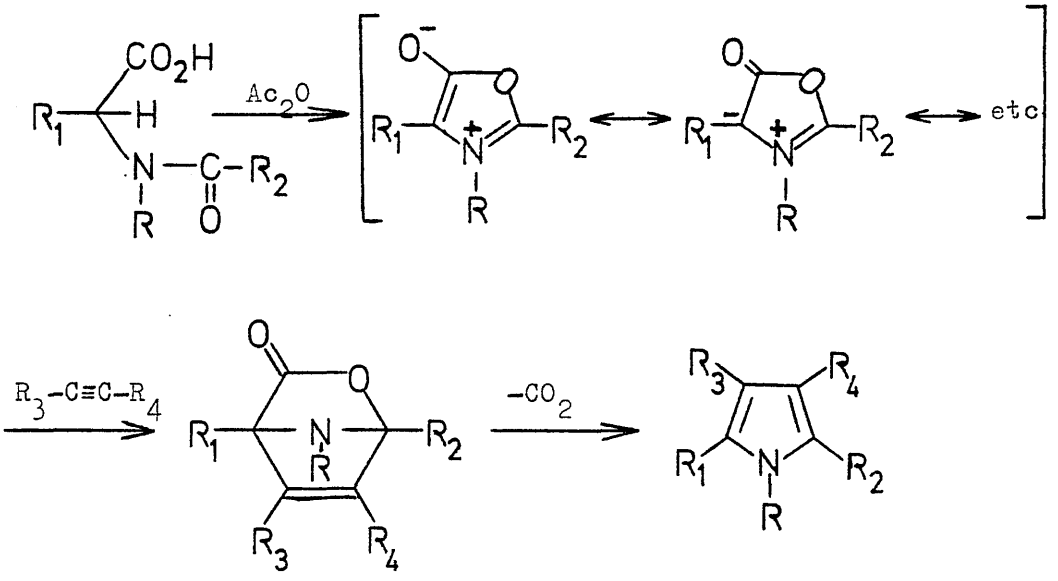


(20)

(b) Synthesis of the Pyrrolizidine Nucleus

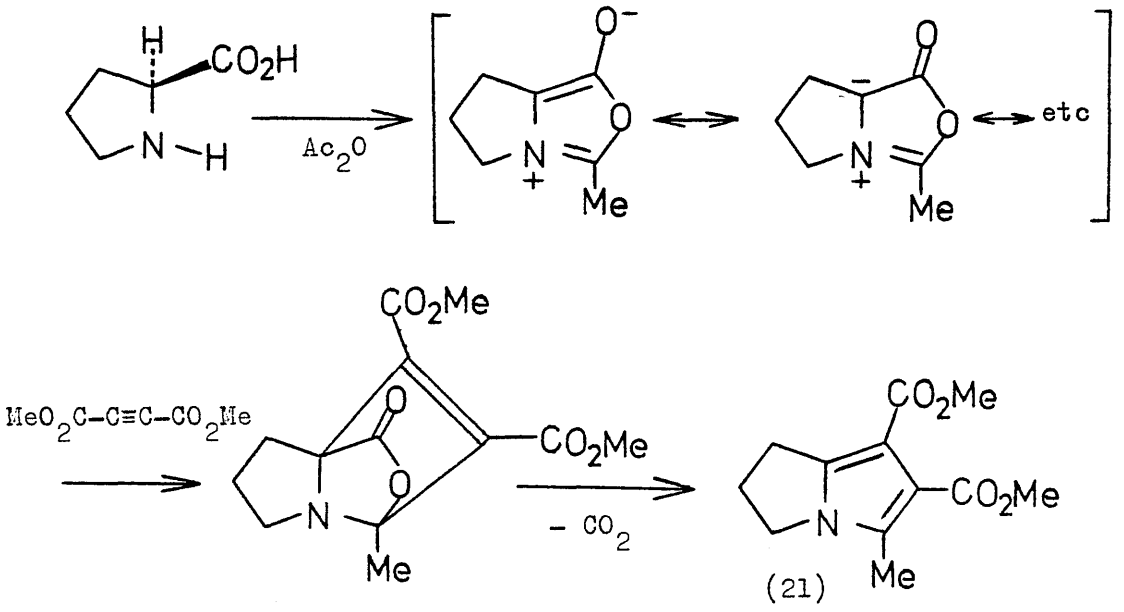
Huisgen and co-workers discovered that N-acyl-α-amino acids gave mesoionic oxazolones (called 'Munchnones') on treatment with acetic anhydride (Scheme 10).[16] These aromatic zwitterions were found to combine with alkynes in a 1,3-dipolar cycloaddition reaction to give adducts, which then eliminated carbon dioxide to produce good yields of substituted pyrrole derivatives.[17]

Scheme 10



When L-proline was heated for 1 hour with acetic anhydride at 130 °C in the presence of dimethyl acetylene dicarboxylate, the dihydropyrrolizine diester (21) was formed by the sequence of N-acetylation, formation of the mixed anhydride, cyclisation to the mesoionic oxazolone, cycloaddition with the dipolarophile and evolution of carbon dioxide (Scheme 11).

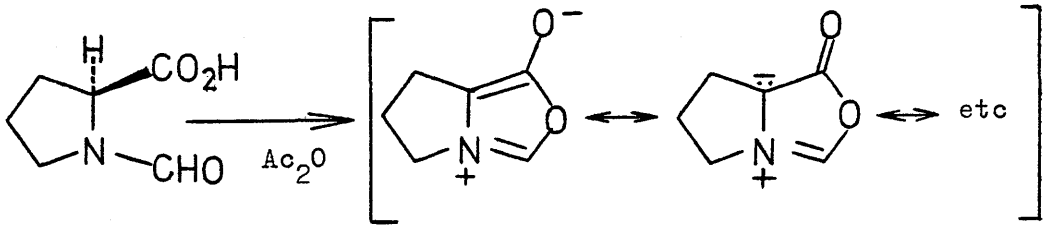
Scheme 11

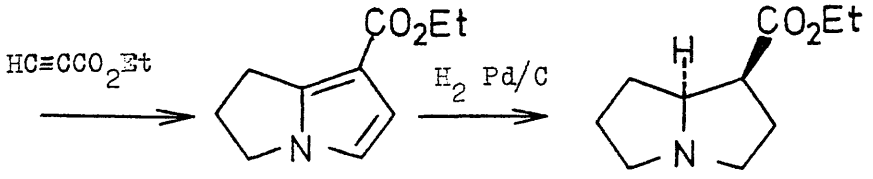


This extremely facile, one-pot reaction proceeded in good yield (76%), without any other noteworthy reactions occurring. An analogous reaction sequence with dimethyl fumarate produced a mixture of tetrahydropyrrolizine diesters, which were not separated and identified.[18]

The potential of this method for the synthesis of natural necine bases was first exploited by Pizzorno and Albonico.[19] Regiospecific 1,3-dipolar cycloaddition of ethyl propiolate to the oxazolium oxide formed from N-formyl-L-proline gave a dihydropyrrolizine ester (7) in 90% yield. Stereospecific catalytic hydrogenation of this ester yielded the saturated pyrrolizidine ester (22) (Scheme 12) in 93% yield. This ester can be converted into the diastereomeric 1-hydroxymethyl-pyrrolizidines (±)-isoretronecanol (9) (reduction) and (±)-trachelanthamidine (10) (epimerisation plus reduction)[20].

Scheme 12

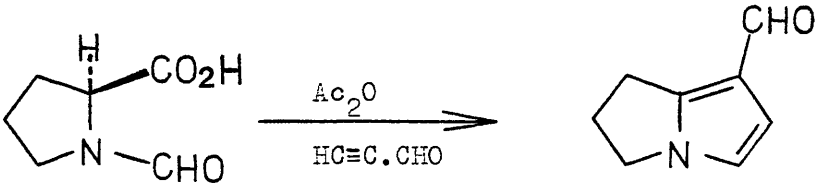




(7) (±)-(22)

Recently, the synthesis of the butterfly pheromone (23) was achieved in a one-pot reaction by Pizzorno and Albonico.[21] N-Formyl-L-proline was heated in acetic anhydride with propargylic aldehyde together with 2,6-di-tert-butyl-4-methylphenol as antioxidant. A 40% yield of the dihydropyrrolizine (23) was obtained (Scheme 13).

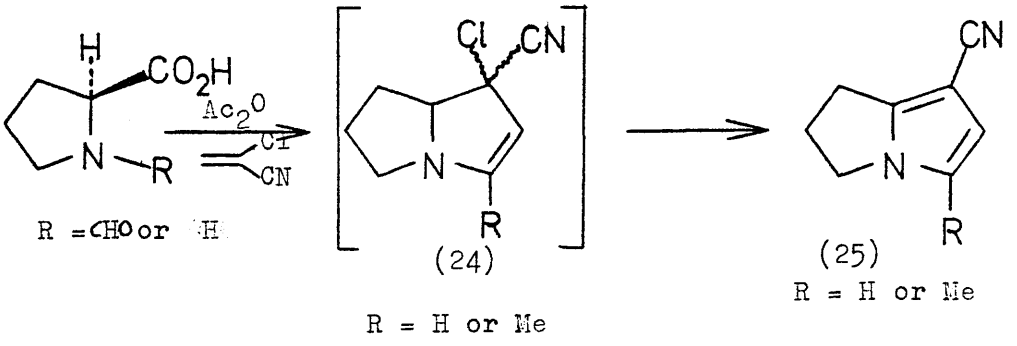
Scheme 13



(23)

Benages and Albonico[22] have also carried out 1,3-dipolar cycloadditions to L-proline derivatives with an olefinic dipolarophile, 2-chloroacrylonitrile (Scheme 14). The intermediates (24) readily lost HCl to generate the cyano-dihydropyrrolizines (25) in 65-70% yield. The same regiospecificity was observed with the olefinic dipolarophile as with ethyl propiolate and propargylic aldehyde.

Scheme 14

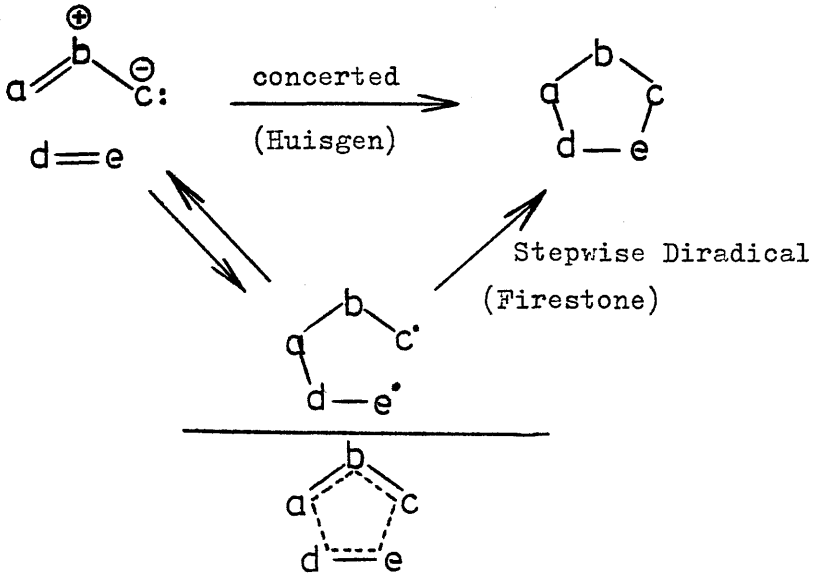


(c) Mechanism of the 1,3-Dipolar Cycloaddition Reaction

A concerted mechanism for 1,3-dipolar cycloadditions was first proposed by the leading worker in the field, Huisgen.[23] An alternative diradical process has been put forward by Firestone[24] (Scheme 15). This has led to considerable arguments in the literature between Huisgen[25] and Firestone[26].

The Huisgen mechanism is a concerted single step, four-centre, "no-mechanism", cycloaddition, in which the two new bonds are both partially formed in the transition state (26),

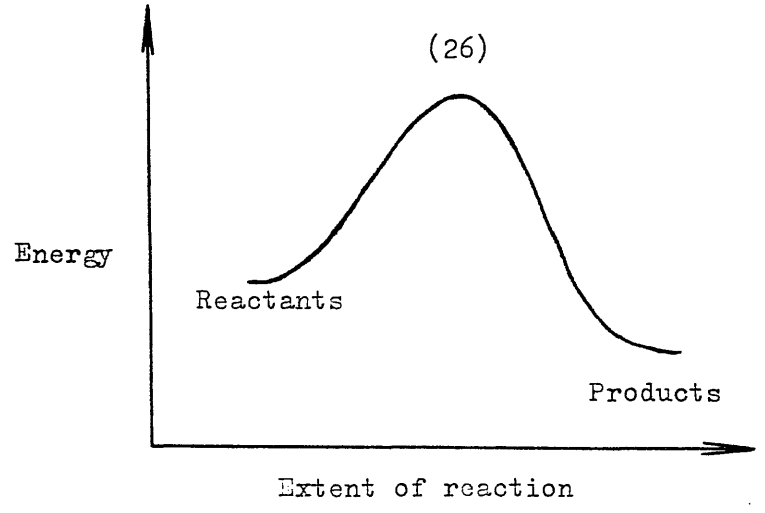
Scheme 15



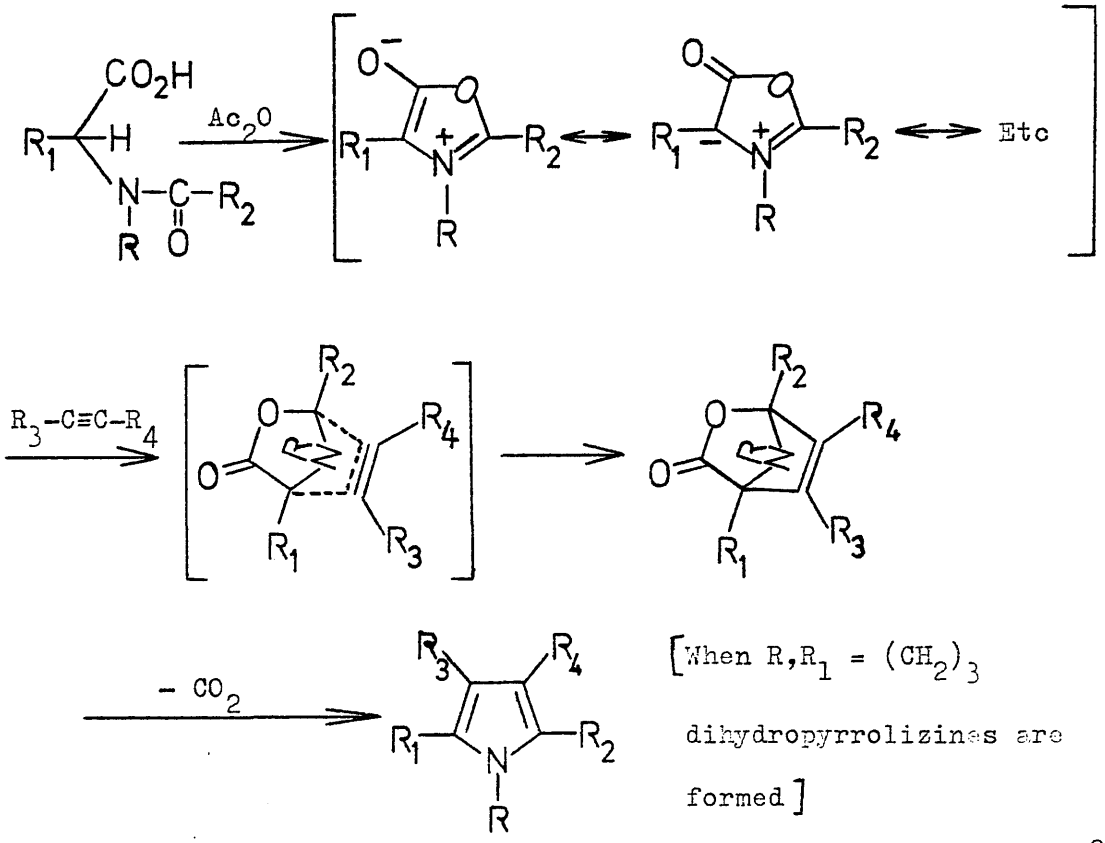
(26)

although not necessarily to the same extent.[23] The reaction co-ordinates are shown in Figure 1. The symmetry-energy correlation diagram for this $[_{\pi}4s + _{\pi}2s]$ process reveals that

Figure 1



Scheme 16



such a cycloaddition reaction is a thermally allowed process.[23,27] This concerted mechanism applied to the synthesis of substituted pyrroles is shown in Scheme 16. Note that in the final step of this sequence, elimination of carbon dioxide is usually considered to be a concerted process, but diradical intermediates could be invoked (Scheme 18).

A different mechanism has been proposed by Firestone.[24,26] He envisages a two-step process involving a spin-paired diradical intermediate (27) (Scheme 17). The reaction co-ordinates for this mechanism are shown in Figure 2. Formation of the transition state (28) is the rate-determining step in this

Scheme 17

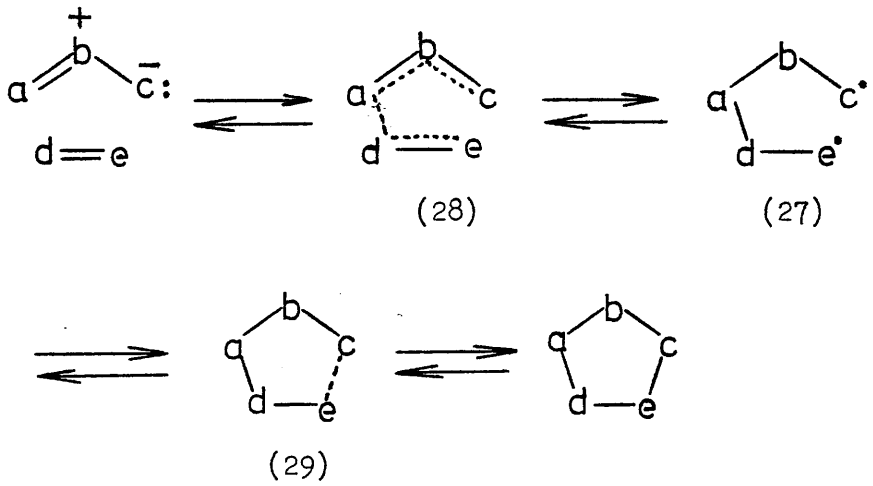
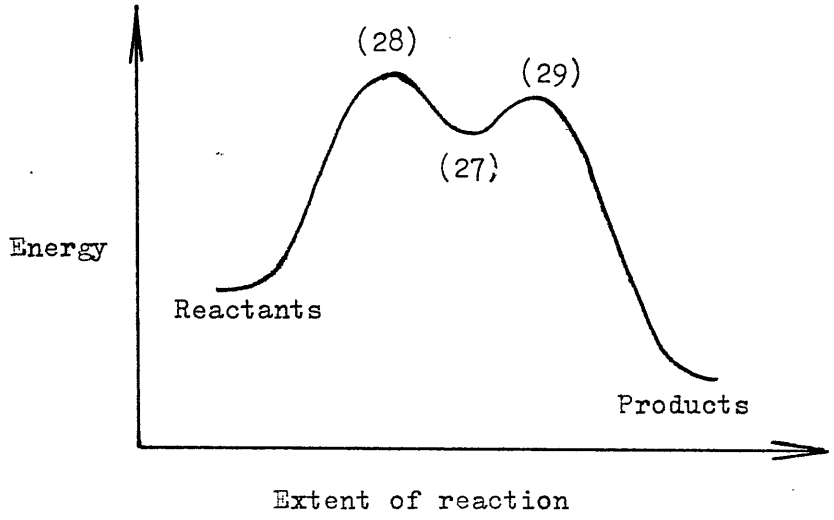


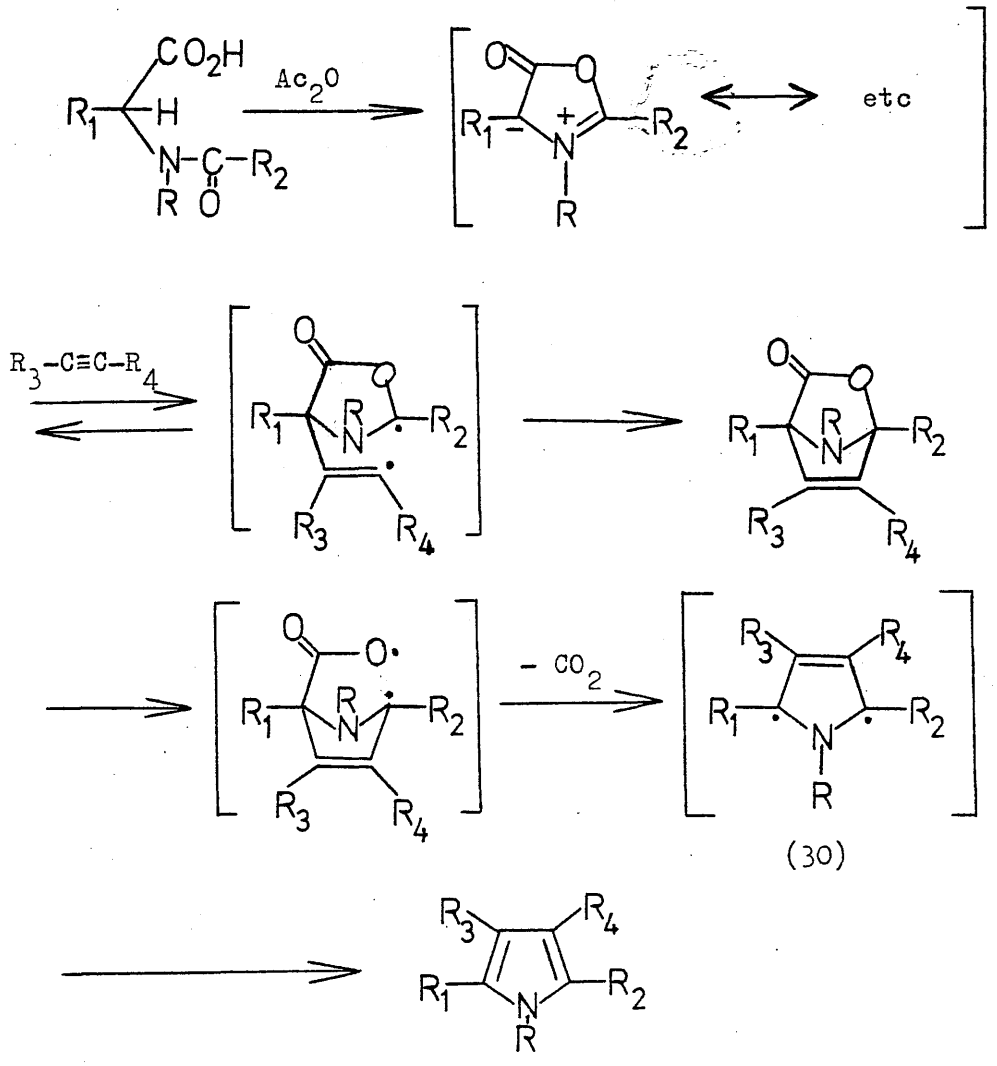
Figure 2



pathway. The diradical mechanism applied to the synthesis of substituted pyrroles, including the carbon dioxide elimination step, is shown in Scheme 18.

Both Huisgen and Firestone argue strongly for their concept of the mechanism of the cycloaddition reaction, and

Scheme 18



recently, Harcourt has suggested that the two opposing camps might be drawn closer together in a "concerted-diradical" mechanism.[28] His approach uses valence-bond theory and stresses the importance of spin-paired diradical structures (20) in describing the electronic structures of 1,3-dipolar molecules.

Cycloadditions of 1,3-dipoles to alkenes are stereo-

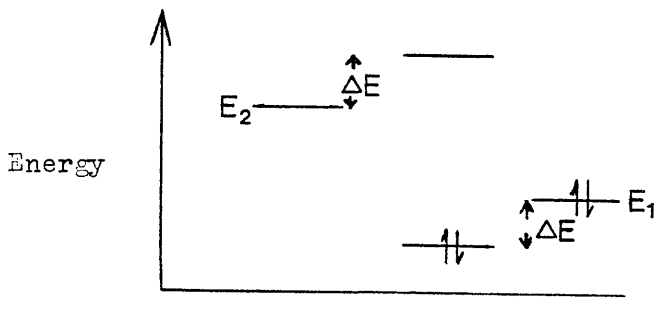
specifically suprafacial, and solvent polarity has little effect on reaction rates. Also small activation enthalpies and large negative activation entropies are generally observed. These facts are considered by most workers in this field[25,27] to be compatible only with the concerted mechanism.

(d) Regioselectivity of 1,3-Dipolar Cycloaddition Reactions

The experimentally observed regioselectivity of most 1,3-dipolar cycloadditions has been the most difficult phenomenon to explain in this field. It is one of the reasons for Firestone's insistence on the stepwise mechanism involving radical intermediates. Recently developed Perturbation Molecular Orbital (PMO) theory has provided a method for understanding the regioselectivity and reactivity of 1,3-dipolar cycloaddition reactions.[29-31]

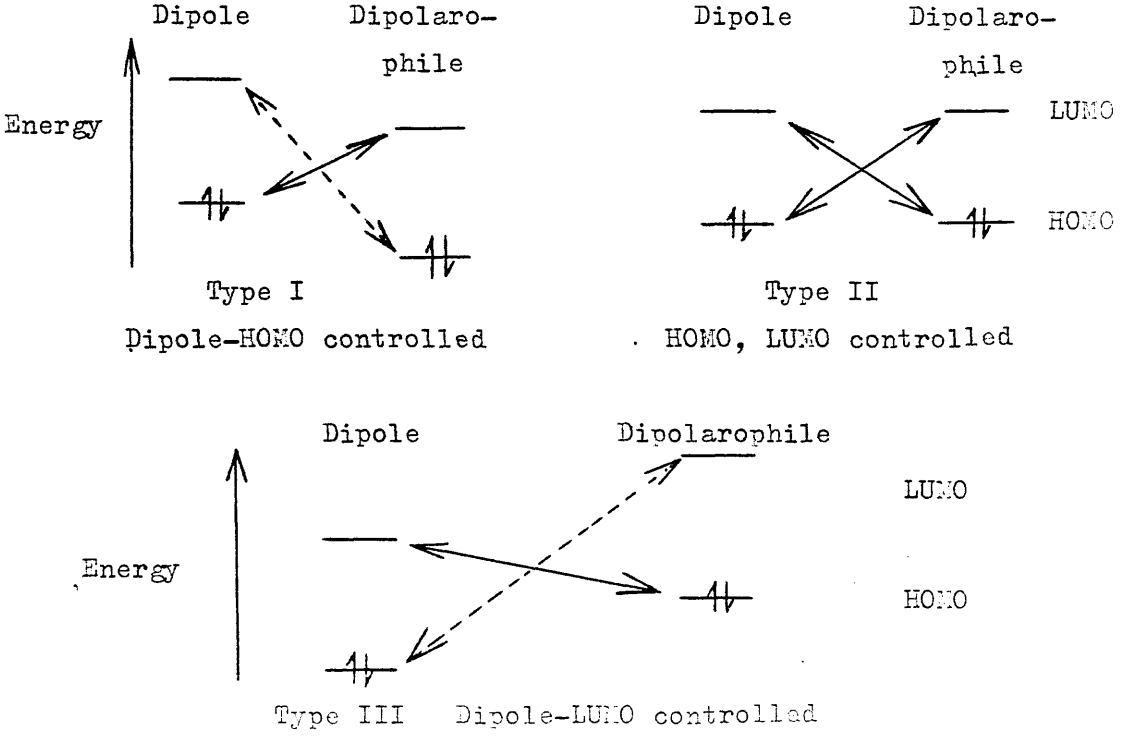
The interaction of two orbitals with energies E_1 and E_2 results in a new set of orbitals (Figure 3), where the energy of the new lower orbital is lowered, and the energy of the higher orbital is raised. Expressions for the difference in energy (ΔE) produced by this interaction have been developed by PMO theory. These show that the closer the energies E_1 and E_2

Figure 3



are in the orbitals, then the more they will interact, and that if the orbitals are of the same symmetry and overlap effectively, then the interaction will be large. In considering the interactions only the key frontier orbitals (highest occupied molecular orbital - HOMO and lowest unoccupied molecular orbital - LUMO) of both reactants are considered.[32] The relative energies of these frontier orbitals can be approximated from first order PMO theory. For 1,3-dipolar cycloadditions, this allows classification into three types depending on the relative energies of the 1,3-dipole and dipolarophile frontier orbital energies (Figure 4).[33] The energy of both HOMO's are increased by the presence of electron-donating substituents, and the energy of both LUMO's are decreased by electron-withdrawing substituents.

Figure 4



All 1,3-dipoles have in common a three atomic orbital π system containing four electrons analogous to an allyl anion. The 1,3-dipole produced from an N-acylproline is an example of an azomethine ylide ($R_2C{=}\overset{+}{N}R\overset{-}{C}R_2$). Ylides are all electron-rich species, characterised by high energy HOMO's and LUMO's. These species react preferentially with electron deficient species (low energy HOMO's and LUMO's), because of the narrow dipole HOMO-dipolarophile LUMO energy gap (Type I in Figure 4). FMO theory indicates that any factors, such as substituents, which decrease this energy gap, either by increasing the dipole HOMO energy or decreasing the dipolarophile LUMO energy will increase reactivity.

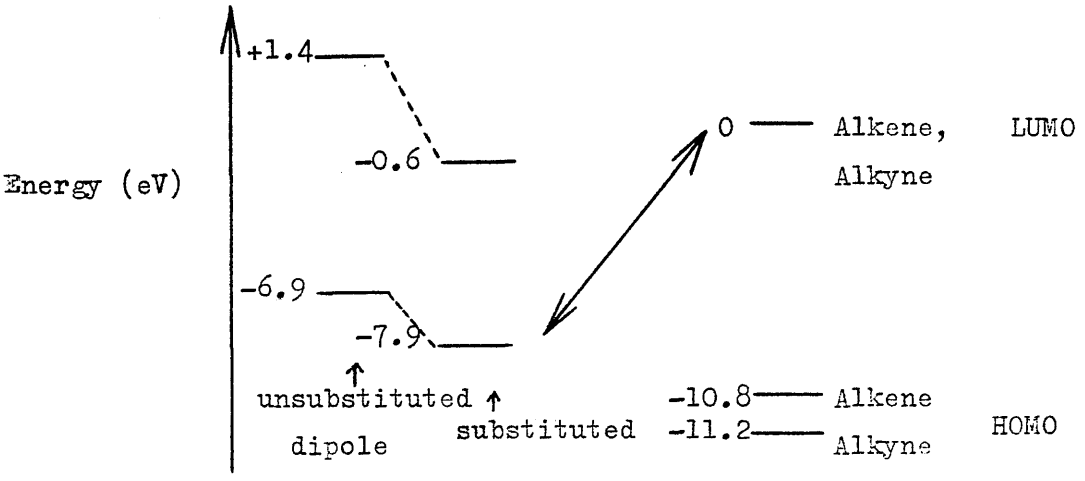
To understand regioselectivity in these cycloaddition reactions, the magnitude of the coefficents (a measure of the contribution of the atomic orbital to the molecular orbital) on the atomic orbitals must be calculated. Substituents on the dipole will cause unequal terminal coefficients on the HOMO and LUMO. The preferred regioisomeric transition state will be the one in which the larger terminal coefficients of the interacting nuclei are united. The degree to which one adduct is preferred will depend on the difference in the squares of the terminal coefficients.[32]

Now, the reaction of N-formylproline with ethyl acrylate or ethyl propiolate will be considered.

(a) Energies of the frontier orbitals of the reactants.

Very little experimental data is available for azomethine

Figure 5



ylides. The approximate energies of the frontier orbitals of the unsubstituted dipole $H_2C{=}\overset{+}{N}H\overset{-}{C}H_2$ are shown in Figure 5. These are average values obtained from several estimates.[30] The effect of the carbonyloxy substituent across the dipole (as in (31)) is to lower the energies of both the frontier orbitals by ~1 eV in the HOMO and ~2 eV in the LUMO [30] (Figure 5). For the dipolarophile, a single electron-withdrawing substituent on an alkene which is also conjugated lowers the HOMO energy relative to ethylene, but has a much larger lowering effect on the LUMO energy giving approximate energy levels for the frontier orbitals shown in Figure 5.[30] The LUMO of an alkyne is roughly the same energy as the corresponding alkene, while the HOMO energy of the alkyne is lower than the corresponding alkene. This results from a decrease in the C-C bond length and larger overlap of the p orbitals in the alkyne, leading to more bonding in the HOMO. Thus it can be seen from Figure 5

that this particular cycloaddition is dipole-HOMO controlled (Type I), because of the smaller energy gap of ~7.7 eV. It can also be predicted that corresponding electron deficient alkenes and alkynes will show roughly the same reactivity in the cycloaddition reaction.

(b) Regioselectivity

It is known that it is the coefficients of the atomic orbitals that influence regioselectivity, and that these coefficients can be estimated from PMO theory. However, very little data is available on this issue, and this leads to some uncertainty in the reasoning. For alkenes and alkynes it has been calculated that the frontier orbitals have larger coefficients on the unsubstituted end of the molecule in both HOMO and LUMO (Figure 6).[32] Calculations have also shown that the HOMO in a 1,3-dipole has the largest coefficient on the anionic carbon (a in figure 7), but when the dipole contains a central N atom, both ends of the dipole bear substantial negative charges (-0.14 to - 0.21 for c, - 0.2 to - 0.40 for a, and + 0.23 to + 0.47 eV for b).[30] Thus, normal expectation from interaction of a 1,3-dipole with an electron deficient alkene or alkyne in a dipole-HOMO controlled reaction would be for the 'anionic' end of the dipole (a) to have the larger coefficient and thus overlap better and become attached to the unsubstituted end of the alkene or alkyne. This would produce the opposite regioselectivity to that observed in these cycloaddition reactions.

Figure 6

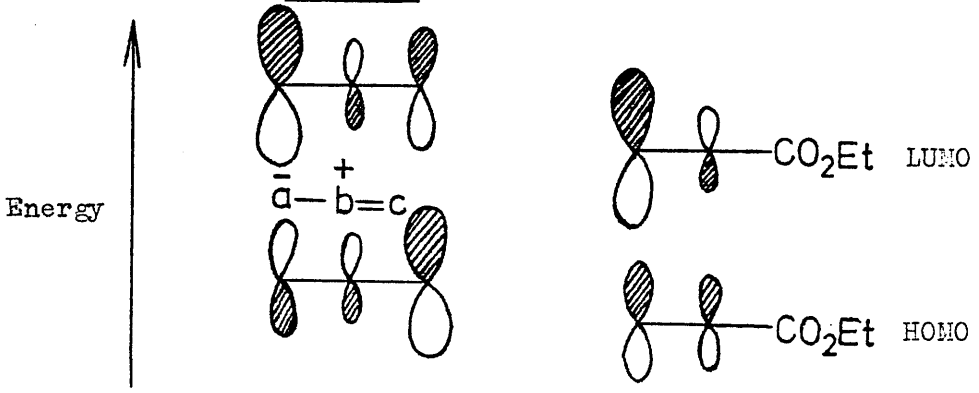
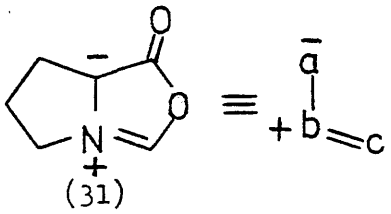


Figure 7



(31)

Therefore it must be argued that the effect of the carbonyloxy substituent is crucially important in determining the regioselectivity. The presence of the carbonyl must lower the coefficient at the anionic end (a) of the dipole, whereas the influence of the oxygen atom attached to c must increase the coefficient at that site in the HOMO (Figure 6). Then overlap occurs at the unsubstituted end of the alkene or alkyne with c to give the regioselectivity observed.

In summary, the reactivity and regioselectivity are the same for alkenes and alkynes in this 1,3-dipolar cycloaddition reaction. The reactivity can be readily understood by

consideration of the relative energies of the frontier orbitals of the reactants. The regioselectivity is not so easy to understand, because not enough calculations have been performed on these systems yet, but it is due to the asymmetry of the dipole frontier orbitals produced by the substituents.

2.3 Synthesis of Pyrrolizidine Ester Alkaloids

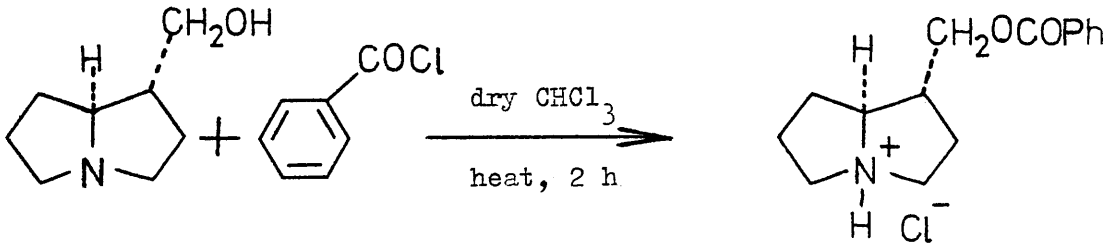
More than one hundred and fifty pyrrolizidine alkaloids of the monoester or diester type have been isolated and characterized. A number of synthetic approaches to these ester alkaloids have been developed, leading to syntheses of some monoester alkaloids and semi-synthetic diesters of retronecine (2). No total syntheses of naturally occurring unsymmetrical diester and macrocyclic diester alkaloids have yet been accomplished.

(a) Monoesters (i) Acylation

The first synthesis of a simple monoester alkaloid was achieved by Gurevich and Men'shikov.[34] They prepared the hydrochloride salt of (±)-trachelanthamidine benzoate as shown in Scheme 19.

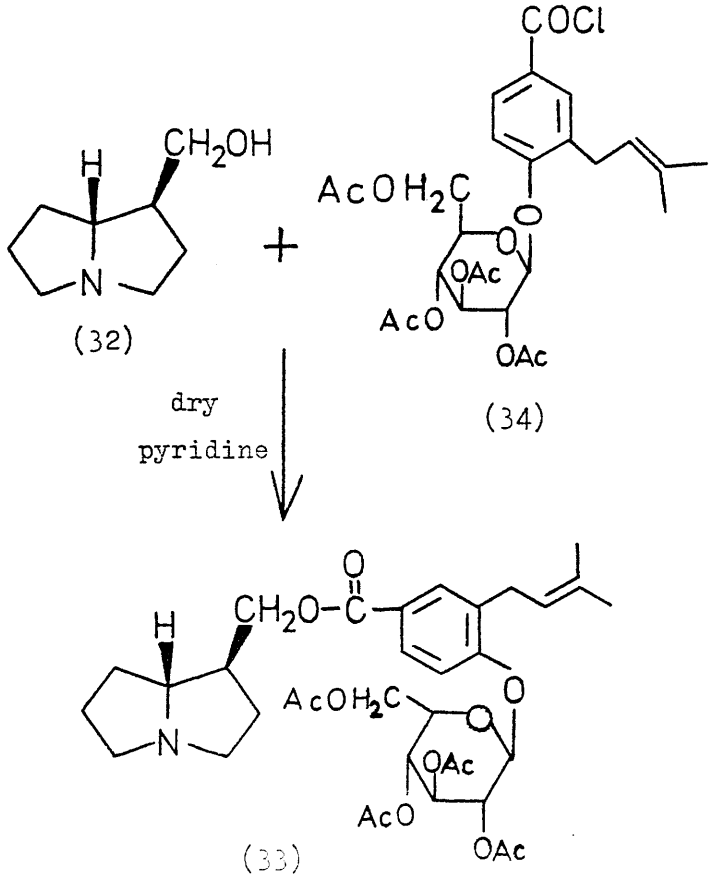
Two simple ester derivatives of (+)-laburnine (32) are naturally occurring. Hart and Lamberton synthesised (+)-

Scheme 19



laburnine benzoate using benzoyl chloride,[35] while Lindstrom and Luning used ketene to prepare (+)-laburnine acetate.[36] The tetraacetyl derivative (33) of the Orchidaceae alkaloid malaxine was synthesised by Tanino et al.,[37] by treatment of (+)-laburnine with the acyl chloride (34) (Scheme 20).

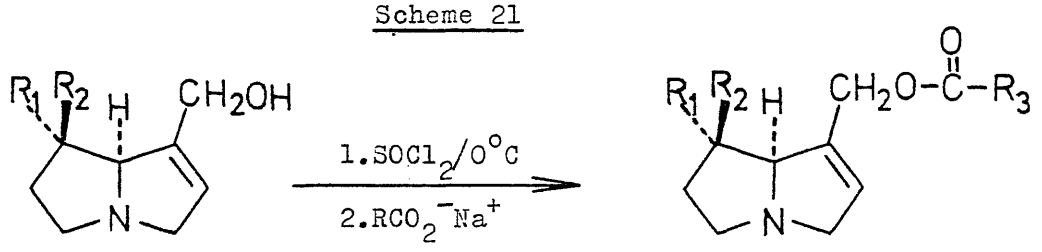
Scheme 20



(ii) Nucleophilic Substitution

A number of naturally occurring pyrrolizidine esters have been synthesised by Culvenor and his co-workers, by converting 1,2-unsaturated necine bases into their chloromethyl derivatives followed by nucleophilic substitution with the appropriate carboxylate anion.[38] In this way, supinine (35) was prepared from (-)-supinidine (36), heliotrine (37) from heliotridine (38), and the diastereoisomers intermedine and lycopsamine (39) from (+)-retronecine (40). The angelate esters (41) and (42) were obtained in a similar fashion (Scheme 21). This method depends upon the high reactivity of the allylic halides towards nucleophilic substitution. With the chloromethyl derivatives of the saturated necine bases, nucleophilic substitution competes with quaternisation and a mixture of products is obtained.

Scheme 21



(36) $R_1=R_2=H$

(38) $R_1=OH$, $R_2=H$

(40) $R_1=H$, $R_2=OH$

(35) $R_1=R_2=H$, $R_3=C(CHMe_2)(OH)CH(OH)Me$

(37) $R_1=OH$, $R_2=H$, $R_3=C(CHMe_2)(OH)CH(OH)Me$

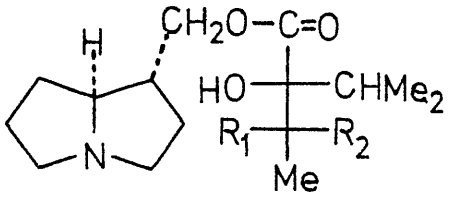
(39) $R_1=H$, $R_2=OH$, $R_3=C(CHMe_2)(OH)CH(OH)Me$

(41) $R_1=H$, $R_2=OH$, $R_3=CMe=CHMe$

(42) $R_1=OH$, $R_2=H$, $R_3=CMe=CHMe$

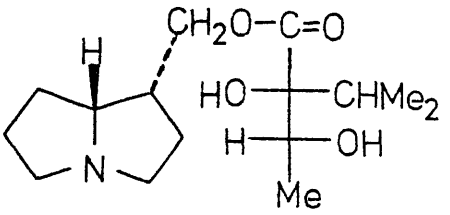
(c) Transesterification

The first total syntheses of the pyrrolizidine monoester alkaloids trachelanthamine (43), viridiflorine (44), and lindelofine (45), were achieved by Russian workers.[39]



(43) R_1=H, R_2=OH

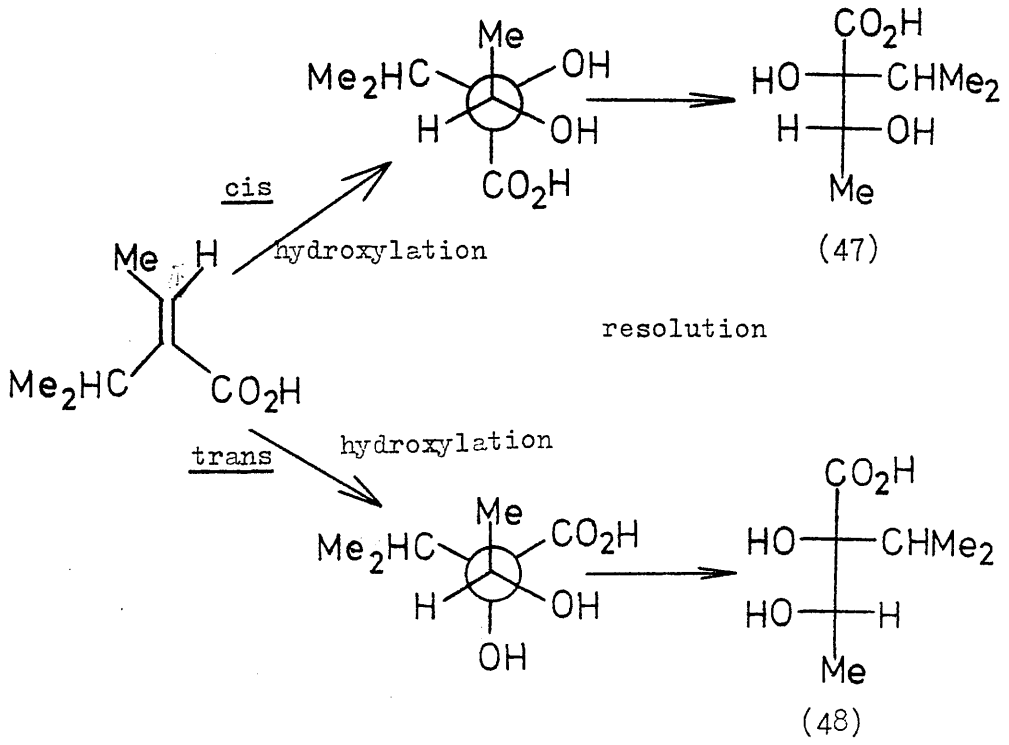
(44) R_1=OH, R_2=H



(45)

This total synthesis requires separate syntheses of optically active necines and optically active acids, followed by their combination. Likhosherstov et al.[40] synthesised a diastereomeric mixture of 1-hydroxymethylpyrrolizidines. Separation of this mixture gave (±)-trachelanthamidine and (±)-isoretronecanol, from which (-)-trachelanthamidine and (-)-isoretronecanol were obtained by resolution via the acid dibenzoyltartrate salts. To produce the acid components of these alkaloids, (±)-trachelanthic acid was synthesised by cis-hydroxylation of trans-2-isopropylcrotonic acid (46) (Scheme 22). After resolution, (+)— threo-2-isopropyl-2,3-dihydroxybutyric

Scheme 22

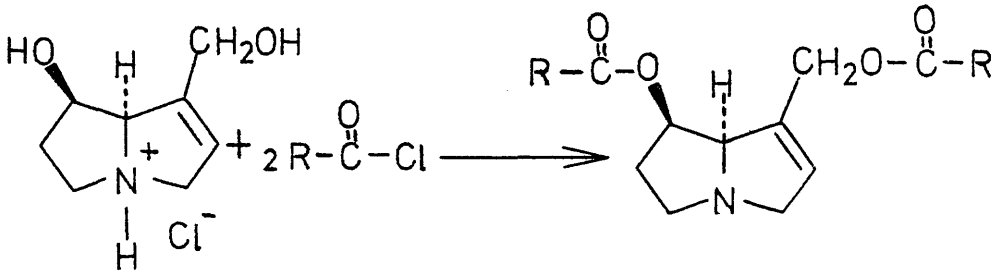


acid ((+)-trachelanthic acid (47)) was obtained. The corresponding erythro-isomer formed by trans-hydroxylation of (46), followed by resolution was (+)-viridifloric acid (48). The optically active acids were converted into their methyl ester di-O-benzyl ether derivatives. Base-catalysed trans-esterification of these derivatives with the appropriate optically active necine, followed by hydrogenolysis of the benzyl ethers gave trachelanthamine (43), viridiflorine (44), and lindelofine (45), in low yield.

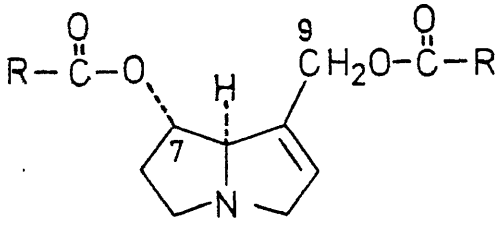
(b) Diesters (i) Use of Acyl Chlorides

A number of diesters of (+)-retronecine were prepared by Mattocks [41] by heating retronecine hydrochloride with various acid chlorides as shown in Scheme 23. Culvenor

Scheme 23



and co-workers [42] also prepared a range of semi-synthetic pyrrolizidine esters in connection with their studies on pyrrolizidine alkaloid toxicity. Diesters (49) were produced by treatment of heliotridine with various acid chlorides. When the acylation was carried out with an equimolar amount



(49)

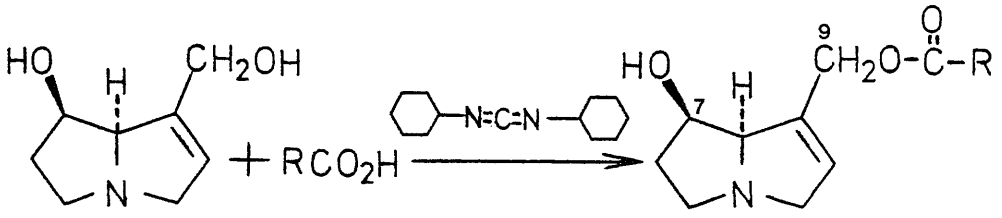
of pivalyl chloride, a mixture of the 7-monoester, 9-monoester, and 7,9-diester (49, R = Me_3C) was produced. Selective formation of 9-monoesters was achieved by conversion of the allylic

alcohol to the 1-chloromethyl derivative followed by nucleophilic substitution with a carboxylate anion as described previously (Section (a)(ii)).

(ii) Use of N,N'-Dicyclohexylcarbodi-imide (DCC) and N,N'-Carbonyldi-imidazole (CDI)

Selective esterification at C-9 of (+)-retronecine (40) has also been achieved by Hoskins and Crout [43] with simple acids using DCC as coupling reagent (Scheme 24). The yields

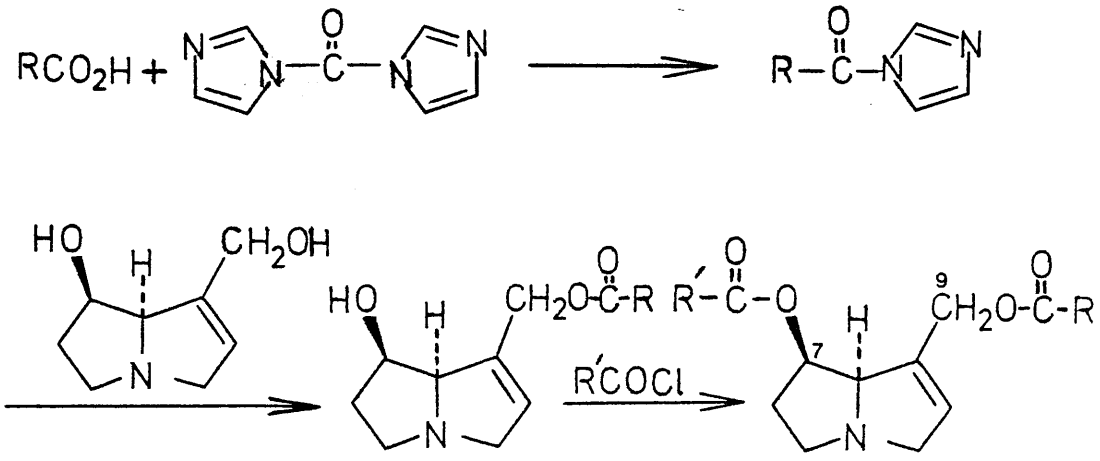
Scheme 24



(40)

were moderate, and some 7-monoester was also usually formed. The selectivity for esterification at C-9 increased with the steric size of the acid, presumably since esterification at the remaining 7-hydroxy group is then hindered. Greater selectivity for formation of 9-monoesters was obtained by the use of CDI. It was necessary to prepare the acylimidazole first (Scheme 25), then addition of (+)-retronecine gave

Scheme 25

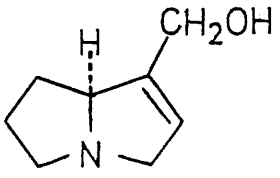


reasonable yields of the 9-monoester. This technique worked well for $\alpha\beta$-unsaturated acids and bulky α-trisubstituted acids (those commonly found in natural pyrrolizidine ester alkaloids). An unsymmetrical diester of retronecine was then prepared by esterification at the 7-position with a suitable acid chloride (Scheme 25).

CHAPTER 3 SYNTHESIS OF (±)-SUPINIDINE

3.1 Synthetic Strategies

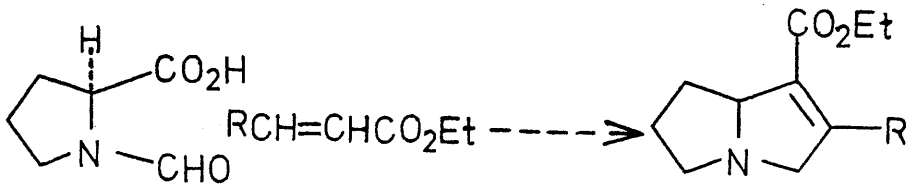
Supinidine (1) is the simplest example of a 1,2-unsaturated necine. Ester alkaloids must contain this unsaturation in order to exhibit physiological activity (Chapter 1.1). At the start of this work, the only synthesis of (±)-supinidine had been carried out by Tufariello and Tette,[13] in a very low overall yield (ca. 3%). The final step of their synthesis required separation of a mixture of products by preparative g.l.c. (Chapter 2.1). An improved synthetic route to this necine base was obviously desirable.



(1) Supinidine

The synthetic method selected for construction of the pyrrolizidine ring system was the 1,3-dipolar cycloaddition of unsaturated esters to N-formyl-L-proline (50) (Chapter 2.2). The initial approach was to carry out the cycloaddition reaction with olefinic esters attempting to construct the desired

Scheme 26

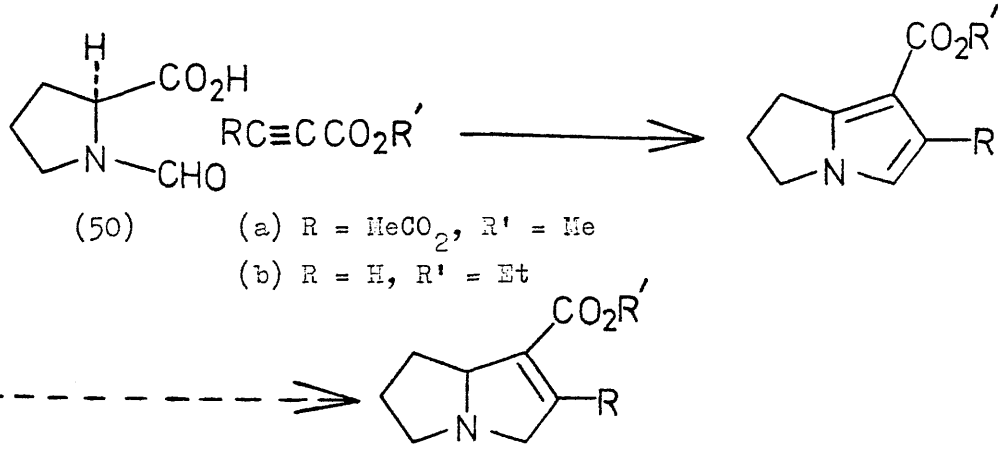


(50) R = H or CO_2Et

1,2-unsaturation directly (Scheme 26).

Because mixtures of double-bond isomers were obtained by this procedure (Chapter 3.2), it was decided to use acetylenic esters in the cycloaddition reaction (Scheme 27) to produce dihydropyrrolizines as this is known to be an efficient procedure (Chapter 2.2). Two of these esters were prepared

Scheme 27



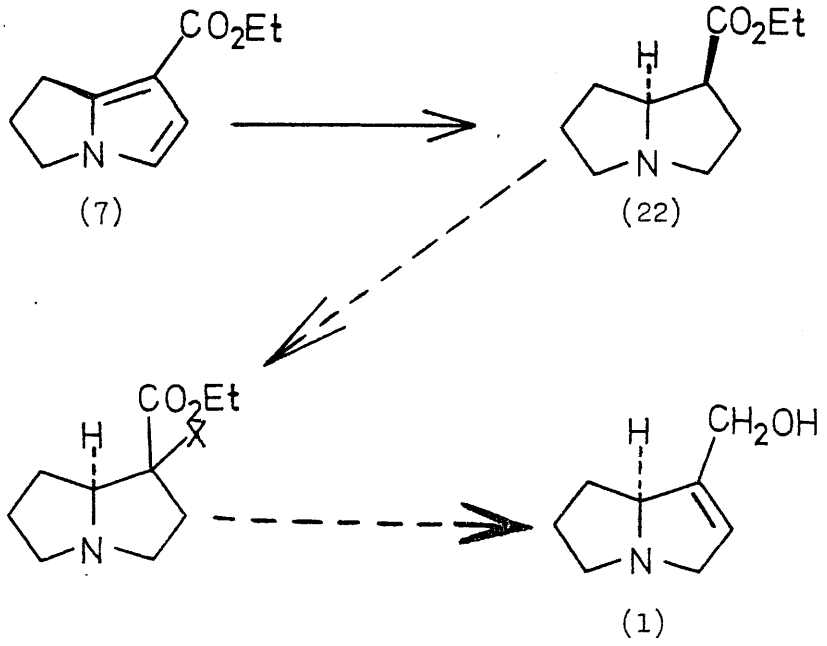
(50) (a) R = $MeCO_2$, R' = Me

(b) R = H, R' = Et

(Chapter 3.3), and then various 1,4-reduction attempts were made on one of them to try to produce the desired 1,2-dehydropyrrolizidine system (Chapter 3.4).

Finally, it was known[19] that the dihydropyrrolizine ester (7) can be hydrogenated to the fully saturated ester (22) (Scheme 28). It was then intended to introduce a good leaving group α to the ester carbonyl group which could subsequently be eliminated to generate the required unsaturation at the 1,2-position (Chapter 3.5).

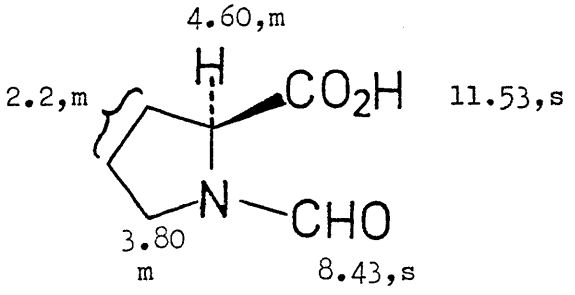
Scheme 28



3.2 Synthesis of Tetrahydropyrrolizine Esters

Gotthardt and Huisgen reported[18] that the 1,3-dipolar cycloaddition of N-formyl-L-proline (50) with dimethyl fumarate produced a mixture of tetrahydropyrrolizine esters, which were not separated and identified (Chapter 2.2). Crystalline N-formyl-L-proline was isolated in 96% yield

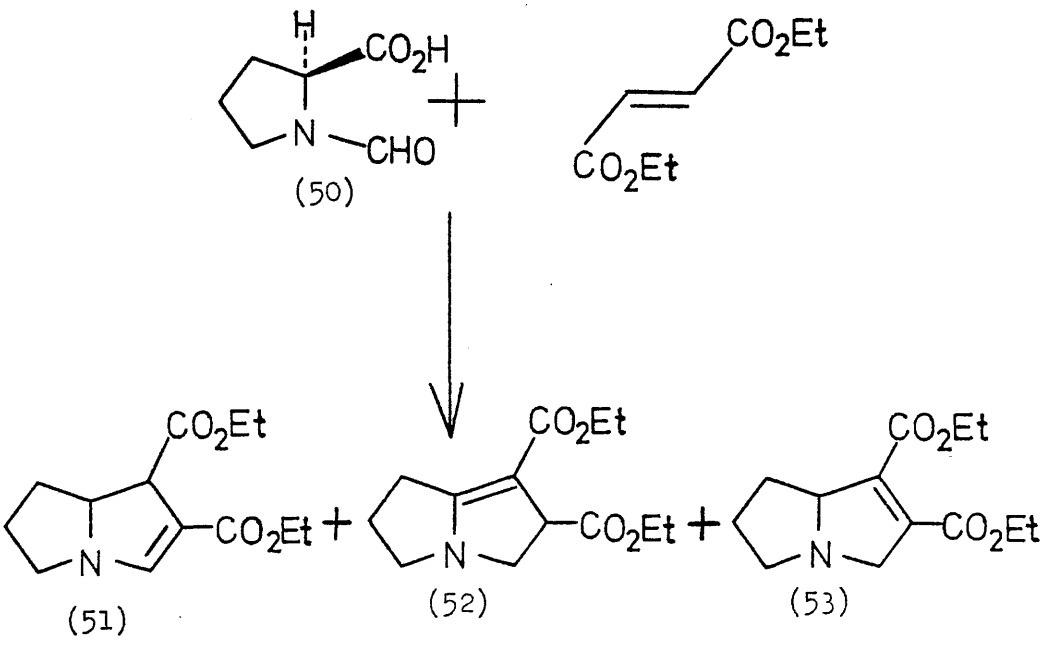
Figure 8



N.m.r. spectrum of N-formyl-L-proline (50) in $CDCl_3$ (δ values)

after treatment of natural L-proline with a solution of formic acid and acetic anhydride at room temperature. The n.m.r. spectrum of the N-formyl-L-proline taken in deuteriochloroform was assigned as shown in Figure 8. The cycloaddition reaction was carried out as before[18] with dimethyl fumarate. Diethyl fumarate and diethyl maleate were also used as dipolarophiles. All these reactions produced similar mixtures of products as judged by t.l.c. analysis. The mixture of products obtained from the reaction with diethyl fumarate (Scheme 29) was separated by preparative t.l.c., using the technique of double development for improved separation of bands with similar R_f values. Three tetrahydropyrrolizine esters were isolated and structures were postulated on the basis of the following spectral characteristics. All three products displayed similar mass spectra with molecular ions at m/e 253, and base peaks at m/e 180 (loss of CO_2Et). The n.m.r spectra were too complex

Scheme 29

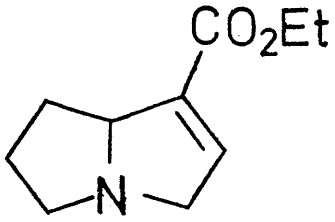


to interpret fully, but a one proton olefinic singlet at δ 7.1 ppm present in only one of the three spectra is considered to be good evidence for structure (51). In their i.r. spectra, compounds (51) and (52) showed both saturated and unsaturated ester carbonyl absorptions, whereas (53) displayed only one band at 1,700 cm^{-1} corresponding to the two unsaturated ester carbonyl groups. Confirmation of the extended conjugation in all these compounds was obtained from the u.v. spectra. Compounds (51) and (52) showed $\lambda_{max.}$ 291 (ε 14,800) and 290 (ε 15,000) nm respectively. This may be compared with the model compound, $CH_3C(NMe_2)=CHCO_2H$, which displays a $\lambda_{max.}$ of 285 nm,[44] noting that acids and their simple ester derivatives

usually show closely similar u.v. characteristics. The nitrogen substituent exerts a large auxochromic shift on the $\lambda_{max.}$ normally associated with an α,β-unsaturated ester (ca. 215-225 nm). The somewhat different chromophore of (53) gave $\lambda_{max.}$ 297 nm (ε 19,500).

Reaction of N-formyl-L-proline with diethyl maleate appeared to give the same mixture of products (t.l.c. data), which was not separated. Similar cycloaddition with dimethyl fumarate (used partly to simplify the n.m.r. spectra obtained) gave three analogous products after separation by preparative t.l.c. It should be emphasized that, since these mixtures did not appear to be promising for development of a synthesis of (±)-supinidine, none of the isolated esters was fully characterised and these structural assignments are only tentative. Nevertheless, it seems clear that tetrahydropyrrolizine esters are being formed, although the mechanism for formation of these isomeric compounds by breakdown of the intermediate tricyclic compound is not fully understood (Chapter 2.2).

The use of ethyl acrylate as the dipolarophile was also investigated. It was hoped that this would produce the key unsaturated ester (19) (plus isomers) on cycloaddition with N-formyl-L-proline. Various reaction conditions were explored, but all reactions produced complex mixtures of basic products and polymers of ethyl acrylate. Even from reactions carried



(19)

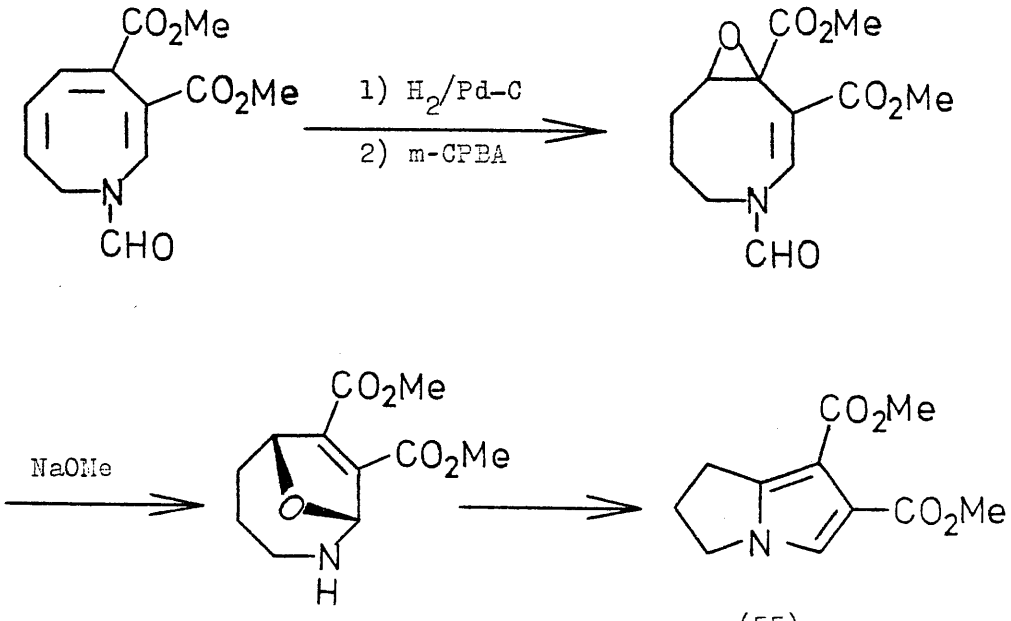
out in the presence of a hydroquinone stabiliser, no identifiable products were obtained.

3.3 Synthesis of Dihydropyrrolizine Esters

(a) Dimethyl 2,3-Dihydro-1H-pyrrolizine-6,7-dicarboxylate (55)

Mariano *et al.*[45] have recently reported a new method for construction of the pyrrolizidine nucleus involving azocine derivatives (Scheme 30). These authors managed to synthesise

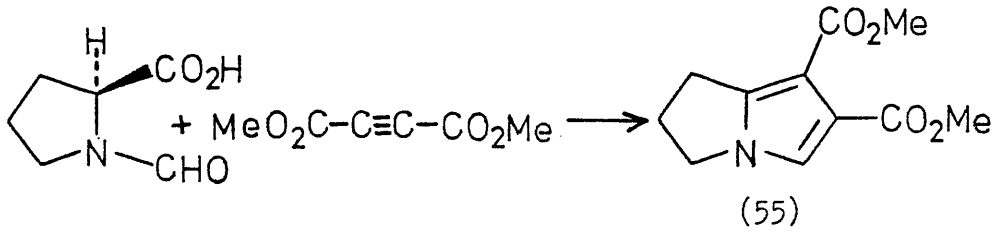
Scheme 30



(55)

only one example (55) of this system as an oil by their rather lengthy procedure in low overall yield (< 3%). This same diester (55) was readily synthesised in a one-pot reaction by 1,3-dipolar cycloaddition of N-formyl-L-proline with dimethyl acetylenedicarboxylate in acetic anhydride (Scheme 31). The product was obtained crystalline in 90% yield and u.v., i.r., n.m.r., and mass spectra were in accord with those published for this diester (55).[45]

Scheme 31

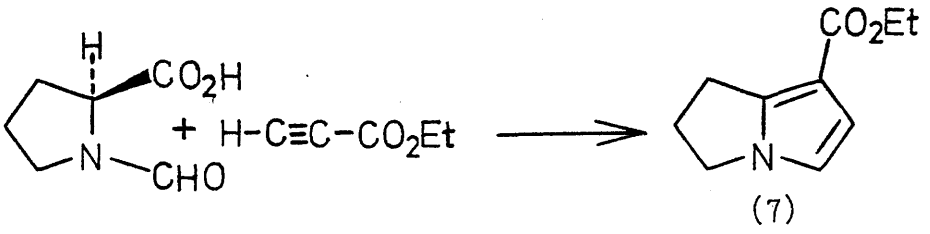


(55)

(b) Ethyl 2,3-Dihydro-1H-pyrrolizine-7-carboxylate (7)

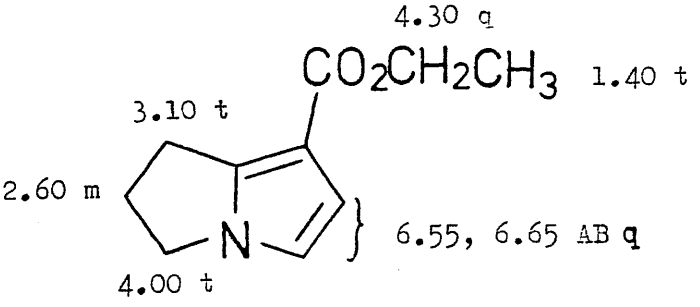
In order to proceed with attempts to synthesise (±)-supinidine (1), the dihydropyrrolizine ester (7) was required. This was prepared by the regiospecific 1,3-dipolar cycloaddition of ethyl propiolate to N-formyl-L-proline as reported previously[19] (Scheme 32). Yields of 83-90% of the ester (7) were obtained by vacuum distillation instead of the chromatographic method used by Pizzorno and Albonico[19]. The mass spectrum of (7) showed a molecular ion at m/e 179

Scheme 32



and main fragment ions at m/e 134 (M-OEt), and 106 ($M-CO_2Et$). The presence of the unsaturated ester system was indicated by the u.v. spectrum ($\lambda_{max.}$ 233 (ε 15,600) and 255 (ε 12,700) nm) and by a band at 1,700 cm^{-1} in the i.r. spectrum. The assignments for the n.m.r. spectrum are shown in Figure 9.

Figure 9

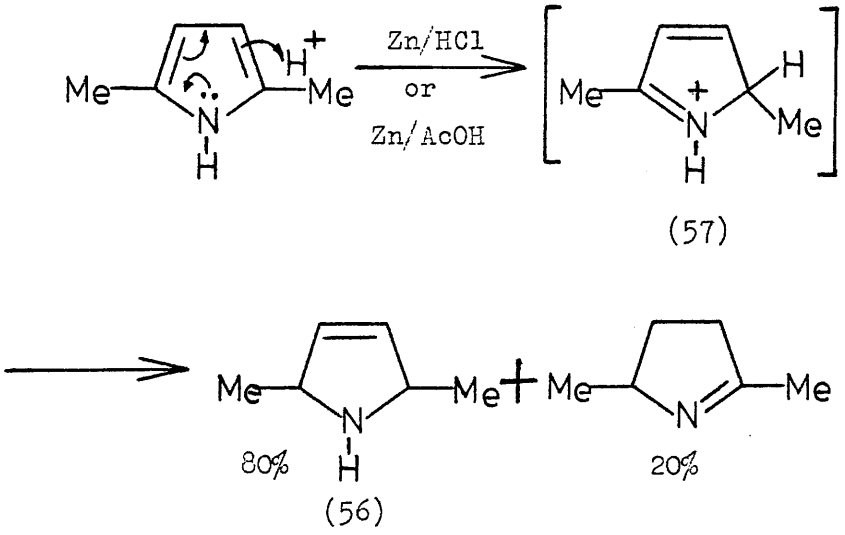


N.m.r spectrum of (7) in $CDCl_3$ (δ values)

3.4 Attempted 1,4-Reduction of Dihydropyrrolizine Ester (7)

Evans has reported[46] that the partial reduction of substituted pyrroles can be accomplished by zinc in acetic acid or dilute hydrochloric acid. For example, with 2,5-dimethylpyrrole, 1,4-addition of hydrogen takes place to give the 3-pyrroline (56) as the major product (Scheme 33). The mechanism presumably involves reduction of an intermediate pyrrolenine salt (57).[47]

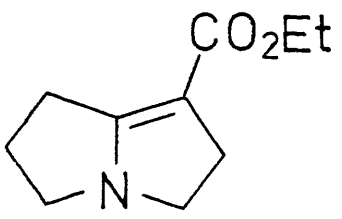
Scheme 33



Ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate (7) is a substituted pyrrole derivative, and therefore its reduction

with zinc under acidic conditions was investigated.

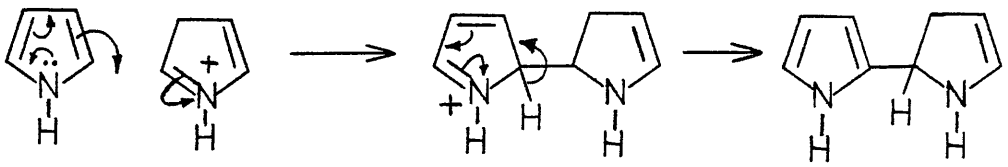
Treatment of the ester (7) with zinc and hydrochloric acid at or below room temperature for various lengths of time produced similar mixtures of products together usually with some unreacted starting material. The best yields of the two major products were obtained by use of hydrochloric acid for 24 hours. These two compounds were separated by preparative t.l.c. The less polar minor component obtained in 18% yield had a molecular ion at m/e 181 corresponding to addition of two hydrogen atoms. The presence of an $\alpha\beta$-unsaturated ester was indicated by the bands at 1,680 and 1,620 cm^{-1} in the i.r. spectrum. However, the chromophore in the u.v. spectrum at $\lambda_{max.}$ 302 nm, ε 11,800, suggested that the $\alpha\beta$-unsaturated ester has a N at the β-position, to account for the large auxochromic shift observed. This u.v. spectrum may be compared with that for the postulated compound (52) which had $\lambda_{max.}$ 291, ε 14,800. Thus the structure (12) is proposed for this compound. The n.m.r spectrum of (12) was complex, yet consistent with this structure. In particular, no olefinic signals were present.



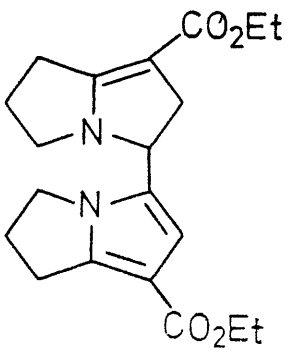
(12)

The major component of this reduction mixture, obtained in 29% yield, was shown to be a dimer from its mass spectrum with a molecular ion at m/e 308. This mass corresponds to reduction of one double bond and formation of a new C-C bond. One interesting feature of the n.m.r. spectrum of this dimer is the presence of only one olefinic proton. Pyrrole is known to form a mixture of polymers with mineral acid, and a trimer can be produced under controlled conditions.[48] However, dimers are readily obtained from α-mono or $\alpha\beta$-dialkylpyrroles, probably by the type of mechanism shown in Scheme 34.[48] A similar mechanism may be operating in this case to produce

Scheme 34



the dimeric pyrrolizidine obtained. A possible structure which fits the spectral data, and can be accounted for by this type of mechanism is (58).



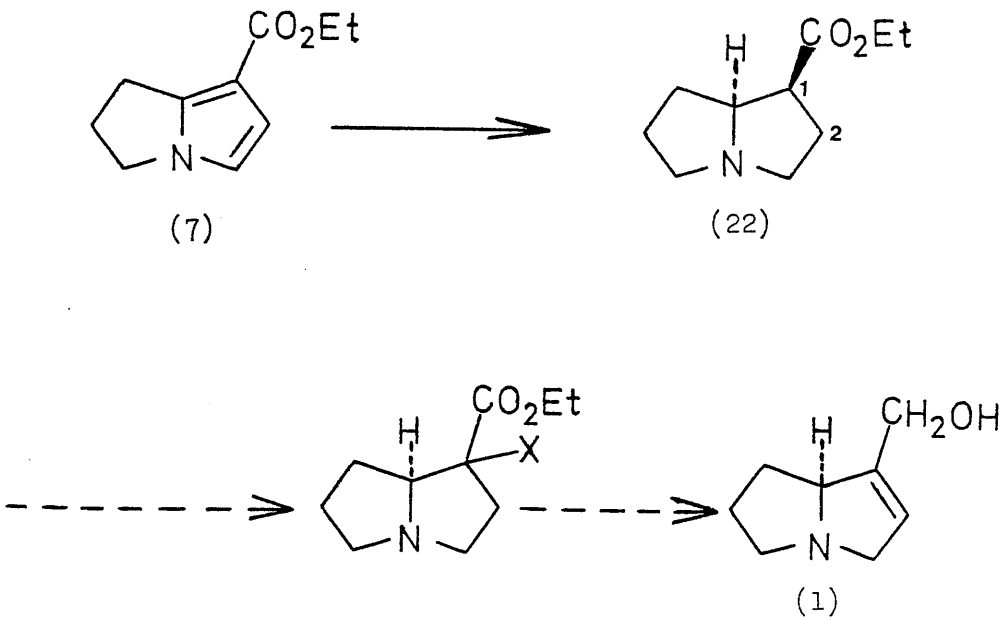
(58)

Although these results are interesting, this line of research was abandoned because no evidence was obtained for the presence of the desired 1,2-unsaturation in the pyrrolizidine ester products.

3.5 Synthesis of (±)-Supinidine (1)

In view of the failure to achieve partial reduction of the dihydropyrrolizine ester (**7**) in the desired manner, it was decided to hydrogenate this ester stereospecifically to the fully saturated pyrrolizidine ester (22) as demonstrated earlier by Pizzorno and Albonico[19] (Scheme 28). Accordingly,

Scheme 28



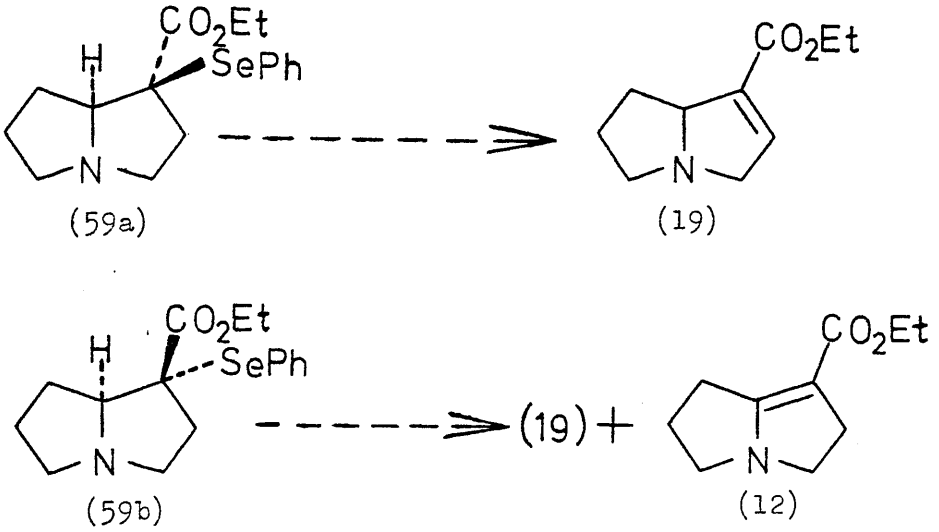
the thermodynamically less stable racemate (22) was prepared in 80% yield by catalytic hydrogenation of (7) over palladised charcoal. Completion of the reaction was indicated by loss of the u.v. chromophore, by the appearance of the saturated ester carbonyl band at 1,735 cm^{-1} in the i.r. spectrum, and by the loss of the olefinic proton in the n.m.r. spectrum. T.l.c. indicated the presence of only one racemate as observed previously.[19] This was characterised as its picrate.

In order to introduce unsaturation at the 1,2-position of (22), it was first necessary to introduce a good leaving group at the 1-position, α to the ester function. Subsequent elimination of HX would then result in generation of unsaturation at either 1,2 or 1,8-positions. Various possibilities were considered for the group X (Scheme 28). With X = halogen or hydroxyl, it was expected that the elimination would take place to give entirely 1,8-unsaturation as was observed earlier by Kochetkov *et al.*[8] for the hydroxy group (Chapter 2.1). The use of X = phenylsulphenyl or phenylselenenyl was then considered. These groups should both be readily introduced under basic conditions. Elimination would then be achieved after oxidation to the phenylsulphoxide and phenylselenoxide respectively. The use of selenium in organic synthesis has developed rapidly over the past few years,[49,50] and in this case it was preferred over sulphur for several reasons. First

selenium (II) is oxidised somewhat more easily to Se (IV), but with more difficulty to Se (VI) than the corresponding sulphur oxidations. The oxidation of selenides to selenoxides is carried out with common oxidising agents (e.g. hydrogen peroxide, peracetic acid), whereas the oxidation of sulphides cleanly to sulphoxides requires selective reagents (typically sodium periodate) to avoid oxidation to sulphones. Secondly, selenium forms weaker σ bonds than sulphur, and so cleavage of the C-Se is more rapid than with the analogous C-S bond. Thus, alkyl selenoxides are known to undergo elimination about 1000 times faster than sulphoxides.[51,52] Selenoxide fragmentations usually occur at approximately 100 °C lower temperature than the corresponding sulphoxide eliminations. Furthermore, selenoxide fragmentations unlike those of sulphoxides, do not seem to be reversible.[52,53] Elimination of the phenylselenoxide group is known to proceed in a stereospecific syn-fashion.[49,54] It should be noted that two diastereomeric phenylselenides (59a) and (59b) could be produced on introduction of the phenylselenenyl group into the ester (22). syn-Elimination of the selenoxide derived from (59a) would produce only 1,2-unsaturated ester (19). With the diastereomeric selenoxide derived from (59b), syn-elimination might be expected to produce both 1,2- and 1,8-didehydropyrrolizidine esters (Scheme 35). However, it is

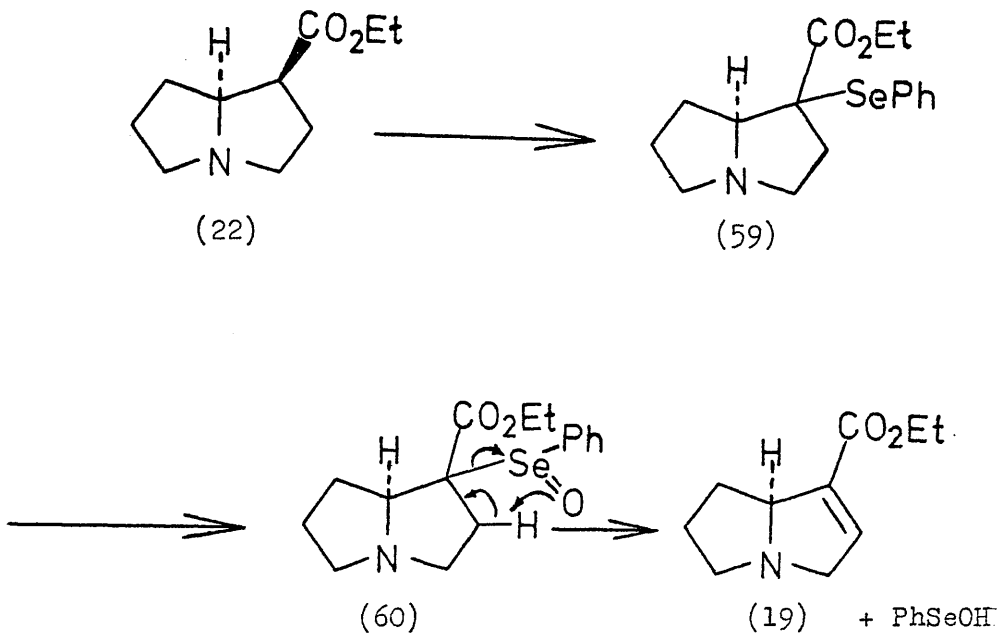
known that elimination of a phenylselenoxide group towards an electron-withdrawing group, such as nitrogen, is moderately disfavoured.[49] Therefore, there is a strong possibility that fragmentation of the selenoxides derived from (59a) or (59b) or a mixture of both will result predominantly in formation of the desired 1,2-unsaturation.

Scheme 35



Introduction of the chosen phenylseleno-group was readily accomplished by treating the lithium enolate derived from the ester (22) with phenylselenenyl chloride at - 78 °C (Scheme 36). Oxidation of this phenylselenenyl ester (59) to the selenoxide (60) was achieved with 27% hydrogen peroxide. The intermediates containing selenium (59) and (60) were not isolated in this sequence. syn-Elimination of the selenoxide (60) took place

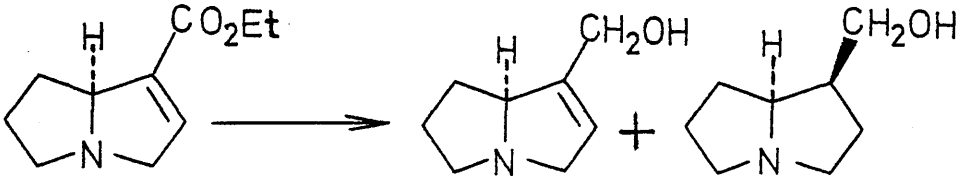
Scheme 36



at room temperature to produce a single compound (shown by g.l.c.), the anticipated 1,2-unsaturated pyrrolizidine ester (19), which was characterised as its picrate. The overall yield for the sequence in Scheme 36 was 60%. The free base had the expected bands in the i.r. spectrum at 1,720 and 1,640 cm^{-1} due to the $\alpha\beta$-unsaturated ester. The key feature in the n.m.r. spectrum was the presence of an olefinic proton multiplet at δ 6.58. However, there was no chromophore in the u.v. spectrum above 220 nm which would be expected for the isomeric 1,8-unsaturation. Indeed, no evidence (u.v. or n.m.r.) for the formation of any 1,8-unsaturated compounds in the reaction mixture could be obtained.

Finally, the 1,2-unsaturated ester (19) was converted into (±)-supinidine (1) using lithium aluminium hydride (Scheme 37). Some reduction of the double bond was also observed as noted previously by Tufariello and Tette.[13]

Scheme 37



(19) (1) (9)

These workers separated their mixture of alcohols by preparative g.l.c. A better procedure was found to be separation by preparative t.l.c. Development of the chromatogram in chloroform - methanol - ammonia (5:4:1) gave two components. The less polar major component, R_f 0.55 (45% yield) was (±)-supinidine (1), characterised as its picrate. The i.r., n.m.r., and mass spectra of (±)-supinidine were in accord with published data.[13,55] Furthermore, the i.r. and n.m.r. spectra of (±)-supinidine picrate were identical with those of a sample of (-)-supinidine picrate, supplied by Dr C.C.J. Culvenor (Figures (10) and (11)). The second component, R_f 0.30 (31% yield) was found to be (±)-isoretronecanol (9), also

Figure 10

I.r. Spectra of Supinidine Picrates (KBr disc)

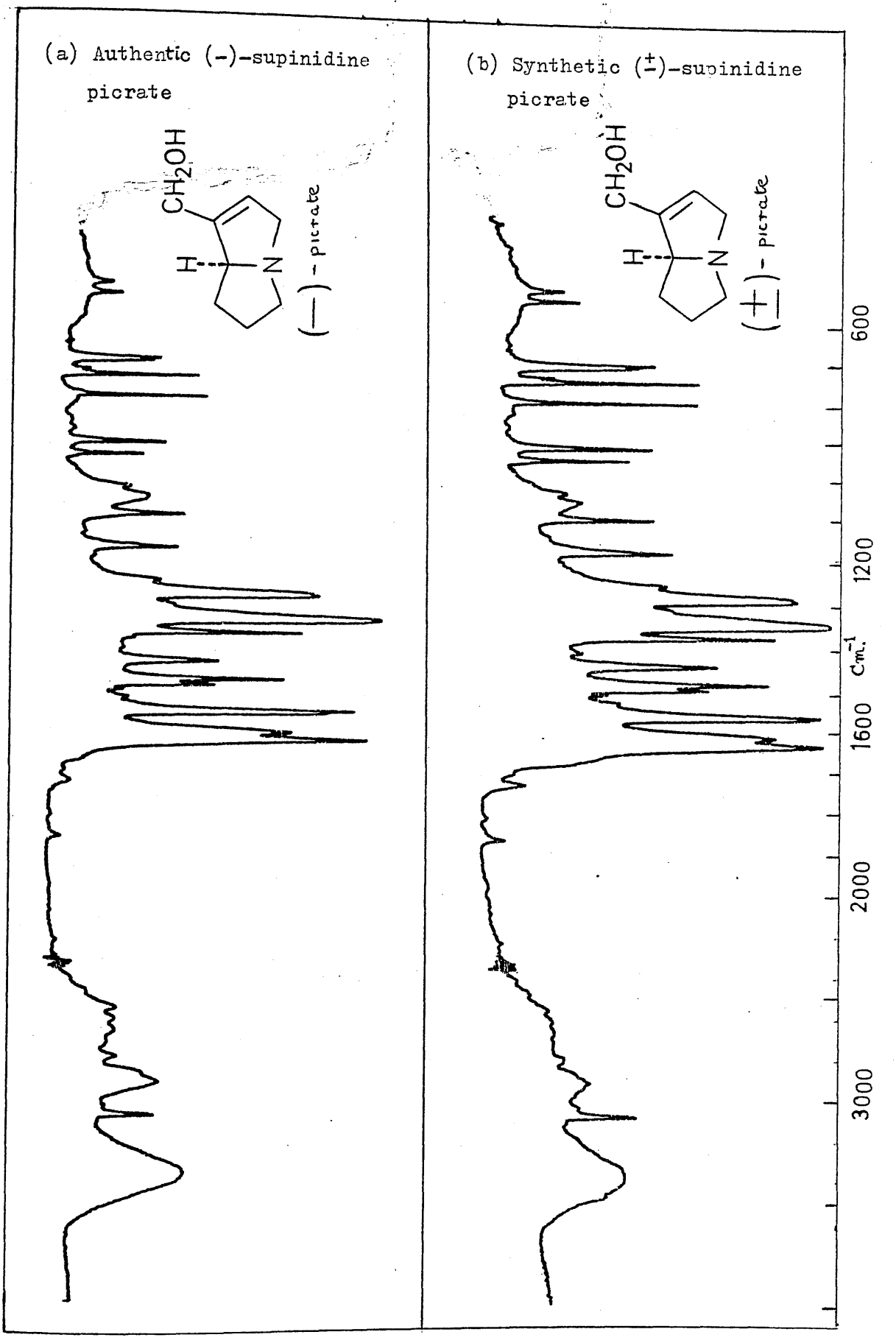


Figure 11(a)

N.m.r. Spectrum of

Authentic (-)-Supinidine

Picrate in $CDCl_3$

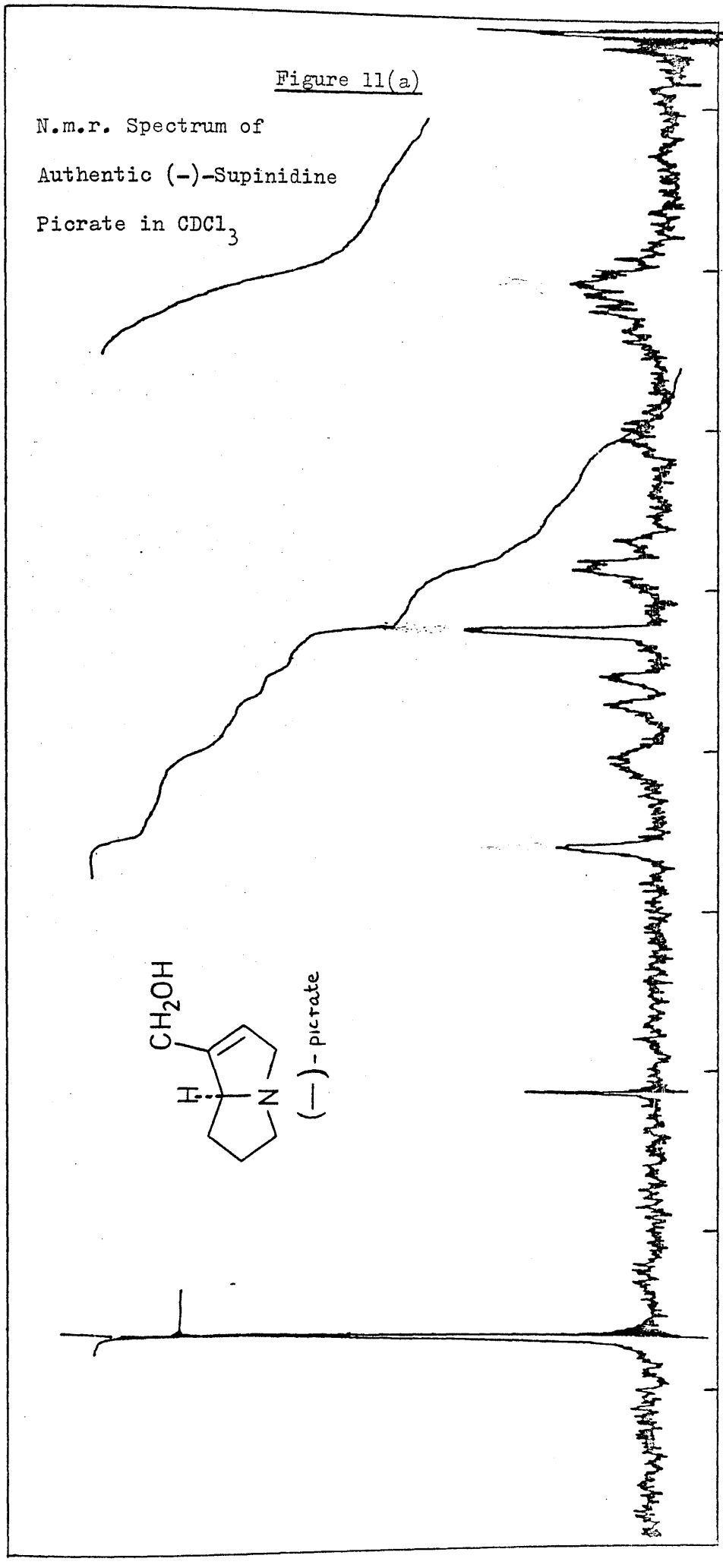
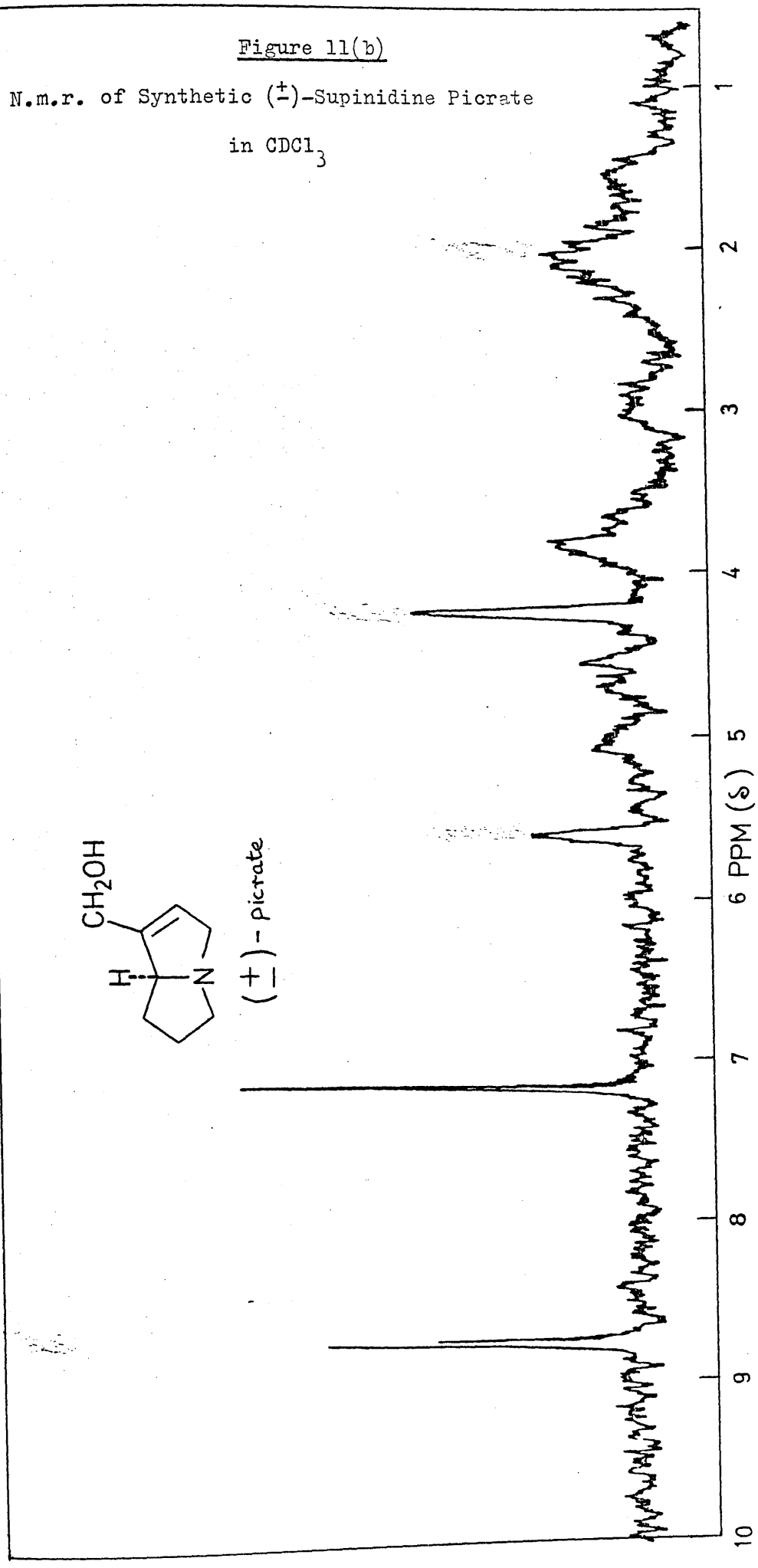


Figure 11(b)

N.m.r. of Synthetic (±)-Supinidine Picrate

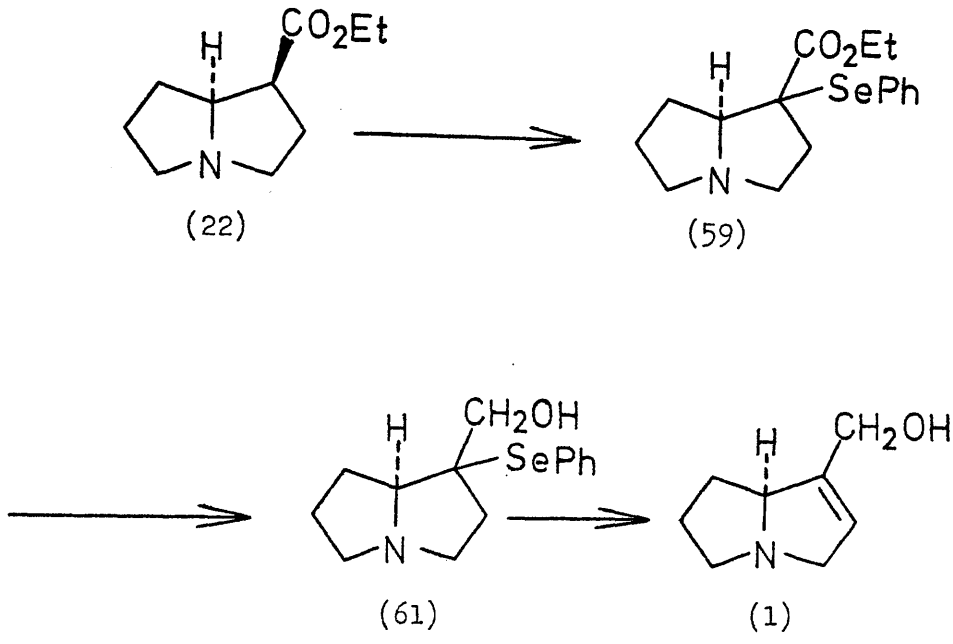
in $CDCl_3$



characterised as its picrate. I.r., n.m.r., and mass spectra of the free base were in accord with reported values for (±)-isoretronecanol.[19,56] It should be noted that higher yields of (±)-isoretronecanol (9) (ca. 70% overall from N-formyl-L-proline) can be obtained by lithium aluminium hydride reduction of the saturated ester (22).[19]

The partial reduction of the double-bond in the ester (19) on treatment with lithium aluminium hydride is a serious disadvantage to this procedure. A means of solving this problem was suggested by the work of Leonard-Coppens and Krief,[57] who showed that phenylseleno-compounds containing an α-carbonyl group can be reduced to β-hydroxyselenides by sodium borohydride or lithium aluminium hydride under mild conditions. Accordingly, phenylseleneylation of the lithium enolate derived from the ester (22) was again carried out, but this time the intermediate phenylseleno-ester (59) was isolated as an oil by preparative t.l.c. in 57% yield, and characterised as its picrate. The free base showed a saturated ester carbonyl band in the i.r. spectrum at 1,720 cm^{-1}. Reduction of the phenylseleno-ester (59) with lithium aluminium hydride took place smoothly to give the phenylseleno-alcohol (61) (Scheme 38), which was purified by preparative t.l.c. (62% yield) and crystallisation. The i.r. spectrum showed OH stretching of the alcohol at 3,400 cm^{-1} and no carbonyl band was observed. Fragmentation of the selenoxide derived from (61) gave (±)-supinidine (1),

Scheme 38



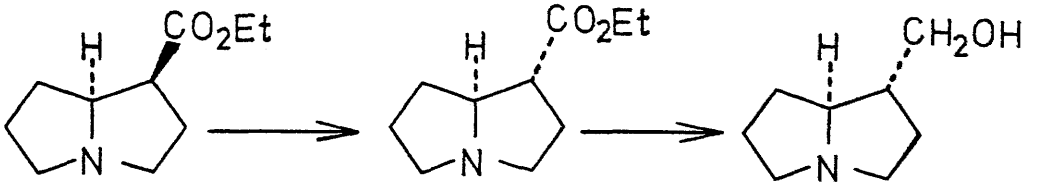
purified by preparative t.l.c. (59% yield). The i.r., n.m.r., and mass spectra were again in accord with published values.[13,55]

The stereochemistry of the intermediate selenide (59) is uncertain. It is possible that addition of the phenylselenenyl group takes place on the less hindered exo-face of the intermediate lithium enolate of the ester (22) to produce the seleno-ester (59b). As discussed earlier, both seleno-esters (59a) and (59b) should yield predominantly 1,2-unsaturated compounds as was in fact observed.

3.6 Synthesis of (±)-Trachelanthamidine (10)

Finally, to complete this Chapter, the diastereomeric alcohol of (±)-isoretronecanol (9) was prepared by established methods. The thermodynamically less stable racemic ester (22) was epimerised[58] with sodium ethoxide in ethanol to give the exo-isomer (62) (Scheme 39). Lithium aluminium hydride reduction of the ester (62) yielded (±)-trachelanthamidine (10), in an overall yield from N-formyl-L-proline of 51%. The i.r., n.m.r., and mass spectra of the free base were consistent with reported values,[56,59] and (±)-trachelanthamidine was characterised as its picrate.

Scheme 39

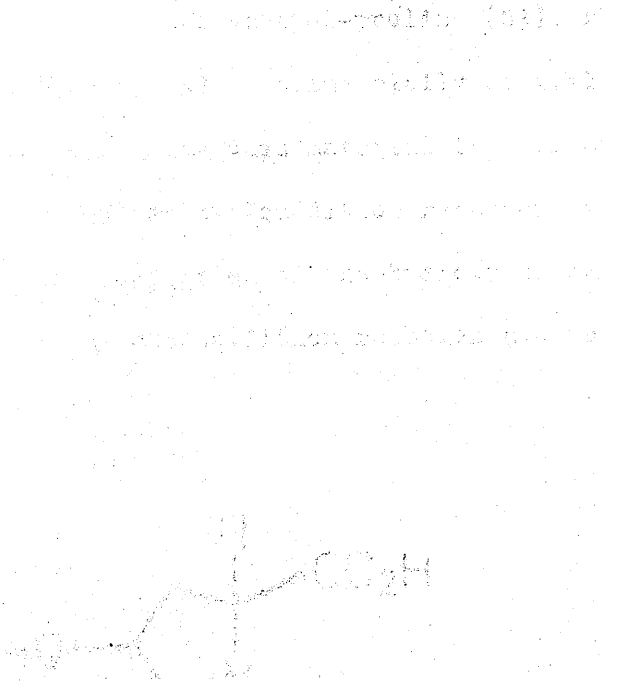


(22) (62) (10)

3.7 Conclusion

The saturated pyrrolizidine ester ethyl (±)-pyrrolizidine-1-endo-carboxylate (22) was readily prepared by regiospecific 1,3-dipolar cycloaddition of ethyl propiolate to N-formyl-L-

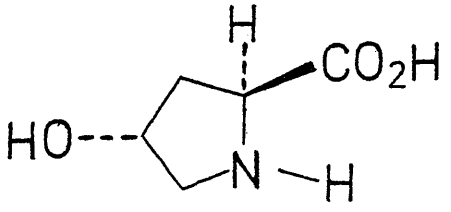
proline followed by stereospecific catalytic hydrogenation. A novel method for the conversion of this saturated ester into its 1,2-didehydro-derivatives has been established. The unsaturation is introduced by phenylselenenylation α to the ester function, followed by syn-elimination of the derived selenoxide. Using this procedure, $(\pm)$-supinidine has been synthesised in ca. 20% overall yield.



CHAPTER 4 SYNTHESIS OF OPTICALLY ACTIVE PYRROLIZIDINE BASES

4.1 Introduction

In view of the successful synthesis of (±)-supinidine (1) described in Chapter 3, it was decided to try to extend this strategy to the synthesis of a more complex necine base, such as retronecine (2). This would require the use of 3-hydroxy-proline in the cycloaddition reaction. As this compound was not readily available in any of its isomeric forms, attention was first focused on (-)-4-hydroxy-L-proline (63), which is a constituent of collagen, and is commercially available in one optically active form. With this material the effect of the hydroxy substituent on the cycloaddition reaction was investigated. The possibilty that elimination of the hydroxy or acyloxy group would occur during the cycloaddition reaction had to be considered.

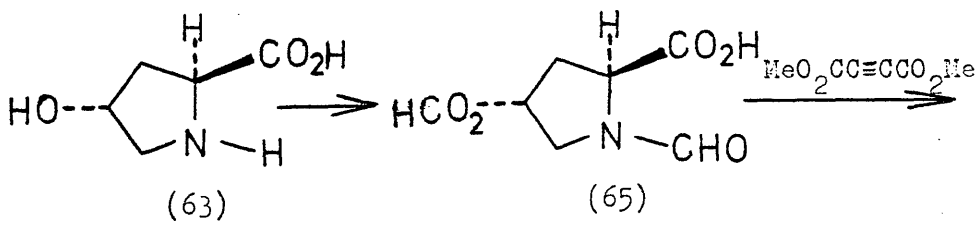


(63)

4.2 Synthesis of Dimethyl (+)-2*d*-Hydroxy-2,3-dihydro-1H-pyrrolizidine-6,7-dicarboxylate (66)

In order to see if the 1,3-dipolar cycloaddition still takes place with an hydroxy substituent on the proline ring, the N,O-diformyl derivative (65) of 4-hydroxy-L-proline was heated with dimethyl acetylenedicarboxylate in acetic anhydride (Scheme 40). One major optically active product was obtained in 87% yield. The expected structure (64) was confirmed on the basis of the spectral data. The u.v. chromophore at 260 nm (ε 12,800) is similar to that observed for the pyrrole diester (55). The n.m.r. spectrum of (64) was assigned as shown in Figure 12. There was no indication of any elimination process

Scheme 40



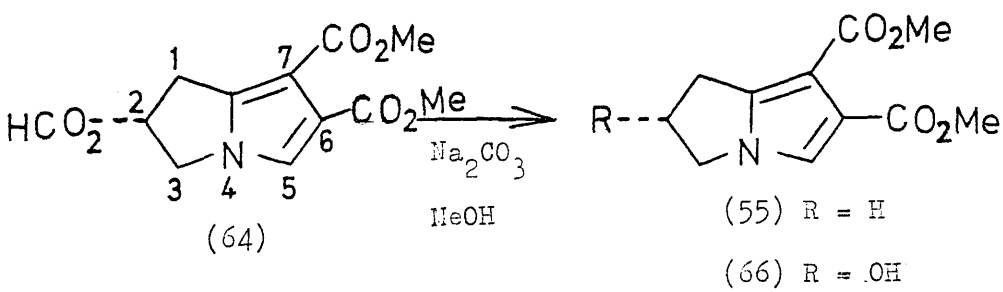
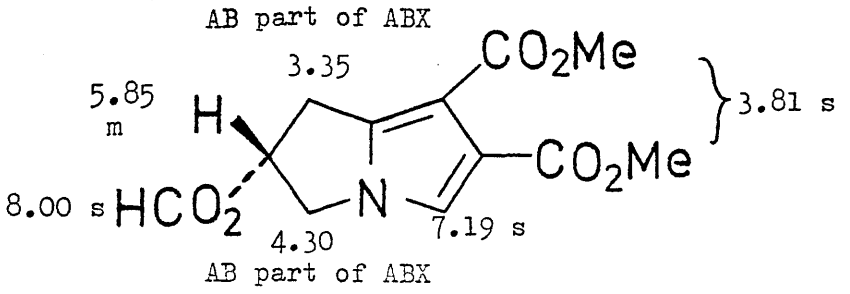


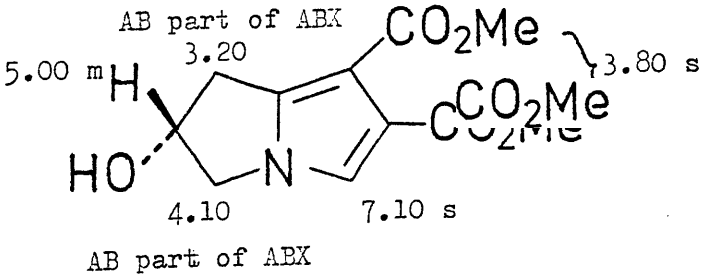
Figure 12



N.m.r. spectrum of (64) in $CDCl_3$ (δ values)

occurring during the cycloaddition leading to pyrrolizine derivatives. Removal of the O-formyl group under basic conditions (Scheme 40), gave a 94% yield of the optically active 2α-hydroxy compound (66). The u.v. spectrum of (66) was very similar to that of (64), and the assignments of the n.m.r. spectrum are shown in Figure 13.

Figure 13

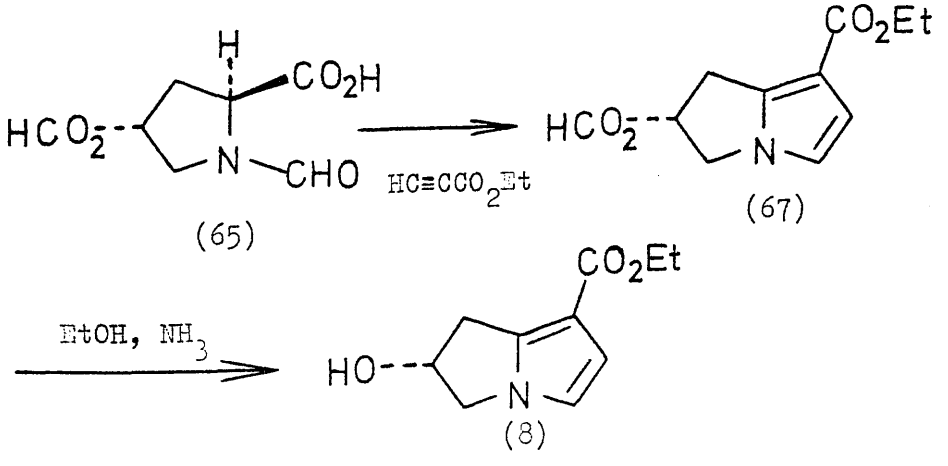


N.m.r. spectrum of (66) in $CDCl_3$ (δ values)

4.3 Synthesis and Hydrogenation of Ethyl (+)-2*d*-hydroxy-2,3-dihydro-1H-pyrrolizine-7-carboxylate (8)

In the previous section, the addition of a symmetrical dipolarophile to the 4-hydroxyproline derivative was discussed. It was anticipated that regiospecific cycloaddition of the unsymmetrical dipolarophile, ethyl propiolate, would be achieved in the same sense as described earlier (Chapters 2.2 and 3). Accordingly, the N,O-diformyl derivative (65) of 4-hydroxy-L-proline was treated with ethyl propiolate in acetic anhydride to produce one optically active major product as needles in 80% yield (Scheme 41). This was formulated as the 7-ester (67) because of the AB quartet in the n.m.r. spectrum (δ 6.60, 6.70, J 3 Hz) due to the adjacent pyrrolic protons. The rest of the spectral data were consistent with this structure (67).

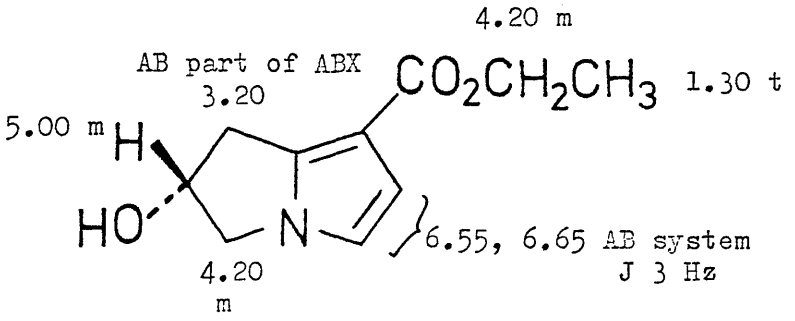
Scheme 41



(65) (67) (8)

Removal of the O-formyl group of (67) under basic conditions gave a quantitative yield of the optically active alcohol (8). This compound showed the presence of the hydroxyl group (3,350 cm^{-1}) and unsaturated ester (1,680 cm^{-1}) in its i.r. spectrum. The n.m.r. spectrum was assigned as shown in Figure 14. Thus, regiospecific 1,3-dipolar addition had been

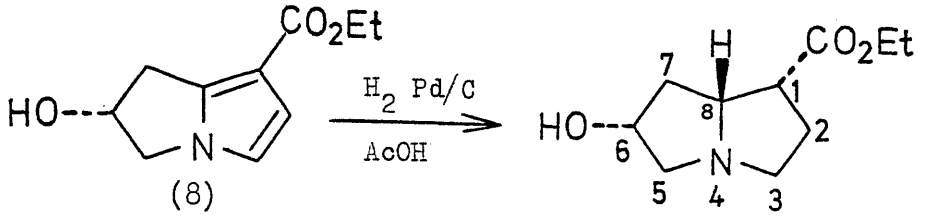
Figure 14



N.m.r. spectrum of (8) in $CDCl_3$ (δ values)

achieved and the optically active ester (8) was produced. Further utility of this compound depended upon its behaviour on catalytic hydrogenation. The completion of the hydrogenation reaction was monitored by loss of u.v. chromophore and the disappearance of the pyrrolic proton signals in the n.m.r. spectrum. A single optically active crystalline ester was formed in 80% yield. This was formulated as (68) (Scheme 42). The relative stereochemistry at C-1 and C-8 is determined by the stereospecific cis-addition of hydrogen and the absolute configuration was presumed to be as shown because of

Scheme 42



(8) (68)

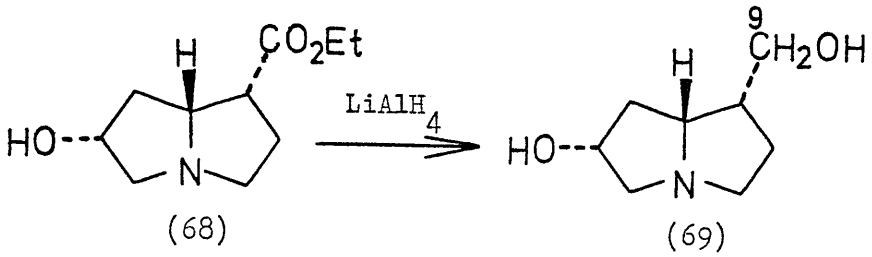
stereospecific addition of hydrogen from the sterically less hindered β face of the unsaturated ester (8). Proof of this stereochemical assignment was obtained by conversion of (68) into a number of known, naturally occurring, optically active necine bases (Chapter 4.5).

4.4 Synthesis of Novel Optically Active Necine Bases

The production of a single stereoisomer (68) in the preceding section was a most important result. Conversion of this compound into a number of novel necine bases was immediately feasible by established techniques.

Reduction of the saturated pyrrolizidine ester (68) with lithium aluminium hydride gave a new, optically active necine base in 89% yield, (+)-6α-hydroxy-1α-hydroxymethyl-3β-pyrrolizidine (69) (Scheme 43), characterised as its picrate. The free base showed the presence of an hydroxyl group in its

Scheme 43

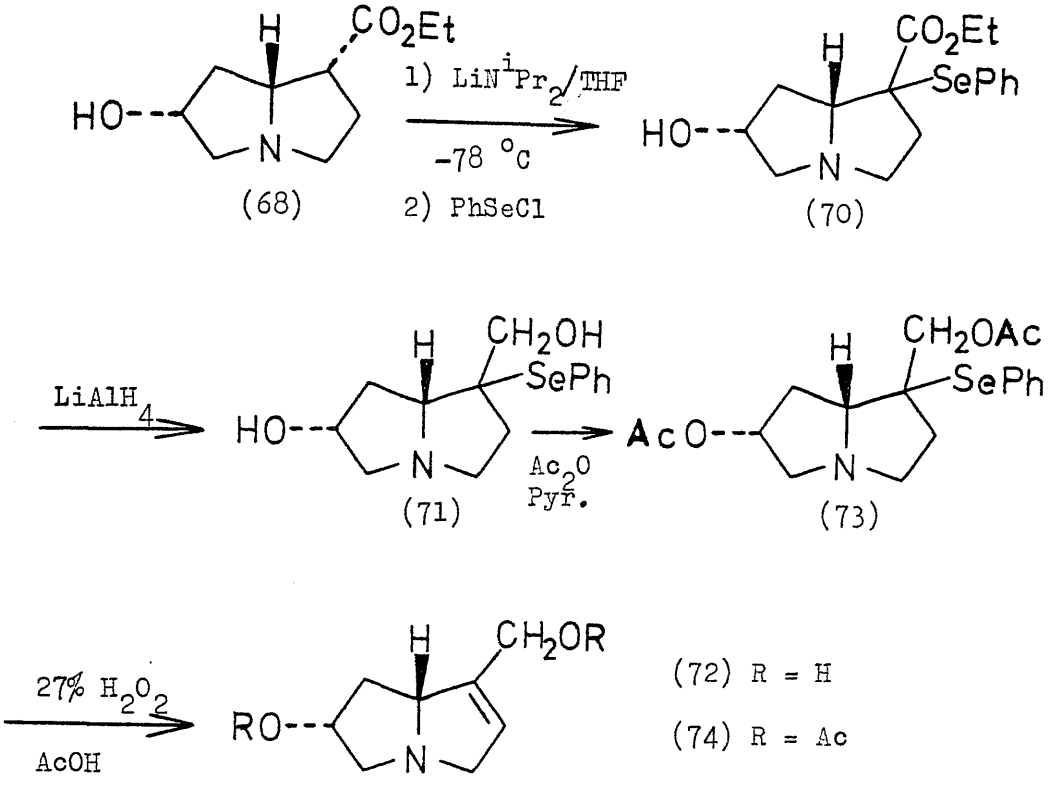


(68) (69)

i.r. spectrum (3,350 cm^{-1}). The n.m.r spectrum of (69) was complex, but a two proton doublet at δ 3.62 was attributable to the C-9 protons.

Conversion of the saturated ester (68) into its 1,2-unsaturated analogue was also feasible by the route discussed in Chapter 3. Phenylselenenylation of the lithium enolate derived from (68) was readily accomplished (Scheme 44). A single product (70) (t.l.c.) was formed in 60% yield. Reduction of this ester with lithium aluminium hydride gave the corresponding alcohol (71) as a crystalline solid in 57% yield, after preparative t.l.c. The i.r. spectrum showed the presence of the hydroxyl group (3,400 cm^{-1}) and absence of any carbonyl absorption. Oxidation of (71) to the selenoxide followed by selenoxide elimination would be expected to produce the allylic alcohol (72). Unfortunately this basic diol was extremely polar and it was not possible to isolate it from the reaction mixture. Therefore the phenylseleno-

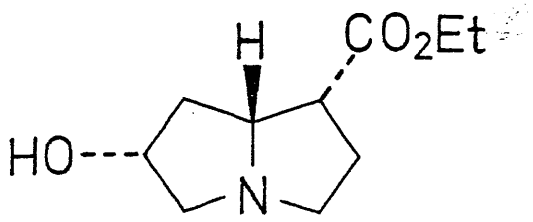
Scheme 44



alcohol (71) was acetylated with acetic anhydride to yield the diacetate (73) as an oil, which was not characterised. Oxidation of this acetate (73), followed by selenoxide elimination gave a single optically active oily product (74), which was characterised as its picrate. The free base showed an ester peak in the i.r. spectrum at 1,750 cm^{-1}. The main feature of the n.m.r. spectrum was the presence of an olefinic proton multiplet at δ 5.75 (H-2).

As with the synthesis of (±)-supinidine, the stereochemistry of the intermediate selenides (70) and (71) is not known (see discussion in Chapter 3.5). Again, no evidence was obtained for any 1,8-unsaturated compound in the selenoxide elimination process.

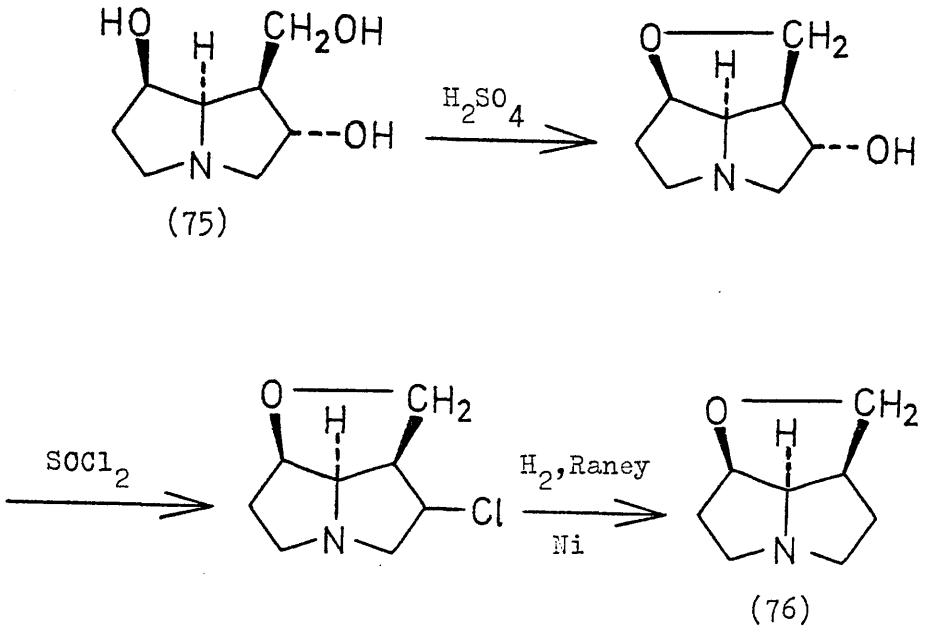
4.5 Synthesis of Natural Optically Active 8β-Pyrrolizidine Bases



(68)

In Section 4.3, the synthesis of the saturated pyrrolizidine ester (68) was described, and a provisional stereochemical assignment for this compound was made. It was known that in a previous stereochemical correlation, rosmarinecine (75) was converted into anhydroplatynecine (76). Removal of the 2-hydroxy group was achieved as shown in Scheme 45.[60] It was considered that analogous removal of the 6-hydroxy group in (68) would lead to an optically active ester, which would then be converted into one of the optically active 1-hydroxymethylpyrrolizidines. All four possible

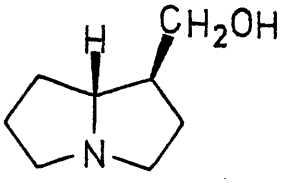
Scheme 45



stereoisomers are known compounds, and their absolute configurations[7] and reported optical rotations are shown in Figure 15. It should be noted that there is some uncertainty in the absolute values for the rotations listed in Figure 15, due in part to the fact that these bases are oily substances and accurate mea surement of the weight of base used in obtaining the optical rotation is difficult. Nevertheless the absolute configuration at C-1 and C-8 of (68) could be established by comparison with the known values of the optical rotations for these stereoisomers.

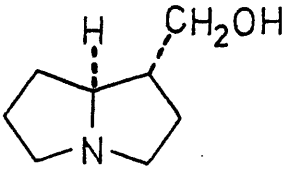
Accordingly, the hydroxypyrrolizidine ester (68) was converted into the 6-chloro compound (79) with thionyl chloride

Figure 15



(32) Laburnine

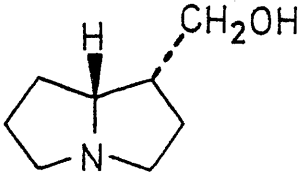
$[\alpha]_D$ + 15.4 ° 35



(77) Trachelanthamidine

$[\alpha]_D$ -13.8 ° 61

-12.94 ° 62

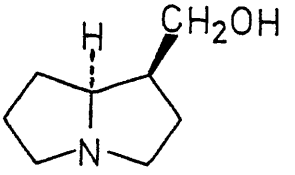


(76) Lindelofidine

or (+)-isoretronecanol

$[\alpha]_D$ + 79.1 ° 63

+ 71.7 ° 64



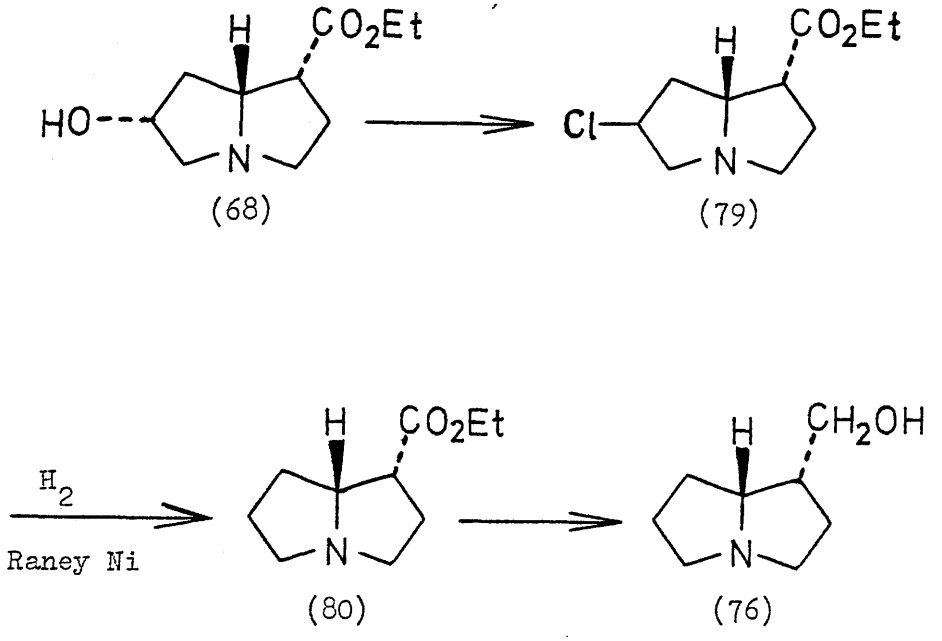
(78) Isoretronecanol

$[\alpha]_D$ - 78.2 ° 65

Stereoisomeric Forms of 1-hydroxymethylpyrrolizidine

in 90% yield (Scheme 46). The chlorine atom was removed by catalytic hydrogenation in 90% yield, and reduction of the ester (80) with lithium aluminium hydride gave a 93% yield of lindelofidine (or (+)-isoretronecanol) (76) $[\alpha]_D$ + 70.2 °, (c.f. Figure 15). The base was characterised as its picrate,

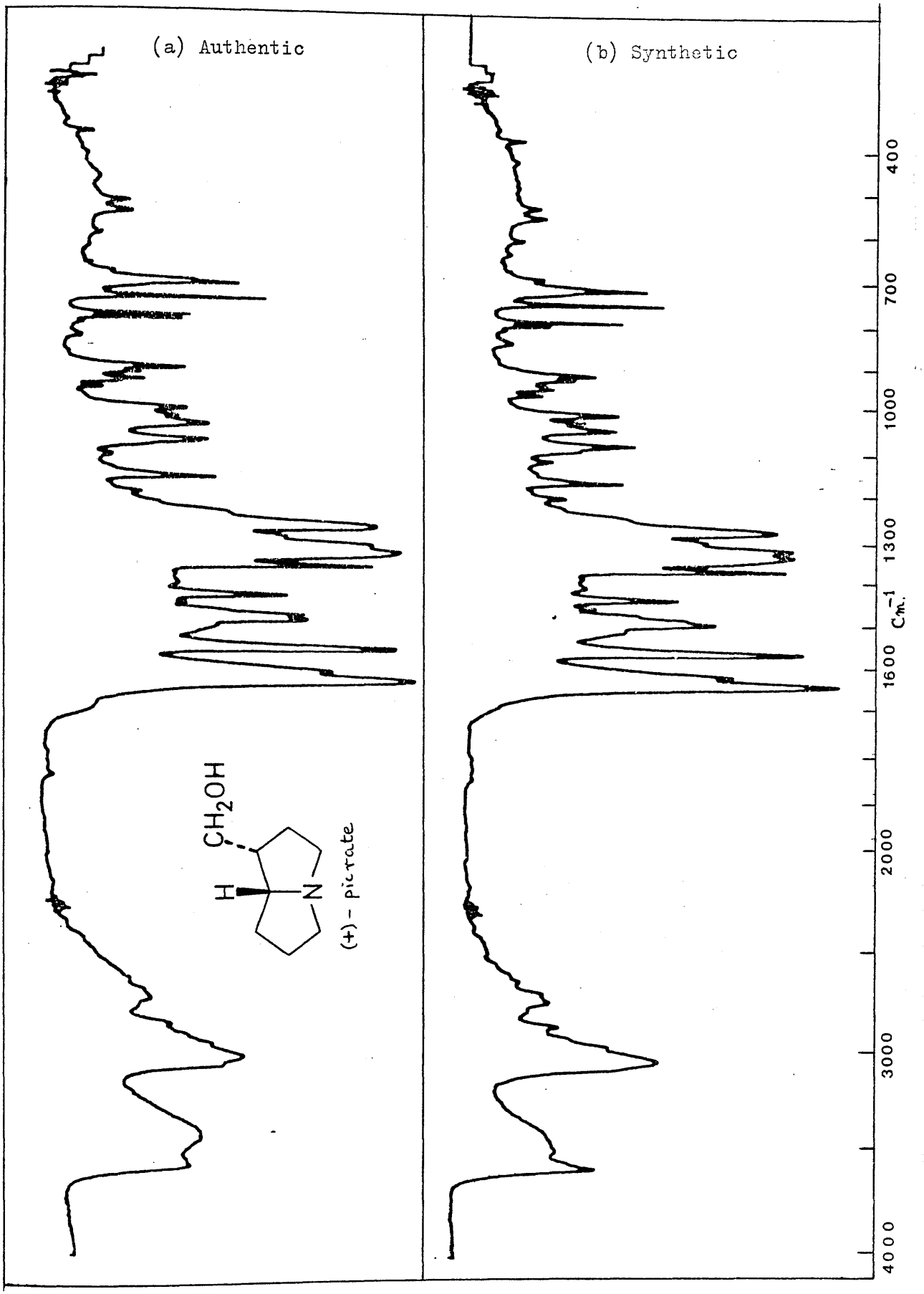
Scheme 46



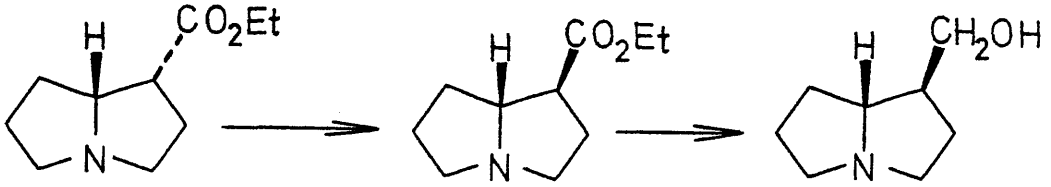
which had an identical i.r. spectrum (Figure 16) and undepressed mixed melting point with an authentic sample of lindelofidine supplied by Dr C.C.J. Culvenor. Thus, the absolute configuration of the novel necines (69) and (74) and the hydroxypyrrolizidine ester (68) is confirmed, and a total synthesis of an optically active necine base has been achieved in an overall yield of 45% from readily available starting materials.

The ester (80) is the thermodynamically less stable compound. Therefore, epimerisation[58] of this ester was carried out with sodium ethoxide in ethanol to give the diastereomeric ester

Figure 16 I.r. Spectra of Lindelofidine Picrates (KBr)



Scheme 47

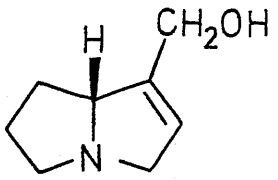


(80) (81) (32)

(81) in 68% yield (Scheme 47). Lithium aluminium hydride reduction of this ester (81) gave a 92% yield of laburnine (32), $[\alpha]_D^{22}$ + 14.6 ° (c.f. Figure 15). The oily base was again characterised as its picrate.

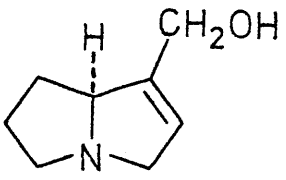
A final confirmation of the absolute configuration at C-8 of the ester (80) was possible by conversion of this ester into an optically active form of supinidine by the route dicussed in Chapter 3.5. Both enantiomers of supinidine are known compounds (Figure 17).

Figure 17



(82)

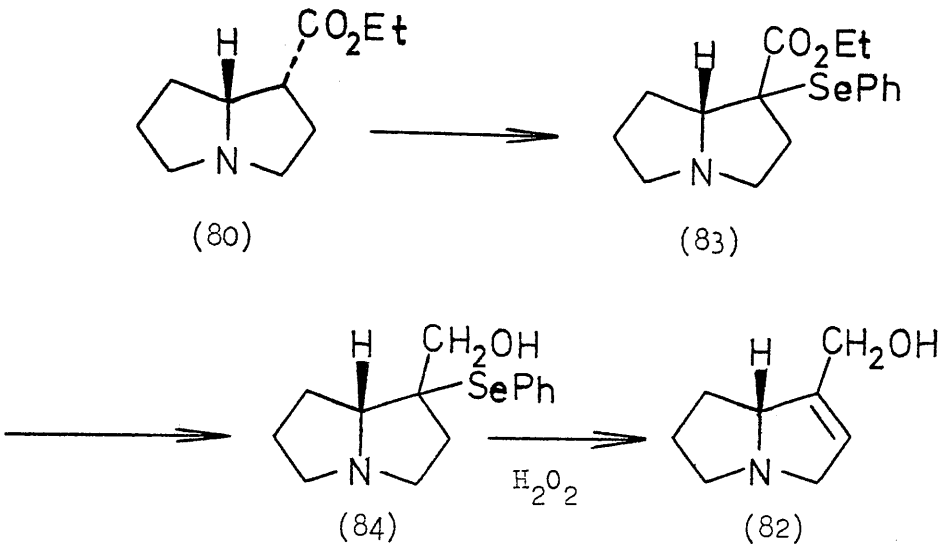
$[\alpha]_D^{20}$ + 9.2 ° 64



(36)

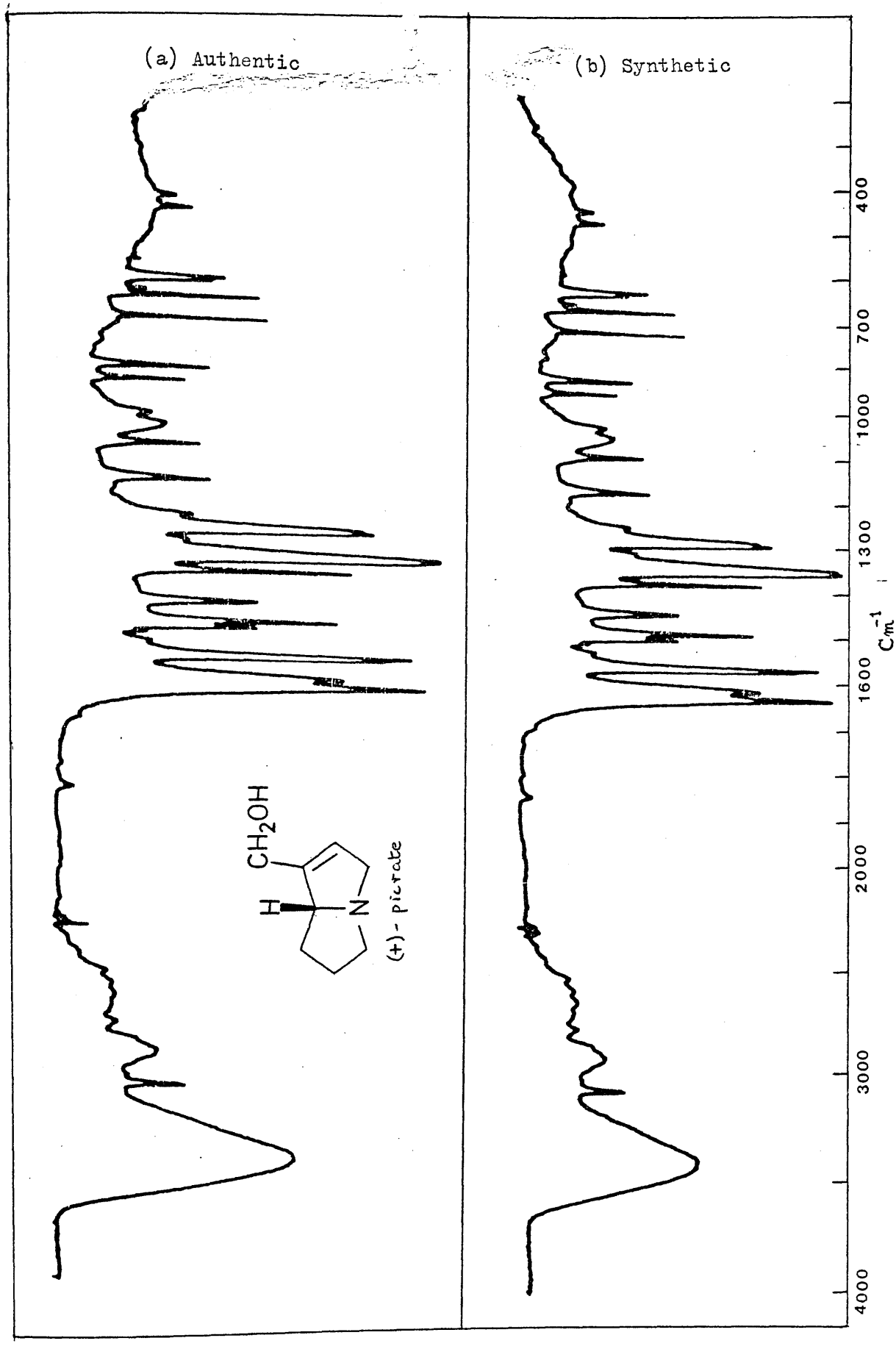
$[\alpha]_D$ - 9.45 ° 66

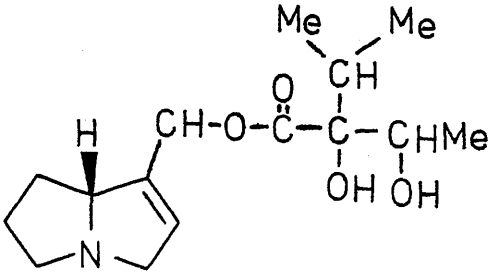
Scheme 48



Phenylselenenylation of the lithium enolate derived from optically active ester (80) gave the phenylselenide (83) in 67% yield (Scheme 48). Reduction of this ester (83) with lithium aluminium hydride gave the crystalline alcohol (84) in 61 % yield. Oxidation to the selenoxide with hydrogen peroxide followed by selenoxide fragmentation gave a 55% yield of supinidine (82) $[\alpha]_D$ + 7.6 ° (c f. Figure 17). (+)-Supinidine was characterised as its picrate, which had an identical i.r. spectrum (Figure 18) and mixed m.p. with an authentic sample of (+)-supinidine picrate supplied by Dr C.C.J. Culvenor. The spectral data for the intermediates in this sequence were in accord with those obtained for the racemic material, discussed in Chapter 3.5.

Figure 18 I.r. Spectra of (+)-Supinidine Picrates (KBr)





(84)

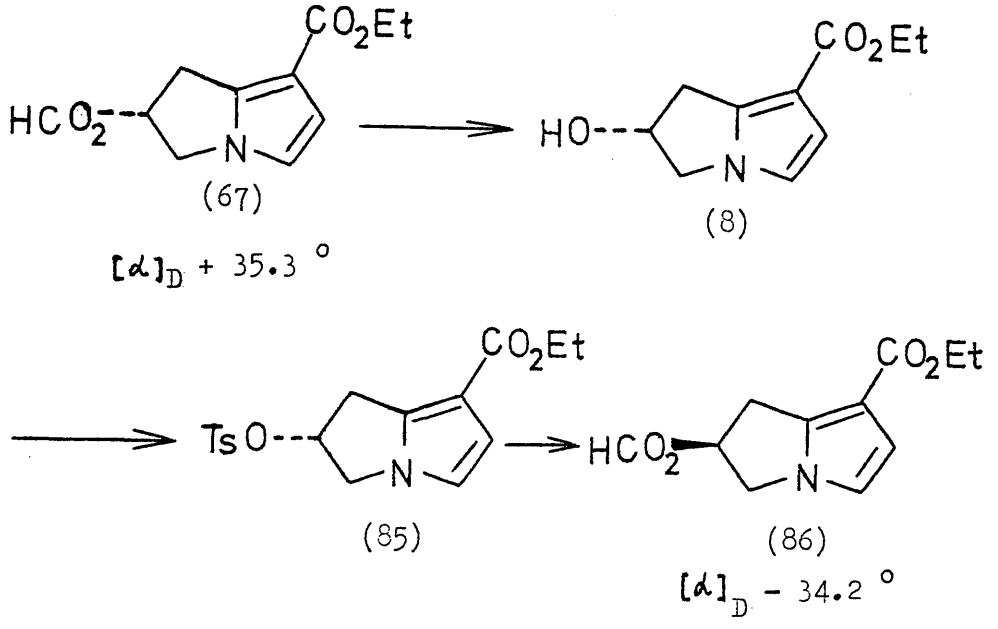
Thus the absolute configuration at C-8 throughout the sequences shown in Schemes 46-8 is confirmed as 8β. (+)-Supinidine was isolated by Culvenor and Smith[64] from cynaustine (84). This is the first occurrence of an allylic alcohol derived from 8β-pyrrolizidine.

Despite the difficulty in obtaining accurate values for the rotations of these oily bases, it is evident from a comparison of the values for the synthetic bases with those reported (Figures 15 and 17) that all the synthetic optically active bases were obtained with a high degree of optical purity (85-100%).

4.6 Synthesis of Natural Optically Active 8α-Pyrrolizidine Bases

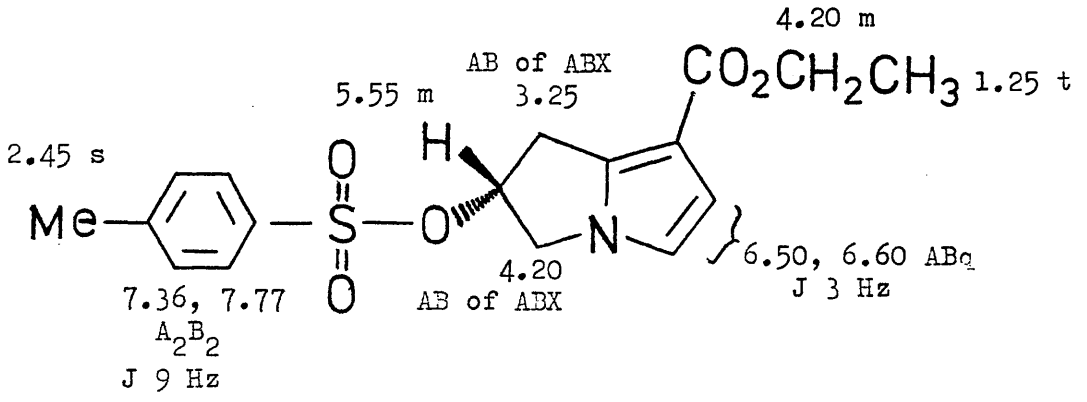
Since natural 4-hydroxy-L-proline (63) can be converted into its enantiomer by epimerisation of both chiral centres,[67] the routes described above could in principle be used to synthesise the 8α-pyrrolizidine bases, (-)-trachelanthamidine (77), (-)-isoretronecanol (78), and (-)-supinidine (36). However,

Scheme 49



inversion of two chiral centres is a lengthy procedure, and a better approach to the synthesis of these 8α-pyrrolizidines was sought. At one stage in the synthesis of 8β-pyrrolizidines an intermediate (8) with only one chiral centre is produced. Therefore, epimerisation of the 2α-hydroxy substituent of this ester (8) was carried out. Treatment of the (+)-ester (8) with p-toluenesulphonyl chloride in pyridine gave the (+)-tosylate (85)(Scheme 49), in 91% yield. The assignments for the n.m.r. spectrum of (85) are shown in Figure 19. Displacement of the tosylate anion by formate anion in an S_N 2 reaction with inversion of stereochemistry was achieved in 84% yield with tetraethylammonium formate in dry acetone. The formate ester (86) had $[\alpha]_D^{23}$ - 34.2 ° compared to the rotation of $[\alpha]_D^{18}$ + 35.3 ° obtained from the product of 1,3-dipolar cycloaddition of

Figure 19



N.m.r. spectrum of (85) (δ values)

ethyl propiolate with the N,O-diformyl derivative of 4-hydroxy-L-proline. Thus complete inversion of stereochemistry has been obtained in 76% overall yield by this simple procedure. Further treatment of the ester (86) as described in Chapter 4.3 and 4.5 will yield the 8*a*-pyrrolizidines, (-)-trachelanthamidine (77), (-)-isoretronecanol (78), and (-)-supinidine (36).

4.7 Conclusion

The use of natural 4-hydroxy-L-proline as a model compound in the 1,3-dipolar cycloaddition reaction with ethyl propiolate has led to the formation of two new optically active necine bases. Confirmation of the absolute configuration of these two bases was achieved by removal of one chiral centre, and conversion to known naturally occurring 8β-pyrrolizidine

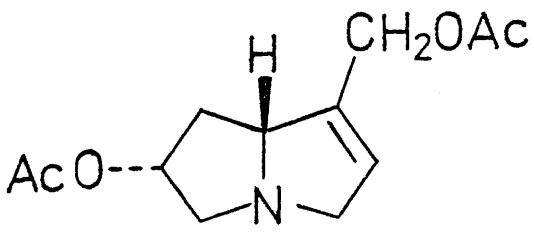
bases, (+)-lindelofidine(76), (+)-laburnine (32) and (+)-supinidine (82). Furthermore, a procedure has been described for the preparation of the corresponding 8a-pyrrolizidines, (-)-trachelanthamdine (77), (-)-isoretronecanol (78), and (-)-supinidine (36).

CHAPTER 5 ATTEMPTED SYNTHESIS OF (±)-RETRONECINE

5.1 Introduction

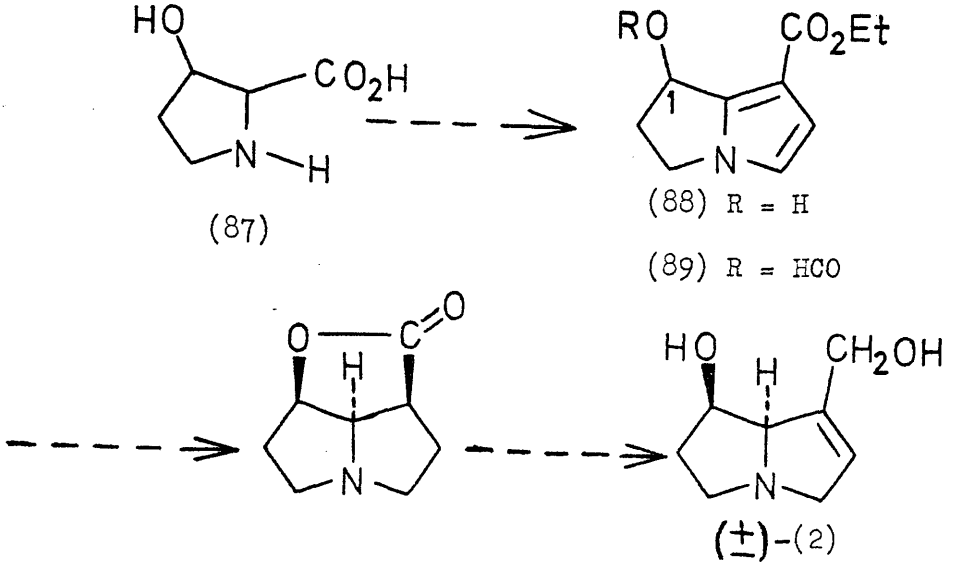
Most of the naturally occurring pyrrolizidine macrocyclic diester alkaloids contain retronecine (2) as the base portion. None of these macrocyclic alkaloids has yet been synthesised. Part of this work involved attempts to synthesise one of these macrocyclic diesters (Chapter 6), and therefore a supply of retronecine (2) was required. Since the amount of natural retronecine which can be obtained from plants is limited, and the existing synthesis of retronecine involves a lengthy procedure with low overall yield (Chapter 2.1), it was decided to investigate new synthetic routes to retronecine.

The successful utilisation of 4-hydroxy-L-proline in the synthesis of the diacetate (74) of the novel 1,2-unsaturated pyrrolizidine base (Chapter 4.4) suggested that the use of 3-hydroxyproline (87) in an analogous manner should be investigated (Scheme 50). It was realised that the presence



(74)

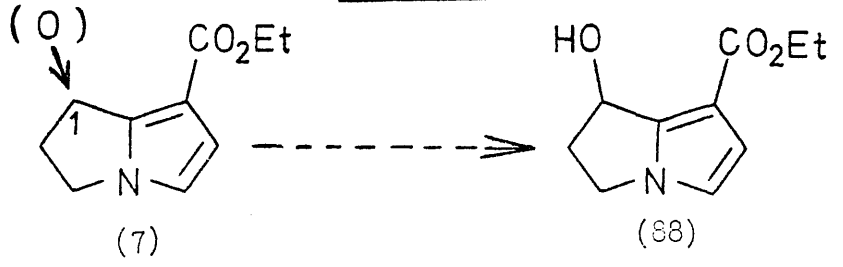
Scheme 50



of hydroxy (88) or formyloxy (89) substituents at the 1-position of the desired dihydropyrrolizine ester might lead to unwanted elimination processes from this activated 'benzylic' position. In that event, introduction of a suitable protecting group for the hydroxy function in 3-hydroxyproline could be investigated (Chapter 5.2 and 5.3).

An alternative approach to the desired intermediate (88) involved the known ester (7). It was anticipated that use could be made of the activated 1-position to attempt to introduce an oxygen function in this position (Scheme 51) (Chapter 5.4).

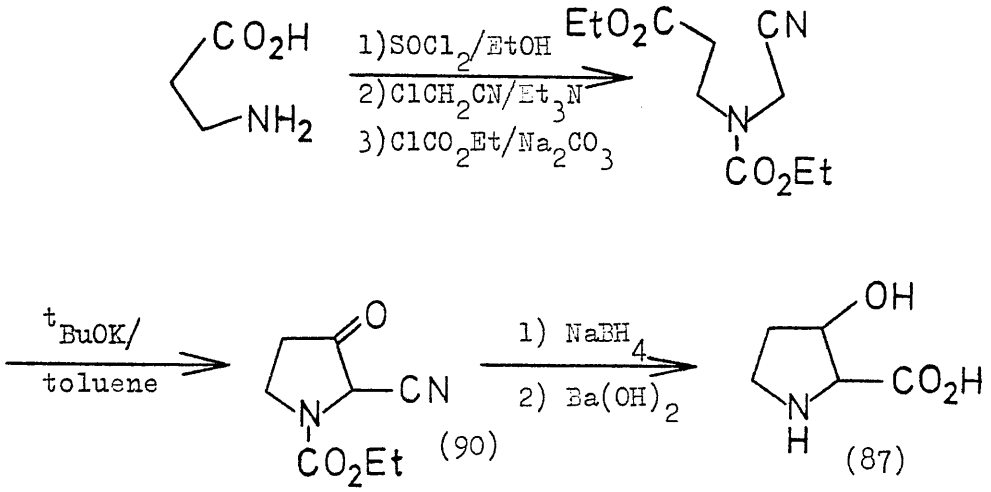
Scheme 51



5.2 Synthesis of 3-Hydroxyprolines

A mixture of the racemic cis- and trans-3-hydroxyprolines (87) were prepared by a modification of the route used by Blake et al.[68] in low overall yield (10%) (Scheme 52).

Scheme 52



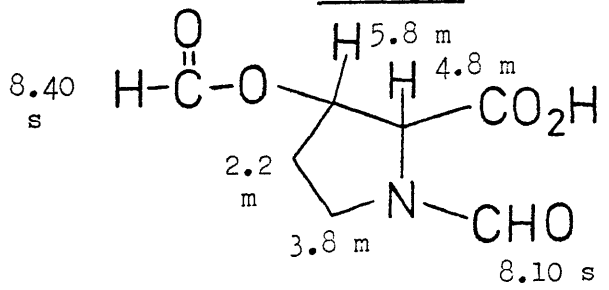
The pyrrolidone (90) was obtained as an extremely viscous oil and was difficult to purify by distillation as reported by Blake et al.[68] Therefore the crude product was reduced with sodium borohydride to the corresponding alcohol. Purification by distillation achieved by Blake et al. was again difficult due to the high boiling point and decomposition of the product, and so the crude product was hydrolysed with barium hydroxide to give a mixture of cis-and trans-3-hydroxy-proline (87). This mixture was purified by ion exchange resins and crystallisation from aqueous ethanol.

The n.m.r. spectrum of the mixture of geometric isomers was in accord with the values reported for the separate isomers.[69] It was not considered necessary to separate this mixture because preparation of the hydroxy ester (88) from this mixture would produce only one racemic compound.

5.3 Attempted Synthesis of Ethyl 1-Hydroxy-2,3-dihydro-1H-pyrrolizine-7-carboxylate (88)

The mixture of cis- and trans-3-hydroxyproline (87) was converted into its N,O-diformyl derivatives (91) with formic acid and acetic anhydride at room temperature. The formylated products did not crystallise. The assignments for the n.m.r. spectrum of this mixture are shown in Figure 20.

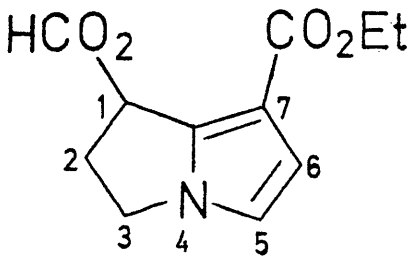
Figure 20



N.m.r. spectrum of cis and trans N-formyl-3-formyloxyproline (91) in $CDCl_3$ (δ values)

The 1,3-dipolar cycloaddition of ethyl propiolate to the mixture of cis-and trans-N-formyl-3-formyloxyproline (91) in

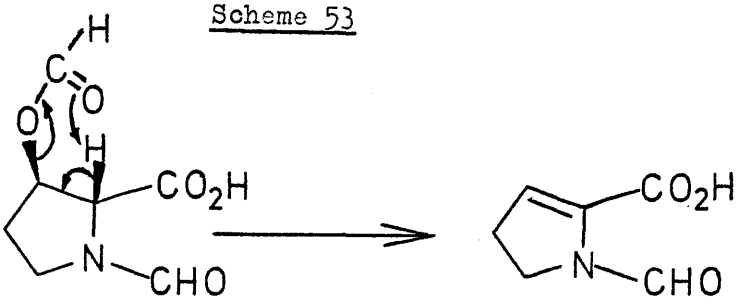
acetic anhydride was investigated. The reaction mixture was heated at various temperatures for different lengths of time (See Experimental Section). Mixtures of products were obtained. Examination of the n.m.r. spectra of the crude products indicated that no dihydropyrrolizine (89) had been formed,



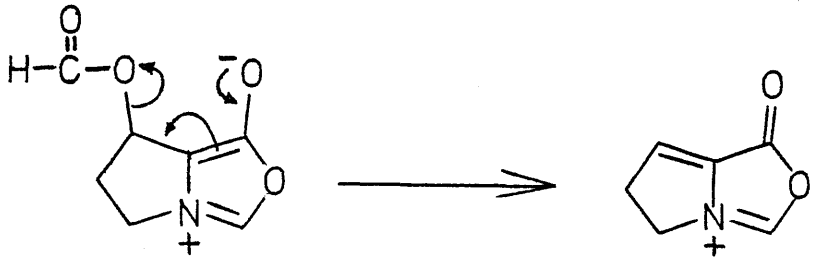
(89)

since the easily recognisable AB quartet due to the pyrrole hydrogens at C-5 and C-6 was always absent. Furthermore, no formyloxy proton was ever observed, suggesting that elimination of formic acid has occurred. Two possibilities for elimination of this formyloxy group can be considered. Firstly, intramolecular elimination of formic acid from trans-N-formyl-3-formyloxyproline (Scheme 53), or secondly, elimination of formate anion from the intermediate azomethine ylide generated by acetic anhydride (Scheme 54) could have occurred. The second possibility is considered more likely in this case. The next logical step was to prepare a range of 3-hydroxyprolines with protecting groups on the hydroxy function and investigate their behaviour in the cycloaddition reaction. Unfortunately, lack of time prevented any progress in this direction.

Scheme 53

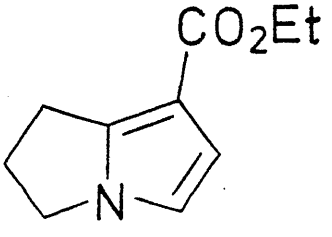


Scheme 54



5.4 Attempts to introduce Oxygen Substituents at the 1-Position of 2,3-Dihydro-1H-pyrrolizine Derivatives

The dihydropyrrolizine ester (7) is readily available from the 1,3-dipolar cycloaddition of ethyl propiolate with the azomethine ylide derived from N-formyl-L-proline (Chapter 3.3).



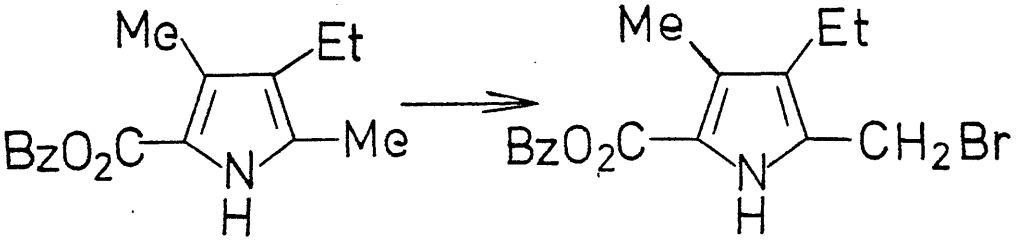
(7)

It was hoped that the 1-position of the ester (7) would show the reactivity of a benzylic methylene group. Several methods for introduction of the 1-hydroxy group were attempted.

(a) Via Halogenation

Ellis et al.[70] reported that bromination of the substituted pyrrole (92) could be achieved at one of the 'benzylic' positions with bromine in dry ether (Scheme 55). They also found that the

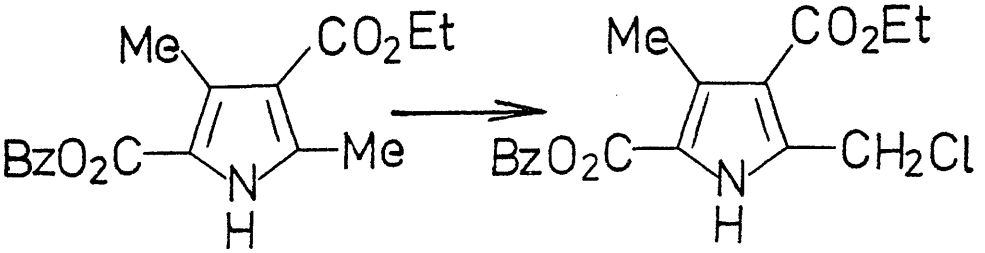
Scheme 55



(92) (93)

related pyrrole (94) could not be brominated under the same conditions, but treatment of (94) with sulphuryl chloride in acetic acid did produce the 5-chloromethyl compound (95) (Scheme 56).

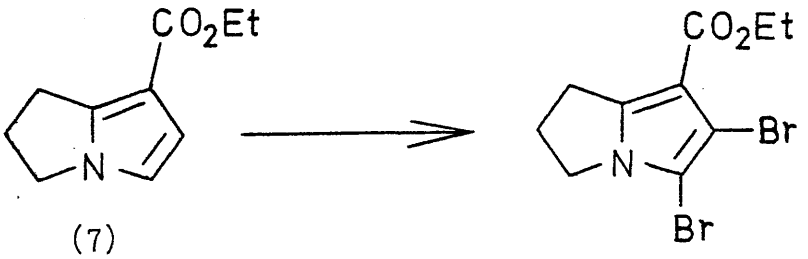
Scheme 56



(94) (95)

Preliminary experiments on the bromination of the ester (7) were carried out with N-bromosuccinimide(NBS) in the presence of benzoyl peroxide. It was found that bromination occurred readily in the aromatic portion of the molecule, and that the amount of NBS used was critical. With one equivalent of NBS, a monobromoester was formed, while with two equivalents of NBS the 5,6-dibromoester (96) resulted (Scheme 57).

Scheme 57



(7) (96)

The use of three equivalents of NBS produced a mixture of products which could not be identified. The dibromoester (96) was crystallised and its structure established by analysis and spectral data. The n.m.r. spectrum showed no pyrrole protons present. The usual triplets for the C-1 and C-3 protons were observed at δ 3.20 and 4.00, respectively, together with a multiplet at δ 2.50 due to the C-2 protons. The facile bromination of the pyrrole ring produced a fully substituted pyrrole derivative which was then used for further studies, since it was anticipated that the two bromine atoms could be readily removed by catalytic hydrogenation after introduction of the 1-substituent.

Attempted bromination or chlorination of the dibromoester (96) with NBS or sulphuryl chloride, respectively, produced a complex mixture of products. This may be due to the instability of the expected halogenated products, since it has been reported that the halogenated pyrroles (93) and (95) are unstable.[70]

(b) Oxidation

The alternative approach to produce 1-hydroxy substituents is to try to introduce oxygen directly into the ester (7) or the dibromoester (96). Treatment of the dibromoester (96) with lead tetra-acetate gave a mixture with two main components, which were separated by preparative t.l.c. Both products were unstable and could not be characterised.

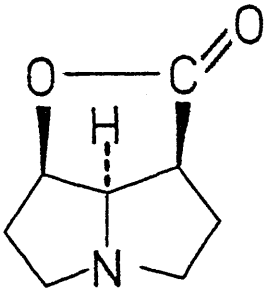
Finally, attempted oxidation of the ester (7) with selenium dioxide and chromium dioxide-pyridine complex[71] gave mainly unchanged starting material. Lack of time prevented further development of this approach.

5.5 Conclusion

The N,O-diformyl derivatives (91) of *cis*-and *trans*-3-hydroxyproline were prepared. Attempts to carry out the cycloaddition reaction with these compounds failed, presumably due to elimination of the formyloxy group. Further progress with this synthetic route depends on the finding of a suitable protecting group for the hydroxy function.

Bromination of the dihydropyrrolizine ester (7) occurred preferentially on the pyrrole ring. Attempts to introduce halogen or oxygen functions into the dibromoester (96) did not produce stable, identifiable products. Further work in this direction would be desirable.

Finally, the lactone (97) required in this synthetic scheme to retronecine (Scheme 50) has been synthesised by Viscontini and co-workers[72]. This could be prepared and then conversion to retronecine could be attempted by the steps established for the synthesis of (±)-supinidine (Chapter 3.5)

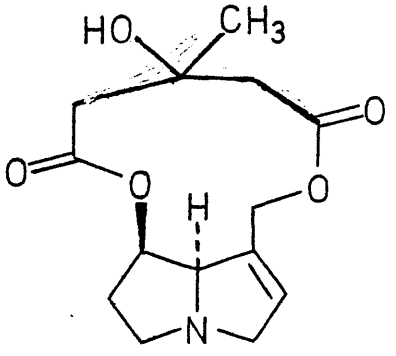


(97)

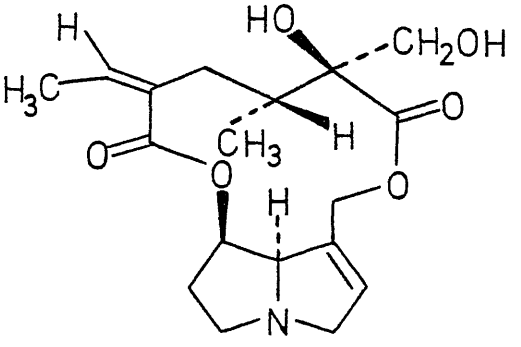
CHAPTER 6 SYNTHESIS OF A MACROCYCLIC PYRROLIZIDINE DIESTER

6.1 Introduction

Approximately one hundred macrocyclic pyrrolizidine diesters have been isolated and characterised.[2,3] Most of these macrocyclic alkaloids contain (+)-retronecine (40) as the base portion, and they can be divided into two main structural groups; those with an 11-membered macro-ring as in dicrotaline (3), and those with a 12-membered macro-ring as in retrorsine (4). None of these macrocyclic diester alkaloids has yet been reconstructed from the constituent necine and dicarboxylic acid. A number of problems are apparent. Many of the dicarboxylic acids are highly substituted and contain a number of chiral centres.



(3)

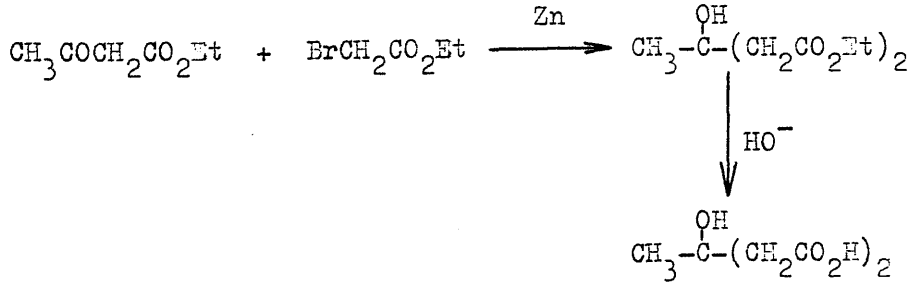


(4)

Thus stereospecific synthesis of these acids, followed by resolution is required before construction of the macro-ring can be attempted. Furthermore, in forming the macro-ring from the necine and the necic acid, there are two possible modes of attachment of the dicarboxylic acid to the dihydroxy-necine, and so mixtures of diastereoisomers are likely to be produced on formation of the macrocyclic ring.

It was therefore decided to attempt the synthesis of dicrotaline (3), which is the simplest 11-membered macrocyclic pyrrolizidine diester alkaloid. Dicrotaline was isolated by Marais in 1944,[73] and the structure of dicrotalic acid (98) was proved by the synthesis of Adams and van Duuren (Scheme 58).[74]

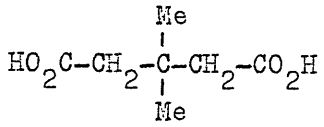
Scheme 58



(98)

Reconstruction of dicrotaline from natural (+)-retronecine (40) and synthetic dicrotalic acid (98) would constitute a total synthesis since (+)-retronecine has been synthesised by Geissman

and Waiss (Chapter 2.1). Dicrotalic acid has no chiral centres, and is simple to prepare. However, formation of the macro-ring would presumably lead to a mixture of C-13 epimers; one of which would be dicrotaline. The absolute configuration at C-13 of dicrotaline is not known. Attempts to synthesise dicrotaline are discussed in Chapter 6.2.



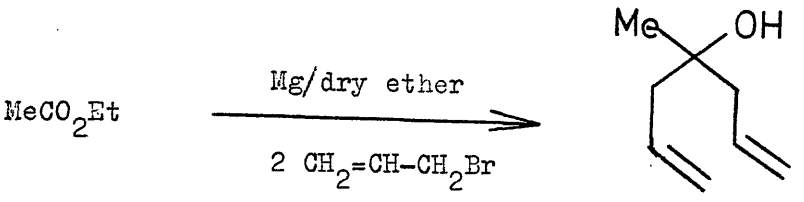
(99)

The production of diastereomers on formation of the macro-ring is avoided if a symmetrical dicarboxylic acid such as 3,3-dimethyl-glutaric acid (99) is employed. The successful reconstruction of an 11-membered macrocyclic diester from (+)-retronecine and (99) is described in Chapter 6.3.

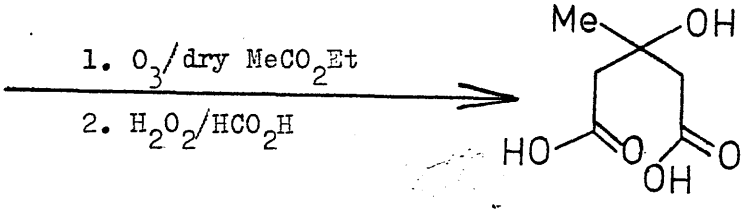
6.2 Attempted Synthesis of Dicrotaline

A supply of (+)-retronecine (40) was available from hydrolysis of the pyrrolizidine alkaloid retrorsine (4), obtained by extraction of Senecio isatideus plants. Dicrotalic acid (98) was synthesised as shown in Scheme 59 by a modification of the

Scheme 59



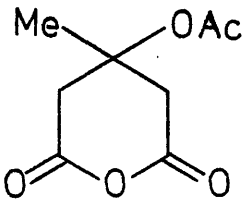
(100)



(98)

route outlined by Klosterman and Smith.[75] Grignard reaction of ethyl acetate with two equivalents of allyl magnesium bromide gave the alcohol (100) in 70% yield. Oxonolysis and oxidation of the dialdehyde produced yielded dicrotalic acid (98) in quantitative yield.

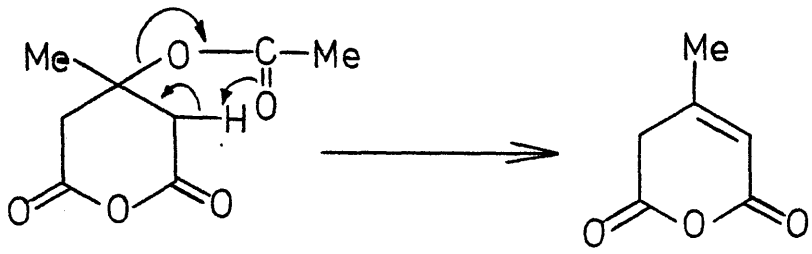
For esterification of dicrotalic acid (98) to (+)-retronecine



(101)

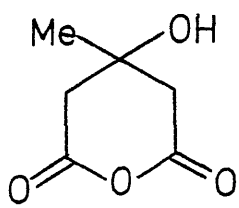
(40) it was decided to use the anhydride of dicrotalic acid, because of difficulties encountered with established methods (see later). However, treatment of dicrotalic acid with acetic anhydride produced the O-acetyl derivative (101) of dicrotalic anhydride. The i.r. spectrum of (101) showed two carbonyl absorptions at 1,810 and 1,760 cm^{-1} due to the anhydride. The ester carbonyl band also occurs at 1760 cm^{-1}. In the n.m.r. spectrum of (101), the methylene protons are non-equivalent giving rise to an AB quartet at δ 3.50 and 2.80 ppm (J 18 Hz). The lowfield proton is deshielded by the adjacent carbonyl of the ester group. The O-acetyl derivative (101) was not considered suitable for use in the synthesis of dicrotaline, because of the possible difficulty in removing the acetyl group after formation of the alkaloid. Furthermore, Adams and van Duuren had shown[74] that the acetate (101) readily lost a molecule of acetic acid on heating at 100 °C for 12 hours. (Scheme 60).

Scheme 60



(101)

To avoid these problems of removal of the acetyl group and possible elimination of acetic acid, a new method for the synthesis of dicrotalic anhydride was devised. Treatment of dicrotalic acid (98) with excess thionyl chloride in dry ether at room temperature overnight gave the crystalline anhydride (102) in 89% yield. The i.r. spectrum of (102) showed hydroxyl

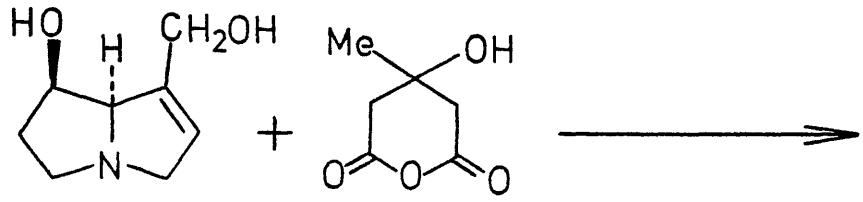


(102)

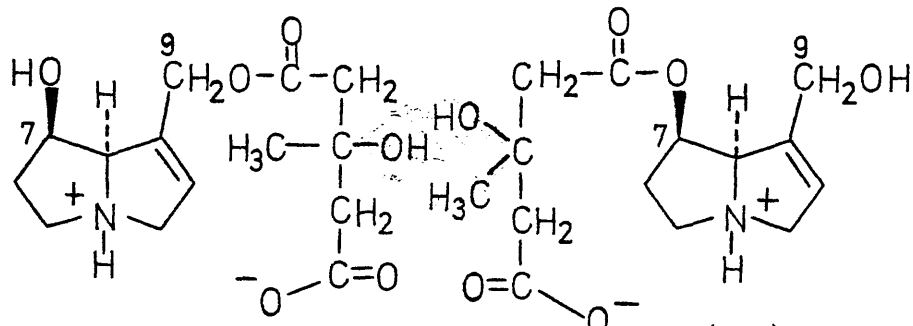
absorption at 3,550 cm^{-1} and the two carbonyl bands at 1,810 and 1,750 cm^{-1}. In the n.m.r. spectrum, the methylene protons appeared as a singlet at δ 2.97 ppm.

The first procedure investigated for the formation of a monoester between (+)-retronecine and dicrotalic acid was the acylimidazole method discussed in Chapter 2.3. The acylimidazole derived from dicrotalic acid and N,N'-carbonyldi-imidazole was reacted with (+)-retronecine, and small scale reactions were followed by t.l.c. Under various conditions, (See Experimental Section) mixtures of products were obtained, and these could not be separated by t.l.c. Because of this disappointing result, the reaction of (+)-retronecine with an equimolar amount of dicrotalic anhydride was studied. The course of the reaction was monitored by n.m.r. spectroscopy using D_6-acetone as solvent. In fact, after one hour, most of the n.m.r. signals had disappeared and a white precipitate had formed. This insoluble product was washed with acetone to remove unreacted reagents and was formulated as a mixture[43] of the 7-monoester (103) and the 9-monoester (104) of retronecine present as zwitterions (Scheme 61), from the n.m.r. spectrum taken in D_4-methanol. The C-7 monoester (103) showed multiplets at δ 4.60 (H-7) and 5.72 (H-2) ppm, while the C-9 monoester (104) displayed a singlet at δ 4.80 (H-9), and a multiplet at 5.85 (H-2) ppm. From the integrations of these signals, the ratio of C-7 to C-9 monoester is 1:2. Methods for the intramolecular acylation of this mixture were then investigated. Treatment of the mixture (103) and (104) with three molar equivalents of thionyl chloride at 5 °C gave a

Scheme 61



(40)



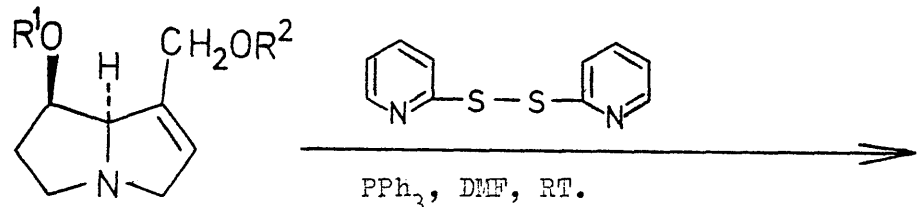
(104) (103)

low yield of a mixture of six components which could be extracted with chloroform from the basified reaction mixture. None of these products could be identified.

A highly efficient method for internal esterification of hydroxyacids has been developed by Corey and Nicolaou.[76] This method utilises a dipyridyl disulphide and triphenylphosphine in a non-polar solvent, under high dilution conditions. Unfortunately, the mixture of C-7 and C-9 monoesters (103) and (104) would not dissolve in any non-polar solvent such as benzene, chlorobenzene, toluene, xylene, carbon tetrachloride, nor in the more polar methylene chloride, chloroform, acetone or acetonitrile.

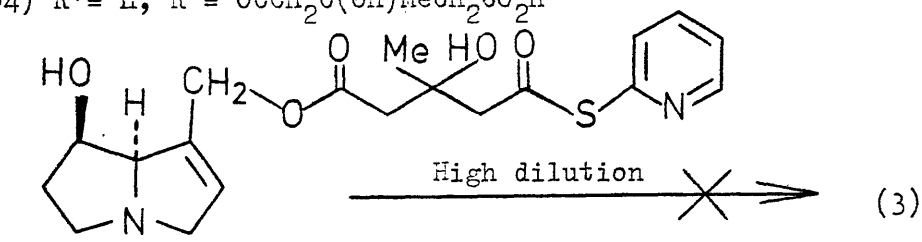
Finally, it was found that dimethylformamide is a suitable solvent. The mixture of C-7 and C-9 monoesters (103) and (104) were converted to their 2-pyridinethiol esters in concentrated dimethylformamide solution (Scheme 62). Lactonisation was then

Scheme 62

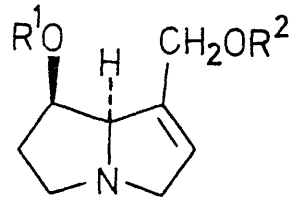


(103) R' = $OCCH_2C(OH)MeCH_2CO_2H$, R^2 = H

(104) R' = H, R^2 = $OCCH_2C(OH)MeCH_2CO_2H$



+ C-7 ester



(105) R^1 = $COCH{=}CMe_2$, R^2 = H

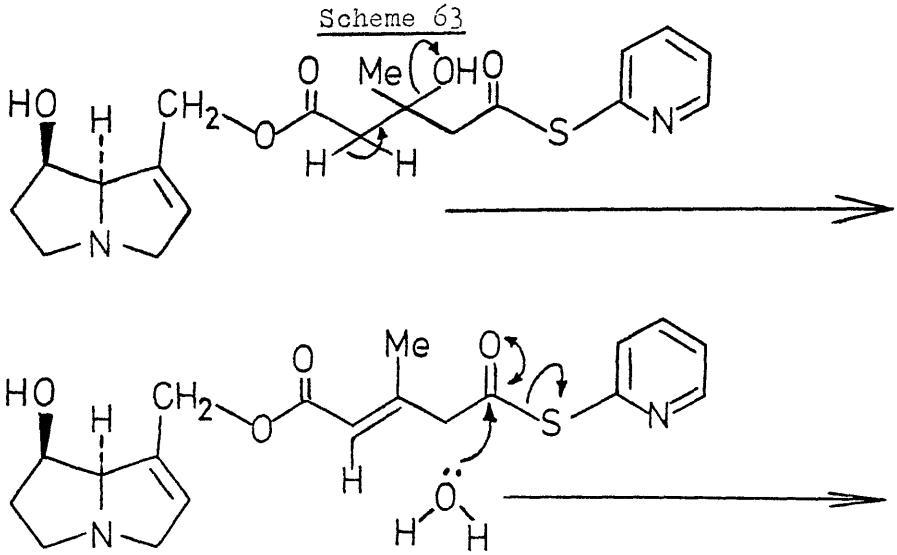
(106) R^1 = H, R^2 = $COCH{=}CMe_2$

attempted by refluxing this mixture in dry dimethylformamide under high dilution conditions. Extraction of the reaction mixture with chloroform from basic solution gave one major component which was purified by preparative t.l.c. This was

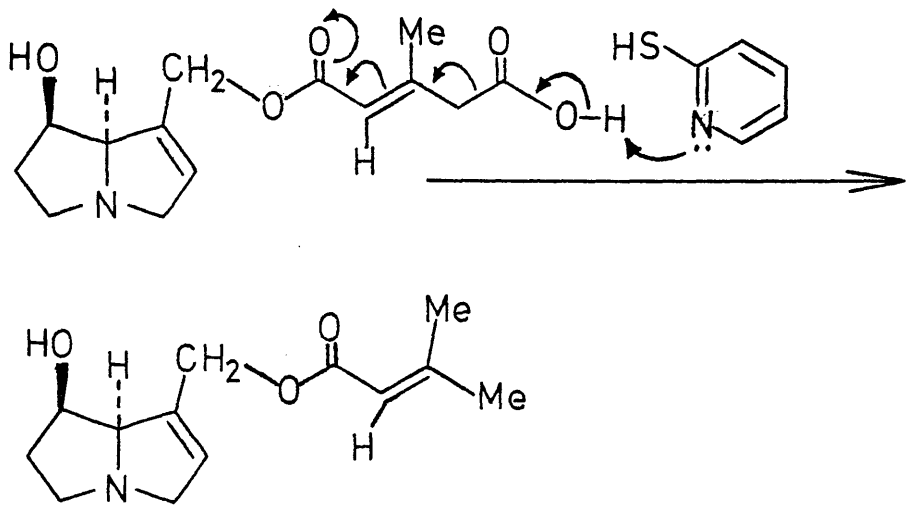
shown to be a mixture of the C-7 monoester (105) and C-9 monoester (106) on the basis of spectral data. A molecular ion at m/e 237 was observed in the mass spectrum, corresponding to loss of carbon dioxide and water from the starting monoesters. The i.r. spectrum indicated an α,β-unsaturated ester with absorption at 1,700 and 1,650 cm^{-1}. The u.v. spectrum confirmed this assignment with $\lambda_{max.}$ 220 nm (ε 10,900). The n.m.r. spectrum showed two methyl singlets at δ 1.90 and 2.18 ppm. The C-7 monoester (105) gave rise to multiplets at δ 5.70 (H-2) and 5.35 (H-7), and the C-9 monoester (106) showed multiplets at δ 5.82 (H-2) and 4.70 (H-9) ppm. From the integration of these signals, the ratio of C-7 to C-9 monoester is again 1:2.

The formation of the senecioyl esters (105) and (106) is readily explained by dehydration and decarboxylation of the monoesters. A possible mechanism (on the C-9 monoester) is shown in Scheme 63.

Scheme 63



Scheme 63 (Continued)



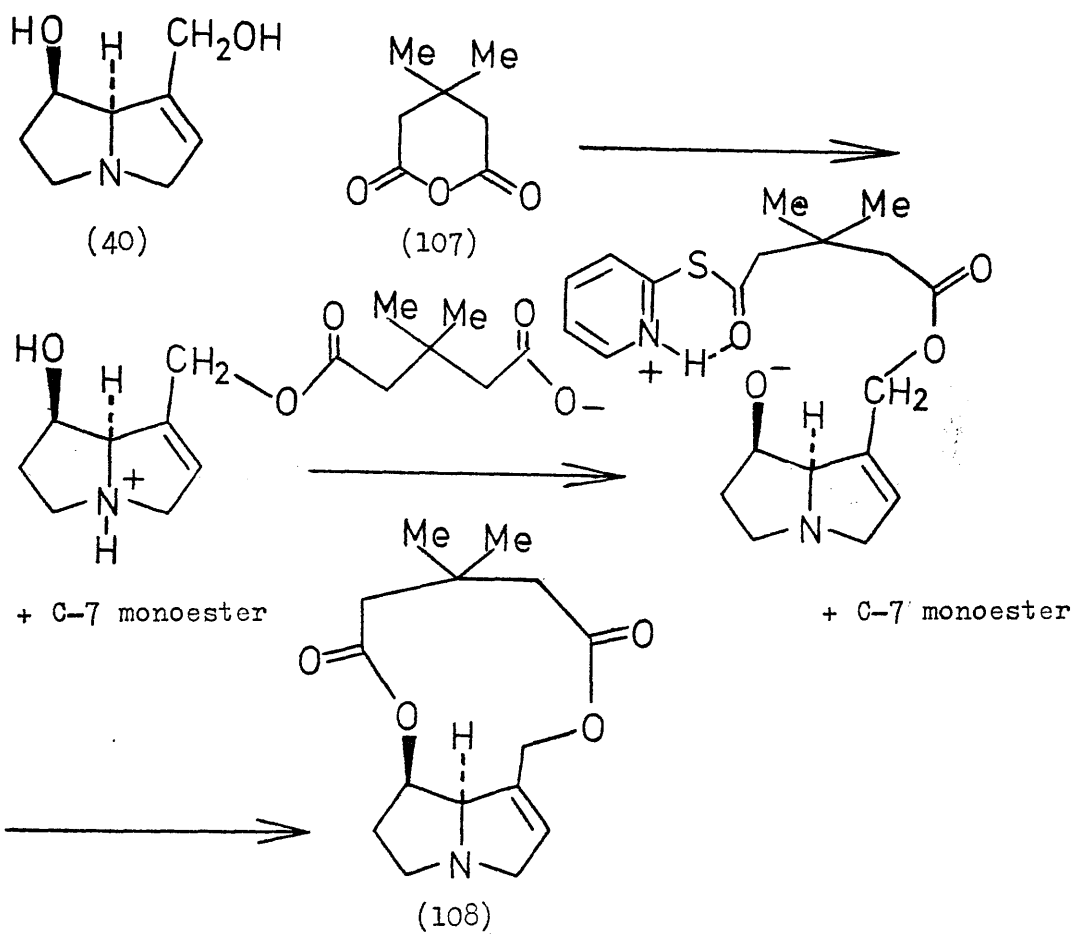
(106)

6.3 Synthesis of 13,13-Dimethyl-1,2-didehydrocrotalanine

The use of dicrotalic anhydride was expected to lead to a mixture of C-13 epimers in the formation of the macrocyclic ring. Instead the presence of the hydroxy group in the acid portion was crucial and under the reaction conditions employed, dehydration and decarboxylation were observed. In order to avoid both these problems of formation of diasteromers and dehydration, a different anhydride, 3,3-dimethylglutaric anhydride (107) was used in order to prepare an analogue of dicrotaline.

Esterification of (+)-retronecine with commercially available 3,3-dimethylglutaric anhydride produced a mixture of C-7 and C-9 monoesters (Scheme 64) in a ratio 1:2 in an exactly analogous fashion to the reaction of (+)-retronecine with

Scheme 64



(40) (107) (108)

+ C-7 monoester + C-7 monoester

dicrotalic anhydride. Internal esterification of this mixture of monoesters was carried out using the Corey-Nicolaou method with 2,2'-dipyridyl disulphide and triphenylphosphine in dimethylformamide. Extraction of the basified reaction mixture with chloroform gave two main components which were separated by preparative t.l.c. The polar minor component was not identified. The major component, obtained in 47% yield as an oil, was characterised as its picrate, and identified as

13,13-dimethyl-1,2-didehydrocrotalanine (108) from its spectral characteristics. The u.v. spectrum indicated that there was no $\alpha\beta$-unsaturated ester present. The i.r. spectrum showed the saturated ester carbonyl absorption at 1,738 cm^{-1}. A molecular ion was observed in the mass spectrum at m/e 279, and a typical fragmentation pattern for a macrocyclic pyrrolizidine diester[2] was present with peaks at m/e 138, 137, 136, 120, 119, 117, 94, 93, 83, and 80. The crucial feature in the n.m.r. spectrum is an AB quartet at δ 4.08 and 5.32 ppm (J 12 Hz) due to the protons at C-9. The chemical shift difference of 1.24 ppm is rather large for an 11-membered macrocyclic diester alkaloid (values of 0.0 to 0.92 ppm have been observed[2]), but comparison with dicrotaline is not possible since no n.m.r. spectral data have been reported for dicrotaline. The characteristic mass spectrum and non-equivalence of the protons at C-9 are taken as good evidence that an 11-membered macrocyclic diester of retronecine has been formed.

6.4 Conclusion

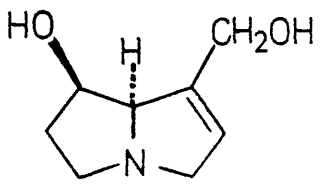
Monoesterification of retronecine with dicrotalic anhydride produced a mixture of C-7 and C-9 monoesters. Attempts to close the macrocyclic diester ring of this monoester mixture to form

dicrotaline resulted in the dehydration and decarboxylation to give a mixture of C-7 and C-9 senecioylretronecine. An analogue of dicrotaline was prepared by using 3,3-dimethylglutaric anhydride under the same esterification conditions. This represents the first synthesis of an 11-membered macrocyclic pyrrolizidine diester.

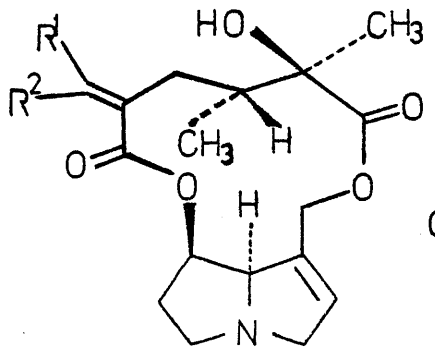
CHAPTER 7 PYRROLIZIDINE ALKALOIDS FROM SENECIO ODORUS SCH. BIP.

7.1 Introduction

Pyrrolizidine alkaloids have been identified from about 150 species of the very large Senecio genus. Very few of the succulent species from this genus have been investigated. Therefore as part of a plan to determine the nature of the alkaloids in a number of succulent Senecio species, the South African succulent plant Senecio odorus was chosen for study. This species was investigated to see if pyrrolizidine alkaloids are present and if so to determine whether they exhibit those structural features considered necessary for hepatotoxic action (Chapter 1.3). As in many studies on the pyrrolizidine alkaloids present in plants, it was expected that chemotaxonomic relationships would be extremely useful in arriving at structures for the alkaloids. It is known that the majority of Senecio species contain pyrrolizidine alkaloids which are macrocyclic (12 membered ring) diesters of retronecine (40). The esterifying acids usually contain ten carbon atoms with the carbon skeleton shown in (109). A typical representative is senecionine (5).

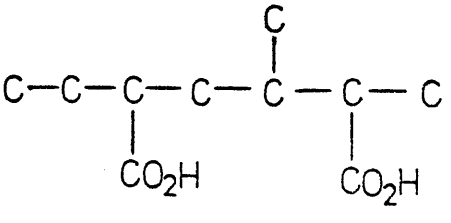


(40)



(5) R^1 = H, R^2 = Me

(110) R^1 = Me, R^2 = H



(109)

7.2 Extraction and Separation of the Alkaloids

Senecio odorus plants were obtained from Mr G. Rowley, Department of Botany, University of Reading and they were grown at the Department of Botany, University of Glasgow to provide sufficient material for extraction. The fresh plant material was extracted continuously with methanol in a Soxhlet apparatus. The concentrated extract was taken up in dilute sulphuric acid and extracted with chloroform. The acid solution was treated with zinc dust to reduce any N-oxides, filtered, basified and extracted with chloroform to give the crude alkaloid mixture in 0.038% yield. Examination of the mixture by tlc showed two bands with the leading component present in greater amount than the second. Separation was readily effected by preparative thin layer chromatography.

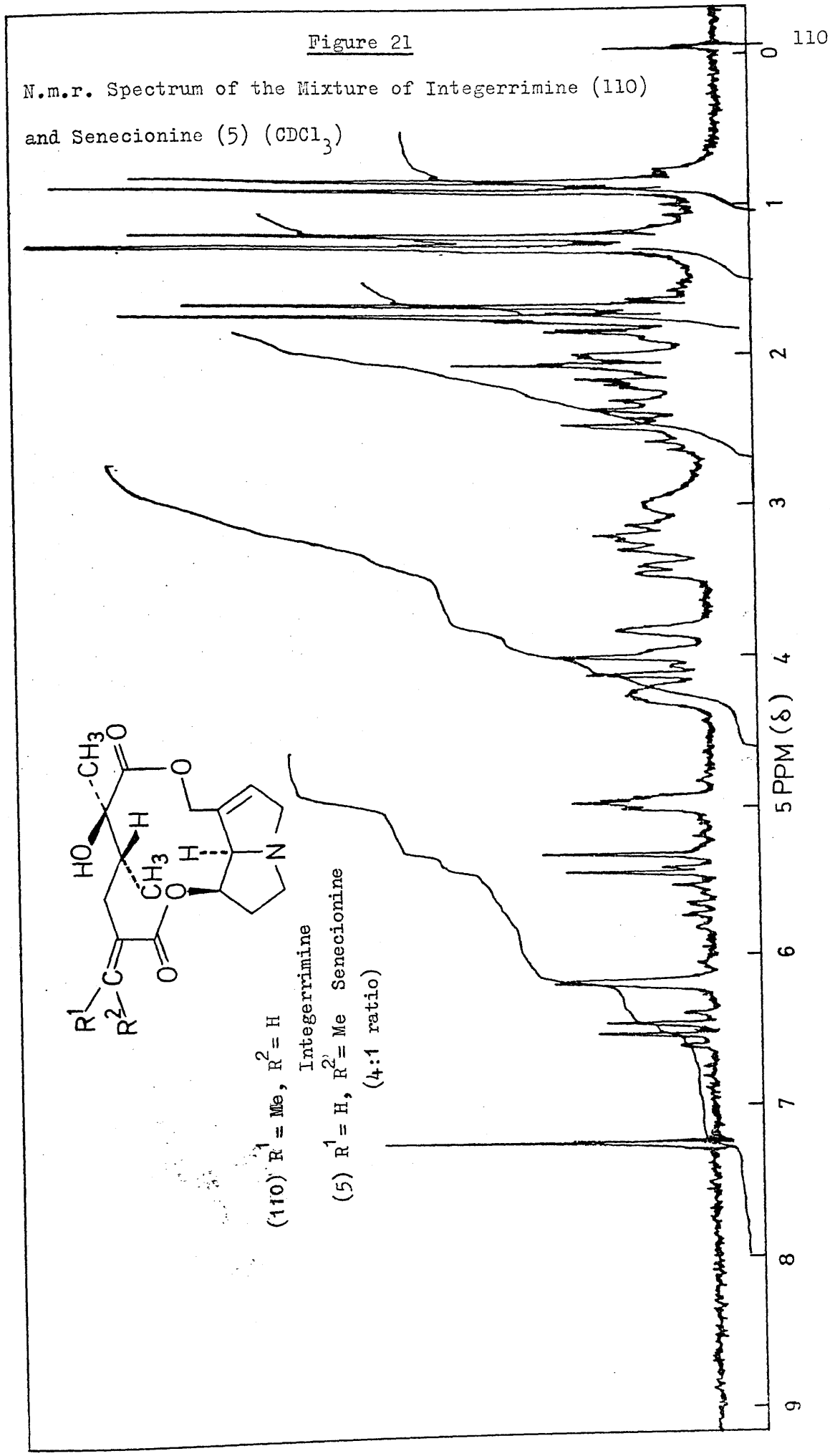
7.3 Identification of Integerrimine (10) and Senecionine (5)

G.l.c. analysis of the leading band (R_f 0.41) indicated the presence of two compounds in the ratio of 4:1 with R_s values* of 1.27 and 1.00 respectively. Comparison with an authentic sample of senecionine showed that the minor constituent was senecionine. From the R_f and R_s values observed the most likely structure for the major component of the mixture was integerrimine which is a geometrical isomer of senecionine. Support for this assignment was obtained by careful consideration of the n.m.r. spectrum of the mixture taken in deuteriochloroform (Figure 21), in comparison with the n.m.r. spectrum of authentic senecionine (Figure 22).

* R_s values are defined on page 123

Figure 21

N.m.r. Spectrum of the Mixture of Integerrimine (110) and Senecionine (5) ($CDCl_3$)

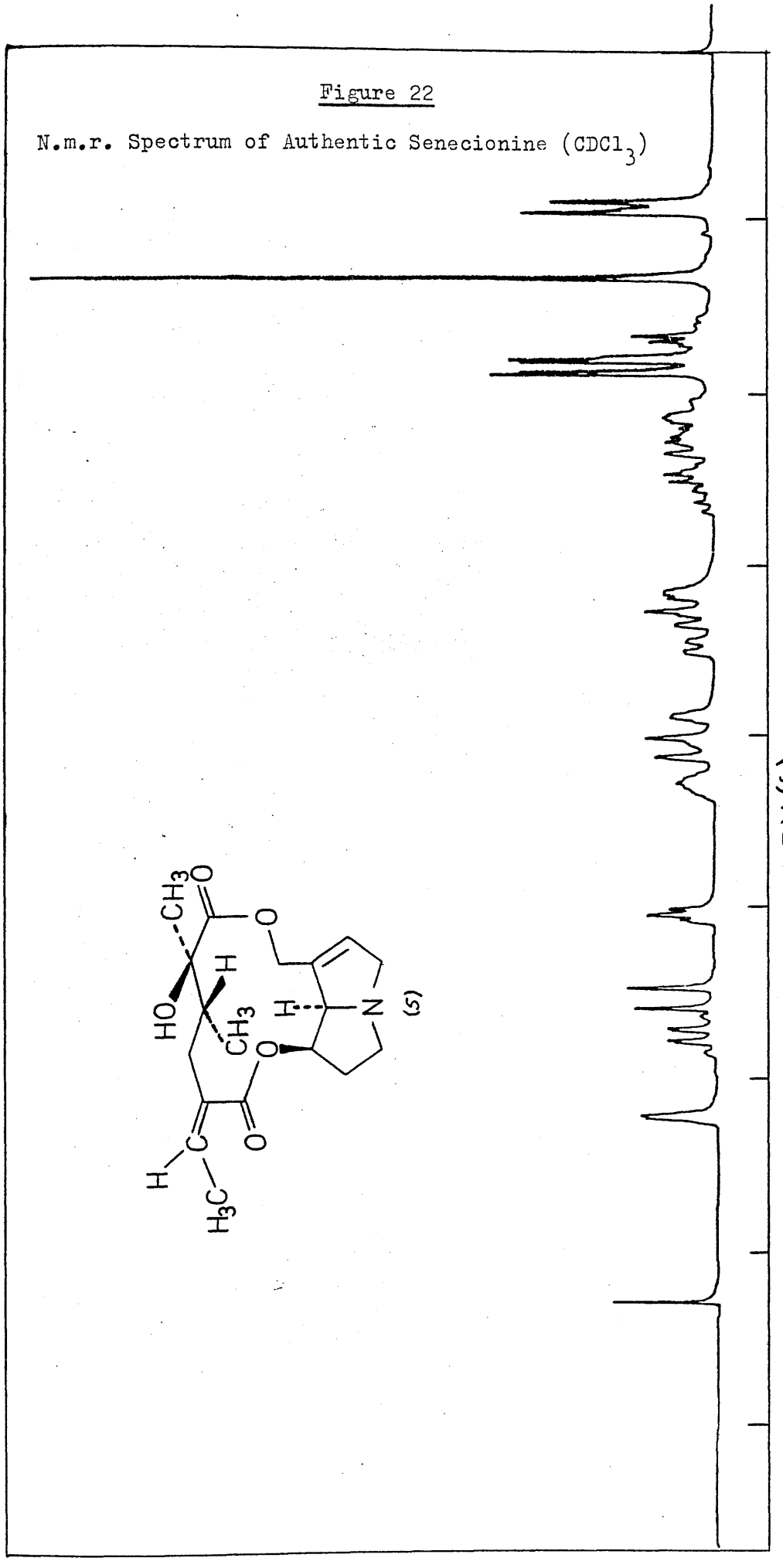


(110) R^1 = Me, R^2 = H
Integerrimine

(5) R^1 = H, R^2 = Me Senecionine
(4:1 ratio)

Figure 22

N.m.r. Spectrum of Authentic Senecionine ($CDCl_3$)



The olefinic proton of integerrimine showed a quartet centred at δ 6.50 (J 7 Hz), which is indicative of a cis-arrangement about the double bond. This is observed at a lower field than a trans-arrangement as in senecionine which has a quartet centred at δ 5.70 (J 7 Hz). The chemical shifts of the protons of the methyl groups on the double bond were also different in the two isomers. Integerrimine had a doublet centred at δ 1.75 (J 7 Hz) whereas senecionine showed a doublet at δ 1.85 (J 7 Hz). The three broad singlets at δ 6.20, 4.98 and 4.25 correspond to the olefinic proton at C-2 and two methine protons at C-7 and C-8 respectively. The signals of the methylene protons at C-9 appeared as an AB system at δ 5.90 and 4.10 (J 11.5 Hz). This chemical shift difference of 1.30 ppm is typical of a twelve-membered macrocyclic diester of retronecine (40).

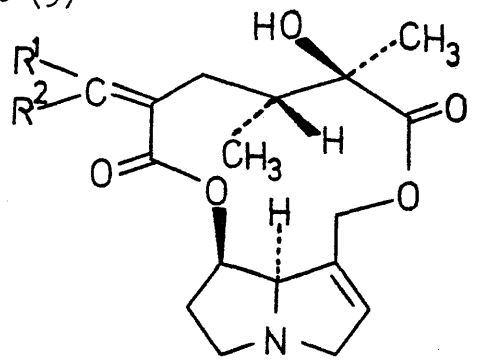
Thus the n.m.r. spectrum corresponds fully with that expected for a 4:1 mixture of integerrimine and senecionine.

The mass spectrum of the mixture of integerrimine and senecionine showed molecular ions at m/e 335 and a fragmentation pattern typical of retronecine macrocyclic diesters. The major fragmentation pathway for macrocyclic pyrrolizidine alkaloids containing an allylic ester involves fission of the allylic ester followed by breakdown of the acid portion until only the base part is left.[2] The identity of the necine base can then usually be determined by its characteristic fragmentation pattern. The probable fragmentation pathway for the mixture of geometric isomers integerrimine and senecionine is shown in Scheme 65.

Because of the small quantity of the mixture of integerrimine and senecionine obtained it was not possible to separate these two

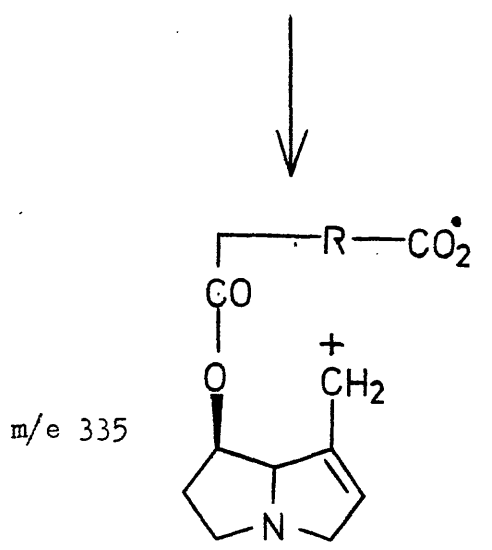
Scheme 65

Probable fragmentation patterns for integerrimine (110) and senecionine (5)

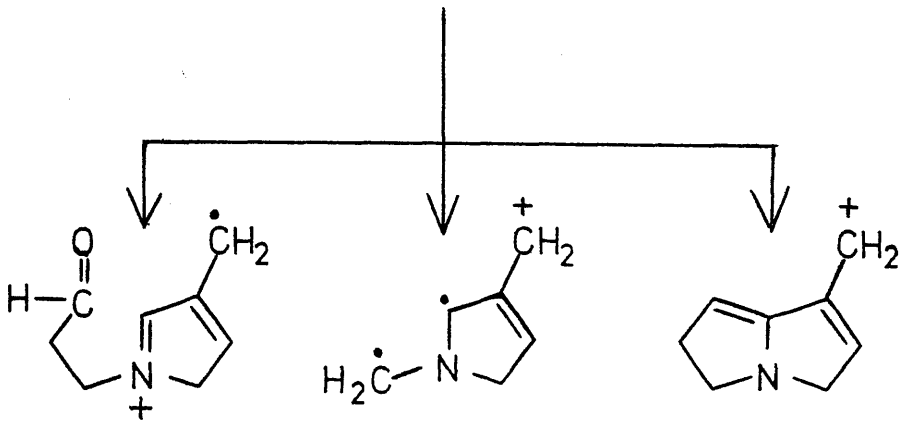


(110) R^1= Me, R^2= H

(5) R^1=H, R^2= Me

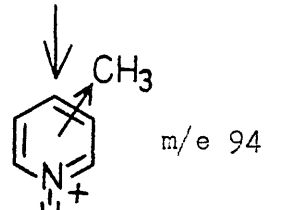


m/e 335



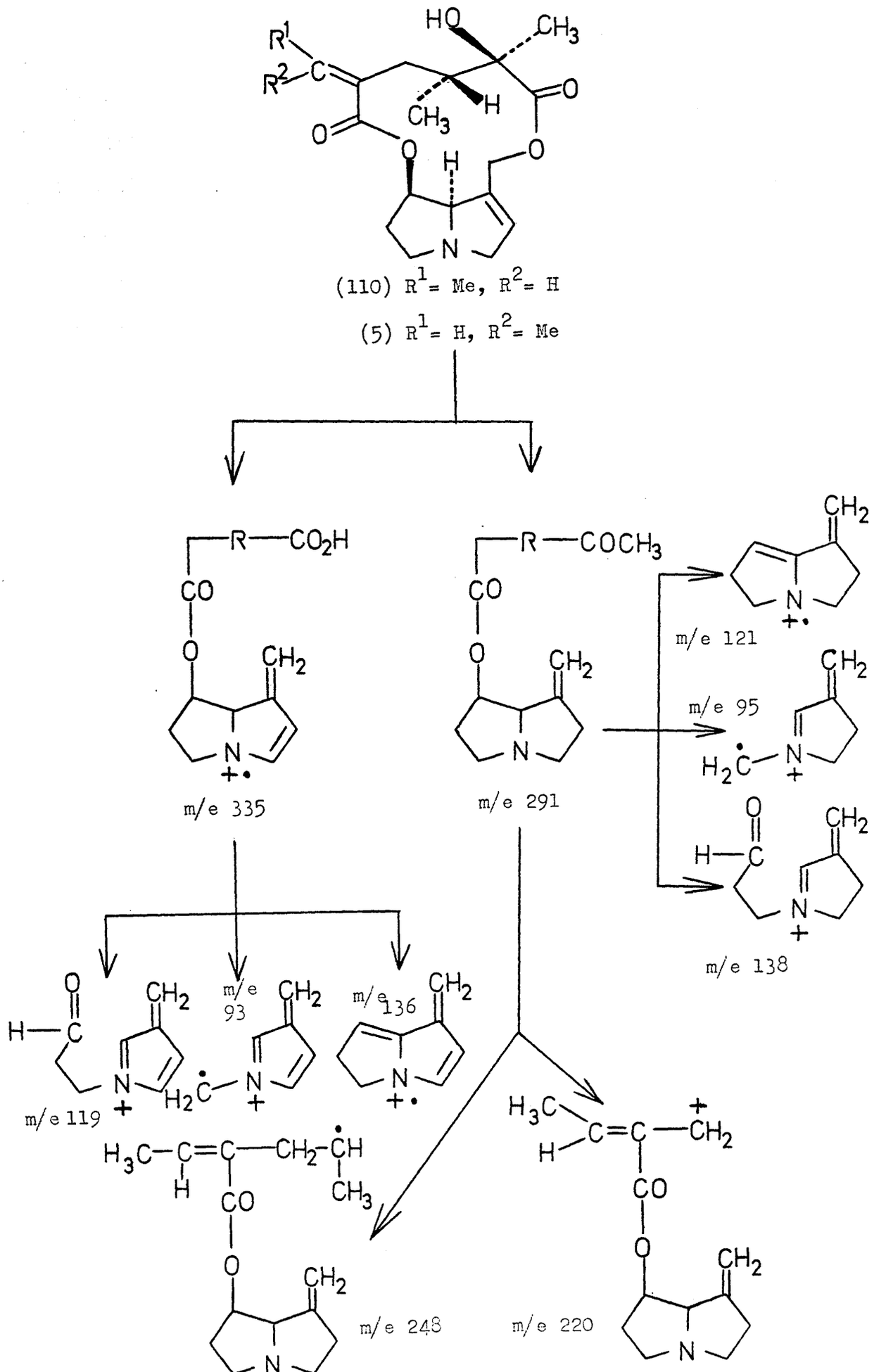
m/e 137

m/e 120



m/e 94

Scheme 65 (Continued)



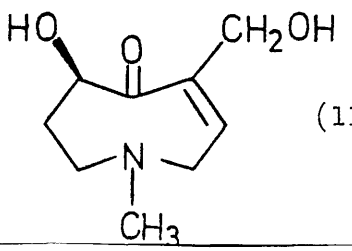
alkaloids to definitely prove their structures.

Senecionine was first isolated long ago from Senecio vulgaris, the common groundsel by Grandval and Lajoux[77] and its structure was elucidated by Kropman and Warren.[78] Integerrimine (squalidine) was first isolated by Barger and Blackie[79] and its structure was also elucidated by Kropman and Warren.[78] The identity of integerrimine with squalidine was established by Santavy et al.[80] The two geometrical isomers senecionine and integerrimine have commonly been found to occur together.

7.4 Identification of Senkirkine (111).

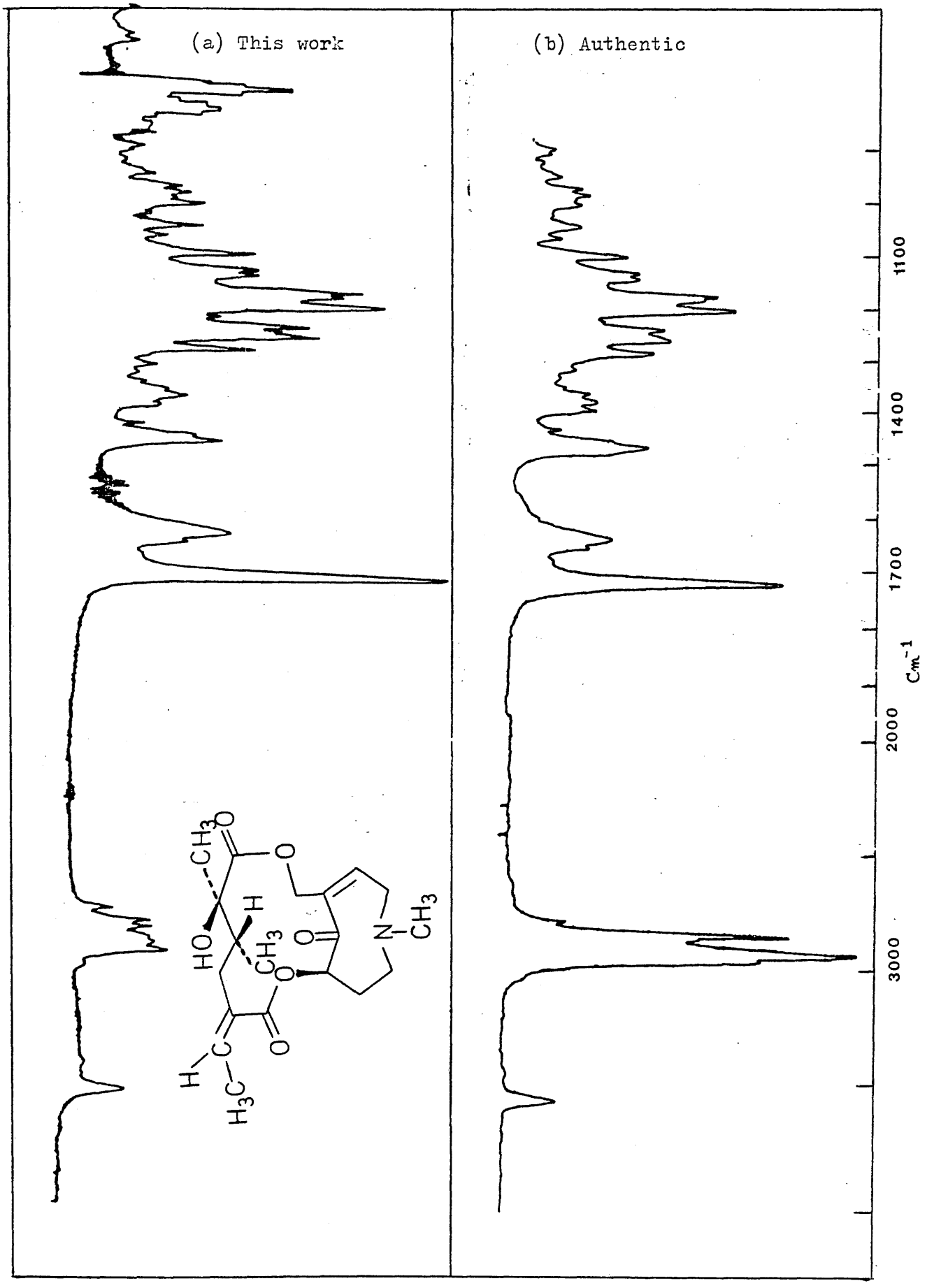
G.l.c. analysis of the second band (R_f 0.23) obtained by thin layer chromatographic separation of the alkaloid mixture showed the presence of one alkaloid (R_s 1.90). The i.r. spectrum (Figure 23, page 116) indicated an $\alpha\beta$-unsaturated ester system and a saturated hydrogen-bonded ester carbonyl both at 1,722 cm^{-1}. The absorption due to the carbon-carbon double bond of the $\alpha\beta$-unsaturated ester function was at 1,660 cm^{-1} and a band at 1,640 cm^{-1} is attributed to the carbonyl absorption of an otonecine ester alkaloid. This occurs at an unusually low wavelength because of the transannular interaction between the nitrogen atom and the carbonyl group ($\overset{\delta+}{N}\cdots\overset{\delta-}{C}\text{-}O$).

The presence of fragment ions in the mass spectrum of the alkaloid at m/e 168, 151, 123, 122, 110 and 94 is characteristic of the diesters of the seco-pyrrolizidine base otonecine (112).[81] The molecular ion and fragmentation pattern shown in Scheme 66 are consistent with the known alkaloid senkirkine (111).



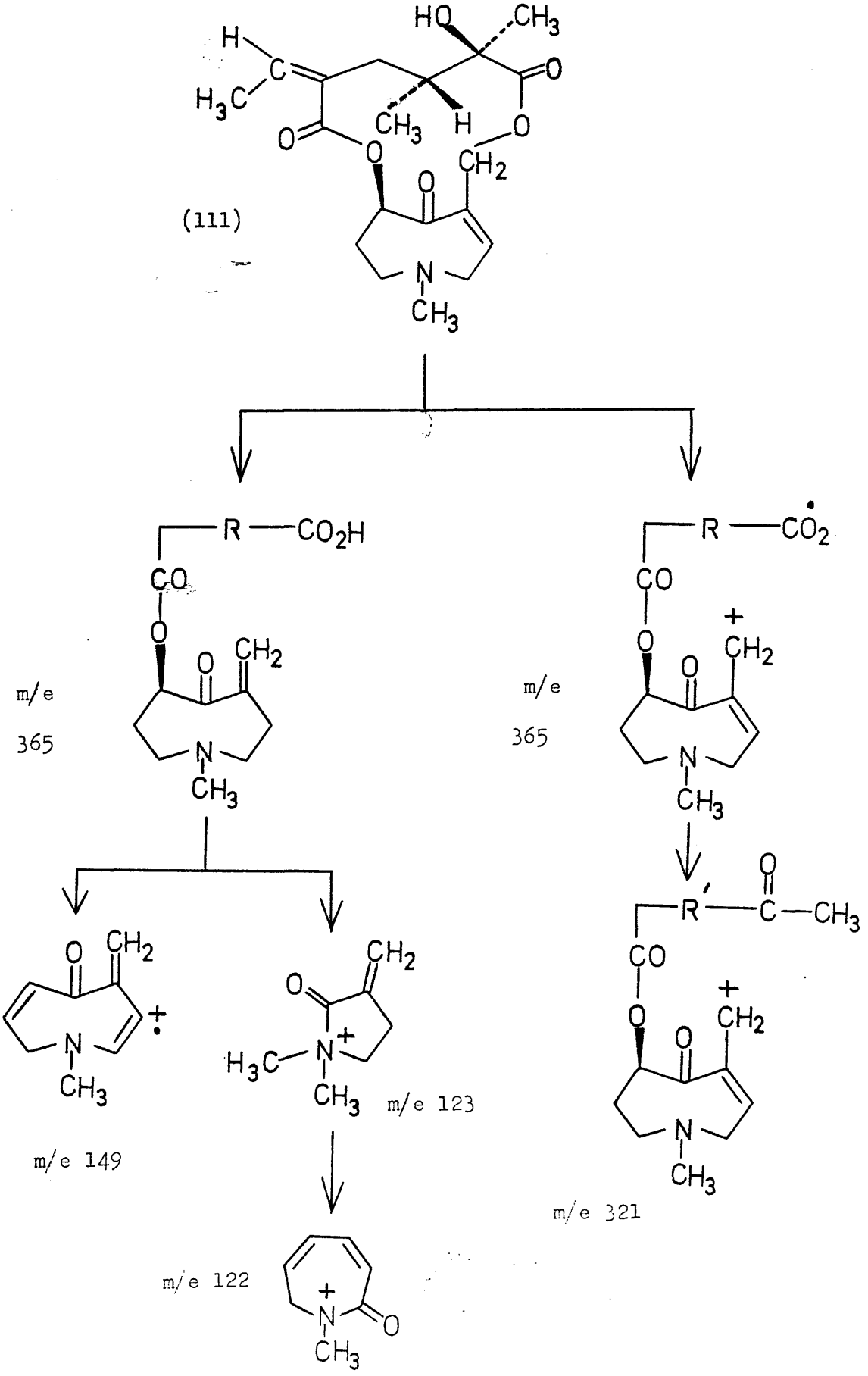
(112)

Figure 23 I.r. Spectra of Senkirkine (111) (CCl_4)

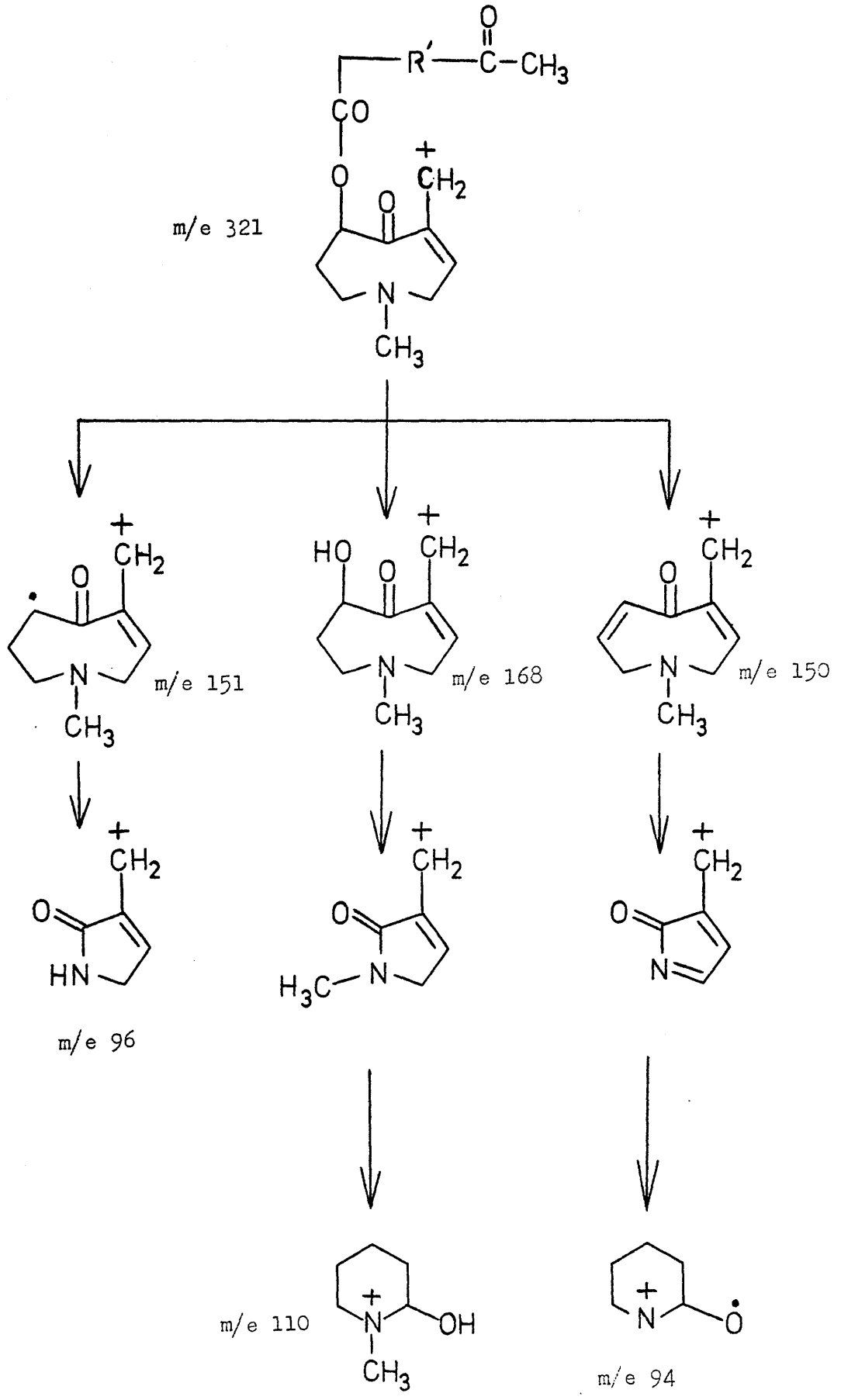


Scheme 66

Probable fragmentation patterns for senkirkine (111)



Scheme 66 (Continued)



The n.m.r. spectrum of this alkaloid in deuteriochloroform (Figure 24 , page120) is similar to that published for senkirkine.[82] Thus an easily recognisable AB pattern at δ 5.46 and 4.39 (J 12Hz) is indicative of a twelve-membered macrocyclic diester ring. The one proton triplet centred at δ 6.15 is assigned to the vinyl proton at C-2. A quartet centred at δ 5.90 (J 8 Hz) arising from the single proton at C-20 is due to coupling to the adjacent methyl group. It shows further allylic coupling (J 1 Hz) to one of the protons of the methylene group at C-14. A one proton triplet at δ 5.00, associated with the proton at C-7, arises from coupling with the adjacent methylene group at C-6. A three proton singlet at δ 2.10 is assigned to the N-methyl group. A doublet at δ 1.90 (J 8 Hz) assigned to the methyl group at C-21, showed further splitting by homoallylic coupling to one of the protons of the methylene group at C-14. The protons on the C-19 methyl group gave a doublet at 0.90 from coupling (J 7 Hz) with the adjacent proton. A three proton singlet at δ 1.35 is assigned to the tertiary C-18 methyl group. A broad one proton singlet at δ 1.25 is due to the hydroxyl group. The complicated peaks at δ 2.20-3.60 are due to methylene protons at C-3, C-5, C-6, and C-14.

The structure of the alkaloid was confirmed by comparison of the g.l.c., i.r. (Figure 23 , page116), n.m.r. (Figure 24 , pp. 120-1), and mass spectrum with an authentic sample of senkirkine supplied by Dr D.H.G.Crout.

Senkirkine was first isolated from the bark and leaves of Senecio kirkii Hook.f. by Briggs et al.,[82] and its structure was elucidated by the same group of workers.

Figure 24(a)

N.m.r. Spectrum of Isolated Senkirkine (111) ($CDCl_3$)

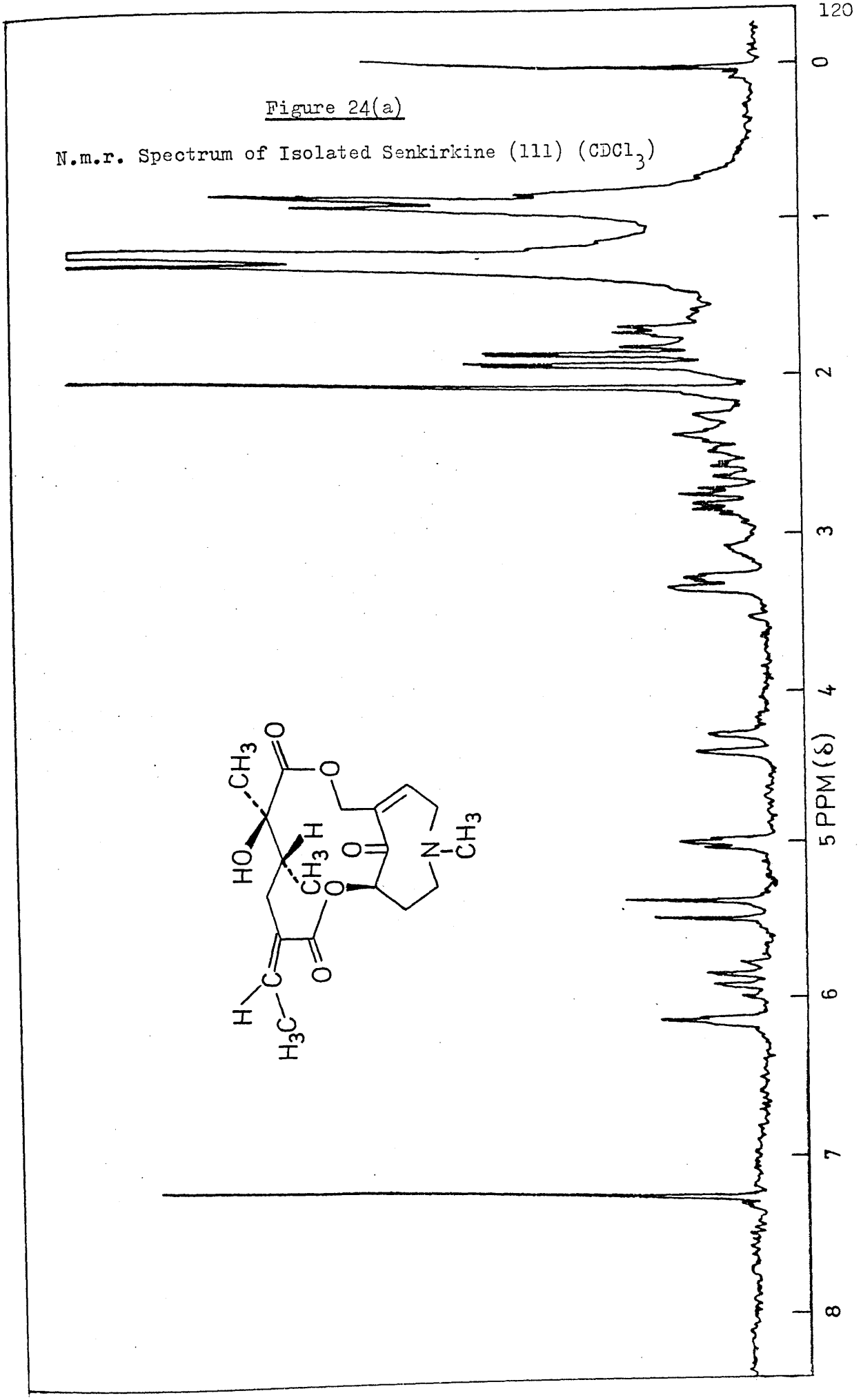
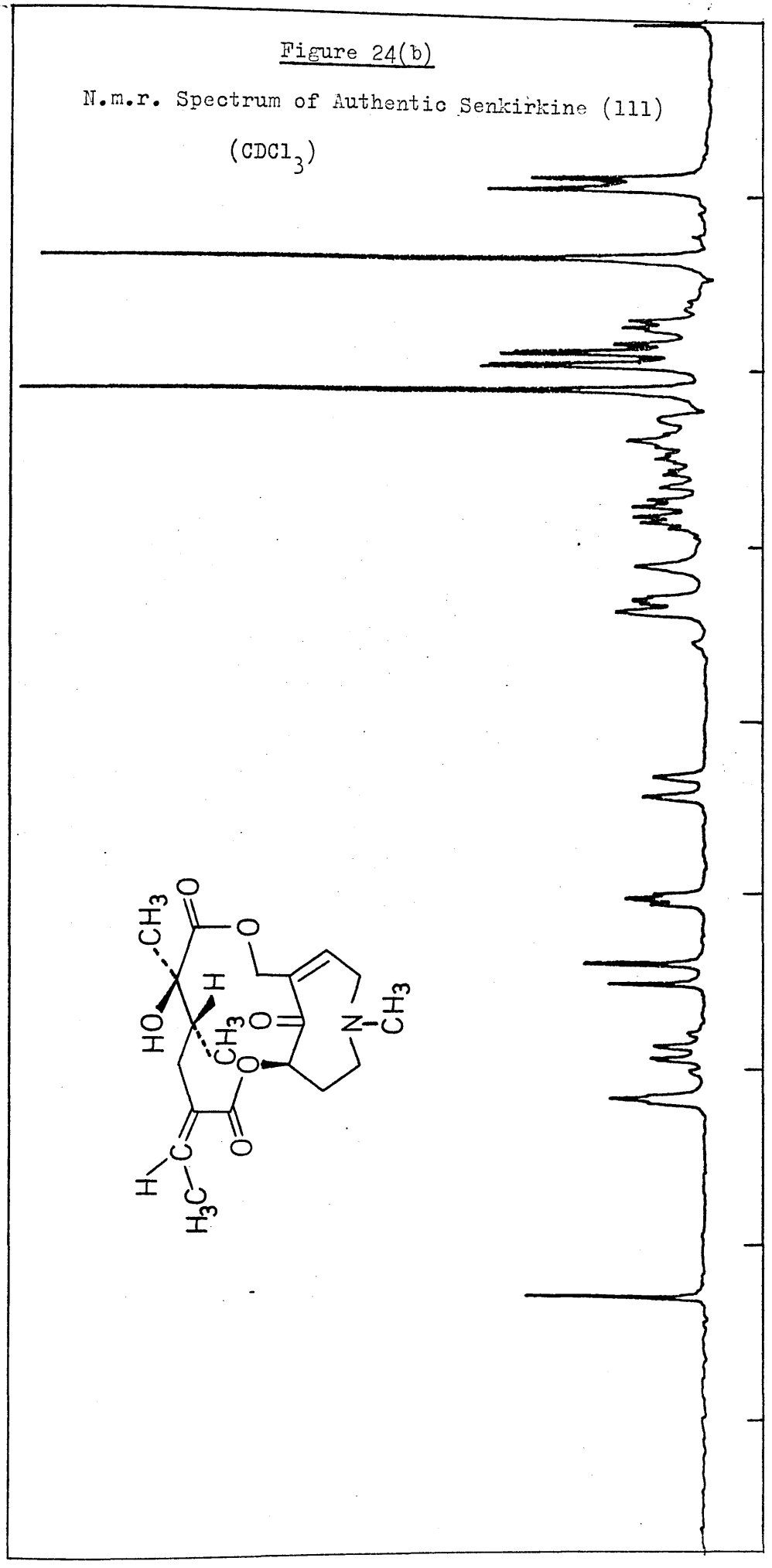


Figure 24(b)

N.m.r. Spectrum of Authentic Senkirkine (111)

($CDCl_3$)



7.5 Conclusion

Senecio odorus was shown to contain three pyrrolizidine alkaloids, integerrimine (110), senecionine (5) and senkirkine (111). The mixture of integerrimine and senecionine was not separated. All these three alkaloids were found to have the structural features considered necessary for the alkaloids to exhibit hepatotoxicity (Chapter 1.3).

CHAPTER 8 EXPERIMENTAL

8.1 General Notes

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

Thin layer chromatography (tlc) of alkaloids was carried out on Kieselgel G (Merck). The alkaloids were located with the modified Dragendorff reagent.[83]

Gas liquid chromatography (glc) was carried out on a Perkin-Elmer F11 Flame Ionization Chromatograph using a 1% SE-30 column at a temperature of 180 °C. Because of the difficulty in reproducing retention times, senecionine was used as standard, and R_s values are quoted, where

$$R_s = \frac{\text{Retention time of alkaloid}}{\text{Retention time of senecionine}}$$

Diethyl ether and tetrahydrofuran were dried over lithium aluminium hydride and distilled prior to use.

8.2 EXPERIMENTAL TO CHAPTER 3

N-Formyl-L-proline (50) - Formic acid (92 g; 2.0 mol) and acetic anhydride (102 g; 1.0 mol) were heated at 40-50 °C with stirring for 2 h, then L-proline (11.5 g; 0.1 mol) in formic acid (50 ml) was added at room temperature. The reaction mixture was stirred at room temperature for 16 h then ice-cold water (200 ml) was added. The mixture was evaporated *in vacuo* to give N-formyl-L-proline (50) as an oil that was crystallised from ethyl acetate (13.75 g; 95.8%), m.p. 89-91 °C (lit.,[19] 88-91 °C); $\nu_{max.}$($CHCl_3$) 3 000b, 1 730, and 1 675 cm^{-1}; δ ($CDCl_3$) 11.53 (1H, s, CO_2H), 8.43 (1H, s, CHO), 4.60 (1H, m, CH-CO_2H), 3.80 (2H, m, NCH_2), and 2.20 (4H, m); m/e 143 (M^+, 8%), 99 (52), 98 (58), 71 (42), 70 (100), 68 (33), 43 (52), 42 (33), and 41 (72).

Attempted Cycloaddition Reaction of N-Formyl-L-proline with Ethyl Acrylate - N-Formyl-L-proline (286 mg; 2 mmol) was dissolved in acetic anhydride (4 ml) and ethyl acrylate (505 mg; 5 mmol) was added. The reaction mixture was stirred and heated at 130 °C for 4 h under nitrogen. The solvents were removed *in vacuo* to give a black tarry residue which was separated by preparative tlc ($CHCl_3/Et_2O$ 1:1 v/v, plate developed twice) and gave a number of components, none of which could be identified. A variety of reaction conditions were investigated using the above reactants.

(a) Reactants were left at room temperature for 20 h.

(b) Reactants were heated at 110-115 °C for 10 h.

(c) Reactants were heated at 80 °C for 20 h.

(d) A catalytic amount of $BF_3.Et_2O$ (10%) was used and the reaction

was heated at 100 °C for 4 h.

(e) Hydroquinone (10%) was added and the reactants heated at 135 °C under nitrogen for 6 h.

(f) Ethyl acrylate was slowly added to a solution of N-formyl-L-proline in acetic anhydride with hydroquinone (10%) and heated at 135 °C for 6 h.

All these reactions gave complex mixtures of products which could not be identified.

Reaction of N-Formyl-L-proline with Diethyl Fumarate - N-Formyl-L-proline (143 mg; 1 mmol) was dissolved in acetic anhydride (3 ml) and diethyl fumarate (344 mg; 2 mmol) was added. The reaction mixture was stirred and heated at 135 °C under nitrogen for 4 h. The solvent was removed *in vacuo* to give a mixture of tetrahydropyrrolizine esters as a red-brown oil which was separated by preparative tlc (Et_2O/ petrol 3:1 v/v). The plate was developed twice to give three components.

The first component (R_f 0.47) as an oil (46 mg; 18%) was tentatively assigned as diethyl 5,6,7,8-tetrahydro-1H-pyrrolizine-1,2-dicarboxylate (51), $\nu_{max.}(CCl_4)$ 1 740, 1 700, 1 600, and 1 180 cm^{-1}; δ (CCl_4) 7.10 (1H, s, olefinic H), 4.20 (4H, m, $CO_2\underline{CH_2}CH_3$), 3.00 (2H, t, J 7Hz, NCH_2), 2.50 (6H, m), and 1.25 (6H, m, CH_3); m/e 253 (12%), 222 (8), 194 (15), 180 (100), 152 (40), 134 (40), 106 (100), and 104 (80); $\lambda_{max.}$(EtOH) 291 nm, ε 14,800.

The second component (R_f 0.36) as an oil (35 mg; 14%) was tentatively assigned as diethyl 2,5,6,7-tetrahydro-3H-pyrrolizine-1,2-dicarboxylate (52); $\nu_{max.}(CCl_4)$ 1 730, 1 710, 1 680, 1 600, and 1 240 cm^{-1}; δ (CCl_4) 4.10 (4H, m, $CO_2\underline{CH_2}CH_3$), 3.50-2.00 (9H, m), 1.15 (6H, m, CH_3); m/e 253 (80%), 222 (12), 206 (30), 180 (100), 152 (76), 134 (82), 108 (100), and 106 (76); $\lambda_{max.}$ (EtOH) 290 nm, ε 15,000.

The third component (R_f 0.23) as an oil (80 mg; 32%) was tentatively assigned as diethyl 5,6,7,8-tetrahydro-3H-pyrrolizine-1,2-dicarboxylate (53); $\nu_{max.}(CCl_4)$ 1 700, 1 620, and 1 240 cm^{-1}; δ (CCl_4) 4.10 (4H, m, $CO_2\underline{CH_2}CH_3$), 4.00-2.25 (9H, m), 1.20 (6H, m, CH_3); m/e 253 (15%), 206 (8), 180 (100), 108 (86), and 106 (72); $\lambda_{max.}$(EtOH) 297 nm, ε 19,500.

Reaction of N-Formyl-L-proline with Diethyl Maleate - N-Formyl-L-proline (143 mg, 1 mmol) was dissolved in acetic anhydride (3 ml) and diethyl maleate (344 mg, 2 mmol) was added. The reaction mixture was stirred and heated at 135 °C for 4 h. The acetic anhydride and diethyl maleate were removed *in vacuo* to give a mixture of tetrahydropyrrolizine diesters which were the same (tlc) as in the case of diethyl fumarate reaction.

Reaction of *N*-Formyl-L-proline with Dimethyl Fumarate - N-Formyl-L-proline (286 mg, 2 mmol) was dissolved in acetic anydride (5 ml) and dimethyl fumarate (576 mg, 4 mmol) was added. The reaction mixture was stirred and heated at 135 °C under nitrogen for 4 h. The acetic anhydride and dimethyl fumarate were removed *in vacuo* to give a mixture of tetrahydropyrrolizine diesters as a red-brown oil which was separated by preparative tlc (Et_2O/petrol 3:1 v/v). The plate was developed twice to give three components.

The first component (R_f 0.50) as an oil (81 mg; 18%) was tentatively assigned as dimethyl 5,6,7,8-tetrahydro-1H-pyrrolizine-1,2-dicarboxylate; $\nu_{max.}(CCl_4)$ 1 740, 1 700, 1 660, 1 600, and 1 200 cm^{-1}; δ (CCl_4) 6.80 (1H, s, olefinic H), 4.30 (1H, m, NCH), 3.80 (2H, m, NCH_2), 3.65 (3H, s, CH_3), 3.60

(3H, s, CH_3), and 3.20-1.80 (5H, m); m/e 225 (M^+, 30%), 194 (22), 166 (100), 134 (100), and 106 (30).

The second component (R_f 0.37) an oil (68 mg; 15%) was tentatively assigned as dimethyl 2,5,6,7-tetrahydo-3H-pyrrolizine-1,2-dicarboxylate; $\nu_{max.}$(CCl_4) 1 740, 1 715, 1 680, 1 600, and 1 250 cm^{-1}; δ (CCl_4) 4.15 (1H, d, J 9 Hz), 3.70 (3H, s, CH_3), 3.60 (3H, s, CH_3), 3.38 (1H, d, J 9Hz), 3.20-2.20 (7H,m); m/e 225 (M^+, 45%), 194 (30), 166 (100), 134 (100), and 106 (30).

The third component (R_f 0.23) isolated as an oil (90 mg, 20%) was tentatively assigned as dimethyl 5,6,7,8-tetrahydro-3H-pyrrolizine-1,2-dicarboxylate; $\nu_{max.}$(CCl_4) 1 740, 1 690, 1 625, and 1 200 cm^{-1}; δ (CCl_4) 4.20-3.90 (4H, m, NCH_2), 3.65 (3H, s, CH_3), 3.55 (3H, s, CH_3), and 3.20-2.12 (5H, m); m/e 225 (M^+, 35%), 194 (12), 166 (100), 134 (100), and 106 (100).

Reaction of N-Formyl-L-proline with Dimethyl Acetylene Dicarboxylate - N-Formyl-L-proline (143 mg; 1 mmol) was dissolved in acetic anhydride (5 ml) and dimethyl acetylene dicarboxylate (284 mg, 2 mmol) was added. The reaction mixture was heated at 135 °C under nitrogen for 2 h. Acetic anhydride and dimethyl acetylene dicarboxylate were evaporated *in vacuo* to give dimethyl 2,3-dihydro-1H-pyrrolizine-6,7-dicarboxylate (55) (203.8 mg; 91.4%) as large prisms, m.p. 88-89 °C (from ethyl acetate) (Lit.,[45] oil), (Found: C, 59.4; H, 5.6; N, 6.15. $C_{11}H_{13}NO_4$ requires C, 59.19; H, 5.83; N, 6.28%) $\nu_{max.}$ (CCl_4) 2 980, 1 730, 1 700, 1 445, 1 065 cm^{-1}; δ ($CHCl_3$) 7.12 (1H, s, H-5), 3.96 (2H, t, J 7 Hz, H-3), 3.80 (6H, s, CO_2Me), 3.04 (2H, t, J 7 Hz, H-1), and 2.50 (2H, m, H-2); m/e 223 (M^+, 50%), 192 (100), 191 (79), 164 (10), 162 (40), 134 (15), 133 (32), 105 (22), and 77 (15). I.r., u.v., and n.m.r., and mass spectra

were in accord with the reported values[45] for this compound (obtained as an oil).

Synthesis of (±)-Supinidine

Reaction of N-Formyl-L-proline with Ethyl Propiolate[19]

N-Formyl-L-proline (1.43 g, 10 mmol) was dissolved in acetic anhydride (10 ml) and ethyl propiolate (4.9 g, 50 mmol) was added. The reaction was heated at 135 °C under nitrogen for 4 h. Excess acetic anhydride and ethyl propiolate were removed *in vacuo* to give a red-brown oil which was distilled through a short-path distillation apparatus to give ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate (7) as an oil (b.p. 100 °C/0.5 mm Hg) (1.485 g; 83%) $\nu_{max.}$ (CCl_4) 1 700 cm^{-1}; δ ($CDCl_3$) 6.55, 6.65 (2H, AB q, J 3 Hz, H-5,6) 4.30 (2H, q, J 7Hz, CH_2CH_3), 4.00 (2H, t, J 7Hz, NCH_2), 3.10 (2H, t, J 7Hz, allylic H's), 2.60 (2H, m, CH_2), and 1.40 (3H, t, J 7Hz, CH_3); m/e 179 (M^+, 20%), 150 (60), 134 (100), 106 (98), 105 (30), 80 (15), 79 (44), 78 (51), and 77 (60); λ_{max}(EtOH) 233 (ε 15,600) and 255 (ε 12.700) nm.

Attempted Partial Reduction of Ethyl 2,3-Dihydro-1H-pyrrolizine-7-carboxylate (7) - Hydrochloric acid (20%, 15 ml) was cooled to 0 °C and zinc dust (3g) was added with vigorous stirring. Ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate (358 mg; 2 mmol) was added slowly. After all the dihydropyrrolizine ester had been added concentrated hydrochloric acid (6 ml) was added and the stirring continued at 0°C for 6 h and at room temperature for 18 h. The remaining zinc was filtered through celite and washed with a little water. The filtrate was basified with concentrated sodium hydroxide solution to pH 10-12 and then extracted with chloroform (4 x 250 ml). The chloroform extracts were dried over anhydrous sodium sulphate and evaporated to give a yellow oil which was separated by preparative tlc (Et_2O/petrol 4:1 v/v).

The plate was developed twice and gave three components.

The first component (R_f 0.55) was starting material (42 mg).

The second component (R_f 0.35), a pale yellow oil, is probably ethyl 2,5,6,7-tetrahydro-3H-pyrrolizine-1-carboxylate (12) (66.2 mg; 18.3%) $\nu_{max.}$(CCl_4) 1 680, 1 620, and 1 250 cm^{-1}; δ (CCl_4) 4.10 (2H, q, J 7Hz, $C\underline{H}_2CH_3$), 3.40-2.20 (10H, m), and 1.30 (3H, t, J 7Hz, CH_3); m/e 181 (M^+, 32%), 136 (75), 108 (100), and 80 (55); $\lambda_{max.}$(EtOH) 302 nm, ε 11,800.

The third component (R_f 0.26) a pale yellow oil was a dimeric pyrrolizidine ester (58)(104.6 mg; 29.2%) $\nu_{max.}$(CCl_4) 1 710, 1 690, 1 675, 1 620, and 1 200 cm^{-1}; δ (CCl_4) 6.18 (1H, s, olefinic H), 4.10 (4H, m, $C\underline{H}_2CH_3$), 3.90-2.20 (15H, m), and 1.20 (6H, m, CH_3); m/e 358 (M^+, 32%), 313 (12), 285 (55), 239 (30), 212 (35), 179 (12), 181 (25), and 106 (100); $\lambda_{max.}$(EtOH) 300 nm (ε 3,140)

Ethyl endo-pyrrolizidine-1-carboxylate (22) - Ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate (7) (1.074 g; 6 mmol) in acetic acid (20 ml) was hydrogenated at 21 torr using 10% Pd/C (1 g) for two days at room temperature. The catalyst was removed by filtration through Celite and evaporation of the solvent gave ethyl endo-pyrrolizidine-1-carboxylate (22) (1.05 g; 96%) as an oil; $\nu_{max.}$(CCl_4) 2 950, 1 735, and 1 250 cm^{-1}; δ ($CDCl_3$) 4.20 (2H, q, J 7Hz, $C\underline{H}_2CH_3$), 3.70-1.80 (12H,m), and 1.30 (3H, t, CH_3); m/e 183 (M^+, 20%), 182 (12), 179 (15), 164 (27), 154 (20), 150 (17), 148 (15), 138 (25), 134 (20), 110 (15), 108 (15), 98 (50), 83 (100), 80 (10), and 70 (70).

The picrate had m.p 119-121 °C (lit.,[19] 119-121 °C and[84] 119.5-120 °C) (Found: C, 46.42; H, 4.55; N, 13.45. $C_{16}H_{20}N_4O_9$ requires C, 46.60; H, 4.85; N, 13.59%).

Ethyl 5,6,7,8-Tetrahydro-3H-pyrrolizine-1-carboxylate (19) - To a solution of lithium di-isopropylamide [prepared from di-isopropylamine (0.62 ml, 5.67 mmol) and 1.7M n-butyl lithium in hexane (3.44 ml; 5.76 mmol)] in dry tetrahydrofuran (10 ml) at -78 °C was added dropwise during 1 h a solution of the ester (22) (732 mg; 4 mmol) in dry tetrahydrofuran (4 ml) under nitrogen. The solution was stirred at -78°C for 1 h then phenyl selenenyl chloride (864 mg; 4.5 mmol) in dry tetrahydrofuran (4 ml) was added rapidly and the solution was stirred for a further 2 h at -78 °C. The reaction mixture was warmed to 0 °C, glacial acetic acid (15 ml) and 27% hydrogen peroxide (4 ml) were added and stirred for 1 h, then at room temperature for 20 h. After basification (pH 10), the mixture was extracted with chloroform (4 x 50 ml), and the chloroform extracts were dried (K_2CO_3), filtered, and concentrated to give a yellow oil. Preparative tlc ($CHCl_3/CH_3OH/NH_3$ 85:14:1) gave the major component ethyl 5,6,7,8-tetrahydro-3H-pyrrolizine-1-carboxylate (R_f 0.57) as an oil (435 mg; 60%) $\nu_{max.}(CCl_4)$ 1 720, 1 640, and 1 250 cm^{-1}; δ (CCl_4) 6.58 (1H, m, olefinic H), 4.15 (2H, q, J 7Hz, $C\underline{H}_2CH_3$), 3.90-1.70 (9H,m), and 1.25 (3H, t, J 7Hz, CH_3); m/e 181 (M^+, 26%), 179 (14), 153 (19), 150 (19), 136 (50), 134 (24), 108 (25), 106 (13), and 80 (38). The picrate had m.p. 156-158 °C (from ethanol) (Found: C, 46.50; H, 4.29; N, 13.66. $C_{16}H_{18}N_4O_9$ requires C, 46.80; H, 4.39; N, 13.67%). G.l.c. on 5% Carbowax, 1% KOH, 20 mesh at 120°C gave 1 peak.

(±)-Supinidine (1) and (±)-Isoretronecanol (9) - A solution of ethyl 5,6,7,8-tetrahydro-3H-pyrrolizine-1- carboxylate (19) (90.5 mg; 0.5 mmol) in anhydrous ether (4 ml) was slowly added to a precooled (-15 °C) suspension of lithium aluminium hydride (38 mg; 1.0 mmol) in ether (4 ml) under nitrogen. The mixture was stirred for 2 h at -15 °C and 1 h at room temperature.

Excess lithium aluminium hydride was destroyed by the slow addition of wet ether followed by 20% sodium hydroxide solution (0.3 ml). The resulting suspension was filtered through Celite, dried (sodium sulphate), filtered, and concentrated to give a yellow oil. Preparative tlc ($CHCl_3/CH_3OH/NH_3$ 5:4:1) gave two major products.

(±)-Supinidine (1) was isolated at R_f 0.55 as an oil, (31 mg; 45%) $\nu_{max.}(CHCl_3)$ 3 320 and 1 600 cm^{-1}; δ ($CDCl_3$) 5.49 (1H, m, H-2), 5.12 (1H, br s, OH), 4.13 (2H, s, H-9), 4.00 (1H, m, H-8), 3.80-3.35 (2H, m, H-3), 3.1 and 2.5 (2H, m, H-5), 2.2-1.5 (4H, m, H-6 and H-7); m/e 139 (M^+, 55%), 138 (15), 122 (50), 121 (35), 120 (73), 111 (20), 108 (42), 106 (40), 94 (18), and 80 (100). I.r., n.m.r. and mass spectra were in accord with reported values for supinidine.[13] The picrate had m.p. 125-126 °C (from ethanol) (lit.,[13] 124-126 °C) (Found: C, 45.5; H, 4.4; N, 15.0. $C_{14}H_{16}N_4O_8$ requires C, 45.6; H, 4.4; N, 15.2%).

The second component, R_f 0.30(22 mg; 31%) was endo-(±)-1-hydroxymethylpyrrolizidine (isoretronecanol)(9) $\nu_{max.}(CHCl_3)$ 3 300 cm^{-1}; δ ($CDCl_3$) 5.25 (1H, br s, OH), and 3.70-1.80 (14H, complex); m/e 141 (M^+, 42%), 140 (29), 124 (29), 110 (27), 108 (27), 97 (76), 84 (29), 83 (100), and 82 (78). I.r., n.m.r., and mass spectra were in accord with reported values for (±)-isoretronecanol[56] . The picrate had m.p. 187-189 °C (from ethanol)(lit.,[19] 187-189 °C and[56] 188-190 °C)(Found: C, 45.3; H, 5.2; N, 15.1. $C_{14}H_{18}N_4O_8$ requires C, 45.4; H, 4.9; N, 15.1%).

Phenylselenation of the Ester (22) - To a solution of lithium di-isopropylamide [prepared from di-isopropylamine (1.24 ml; 11.52 mmol) and 1.7M n-butyl lithium in hexane (6.8 ml, 11.52 mmol)] in dry tetrahydrofuran (10 ml) at -78°C, a solution of the ester (22) (1.464 g, 8 mmol) in dry tetrahydrofuran (4 ml) was added

dropwise during 1 h under nitrogen. The solution was stirred at -78 °C for a further 1 h then phenylselenenyl chloride (1.728 g, 9 mmol) in dry tetrahydrofuran (4 ml) was added rapidly and the solution stirred at -78 °C for 3 h and then poured into water (50 ml). The mixture was extracted with ether (80 ml) and chloroform (3 x 80 ml). After drying (sodium sulphate), filtering and concentrating, the organic layers yielded a brown oil containing one major component, R_f 0.55 ($CHCl_3/CH_3OH/NH_3$ 85:14:1). Preparative tlc afforded ethyl 1-phenylselenenylpyrrolizidine-1-carboxylate (59) as a pale yellow oil (1.55 g, 57%) $\nu_{max.}$ (CCl_4) 1 720 and 1 580 cm^{-1}; δ ($CDCl_3$) 7.50 (5H, m), 4.20 (2H, q, J 7Hz), 3.8-2.0 (11H, complex,m), and 7.20 (3H, t, J 7Hz); m/e 339 (43), 337 (20), 182 (100), 181 (50), 158 (23), 154 (26), 136 (29), 110 (20), 108 (26), 83 (50). The picrate had m.p. 155-157 °C (from ethanol)(Found: C, 46.3; H, 4.1; N, 9.8. $C_{22}H_{24}N_4O_9Se$ requires C, 46.6; H, 4.2; N, 9.9%).

Reduction of the phenylselenenyl ester (59)[57] - A solution of the ester (59) (339 mg, 1 mmol) in dry ether (4 ml) was added to a cooled (-15 °C) suspension of lithium aluminium hydride (76 mg, 2 mmol) in dry ether (4 ml) under nitrogen. The mixture was stirred for 2 h at -15°C and 1 h at room temperature. Excess lithium aluminium hydride was destroyed by the slow addition of wet ether followed by 20% sodium hydroxide solution (0.5 ml). The resulting suspension was filtered through Celite, dried (sodium sulphate), filtered, and concentrated to yield a yellow oil. Preparative tlc ($CHCl_3/CH_3OH/NH_3$ 85:14:1) gave the major component 1-hydroxymethyl-1-phenylselenenylpyrrolizidine (61) at R_f 0.25 as a pale yellow oil (183.4 mg, 62%) which afforded crystals from ethyl acetate, m.p. 125-127 °C; $\nu_{max.}$ (KBr) 3 400 and 1 580 cm^{-1};

δ ($CDCl_3$) 7.40 (5H, m), 3.50 (1H, s, OH), 3.60-2.00 (13H, complex); m/e 297 (M^+,8%), 158 (40), 139 (60), 130 (40), 122 (45), 120 (60), 108 (30), 83 (100), and 78 (100). (Found: C, 56.6; H, 6.4; N, 4.5. $C_{14}H_{19}NOSe$ requires C, 56.7; H, 6.4; N, 4.7%).

(±)-Supinidine (1) - The phenylselenenyl alcohol (61) (183.4 mg; 0.61 mmol) in acetic acid (5 ml) was mixed with 27% hydrogen peroxide (1 ml) and stirred at 0 °C for 1 h and at room temperature for 12 h. After basification (pH 10), the mixture was extracted with chloroform (4 x 25 ml) and the extracts were dried (potassium carbonate), filtered, and concentrated to give a pale yellow oil. Preparative tlc ($CHCl_3/CH_3OH/NH_3$ 5:4:1) gave the major component as an oil (R_f 0.55), (50 mg; 59%) $\nu_{max.}$ ($CHCl_3$) 3 320 and 1 600 cm^{-1}; δ ($CDCl_3$) 5.49 (1H, m, H-2), 5.12 (1H, br s, OH), 4.13 (2H, s, H-9), 4.0 (1H, m, H-8), 3.80-3.35 (2H, m, H-3), 2.50 and 3.10 (2H, m, H-5), 2.20-1.50 (4H, complex, H-6 and H-7); m/e 139 (M^+, 55%), 138 (15), 122 (50), 121 (35), 120 (73), 108 (42), 106 (40), 94 (18), 80 (100).
The picrate had m.p. 125-126 °C (from ethanol) (lit.,[13] 124-126 °C) (Found: C, 45.5; H 4.4; N, 15.0. $C_{14}H_{16}N_4O_8$ requires C, 45.6; H, 4.4; N, 15.2%).

Ethyl (±)-Pyrrolizidine-1-exo -carboxylate (62) - Ethyl (±)- pyrrolizidine-1-endo-carboxylate (22) (91.5 mg; 0.5 mmol) was added to the reaction mixture of sodium (12 mg; 0.5 mmol) and absolute ethanol (5 ml). After 12 h at 60 °C, the mixture was cooled and poured into cold dilute hydrochloric acid, the pH ajusted to 3-4, and washed with chloroform. The epimerisation product was then extracted from the alkaline aqueous layer with chloroform.

The combined extracts were dried over anhydrous sodium sulphate, filtered, and concentrated in vacuo to give ethyl (±)-pyrrolizidine-1-exo-carboxylate (62) as a pale yellow oil (64 mg; 70%) $\nu_{max.}$ (CCl_4) 1 735 and 1 180 cm^{-1}; δ ($CDCl_3$) 4.15 (2H, q, J 7Hz, CH_2CH_3), 3.65 (1H, m, H-8) 3.20-1.70 (11H, complex), and 1.25 (3H, t, J 7Hz, CH_3); m/e 183 (M^+, 10%), 138 (16), 136 (12), 110 (24), 108 (48), 83 (100), 82 (80), and 80 (48). No evidence for formation of the endo-isomer was obtained by tlc in several solvent systems. The picrate had m.p. 141-143 °C (from ethanol) (Found: C, 46.47; H, 4.88; N, 13.66. $C_{16}H_{20}N_4O_9$ requires C, 46.60; H, 4.85; N, 13.59).

(±)-Trachelanthamidine (10) - A solution of ethyl (±)-pyrrolizidine-1-exo-carboxylate (62) (40 mg; 0.4 mmol) in dry ether (5 ml) was added to a suspension of lithium aluminium hydride (30 mg; 0.8 mmol) in dry ether (4 ml) under nitrogen. The mixture was stirred for 2 h at room temperature. Excess hydride was destroyed by slow addition of wet ether followed by 1M sodium hydroxide solution (0.1 ml). The resulting suspension was filtered through Celite, washed with chloroform and the combined solvents dried (sodium sulphate), filtered and evaporated in vacuo to yield (±)-trachelanthamidine (10) as an oil (28 mg, 97%) δ ($CDCl_3$) 3.62 (2H, d, J 7Hz, H-9), 2.55 (1H, br s, OH), 3.30-1.20 (12 H, complex). m/e 141 (M^+,35%), 140 (30), 124 (25), 113 (10), 110 (25), 108 (10), 97 (20), 83 (100), 82 (100), and 80 (20). The picrate had m.p. 173-175 °C (lit.,[84] 174-175°C) $\nu_{max.}$(KBr) 3 560, 3 050, 1 640, 1 565 cm^{-1}. The n.m.r. and mass spectra of trachelanthamidine were consistent with reported values.[56,59]

8.3 EXPERIMENTAL TO CHAPTER 4

(2S, 4R)-N-Formyl-4-formyloxyproline (65) - Formic acid (69 g; 1.5 mol) and acetic anhydride (76 g; 0.75 mol) were stirred at room temperature for 1 h. (2S, 4R)-4-hydroxyproline [naturally occurring 4-hydroxyproline $[\alpha]_D^{25}$ -75.3 ° (c 20, water)] (10 g; 0.076 mol) in formic acid (20 ml) was added. The reaction mixture was stirred at room temperature for 12 h. The solvent was removed *in vacuo* to give (2S,4R)-N-formyl-4-formyloxyproline (65) as crystals (12.93 g; 91%) m.p. 171-173 °C (from methanol) (Found: C, 44.76; H, 4.71; N, 7.27. $C_7H_9NO_5$ requires C, 44.92; H, 4.87; N, 7.48%) $[\alpha]_D^{18.4}$ -74.04 ° (c 5, methanol); $\nu_{max.}$ (KBr) 3 000, 1 765, 1 718, and 1 620 cm^{-1}; δ (CD_3OD) 8.35 (1H, s, CHO), 8.15 (1H, s, CHO), 5.50 (1H, m, H-4), 4.60 (1H, m, H-1), 3.70 (2H, m, H-3), and 2.50 (2H, m, H-5); m/e 187 (M^+, 5%), 169 (9), 143 (22), 142 (9), 141 (12), 113 (9), 97 (25), 96 (75), 71 (18), 69 (9), and 68 (100).

Dimethyl (+)-2α-formyloxy-2,3-dihydro-1H-pyrrolizine-6,7-dicarboxylate (64) - A solution of (2S,4R)-N-formyl-4-formyloxyproline (374 mg; 2 mmol) and dimethyl acetylene dicarboxylate (568 mg; 4 mmol) in acetic anhydride (6 ml) was heated at 140 °C for 4 h under nitrogen. Excess reagents were evaporated *in vacuo* to give dimethyl (+)-2α-formyloxy-2,3-dihydro-1H-pyrrolizine-6,7-dicarboxylate (64) (465 mg; 87.1%) as large prisms m.p. 85-86 °C (from ethyl acetate) $[\alpha]_D^{25}$ +31.4° (c 5, $CHCl_3$) (Found: C, 53.86; H, 5.07; N, 5.14. $C_{12}H_{13}NO_6$ requires C, 53.93; H, 4.87; N, 5.24%) $\nu_{max.}$ (KBr) 1 720, 1 620, 1 280, and 1 190 cm^{-1}; δ ($CDCl_3$) 8.00 (1H, s, CHO), 7.19 (1H, s, H-5), 5.85 (1H, m, H-2), 4.30 (2H, m, H-3), 3.81 (6H, s, CH_3), and 3.35 (2H, m, H-1); $\lambda_{max.}$ (MeOH) 260 nm, ε 12,300.

m/e 267 (M^+, 18%), 236 (20), 220 (70), 189 (100), 188 (80), 161 (50), 159 (28), 132 (20), 131 (18), 117 (16), and 110 (28).

Dimethyl (+)-2α-hydroxy-2,3-dihydro-1H-pyrrolizine-6,7-dicarboxylate (66) - Dimethyl (+)-2α-formyloxy-2,3-dihydro-1H-pyrrolizine-6,7-dicarboxylate (64) (267 mg, 1 mmol) was dissolved in methanol (10 ml) and 50% sodium hydroxide solution (10 ml) was added. The reaction mixture was stirred at room temperature for 10 h, then extracted with chloroform (3 x 30 ml). The chloroform extracts were dried (sodium sulphate), filtered, and evaporated to give dimethyl (+)-2α-hydroxy-2,3-dihydro-1H-pyrrolizine-6,7-dicarboxylate (66) (225 mg; 94%) as crystals m.p. 89-90 °C (from ethyl acetate) $[\alpha]_D^{25}$ +32.62° (c 5, $CHCl_3$) (Found: C, 55.23; H, 5.54; N, 5.63. $C_{11}H_{13}NO_5$ requires C, 55.23; H, 5.44; N, 5.86%) $\nu_{max.}$ (KBr) 3 480, 3 300, and 1 720 cm^{-1}; δ ($CDCl_3$) 7.10 (1H, s, H-5), 5.00 (1H, m, H-2), 4.10 (2H, m, H-3), 3.80 (6H, s, CH_3), and 3.20 (2H, m, H-1); m/e 239 (M^+, 60%), 208 (100), 207 (57), 190 (90), 178 (37), 164 (27), 160 (15), 134 (17); $\lambda_{max.}$ (EtOH) 260 nm, ε 11,300.

Ethyl (+)- 2α-formyloxy-2,3-dihydro-1H-pyrrolizine-7-carboxylate (67) - A solution of (2S,4R)-N-formyl-4-formyloxy-L-proline (65) (1.87 g; 10 mmol) and ethyl propiolate (4.9 g, 50 mmol) in acetic anhydride (10 ml) was heated at 140 °C for 10 h under nitrogen. Excess reagents were evaporated in vacuo leaving a red-brown oil which was distilled through a short path distillation apparatus b.p. 138-140 °C (0.8 mm Hg) to give an oil which crystallised from ether as needles (1.792 g, 80.4 %) m.p. 62.5-63.5 °C (Found: C, 59.27; H, 5.70; N, 5.98. $C_{11}H_{13}NO_4$ requires C, 59.19; H, 5.83; N, 6.28%) $[\alpha]_D^{18.4}$ +35.3° (c 4, $CHCl_3$); $\nu_{max.}$ (CCl_4) 1730, 1 715, 1 530 and 1 170 cm^{-1}; $\lambda_{max.}$ (EtOH) 261 nm, ε 10,250; δ ($CDCl_3$)

8.10 (1H, s, CHO), 6.60, 6.65 (2H, ABq, J 3Hz, H-5,6) 5.90 (1H, m, H-2), 4.30 (4H, m, H-3 and $C\underline{H}_2CH_3$), 3.40 (2H, m, H-1), and 1.35 (3H, t, J 7Hz, CH_3); (Found: M^+, 223.08483. $C_{11}H_{13}NO_4$ requires M, 223.08444); m/e 223 (M^+, 9%), 179 (6), 178 (7), 177 (16), 123 (84), 92 (16), 93 (100), 85 (19), and 83 (28).

Ethyl (+)-2α-hydroxy-2,3-dihydro-1H-pyrrolizine-7-carboxylate (8) - A solution of ethyl (+)-2α-formyloxy-2,3-dihydro-1H-pyrrolizine-7-carboxylate (67) (1.784 g; 8 mmol) in ethanol (100 ml) with concentrated ammonia solution (10 ml) was stirred at room temperature for 16 h. The solvent was removed *in vacuo* and the residue dissolved in chloroform (50 ml) which was washed with water (2 x 30 ml) and dried (sodium sulphate) then filtered and evaporated to give ethyl (+)-2α-hydroxy-2,3-dihydro-1H-pyrrolizine-7-carboxylate (8) as a pale yellow oil in quantitative yield. $[\alpha]_D^{18.4}$ +34.6° (c 4, $CHCl_3$) $\nu_{max.}$ (Film) 3 350, 1 680, and 1 560 cm^{-1}; δ ($CDCl_3$) 6.60, 6.50 (2H, ABq, J 3 Hz, H-5,6), 5.00 (1H, m, H-2), 4.20 (4H, m, H-3 and $C\underline{H}_2CH_3$), 3.20 (2H, m, H-1), and 1.30 (3H, t, J 7Hz, $C\underline{H}_3$); (Found: M^+, 195.08968. $C_{10}H_{13}NO_3$ requires M, 195.08953) m/e 195 (M^+, 95%), 177 (15), 150 (100), 132 (50), 122 (40), and 94 (40). $\lambda_{max.}$ (EtOH) 252 nm, ε 11,370.

Ethyl (+)-6α-hydroxy-8β-pyrrolizidine-1α-carboxylate (68) - A solution of ethyl (+)-2α-hydroxy-2,3-dihydro-1H-pyrrolizine-7-carboxylate (8) (1.95 g; 10 mmol) in acetic acid (30 ml) was hydrogenated at 21 torr using 10% Pd/C (2 g) as catalyst. The reaction was shaken at room temperature for 2 days. The catalyst was removed by filtration through Celite and the filtrate was concentrated *in vacuo* to give a residue which was dissolved in

chloroform (100 ml). This solution was dried (potassium carbonate), filtered, and concentrated *in vacuo* to yield ethyl (+)-6α-hydroxy-8β-pyrrolizidine-1α-carboxylate (68) as needles (1.602 g; 80.5%) m.p. 109-110 °C (from ethyl acetate) $[\alpha]_D^{18.4}$ +73.4°(c 4, $CHCl_3$) $\nu_{max.}$(KBr) br3 100, and 1 720 cm^{-1}; δ ($CDCl_3$) 4.38 (1H, m, H-6), 4.15 (2H, q, J 7Hz, $C\underline{H}_2CH_3$), 3.75 (1H, q, J 7Hz, H-8), 3.25-2.00 (10H, complex), and 1.25 (3H, J 7Hz, CH_3); m/e199 (M^+, 12%), 155 (16), 154 (19), 126 (6), 106 (28), 99 (47), and 82 (100); (Found: C, 60.40; H, 8.38; N, 7.10. $C_{10}H_{17}NO_3$ requires C, 60.30; H, 8.54; N, 7.03%).

Ethyl (+)-6α-hydroxy-1-phenylseleno-8β-pyrrolizidine-1-carboxylate (70) - A solution of ethyl (+)-6α-hydroxy-8β-pyrrolizidine-1α-carboxylate (68) (597 mg; 3 mmol) in tetrahydrofuran (10 ml) was added dropwise during 30 min at -78 °C to a solution of di-isopropylamine (0.71 ml; 6.48 mmol) and 1.7 M n-butyl lithium in hexane (3.87 ml; 6.48 mmol) in dry tetrahydrofuran (10 ml). After stirring at -78°C for 1 h, phenylselenenyl chloride (1.243 g; 6.48 mmol) in dry tetrahydrofuran (3 ml) was added rapidly at -78 °C. The reaction mixture was stirred at this temperature for 3 h then poured into water (50 ml) and extracted with ether (50 ml), followed by chloroform (3 x 50 ml). The solution was dried (sodium sulphate), filtered, and concentrated *in vacuo* to give a yellow oil. Preparative tlc ($CHCl_3/CH_3OH/NH_3$ 85:14:1) gave ethyl (+)-6α-hydroxy-1-phenylseleno-8β-pyrrolizidine-1-carboxylate (70) (R_f 0.45) as a pale yellow oil (639 mg; 60.1%) $[\alpha]_D^{18.4}$ +36.9°(c 4, $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 400, 1 720, and 1 580 cm^{-1}; δ ($CDCl_3$) 7.50 (5H, m, Ph), 4.40 (1H, m, H-6), 4.10 (2H, m, $C\underline{H}_2CH_3$), 3.80-1.80 (10H, complex),

and 1.20 (3H, m, CH_3); m/e 355 (M^+, 24%), 353 (12), 198 (40), 197 (24), 158 (28), 154 (33), 152 (30), 125 (15), 126 (17), 106 (40), 99 (100), and 78 (52).

Ethyl (-)-6α-hydroxy-1-hydroxymethyl-1-phenylseleno-8β-pyrrolizidine (71) - A solution of ethyl (+)-6α-hydroxy-1-phenylseleno-8β-pyrrolizidine-1-carboxylate (70) (500 mg; 1.412 mmol) in dry ether (10 ml) was added slowly to a cold (-15 °C) suspension of lithium aluminium hydride (69.2 mg; 2.82 mmol) in dry ether (10 ml) under nitrogen. The reaction mixture was stirred at -15 °C for 2 h then at room temperature for 1 h. Wet ether (10 ml) followed by 1M sodium hydroxide solution (0.5 ml) were slowly added. The resulting suspension was filtered through celite which was then washed with chloroform. The combined solutions were dried (sodium sulphate), filtered, and evaporated in vacuo to give a yellow oil. Preparative tlc ($CHCl_3/CH_3OH/NH_3$ 85:14:1) afforded ethyl (-)-6α-hydroxy-1-hydroxmethyl-1-phenylseleno-8β-pyrrolizidine (71) (253 mg; 57.5%) as crystals m.p. 148-150 °C (from chloroform) $[\alpha]_D^{18.4}$ -32.14° (c 5, $CHCl_3$) $\nu_{max.}$ ($CHCl_3$) 3 400 and 1 580 cm^{-1}; δ ($CDCl_3$) 7.50 (5H, m, Ph), 4.50 (1H, m, H-6), 4.15 (2H, s, H-9), 3.75-1.20 (11H, complex) m/e 313 (M^+, 10%), 311 (6), 156 (18), 155 (50), 139 (20), 112 (30), 99 (100), 78 (30), and 77 (16); (found: C, 53.69; H, 6.14: N, 4.20. $C_{14}H_{19}NO_2Se$ requires C, 53.85; H, 6.09; N, 4.49%).

(-)-6α-Acetoxy-1-acetoxymethyl-5,6,7,8 -tetrahydro-3H-pyrrolizine (74) - A solution of ethyl (-)-6α-hydroxy-1-hydroxymethyl-1-phenylseleno-8β-pyrrolizidine (71) (172 mg; 0.55 mmol) in acetic anhydride (6 ml) and pyridine (2 ml)

was stirred at room temperature for 16 h. The solvent was removed in vacuo to give the diacetate which without purification was treated with 27% hydrogen peroxide solution (2 ml) in acetic acid (5 ml). The reaction mixture was stirred at room temperature for 16 h then basified with saturated sodium carbonate solution to pH 10 and then extracted with chloroform (4 x 30 ml). The extracts were dried (sodium sulphate), filtered, and concentrated in vacuo to give a pale yellow oil. Preparative tlc ($CHCl_3$/CH_3OH/NH_3 85:14:1) afforded (-)-6α-acetoxy-1-acetoxymethyl-5,6,7,8-tetrahydro-3H-pyrrolizine (74) (R_f 0.28) as an oil (82.4 mg; 62.5%) $[\alpha]_D^{18.4}$ -45.0° (c 5, $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 750 and 1 230 cm^{-1}; δ ($CDCl_3$) 5.75 (1H, m, H-2), 5.50 (1H, m, H-6), 4.65 (2H, s, H-9), 4.60-4.20 (5H, complex), 3.90-2.90 (2H, m, H-7), 2.08 (3H, s, CH_3), and 2.00 (3H, s, CH_3); m/e 239 (M^+, 3%), 155 (25), 112 (25), 111 (75), 94 (30), 93 (25), and 80 (100).

The picrate had m.p. 115-117 °C (from ethanol) (Found: C, 46.06; H, 4.20; N, 11.99. $C_{18}H_{20}N_4O_{11}$ requires C, 46.15; H, 4.27; N, 11.96%).

(+)-6α-hydroxy-1α-hydroxymethyl-8β-pyrrolizidine (69) - Ethyl (+)-6α-hydroxy-8β-pyrrolizidine-1α-carboxylate (68) (119.4 mg; 0.6 mmol) in dry ether (10 ml) was added to a suspension of lithium aluminium hydride (45.6 mg; 1.2 mmol) in dry ether (10 ml) under nitrogen at room temperature. The reaction mixture was stirred at room temperature for 2 h, then wet ether (10 ml) was slowly added followed by 1M sodium hydroxide solution (0.5 ml). The ether was removed and the residue was extracted with hot chloroform (5 x 20 ml). The combined solutions were dried (sodium sulphate), filtered and evaporated in vacuo to give (+)-6α-hydroxy-1α-hydroxymethyl-8β-pyrrolizidine (69) (83.7 mg; 88.85%) as an oil $[\alpha]_D^{18.4}$

+68.2 ^{o} (c 4, methanol) $\nu_{max.}$(film) 3 350 cm^{-1}; δ (CD_3OD) 4.80 (2H, broad s, OH), 4.30 (1H, m, H-6), 3.62 (2H, d, J 7Hz, H-9), 3.50-1.20 (10H, complex); m/e 157 (M^+, 30%), 156 (25), 140 (20), 113 (40), 106 (40), 99 (55), 85 (30), 93 (40), 82 (100), and 80 (25).

The picrate had m.p. 151-152 ^{o}C (Ethanol) (Found: C, 43.20; H, 4.62; N, 14.27. $C_{14}H_{18}N_4O_9$ requires C, 43.50; H, 4.86; N, 14.50%).

Ethyl 6-chloro-8β-pyrrolizidine-1α-carboxylate (79) - Ethyl (+)-6α-hydroxy-8β-pyrrolizidine-1α-carboxylate (68) (995 mg; 5 mmol) was treated with thionyl chloride (40 ml) at 5 ^{o}C and then heated at reflux for 5 h. Excess of the reagent was evaporated *in vacuo* to give ethyl 6-chloro-8β-pyrrolizidine-1α-carboxylate HCl (1.144 g; 90.1%) as crystals m.p. 172-174 ^{o}C (from acetone) $\nu_{max.}$ (KBr) 2 515, 2 300, and 1 740 cm^{-1}; δ ($CDCl_3$) 4.90 (1H, m, H-6), 4.20 (2H, q, J 7Hz, CH_2CH_3), 4.00-3.40 (6H, complex), 2.40 (4H, complex), and 1.22 (3H, t, J 7Hz, CH_3); (Found M-Cl, 217.08693. $C_{10}H_{17}NO_2Cl$ requires M-Cl, 217.08693) m/e 254 (M^+), 219 (3), 217 (9), 182 (65), 174 (8), 172 (24), 136 (24), 115 (29), 117 (88), 108 (33), 106 (33), 82 (100), and 80 (40).

Ethyl (+)-8β-pyrrolizidine-1αcarboxylate (80) - The hydrochloride salt of ethyl 6-chloro-8β-pyrrolizidine-1α-carboxylate (79) (635 mg; 2.5 mmol) was hydrogenated at 15torr in ethanol using Raney-nickel as catalyst at room temperature. When hydrogen uptake ceased, the solution was filtered through Celite and the filtrate was concentrated *in vacuo* to give an oil which was basified with saturated sodium carbonate solution (20 ml) and

extracted with chloroform (3 x 50 ml). The chloroform extracts were dried (sodium sulphate), filtered and evaporated *in vacuo* to give ethyl (+)-8β-pyrrolizine-1α-carboxylate (80) (413 mg; 90.3%) as an oil $[\alpha]_D^{18.4}$ +61.24 ° (c 5, ethanol) $\nu_{max.}(CCl_4)$ 1 735 and 1 250 cm^{-1}; δ ($CDCl_3$) 4.15 (2H, q, J 7 Hz, CH_2CH_3), 3.75 (1H, q, J 7Hz, H-8), 3.30-1.80 (11H, complex), and 1.25 (3H, t, J 7 Hz, CH_3); m/e 183 (M^+, 25%), 138 (30), 136 (22), 134 (15), 110 (16), 109 (18), 108 (30), 106 (18), 83 (100), 82 (60), and 80 (15).

The picrate had m.p. 111-112 °C (ethanol) (Found: C, 46.4; H, 4.74; N, 13.3. $C_{16}H_{20}N_4O_9$ requires C, 46.60; H, 4.85; N, 13.59%)

(+)-Isoretronecanol (76) - A solution of ethyl (+)-8β-pyrrolizidine-1α-carboxylate (80) (183 mg; 1 mmol) in dry ether (10 ml) was added to a suspension of lithium aluminium hydride (76 mg; 2 mmol) in dry ether (10 ml) under nitrogen. The reaction mixture was stirred at room temperature for 2 h, then wet ether (10 ml) and 1N sodium hydroxide solution (0.3 ml) were added slowly. The resulting suspension was filtered through Celite, dried (sodium sulphate), filtered, and evaporated *in vacuo* to give (+)-isoretronecanol (76) (132 mg; 93.6%) as an oil $[\alpha]_D^{25}$ +70.24 ° (c 5, Ethanol) (lit.[63] $[\alpha]_D$ +79.1°(ethanol) and[64] $[\alpha]_D^{20}$ +71.7 ° (c 1.04 in ethanol)) $\nu_{max.}(CHCl_3)$ 3 350 cm^{-1}; δ ($CDCl_3$) 4.32 (1H, br s, OH), 3.62 (2H, d, J 7Hz, H-9), 3.20-1.50 (12H, complex); m/e 141 (M^+, 35%), 140 (20), 124 (30), 113 (7), 110 (30), 108 (22), 107 (7), 98 (8), 97 (10), 95 (10), 83 (35), 83 (100), 82 (76), and 80 (30). The picrate had m.p.193-194°C (lit.,[63] 193-194°C) Mixed m.p. with authentic sample (supplied by Dr C.C.J.Culvenor) was undepressed. I.r. spectra of both samples were almost identical (Found: C, 45.30; H, 4.77; N, 14.85. $C_{14}H_{18}N_4O_8$ requires C, 45.40; H, 4.86; N, 15.13%).

Ethyl (+)-1-phenylseleno-8β-pyrrolizidine-1-carboxylate (83) - A solution of ethyl (+)-8β-pyrrolizidine-1α-carboxylate (80) (549 mg; 3 mmol) in dry tetrahydrofuran (3 ml) was added dropwise during 1 h at -78 °C to a solution of lithium di-isopropylamide [prepared from di-isopropylamine (0.46 ml; 4.32 mmol) and 1.7M n-butyl lithium in hexane (2.58 ml; 4.32 mmol)] in dry tetrahydrofuran (15 ml) under nitrogen. The solution was stirred at -78 °C for 1 h, then phenylselenenyl chloride (720 mg; 3.75 mmol) in dry tetrahydrofuran (5 ml) was added rapidly and the mixture was stirred for a further 3 h at -78 °C. The reaction mixture was poured into water (25 ml) then extracted with ether (60 ml) and chloroform (3 x 60 ml). The organic extracts were dried (sodium sulphate) filtered, and the solvent removed *in vacuo* to yield a yellow oil containing one major product (R_f 0.55) ($CHCl_3/CH_3OH/NH_3$ 85:14:1). Preparative tlc afforded ethyl (+)-1-phenylseleno-8β-pyrrolizidine-1-carboxylate (83) (628 mg; 61.9%) as a pale yellow oil $[\alpha]_D^{18.4}$ +42.1 ° (c 5, $CHCl_3$) $\nu_{max.}$ (CCl_4) 1 720 and 1 580 cm^{-1}; δ ($CDCl_3$) 7.40 (5H, m, Ph), 4.10 (2H, m, $C\underline{H}_2CH_3$) 3.80-1.90 (11H, complex), and 1.15 (3H, m, CH_3) (Found: M^+, 339.07322. $C_{16}H_{21}NO_2Se$ requires M, 339.07371); m/e 339 (M^+, 30%), 337 (15), 182 (70), 180 (35), 157 (45), 154 (70), 136 (38), 109 (45), 108 (75), 84 (38), 83 (100), and 80 (45).

(-)-1-Hydroxymethyl-1-phenylseleno-8β-pyrrolizidine (84) - A solution of ethyl (+)-1-phenylseleno-8β-pyrrolizidine-1-carboxylate (83) (508.5 mg; 1.5 mmol) in dry ether (10 ml) was slowly added to a cooled (-15 °C) suspension of lithium aluminium hydride (114 mg; 3 mmol) in dry ether (10 ml) under nitrogen. The reaction mixture was stirred for 2 h at -15 °C and 1 h at

room temperature. Excess lithium aluminium hydride was destroyed by the slow addition of wet ether (15 ml) and 1M sodium hydroxide solution (0.5 ml). The resulting suspension was filtered through Celite, which was washed with chloroform. The combined organic extracts were dried (sodium sulphate), filtered and concentrated in vacuo to give a pale yellow oil containg one major component R_f 0.25 ($CHCl_3/CH_3OH/NH_3$ 85:14:1). Preparative tlc afforded (-)-hydroxymethyl-1-phenylseleno-8β-pyrrolizidine (84) (271 mg; 60.8%) as crystals m.p. 142-144 °C (from chloroform) $[\alpha]_D^{18.4}$ -22.87° (c 3, $CHCl_3$) $\nu_{max.}$ (KBr) 3 420 and 1 440 cm^{-1}; δ ($CDCl_3$) 7.55-7.30 (5H, m, Ph), 3.70-2.80 (8H, complex), and 1.90 (6H, m); m/e 297 (M^+, 10%), 141 (25), 140 (65), 139 (10), 122 (45), 122 (45), 120 (25), 108 (40), 83 (100), 80 (30), 78 (40), and 77 (40); (Found: C, 56.77; H, 6.54; N, 5.13. $C_{14}H_{19}NOSe$ requires C, 56.75; H, 6.42; N, 4.73%).

(+)-Supinidine - (-)-1-Hydroxymethyl-1-phenylseleno-8β-pyrrolizidine (84) (200 mg, 0.67 mmol) in acetic acid (5 ml) was mixed with 27% hydrogen peroxide (2 ml) and stirred at 0°C for 1 h, then at room temperature for 12 h. After basification (pH 10), the mixture was extracted with chloroform (4 x 30 ml). The extracts were dried (potassium carbonate), filtered, and evaporated in vacuo to give a pale yellow oil containing one major component R_f 0.55 ($CHCl_3$:CH_3OH:NH_3 5:4:1). Preparative tlc afforded (+)-supinidine (82) (51 mg, 54.7%) as an oil $[\alpha]_D^{18}$ +7.60 ° (c 3, EtOH) [lit.[64], $[\alpha]_D^{20}$ + 9.2 ° (c 2.07, EtOH)] $\nu_{max.}$ ($CHCl_3$) 3 320, 1 600, and 1 450 cm^{-1}; δ ($CDCl_3$) 5.50 (1H, m, H-2), 5.20 (1H, s, OH), 4.18 (2H, s, H-9), 4.35 (1H, m, H-8), 3.90-3.45 (2H, m, H-3), 3.30-2.60 (2H, m, H-5), 2.10-1.50 (4H, complex, H-6 and H-7); m/e 139 (M^+, 48%), 138 (16),

122 (44), 120 (24), 111 (20), 110 (16), 108 (42), 94 (16), 93 (16), 91 (8), and 80 (100). The i.r.,n.m.r., and mass spectra were in accord with the reported values[63] for (±)-supinidine. The picrate had m.p. 144-145 °C (EtOH) (lit.,[64] 144-145 °C) (Found: C, 45.83; H, 4.35; N, 15.22. $C_{14}H_{16}N_4O_8$ requires C, 45.65; H, 4.35; N, 15.22%). The mixed m.p. was undepressed with an authentic sample supplied by Dr C.C.J.Culvenor. The i.r. spectra of both samples were identical (p 79).

Ethyl (+)-8β-pyrrolizidine-1β-carboxylate (81) - A solution of ethyl (+)-8β-pyrrolizidine-1α-carboxylate (80) (183 mg, 1 mmol) in absolute ethanol (2 ml) was added to a solution of sodium (35 mg, 1.5 mmol) in absolute ethanol (5 ml). After 12 h at 60 °C the mixture was cooled and poured into cold 1M hydrochoric acid (5 ml). The pH of the solution was adjusted to 2 and it was then washed with chloroform (4 ml). The aqueous layer was basified and the epimerisation product was extracted with chloroform (4 x 15 ml). The combined chloroform extracts were dried (sodium sulphate), filtered and evaporated in vacuo to give ethyl (+)-8β-pyrrolizidine-1β-carboxylate (81) (125 mg, 68.3%) as an oil. $[\alpha]_D^{23}$ + 39.1° (c 3, chloroform) $\nu_{max.}$ 1 735 and 1 180 cm^{-1}; δ ($CDCl_3$) 4.20 (2H, q, J 7 Hz, CH_2), 3.70 (1H, m, H-8), 3.20-1.80 (11H, complex), 1,25 (3H, t, J 7 Hz, CH_3); m/e 183 (M^+, 22%), 154 (20), 138 (20), 136 (20), 134 (14), 117 (18), 108 (26), 106 (46), 83 (100), and 82 (60).

The picrate had m.p. 179-181 °C (EtOH) (Found: C, 46.52; H, 4.87; N, 13.49. $C_{16}H_{20}N_4O_9$ requires C, 46.60; H, 4.85; N, 13.59%)

Laburnine (32) - A solution of ethyl (+)-8β-pyrrolizidine-1β-carboxylate (81) (91.5 mg, 0.5 mmol) in dry ether (7 ml) was added to a suspension of lithium aluminium hydride (38 mg, 1 mmol) in dry ether (7 ml) under nitrogen. The reaction mixture was stirred at room temperature for 2 h, then wet ether (6 ml) and 1M sodium hydroxide solution (0.1 ml) were slowly added. The resulting suspension was filtered through celite, dried (sodium sulphate), filtered and evaporated in vacuo to give laburnine (32) (65 mg, 92.2%) as an oil. $[\alpha]_D^{22}$ + 14.6° (c 3.25, EtOH) (Lit.,[35] $[\alpha]_D$ + 15.4° (c 1.44, EtOH)) $\nu_{max.}$ ($CHCl_3$) 3 350 cm^{-1}; δ ($CDCl_3$) 3.90 (1H, br s, OH), 3.65 (2H, d, J 7 Hz, H-9), 3.20-1.60 (12H, complex); m/e 141 (M^+, 28%), 140 (28), 124 (40), 110 (28), 97 (18), 96 (20), 83 (100), and 82 (80).

The picrate had m.p. 174-175 °C (lit.,[35] m.p. 174-175 °C) (Found: C, 45.68; H, 4.65; N, 14.82. $C_{14}H_{18}N_4O_8$ requires C, 45.40; H,4.86; N, 15.13%).

Ethyl (+)-2α-O-tosyl-2,3-dihydro-1H-pyrrolizine-7-carboxylate (85) - Ethyl 2α-hydroxy-2,3-dihydro-1H-pyrrolixine-7-carboxylate (8) (195 mg, 1 mmol) in dry pyridine (5 ml) was treated with a solution of p-toluenesulphonyl chloride (572 mg, 3 mmol) in pyridine (2 ml). The reaction mixture was stirred at room temperature for 18 h, then sufficient 1M hydrochloric acid was added to make the reaction mixture slightly acidic. The mixture was extracted with chloroform (3 x 50 ml). The chloroform extracts were washed with 1M hydrochloric acid and brine and dried (sodium

sulphate), filtered and concentrated in vacuo to give ethyl (+)- 2α-O-tosyl-2,3-dihydro-1H-pyrrolizine-7-carboxylate (85) (318mg, 91%) as crystals m.p. 123-125 °C (from ether-petrol) $[\alpha]_D^{25}$ + 28.82°(c 5, $CHCl_3$) (Found: C, 58.63; H, 5.25; N, 4.00; S, 9.40. $C_{17}H_{19}NO_5S$ requires C, 58.45; H, 5.44; N, 4.01; S, 9.17%) $\nu_{max.}$(KBr) 1 705, 1695, 1 600, 1 570, and 1 255 cm^{-1}; δ ($CDCl_3$) 7.77,7.36 (4H, AB q, J 9 Hz, Ar H's), 6.60,6.50 (2H, ABq, H-5,6), 5.55 (1H, m, H-2), 4.20 (4H, m, H-3 and $CO_2\underline{CH}_2CH_3$), 3.25 (2H, m, H-1), 2.45 (3H, s, Ar-CH_3), and 1.25 (3H, t, J 7 Hz, CH_3); m/e 349 (M^+, 8%), 304 (10), 191 (12), 177 (100), 149 (30), 148 (18), 132 (32), 118 (12), 105 (22), 104 (70), and 91 (35). $\lambda_{max.}$(EtOH) 252 nm, ε 15, 350.

Ethyl (-)-2β-O-formyl-2,3-dihydro-1H-pyrrolizine-7-carboxylate (86) - Tetraethylammonium formate (523 mg, 3.0 mmol) was added to ethyl (+)-2α-O-tosyl-2,3-dihydro-1H-pyrrolizine-7-carboxylate (85) (174.5 mg, 0.5 mmol) in dry acetone (10 ml). The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated in vacuo. Water (10 ml) was added to the residue and this was then extracted with chloroform (2 x 20 ml). The chloroform extracts were washed with water (2 x 20 ml) and saturated sodium bicarbonate solution (20 ml), dried (sodium sulphate), filtered and evaporated in vacuo to give a brown oil containing one major component, R_f 0.33 ($CHCl_3/Et_2O$ 1:1). Preparative tlc afforded ethyl (-)-2β-O-formyl-2,3-dihydro-1H-pyrrolizine-7-carboxylate (86) (94 mg, 84.3%) as needles, m.p. 62-63 °C (from ether) $[\alpha]_D^{23}$ - 34.2° (c 4, $CHCl_3$) $\nu_{max.}$(CCl_4) 1 735, 1 715, 1 620, and 1 580 cm^{-1}; δ ($CDCl_3$) 8.00 (1H, s, CHO), 6.60, 6.50 (2H, ABq, J 3Hz, H-5,6), 5.85 (1H, m, H-2), 4,20 (4H, m, H-3 and $CO_2\underline{CH}_2CH_3$), 3.30 (2H, m, H-1),

and 1.28 (3H, t, J 7 Hz, CH_3); m/e 223 (M^+, 16%), 179 (24). 178 (60), 148 (30), 149 (22), 131 (65), 102 (30), and 103 (100). (Found: C, 59.26; H, 6.1; N, 6.22. $C_{11}H_{13}NO_4$ requires C, 59.19; H, 5.8; N, 6.28%).

8.4 EXPERIMENTAL TO CHAPTER 5

8.4.1 Attempts to introduce O substituents at the 1-position of 2,3-dihydro-1H-pyrrolizine derivatives.

Ethyl 5,6-dibromo-2,3-dihydro-1H-pyrrolizine-7-carboxylate (96) - Benzoyl peroxide (100 mg) was added to a vigorously stirred solution of ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate (7) (3.222 g; 18 mmol) and N-bromosuccinimide (6.408 g; 36 mmol) in carbon tetrachloride (120 ml). The reaction mixture was stirred at room temperature for 3 h, then animal charcoal was added to the mixture which was then filtered through Celite. The solvent was removed *in vacuo* to give ethyl 5,6-dibromo-2,3-dihydro-1H-pyrrolizine-7-carboxylate (96) (4.587 g; 75.6%) as needles, m.p. 117-118 °C (from hexane) (Found: C, 35.42; H, 3.52; N, 4.40; Br, 47.1. $C_{10}H_{11}NO_2Br_2$ requires C, 35.61; H, 3.26; N,4.15; Br, 47.4%) $\nu_{max.}$(KBr) 1 710, 1 660, 1540, and 1 210 cm^{-1}; δ ($CDCl_3$) 4.35 (2H, q, J 7Hz, $C\underline{H}_2CH_3$), 4.00 (2H, t, J 7Hz, H-3), 3.20 (2H, t, J 7 Hz, H-1), 2.50 (2H, m, H-2), and 1.35 (3H, t, J 7Hz, CH_3); m/e 337 (M^+, 10%), 335 (5), 292 (20), 122 (45), 105 (72), 99 (100), 80 (88), and 70 (88).

Attempted 'Benzylic' Bromination of Ethyl 5,6-dibromo-2,3-dihydro-1H-pyrrolizine-7-carboxylate (96) - Benzoyl peroxide (10 mg) was added to a vigorously stirred solution of ethyl 5,6-dibromo-2,3-dihydro-1H-pyrrolizine-7-carboxylate (96) (50 mg; 0.15 mmol) and N-bromosuccinimide (26.7 mg; 0.15 mmol) in dry carbon tetrachloride (10 ml). The reaction mixture was stirred at room temperature for 6 h, filtered through Celite, and the solvent was removed *in vacuo* to give a mixture of products which could not be identified.

Attempted 'Benzylic' Chlorination of Ethyl 5,6-dibromo-2,3-dihydro-1H-pyrrolizine-7-carboxylate (96) - Ethyl 5,6-dibromo-2,3-dihydro-1H-pyrrolizine-7-carboxylate (96) (168.5 mg; 0.5 mmol) in glacial acetic acid (10 ml) was heated to 50-60 °C during the addition of sulphuryl chloride (67.5 mg; 0.5 mmol) in glacial acetic acid (1 ml) with stirring. The reaction mixture was heated at 70 °C for a further 30 min under nitrogen. The solvent was removed *in vacuo* to give a residue which was dissolved in chloroform (20 ml), washed with saturated sodium bicarbonate solution (3 x 20 ml), dried (sodium sulphate), filtered, and evaporated *in vacuo* to give a mixture of products that could not be identified.

Attempted 'Benzylic' Oxidation of Ethyl 5,6-dibromo-2,3-dihydro-1H-pyrrolizine-7-carboxylate (96) - Ethyl 5,6-dibromo-2,3-dihydro-1H-pyrrolizine-7-carboxylate (96) (197.5 mg; 0.5 mmol) in acetic acid (5 ml) was added dropwise to a stirred solution of lead tetraacetate (445 mg, 1 mmol) in acetic acid (10 ml) under nitrogen. The reaction mixture was heated at 80 °C for 1 h. The solvent was removed *in vacuo* to give a residue which was dissolved in chloroform (30 ml) and washed with saturated sodium bicarbonate solution (3 x 20 ml). The chloroform extract was dried (sodium sulphate), filtered, and concentrated *in vacuo* to give a brown oil. Separation by preparative tlc (chloroform/ether 1:1) gave two unstable components which could not be identified.

Attempted 'Benzylic' Oxidation of Ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate (7) with CrO_3/pyridine complex - A slurry of CrO_3/pyridine complex[71] (3.5g; 13.5 mmol) in methylene choride (10 ml) was added to a solution of ethyl 2,3-dihydro-1H-

pyrrolizine-7-carboxylate (7) (179 mg; 1 mmol) in methylene choride (30 ml). The reaction mixture was stirred at room temperature for 24 h. Ether (50 ml) was added and the resulting solution was washed with saturated sodium bicarbonate solution (6 x 50 ml), 5% hydrochoric acid (3 x 40 ml), 5% sodium bicarbonate solution (40 ml), and saturated sodium chloride solution (40 ml), dried (sodium sulphate), filtered, and evaporated *in vacuo* to give mainly starting material.

Attempted 'Benzylic' Oxidation of Ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate (7) with selenium dioxide - Powdered sublimed selenium dioxide (333 mg; 3 mmol) was added to a solution of ethyl 2,3-dihydro-1H-pyrrolizine-7-carboxylate (7) (179 mg; 1 mmol) in acetic acid (10 ml) and acetic anhydride (2 ml). The reaction mixture was heated under reflux for 2 h, cooled and filtered. The solvent was removed *in vacuo* to give an oil which was dissolved in chloroform (20 ml). The resulting solution was washed with saturated sodium bicarbonate solution (3 x 15 ml), dried (sodium sulphate), filtered and concentrated *in vacuo* to give mainly starting material.

8.4.2 Synthesis of cis- and trans-3-hydroxyproline (87)[68]

Ethyl 3-aminopropionate hydrochloride - β-Alanine (89.1 g, 1 mol) was dissolved in absolute alcohol (900 ml) and cooled to -5 °C. Thionyl chloride (238 g; 2 mol) was added slowly over a period of 1 h, and the reaction mixture was heated under reflux for 4 h. Dry ether was added to the reaction mixture until turbidity appeared. The mixture was kept at 0 °C

for a few h, during which time ethyl 3-aminopropionate hydrochloride crystallized (145 g, 94.5%) m.p. 68–70 °C (lit.,[84] 69–70 °C) $\nu_{max.}$(KBr) 3 350, 3 000, 1 730, 1 580, 1 500, and 1 210 cm^{-1}; δ (CD_3OD) 4.10 (2H, q, J 7Hz, $C\underline{H}_2CH_3$), 3.20 (2H, m, NCH_2), 2.80 (2H, t, J 7Hz, $C\underline{H}_2CO_2Et$), and 1.20 (3H, t, J 7Hz, CH_3); m/e 117 (M–HCl, 92%), 88 (42), 72 (100), 70 (94), and 60 (46).

N-Cyanomethyl-β-alanine ethyl ester – A solution of ethyl 3-aminopropionate hydrochloride (122.8 g; 0.8 mol), triethylamine (161.6 g; 1.6 mol) and chloroacetonitrile (60.4 g; 0.8 mol) in absolute ethanol (900 ml) was stirred at room temperature for 48 h. The solvent was removed in vacuo and dry ether (500 ml) was added to the residue. The triethylamine hydrochloride was removed by filtration and the ether solution was washed with water (2 x 400 ml), dried (sodium sulphate), and concentrated in vacuo to give an oil. Distillation at 98–99 °C/0.5 mm Hg (lit.,[85] b.p. 98–99 °C/0.5 mm Hg) yielded N-cyanomethyl-β-ananine ethyl ester (89 g, 71.3%) as an oil; $\nu_{max.}$(CCl_4) 3 340, 2 230, and 1 730 cm^{-1}; δ ($CDCl_3$) 4.20 (2H, q, J 7 Hz, $C\underline{H}_2CH_3$), 3.60 (2H, s, CH_2CN), 3.00 (2H, m, NCH_2), 2.50 (2H, t, J 7 Hz, $C\underline{H}_2CO_2Et$), and 1.20 (3H, t, J 7Hz, CH_3); m/e 156 (M^+, 12%), 129 (12), 116 (30), 111 (12), 109 (9), 102 (5), 100 (9), 99 (5), 88 (5), 84 (14), 82 (9), and 69 (100).

N-Cyanomethyl-N-ethoxycarbonyl-β-alanine ethyl ester

Ethylchloroformate (52.1 g; 0.48 mol) was added dropwise to a mixture of N-cyanomethyl-β-alanine ethyl ester (75 g; 0.48 mol) and sodium carbonate (50.1 g; 0.48 mol) in water (300 ml) with cooling in an ice bath. The mixture was stirred vigorously at room temperature for 3 h. Water (200 ml) was added and the mixture

was extracted with chloroform (3 x 600 ml). The chloroform extracts were dried (sodium sulphate), filtered and concentrated *in vacuo* to give N-cyanomethyl-N-ethoxycarbonyl-β-alanine ethyl ester (96.6 g; 91.4%) b.p. 120 °C/0.5 mm Hg (lit.,[68] b.p. 128–130 °C/0.8 mm Hg) as an oil; $\nu_{max.}$ (CCl_4) 2 230 and 1 720 cm^{-1}; δ ($CDCl_3$) 4.32 (2H, s, CH_2CN), 4.18 (4H, m, $C\underline{H}_2CH_3$), 3.60 (2H, t, J 7Hz, NCH_2), 2.65 (2H, t, J 7Hz, $C\underline{H}_2CO_2Et$), and 1.20 (6H, m, CH_3); m/e 228 (M^+, 30%), 183 (80), 182 (100), 155 (50), 154 (50), 141 (70), 140 (30), 128 (18), 116 (18), 109 (50), 100 (25), 82 (35), and 69 (90).

2-Cyano-1-ethoxycarbonyl-3-pyrrolidone (90) - N-Cyanomethyl-N-ethoxycarbonyl-β-alanine ethyl ester (60.2 g; 0.264 mol) in toluene (400 ml) was added over a period of 1 h to a mixture of potassium t-butoxide (29.6 g, 0.264 mol) in toluene (1000 ml) at 0 °C under nitrogen. The mixture was stirred for 2 h and then extracted with ice-cold phosphate buffer (pH 7.1) (1 x 800 ml, 1 x 400 ml, 2 x 100 ml). The combined aqueous extracts were acidified to pH 3.8 and extracted with chloroform (1 x 800 ml, 5 x 100 ml). The combined chloroform extracts were dried (sodium sulphate), filtered and concentrated *in vacuo* to give 2-cyano-1-ethoxycarbonyl-3-pyrrolidinone (90) (32.65 g; 68%) which was used in the next step without further purification; $\nu_{max.}$($CHCl_3$) 2 300 and 1 715 cm^{-1}; δ ($CDCl_3$) 4.90 (1H, s, H-2), 4.30 (2H, q, J 7 Hz, $C\underline{H}_2CH_3$), 4.00 (2H, t, J 7 Hz, H-5), 2.90 (2H, t, J 7 Hz, H-4), and 1.30 (3H, t, J 7 Hz, CH_3); m/e 182 (M^+, 100%), 154 (100), 140 (60), 137 (60), 110 (22), 100 (22), 83 (45), and 81 (70).

2-Cyano-1-ethoxycarbonyl-3-pyrrolidinol - 2-Cyano-1-ethoxycarbonyl-3-pyrrolidinone (90) (22.2 g) in ethanol (200 ml)

was rapidly added to a solution of sodium borohydride (37 g) and dipotassium hydrogen phosphate (24 g) in water (400 ml) at 5 °C. The mixture was stirred at 5–10 °C for 10 min, then 1M dilute sulphuric acid was added to pH 2.5. The solution was stirred for 5 min then neutralised to pH 6 and concentrated *in vacuo* to 400 ml. The solution was extracted with chloroform (3 x 400 ml) and the extracts dried (sodium sulphate), filtered and concentrated *in vacuo* to give 2-cyano-1-ethoxycarbonyl-3-pyrrolidinol (20.82 g; 92.7%) as a pale yellow oil which was used in the next step without further purification; $\nu_{max.}(CCl_4)$ 3 460, 2 220, and 1 715 cm^{-1}; δ ($CDCl_3$) 4.80 (1H, m, H-3), 4.30 (1H, m, H-2), 4.20 (2H, q, J 7 Hz, CH_2CH_3), 3.65 (2H, t, J 7 Hz, H-5), 2.20 (2H, m, H-4), and 1.30 (3H, t, J 7 Hz, CH_3); m/e 184 (M^+, 23%), 166 (58), 140 (26), 121 (15), 107 (25), 94 (100), 93 (78), and 83 (45).

Cis- and *trans*-3-hydroxyproline – A mixture of 2-cyano-1-ethoxycarbonyl-3-pyrrolidinol (10.5 g, 57 mmol) and barium hydroxide (55.8 g, 295 mmol) in water (700 ml) was boiled under nitrogen for 46 h. The mixture was cooled, filtered and the filtrate neutralised with sulphuric acid then filtered through celite. The filtrate was concentrated *in vacuo* to about 20 ml and then applied to a column of Dowex 50W-X8 (H^+ form) (50 g). The column was washed thoroughly with water until the eluate was neutral and the the amino acid was eluted with 2M ammonium hydroxide solution. Removal of the solvent *in vacuo* gave *cis*- and *trans*-3-hydroxyproline (87) as a brown oil. Recrystallisation of this mixture of isomers from ethanol/water gave off-white crystals (2.2 g, 29%), m.p. 230–233 °C, $\nu_{max.}$(KBr) 3 310, 3 025, 2 710, 1 620, and 1 580 cm^{-1}; δ (D_2O)

5.00 (1H, m, H-3), 4.45 (1H, t, J 5 Hz, H-2), 3.85 (2H, m, H-5), and 2.45 (2H, m, H-4); m/e 131 (M^+, 5%), 87 (45), 86 (100), 74 (25), 69 (55), 68 (20), 59 (20), 58 (22), 57 (25), and 41 (65); (Found: C, 45.69; H, 6.91; N, 10.56. $C_5H_9NO_3$ requires C, 45.80; H, 6.87; N, 10.69%).

Cis- and trans-N-Formyl-3-formyloxyproline (91) - Formic acid (6.9 g, 0.15 mol) and acetic anhydride (7.6 g, 0.075 mol) were stirred at room temperature for 1 h. Cis and trans 3-hydroxyproline (1 g, 0.0075 mol) were added at room temperature, and stirred for 12 h. The reagents were removed by evaporation in vacuo to give cis- and trans-N-formyl-3-formyloxyproline (91) in quantitative yield as a colourless viscous oil $\nu_{max.}$ (nujol) br. ca. 3 000, 1 735, and 1 650 cm^{-1}. δ (CD_3OD) 8.40 (1H, s, OCHO), 8.10 (1H, s, NCHO), 5.80 (1H, m, H-3), 4.80 (1H, m, H-2), 3.80 (2H, m, H-5), and 2.20 (2H, m, H-4); (Found: M^+, 187.04803. $C_7H_9NO_5$ requires M, 187.048066) m/e 187 (M^+, 7%), 159 (8), 156 (8), 142 (40), 141 (22), 115 (50), 114 (90), 113 (50), 99 (90), 98 (60), 86 (60), and 63 (100).

Attempted 1,3-dipolar cycloaddition of cis-and trans-N-formyl-3-formyloxyproline (91) to ethyl propiolate - A solution of cis and trans N-formyl-3-formyloxyproline (91) (748 mg, 4 mmol) and ethyl propiolate (1.96 g, 20 mmol) in acetic anhydride (8 ml) was heated at 135 °C for 6 h under nitrogen. Excess reagents were removed by evaporation in vacuo to give a residue which was separated by preparative t.l.c. ($CHCl_3$:Et_2O, 60:40) to give three components, none of which could be identified.

A variety of reaction conditions was investigated

using the above reactants.

(a) Reactants were left at room temperature overnight to give mainly starting materials.

(b) Reactants were heated at 80 °C for 24 h to give a mixture of unidentifiable products and starting materials.

(c) Reactants were heated at 135 °C for 3 h to give a similar mixture as in (b).

8.5 EXPERIMENTAL TO CHAPTER 6

4-Hydroxy-4-methylhepta-1,6-diene (100) - Allyl bromide (30.25 g; 0.25 mol) in ether (10 ml) was added in portions over a period of 30 min to magnesium (6.0 g; 0.25 mol) and iodine (0.1 g) in ether (80 ml). The reaction mixture was heated at reflux for 30 min. Dry ethyl acetate (8.8 g, 0.1 mol) was added during 30 min and the mixture was allowed to stand at room temperature for 30 min. The reaction mixture was poured into ice-cold water (100 ml) and the precipitate dissolved by adding 20% sulphuric acid (20 ml). The aqueous layer was extracted with ether (3 x 100 ml) and the combined organic layers were dried (sodium sulphate), filtered and concentrated *in vacuo* to give an oil that was distilled at 60 °C/15 mm (lit.,[75] b.p. 56-57 °C/14 mm Hg) to give 4-hydroxy-4-methylhepta-1,6-diene (100) (7.68 g; 61.74%) $\nu_{max.}$ (film) 3 410 and 1 640 cm^{-1}; δ ($CDCl_3$) 6.00-5.00 (6H, complex, olefinic H's), 2.30 (2H, s, CH_2), 2.10 (2H, s, CH_2), and 1.10 (3H, s, CH_3).

3-Hydroxy-3-methylglutaric acid (dicrotalic acid) (98) - A solution of 4-hydroxy-4-methylhepta-1,6-diene (100) (6.30 g; 50 mmol) in dry ethyl acetate (120 ml) was treated with ozone at -78 °C until a blue colour persisted in the reaction mixture. When the reaction mixture had reached room temperature excess ozone was removed by passage of nitrogen until a negative peroxide test was obtained with starch-potassium iodide paper. Evaporation of the solvent *in vacuo* gave a viscous oil which was dissolved in 98% formic acid (100 ml) and 27% hydrogen peroxide (40 ml). A vigorous reaction occurred on gentle warming. After

the spontaneous reaction had ceased (30–40 min), the reaction mixture was heated under reflux for 30 min. The solvent was removed *in vacuo* to give 3-hydroxy-3-methylglutaric acid (98) in quantitative yield as crystals m.p. 108–109 °C (ether/petrol b.p. 40–60 °C) (Lit.,[74,75] m.p. 108–109 °C) $\nu_{max.}$(nujol) 3 250 and 1 700 cm^{-1}; δ [$(CD_3)_2CO$] 8.20 (2H, br s, CO_2H), 2.70 (4H, s, CH_2), and 1.40 (3H, s, CH_3); m/e 162 (M^+, 5%), 144 (20), 126 (75), 100 (100), 84 (55), and 82 (100).

3-Acetoxy-3-methylglutaric anhydride (101) - A solution of 3-hydroxy-3-methylglutaric acid (98) (510 mg; 5 mmol) in acetic anhydride (10 ml) was heated under reflux for 1 h. The solvent was removed *in vacuo* to give 3-acetoxy-3-methylglutaric anhydride (101) (565.2 mg, 60.3%) as prisms, m.p. 84–85 °C (from ether) (lit.,[74] m.p. 85 °C); $\nu_{max.}$(nujol) 1 810, 1 760, and 1 280 cm^{-1}; δ ($CDCl_3$) 3.50–2.80 (4H, AB q, J 18 Hz, CH_2), 2.10 (3H, s, CH_3CO), and 1.70 (3H, s, CH_3); m/e 186 (M^+, 5%), 144 (10), 142 (18), 100 (40), 82 (100), 72 (50), 58 (50), and 55 (80).

3-Hydroxy-3-methylglutaric anhydride (102) - 3-Hydroxy-3-methyl glutaric acid (98) (810 mg; 5 mmol) was dissolved in dry ether (50 ml) and thionyl chloride (2.38 g; 20 mmol) was added. The reaction mixture was stirred at room temperature for 16 h. The solvent was removed *in vacuo* to give 3-hydroxy-3-methylglutaric anhydride (102) (640 mg; 89%) as needles, m.p. 97–99 °C (from dry acetone/ether) (Found: C, 50.25; H, 5.73. $C_6H_8O_4$ requires C, 50.00, H, 5.60%) $\nu_{max.}$(nujol) 3 550, 1 810, and 1 750 cm^{-1}; δ [$(CD_3)_2CO$] 5.10 (1H, s, OH), 2.97 (4H, s, CH_2), and 1.50 (3H, s, CH_3); m/e 144 (M^+, 5%), 129 (20), 100 (30), 85 (50), 72 (100), 57 (100), and 55 (80).

(+)-Retronecine (40) - Retronecine was prepared by hydrolysis of the naturally occurring retrorsine* (4) with barium hydroxide as reported by Crout and Hoskins[43] and purified by sublimation at 90 °C/0.05 mm Hg to give crystals m.p. 118–120 °C (lit.,[43] 120–121 °C from acetone) $\nu_{max.}$(KBr) 3 320 and 1 660 cm^{-1}; δ (CD_3OH) 5.72 (1H, m, H-2), 4.30 (3H, m, H-7, H-9), 3.90–3.50 (3H, complex, H-3, H-8), 3.30–2.80 (2H, complex, H-5), 2.10–1.80 (2H, complex, H-6); m/e 155 (M^+, 25%), 111 (60), 94 (20), 93 (18), 82 (18), 81 (20), and 80 (100).

Attempted esterification of retronecine (40) with 3-hydroxy-3-methylglutaric Acid (98) - A mixture of 3-hydroxy-3-methylglutaric acid (98) (16.2 mg; 0.1 mmol) and N,N'-carbonyldiimidazole (32.4 mg; 0.2 mmol) in dry chloroform (10 ml) was stirred for 1 h (CO_2 off) then a solution of retronecine (15.5 mg; 0.1 mmol) in dry chloroform (2 ml) was added. The reaction mixture was stirred at R.T. and the reactionwas followed by tlc ($CHCl_3$/CH_3OH/NH_3 85:14:1); aliquots were withdrawn at 2 h, 4 h, 8 h, 16 h, 32 h and 48 h. The results were inconclusive.

A variety of reaction conditions was investigated using the above reactants:

(a) Reactants were heated at 60 °C for 24 h.

(b) A catalytic amount of sodium ethoxide was added and the reaction stirred at room temperature for 24 h.

(c) As for (b) but the reaction was heated at 60 °C for 24 h.

All these reactions gave complex mixtures of products that could not be separated by silica gel or cellulose layer chromatography using a range of solvent systems.

* We thank Dr D.H.G.Crout, Dept of Chemistry, University of Exeter for a generous sample of retrorsine.

Esterification of (+)-retronecine with Dicrotalic anhydride (102) - Retronecine (40) (50.375 mg, 0.325 mmol) was dissolved in dry acetone (10 ml) and then dicrotalic anhydride (102) (46.8 mg, 0.325 mmol) was added, and the solution was stirred at room temperature for 24 h. The acetone solvent was removed to leave a viscous oil which was washed by extraction with acetone 3 times. The residual solvent was removed *in vacuo* to leave an oil which was shown to be a mixture of C-7 monoester (103) and C-9 monoester (104) in ratio 1:2 in quantitative yield. $\nu_{max.}$ (neat) br 3 400, br 3 000, and l 735 cm^{-1}; δ (CD_3OD) 4.60 (m, H-7 of (103)), 4.80 (s, H-9 of (104)), 5.72 (m, H-2 of (103)), 5.85 (m, H-2 of (104)); (Found: M^+ 299.13683. $C_{14}H_{21}NO_6$ requires M, 299.13687); m/e 299 (M^+, 6%), 155 (22), 139 (30), 138 (35), 137 (22), 120 (22), 113 (36), 95 (50), 94 (100), and 80 (82).

Intramolecular Esterification of C-7 and C-9 monoesters using Thionyl Chloride - The crude product mixture of C-7 and C-9 monoesters (103) and (104) (97 mg, 0.325 mmol) was dissolved in dry chloroform (10 ml) at 5 °C and thionyl chloride (128.5 mg, 1.075 mmol) and the reaction mixture was stirred at 5 °C for 6 h and at room temperature for 2 h. Dry chloroform (10 ml) was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated *in vacuo* to leave an oil which was basified with concentrated ammonia

solution (10 ml). The basic solution was then extracted with chloroform (4 x 15 ml). The combined chloroform extracts were dried (sodium sulphate), and evaporated *in vacuo* to give a mixture of products (23 mg). Preparative t.l.c. (CH_3Cl/MeOH/NH_3 = 85:14:1) gave six components, none of which could be identified. Most of the starting materials were retained in the basic solution.

Intramolecular Esterification of the crude C-7 monoester (103) and C-9 monoester (104) by the Corey-Nicolaou method[76] - The crude mixture of C-7 (103) and C-9 monoesters (104) (97 mg, 0.325 mmol) was dissolved in dry dimethylformamide (15 ml) at 20 °C under argon and 2,2'-dipyridyl disulphide (88 mg, 0.4 mmol) and triphenyl phosphine (104.8 mg, 0.4 mmol) were added and stirred for 24 h. This reaction mixture was diluted with dry oxygen-free dimethylformamide (10 ml) and added during 6 h to dimethylformamide which was heated under reflux under argon. Heating was continued for an additional 20 h. The solvent was removed *in vacuo* to give an oil which was acidified with 1 N sulphuric acid (10 ml), which was then washed with chloroform (2 x 10 ml), basified with cold concentrated sodium hydroxide solution (10 ml) to pH 10-12. The basic solution was extracted with chloroform (4 x 20 ml), and the combined chloroform extracts were dried (sodium sulphate), filtered and evaporated *in vacuo* to give an oil which was purified by preparative t.l.c. ($CHCl_3$/MeOH/NH_3 = 85:14:1)

to give one major component (23 mg) as a pale yellow oil (R_f 0.33) which was a mixture of C-7 (105) and C-9 senecioyl-retronecine (106); $\lambda_{max.}$(EtOH) 220 nm (ε 10,900); $\nu_{max.}$($CHCl_3$) 3 450, 1 720, 1 700, and 1 650 cm^{-1}; δ ($CDCl_3$) 5.82 (m, H-2 of (106)), 5.70 (m, H-2 of (105)), 5.35 (m, H-7 of (105)), 4.70 (s, H-9 of (106)), from integrations of these signals, the ratio of the C-7 monoester (105) to C-9 monoester (106) was 1:2, other signals, 4.40-4.10 (m), 3.90 (m, H-8), 3.50-1.85 (complex), 2.18 (s, H-14), 1.90 (s, H-15), and 1.22 (s, impurity); m/e 237 (M^+, 7%), 155 (20), 154 (27), 138 (37), 137 (65), 136 (30), 117 (33), 111 (22), 106 (25), 94 (60), 93 (100), 83 (55), and 80 (62).

Esterification of (+)-Retronecine (40) with 3,3-Dimethylglutaric Anhydride (107) - (+)-Retronecine (40) (50.375 mg, 0.325 mmol) was dissolved in dry chloroform (10 ml), 3,3-dimethylglutaric anhydride (107) (46.15 mg, 0.325 mmol) was added, and the reaction mixture was stirred at room temperature for 24 h. The chloroform solvent was evaporated in vacuo to give a mixture of the C-7 and C-9 monoesters in quantitative yield as an oil which could not be crystallised, $\nu_{max.}$($CHCl_3$) 3 300, 3 000, and 1 725 cm^{-1}; δ ($CDCl_3$) 4.52 (m, H-7 of C-7 ester), 4.71 (s, H-9 of C-9 ester), 5.68 (m, H-2 of C-7 ester), 5.76 (m, H-2 of C-9 ester), from the integrations of these signals the ratio of the C-7 monoester to C-9 monoester was 1:2; m/e 297 (M^+, 5%), 155 (28), 143 (80), 137 (8), 138 (8), 139 (12), 111 (75), 101 (28), 94 (24), 83 (100), and 80 (80).

Intramolecular Esterification of the crude C-7 and C-9 Monoesters by Corey-Nicolaou Method[76] - The crude mixture of C-7 and C-9 monoesters (96 mg, 0.325 mmol) was dissolved in dry dimethylformamide (15 ml) under argon and 2,2'-dipyridyl disulphide (88 mg, 0.4 mmol) and triphenyl phosphine (104.8 mg, 0.4 mmol) was added. The reaction mixture was stirred at room temperature for 24 h under argon. This reaction mixture was then diluted with dry oxygen-free dimethylformamide (10 ml) and added slowly over 6 h to dry dimethylformamide (20 ml) which was heated under reflux under argon. Heating was continued for an additional 20 h. The solvent was removed in vacuo to give an oil which was acidified with 1 M sulphuric acid (10 ml), washed with chloroform (2 x 10 ml), basified with cold concentrated sodium hydroxide solution (10 ml) to pH 10-12. The basic solution was extracted with chloroform (4 x 20 ml), and the combined extracts were dried (sodium sulphate), filtered and evaporated in vacuo to give an oil which was purified by preparative t.l.c. ($CHCl_3$/MeOH/NH_3 = 85:14:1) to give 13,13-dimethyl-1,2-didehydrocrotalanine (108) (R_f 0.58) (42.6 mg, 47%) $[\alpha]_D^{22}$ + 42.4° (c 4.26, $CHCl_3$); $\nu_{max.}$ (CCl_4) 1 738 and 1 655 cm^{-1}; δ ($CDCl_3$) 5.88 (1H, m, H-2), 5.32 and 4.08 (2H, AB q, J 12 Hz, H-9), 5.14 (1H, m, H-7), 4.35 (1H, m, H-8), 3.89-3.30 (2H, complex, H-3), 3.10-2.50 (2H, complex, H-5), 2.10-2.40 (2H, complex, H-6), 2.22 and 2.03 (4H, AB q, J 13.5 Hz, H-12, and H-14), 1.22 (3H, s, CH_3), and 1.18 (3H, s, CH_3). m/e 279 (M^+, 28%), 138 (10), 137

(27), 136 (88), 120 (40), 119 (100), 117 (38), 94 (42), 93 (69), 83 (22), and 80 (32). The picrate had m.p. 191-192 °C (Ex EtOH) (Found: C, 49.46 ; H, 4.87 ; N, 11.13 %. $C_{21}H_{24}N_4O_{11}$ requires C, 49.61; H, 4.72; N, 11.02%)

The second component was not identified (R_f 0.36).

8.6 EXPERIMENTAL TO CHAPTER 7

Integerrimine (110) and Senecionine (5) - Fresh plant material from Senecio odorus (450 g) was extracted continuously with methanol in a Soxhlet apparatus for 48 h. The methanol extract was concentrated in vacuo and the residue dissolved in chloroform (500 ml). The chloroform solution was extracted with 1M sulphuric acid (2 x 150 ml) and the acid solution was washed with chloroform until the washings were colourless (8 x 500 ml), then filtered through Kieselguhr. The acid extract was stirred with zinc dust for 2 h, filtered, washed with chloroform (2 x 300 ml), basified with concentrated sodium hydroxide solution (pH 10) and extracted with chloroform (4 x 600 ml). The chloroform extracts were dried and concentrated to give the crude alkaloid mixture, (152 mg; 0.038%)

Examination of the crude mixture by tlc ($CHCl_3/CH_3OH/NH_3$ 85:14:1) showed two components at R_f 0.41 (major) and 0.23 (minor). The mixture was separated by preparative tlc, the first band (R_f 0.41) was shown to contain two alkaloids (4:1) by glc. (38 mg) $\nu_{max.}$ (CCl_4) 3 520, 1 720, 1 660, 1 450, and 1 270 cm^{-1}; δ ($CDCl_3$) 6.50 (1H, q, J 8 Hz, H-20 of (110)), 6.18 (1H, br , H-2), 5.70 (1H, q, J 8 Hz, H-20 of (5)), 5.40 and 4.10 (2H, AB, J 11.5 Hz, H-9), 4.98 (1H, m, H-7), 4.30 (1H, br , H-8), 3.82 (1H, br , H-3), 3.45 (1H, br , H-3), 3.20-2.00 (7H, complex, H-5, H-14, H-13, and H-6), 1.80 (1H, d, J 8 Hz, H-21 of (5)), 1.75 (1H, d, J 8 Hz, H-21 of (110)), 1.25 (3H, d, J 8 Hz, H-18), and 0.90 (3H, d, J 8 Hz, H-19); m/e 335 (M^+, 12%), 291 (19), 248 (12), 220 (26), 138 (52), 137 (26), 136 (96), 121 (75), 120 (100), 119 (100), 95 (60), 94 (71), 93 (87), and 80 (42).

The n.m.r. spectrum of the mixture is shown in Figure 21, p 110.

Senkirkine (11) - The alkaloid from the second band (R_f 0.23) obtained from thin layer chromatography as crystals (26 mg) had m.p. 197-199 °C (lit.,[82] m.p. 198 °C) $\nu_{max.}(CCl_4)$ 3 520, 1 722, 1 660, 1 640, and 1 450 cm^{-1}; δ ($CDCl_3$) 6.15 (1H, m, H-2), 5.90 (1H, q, J 8 Hz, H-20), 5.46 and 4.39 (2H, AB system, J 12 Hz, H-9), 5.00 (1H, m, H-7), 3.60-2.20 (9H, complex, H-3, H-5, H-6, H-14, and H-13), 2.10 (3H, s, NCH_3), 1.90 (3H, q, J 8 and 1.5 Hz, H-21), 1.70 (1H, m, H-13), 1.35 (3H, s, H-18), 1.25 (1H, br s, OH), and 0.90 (3H, d, J 7 Hz, H-19); m/e 365 (M^+, 22%), 337 (30), 321 (42), 294 (40), 266 (55), 250 (42), 222 (32), 211 (270), 168 (80), 151 (80), 153 (80), 122 (90), 123 (80), 110 (90), 96 (80), 94 (90), 82 (90), and 81 (100).

This alkaloid had identical i.r., n.m.r. (Figs. 23,24, pp 116, 120-1), mass spectrum and mixed melting point with an authentic sample of senkirkine.

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