SYNTHESIS AND BIOLOGICAL ACTIVITY OF MACROCYCLIC DIESTERS OF SYNTHANECINE A

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

by

Robert Howie Barbour

Department of Organic Chemistry
University of Glasgow

June 1986

Acknowledgements

I wish to express my sincere thanks to my supervisor, Dr D.J. Robins, for his help, advice and encouragement during the course of this work and in the preparation of this thesis.

I should also like to thank Dr K. Brown and Dr M. Burton for their assistance and helpful discussions.

Thanks go to the chemistry department analytical services. To Dr D.J. Rycroft, Mr J. Gall and Mr R. Sharp for n.m.r. spectra; to Mr A. Ritchie and Mr P. Stewart for mass spectra; to Mrs W. Harkness and Mrs K. Coombe for microanalyses; and to Mrs F. Lawrie, Miss E. Gill and Linda for i.r. spectra.

I am grateful to Prof. G. Sim and Dr A.A. Freer for X-ray analyses.

For their assistance during my time at the MRC Toxicology Unit, Carshalton, I should like to thank Dr A.R. Mattocks and Miss J. Brown.

Financial support from the SERC and MRC is gratefully acknowledged.

Thanks also go to Mrs M. Palmer for typing this thesis.

Finally, thanks go to my mother and father for their encouragement and to my wife, Linda, for her endless assistance and encouragement throughout this period.

TABLE OF CONTENTS

Summary

This thesis covers three areas of research relating to pyrrolizidine alkaloids, (a) Synthesis of macrocyclic pyrrolizidine alkaloid analogues based on synthanecine A; (b) Biological activity of these pyrrolizidine alkaloid analogues, and (c) Structural studies.

(a) Synthesis of macrocyclic pyrrolizidine alkaloid analogues based on synthanecine A. The synthesis of macrocyclic pyrrolizidine alkaloid analogues based on synthanecine A [(±)-2,3-bishydroxymethyl-1-methyl-3-pyrroline] has been achieved by three routes. In the first, treatment of synthanecine A with various succinic and glutaric derivatives selectively yielded 6-monoester hydroxy acids which were lactonised *via* their corresponding pyridine-2-thiol esters (Corey-Nicolaou lactonisation). The macrocyclic nature of these 10- and 11-membered pyrrolizidine alkaloid analogues was established by spectroscopic studies.

In the second route, (±)-3-chloromethyl-2-hydroxymethyl-1-methyl-3-pyrrolinium chloride, prepared by the reaction of synthanecine A and thionyl chloride, was treated with a series of aliphatic, olefinic and aromatic 5- and 6-membered anhydrides, in the presence of base, to yield macrocyclic diesters. Lactonisation was effected by the intramolecular nucleophilic substitution of the allylic chloride by carboxylate anion. This simple and efficient procedure resulted in higher yields of the 10- and 11-membered pyrrolizidine alkaloid analogues than by the first procedure.

In the final route, macrocyclic diesters of synthanecine

A with 12- to 16-membered rings were prepared by a combination of the above two methods. Treatment of (±)-3-chloromethyl-2-hydroxymethyl-1-methyl-3-pyrrolinium chloride with the appropriate diacid, in the presence of base, gave a monoester hydroxy acid which was lactonised by the Corey-Nicolaou method. Attempts to prepare these analogues *via* the non-hydrolytic conversion of an intermediate monoester phenyl ester into a monoester *N*-acylimidazolide failed; oligomeric products were probably formed.

(b) *Biological activity of pyrrolizidine alkaloid analogues*. The biological activity of a series of these analogues based on synthanecine A was examined. Dilactones of synthanecine A are metabolised in a similar manner to pyrrolizidine alkaloids and thus are hepatotoxic if they are resistant to esterase hydrolysis. The structure of the diacid moiety was seen to have a significant influence on the metabolic rate of these macrocyclic diesters.

(c) *Structural analysis*. The structure of emiline, a macrocyclic pyrrolizidine alkaloid, has been revised. The original structure proposed, containing an 11-membered ring, was inconsistent with ^{1}H and ^{13}C n.m.r. spectroscopic data. The use of advanced n.m.r. spectroscopic techniques was invaluable in this structural revision, which showed that emiline contains a 12-membered ring.

Abbreviations

CDI	-	N,N-carbonyl diimidazole
COSY	-	correlation spectroscopy
18-Crown-6	-	1,4,7,10,13,16-hexaoxacyclooctadecane
d	-	doublet
DBN	-	1,5-diazabicyclo[4,3,0]non-5-ene
DBU	-	1,8-diazabicyclo[5,4,0]undec-7-ene
DCC	-	dicyclohexylcarbodiimide
DEAD	-	diethyl azodicarboxylate
DEPT	-	distortionless enhancement by polarisation transfer
DIBAL	-	di-isobutylaluminium hydride
DMAP	-	4-N,N-dimethylaminopyridine
DMF	-	N,N-dimethylformamide
DME	-	1,2-dimethoxyethane
Hunig's base	-	diisopropylethylamine
Im	-	imidazole
i.r.	-	infra red
MOM	-	methoxy methyl
m.s.	-	mass spectrometry
MSM	-	methane sulphonyl methyl
MTM	-	methane thio methyl
n.m.r.	-	nuclear magnetic resonance
s	-	singlet
t	-	triplet
TEA	-	triethylamine
THF	-	tetrahydrofuran
t.l.c.	-	thin layer chromatography
TMS	-	trimethylsilyl
TMS Im	-	1-(trimethylsilyl)imidazole
TOCP	-	triorthocresyl phosphate
u.v.	-	ultra violet

Publications

Sections of this thesis have been presented for publication, as shown below.

Pyrrolizidine Alkaloid Analogues. Synthesis of 11-Membered Macrocyclic Diesters of (±)-Synthanecine A. Robert H. Barbour and David J. Robins, J. Chem. Soc. Perkin Trans. 1, 1985, 2475.

Metabolism and Toxicity of Synthetic Analogues of Macrocyclic Diester Pyrrolizidine Alkaloids. A.R. Mattocks, H.E. Driver, R.H. Barbour and D.J. Robins, Chem. Biol. Interact., in press.

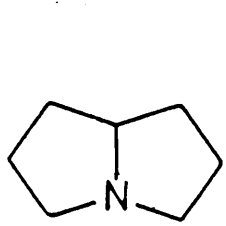
CHAPTER 1

INTRODUCTION

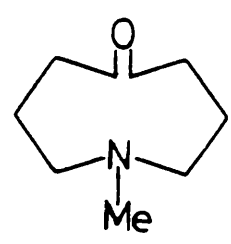
1.1 Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids constitute a large class of natural products.[1] Over 200 have now been isolated. Alkaloids of this type have grown in importance over the years due to their widespread occurrence and known hepatotoxicity.[1] The health and economic hazards of pyrrolizidine alkaloids will be discussed in Chapter 1.3 and the likely mechanism for toxicity is detailed in Chapter 1.4. Pyrrolizidine alkaloid containing plants are widespread both geographically and botanically. They occur on all continents and are represented in at least 60 genera (the three most important being Senecio, Crotalaria, and Heliotropium) in around twelve plant families.[2] In fact it has been estimated that 3% of the world's flowering plants contain pyrrolizidine alkaloids.[3]

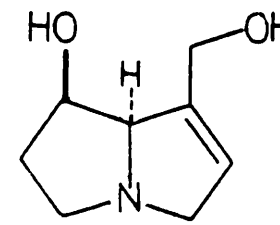
As their name implies these alkaloids contain the 1-azabicyclo[3,3,0]octane (pyrrolizidine) nucleus (1), or a closely related system (2). This nucleus is often found as part of a diol system, as in retronecine (3).



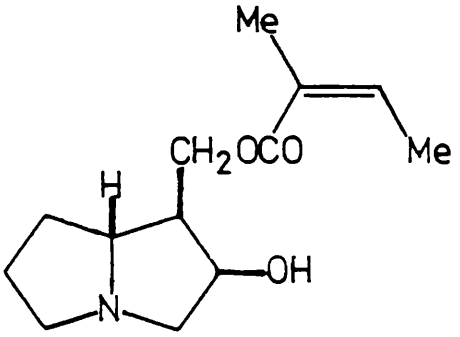
(1)



(2)

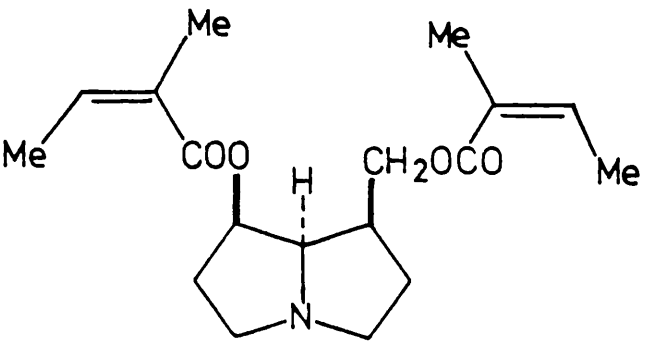


(3)



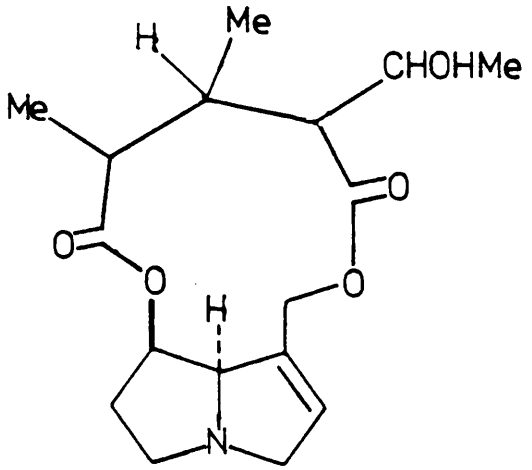
macrophylline

(4)



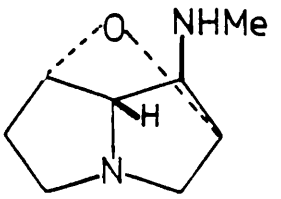
sarracine

(5)



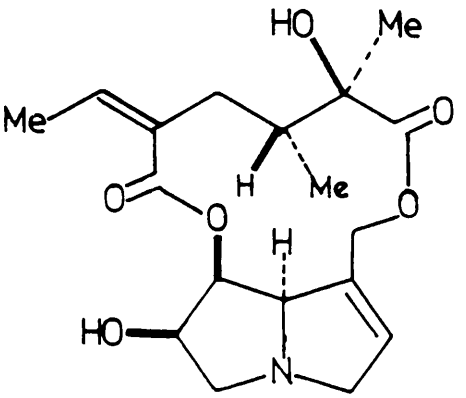
cronaburmine

(6)



loline

(7)



anacrotine

(8)

The most common pyrrolizidine alkaloids are esters.[2] These may be monoesters [such as (4)], diesters [like (5)] or macrocyclic diesters [like (6)]. The amino alcohol portions (necines) differ in the number and position of alcohol substituents, stereochemistry and degree of unsaturation in the right hand ring. The acid moiety of the alkaloids, the necic acids, contain various branched chains and are often hydroxylated and unsaturated. They normally have five to ten carbon atoms and include mono- and dicarboxylic acids. A small collection of simple non-ester pyrrolizidine alkaloids, such as loline (7), have also been isolated.

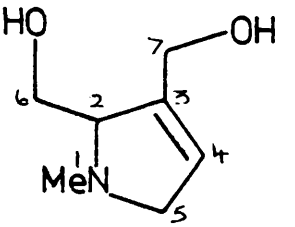
Macrocyclic diester pyrrolizidine alkaloids in which a pyrrolizidine diol [e.g. retronecine (3)] is esterified with a diacid, have attracted most attention since they are the most hepatotoxic.[4] The most common ring sizes in these macrocyclic dilactones are 11- (6) and 12-membered (8). However, a few examples with 13-[5] and 14-membered rings[6] have also been isolated.

For further information on the occurrence,[2,7] chemistry,[8] and pharmacology[4,9] of pyrrolizidine alkaloids a number of reviews are available. A comprehensive book by Bull et al.[1] and the annual reviews presented in the Specialist Periodical Reports[10] and Natural Product Reports[11] are also recommended.

1.2 Synthanecine A

Synthanecine A (9) is a monocyclic analogue of the most common base portion of pyrrolizidine ester alkaloids, namely

retronecine (3). (±)-Synthanecine A was originally prepared by Mattocks[12,13] to further his investigations



(±)-synthanecine A
(9)

into the structural features required for the hepatotoxicity of pyrrolizidine alkaloids.[4,14]

This necine analogue (9) has been employed in our studies because of its structural similarities to retronecine (3) and its ease of synthesis (see Chapter 2.4). Since synthanecine A (9) can be obtained in reasonable quantities, the synthesis of a range of macrocyclic diester analogues could be envisaged. Methods available for the synthesis of macrocyclic diester pyrrolizidine alkaloids are discussed in Chapter 2.3. The synthesis of a number of pyrrolizidine alkaloid analogues with a range of ring sizes, containing synthanecine A is described in Chapters 3 - 5.

The metabolic fate and hepatotoxicity of a series of synthanecine A dilactones were assessed at the M.R.C. Toxicology Unit in Carshalton. The results from the in vivo studies are presented in Chapter 6. The susceptibility of these dilactones to basic hydrolysis has also been studied and these results are included in Chapter 6. A detailed account of the main routes of metabolism and the hazards of

hepatotoxic pyrrolizidine alkaloids can be found in Chapter 1.4 and 1.3 respectively.

Macrocyclic diesters adopt specific conformations, which may have an effect on their hepatotoxicity. Information on the conformation of these systems can be obtained from ^{1}H n.m.r. spectral data and X-ray measurements as discussed in Chapter 1.5.

Attempts will be made to relate metabolism and toxicity to the structure and conformation of the macrocyclic diester analogues prepared.

1.3 Health and Economic Hazards of Pyrrolizidine Alkaloids

Livestock owners in some countries recognised that poisoning of their animals by fodder from meadows, was due to certain weeds and it was during a chemical investigation of one of these weeds that the first pyrrolizidine alkaloids were isolated.[15] When tested on animals these alkaloids were shown to produce liver degradation characteristic of the disease.[16] Other investigations have shown that the symptoms observed for pyrrolizidine alkaloid poisoning depend upon the animal species involved, the plant species ingested and the duration of exposure.[1] However, the main lesion in each case is cirrhosis of the liver. Other organs affected are the lungs, heart, and occasionally the kidneys. The characteristic feature of pyrrolizidine poisoning is a megalocytosis of the liver in which the liver becomes composed of a small number of giant cells. In addition to this hepatotoxic activity some pyrrolizidine

alkaloids are believed to be carcinogenic.[17]

Due to the complexity of this poisoning it has been 'rediscovered' on a number of occasions and is described under a variety of names (e.g. Missouri River Bottom Disease, Pictou Disease, Winton Disease etc.).[1]

Ingestion of pyrrolizidine alkaloids is a major human health hazard. Human poisoning can occur by two methods. (1) Deliberate ingestion. In Jamaica[18] and other countries,[19] plants containing pyrrolizidine alkaloids are often used in herbal remedies and in the preparation of bush teas. (2) Accidental contamination. Pyrrolizidine alkaloid poisoning epidemics in Afghanistan[20] and India[21] in 1975 were due to the consumption of bread prepared from grain contaminated with seeds which contained pyrrolizidine alkaloids. The transfer of pyrrolizidine alkaloids from plants into the milk of cows and goats,[22] and the honey collected by bees,[23] could also affect man.

The extent of this poisoning is not yet fully appreciated and to this day remains a serious health and economic problem.

1.4 Metabolism of Pyrrolizidine Alkaloids

Early experimental studies showed that some pyrrolizidine alkaloids are relatively non toxic, while others are potent hepatotoxins. This led Schoental[24] to suggest that the structural requirements for hepatotoxicity are a double bond in the 1,2-position and the presence of

the ester function, as indicated in Figure 1.

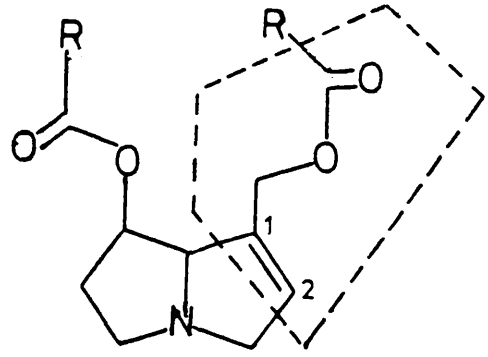
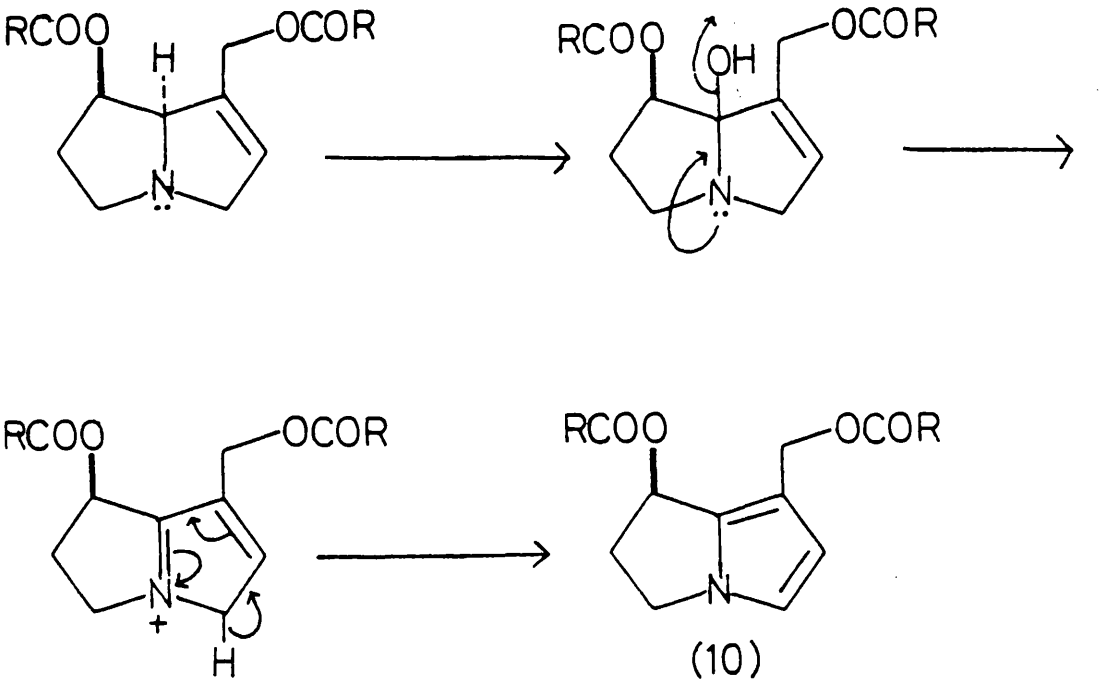


Figure 1

Culvenor[25] then proposed that the way in which the hepatotoxic pyrrolizidine alkaloids act on cell nuclei is an alkylation process. This was based on the known tendency of the allylic ester group to undergo alkyl-oxygen fission. However, it was the outstanding results obtained by Mattocks[26,27] which finally provided evidence for the mechanism of toxicity and the toxic species involved. Mattocks proposed that the alkaloids themselves were not responsible for the toxicity, and suggested that the active toxins were pyrrolic derivatives (10) (dihydropyrrolizines). During his investigations, Mattocks found evidence for these pyrrole metabolites in several organs, primarily the liver, and in the urine of animals dosed with pyrrolizidine alkaloids. Dihydropyrrolizines were also produced from pyrrolizidine alkaloids in vitro using liver slices, giving further evidence that the metabolic activation occurs in the liver.

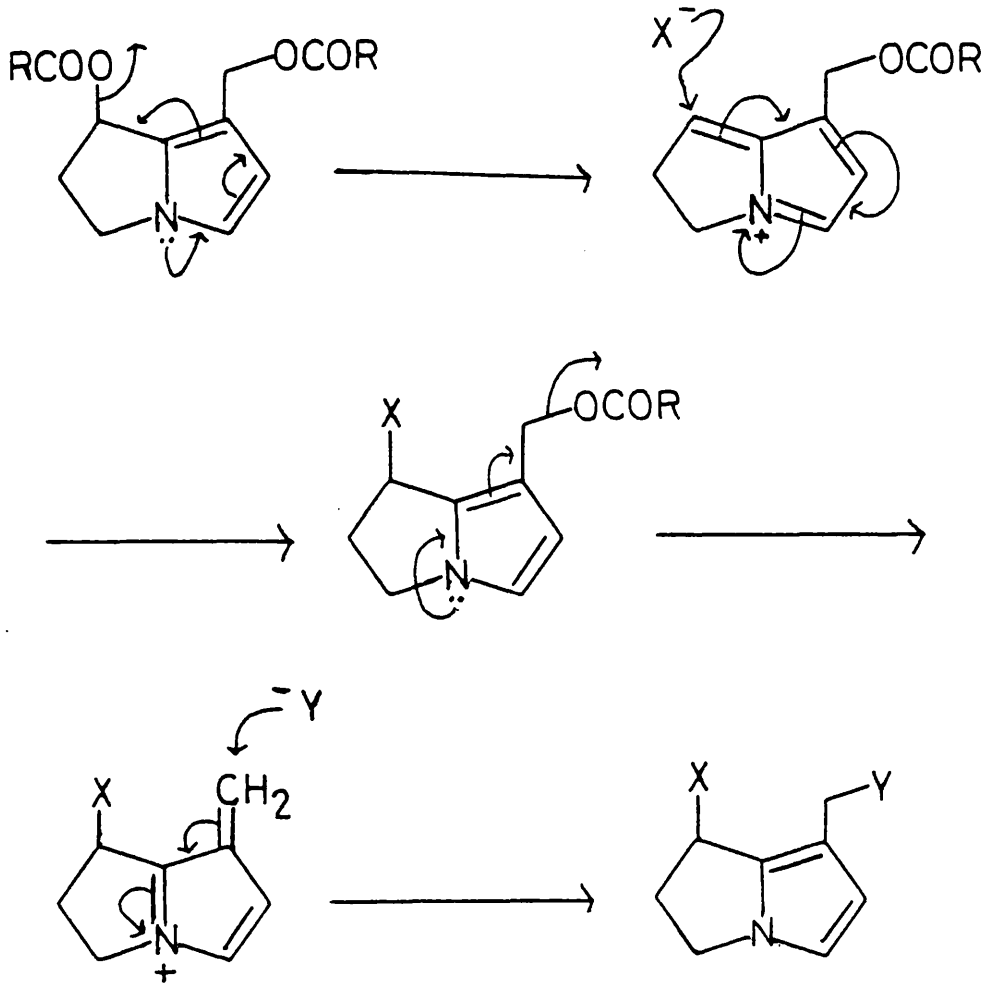
It is now known that the pyrroles are produced by

the action of a hepatic microsomal oxidase enzyme on the alkaloid. This is believed to occur by a hydroxylation - dehydration process, as shown in Scheme 1.



SCHEME 1

Furthermore, Mattocks proposed that the likely toxic mechanism is an alkylation between the pyrrole metabolites and nucleophilic tissue constituents such as nucleic acids or the sulphydryl groups of proteins. The pyrroles can act as bifunctional alkylating agents through the activation of the two ester groups by conjugation with the lone pair of electrons on the pyrrole nitrogen (see Scheme 2). Therefore,



SCHEME 2

this confirms that the structural features required for hepatotoxicity are, as suggested by Schoental,[24] a double bond in the 1,2-position and esterification of one or more of the alcohols.

Previously, Schoental and Mattocks had proposed that a branched chain necic acid, which they believed might cause the alkaloids to interfere with steroid synthesis, was necessary for hepatotoxicity.[28] However, it is now clear that this feature has only a secondary effect on the toxicity.[29]

Chain branching, especially on the α-position of the acid, is reported to cause steric hindrance around the ester groups.[9] This enhances the toxicity of alkaloids by reducing their susceptibility to detoxification by esterase hydrolysis.[30] For this reason macrocyclic diesters are generally more toxic than simple diester alkaloids.

The water solubility and base strength of the alkaloid also has an effect on its hepatotoxicity.

The three principal metabolic processes of pyrrolizidine alkaloids are pyrrole formation and N-oxidation by microsomal oxidases, and hydrolysis by esterases (see Figure 2).[4] The first process produces toxic metabolites, as

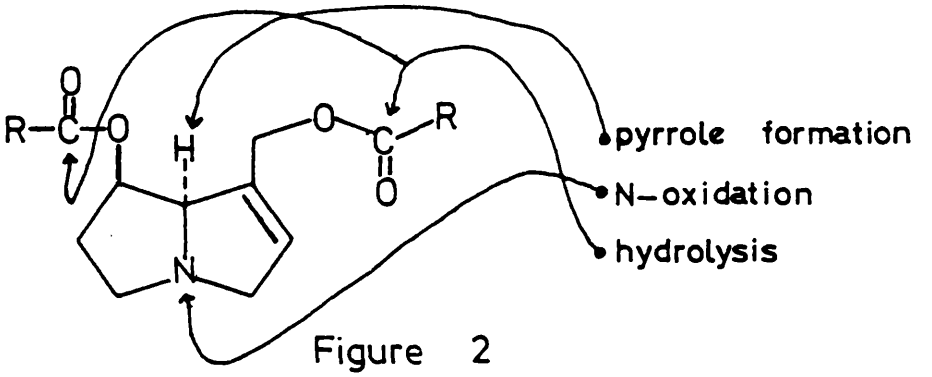
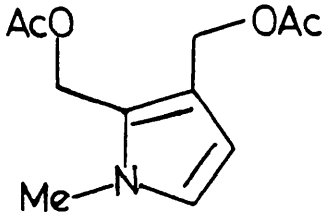


Figure 2

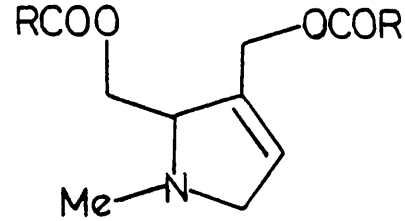
described previously, while the other two are detoxifying, as the highly water soluble products are rapidly excreted.

Mattocks has shown that compounds containing a single pyrrole ring with appropriate substitution, such as in (11), can exhibit hepatotoxic properties.[4,14] This indicates that

the saturated left hand ring of the necine portion is not a structural requirement for hepatotoxicity. Simple pyrroline esters like (12) should therefore be hepatotoxic provided that their ester groups are resistant to hydrolysis.



(11)



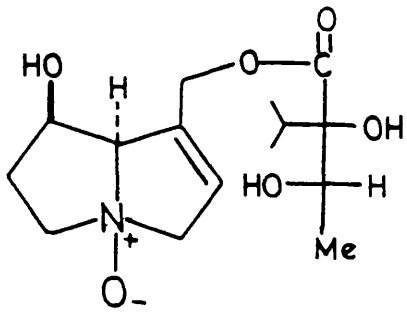
(12)

1.5 Therapeutic Properties of Pyrrolizidine Alkaloids

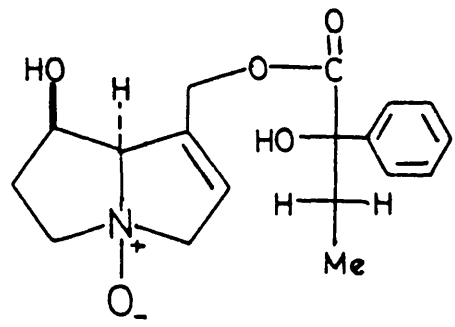
The pyrrolizidine nucleus is not solely associated with hepatotoxicity as some of its derivatives display therapeutic properties.

Indicine N-oxide (13) was shown to be the antitumour constituent of *Heliotropium indicum*.[31] As this pyrrolizidine monoester N-oxide (13) does not exhibit the hepatotoxicity normally associated with pyrrolizidine alkaloids it has been used in clinical trials. These showed that it is effective against advanced gastro-intestinal cancer,[32] and in cases of leukaemia and melanoma. A semi-synthetic pyrrolizidine alkaloid (14),

prepared by Zalkow et al,[33] was shown to be a more active antitumour agent than indicine N-oxide (13).

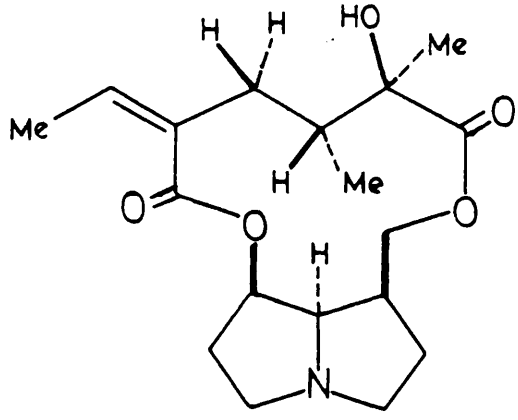


(13)



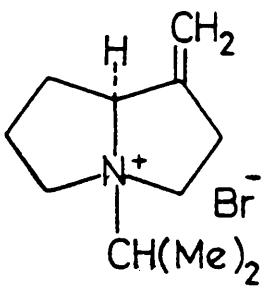
(14)

In the U.S.S.R. platyphylline (15) is widely used for the treatment of hypertension and internal ulcers.[1] Since this macrocyclic diester does not contain a 1,2-double bond it should not be hepatotoxic.

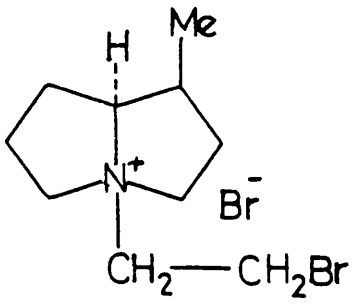


(15)

A series of semi-synthetic quaternary pyrrolizidine derivatives have been the subject of pharmacological studies.[34] The quaternary ammonium derivatives (16) and (17) were shown to exhibit ganglion and neuromuscular blocking activities respectively. Atal et al. also showed

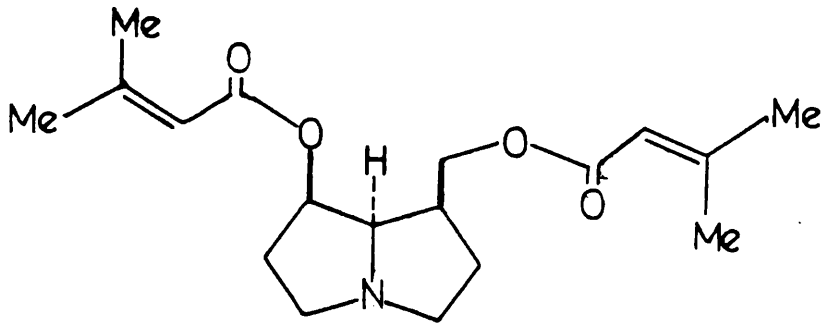


(16)



(17)

that the diester (18) of platynecine has a local anaesthetic activity.

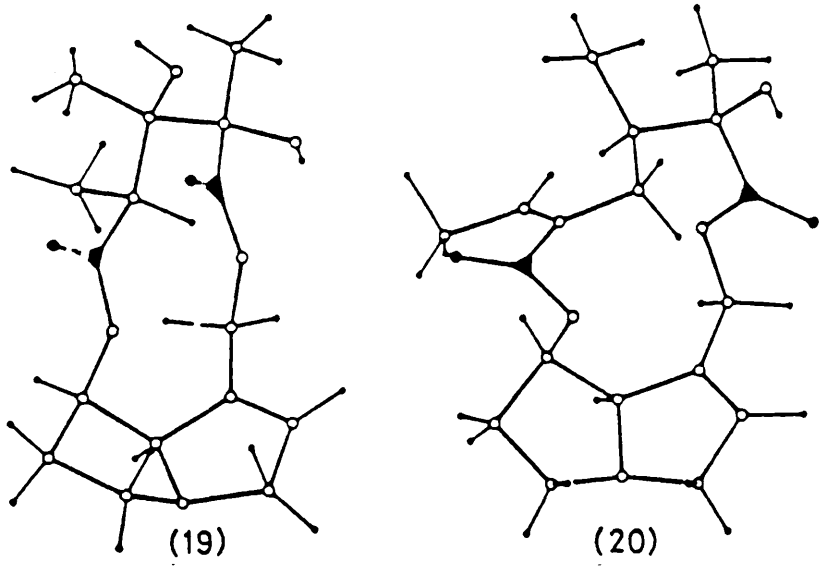


(18)

1.6 Conformational Aspects of Macrocyclic Diester Pyrrolizidine Alkaloids

Knowledge of the preferred conformation of macrocyclic pyrrolizidine alkaloids might lead to a better understanding of the precise structure/activity relationships involved in their metabolism. X-Ray crystallographic and ^{1}H n.m.r. spectral data[35] have provided information on the conformation of these alkaloids in the solid state and organic solution respectively.[1,23]

Examination of X-ray data on a series of 11- and 12-membered macrocyclic diesters containing retronecine has led to some interesting generalisations. All of the natural 11-membered dilactones which have been analysed, except trichodesmine, contain ester carbonyl groups which are syn-parallel and directed downward from the macro-ring as shown in Figure 3 for monocrotaline (19). However, in all of the 12-membered dilactones which have been subjected to X-ray analysis, the ester carbonyl groups are anti-parallel with the C-11 carbonyl directed above the macro-ring [c.f. Figure 3, senecionine (20)].



Crystal structures of monocrotaline (19) and senecionine (20)

Figure 3

^{1}H N.m.r. spectral data have provided information on the conformations of macrocyclic diester alkaloids in organic solutions. In these alkaloids rotation of the C^1-C^9H_2-C^{10}-$C^{11}OR$ sections is limited and the conformation of this part of the molecule can be deduced from the chemical shift differences of the diastereotopic C-9 protons,

$\Delta\delta$H-9. This value has been observed to range from ~0 - 1.53 p.p.m. (see Table 1). When $\Delta\delta$H-9 is large, as for senecionine (20), the downfield proton is subjected to near

Chemical shift differences of the diastereotopic C-9 protons ($\Delta\delta$H-9) of macrocyclic pyrrolizidine alkaloids

11-membered macrocycles		12-membered macrocycles	
	$\Delta\delta$H-9 (p.p.m.)		$\Delta\delta$H-9 (p.p.m.)
crispatine	0.32	integerrimine	1.25
fulvine	0.73	jacobine	1.53
monocrotaline	0.16	otosenine	1.14
retusamine	0.92	senecionine	1.47

Table 1

maximum deshielding from the 1,2-double bond and the ester carbonyl, and therefore lies in the plane of these unsaturated bonds (see Figure 3). When the $\Delta\delta$H-9 value is small [e.g. monocrotaline (19) 0.16 p.p.m.] the C-9 protons must be more symmetrically placed about the plane of the carbonyl group and the more downfield proton is closer to the 1,2-double bond.

Further conformational data have been obtained by examining the allylic and homoallylic couplings between the C-2, C-3 and C-9 protons.[1]

The conformations derived from the ^{1}H n.m.r. spectral data for monocrotaline (19) and senecionine (20) appear to be consistent with the crystal structures shown in Figure 3. Therefore ^{1}H n.m.r. spectroscopy is a useful technique when

considering the conformational aspects of macrocyclic diesters.

It must however be noted that the conformations adopted by the alkaloids in the solid state and in organic solutions are not necessarily the same as those present when the alkaloid is metabolised in vivo, i.e. in dilute aqueous media.

A rough guide to the ring size of a macrocyclic diester containing retronecine can also be obtained from the $\Delta\delta$H-9 values (see Table 1). For 11-membered macrocycles, the difference in chemical shifts is typically 0 - 0.73 p.p.m. whereas for 12-membered macrocycles, the range is usually from 1.25 - 1.53 p.p.m.

CHAPTER 2

SYNTHESIS OF PYRROLIZIDINE ALKALOIDS AND ANALOGUES

2.1 Introduction

Although a great many syntheses of necines and necic acids have been reported, the following chapter is primarily concerned with the combination of these moieties to prepare pyrrolizidine ester alkaloids. The synthesis of semi-synthetic and synthetic analogues will also be considered.

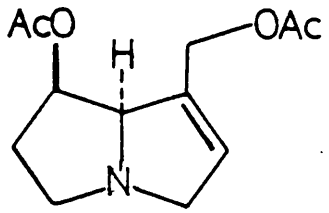
The most outstanding achievement in this area has been the synthesis of diester alkaloids of the macrocyclic type. This has been achieved, so far, by three groups and will be discussed in section 2.3. This section will include details on lactonisation methods and some recent developments in this area. Most of the early synthetic studies were focussed on the preparation of monoester and simple diester alkaloids, and this will be considered in section 2.2. Another area of research which is of synthetic as well as toxicological interest is the preparation of semi-synthetic and synthetic analogues. The semi-synthetic analogues will be considered in their relevant sections whereas the totally synthetic analogues will be dealt with separately in section 2.4.

2.2 Synthesis of Monoesters and Diesters

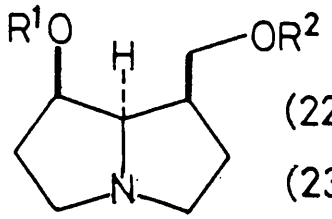
(a) Esterification by Conventional Methods

One of the earliest examples of pyrrolizidine alcohol acylation was the synthesis of retronecine diacetate (21). In their attempts to elucidate the structure of retronecine

(3), Barger et al.[36] prepared the diacetate (21) using acetic anhydride. A similar investigation by Konovalova and Orekhov[37] showed that platynecine (22) formed mono- (23) and dibenzoate (24) derivatives when treated with benzoyl chloride.



(21)

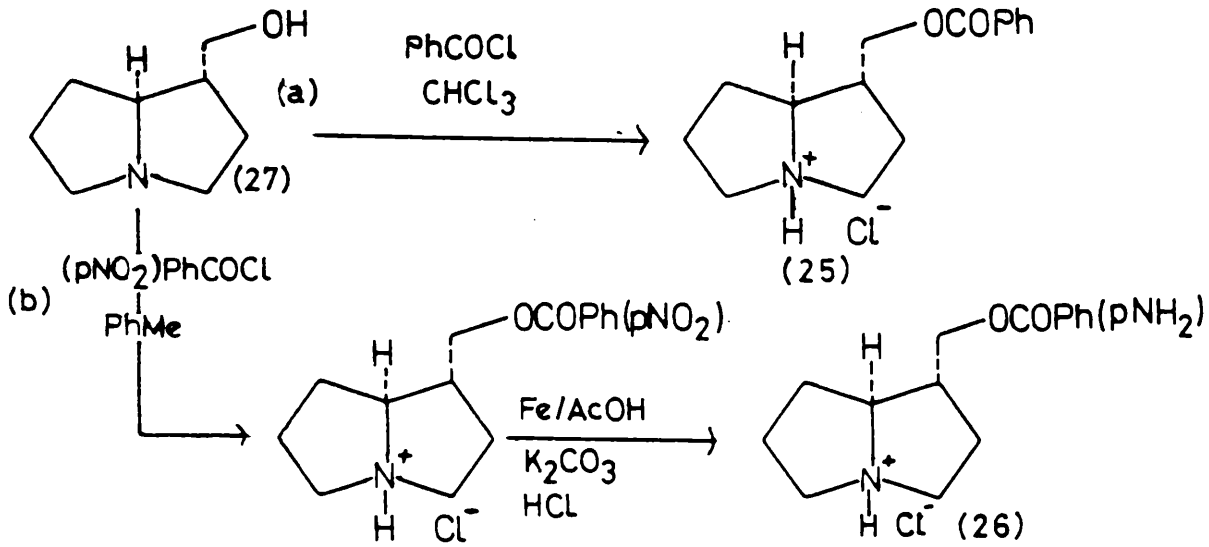


(22) $R^1 = R^2 = H$

(23) $R^1 = H$, $R^2 = PhCO$

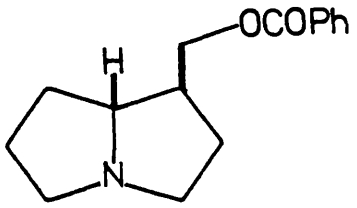
(24) $R^1 = R^2 = PhCO$

The first deliberate synthesis of a pyrrolizidine monoester analogue was by Gurevich and Men'shikov.[38] They prepared the hydrochloride salts of the benzoate (25) and p-aminobenzoate (26) of trachelanthamidine (27) using acid chlorides, as shown in Scheme 3, for subsequent pharmacological studies. The benzoate (25) is a feeble anaesthetic whereas the p-aminobenzoate (26) has comparable potency to cocaine.



SCHEME 3

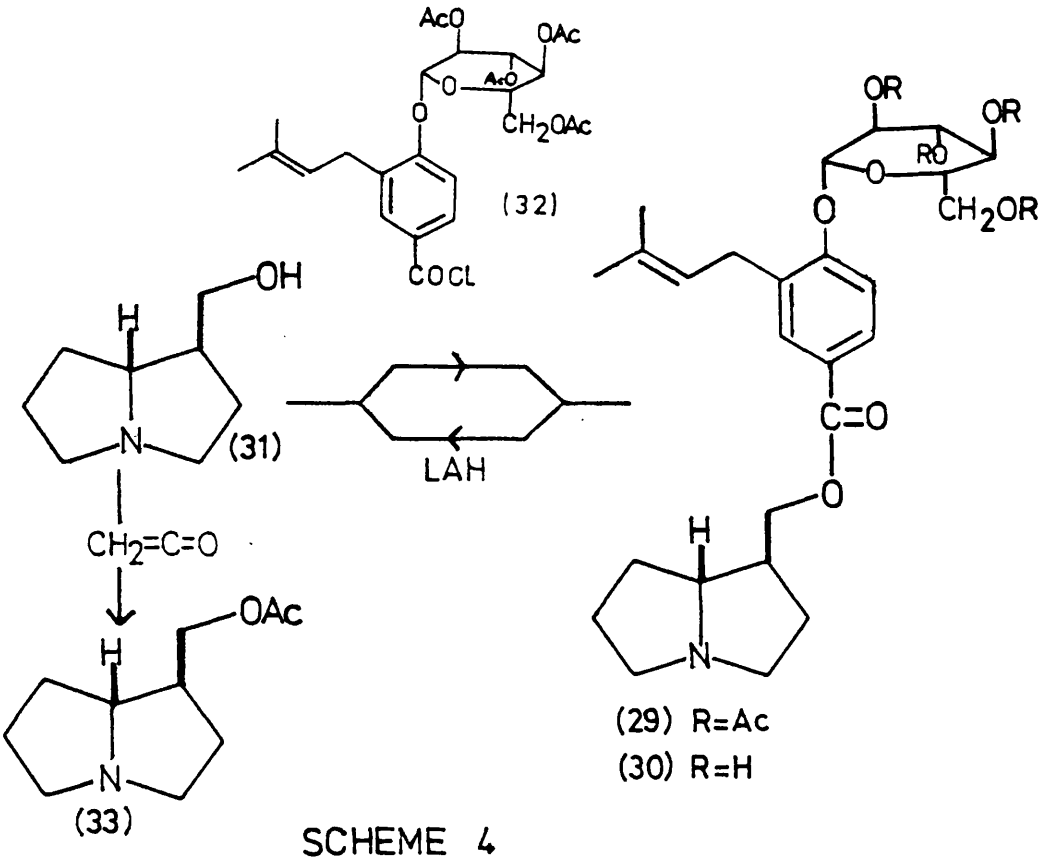
Not surprisingly, acid chlorides were used on numerous occasions for the preparation of pyrrolizidine ester alkaloids and analogues. For example, method (a) (Scheme 3) was employed by Hart and Lamberton[39] to prepare laburnine benzoate (28), for spectroscopic and chromatographic comparison with the natural product.



(28)

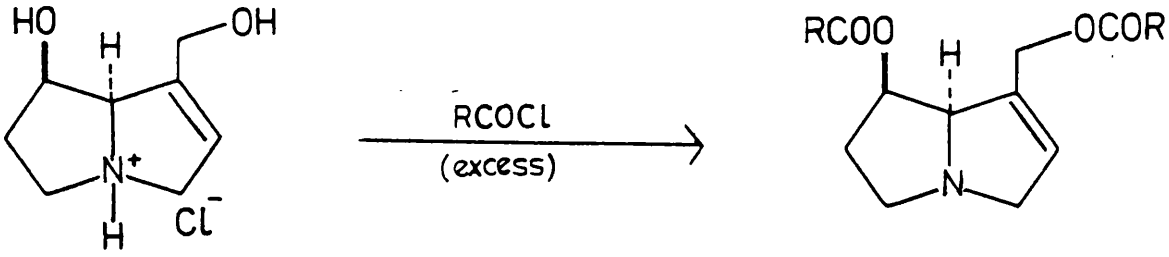
The tetraacetyl derivative (29) of malaxine (30), an alkaloid isolated from Liparis species, was synthesised by Tanino et al.[40] The final step in this synthesis involved the treatment of laburnine (31), in dry pyridine, with the acid chloride (32), as shown in Scheme 4.

Laburnine (31), obtained by the reductive cleavage of malaxine (30), was acetylated by Lindstrom and Luning[41] using ketene (see Scheme 4). Both products, tetraacetylmalaxine (29) and laburnine acetate (33), were prepared to characterise these new alkaloids.



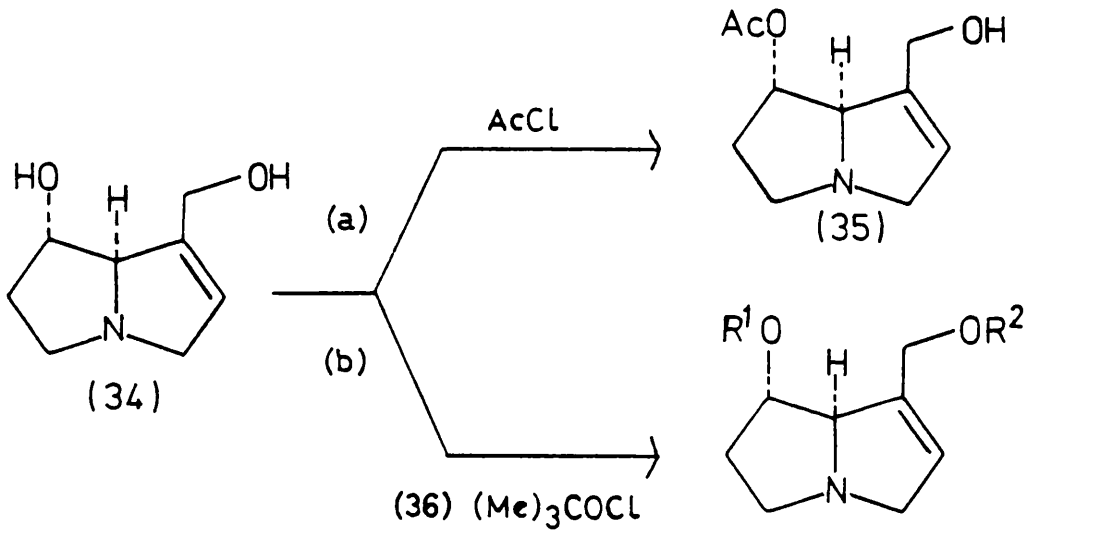
SCHEME 4

Mattocks[42] and Culvenor et al.[43] also employed acid chlorides to prepare semi-synthetic ester alkaloids for subsequent toxicological studies. Mattocks prepared a series of diesters of (+)-retronecine (3) by heating retronecine hydrochloride with various acid chlorides, as shown in Scheme 5. Monoester and diester derivatives of heliotridine (34) were prepared by Culvenor et al. 7-Acetylheliotridine (35) was prepared by treating heliotridine with acetyl chloride at room temperature



SCHEME 5

(Scheme 6a). However,when a solution of heliotridine, in dry chloroform, was treated with a molar equivalent of pivaloyl chloride (36), at room temperature, a mixture of the 7-(37) monoester, 9-(38) monoester and 7,9-diester (39) products was obtained (see Scheme 6b). 7,9-Diester analogues were obtained exclusively by heating heliotridine hydrochloride with various acid chlorides (c.f. Scheme 5).



SCHEME 6

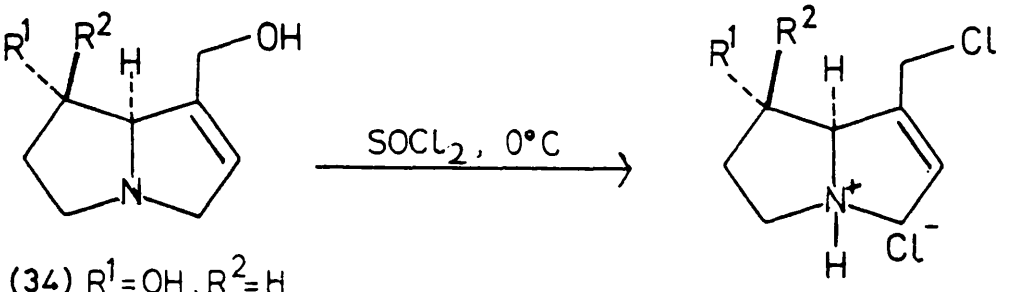
(b) Nucleophilic Displacement

(i) Nucleophilic Displacement of Allylic Chlorides.

This method relies upon the high reactivity of allylic chlorides towards nucleophiles. If the nucleophilic species is a carboxylate anion the product is an allylic ester, which is a functionality commonly observed in pyrrolizidine ester alkaloids.

Culvenor et al.[44] devised this method to prepare some naturally occurring 9-monoesters of the unsaturated hydroxy necines; heliotridine (34), supinidine (40) and retronecine (3). These bases were transformed into the hydrochloride salts of their 1-chloromethyl derivatives, (41), (42) and (43) respectively, using thionyl chloride at 0 ^{o}C. The required ester alkaloids were then prepared by heating these allylic chlorides with the appropriate sodium carboxylate at reflux in aqueous ethanol, as shown in Scheme 7. Poor yields of the products were obtained, especially when using saturated bases, owing to the strong tendency of the chloro compound to form quaternary ammonium derivatives.

Heliotrine (44) from heliotridine (34) and supinine (45) from supinidine (40), were the first two monoester alkaloids prepared by this method.[44] In 1966, Culvenor et al.[45] reported the first formal synthesis of hepatotoxic pyrrolizidine alkaloids, namely intermidine (46) and lycopsamine (47). These diastereomeric 9-monoesters of retronecine were prepared via the 1-chloromethyl derivative (43) of retronecine (see Scheme 7). 9-Angelylretronecine



(34) $R^1 = OH, R^2 = H$

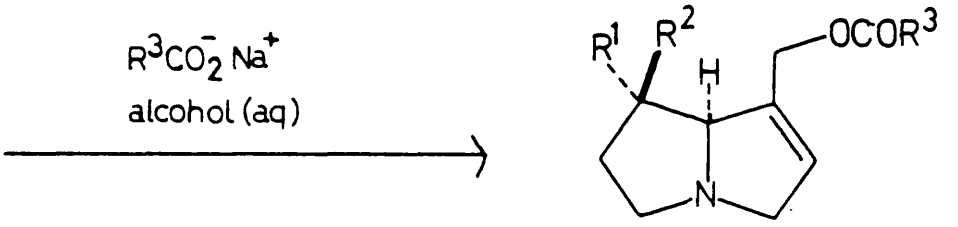
(40) $R^1 = R^2 = H$

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

(41) $R^1 = OH, R^2 = H$

(42) $R^1 = R^2 = H$

(43) $R^1 = H, R^2 = OH$

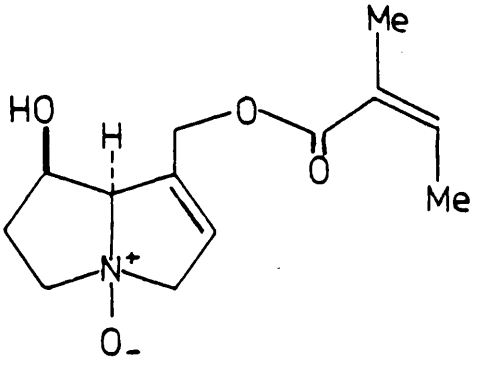


(44) $R^1 = OH$, $R^2 = H$, $R^3 = C(CHMe_2)(OH)CH(OH)Me$

(45) $R^1 = R^2 = H$, $R^3 = C(CHMe_2)(OH)CH(OMe)Me$

(46) and (47) $R^1 = H, R^2 = OH$, $R^3 = C(CHMe_2)(OH)CH(OH)Me$

SCHEME 7



(48)

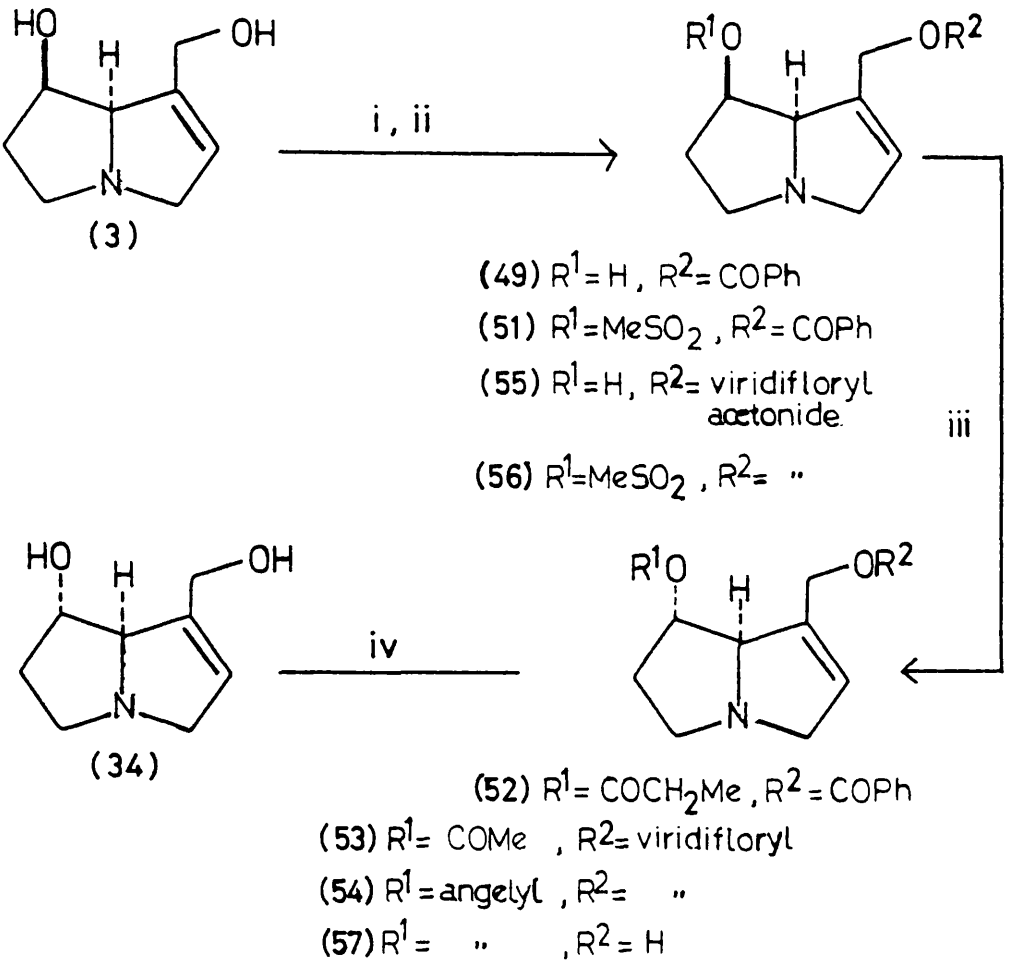
N-oxide (48) was prepared similarly, to confirm the structure of this new alkaloid.[46]

(ii) Nucleophilic Displacement of Secondary Mesylates.

Glinski and Zalkow[47] have reported an efficient method for the transformation of retronecine (3) into heliotridine (34), utilising the reactivity of secondary mesylates towards displacement by cesium carboxylates.[48] This route also permitted the first syntheses of diester pyrrolizidine alkaloids containing different ester groups.

The conversion of retronecine into heliotridine requires the inversion of configuration of a secondary alcohol. This was achieved as outlined in Scheme 8. 9-Benzoylretronecine (49) was synthesised by the site specific coupling of retronecine with benzoic acid, using N,N-carbonyldiimidazole (CDI) (50) (see Chapter 2.2d). This monoester was in turn converted into the key intermediate (51) using methanesulphonyl chloride and triethylamine at -2 °C. The stereochemical inversion was then accomplished by the nucleophilic displacement of the mesylate (51) with various cesium carboxylates in N,N-dimethylformamide (DMF). Cesium propionate was found to give the most promising result. Finally, heliotridine (34) was obtained by the basic hydrolysis or reductive cleavage of the diester (52).

The naturally occurring diesters, 7-acetylechinatine (53) and 7-angelyl-9-(viridifloryl)heliotridine (54), were prepared by an analogous route (see Scheme 8). Esterification of retronecine at C-9 with the acetonide of (-)-viridifloric



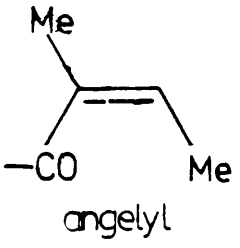
REAGENTS

i $PhCO_2H$ (1eq) , CDI (1·1 eq), THF , r.t. , 16h. , 95% ;

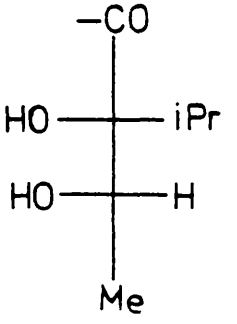
ii $MeSO_2Cl$ (1·3eq) , Et_3N (1·5eq), CH_2Cl_2 , -2°C , 1·5h ;

iii $RCO_2^- Cs^+$ (4eq) , DMF ;

iv $Ba(OH)_2$ aq , r.t. , 87% .



angelyl



viridifloryl

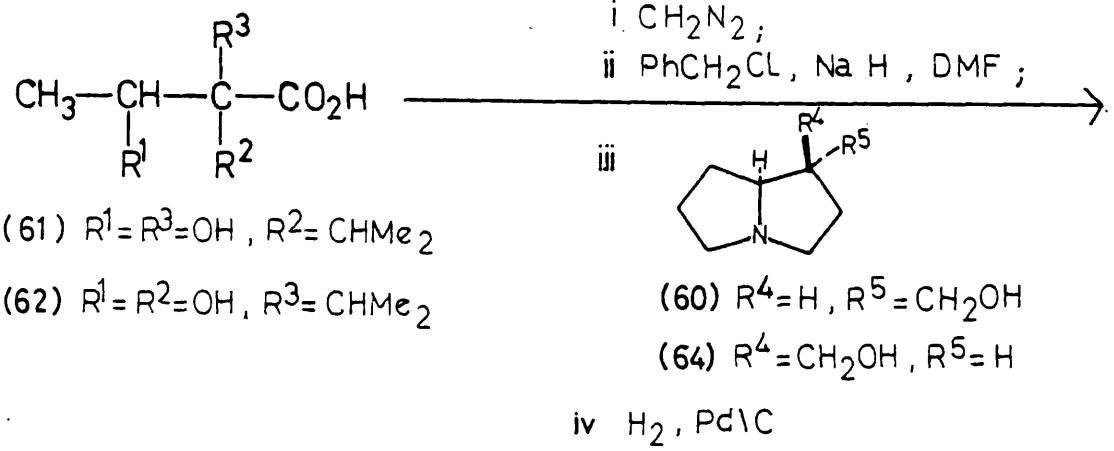
SCHEME 8

acid, using CDI, produced the monoester (55) which was then converted into the 7-mesylate (56). Nucleophilic substitution of (56) with cesium acetate or cesium angelate and deprotection, yielded the required products (53) and (54) respectively. Oxidative cleavage of (54) or its acetonide with periodic acid gave 7-angelylheliotridine (57).

(c) Transesterification.

The first total syntheses of pyrrolizidine monoester alkaloids were accomplished by Kochetov et al.[49] These syntheses included the preparation of optically active necines, the synthesis of optically active acids and esterification of the necines with the corresponding acids. Trachelanthamine (58) and viridiflorine (59) were prepared by the combination of (-)-trachelanthamidine (60) with (+)-trachelanthic acid (61) and (-)-viridifloric acid (62) respectively. Lindelofine (63) was prepared from lindelofidine (64) and (+)-trachelanthic acid (61), as shown in Scheme 9.

The optically active acids were esterified and converted into their di-O-benzylether derivatives, which were then subjected to base catalysed transesterification with the appropriate 1-hydroxymethylpyrrolizidines. Removal of the benzyl ethers, by hydrogenolysis over palladium on charcoal, afforded the required products (58), (59) and (63).

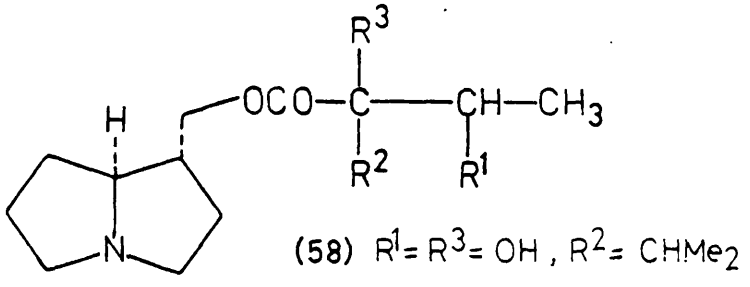


(61) $R^1 = R^3 = OH$, $R^2 = CHMe_2$

(62) $R^1 = R^2 = OH$, $R^3 = CHMe_2$

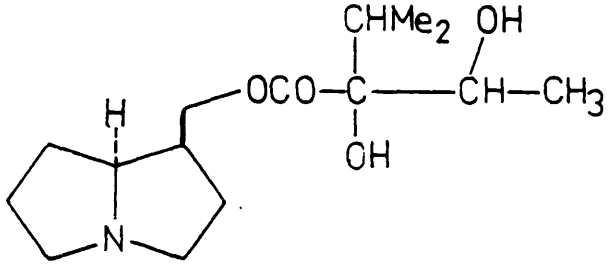
(60) $R^4 = H$, $R^5 = CH_2OH$

(64) $R^4 = CH_2OH$, $R^5 = H$



(58) $R^1 = R^3 = OH$, $R^2 = CHMe_2$

(59) $R^1 = R^2 = OH$, $R^3 = CHMe_2$



(63)

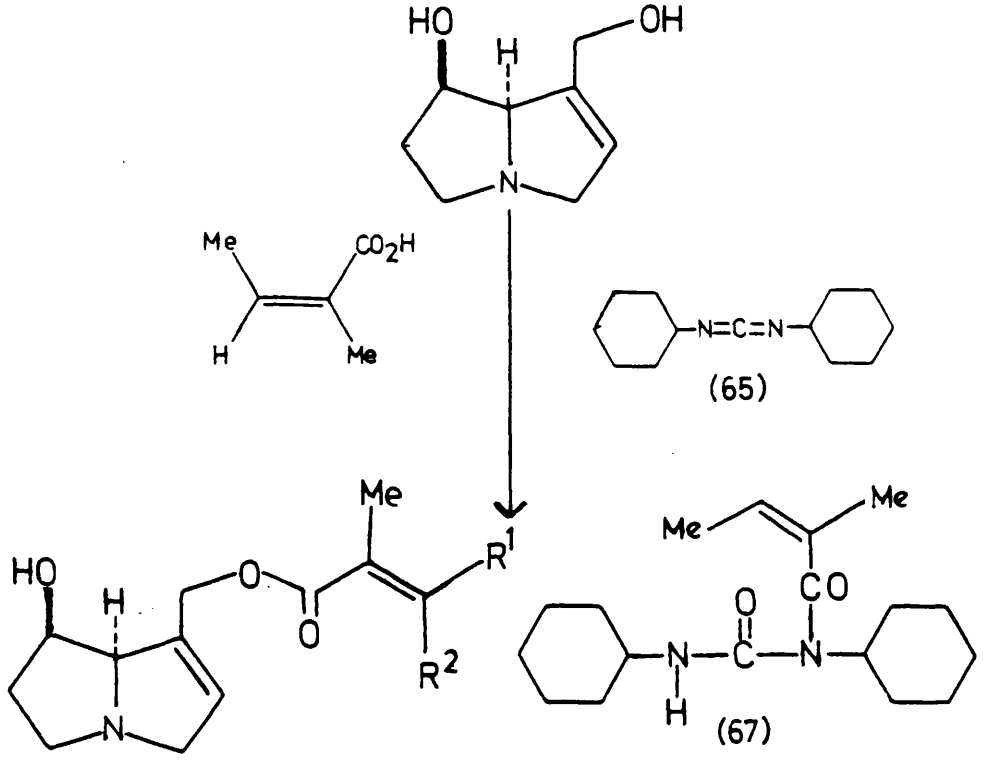
SCHEME 9

(d) Dicyclohexylcarbodiimide (DCC) (65) and N,N-carbonyldiimidazole (CDI) (50)

Hoskins and Crout[50] assessed the potential of DCC (65) and CDI (50) as reagents for the selective esterification

of dihydroxynecine bases, such as retronecine. They also investigated the esterification of necines with α,β-unsaturated acids and with hindered α-hydroxyacids.

Esterification by DCC provided a reasonably selective route for the formation of 9-monoesters of retronecine. The amount of diester formation was roughly inversely proportional to the magnitude of the acid, presumably because the increasing steric hindrance makes the remaining hydroxyl less susceptible to further esterification. Synthesis of 9-angelylretronecine (66), as shown in Scheme 10, gave low yields [due to competing rearrangement to the N-acylurea (67)] and partial geometrical isomerisation giving 9-tiglylretronecine (68). This indicated that DCC was not a suitable reagent for the esterification of retronecine with α,β-unsaturated acid.

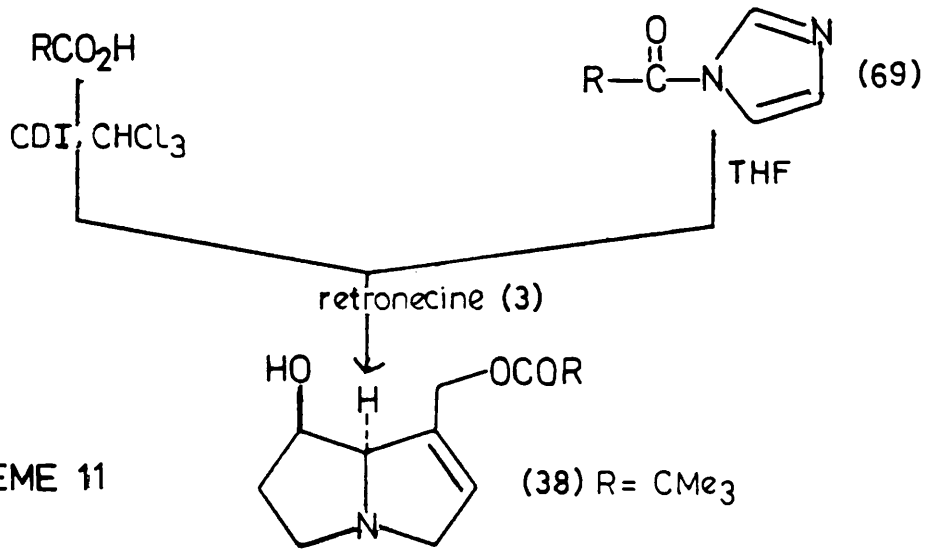


(66) R^1 = H, R^2 = Me

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

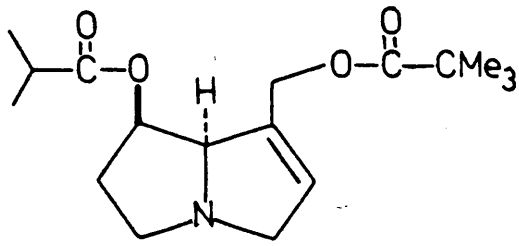
SCHEME 10

Esterification using CDI, or with previously formed N-acylimidazolides, provided regiospecific routes to 9-monoesters of retronecine. Treatment of retronecine with equimolar amounts of N-acylimidazolides (69) for 24 h gave 9-monoesters (38) directly, in good yields. Comparable yields were obtained by adding retronecine directly to a solution of the carboxylic acid and CDI in ethanol free chloroform (see Scheme 11).



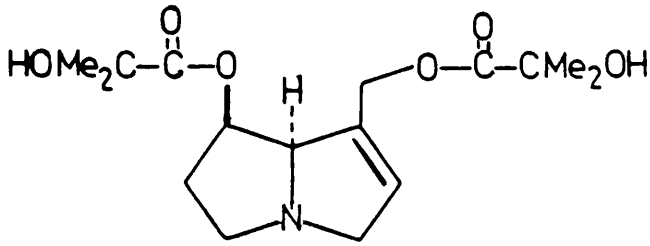
SCHEME 11

This method permitted the synthesis of the α,β unsaturated ester, 9-tiglylretronecine (68), in reasonable yield. Furthermore, the unsymmetrical diester (70) of retronecine was prepared by esterifying the allylic alcohol using pivaloylimidazole and subsequent esterification of the remaining alcohol with the appropriate acid chloride. Finally, the diesterification of retronecine with a carboxylic acid (71) having α-hydroxy-α,α-dialkyl substitution was also achieved using CDI. This substitution pattern is commonly observed in naturally occurring necic acids.

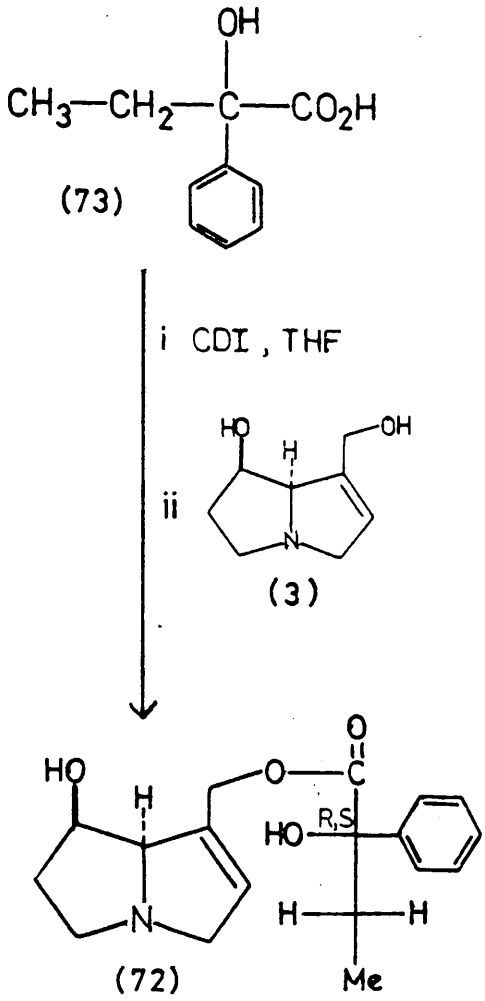


(70)

More recently, Zalkow and co-workers[33] employed CDI to prepare the 9-monoester (72). 9-O-[(±)-2-hydroxy-2-phenylbutyryl]retronecine (72) was formed by the addition of retronecine (3) directly to a solution of the appropriate acid (73) and CDI in dry chloroform (see Scheme 12). The N-oxide (14) of the monoester (72) was shown to be a more active anti-tumour agent than indicine N-oxide (13) (see Chapter 1.5).



(71)



SCHEME 12

2.3 Synthesis of Macrocyclic Diesters

(a) Eleven membered macrocyclic diesters

Macrocyclic diester pyrrolizidine alkaloids have proved to be formidable synthetic targets. In fact it was not until the beginning of this decade that the first promising results were published. Robins and Sakderat[51] reported the synthesis of an eleven membered macrocyclic diester (74) of retronecine.

The key step of this synthesis involved the Corey-Nicolaou

"double activation" method for lactonisation.[52] This method requires the formation of a pyridine-2-thiol ester,[53] which is doubly activated towards lactonisation by the internal transfer of a proton from the hydroxyl to the carbonyl as shown in Figure 4. The transfer of this proton

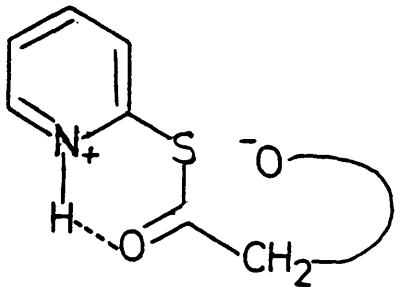
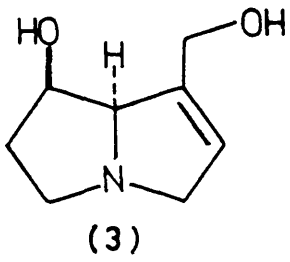


Figure 4

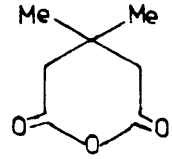
is facilitated by the basic nitrogen of the pyridine nucleus.

Full details of the synthesis of (74) and of a series of similar alkaloid analogues [(75)-(78)] were published soon after (see Scheme 13).[54] Treatment of (+)-retronecine (3) with an equimolar amount of 3,3-dimethylglutaric anhydride (79) in chloroform gave a quantitative mixture of the corresponding 7-(80) and 9-monoesters (81). Esterification occurred predominantly at the primary allylic hydroxyl and no 7,9-diester formation was observed because the initial zwitterionic monoester products had precipitated. The 7- and 9-monoester hydroxyacids were converted into their pyridine-2-thiol ester derivatives, (82) and (83) respectively, with triphenylphosphine (84) and 2,2'-dithiopyridine (85) at room temperature. Vigorous stirring was required to effect the dissolution of the zwitterionic monoesters. Slow addition of the pyridine-2-thiol esters

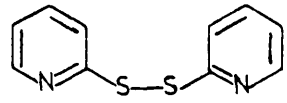


(3)

i

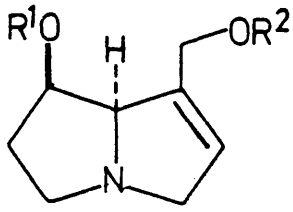


ii



(85)

Ph_3P (84)

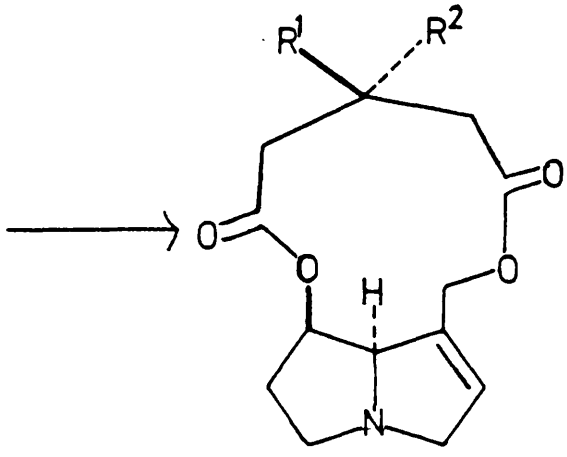


(80) $R^1 = CO\,CH_2CMe_2CH_2CO_2H$, $R^2 = H$

(81) $R^1 = H$, $R^2 = CO\,CH_2CMe_2CH_2CO_2H$

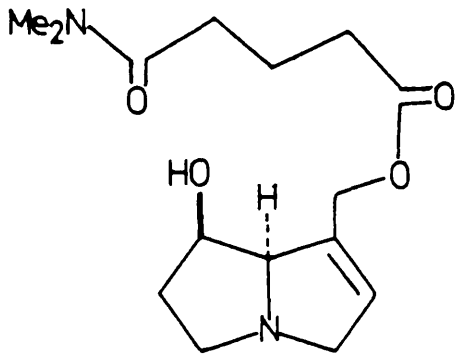
(82) $R^1 = CO\,CH_2CMe_2CH_2COSPy$, $R^2 = H$

(83) $R^1 = H$, $R^2 = CO\,CH_2CMe_2CH_2COSPy$



	R^1	R^2
(74)	Me	Me
(75)	$(CH_2)_4$	
(76)	H	H
(77)	Ph	Ph
(78)	H	Me
	Me	H
(88)	OH	Me
(89)	Me	OH

SCHEME 13



(86)

+ C-7 isomer

(87)

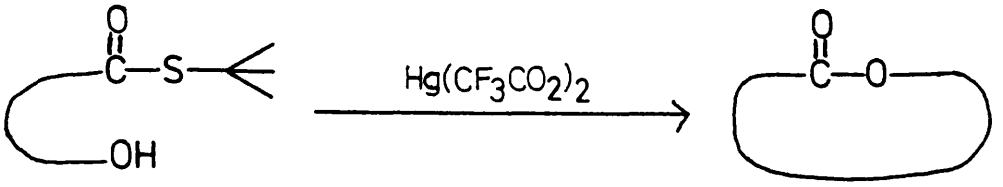
(82) and (83) to refluxing chloroform, followed by 12 hours heating at reflux yielded the required product (74). Macrocyclic diesters [(75)-(78)] were prepared similarly using the appropriately substituted glutaric anhydrides. When N,N-dimethylformamide (DMF) was used instead of chloroform, to solubilise the 7- and 9-zwitterionic monoesters, by products (86) and (87) were isolated. These N,N-dimethylamides were presumed to be formed by the reaction of dimethylamine (from the breakdown of DMF) with pyridine-2-thiol esters.

Soon after, Robins et al.[55] successfully employed this route for the first synthesis of a macrocyclic diester pyrrolizidine alkaloid. Dicrotaline (88), the simplest of the eleven membered macrocyclic diesters of (+)-retronecine (3), and its C-13 epimer (89), were prepared from the trimethylsilylether of 3-hydroxy-3-methylglutaric anhydride and (+)-retronecine in a similar manner to that described above (see Scheme 13). No deprotection step was required as the protecting group was removed in the work-up. The mixture of diastereomers was separated by preparative thin layer chromatography, and the absolute configuration at C-13 in both compounds was established by a sequence of selective reactions on each epimer, yielding optically active mevalonolactone.

The Corey-Nicolaou procedure for lactonisation has been applied successfully in a number of other syntheses of natural products.[56]

Another procedure, developed by Masamune,[57] utilises

t-butylthiol esters to effect lactonisation, as shown in Scheme 14. The soft thiophilic mercury(II)ion (as its



(90) (91)

SCHEME 14

trifluoroacetate or methanesulphonate) is required to catalyse the conversion of the thiol ester (90) into the lactone (91). The mechanism for this conversion is thought to proceed through the intermediate shown in Figure 5. Other thiophilic cations such as Cu(I), Cu(II) and Ag(I) can also be used. This procedure provides an

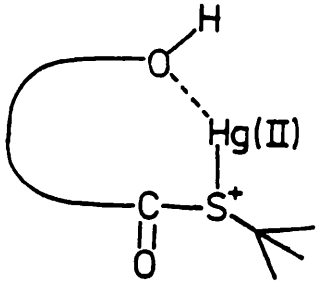


Figure 5

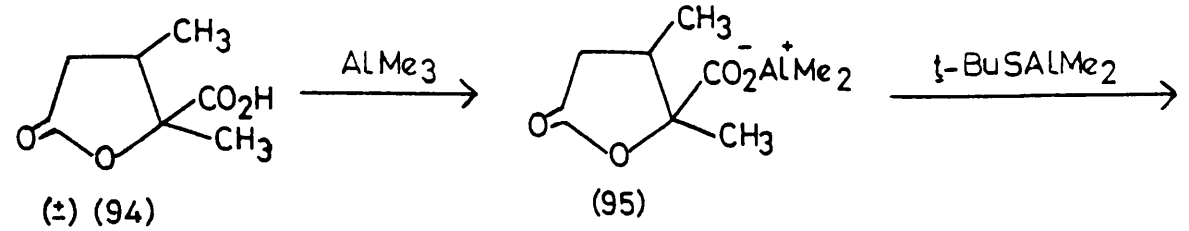
attractive means for protecting and subsequently activating a carboxyl group.

The O-acetyl derivative (92) of crobarbatine (93), another eleven membered macrocyclic diester of (+)-retronecine, and a diastereomer have been synthesised by Huang and Meinwald[58] employing this strategy (see Scheme 15).

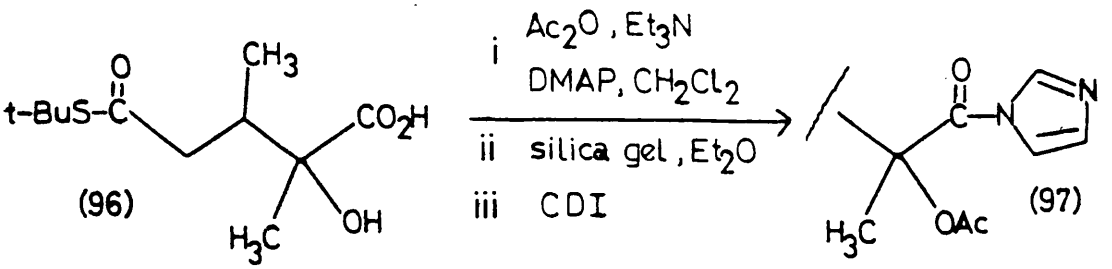
Crobarbatic acid lactone (94), shown to be the trans-

isomer, was converted into the methylene chloride soluble dimethylaluminium salt (95) which was subsequently treated with dimethylaluminium t-butylsulphide affording the required t-butylthiol ester (96). Protection of the tertiary hydroxyl of compound (96), as the acetate, was followed by activation of the free acid as the acylimidazolide (97). The suitably derivatised crobarbatic acid (97) was coupled with retronecine, using a catalytic amount of sodium hydride, yielding a mixture of diastereomeric monoesters (98). Various attempts to lactonise (98) failed. When 6 equivalents or less of mercuric trifluoroacetate or a mixture of mercuric chloride and cadmium carbonate were used, starting material was recovered and when a large excess of mercuric trifluoroacetate or copper(I) trifluoroacetate was used, the carboxylic acid (99) was isolated. Finally, copper(I) trifluoromethanesulphonate - benzene complex was found to effect the crucial lactonisation in good yield. The mixture of diastereomeric cyclic diesters (92), obtained in 62% yield, was separated by column chromatography.

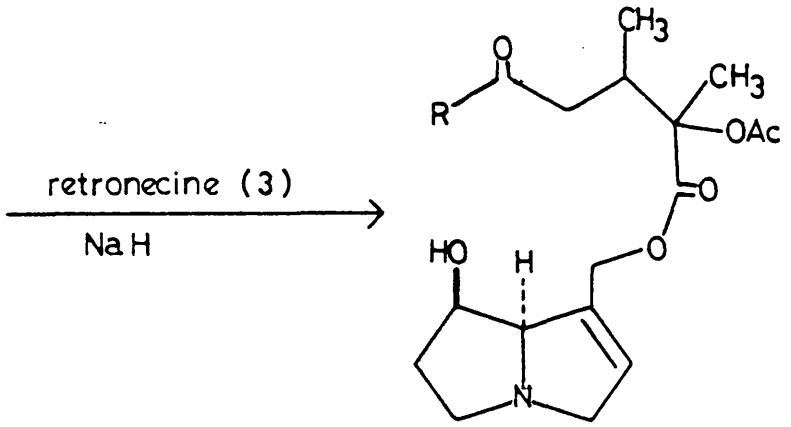
The configurations of the acid portions of the dilactones (92a) and (92b) were established by acid hydrolysis of each diastereomer, and analysis of the Cotton effect exhibited by each lactonic acid (see Figure 6). Attempts to deacetylate (92a) and (92b) by base catalysed hydrolysis, even under mildly basic conditions, produced retronecine. Under acid catalysed conditions only (92a) yielded the deacetylated product (93a) in low yield. However, the authors could not prove that (93a) corresponded to natural crobarbatine.



(±) (94) (95)



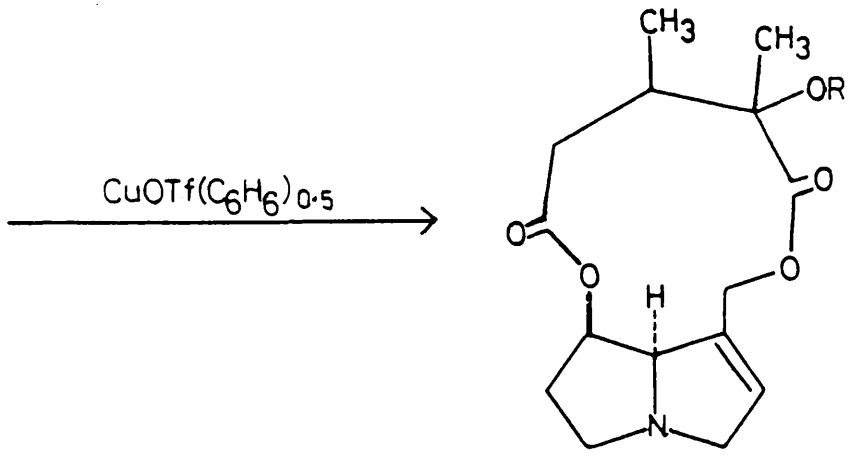
(96) (97)



diastereomeric mixtures

(98) R = t-BuS

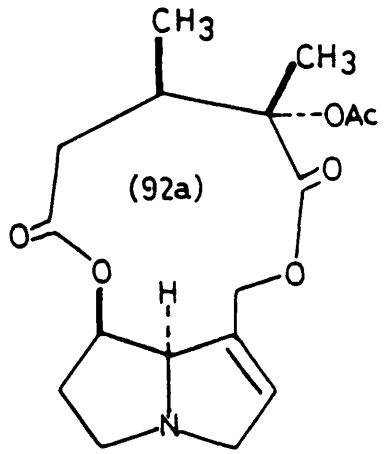
(99) R = OH



(92) R = Ac

(93) R = H

SCHEME 15



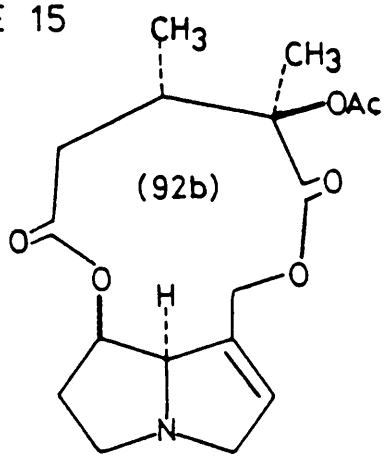
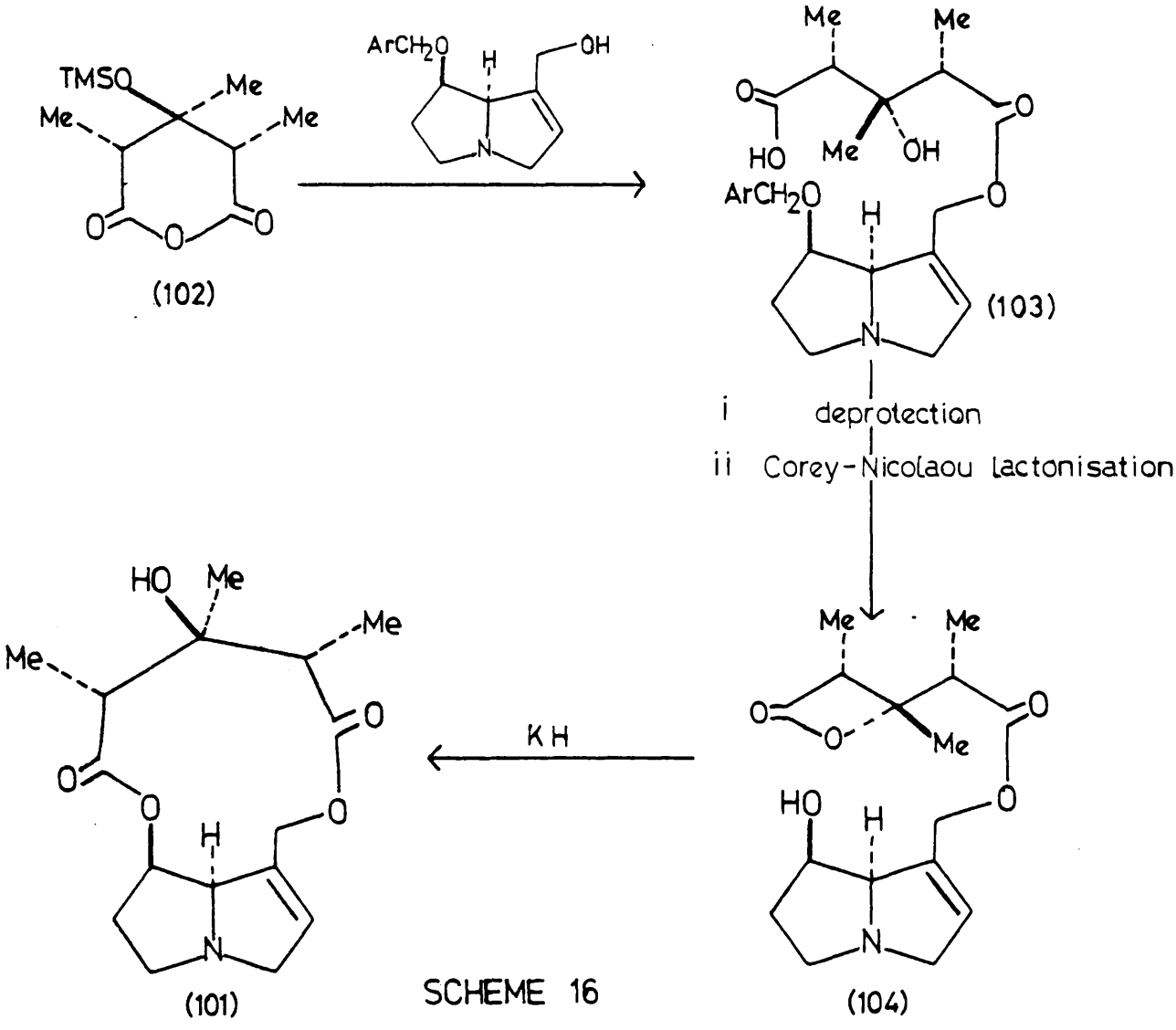


Figure 6

Vedejs and Larsen attempted both Corey-Nicolaou (pyridine-2-thiol ester) and Masamune (t-butylthiol ester) procedures for lactonisation, while trying to prepare (±)-crispatine (100) and (±)-fulvine (101), without much success.

A strategy similar to that applied by Robins et al. for the synthesis of dicrotaline was initially attempted by Vedejs[59] (see Scheme 16). Suitably protected

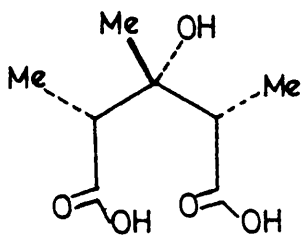


(±)-fulvinic anhydride (102) and (±)-retronecine (3) were combined affording the required monoester (103) and a

diastereomer. Deprotection of the monoesters followed by lactonisation by the Corey-Nicolaou method yielded the β-lactone (104) instead of the macrocyclic dilactone (101). A low yield of the required product (101) was obtained by treating the monoester (104) with potassium hydride.

Improved syntheses of (±)-crispatine (100) and (±)-fulvine (101), reported by Vedejs and Larsen,[60] involved a simple alternative cyclisation method, based on the high reactivity of allylic mesylates towards carboxylate anions (see Scheme 17). A similar method had been used earlier, by Culvenor et al., to prepare 9-monoesters of unsaturated necine bases (see Section 2.2.b.i).

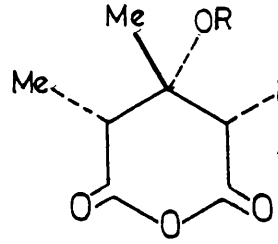
Crispatic anhydride (106), prepared from crispatic acid (105), was protected as its methoxymethylether (107) Opening of the anhydride afforded the carboxyl protected crispatic derivative (108), and activation of the free acid was achieved by preparing the mixed phosphoric anhydride (109). Coupling of the activated acid (109) with the lithium alkoxide (110) of synthetic monosilylated (±)-retronecine gave a 1:1 mixture of the monoester (111a) and a diastereomer. Lactonisation was accomplished by the following deprotection, activation and deprotection sequence. Desilylation of the monoesters (111) liberated the primary allylic hydroxyl (112) which was then transformed into the mesylate (113). This mesylate was not isolated but diluted with acetonitrile and added, over a period of approximately 3 hours, to excess tetra-n-butylammonium fluoride hydrate in acetonitrile. Lactonisation occurred spontaneously to give diasteromers



(105)

DCC

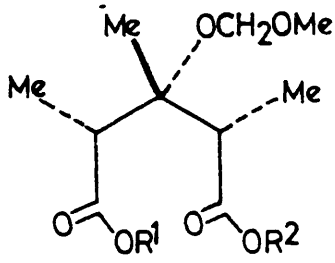
P_2O_5, $(MeO)_2CH_2$



(106) R=H

(107) R=CH_2OMe

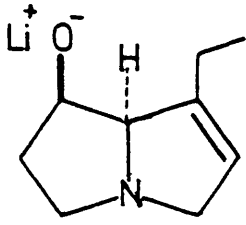
$Me_2AlOCH_2CH_2SiMe_3$



(108) R^1=H, R^2= $CH_2CH_2SiMe_3$

(109) R^1= $PO(OEt)_2$, R^2= $CH_2CH_2SiMe_3$

(109) and

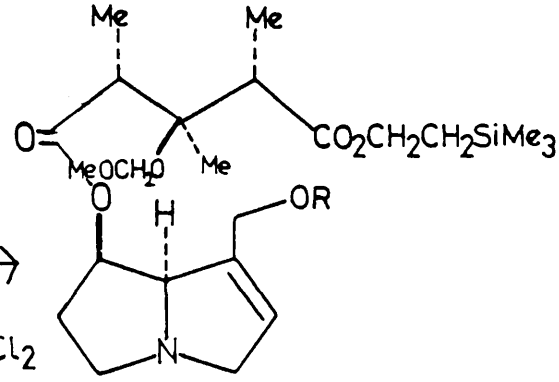


(110)

i DMAP

ii H_2O-HF, THF

iii $MeSO_2Cl$, Et_3N, CH_2Cl_2



(111a) R = TBDMS

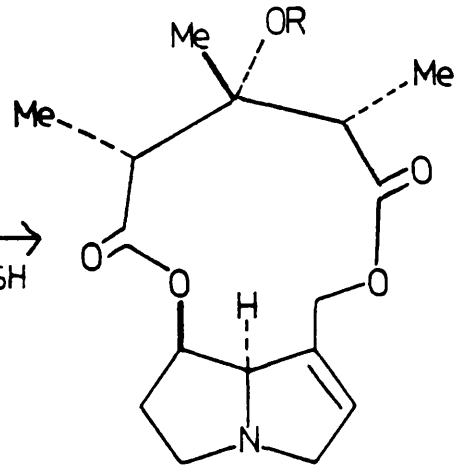
(112a) R = H

(113a) R = SO_2Me

+ a diastereoisomer 'b'

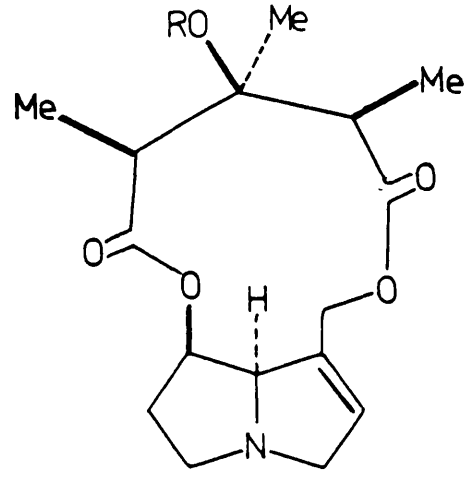
i n-$Bu_4N^+F^-$

ii $BF_3.Et_2O$ -EtSH



(114) R = CH_2OMe

(100) R = H



(115) R = CH_2OMe

(116) R = H

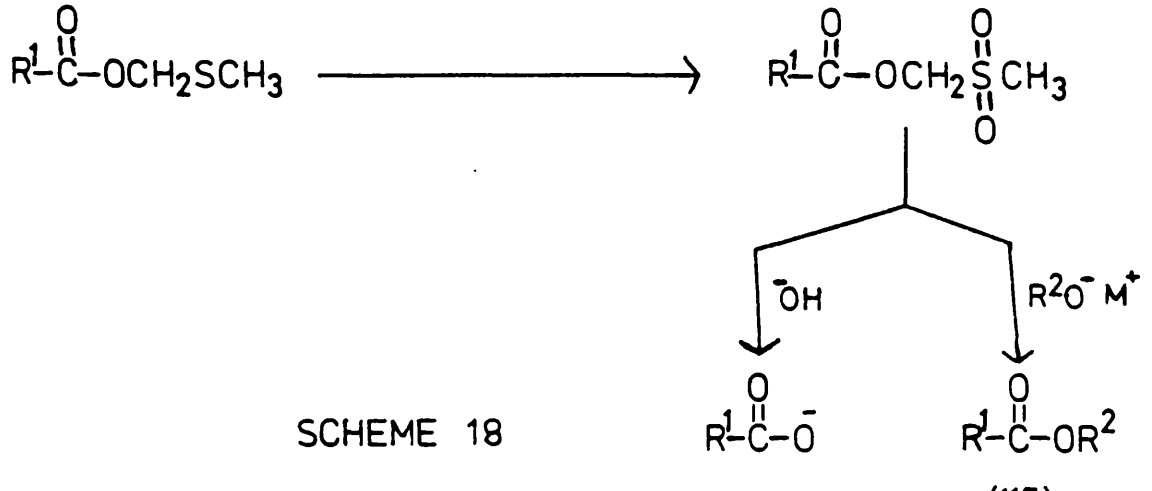
SCHEME 17

(114) and (115) which were separated. Quantitative deprotection of (114) gave (±)-crispatine (100) which was chromatographically and spectroscopically identical with a sample of the natural product. Deprotection of (115) gave (±)-isocrispatine (116). An analogous route was used to prepare (±)-fulvine (101) and (±)-isofulvine.

(b) Twelve membered macrocyclic diesters

Integerrimine (130) is the only twelve membered macrocyclic diester pyrrolizidine alkaloid which has been synthesised. The synthesis, accomplished by Narasaka et al.,[61] includes a novel lactonisation procedure which employs a methylthiomethyl ester ($CO_2CH_2SCH_3$) as a protecting group which can be readily activated.

The methylthiomethyl group is a well known protecting group for alcohols and carboxylic acids. Deprotection is generally carried out with heavy metal salts or oxidising agents. The latter converts the methylthiomethyl group into a methylsulphonylmethyl group which is readily hydrolysed by aqueous alkali (see Scheme 18). The authors expected that an ester (117) would be formed if the methylsulphonylmethyl ester was treated with a metal alkoxide instead of aqueous alkali.



SCHEME 18

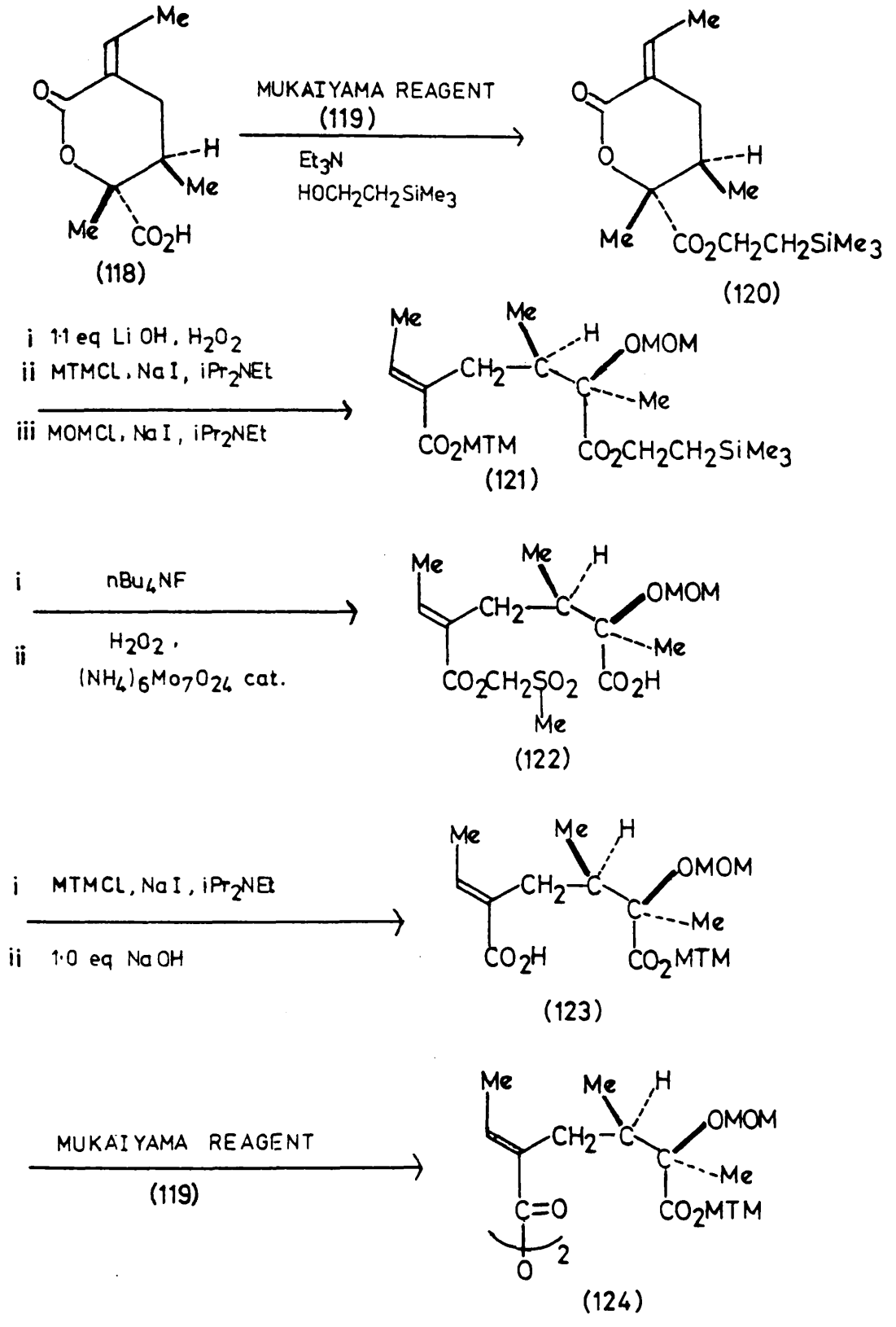
(117)

The synthesis of integerrimine (130) includes the preparation of suitably protected retronecine and integerrinecic acid, coupling of these moieties followed by lactonisation.

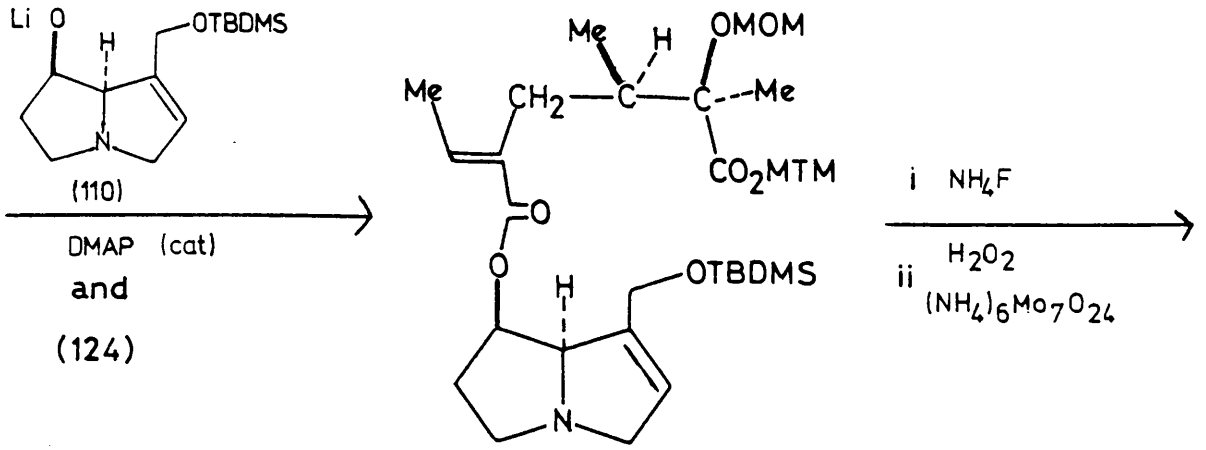
Retronecine was prepared by a modification of the Geissman route and selectively protected as the t-butyldimethylsilyl ether (110).

(±)-Integerrinecic acid, prepared as reported,[61] was suitably derivatised as shown in Scheme 19a. Firstly, the tertiary carboxylic acid of integerrinecic acid lactone (118) was protected as the trimethylsilylethyl ester (120) using the Mukaiyama reagent, 1-chloro-2-methylpyridinium iodide (119). Selective hydrolysis of the δ-lactone, followed by protection of the free acid, as the methylthiomethyl ester, and of the tertiary hydroxyl as the methoxymethyl ether, afforded compound (121). Desilylation of this intermediate (121) preceded the oxidation of the sulphide to the sulphone (122). Protection of the tertiary acid, as the methylthiomethyl ester, followed by hydrolysis of the sulphone yielded the required derivatised integerrinecic acid (123). This was transformed into the acid anhydride (124) by treatment with the coupling reagent (119).

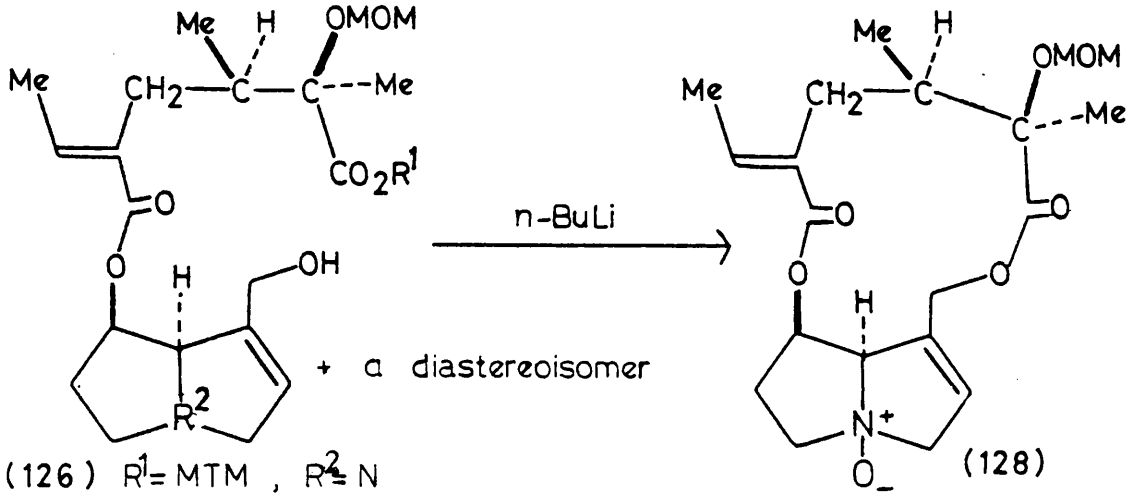
The necine and necic acid moieties were coupled by treating the lithium alkoxide (110) of the silylated retronecine with the acid anhydride (124), in the presence of a catalytic amount of N,N-4-dimethylaminopyridine (DMAP).


MUKAIYAMA REAGENT
(119)
Et3N
HOCH2CH2SiMe3
(118)
(120)
i 1·1 eq LiOH, H2O2
ii MTMCl, NaI, iPr2NEt
iii MOMCl, NaI, iPr2NEt
(121)
i nBu4NF
ii H2O2, (NH4)6Mo7O24 cat.
(122)
i MTMCl, NaI, iPr2NEt
ii 1·0 eq NaOH
(123)
MUKAIYAMA REAGENT
(119)
(124)

SCHEME 19a



(125) + a diasteoisomer



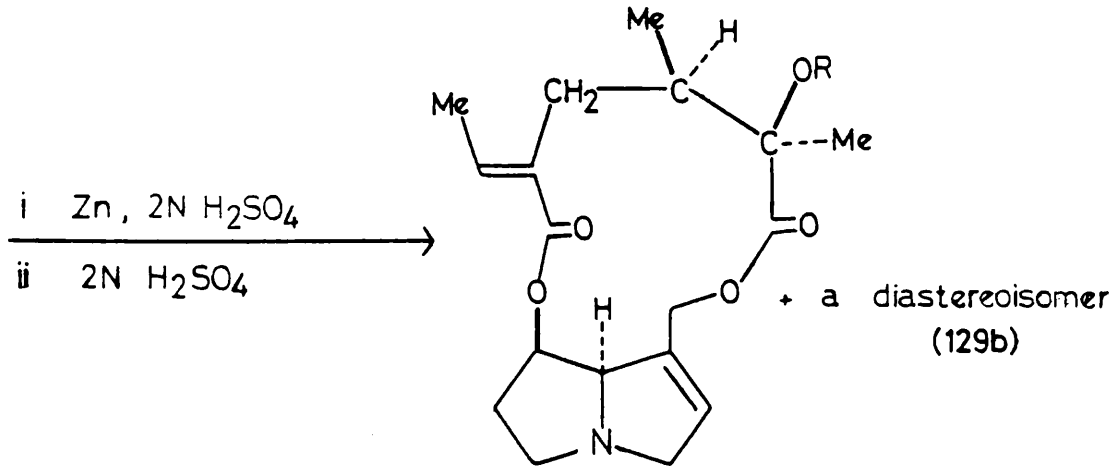
(126) R^1 = MTM , R^2 = N

(127) R^1 = MSM , R^2 = N^+–O^-

+ a diastereoisomer

(128)

+ a diastereoisomer



+ a diastereoisomer

(129b)

(129a) R = MOM

(130) R = H

MOM = CH_2OCH_3

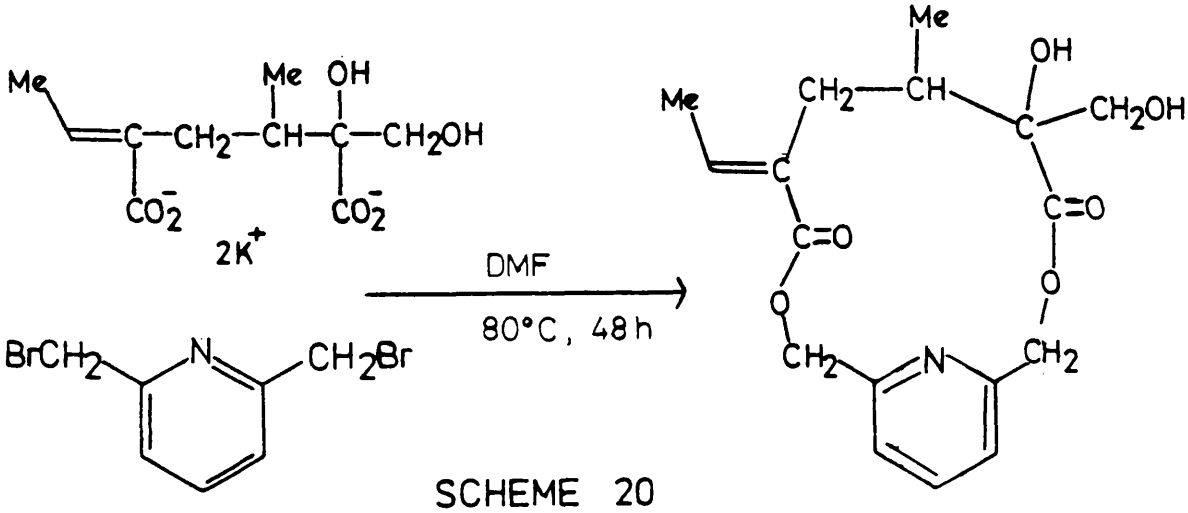
MTM = CH_2SCH_3

MSM = $CH_2SO_2CH_3$

SCHEME 19b

The following sequence of reactions was carried out on the diastereomeric mixture of products (125). The monoesters (125) were desilylated and oxidation of the sulphides (126) gave the corresponding sulphones (127). Lactonisation of the activated acids (127) was achieved by the intramolecular nucleophilic displacement of the methanesulphonylmethyl group to afford the dilactones (128). Reduction of the N-oxides (128) yielded a diastereomeric mixture of bases [(129a) and (129b)] which were separated. Removal of the methoxymethyl ether from (129a) afforded (±)-integerrimine (130) which was spectroscopically and chromatographically identical to a natural sample (see Scheme 19b).

A pyrrolizidine alkaloid analogue (132) with a 12-membered macrocycle has been prepared, by Drewes and Pitchford,[62] by the reaction of 2,6-bis(bromomethyl)pyridine with the dipotassium salt of the C_{10} necic acid, retronecic acid (see Scheme 20). The authors claim that the macrocyclic



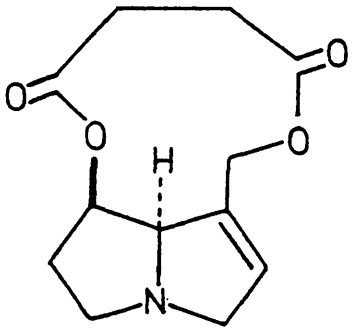
SCHEME 20

diester (132) causes cytotoxic effects similar to pyrrolizidine alkaloids although it is difficult to see how such an analogue could be metabolised to produce these effects.

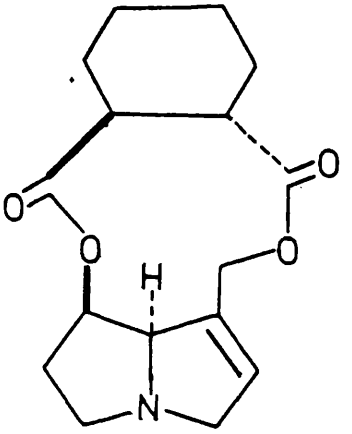
(c) Ten-membered macrocyclic diester analogue

Macrocyclic pyrrolizidine diesters generally have 11- or 12-membered rings. No 10-membered macrocyclic diesters have been isolated, although succinic acid derivatives are common plant constituents.

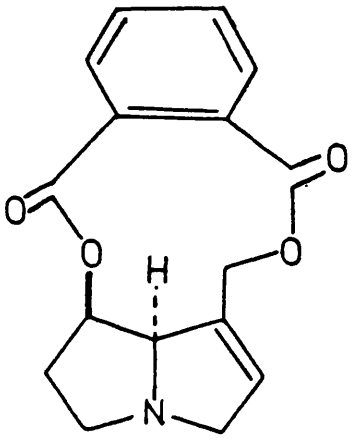
A series of 10-membered macrocyclic diesters [(133)-(135)] of (+)-retronecine have been prepared by Robins and Burton.[63] The compounds (133)-(135) were prepared by the treatment of (+)-retronecine with succinic anhydride, (±)-trans-cyclohexane-1,2-dicarboxylic anhydride and phthalic anhydride respectively. The resulting monoester hydroxy-acids were lactonised via their pyridine-2-thiol esters. The diastereomers (134) were not separated, but were shown to be present in a ratio of approximately 4:1, by n.m.r. spectroscopy. X-ray data for the succinate dilactone (133) showed that the 10-membered macrocycle adopts a solid state conformation in which the carbonyl groups are antiparallel.



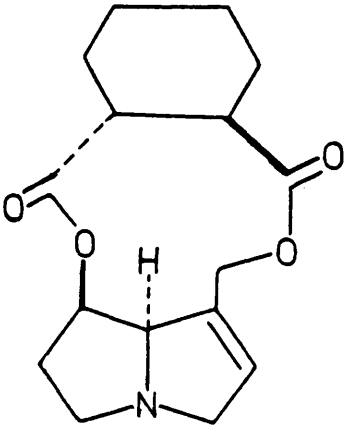
(133)



(134a)



(135)



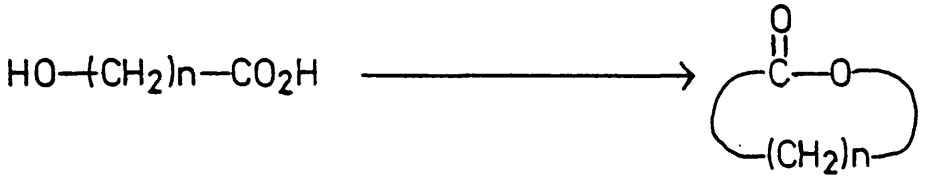
(134b)

Lactonisation

Synthetic methodology for the construction of large ring lactones has been developed over recent years due to the discovery of macrolide natural products with antibiotic and other therapeutic properties.

In this subsection the principles of, and commonly used procedures for, lactonisation will be discussed. Some recent advances in this area will also be detailed. However, for a more comprehensive review of lactonisation procedures, the listed publications are recommended.[64-67]

Lactonisation is the process by which a hydroxy-carboxylic acid is intramolecularly esterified producing a cyclic ester (lactone), as shown in Scheme 21. The rate



SCHEME 21

of this and other cyclisations depends upon the following two factors. Firstly, the atoms at the end of the chain have to come within reacting distance (entropy factor) and secondly, steric and stereoelectronic interactions may hinder the ring closure when the two ends meet (enthalpy factor).

Specific structural features can enhance the rate of cyclisation. For example, the presence of unsaturation in a medium sized carbon chain will reduce the rotational possibilities available and eliminate transannular interactions, thereby promoting an intramolecular process.[68]

The rate of cyclisation is also dependent on the length of carbon chain involved as shown in Figure 7. From this graph, based on the work of Illuminati et al.,[69] it can be seen that 5-membered lactones are formed 10^6 times more readily than 8-membered lactones.

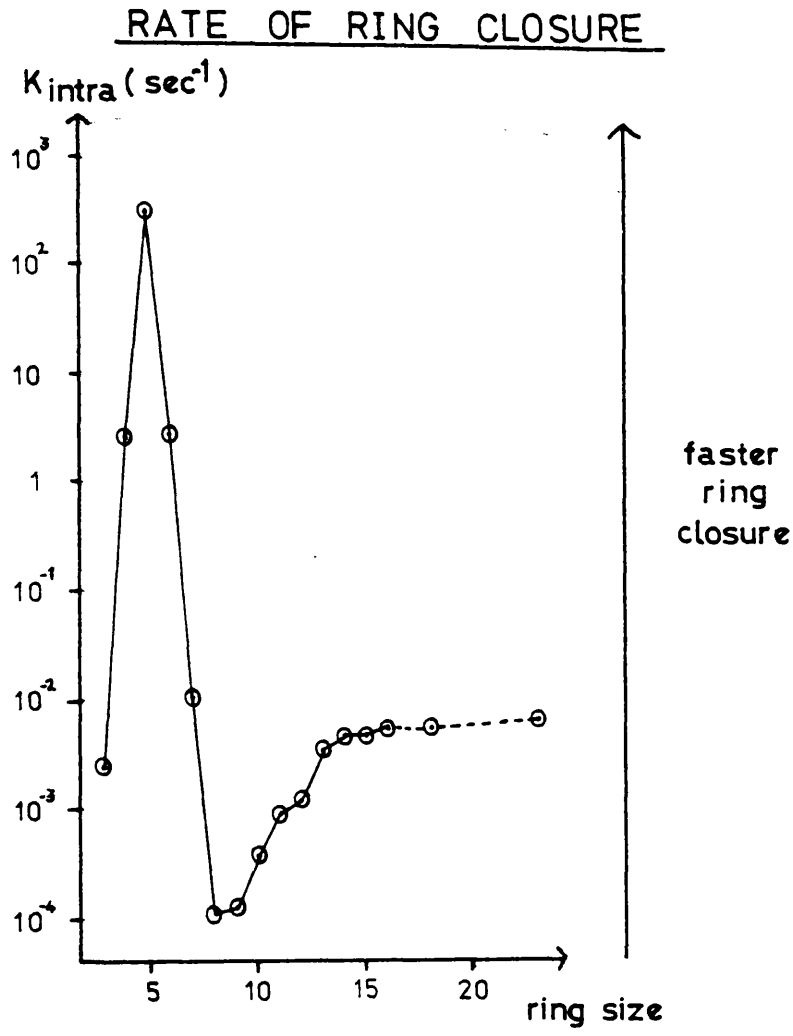


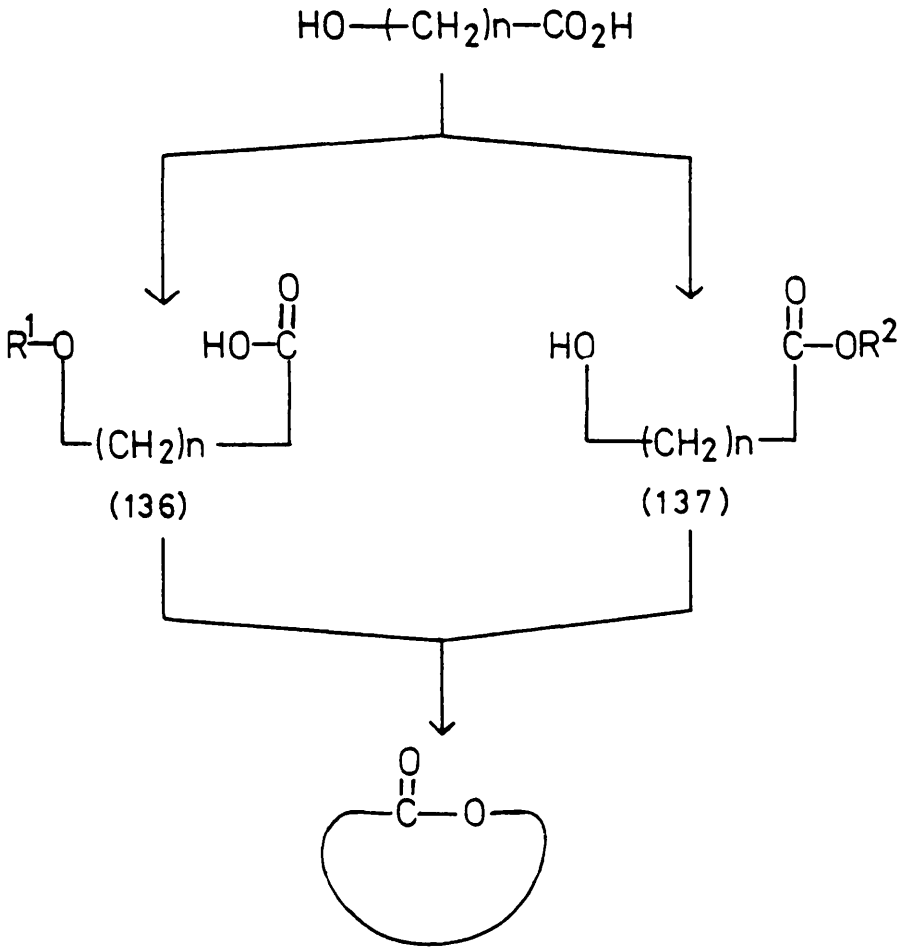
Figure 7

Macrocyclic lactones are prepared by the lactonisation of long chain hydroxycarboxylic acids. Although the steric and stereoelectronic interactions involved may be negligible this process is disfavoured on the basis of the entropy factor. A competing intermolecular process (esterification) exists from which oligomeric products are formed (see Scheme 22).

SCHEME 22

This process is favoured when long chain ω-hydroxycarboxylic acids are reacted under normal reaction conditions. However, since lactonisation is a unimolecular process and esterification a bimolecular process, the former process can be enhanced and the latter minimised by reacting the ω-hydroxyacid under dilute conditions.

The preparation of a macrocyclic lactone from a long chain hydroxyacid is generally achieved by the following procedure (see Scheme 23).



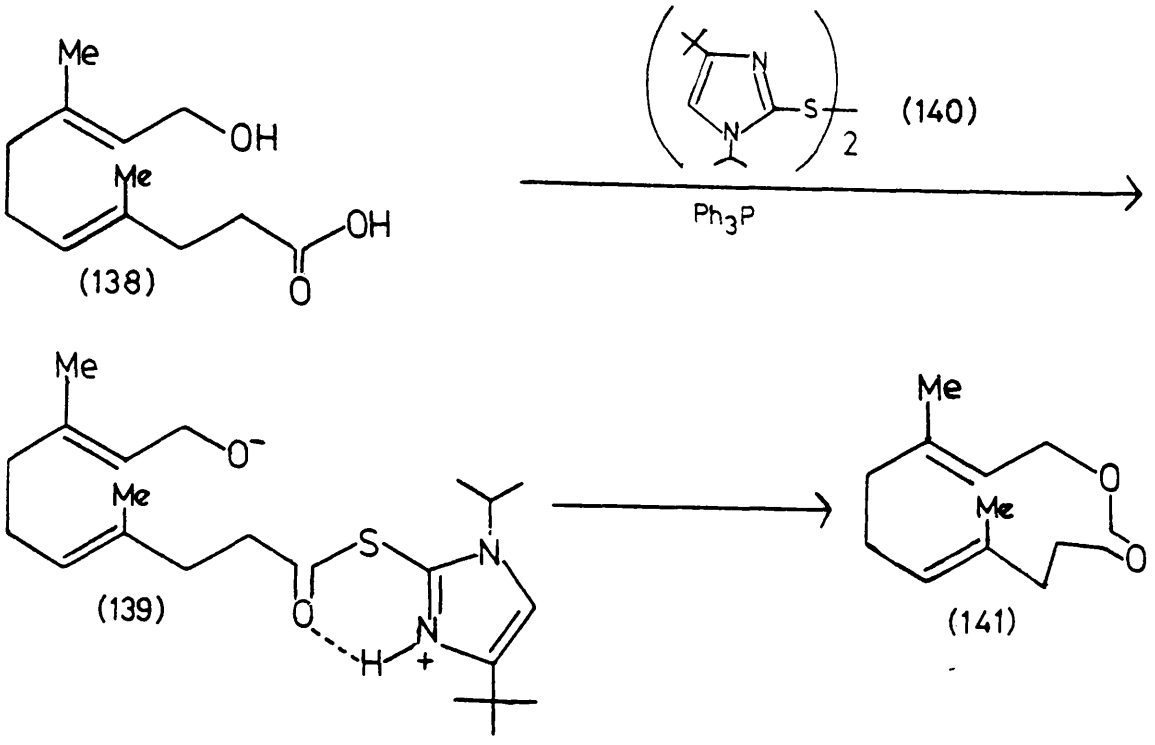
SCHEME 23

Firstly the hydroxyacid is converted into a hydroxyl (136) or carboxyl (137) activated intermediate by treatment with suitable reagents. Lactonisation is then effected by adding a solution of the reactive intermediate [(136) or (137)] dropwise, over a period of time, to solvent heated at reflux.

A number of lactonisation reagents have been developed over the past dozen years. The widely used Corey[52] and Masamune[57] methods which utilize intermediate thiol esters to promote lactonisation, have already been discussed in the preceding subsection. Other commonly used procedures are

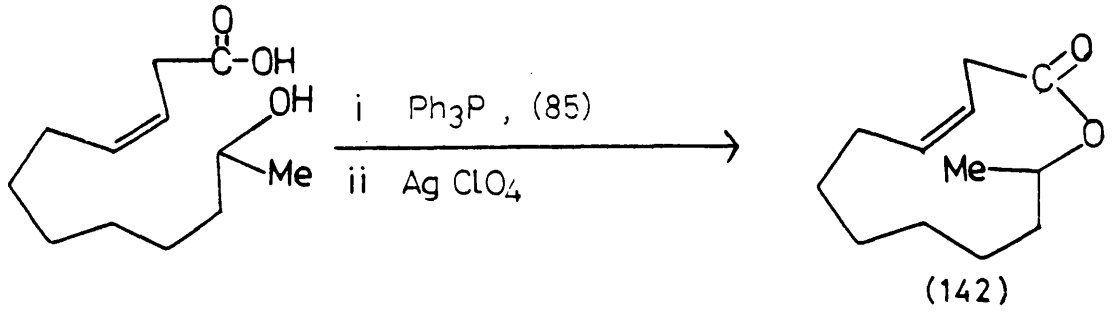
1. Modifications to the Corey Method

In the syntheses of two macrolide pheromones (141) and (142), published by Oehlschlager et al.[70] the crucial lactonisation steps were effected by modified Corey methods as shown in Schemes 24 and 25. In the first, the hydroxy-acid (138) was transformed into the thiol ester (139) by treatment with the disulphide (140) and triphenylphosphine (84) (c.f. preparation of dicrotaline, Scheme 13). Cyclisation to the required lactone was promoted through the double activation of the thiol ester. Imidazolethiol esters, such as (139), are reported[71] to be more reactive



SCHEME 24

intermediates than the corresponding pyridine analogues, as lactonisation can occur at room temperature. In the second, the lactone (142) was prepared <u>via</u> a pyridine-2-thiol ester

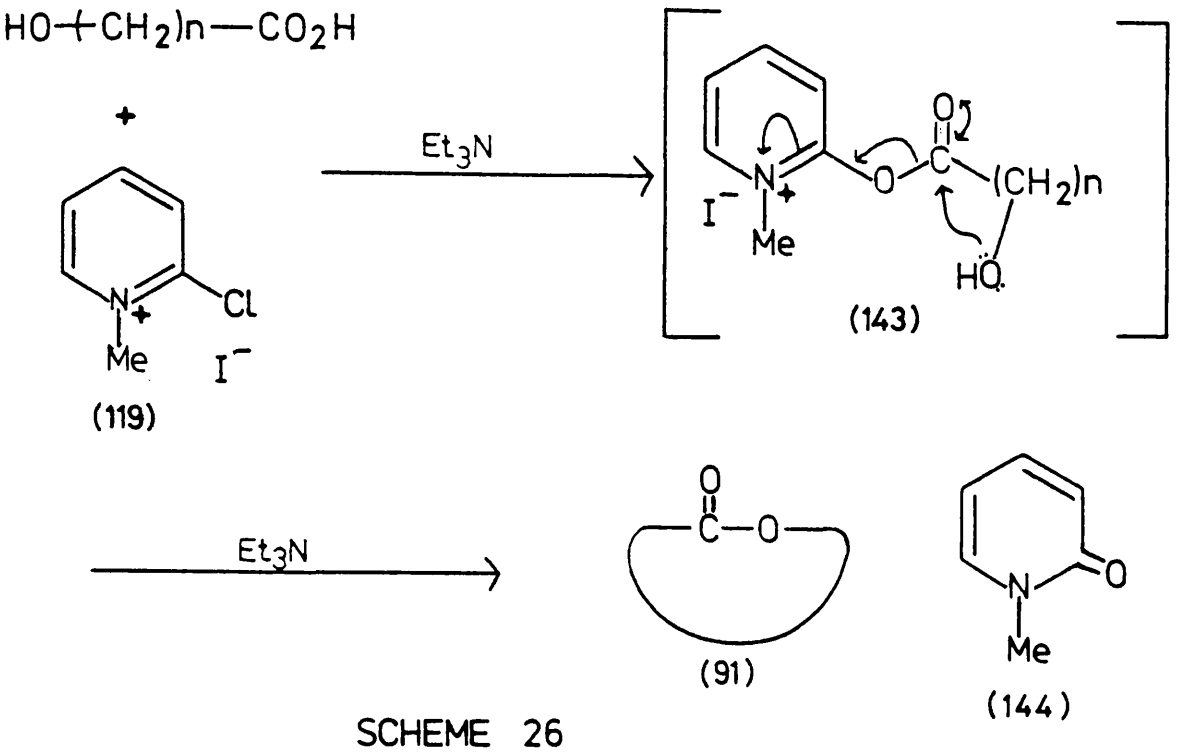


SCHEME 25

in the presence of silver ions (as the perchlorate). Silver ions are used to activate the thiol ester by complexation.[72]

2. Mukaiyama Method

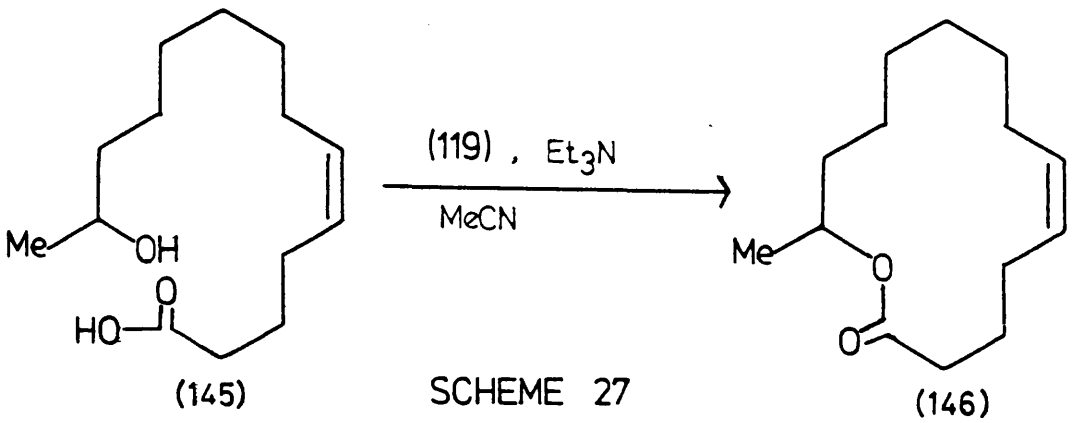
The cyclisation of ω-hydroxyacids using 1-methyl-2-chloropyridinium iodide (119) in the presence of triethylamine (TEA), was reported by Mukaiyama.[73] The proposed mechanism is shown in Scheme 26. Nucleophilic attack of



SCHEME 26

the carboxylate ion on the chloropyridinium salt (119) generates an active acylating agent (143) which rapidly cyclises as a result of the intramolecular attack of the hydroxyl group. The 2-pyridone (144) is the by-product.

The macrolide (146) was prepared, by Oehlschlager et al.,[74] in a 49% yield, using this method (see Scheme 27). A solution of the hydroxyacid (145) and TEA in acetonitrile was added dropwise to a solution of the Mukaiyama reagent (119) in acetonitrile heated at reflux.

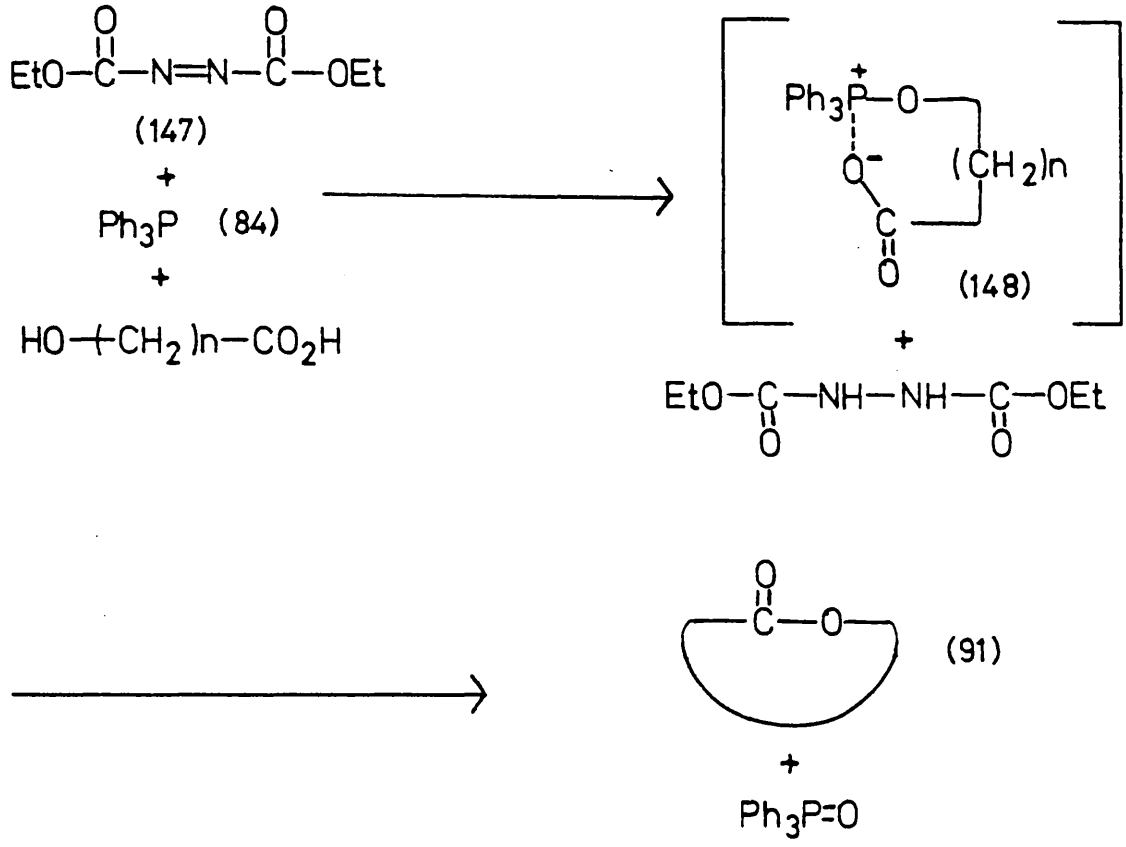


(145) SCHEME 27 (146)

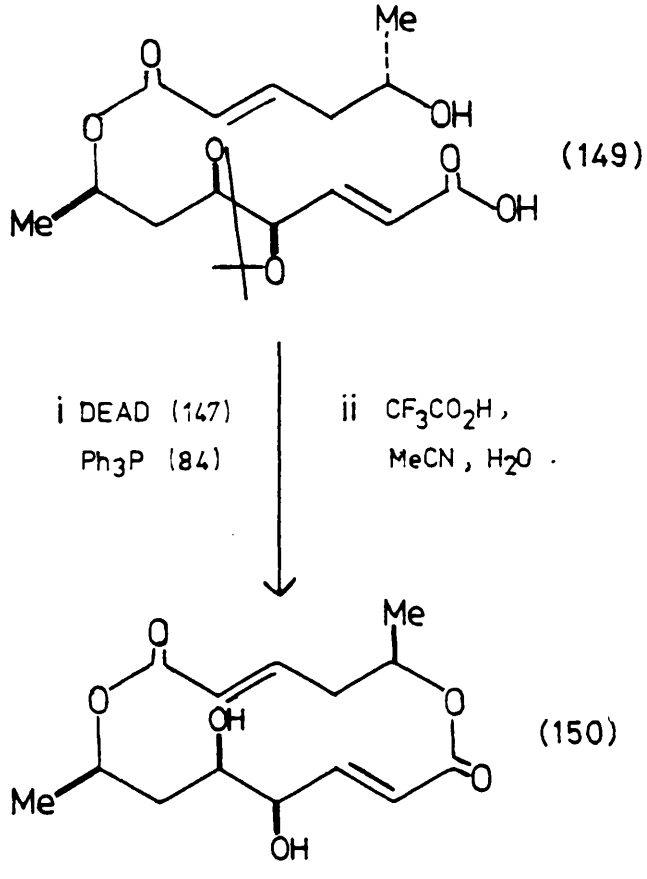
3. Mitsunobu Method

The procedures already mentioned, attributed to Corey, Masamune and Mukaiyama, have involved activated carboxyl intermediates. The following method, reported by Mitsunobu,[75] employs the "reverse activation," wherein the hydroxyl group is activated (see Scheme 28). The hydroxyacid is converted into the reactive intermediate (148) upon treatment with diethyl azodicarboxylate (DEAD) (147) and triphenylphosphine (84). Then, nucleophilic attack of the carboxylate ion, of intermediate (148), on the carbinol carbon afforded the desired lactone (91). Inversion of configuration is observed if the carbinol carbon is chiral.

The synthesis[76] of colletodiol (150) was achieved using this procedure (see Scheme 29). Treatment of the hydroxyacid (149) with reagents (147) and (84) at -10 °C generated an activated hydroxyl intermediate which spontaneously cyclised at 0 °C. Deprotection yielded the required macrolide (150).



SCHEME 28



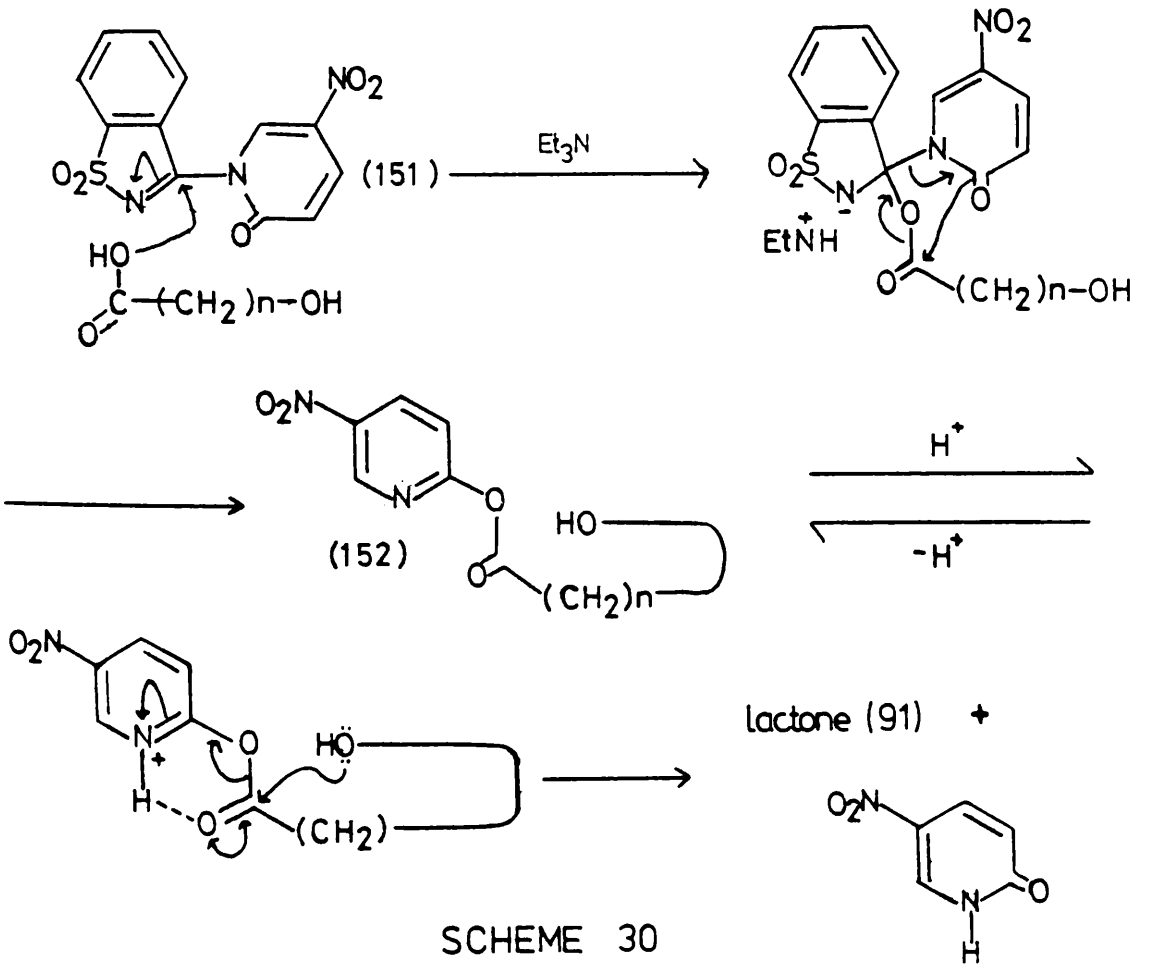
SCHEME 29

4. Other Methods

There are a number of other types of activated intermediates, e.g. mixed acid anhydrides and N-acyl-imidazolides, which have been employed in macrolide syntheses. For further information and these and other methods of lactonisation, the recommended reviews[64-67] should be consulted.

Recently Developed Lactonisation Procedures

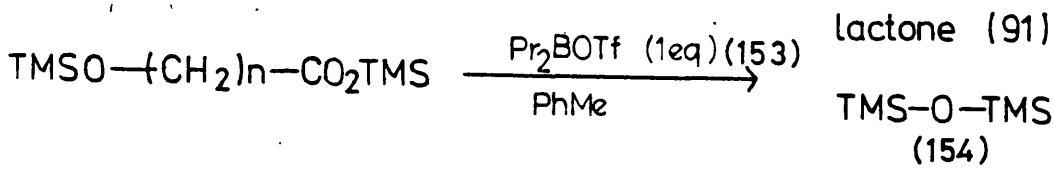
A new lactonisation reagent was reported by Kinoshita et al.[77] 3-(5-Nitro-2-oxo-1,2-dihydro-1-pyridyl)-1,2-benzisothiazole-1,1-dioxide (151) promotes lactonisation



SCHEME 30

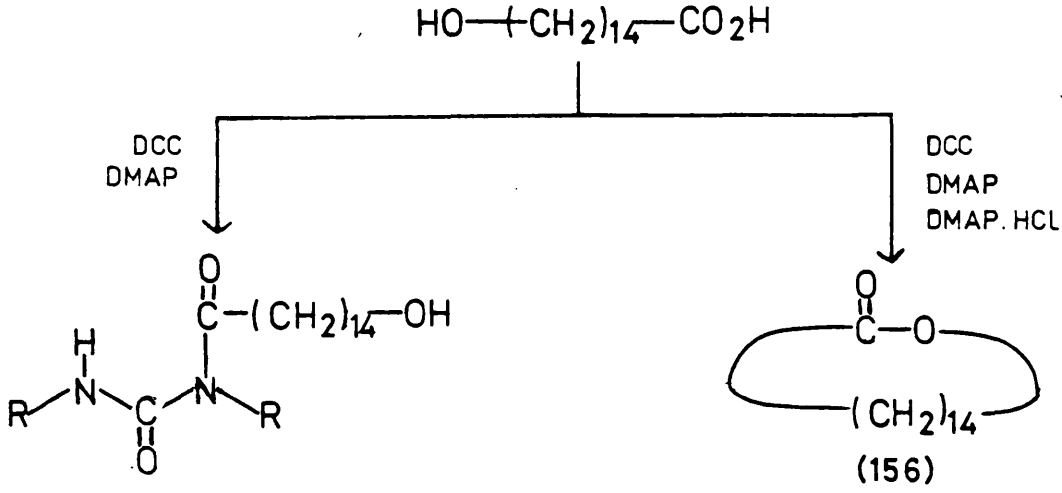
via the ester (152). Nucleophilic attack by the hydroxyl, yielding the macrolide (91), is catalysed by p-toluene-sulphonic acid (see Scheme 30).

The same group has also published[78] a procedure for the lactonisation of 0,0'-disilylated hydroxycarboxylic acids, as shown in Scheme 31. The presence of dipropylboryltriflate (153) promotes the condensation liberating hexamethyldisiloxane (154).



SCHEME 31

The Steglich procedure for esterification (DCC/DMAP/DMF) has been modified by Keck[79] for the synthesis of macrocyclic lactones (see Scheme 32). When the Steglich method was employed



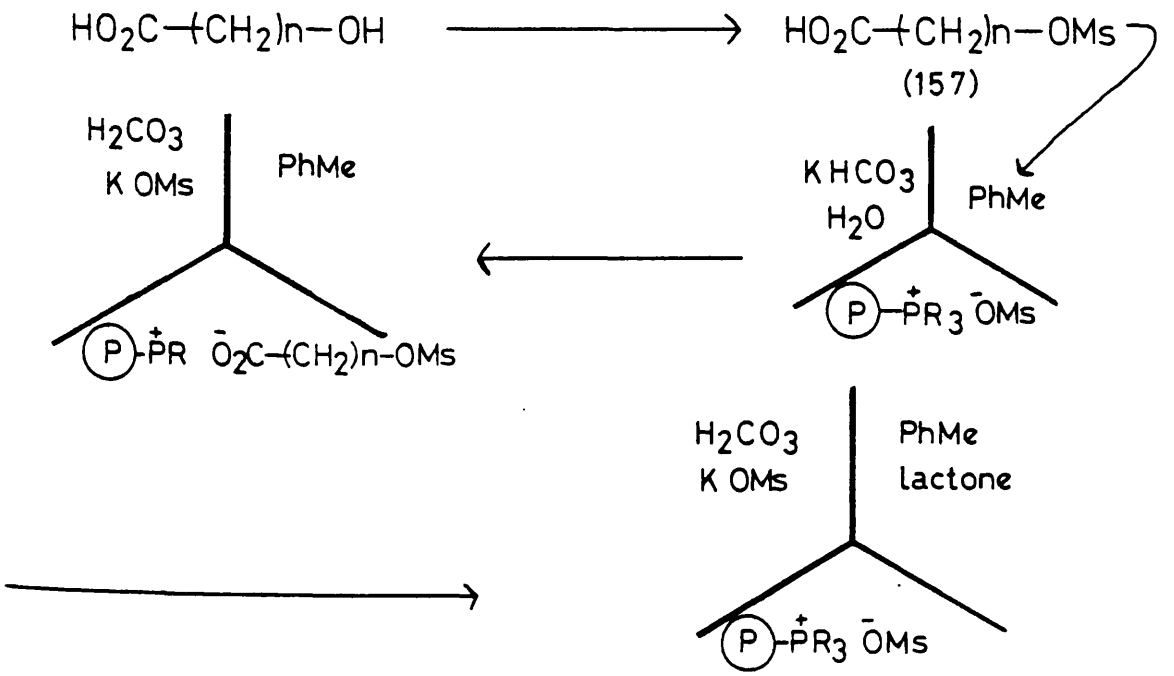
SCHEME 32

under the high dilution conditions generally required for lactonisation a near quantitative yield of N-acylurea (155) was obtained. However when 1 equivalent of DMAP.HCl was present a high yield of the required macrocyclic lactone (156) was afforded.

Failure of the Steglich method, under high dilution conditions, was due to the low effective concentration of proton sources.

One of the major problems associated with the formation of macrolides is the requirement for relatively high dilutions. In their attempts to overcome this problem some groups have employed polymer supported reagents to achieve pseudodilute conditions.

Polymer supported thiol esters[80] and polystyryldiphenylphosphine,[81] used in the Mitsunobu procedure, only produced low yields of macrocyclic lactones. However, a triphase catalytic cyclisation method, reported by Regen and Kimura,[82] afforded macrocyclic lactones in yields comparable with those obtained using the Corey-Nicolaou[52] and



SCHEME 33

Kruizinga-Kellog[83] (Cs_2CO_3, DMF, ω-iodoacids) methods (see Scheme 33). The hydroxyacid was transformed into the mesylate derivative (157) which was added to the triphase system. An acid-base reaction was followed by an exchange of ions. Upon heating, lactonisation occurred and the mesylate form of the resin was regenerated.

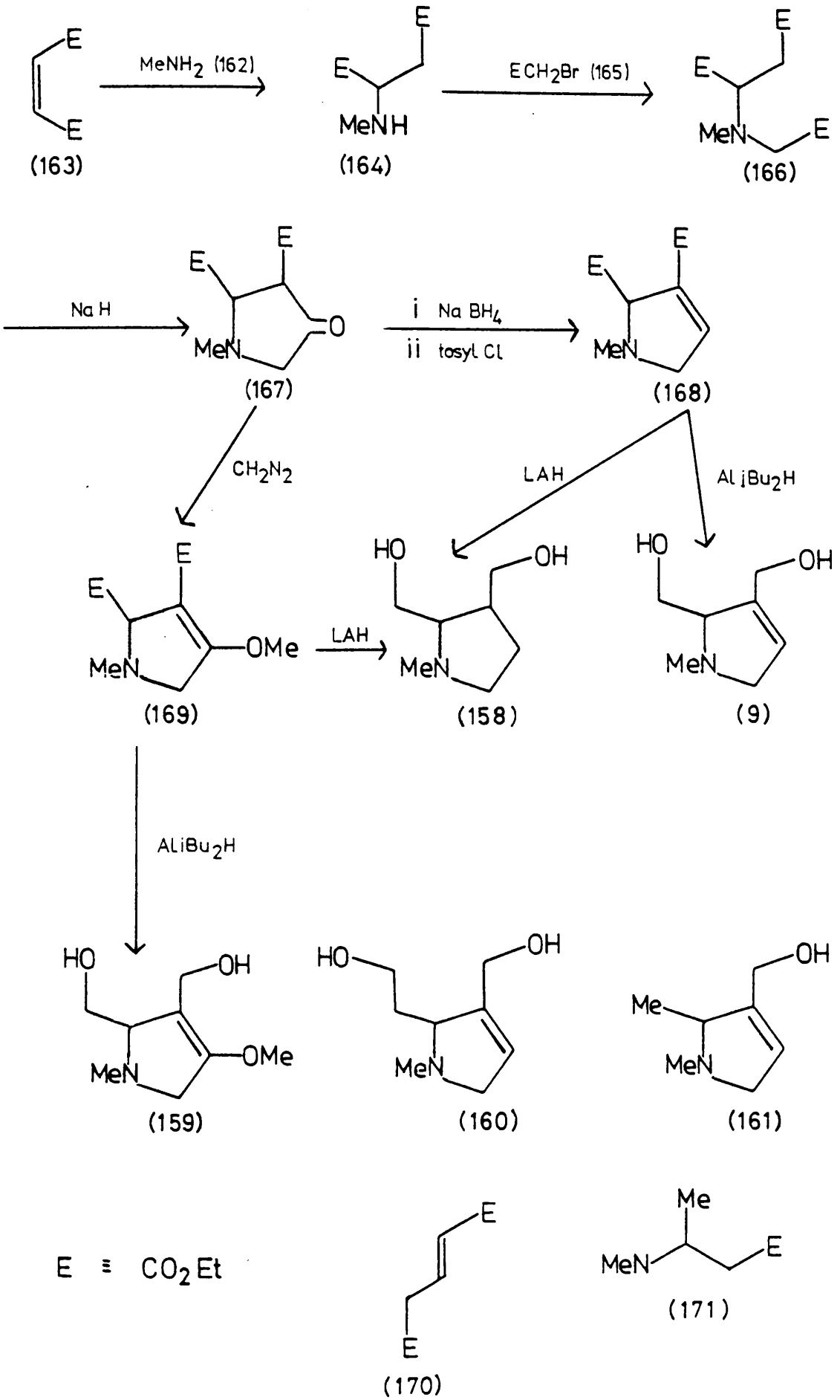
Purification of complex reaction mixtures is also simplified by using polymer supported reagents.

During the planning of the synthesis of novel lactonic systems a number of factors have to be considered. The choice of lactonisation procedure is clearly of importance. The size of ring which is to be prepared, the flexibility of the carbon chain, and the steric interactions which may hinder the ring closure, must also be taken into account.

2.4 Synthesis of Pyrrolizidine Alkaloid Analogues

An important series of pyrrolizidine alkaloid analogues, prepared by Mattocks,[12,13] are the synthanecines A - E [(9) - (158) - (161)]. These monocyclic analogues of the bicyclic necine bases were prepared for metabolic and toxicological studies. Synthanecine A, analogous to retronecine (3), was used extensively in these studies. The synthesis of synthanecine A was first reported in 1974.[12] However, an improved route (see Scheme 34) followed soon after.[13]

Condensation of methylamine (162) and diethyl maleate (163) afforded the diester (164), which was then converted into the triester (166), using ethylbromoacetate(165). Dieckmann

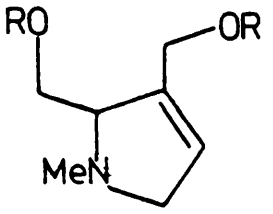

MeNH2 (162)
ECH2Br (165)
(163)
(164)
(166)
Na H
(167)
i Na BH4
ii tosyl Cl
(168)
CH2N2
LAH
Al iBu2H
(169)
(158)
(9)
ALiBu2H
(159)
(160)
(161)
E ≡ CO2Et
(170)
(171)

SCHEME 34

cyclisation of compound (166) gave the ketone (167). Selective reduction of the ketone followed by dehydration introduced the required unsaturation. Finally, reduction of the diester (168) to the diol, synthanecine A (9), was achieved using di-isobutylaluminium hydride.

Synthanecine B (158) was prepared by reduction of the olefinic diester (168) or the enol ether (169). This enol ether (169) was prepared by treating the ketone (167) with diazomethane. Selective reduction of the enol ether (169) gave synthanecine C (159). By exchanging diethyl maleate (163) for diethyl glutaconate (170), in Scheme 34, synthanecine D (160) was prepared instead of synthanecine A. Synthanecine E (161) was prepared using ethyl-3-methylamino-butyrate (171), instead of the diester (165), in an analogous route.

A series of diesters and dicarbamates [(172) - (176)]



(172) R = COC(Me)(Et)OMe
(173) R = $PO(OEt)_2$
(174) R = CONHEt
(175) R = $CONMe_2$
(176) R = $CONEt_2$
(177) R = CO_2Ph

of synthanecine A was prepared by conventional methods. The bis-2-methoxy-2-methylbutyrate (172) and the bis-diethylphosphate (173) diesters were prepared *via* their corresponding acid chloride and phosphoryl chloride respectively. The *N*-ethyl dicarbamate (174) was prepared from synthanecine A and ethyl isocyanate; the amino alcohol itself, acted as a base in this reaction. Finally, the dicarbamates (175) and (176) were prepared from the bis-phenylcarbonate (177) and the appropriate amine.

The results from biological tests showed that these diester derivatives were metabolised to pyrrole derivatives which exhibited a hepatotoxicity similar to, if not identical to, that of pyrrolizidine alkaloids. The *N*-ethyl-carbamate diester (174) is more toxic than the common natural alkaloid monocrotaline (19). However, simple diesters are less toxic due to esterase hydrolysis (see Chapter 1.4).

At the start of this project no macrocyclic diesters of synthanecine A had been prepared. In this project, we have investigated methods for preparing such derivatives (Chapters 3 - 5). Some macrocyclic diesters of synthanecine A have been subjected to biological tests to assess their hepatotoxicity (Chapter 6).

CHAPTER 3

SYNTHESIS OF TEN AND ELEVEN MEMBERED MACROCYCLIC DIESTERS OF SYNTHANECINE A BY THE COREY-NICOLAOU METHOD

3.1 Introduction

Macrocyclic diester pyrrolizidine alkaloids generally have 11- or 12-membered rings and are more hepatotoxic than simple monoester or diester alkaloids [e.g. In young male rats retrorsine, a 12-membered dilactone, has an LD_{50} of 34 mg, whereas the LD_{50} of heliotrine (44) is 280 mg.[4] An LD_{50} (lethal dose 50%) is the quantity of a substance necessary to kill 50% of exposed animals in laboratory tests within a specified time]. The presence and conformation of a macrocycle may be important factors in reducing the susceptibility of the ester groups to esterase hydrolysis. Also, oxidation of the pyrroline ring may be favoured rather than N-oxidation (see Chapter 1.4).

A series of semi-synthetic eleven-membered[54] and ten membered[63] macrocyclic diesters of (+)-retronecine have been prepared by Robins and co-workers. Lactonisation of the hydroxycarboxylic acid intermediates was accomplished by the Corey-Nicolaou method (see Scheme 13). As (+)-retronecine (3) is not readily available, by synthesis or from natural sources, the quantities of these derivatives prepared were limited. Since reasonable amounts of the most toxic macrocyclic alkaloids are needed for further metabolic and toxicological studies a more accessible necine or necine analogue was required. Synthanecine A (9), a monocyclic

analogue of retronecine, was selected because it is readily available *via* a short and efficient route (see Chapter 2.4).[12,13]

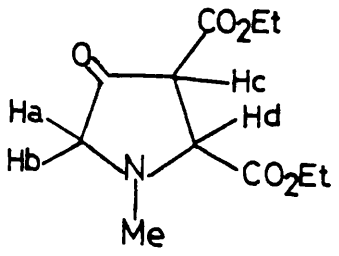
The synthesis of a series of 10- and 11-membered macrocyclic diesters of synthanecine A, in suitable quantities for toxicological testing, was achieved employing the widely used Corey-Nicolaou method for lactonisation. All toxicity tests were performed at the M.R.C. Toxicology Unit, Carshalton (see Chapter 6).

3.2 Synthesis of Synthanecine A.

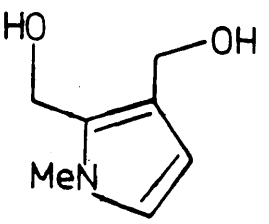
Synthanecine A was prepared by a slightly modified version of the route published by Mattocks (c.f. Scheme 34).[12,13]

The addition of methylamine (162) to diethyl maleate (163) occurred readily affording a 93% yield of the desired diester (164). Purification of the diester was achieved by an acid-base recycle (see experimental), rather than by the published distillation at reduced pressure. *N*-Alkylation of the diester (164) using ethyl bromoacetate (165) and potassium carbonate with ethanol as the solvent, as reported, gave a low yield (25%) of the triester (166). When the reaction mixture was heated at reflux for longer or for shorter periods no improvement in this yield was observed. The use of *n*-butanol, a higher boiling solvent, resulted in a 2.5% yield. A better yield (70%) was obtained using acetone, containing 7% water, as the solvent. This yield

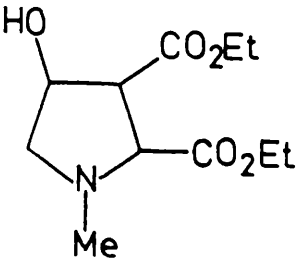
was improved further by employing an efficient mechanical stirrer. The triester (166) was cyclised by the reported Dieckmann procedure giving a 90% yield of the required pyrrolidone (167). The presence of only two AB quartets in the ^{1}H n.m.r. spectrum of compound (167) [geminal coupling $J_{Ha\,Hb}$ = 18 Hz and vicinal coupling $J_{Hc\,Hd}$ = 8 Hz] suggests that there is only one racemate present. Application of the Karplus equation to the vicinal AB system resulted in possible dihedral angles of 25° or 135°. This did not allow definite assignment of stereochemistry to the pyrrolidone (167).



(167)



(179)



(178)

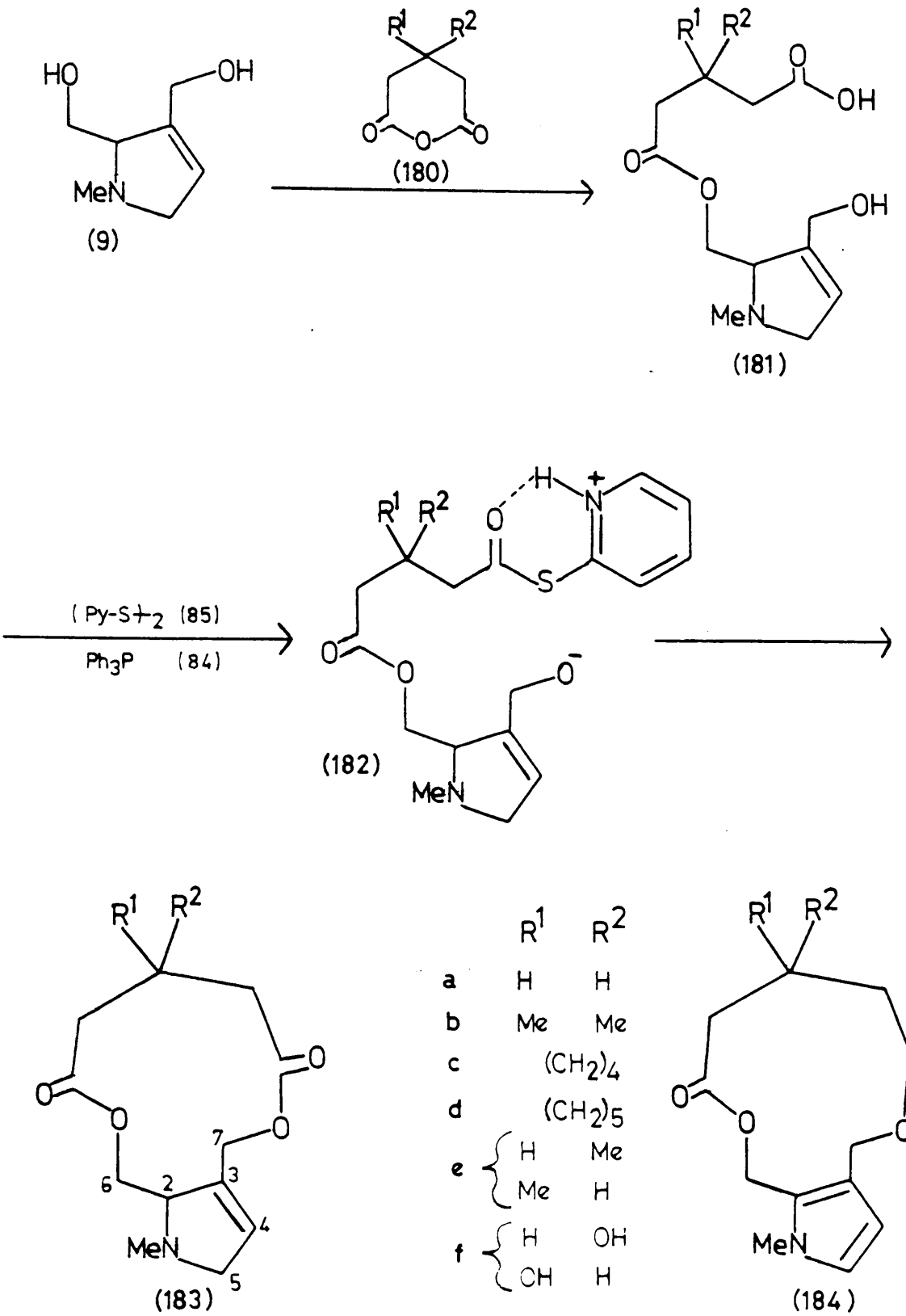
Unfortunately the low yield (52%) of alcohol (178) obtained from reduction of the ketone (167) could not be improved. Dehydration of the hydroxypyrrolidine (178) was achieved using p-toluenesulphonyl chloride in pyridine (88%). The last traces of pyridine were removed by azeotroping with water, then with benzene. Finally, selective reduction of the diester (168) as reported, gave a mixture (94%) of synthanecine A (9) and the less polar by-product, 2,3-bishydroxymethyl-1-methylpyrrole (synthanecine A pyrrole) (179) in a ratio of 85:15 (determined by ^{1}H n.m.r.

spectroscopy). Attempted purification of synthanecine A by the reported acid-base recycle, which employs a citrate-phosphate buffer (pH 4.3), afforded a 50% recovery of amine which was still contaminated with the pyrrolic by-product (179). Synthanecine A was successfully purified by column chromatography on silica gel, eluting with chloroform: methanol:triethylamine (85:14:1). The pure necine analogue (9) was characterised as the picrolonate, which has a melting point of 175° - 176 °C (Literature value 176 °C). Synthanecine A was stored under dry argon at 0 °C to prevent oxidation to the corresponding pyrrole diol (179).

3.3 Synthesis of 11-membered Dilactones

A supply of (±)-synthanecine A was prepared using the synthetic procedure described above. For the diacid component, we first chose to use symmetrically substituted glutaric anhydride derivatives to avoid the problem of the formation of diastereomers.

Thus, treatment of synthanecine A with equimolar amounts of a series of 3,3-disubstituted glutaric anhydride derivatives (180 a - d), in dry 1,2-dimethoxyethane, gave quantitative yields of the corresponding 6-monoesters (181 a - d) of synthanecine A (see Scheme 35). This regio-selective esterification was indicated by a downfield shift of ca. 0.4 p.p.m. for the protons at C-6 in the ^{1}H n.m.r. spectrum of the monoesters compared with the signal for synthanecine A. The chemical shifts of the C-7 protons of the monoesters remained unchanged when compared with those



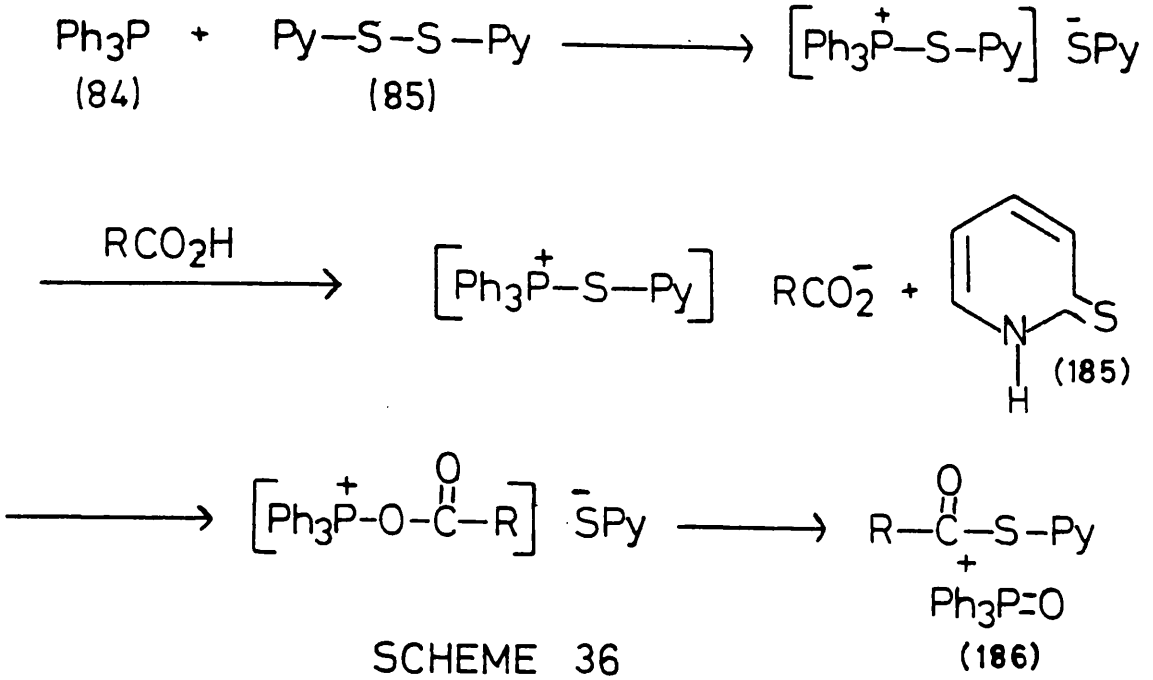
	R^1	R^2
a	H	H
b	Me	Me
c	$(CH_2)_4$	
d	$(CH_2)_5$	
e	H	Me
	Me	H
f	H	OH
	OH	H

SCHEME 35

in synthanecine A. No 6,7-diester formation was observed (^{1}H n.m.r. and mass spectral data) probably because the 6-monoesters formed initially are zwitterionic and precipitated from the reaction mixture. The extent of these reactions was followed by the disappearance of synthanecine A (9) by thin layer chromatography (t.l.c.)(see Section 3.5). These reactions were generally complete after ca. 10 h at room temperature although longer reaction times did not lead to degradation of the products and ensured maximum yields. The occurrence of this regioselective esterification shows that the primary hydroxyl is a stronger nucleophile than the primary allylic hydroxyl. This difference in reactivity was exploited in another procedure for preparing macrocyclic diesters (see Chapter 4). With reference to retronecine (3), Robins and co-workers[54] found that the primary allylic hydroxyl was a more reactive nucleophile than the secondary hydroxyl and C-9 monoesterification predominated.

The Corey-Nicolaou "double activation" method[52] was selected for the lactonisation step. The pyridine-2-thiol esters (182 a - d) were prepared by adding 2,2'-dithiopyridine (85) and triphenylphosphine (84) to suspensions of the 6-monoesters of synthanecine A. Vigorous stirring gradually effected dissolution, and the mixtures were stirred until formation of the pyridine-2-thiol esters was complete (t.l.c.). Again prolonged reaction times ensured maximum yields. This method for preparing thiol esters, reported by Mukaiyama[53], employs 2,2'-dithiopyridine as a hydrogen acceptor and triphenylphosphine as an oxygen

acceptor (see Scheme 36).

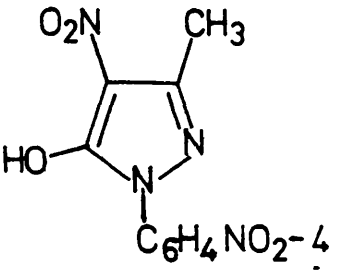


SCHEME 36

The pyridine-2-thiol esters underwent lactonisation when heated at reflux in 1,2-dimethoxyethane. Excessive refluxing resulted in the formation of less polar by-products which gave a positive result (purple colouration) with Ehrlich's reagent (see Section 3.5). These by-products are presumably the corresponding pyrrolic derivatives (184 a - d). The high dilution conditions generally required for macrolactonisations were achieved by diluting the reaction mixture (ca. 2x) before heating at reflux, or by adding the reaction mixture dropwise to refluxing DME. On no occasion was dilide formation observed.

The product mixture was initially subjected to an acid base recycle to remove the bulk of the pyridthione (185) and oxide (186) by-products. Final purification of the compounds was achieved by column chromatography on basic alumina. The 11-membered macrocyclic diester (183a) was

obtained as an oil, and characterised as the salt with picrolonic acid (187). The other three macrocyclic



(187)

products (183 b - d) were crystallised (each ca. 25% overall yield). Correct accurate mass measurements were obtained for the four racemic macrocyclic products (183 a - d), and similar fragmentation patterns were observed in their mass spectra with main peaks at m/z 123, 107, 94, 82 and 80. This fragmentation pattern appears to be analogous to that observed for macrocyclic diesters of retronecine (see Chapter 3.5.2).

In the ^{1}H n.m.r. spectra of the cyclised products (183 a - d) recorded in deuteriochloroform, the protons assigned to C-7 had shifted downfield by ca. 0.45 p.p.m. relative to the signal in synthanecine A, and appeared as an AB quartet. The chemical shift difference between these protons ($\Delta\delta$ H-7) was small [0.10 - 0.19 p.p.m. for (183 b - d), and ca. 0 p.p.m. for (183a)]. Values of 0 - 1.24 p.p.m. for the chemical shift difference between the allylic protons at C-9 [as in (88)] have been observed for 11-membered

macrocyclic diesters of retronecine. The two protons at C-6 of the macrocyclic compounds (183 a - d) are also diastereotopic and chemical shift differences of 0.07 - 0.18 p.p.m. were observed for these protons in their ^{1}H n.m.r. spectra (see Chapter 3.6.1). The distinctive mass spectra of compounds (183 a - d) and the non-equivalence of the protons at C-6 and C-7 in the ^{1}H n.m.r. spectra indicate that 11-membered macrocyclic diesters of synthanecine A have been produced.

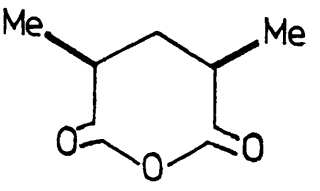
Finally, the i.r. spectra of the macrocyclic dilactones (183 a - d) contained absorptions at ca. 1740 cm^{-1} corresponding to the saturated ester carbonyls. No evidence was found for the presence of hydroxyl or carboxylic acid groups.

When synthanecine A was treated with 3-methylglutaric anhydride, subsequent lactonisation afforded two macrocyclic diastereomeric racemates (183e). The doubling of some of the signals in the ^{13}C n.m.r. spectrum of the mixture indicated that the diastereomeric racemates (183e) were present in a 1:1 ratio. This shows that the coupling of the two moieties (alcohol and anhydride) proceeds in a non-selective manner. The diastereomers (183e) could not be separated by t.l.c. using a variety of solvent systems. Full characterisation data, for the mixture, were consistent with the formation of the macrocyclic dilactones (183e).

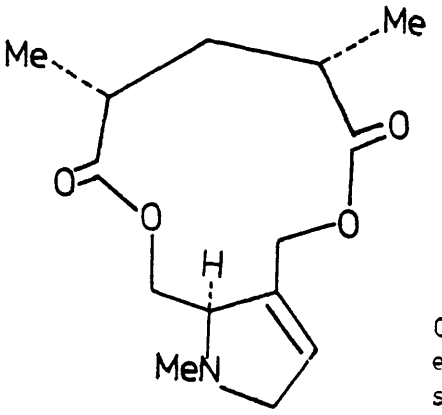
A pyrrolizidine alkaloid analogue with the same diacid portion as dicrotaline (88) was also prepared. Reaction of

3-hydroxy-3-methylglutaric anhydride (180f) with synthanecine A (9), followed by lactonisation of the monoesters again produced two diastereomeric racemates (183f) in ca. 1:1 ratio as judged by their ^{13}C n.m.r. spectrum. Separation of the diasteromers could not be effected by t.l.c. Therefore, spectroscopic data were obtained for the mixture. The ^{1}H n.m.r., ^{13}C n.m.r. and mass spectral data indicated that the alkaloid analogue, and a diastereomer had been prepared.

Finally, efforts were made to prepare macrocyclic dilactones with substituents on the α positions to the ester carbonyls, since steric hindrance around the ester groups is believed to enhance the toxicity of the alkaloids, by increasing their resistance to esterase hydrolysis.[9] Therefore synthanecine A was treated with 2,4-meso-dimethylglutaric anhydride (188) and the resultant monoesters were lactonised as described above to give two diastereomeric racemates (189) and (190). These racemates were purified by column chromatography, and were then separated by p.l.c. Both racemates displayed fragmentation patterns in their mass spectra similar to those shown by the analogues (183 a - d). An X-ray structure determination carried out by Professor G.A. Sim,[84] on the more polar base confirmed its macrocyclic nature and established the relative configuration of the three chiral centres, to be as shown in (190) (See Section 3.7).

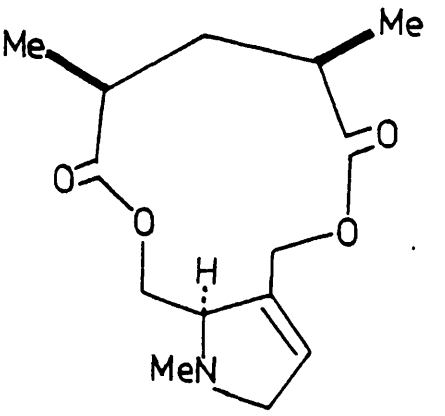


(188)



(189)

Only one enantiomer shown

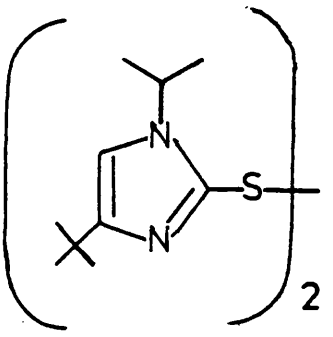


(190)

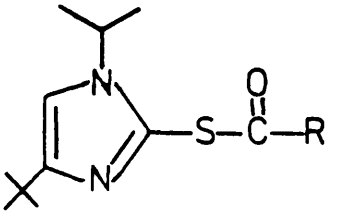
The ^{1}H n.m.r. spectra of the two racemates (189) and (190) showed some differences from the analogues (183 a - d). In particular, higher chemical shift differences were observed for the protons at C-6 [0.48 for (189) and 0.51 p.p.m. for (190)] and for those at C-7 [0.20 for (189) and 0.40 p.p.m. for (190)]. This suggests that the conformations of the macrocyclic rings in (189) and (190) in deuteriochloroform solution, may be different from those of the analogues (183 a - d), due to the presence of the methyl groups at C-10 and C-12 in (189) and (190) (see Chapter 3.7).

2,2,4,4-Tetramethylglutaric anhydride (191) was also treated with synthanecine A. The monoester precipitate afforded was subsequently transformed into the corresponding pyridine-2-thiol ester. All attempts to lactonise this thiol ester failed. The cyclisation was possibly hindered by the presence of the substituents α to the thiol ester carbonyl.

As this procedure for the synthesis of macrocyclic diesters did not appear to produce any major by-products and did not require the isolation of any intermediates it was disappointing that only ca. 25% yields of the products were obtained. It was considered that these yields might be increased by employing the imidazole disulphide (140). Imidazole thiol esters, such as (192), are reported[71] to be ca. 100 times more reactive than the corresponding pyridine analogues.



(140)



(192)

The C-6 monoester produced by the reaction of synthanecine A with 3,3-dimethylglutaric anhydride was treated with the disulphide (140) and triphenylphosphine at room temperature. Some lactonisation did occur at room temperature but heating at reflux in DME was necessary to complete the reaction. No increase in yield was observed using the imidazole disulphide (140).

Another modification to the Corey procedure, is the use of the silver ions (as the perchlorate) to activate pyridine-2-thiol esters towards lactonisation.[72] When a solution of the pyridine-2-thiol ester (182b) in DME was

added dropwise to a solution of silver perchlorate in DME which was heated at reflux, no improvement in the yield was observed.

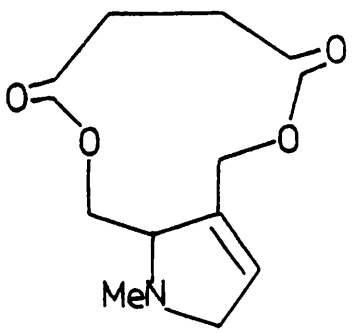
It may be that low yields obtained in the lactonisations are due to a combination of factors. One likely factor is the instability of the cyclised products towards the conditions involved in the purification procedure. Hydrolysis of the dilactones could occur readily during the acid base recycle. When a pure sample of the macrocyclic diester (183b) was subjected to an acid-base recycle at room temperature, only a 40% recovery of product was obtained. Repeating this procedure, but keeping all apparatus and solutions as cool as possible, resulted in a 51% recovery of the macrocyclic product (183b). A possible solution to this problem would be to devise a synthetic procedure that did not involve an acid-base recycle (see Chapter 4).

Another limitation of this procedure results from the persistence of triphenylphosphine oxide (186) which contaminates the final product. It was thought that this problem might be overcome by employing a polymer supported triphenylphosphine. Unfortunately, as time was not available the use of polystyryldiphenylphosphine instead of triphenylphosphine in the Corey-Nicolaou procedure was not investigated.

3.4 Synthesis of 10-membered Dilactones.

The formation of a 10-membered macrocyclic diester of synthanecine A was of synthetic as well as toxicological interest. Since a molecular model of the 10-membered macrocycle (193) appeared to display an increase in ring strain, it was considered that the ring formation might be less favourable than for 11-membered rings. This additional ring strain might also have an influence on the biological activity of the dilactone if it could be formed.

When synthanecine A was treated with an equimolar amount of succinic anhydride in dry 1,2-dimethoxyethane (DME) a monoester precipitate was observed. This monoester was transformed into the corresponding pyridine-2-thiol ester, using 2,2'-dithiodipyridine (85) and triphenylphosphine (84) (c.f. Scheme 35). The thiol ester was then added dropwise to refluxing DME to effect lactonisation. The desired

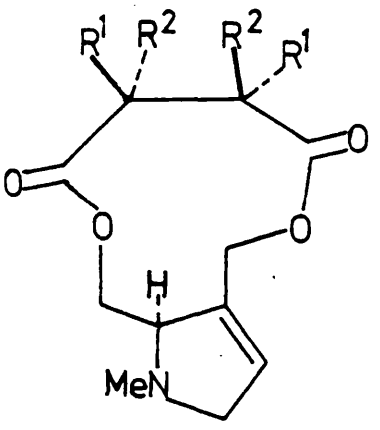


(193)

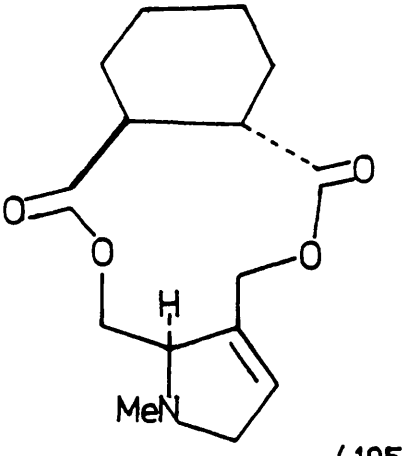
10-membered macrocyclic diester (193) was isolated as white crystals in ca. 30% yield. The high resolution mass spectrum

of the free base displayed a molecular ion corresponding to $C_{11}H_{15}NO_4$. In addition a typical fragmentation pattern for a synthanecine A dilactone was observed. The i.r. spectrum showed a saturated ester carbonyl absorption at 1736 cm^{-1}. In the ^1H n.m.r. spectrum of the macrocyclic diester (193) large chemical shift differences for the C-6 (0.42 p.p.m.) and C-7 protons (0.68 p.p.m.) were observed.

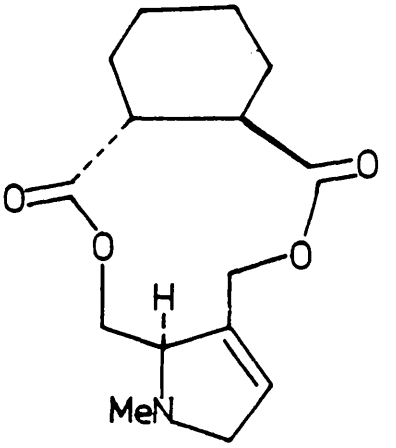
Ten-membered macrocyclic diesters with α,α-disubstitution [(194) and (195)] were prepared to see what effect steric hindrance at the α position to the ester carbonyl would have on the biological activity of 10-membered macrocycles. Synthanecine A was treated with (±)-trans-2,3-dimethyl-



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



(195)

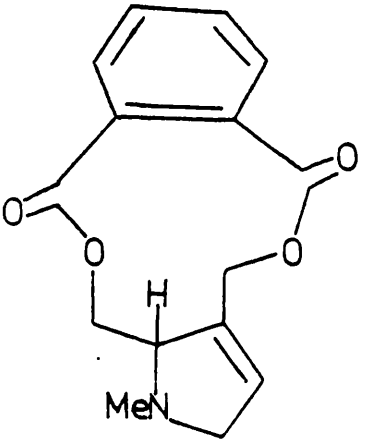


Only one enantiomer shown

succinic anhydride and (±)-trans-cyclohexane-1,2-dicarboxylic anhydride respectively and the corresponding monoesters were cyclised by the Corey-Nicolaou method (c.f. Scheme 35), in yields of 32% and 23% respectively. In each case the two

diastereomeric racemates which were obtained could not be separated by t.l.c. Therefore characterisation data were obtained for the mixtures. The high resolution mass spectra of both mixtures displayed characteristic fragmentation patterns and molecular ions corresponding to $C_{13}H_{19}NO_4$ for (194) and $C_{15}H_{21}NO_4$ for (195) were observed. The i.r. spectra of (194) and (195) contained carbonyl absorptions (ca. 1735 cm^{-1}) consistent with the presence of saturated esters. The ^1H and ^{13}C n.m.r. spectra of the diastereomeric mixtures were complicated due to the doubling of peaks. However, from the H-4 signal in their ^1H n.m.r. spectra it was possible to estimate that in both of the dilactone mixtures [(194) and (195)] the diastereomeric racemates were present in ratios of ca. 1:1.6. Large chemical shift differences for the C-6 and C-7 protons were also observed in the ^1H n.m.r. spectra ($\Delta\delta$ H-6 ≃ 0.6 p.p.m. and $\Delta\delta$ H-7 ≃ 0.9 p.p.m.).

To test the scope of this procedure it was repeated



(196)

with an aromatic anhydride. When phthalic anhydride was reacted with synthanecine A a low yield (ca. 16%) of the desired macrocyclic diester was obtained. The aromatic dilactone (196) was obtained as white prisms and characterised as the free base. The high resolution mass spectrum of the racemate (196) showed a correct accurate mass measurement, corresponding to $C_{15}H_{15}NO_4$, and a fragmentation pattern indicative of a synthanecine A dilactone was observed. A carbonyl absorption at 1720 cm^{-1} that is characteristic of the additional conjugation, was displayed in the i.r. spectrum of (196). In the ^1H n.m.r. spectrum of dilactone (196) a complex array of signals between 7.50 - 7.85 p.p.m. was observed, corresponding to the four aromatic protons. Also, the signals corresponding to the C-6 and C-7 protons are shifted downfield by ca. 0.45 p.p.m., and split into a doublet of AB quartets ($\Delta\delta$ H-6 = 0.36 p.p.m.) and an AB quartet ($\Delta\delta$ H-7 = 0.48 p.p.m.) when compared to the signals for synthanecine A.

When 3,4,5,6-tetrachlorophthalic anhydride was treated in a similar fashion with synthanecine A, none of the desired cyclised product was isolated. Also, reaction of maleic anhydride with synthanecine A by the above procedure, failed to produce any cyclised product.

3.5 Chromatographic Analysis of this Synthetic Route.

The macrocyclic diesters [(183 a - f), 189, 190, (193 - 196)] were prepared by a "one-pot" reaction, wherein none of the intermediates were isolated. Therefore, to ensure

maximum yields of the macrocycles it was important that all stages of this synthesis were carefully monitored to completion before the next stage was attempted. Since the intermediates have well defined Rf values, the reaction sequence was simply and efficiently monitored by thin layer chromatography (t.l.c.). When a sample of the reaction mixture was applied to a silica gel plate which was eluted with chloroform-methanol-conc. ammonia (85:14:1), the Rf values of the intermediates were as shown in figure 8.

Synthanecine A (9) has an Rf value of 0.1 whereas the highly polar zwitterionic monoester (cf. Scheme 36) does not move from the baseline. Transformation of the monoester into the pyridine-2-thiol ester was indicated by the disappearance of the baseline spot and formation of a u.v. active product with Rf value ca. 0.3. Lactonisation to the cyclic diester yielded a less polar product with Rf 0.5 - 0.6.

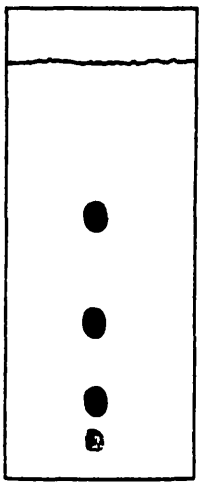
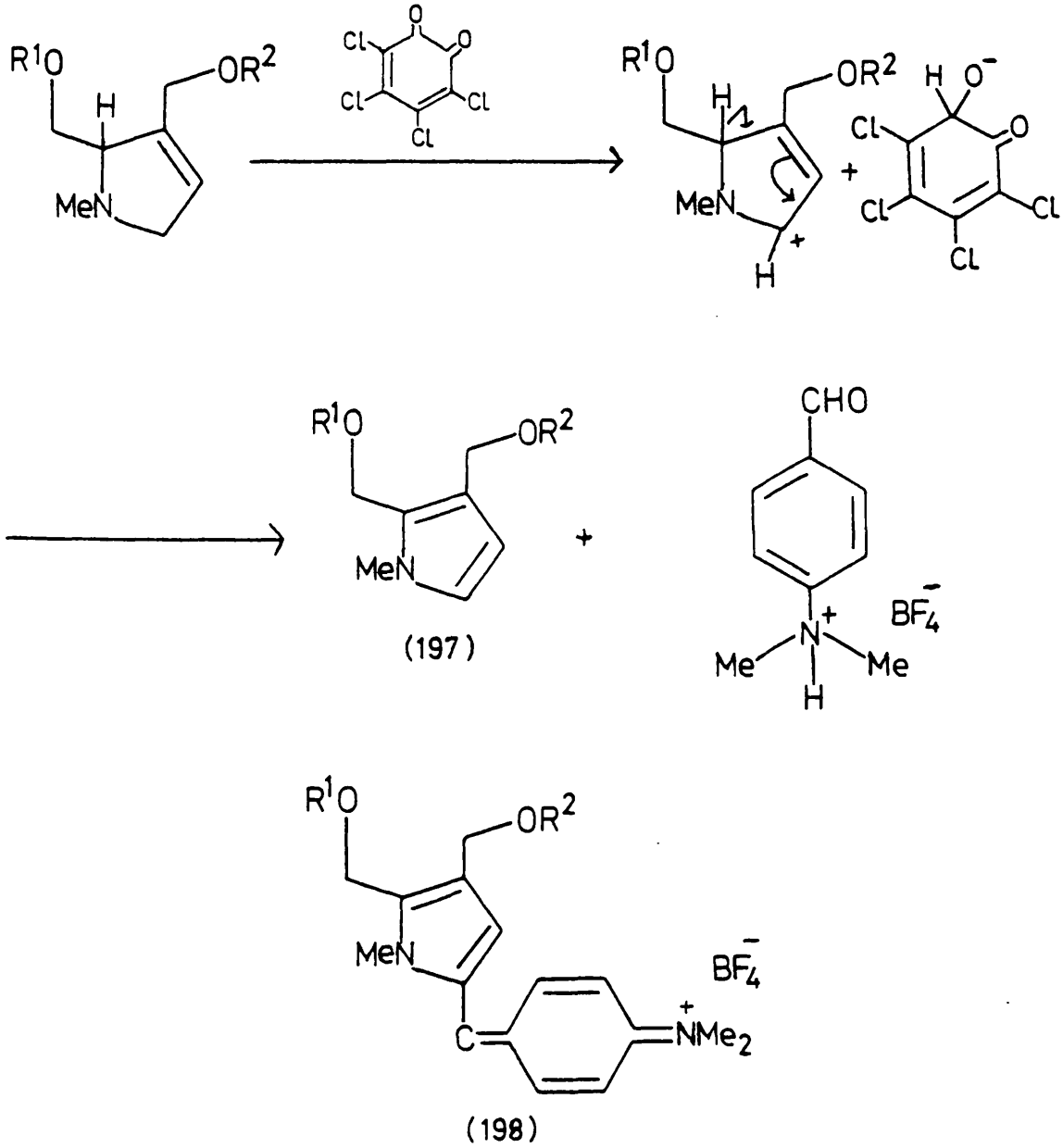


Figure 8

These differences in polarity are due to the number of hydroxyls present in these molecules. The diol (9) has a small Rf value whereas the diesters have much higher Rf values.

The pyrroline-containing compounds were detected on the thin layer chromatogram using the method reported by the groups of Molyneux and Roitman[85] (see Scheme 37). The



SCHEME 37

chromatogram was sprayed with a 1% solution of o-chloranil in toluene, and upon heating the pyrroline intermediates were oxidised to their corresponding pyrroles (197). On subsequent spraying with Ehrlich's reagent (2% p-dimethyl-aminobenzaldehyde in ethanol containing 2% borontrifluoride etherate) these pyrroles were converted into conjugated adducts (198) which have a characteristic deep purple colour. Detection of any pyrrolic by-products was achieved by spraying the chromatogram with Ehrlich's reagent only.

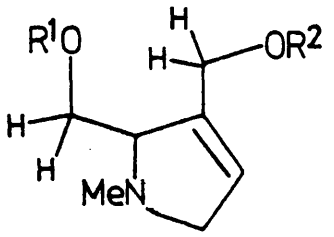
The reaction with Ehrlich's reagent is the basis of the method for the detection of pyrrole derivatives which were extracted from the livers and lungs of rats during the toxicological tests.

3.6 Spectroscopic Analysis of these Macrocycles.

^{1}H N.m.r. spectroscopy and mass spectrometry have provided essential data for the characterisation of these macrocyclic synthanecine A dilactones.

3.6.1 ^{1}H N.m.r. Spectroscopy.

The important features in the ^{1}H n.m.r. spectra of these synthanecine A derivatives, are the signals due to the protons bonded to the C-6 and C-7 carbinol carbons. The chemical shifts and splittings of these signals provide vital information on the state of esterification of this system. When the C-6 and C-7 hydroxyls are not esterified, as in synthanecine A (9), these diastereotopic protons give rise



(9) $R^1 = R^2 = H$
(199) $R^1 = R^2 = COMe$
(193) $R^1, R^2 = COCH_2CH_2CO$
(183c) $R^1, R^2 = COCH_2C[(CH_2)_4]CH_2CO$

to signals with chemical shifts of 3.66 p.p.m. and 4.20 p.p.m. respectively. The signals corresponding to the C-6 protons appear as a broad doublet, due to splitting by the C-2 proton and a broad singlet is observed for the C-7 protons as the adjoining carbon (C-3) is quaternary (see Figure 9). When these hydroxyls are esterified, as in the diacetate (199), the signals for the C-6 and C-7 protons are shifted downfield, but their appearance remains the same (see Figure 10). The deshielding of protons attached to the α carbon atom of the alcohol moiety of esters, as compared with the same protons in the free hydroxyls, is called the "acylation shift".[86] Generally, the acylation shift for secondary alcohols is between 1.0 - 1.15 p.p.m. whereas for primary alcohols the range is from 0.40 - 0.60 p.p.m. The acylation shifts for the C-6 and C-7 protons of synthanecine A diacetate (199) [c.f. Figures 9 and 10] are ca. 0.45 p.p.m. which is indicative of primary alcohol acylation. This phenomenon was utilized when studying the monoesterification of synthanecine A. When synthanecine A was treated with one equivalent of an acylating agent (acid anhydride or N-acylimidazolide) the broad doublet at 3.66 p.p.m.,

corresponding to the C-6 protons, was shifted downfield by ca. 0.45 p.p.m. and the broad singlet at 4.20 p.p.m. assigned to the C-7 protons remained constant. This indicated that monoesterification occurred at the more nucleophilic primary hydroxyl rather than the primary allylic hydroxyl.

The conformations of macrocyclic diesters of synthanecine A are influenced by the presence of the fused pyrroline ring and the two ester groups. These constraints on the molecule may lead to the macrocycle adopting a conformation in which the diastereotopic protons at C-6 and C-7 are influenced to different extents by the ester carbonyls and, in the case of the C-7 protons, by the 1,2-double bond. In the simplest case where these protons are nearly magnetically equivalent, they give rise to broad signals, similar to those observed for the diacetate (199). As the degree of non-equivalence becomes greater, the broad singlet due to the C-7 protons is resolved into an AB quartet and the broad doublet corresponding to the C-6 protons is split into a doublet of AB quartets (see Figures 11 and 12). The differences in the chemical shifts of the signals for the two protons at C-7 ($\Delta\delta$ H - 7) and for the two protons at C-6 ($\Delta\delta$ H - 6), are a measure of the degree of non-equivalence of these protons. The occurrence of significant $\Delta\delta$ H - 7 and $\Delta\delta$ H - 6 values is considered to be convincing evidence for the presence of a macrocyclic diester. As a range of values have been observed for $\Delta\delta$ H - 6 and $\Delta\delta$ H - 7 in macrocyclic diesters of synthanecine

Figure 9

^{1}H n.m.r. spectrum of

(±)-Synthanecine A.

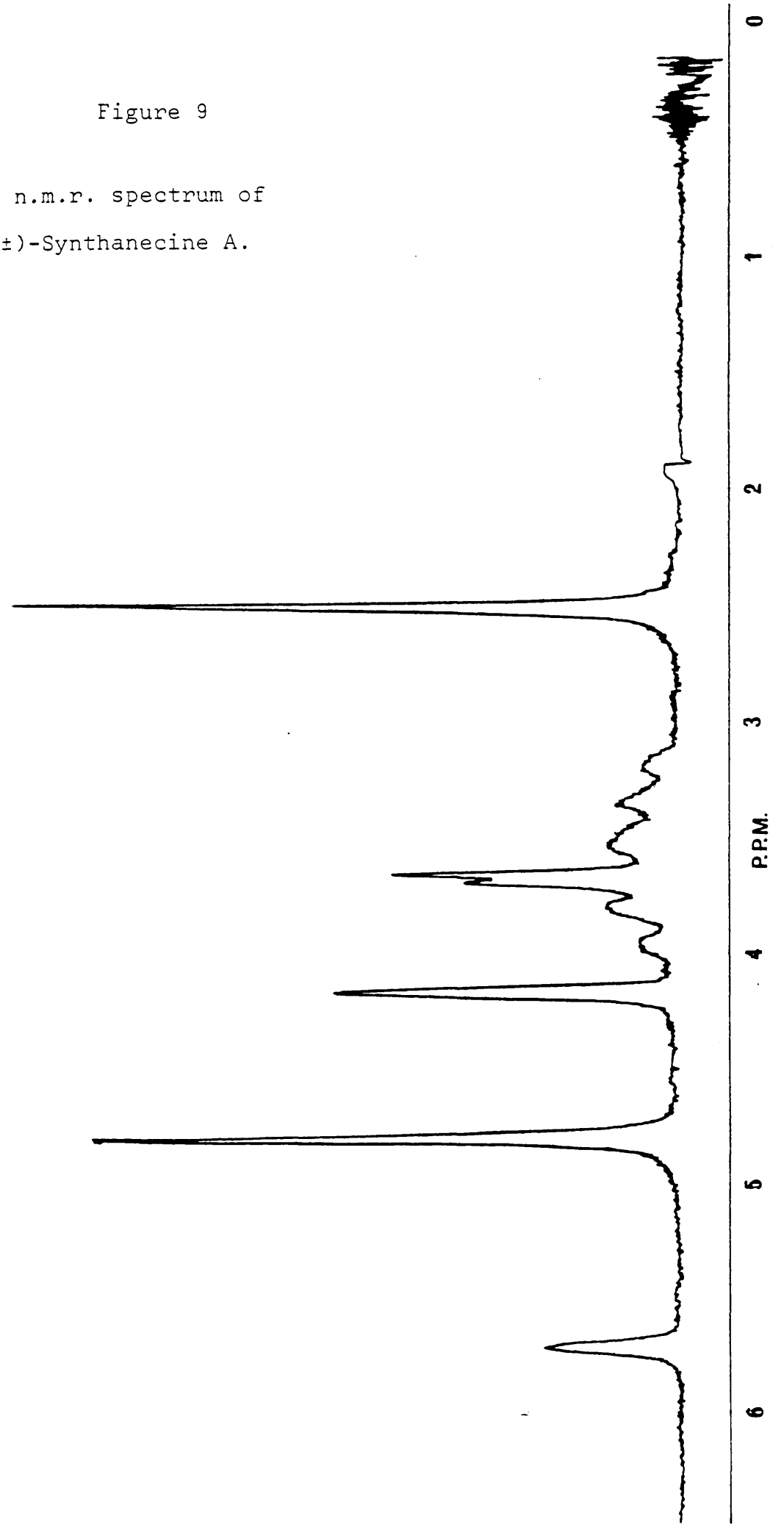


Figure 10

^{1}H n.m.r. spectrum of

(±)-6,7-O,O-(Diacetyl)synthanecine A (199).

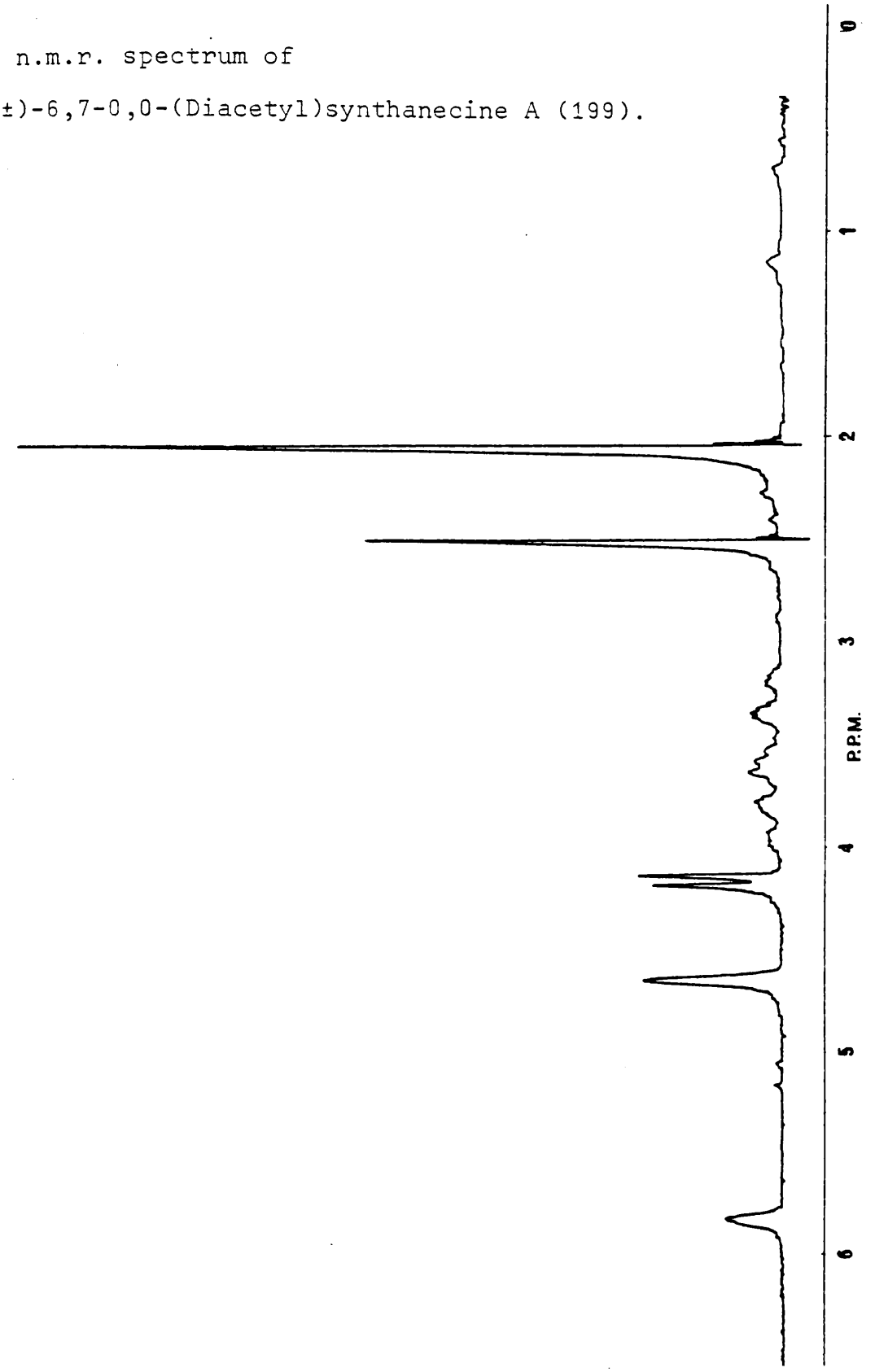


Figure 11

^{1}H n.m.r. spectrum of (±)-6,7-O,O-(Succinyl)synthanecine A (193).

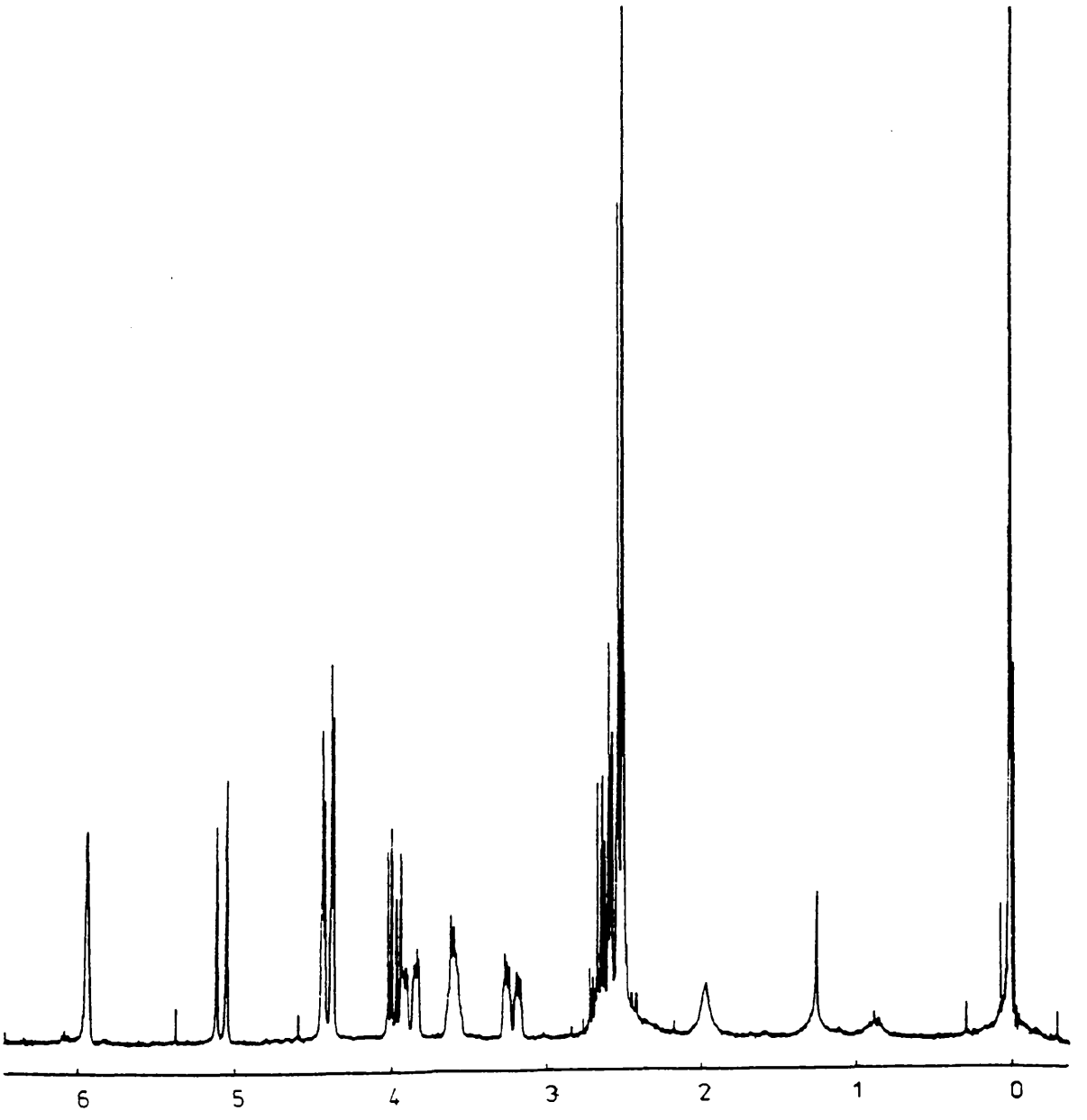
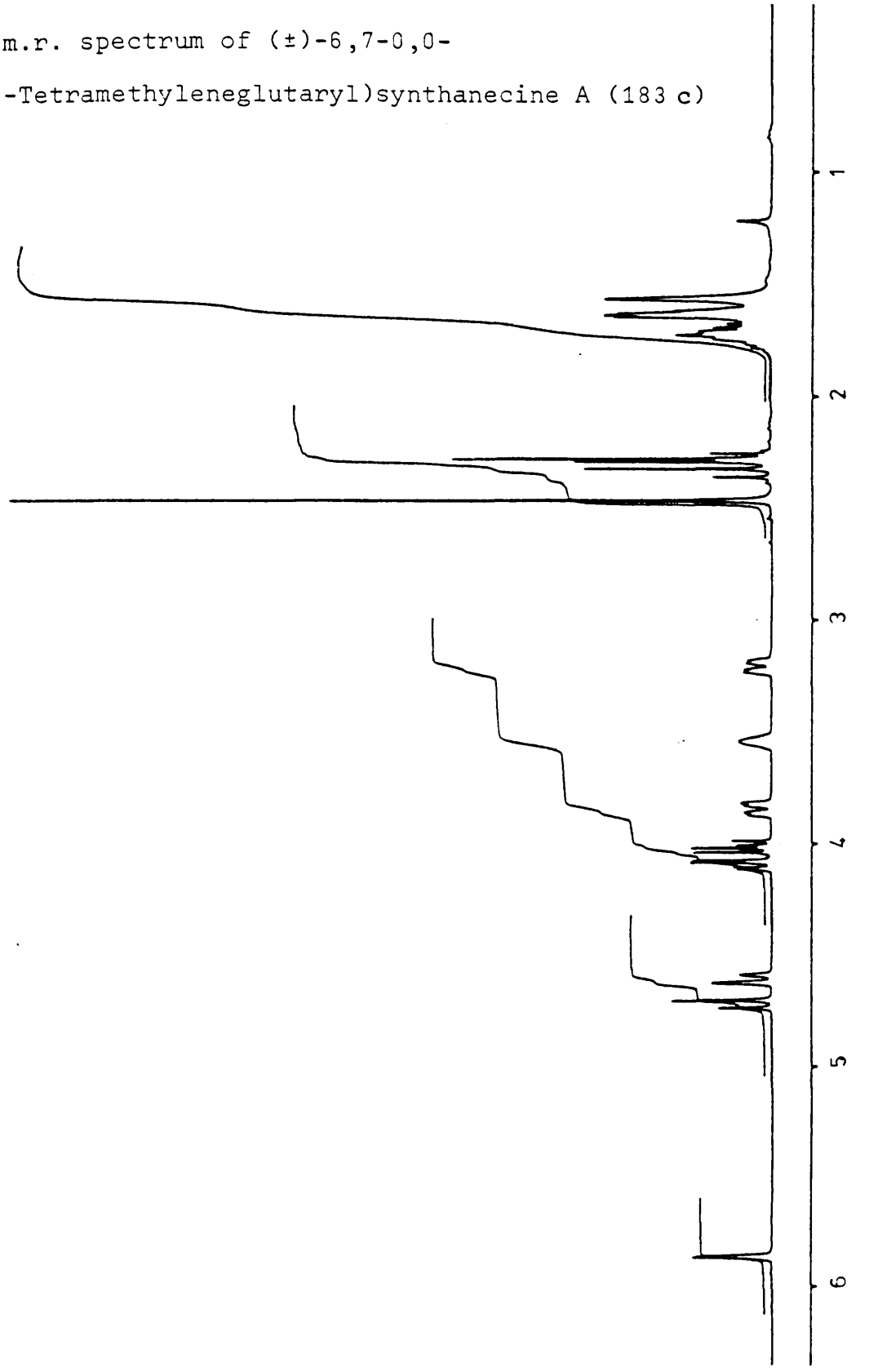


Figure 12

^{1}H n.m.r. spectrum of (±)-6,7-O,O-(3,3-Tetramethyleneglutaryl)synthanecine A (183 c)



A it appears that these macrocycles have adopted different conformations (see Chapter 3.7).

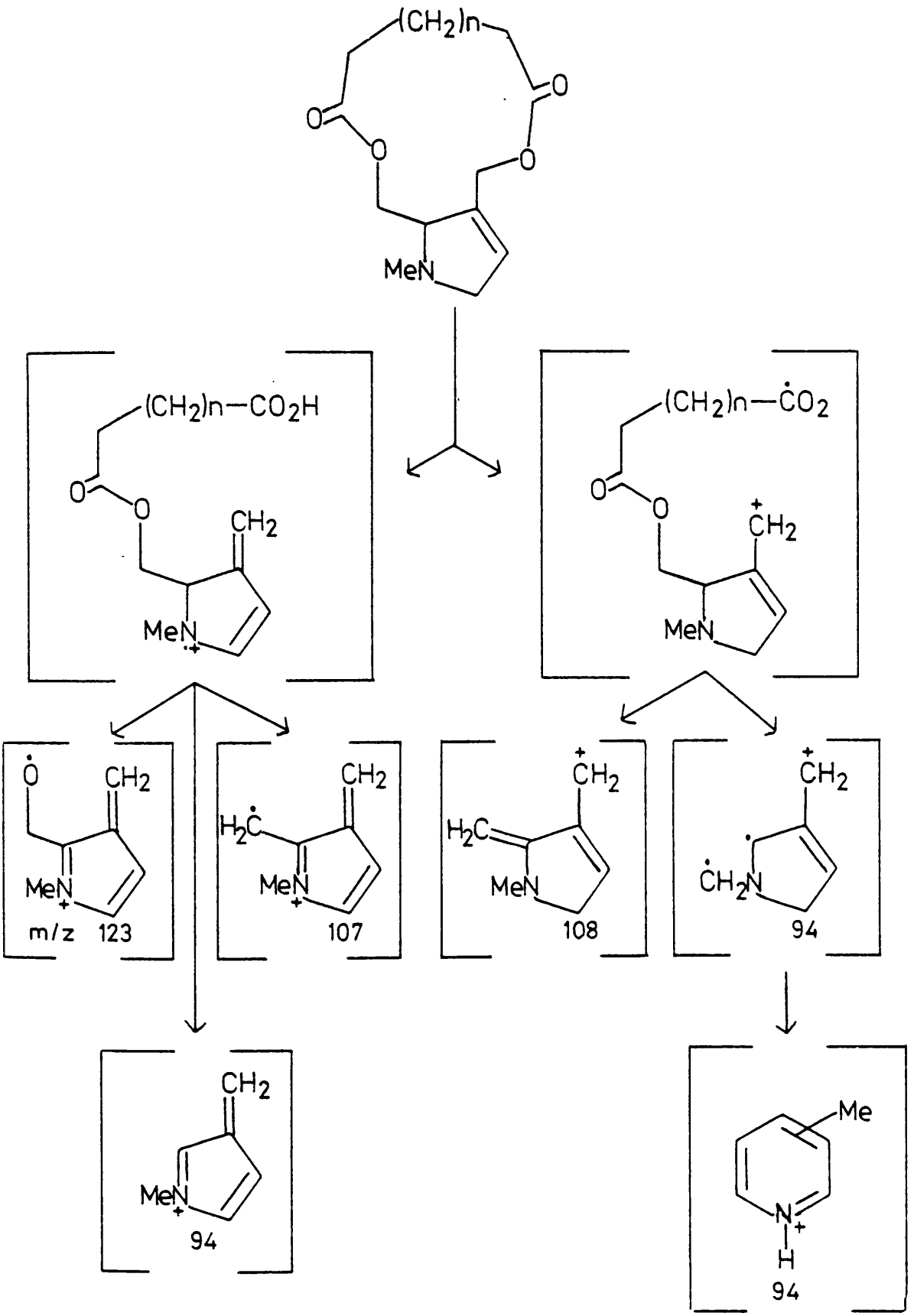
3.6.2 Mass Spectrometry.

Analysis of the mass spectra of the macrocyclic diesters [(183 a-f), 189, 190, (193 - 196)] reveals that they have similar fragmentation patterns. The main ions are at m/z 123, 107, 94, 82 and 80. It is known that the characteristic fragmentation pattern of retronecine diesters (m/z 136, 120, 94, 82 and 80) probably arises by cleavage of the allylic ester followed by loss of the diacid proton.[1] The fragmentation patterns for diesters of retronecine and synthanecine A are similar. The ions at m/z 136 and 120 of retronecine diesters contain an additional CH in the base fragment compared to the analogous peaks in the mass spectra of synthanecine A derivatives. A possible fragmentation pattern for the macrocyclic synthanecine A dilactones, based on this similarity, is shown in Scheme 38.

3.7 Conformational Aspects of Synthanecine A Dilactones.

The conformation of macrocyclic pyrrolizidine alkaloids in the solid state and in organic solutions can be evaluated from X-ray crystallographic and ^{1}H n.m.r. spectroscopic data respectively, and for senecionine (20) these conformations appear to be similar (see Chapter 1.6).

^{1}H N.m.r. spectroscopy can provide data on the magnetic non-equivalence of the C-6 ($\Delta\delta$ H - 6) and the C-7 ($\Delta\delta$ H - 7)


(CH2)n
O
O
O
O
MeN
(CH2)n—CO2H
CH2
MeN
(CH2)n—ĊO2
CH2
MeN
Ö
CH2
MeN
m/z 123
CH2
H2Ċ
MeN
107
CH2
H2C
MeN
108
CH2
ĊH2N
94
CH2
MeN
94
Me
N
H
94

SCHEME 38

diastereotopic protons of synthanecine A dilactones. The extent of this non-equivalence is indicative of the conformation adopted by the macrocycle in organic solution, as discussed in Chapter 1.6 (i.e. A small $\Delta\delta$ H value indicates that the macrocycle adopts a conformation in which the diastereotopic protons are symmetrically distributed about the plane of the neighbouring carbonyl group and a large $\Delta\delta$ H value indicates that in the adopted conformation one of the diastereotopic protons is located closer to the plane of the neighbouring ester carbonyl than the other). A range of $\Delta\delta$ H-7 and $\Delta\delta$ H- 6 values have been observed for this series of analogues as shown in Table 2. For macrocycles

TABLE 2.

	11-membered dilactones	$\Delta\delta$ H - 7 (p.p.m.)	$\Delta\delta$ H - 6 (p.p.m.)
(183 a)	glutarate	0	0.07
(183 b)	3,3-dimethylglutarate	0.16	0.17
(183 c)	3,3-tetramethyleneglutarate	0.10	0.07
(183 d)	3,3-pentamethyleneglutarate	0.13	0.13
(189)	2,4-dimethylglutarate	0.20	0.48
(190)	2,4-dimethylglutarate	0.40	0.51
	10-membered dilactones		
(193)	succinate	0.68	0.42
(196)	phthalate	0.48	0.36

(183 a - d) the $\Delta\delta$ H - 7 values are between 0 and 0.17 p.p.m. Whereas dilactones (189) and (190) exhibit $\Delta\delta$ H - 7 values of 0.2 and 0.4 p.p.m. respectively. A similar trend is observed for the $\Delta\delta$ H - 6 values of these 11-membered macrocyclic diesters. Therefore, it is possible that macrocycles (183 a-d) adopt different organic solution conformations from dilactones (189) and (190). This may be a consequence of the different patterns of substitution of their diacid components.

To confirm the macrocyclic nature of these products the structures of dilactones (183b), (183c) and (190) were elucidated by Professor G.A. Sim from X-ray crystallographic data (see Figures 13, 14 and 15 respectively).[84] It is clear that compounds (183b) and (183c), which exhibit small $\Delta\delta$ H - 6 and - 7 values in the ^{1}H n.m.r. spectra, are macrocyclic and adopt solid state conformations in which their ester carbonyl groups are anti-parallel, with the allylic ester carbonyl directed below the plane of the macro-ring. On the other hand dilactone (190), which has large $\Delta\delta$ H - 6 and $\Delta\delta$ H - 7 values, adopts a conformation in which its ester carbonyl groups are syn-parallel and directed above the plane of the macro-ring. Most 11-membered macrocyclic diesters of retronecine have ester carbonyl groups that are syn-parallel and directed below the plane of the ring.

The X-ray structure of the succinate dilactone (193) (Figure 16) calculated by Dr A.A. Freer, shows that this 10-membered macrocycle adopts a conformation in which its ester carbonyl are anti-parallel and directed outwards from

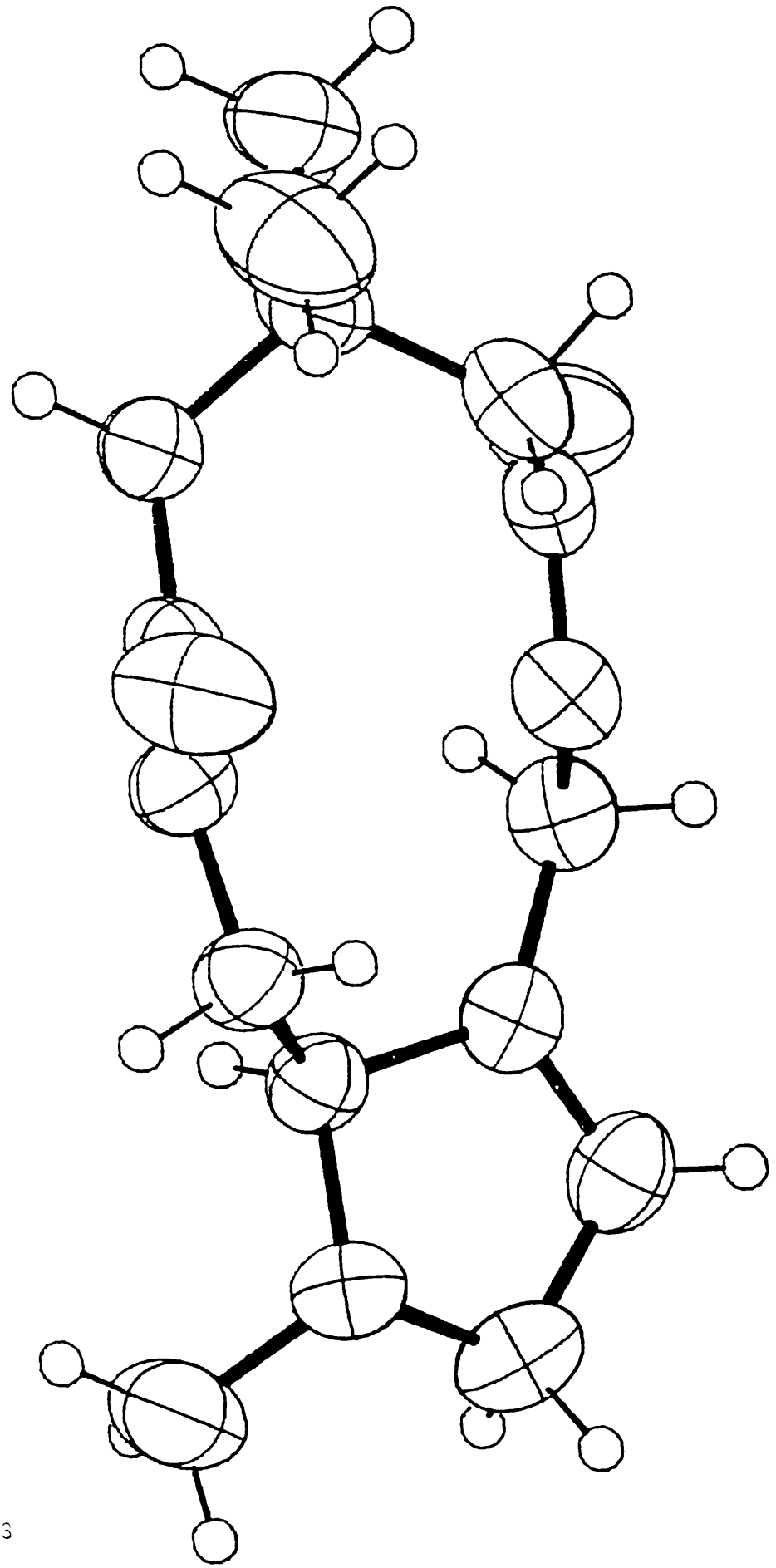


Figure 13

X-ray structure of (±)-6,7-O,O-(3,3-dimethyl-glutaryl)synthanecine A (183 b).

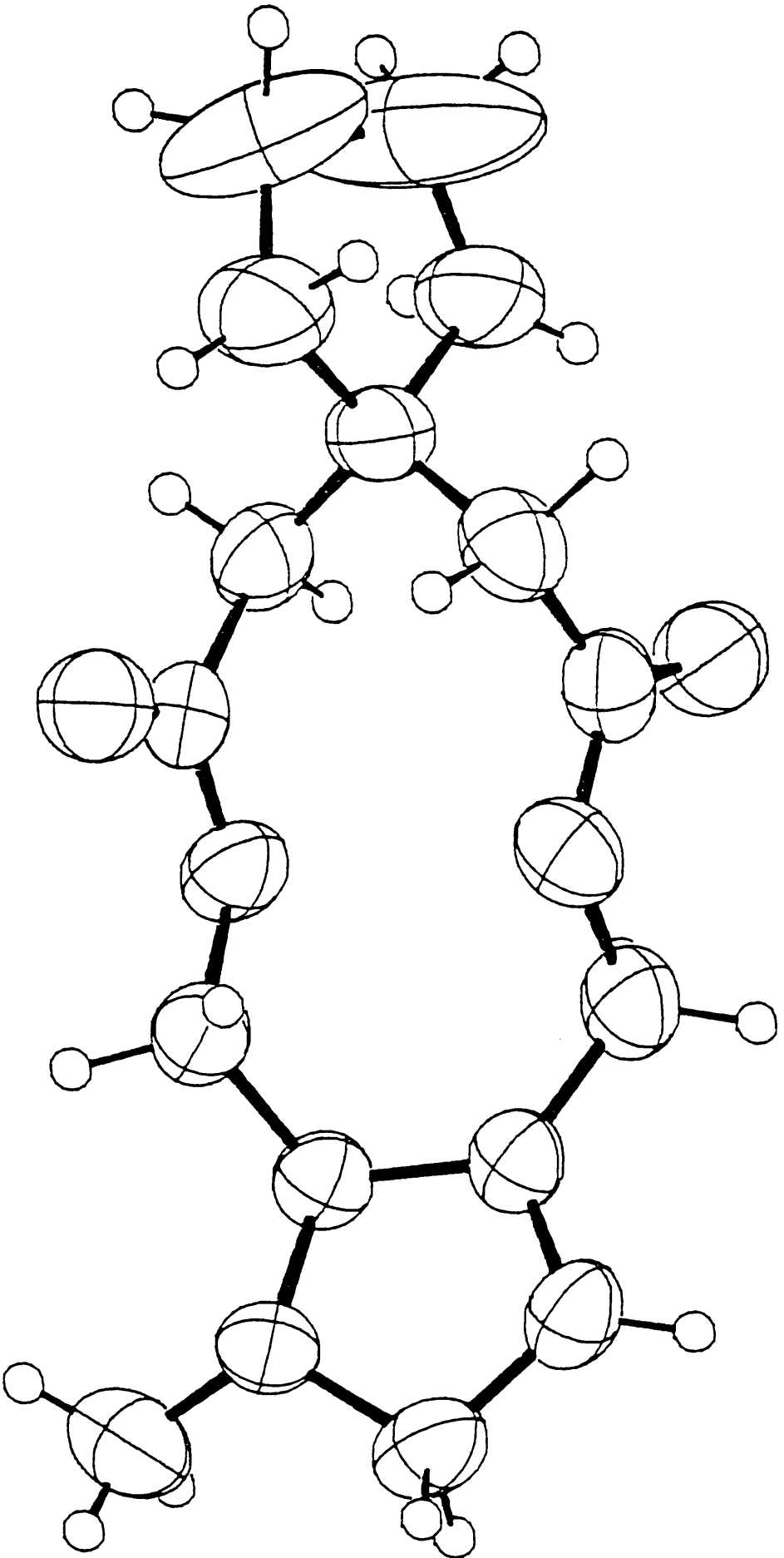


Figure 14

X-ray structure of (±)-6,7-O,O-(3,3-tetramethylene-glutaryl)synthanecine A (183 c).

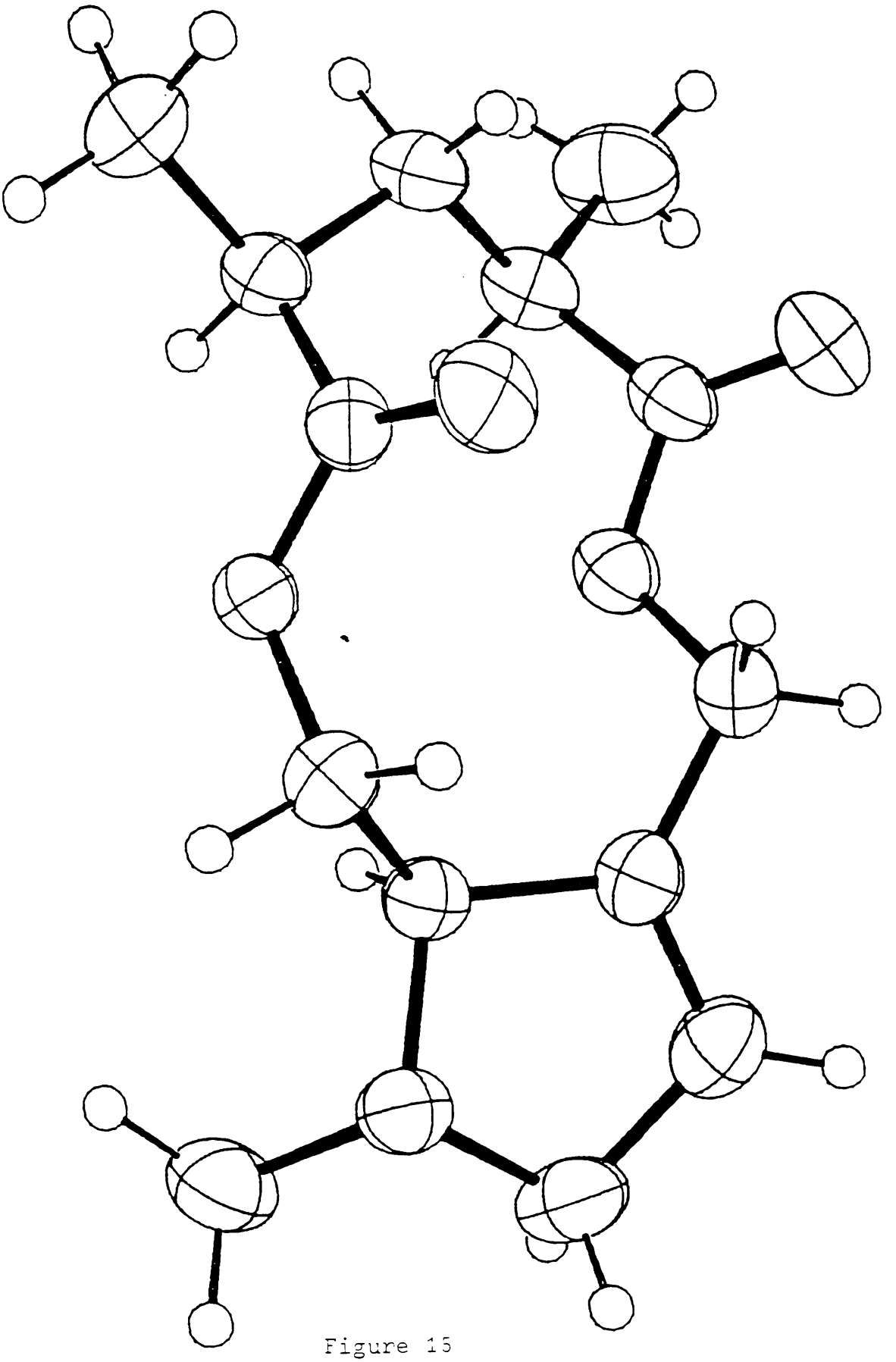


Figure 15

X-ray structure of (±)-6,7-O,O-(meso-2,4-dimethyl-glutaryl)synthanecine A (190).

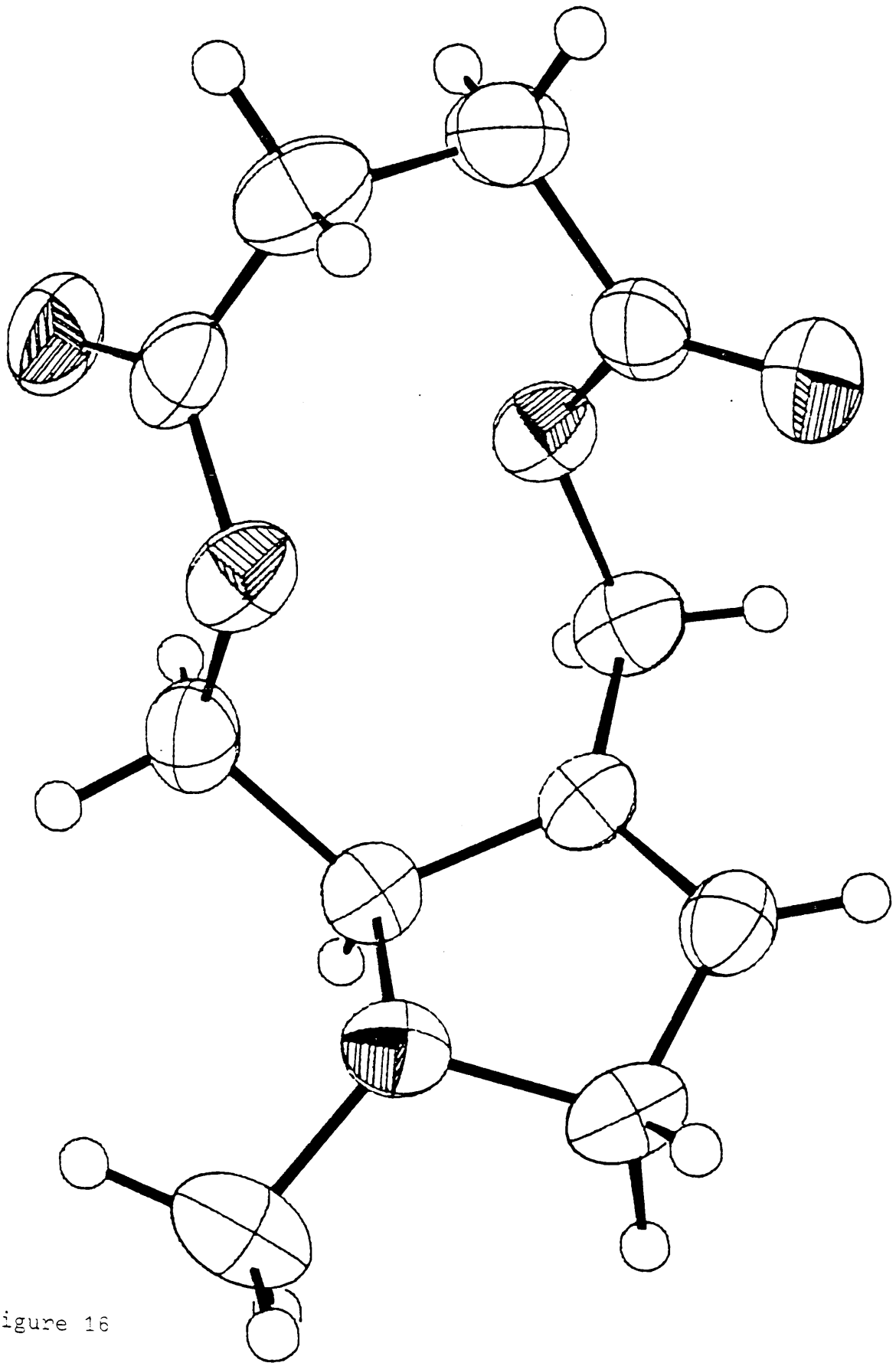


Figure 16

X-ray structure of (±)-6,7-O,O-(succinyl)-synthanecine A (193).

the macro-ring. This is the same orientation as observed for the 10-membered macrocyclic diester (133) containing retronecine.

Some interesting differences have emerged between the X-ray structures of these synthanecine dilactones. Further X-ray studies should be carried out on other dilactones of synthanecine A to discover if any general trends will emerge.

3.8 Conclusions.

A series of 10- and 11-membered macrocyclic diesters of synthanecine A [(183 a - f), 189, 190, (193 - 196)] have been prepared using the Corey-Nicolaou procedure to effect the crucial lactonisation step. High resolution mass spectrometry and ^1H n.m.r. spectroscopy have provided the essential data for the characterisation of these dilactones (see Chapter 3.6). X-ray spectral data of (183b), (190), (183c) and (193) show that the macrocycles adopt different conformations. This is also indicated by the ^1H n.m.r. spectroscopic data.

Some of these macrocyclic diesters have been tested at the M.R.C. Toxicology Unit to assess their hepatotoxicity (see Chapter 6).

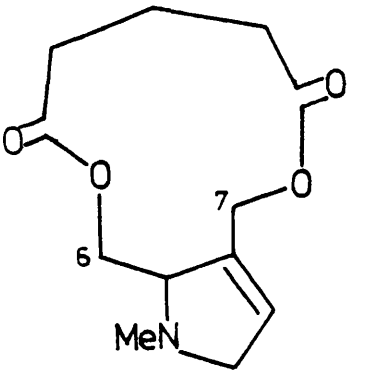
The fact that these dilactones were obtained in low yields, may be a consequence of the lengthy purification procedure. A more efficient synthetic method would not involve large quantities of coupling reagents and the undesirable acid-base recycle for purification (see Chapter 4).

CHAPTER 4

SYNTHESIS OF A SERIES OF ALIPHATIC, OLEFINIC AND AROMATIC SYNTHANECINE A DILACTONES VIA THE ALLYLIC CHLORIDE (200).

4.1 Introduction.

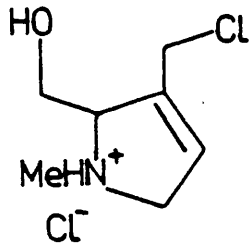
Although the synthesis of a series of macrocyclic diesters of synthanecine A was accomplished using the Corey-Nicolaou lactonisation procedure, only low yields of these products were obtained (see Chapter 3). In order to produce sufficient material for further studies of their biological activity, a more efficient method for the preparation of macrocycles, such as (183a), was required. Use of a different method might also enable a wider range of analogues to be prepared.



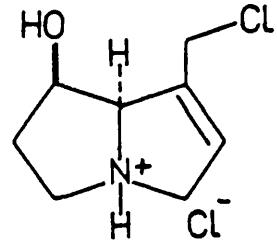
(183a)

During our studies on the formation of macrocyclic diesters (see Scheme 35) it was observed that monoesterification of synthanecine A, using an anhydride, occurred selectively at the C-6 hydroxyl. Cyclisation was then effected by the intramolecular condensation of the free acid and the primary allylic hydroxyl.

A method for the specific esterifcation of the allylic hydroxyl of retronecine was developed by Culvenor and co-workers[44] (see Scheme 7). This method relies upon the high reactivity of allylic chlorides towards nucleophilic displacement by a carboxylate anion. A related reaction was reported by Vedejs and Larsen[60] in which lactonisation of a hydroxyacid was accomplished by the carboxylate displacement of an allylic mesylate (see Scheme 17). We have combined these methods to develop a convenient and efficient route to synthanecine A dilactones containing aliphatic, olefinic and aromatic diacids. This procedure is based upon the nucleophilicity of the primary hydroxyl and the electrophilic reactivity of the allylic chloride moieties of the key intermediate (200).



(200)

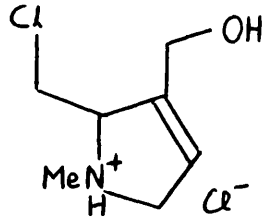


(201)

A similar procedure has been developed, by Robins and Burton,[87] employing the hydrochloride salt (201), for the preparation of macrocyclic diesters of retronecine.

Compound (200) could also arise by initial attack of the more nucleophilic hydroxy group at C-6 of synthanecine A (9) on thionyl chloride, followed by formation of a cyclic sulphite ester, and subsequent attack by chloride to give the product (200).

An alternative structure for this product (200) which is also consistent with the available n.m.r. spectroscopic evidence is the structural isomer (200a). Further data will be required to establish the correct structure of this reaction product.

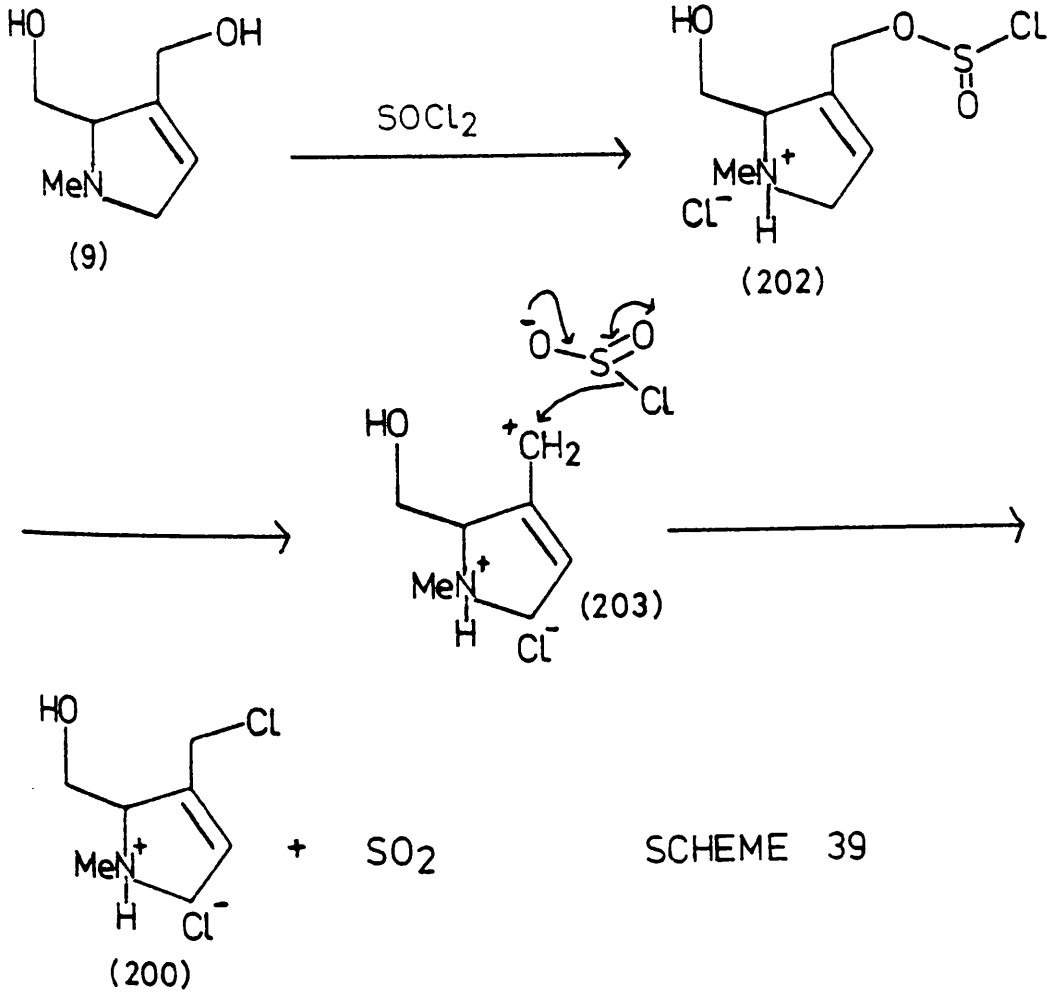


(200a)

4.2 Synthesis of 3-Chloromethyl-2-Hydroxymethyl-1-Methyl-3-Pyrrolinium Chloride (200).

Since Adams and Van Duuren[88] prepared (7R,8R)-1-chloromethyl-7-hydroxy-1,2-didehydropyrrolizidine as the hydrochloride salt (201) by treating (+)-retronecine with thionyl chloride at 0°C, it was anticipated that the allylic chloride (200) could be prepared similarly.

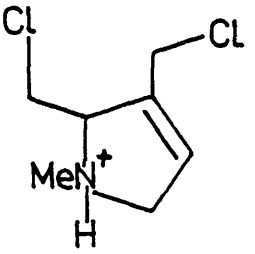
When synthanecine A (9) was treated with thionyl chloride at 0°C the desired allylic chloride (200) was produced. This chlorination possibly proceeded *via* the mechanism shown in Scheme 39. The chlorosulphite (202), produced by the reaction of synthanecine A and thionyl



SCHEME 39

chloride, dissociates into an intimate ion pair (203), the anion of which immediately collapses to give sulphur dioxide and chloride ion. Subsequent attack of the latter on the carbonium ion gives the desired allylic chloride (200).

When the reaction was complete (45 min) the excess thionyl chloride was removed under reduced pressure at $0^{\circ}C$ to prevent further chlorination which would give the 6,7-dichloro derivative (204). The crude product was initially



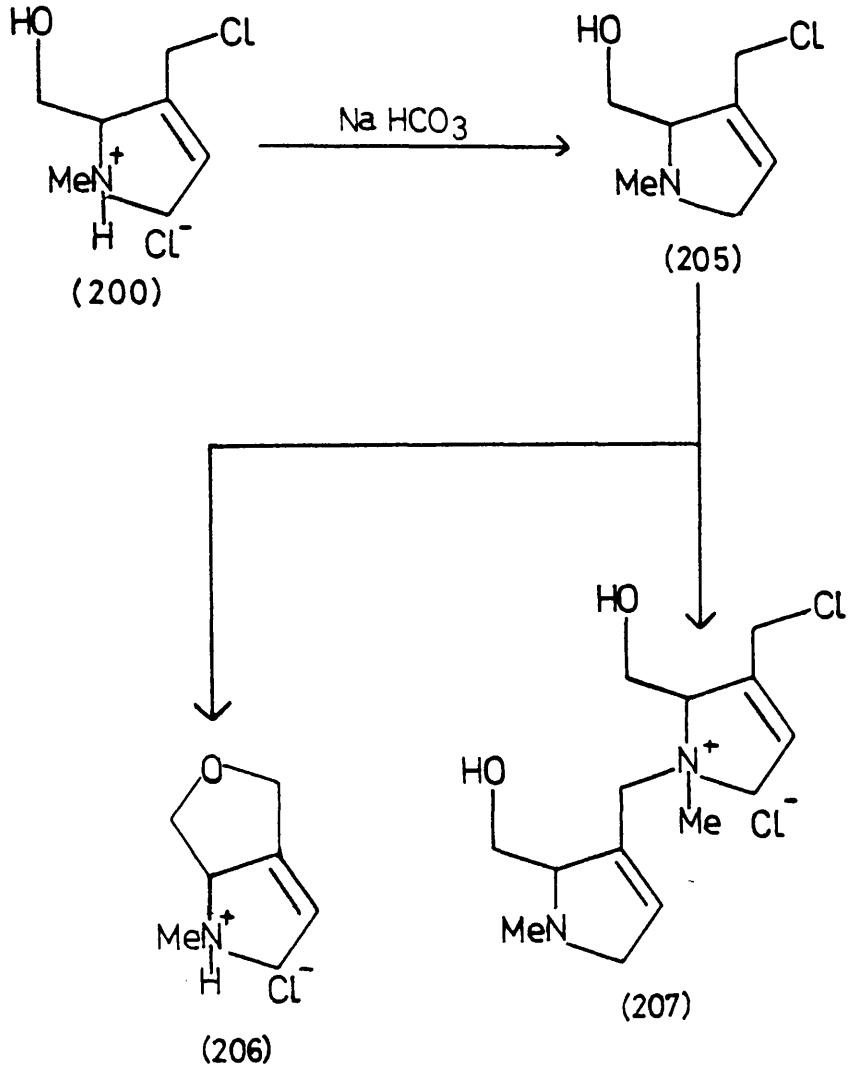
(204)

obtained as a purple gum. Subsequently, purification and crystallisation afforded the hydrochloride salt (200) as white crystals (55%) and a brown oil (35%). The brown oil could be used to prepare synthanecine A dilactones, however lower yields (ca. 60% of the reported yields) of the macrocyclic products were obtained.

Correct microanalytical data were obtained for the crystalline hydrochloride salt (200). The accurate mass spectrum of the allylic chloride (200) did not display a molecular ion, however ions at m/z 132 and 130 correspond to a molecular ion minus CH_3OH. The presence of chlorine in the molecule was indicated by its characteristic isotope pattern. The ^1H n.m.r. spectrum of the chloromethyl

derivative (200), recorded in deuterium oxide (D_2O), displayed a sharp singlet at 3.16 p.p.m. due to the N-Me group. This is a downfield shift of ca. 0.6 p.p.m. relative to the signal for synthanecine A and is due to the presence of the quaternised nitrogen. Downfield shifts for the signals corresponding to the N-Me and C-2 and an upfield shift for the signal assigned to C-7, were observed in the ^{13}C n.m.r. spectrum of the hydrochloride salt (200), when compared to that of synthanecine A. The downfield shifts are due to the presence of the quaternary ammonium salt and the observed upfield shift is indicative of chlorination at C-7.

The allylic chloride (200) was also obtained when a solution of synthanecine A in dry DME at $-5^{o}C$ was treated with thionyl chloride. The free base, 3-chloromethyl-2-hydroxymethyl-1-methyl-3-pyrroline (205), was obtained by neutralising the hydrochloride salt (200) as shown in Scheme 40. When the free base (205) was stored, even at $0^{o}C$, it decomposed into an intractable gum. The basic nitrogen of (205) may assist the primary hydroxyl towards intramolecular nucleophilic displacement of the allylic chloride to produce the cyclic ether (206), or intermolecular attack may yield polyether products. Quaternisation of the free amine by the allylic chloride to form compound (207), or a polymer is also possible (see Scheme 40). The allylic chloride was therefore stored as the hydrochloride salt (200).

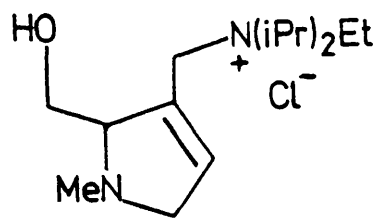


SCHEME 40

4.3 Synthesis of Macrocyclic Diesters of Synthanecine A.

In our initial investigations into the preparation of synthanecine A dilactones from the allylic chloride (200), substituted glutaric anhydride derivatives were chosen for the diacid moiety as this allowed a comparison to be made with the procedure discussed in Chapter 3 that involved Corey-Nicolaou lactonisation. A solution of the hydroxy-

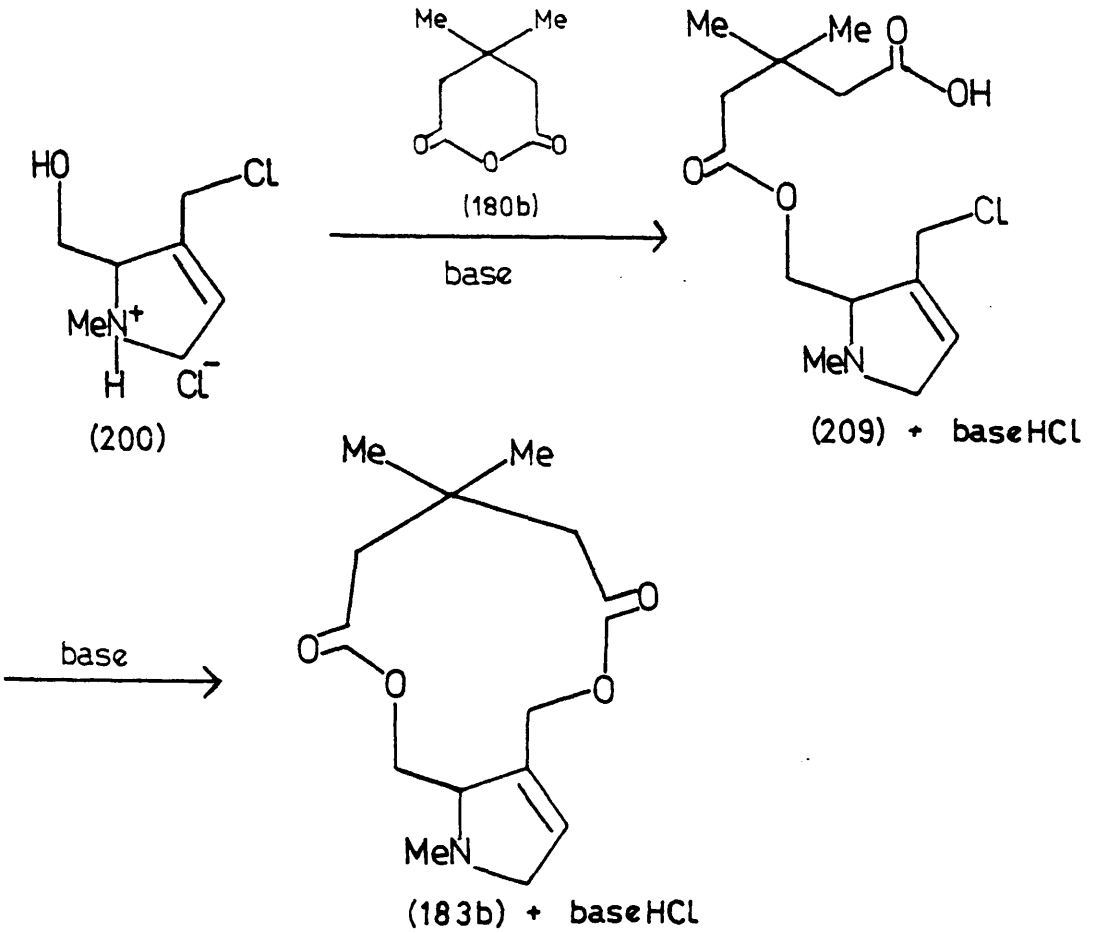
allylic chloride (200) in DMF was treated with an equimolar amount of 3,3-dimethylglutaric anhydride (180b) in the presence of 2.1 equivalents of various bases. The first equivalent of base is employed to neutralise the hydrochloride salt (200) and the second is used to generate the carboxylate anion of the initial monoester product (209) (see Scheme 41). When the hindered base, diisopropylethylamine (Hunig's base) (210) was used, no cyclised product was formed and the allylic chloride (200) slowly decomposed (t.l.c.). Possible decomposition products are the cyclic ether (206) and the quaternary ammonium derivatives (207) and (208) (see Scheme 40). With 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU) (211) as the base a product was observed



(208)

at the expected Rf value on t.l.c. (The reactions discussed in this chapter were monitored by t.l.c. using the method reported in Chapter 4.4). The starting material (200) had disappeared after 5 h at room temperature, but the reaction mixture was left for a further ca. 20 h to ensure a maximum yield. After this time the solvent (DMF) was removed under reduced pressure and the resulting oil was purified by column chromatography on basic alumina. A 53%

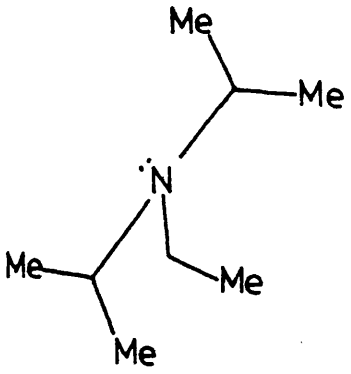
yield of a white crystalline product was obtained. This product was spectroscopically and chromatographically identical with a known sample of the dilactone (183 b).



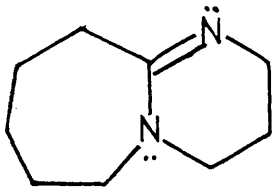
using (210) as base — no reaction
(211) — 53% yield
(212) — 37% yield

SCHEME 41

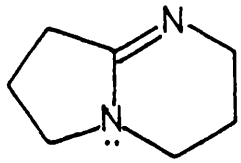
No improvement on this yield was obtained when another stronger base, 1,5-diazabicyclo[4,3,0]non-5-ene (DBN) (212) was employed. Other glutaric anhydride derivatives were treated with the allylic chloride (200) in a similar fashion, using DBU (211) as base. In all cases the yields



(210)



(211)

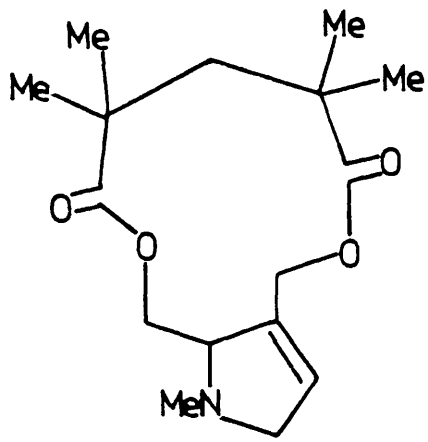


(212)

of cyclised products obtained were better than those achieved by the Corey-Nicolaou method (Chapter 3). The increased yields may be partially due to the simple and convenient purification procedure that was used. Since this route does not involve large quantities of coupling reagents, the crude reaction mixture was subjected to column chromatography without initial purification by a potentially destructive acid base recycle. The reaction of glutaric anhydride (180a) and 1,1-cyclohexanediacetic anhydride (180d) with allylic chloride (200) and DBU afforded the corresponding macrocyclic diesters (183a) and (183d) in 37% and 66% yields respectively. Other macrocyclic diesters of interest were also prepared by the method discussed above. A mixture of the diasteromeric racemates (183f) which are analogues of the pyrrolizidine alkaloid dicrotaline (88) were prepared in 52% yield. The diasteromeric racemates (189) and (190) were also prepared in a yield of 77%. All of the 11-membered macrocyclic diesters prepared by the procedure described above, displayed spectroscopic and chromatographic properties which were identical to those

obtained for known samples.

Synthesis of a new macrocyclic dilactone (213) containing 2,2,4,4-tetramethylglutaric acid and synthanecine A was also accomplished by this method in 44% yield. An accurate mass measurement of the racemate (213) gave a molecular formula of $C_{16}H_{25}NO_4$ and the mass spectral fragmentation pattern observed was typical of a macrocyclic diester containing synthanecine A. The i.r. spectrum of (213) displayed an ester carbonyl absorption at 1730 cm^{-1}. In the ^1H n.m.r. spectrum of the sterically hindered dilactone (213), compared to that of synthanecine A, the signals assigned to the C-6 protons were shifted downfield by 0.55 p.p.m. and appeared as a doublet of AB quartets; a $\Delta\delta$ H-6 value of 0.53 p.p.m. was observed. The C-7 diastereotopic protons were shifted downfield by 0.46 p.p.m. and were magnetically equivalent. Another interesting feature in the



(213)

^1H n.m.r. spectrum of (213), which is also consistent with the formation of a macrocycle, is the distinct non

equivalence of the C-11 protons. Previous attempts to prepare the 11-membered dilactone (213) by the Corey-Nicolaou method had been unsuccessful. Due to the increased steric hindrance around its ester carbonyls, the 2,2,4,4-tetramethylglutarate dilactone (213) may have enhanced hepatotoxicity (see Chapter 1.4). The hepatotoxicity of this dilactone (213) will be assessed at the M.R.C. Toxicology Unit, Carshalton.

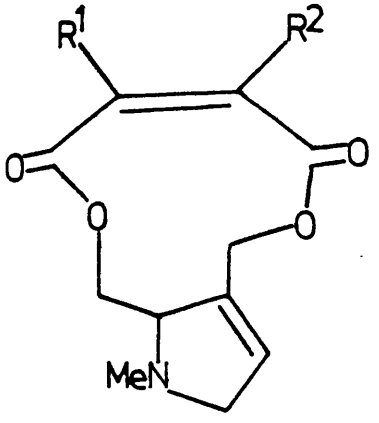
For further comparisons with the Corey-Nicolaou method a series of 10-membered aliphatic macrocyclic diesters were prepared. Treatment of the hydrochloride salt (200) with succinic anhydride and DBU gave a 10% yield of the dilactone (193). This reaction proceeded very slowly and even after a few days at room temperature some unreacted allylic chloride (200) was observed. T.l.c. also indicated the presence of a major by-product with Rf value of 0.0. An increase in the yield of the succinate cyclic diester (193) was obtained when the reaction mixture was heated at 70 C for 6 h. When (±)-2,3-dimethylsuccinic anhydride was treated with the allylic chloride (200) and DBU at room temperature a 17% yield of the 10-membered diastereomeric racemates (194) was obtained. This reaction also occurred slowly. The reactions discussed above may be unfavourable due to the increased strain present in 10-membered macrocycles and the orientation of the attacking carboxylate anion. It appears that low yields of macrocyclic products are obtained when the rate of reaction is slow. One possible reason for this is that the starting material (200) is decomposed under the

basic reaction conditions. Preparation of the 10-membered macrocycles (195) occurred more readily and a significant increase in yield was observed (51%). The presence of the cyclohexane ring reduces the flexibility of the attacking carboxylate, thus making the cyclisation more favourable. The 10-membered macrocycles (193) - (195) displayed ^{1}H n.m.r. spectra which were identical with those obtained for known samples.

Deslongchamps[68] has reported that the rate of cyclisation of a 10-membered carbon chain is facilitated by the presence of unsaturation with the appropriate stereochemistry. This unsaturation reduces the degrees of freedom and transannular interactions of the system, therefore making the cyclisation more favourable on the basis of both the entropy and enthalpy factors (see Chapter 2.3).

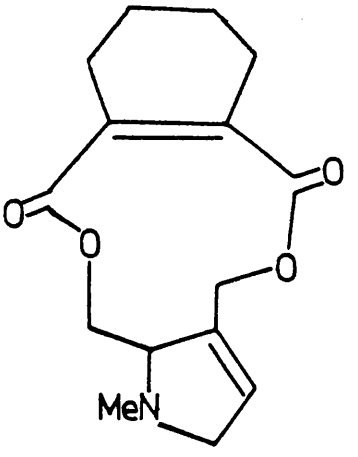
When maleic anhydride, the unsaturated derivative of succinic anhydride, was treated with the hydrochloride salt (200) in the presence of DBU, the racemate (214) was formed after a few hours and was isolated in a 49% yield. Since the succinate dilactone (193) was isolated in only 10% yield after 3 days under identical conditions it appears that the presence of the double bond, with cis stereochemistry, has increased the rate of lactonisation and led to a higher yield of product. By comparing the rate of formation and yield of this olefinic dilactone (214) with those of the saturated dilactone (195) that contains a cyclohexane ring, it would appear that the fused ring has the same influence as that of the double bond. Treatment of

2,3-dimethylmaleic anhydride in a similar manner afforded the corresponding racemate (215) in 33% yield. The rate



(214) $R^1 = R^2 = H$

(215) $R^1 = R^2 = Me$



(216)

of reaction of this anhydride with the allylic chloride (200) in the presence of DBU, was noticeably slower than the corresponding reaction using maleic anhydride. An increased reaction rate was observed when 3,4,5,6-tetrahydrophthalic anhydride was treated similarly. As a consequence of this increased rate of reaction the racemate (216) was obtained in a 77% yield, which indicates that the unsaturation and the carbocyclic ring have had a combined beneficial influence on this reaction.

The three new olefinic dilactones (214), (215) and (216) were fully characterised as free bases. When they were purified by recrystallisation, the white crystalline solids gave correct microanalytical data. High resolution mass spectra data on the olefinic dilactones (214), (215)

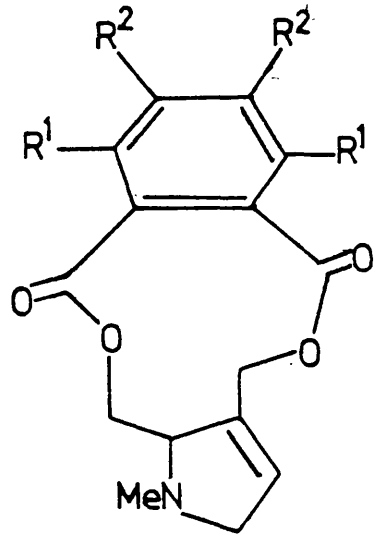
and (216) display molecular ions corresponding to $C_{11}H_{13}NO_4$, $C_{13}H_{17}NO_4$ and $C_{15}H_{19}NO_4$ respectively. In all cases the fragmentation patterns exhibited were indicative of dilactones containing the synthanecine moiety (see Chapter 3.6.2). The i.r. spectra showed α,β-unsaturated ester carbonyl absorptions at ca. 1725 cm^{-1}, and no hydroxyl or carboxylic acid absorptions were observed. The key features in the ^1H n.m.r. spectra of the macrocyclic diesters (214), (215) and (216) were the AB quartets (ca. 4.85 p.p.m.) corresponding to the C-7 protons and the doublet of AB quartets (ca. 4.25 p.p.m.) assigned to the C-6 protons. The acylation shifts for these signals (ca. 0.6 p.p.m.) were consistent with the esterification of primary alcohols. As the extent of non-equivalence of the C-6 and C-7 diastereotopic protons is about the same in each case (see Table 3) it would appear that these macrocycles adopt similar conformations.

Synthanecine A dilactone	Δδ H-6 (p.p.m.)	Δδ H-7 (p.p.m.)	Reaction Yield (%)
(214) maleate	0.36	0.53	49
(215) 2,3-dimethylmaleate	0.44	0.53	33
(216) 3,4,5,6-tetra-hydrophthalate	0.46	0.60	77

Table 3

We then decided to prepare the 10-membered aromatic dilactone (196) to see if the aromatic ring would have an influence on the rate of reaction and the isolated yield. Previous attempts to prepare the phthalate derivative (196) by the Corey-Nicolaou procedure only gave low yields (ca. 16%) of the product. The hydrochloride salt (200) in DMF was treated with phthalic anhydride in the presence of DBU. The starting material (200) had completely disappeared after 10 min. and the presence of cyclised product (Rf 0.72) and a highly polar by-product (Rf 0.0) were indicated by t.l.c. After 6 h the reaction mixture was worked up in the usual manner to give an 86% yield of the macrocyclic diester (196). In view of this encouraging result, this procedure was repeated with a series of symmetrically halogenated phthalic anhydrides. The halogenated derivatives (217), (218) and (219) were formed at approximately the same rate as the phthalate dilactone (196) and in comparable yields (see Table 4).

The phthalate dilactone (196) was spectroscopically and chromatographically identical with a known sample. The novel halogenated phthalate derivatives (217), (218) and (219) were obtained as white crystalline solids and characterised as free bases. Appropriate molecular ions were observed in their high resolution mass spectra and the presence of the expected isotope abundance patterns confirmed the presence of halogens. The mass spectral fragmentation patterns of the new analogues were typical of synthanecine A dilactones with main peaks at m/z 123, 107, 94 and 82.



(196) $R^1 = R^2 = H$

(217) $R^1 = H$, $R^2 = Cl$

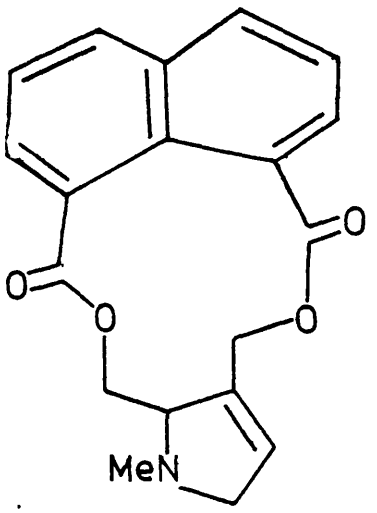
(218) $R^1 = R^2 = Cl$

(219) $R^1 = R^2 = Br$

The ^1H n.m.r. spectra of the aromatic dilactones (217), (218) and (219) displayed the characteristic AB quartets and doublet of AB quartets assigned to the C-7 and C-6 diastereotopic protons respectively. Primary alcohol acylation shifts of ca. 0.75 p.p.m. abd 0.85 p.p.m. were observed for the C-6 and C-7 protons. The chemical shift difference of these protons (see Table 4) shows more variation than the olefinic series (see Table 3). This may be due to the presence of different conformations as a consequence of the steric effects of the halogens.

Synthanecine A dilactones	Δδ H-6 (p.p.m.)	Δδ H-7 (p.p.m.)	Yields (%)
(196) phthalate	0.36	0.48	86
(217) 4,5-dichlorophthalate	0.34	0.47	91
(218) 3,4,5,6-tetrachloro-phthalate	0.23	0.23	67
(219) 3,4,5,6-tetrabromo-phthalate	0.13	0.15	81

Table 4



(220)

Finally, treatment of the allylic chloride (200) with naphthalene-1,8-dicarboxylic anhydride and DBU produced an 11-membered aromatic dilactone (220). The formation of this analogue (220) occurred readily and a 65% yield was obtained. The 11-membered macrocyclic diester (220) was characterised as the free base. Comparison of ^{1}H n.m.r. spectra of the dilactone (220) and synthanecine A showed that the protons on C-7 had shifted downfield by ca. 0.8 p.p.m. and appeared as an AB quartet. The signals for the protons on C-6 had also shifted downfield by ca. 0.8 p.p.m. and appeared as a doublet of AB quartets. The i.r. spectrum of the 11-membered dilactone (220) showed a conjugated ester carbonyl at 1725 cm^{-1}. A weak absorption at 3020 cm^{-1} corresponded to the aryl C-H stretch. Correct accurate mass measurement and microanalytical data were obtained for the free base (220).

The rates of formation and yields obtained of these macrocyclic diesters of synthanecine A, appeared to be influenced by changes in the diacid moieties. Saturated 11-membered macrocycles were more readily formed than saturated

10-membered ones. Also, the presence of any structural feature that reduced the flexibility of the intermediate carboxylate anion, increased the rate of cyclisation which consequently led to an increase in the isolated yield. [This is because reaction times lead to decomposition of some of the starting allylic chloride (200)]. For example, the succinate product (193) was formed slowly and in very low yield (10%) (see Table 5). When unsaturation was introduced into the 2,3-position (maleic anhydride) the reaction proceeded much more readily and the product (214) was obtained in a 49% yield. By introducing an aromatic ring (phthalic anhydride) the rate of reaction and yield were increased further.

The rate of reaction and yields of macrocyclic diesters obtained by this route were also dependent on the base employed (see Table 6).

Percentage Yields of Macrocyclic Diesters using Different Bases

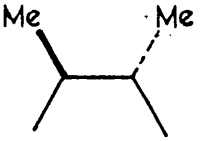
macrocyclic diester		base		
		HUNIG'S (210)	DBN (212)	DBU (211)
(183b)	3,3-dimethyl-glutarate	x	37%	53%
(214)	maleate	x	x	49%
(218)	3,4-dichloro-phthalate	40%	24%	91%

x no reaction

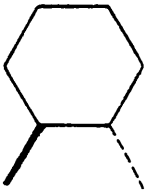
Table 6

Percentage yields of 10 membered macrocyclic diesters of synthanecine A obtained when DBU was used as base.

10

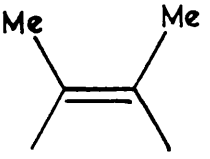


17

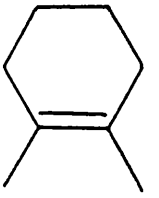


51

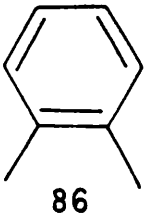
49



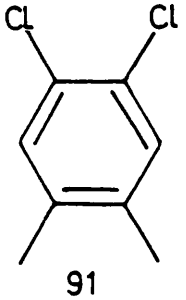
33



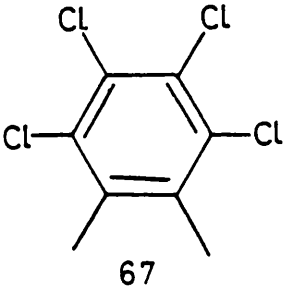
77



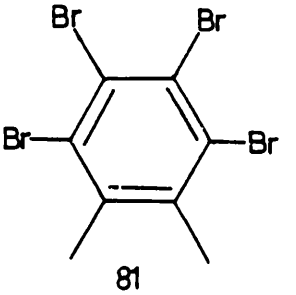
86



91



67



81

Table 5

The best results were obtained when DBU was utilised. Time did not permit study of this reaction with other bases. However it is clear that good yields were obtained in the lactonisation by using a strong amine base.

4.4 Chromatographic analysis of this route.

The formation of these macrocyclic diesters was monitored by t.l.c. using the same procedure as described in Chapter 3.5. The disappearance of the allylic chloride (200) and the formation of macrocyclic diesters were observed, by t.l.c., without difficulty (see Figure 17). However, the

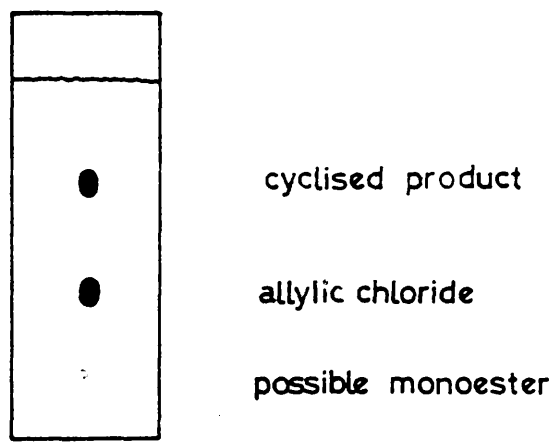


Figure 17

likely monoester intermediate may be obscured by baseline by-products or it may react rapidly to produce the cyclised product. Therefore these reactions were not terminated after the disappearance of the allylic chloride (200), but were routinely continued for 24 h to ensure a maximum conversion. Formation of the 10-membered dilactones (193), (194) and (215) normally required 48 h.

4.5 Conclusions.

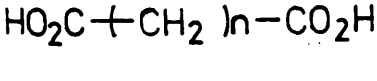
A series of aliphatic, olefinic and aromatic synthanecine A dilactones were prepared in DMF, from the allylic chloride (200), the appropriate cyclic anhydride and 2 equivalents of DBU as base. This new method has advantages over the Corey-Nicolaou method (Chapter 3). First, a wider variety of dilactones can be prepared under mild conditions. Secondly, the procedure is simple and can probably be scaled up to produce larger quantities of the macrocyclic products, for testing purposes. Thirdly, the work-up procedure does not involve a potentially destructive acid-base recycle and better yields of all the macrocyclic products except (193) and (194) were obtained. [10-membered dilactones (193) and (194) were formed in better yields by the Corey-Nicolaou method, presumably because their intermediate carboxylates are not properly orientated for the efficient displacement of the allylic chloride].

CHAPTER 5

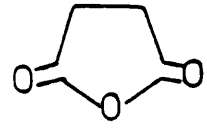
SYNTHESIS OF 12-MEMBERED AND LARGER MACROCYCLIC DIESTERS OF SYNTHANECINE A

5.1 Introduction

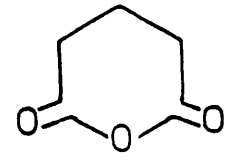
A number of 10- and 11-membered synthanecine A dilactones have been prepared by the two routes discussed in Chapters 3 and 4. In one of these procedures monomeric diacid anhydrides were treated with synthanecine A to form intermediate monoesters which were then lactonised by the Corey-Nicolaou method (Chapter 3). In the second, initial esterification of the hydroxy allylic chloride (200) was also accomplished using monomeric diacid anhydrides, and then the lactonisation step was effected by carboxylate displacement of the allylic chloride (Chapter 4). Cyclic anhydrides are in most cases only formed by derivatives of succinic (221) and glutaric acids (222) since they form stable 5- (225) and 6-membered (180 a) rings. Therefore the methods already described are mainly suitable for the preparation of 10- and 11-membered macrocyclic diesters of synthanecine A.



(221) n = 2
(222) n = 3
(223) n = 4
(224) n = 5

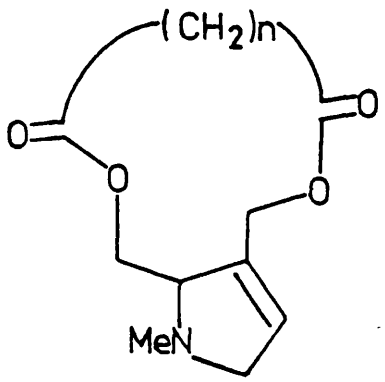


(225)



(180a)

Macrocyclic pyrrolizidine alkaloids occur most frequently with 11- and 12-membered rings. A few 13-membered pyrrolizidine dilactones have also been discovered. Therefore, our next objective was to prepare 12- (226) and 13-membered (227) macrocyclic diesters of synthanecine A. Such compounds are derivatives of adipic (223) and pimelic (224) acids. Since these diacids form linear polymeric



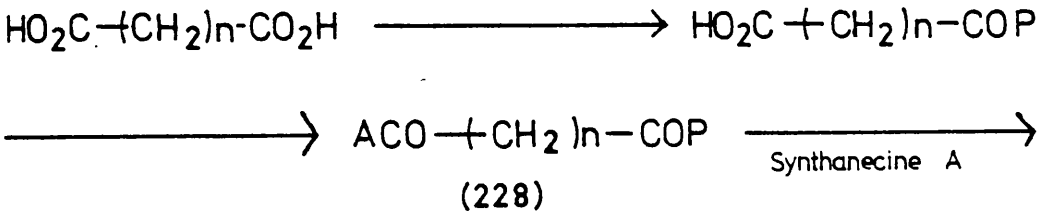
(226) n = 4
(227) n = 5

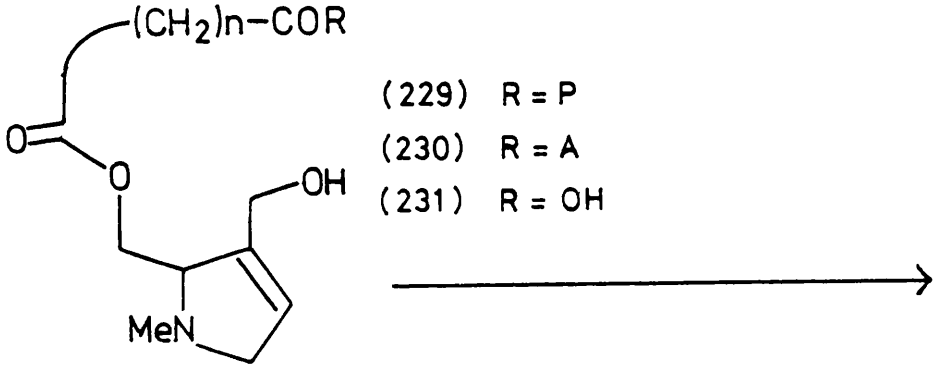
anhydrides rather than cyclic monomeric ones a new route was required in which a different method for monoesterification was used. Novel pyrrolizidine alkaloid analogues with larger rings might also be prepared by this new route.

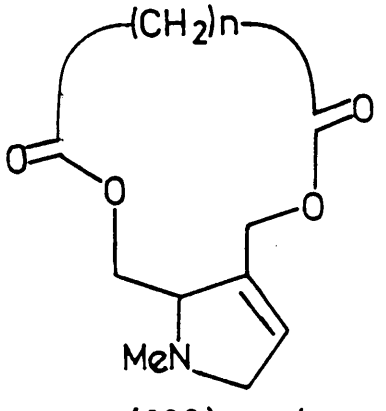
5.2 Attempted Synthesis of Macrocyclic Diesters With 12-Membered and Larger Rings

The combination of a diacid and a diol, to produce an intermediate monoester must be selective otherwise a complex mixture of oligomers would be formed. The selective mono-esterification of a diacid with synthanecine A might be achieved after protection of one of the carboxyl groups

followed by activation of the other (see Scheme 42). Treatment of synthanecine A with the activated and protected diacid (228) should give the C-6 monoester (229) of synthanecine A selectively. To complete the synthesis, the protected acid (229) has to be transformed into an activated acid (230) which can then undergo lactonisation to give







(226) n = 4

P = Protecting group

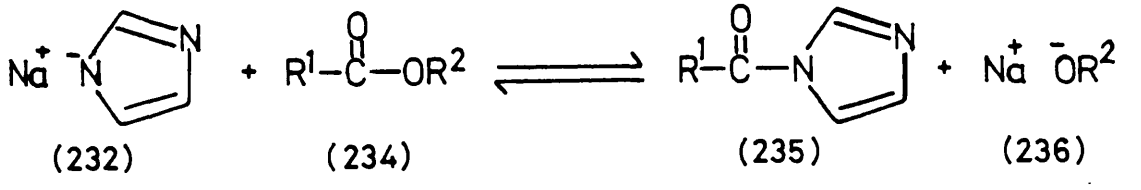
A = Activating group

SCHEME 42

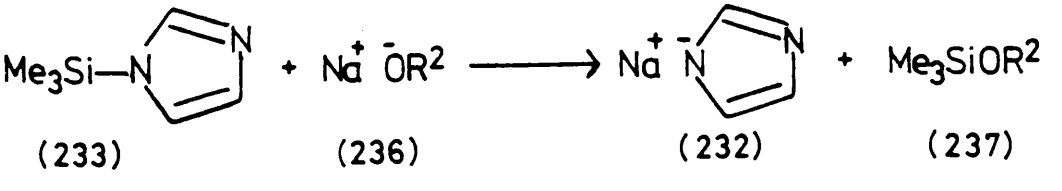
the desired macrocyclic product (226). Removal of the protecting group from the intermediate (229) would afford a

hydroxy acid (231) that would probably not be readily manipulated due to its zwitterionic properties. Therefore, a non hydrolytic route from the protected acid to the activated acid would be preferable. This activation process should also be selective and not interfere with the ester linkage to synthanecine A.

In 1976 Masamune and co-workers[89] reported a procedure for the direct conversion of phenyl and 2,2,2-trifluoroethyl esters into N-acylimidazolides as shown in Scheme 43. Imidazolyl sodium (232), produced by the reaction of 1-(trimethylsilyl)imidazole (233) with a catalytic quantity of sodium phenoxide, reacted with the ester (234) to produce the required N-acylimidazolide (235) and the sodium alkoxide (236).



(232) (234) (235) (236)



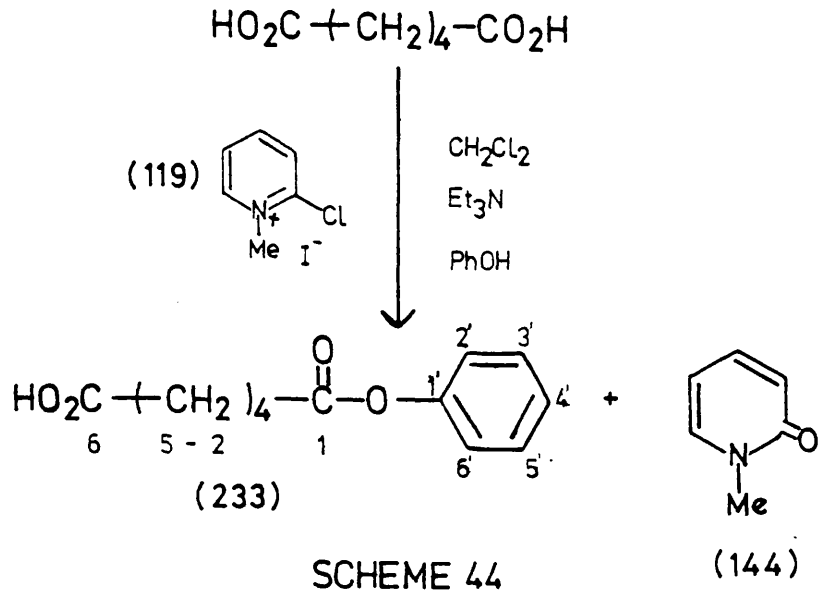
(233) (236) (232) (237)

R^2 = Ph or CH_2CF_3

SCHEME 43

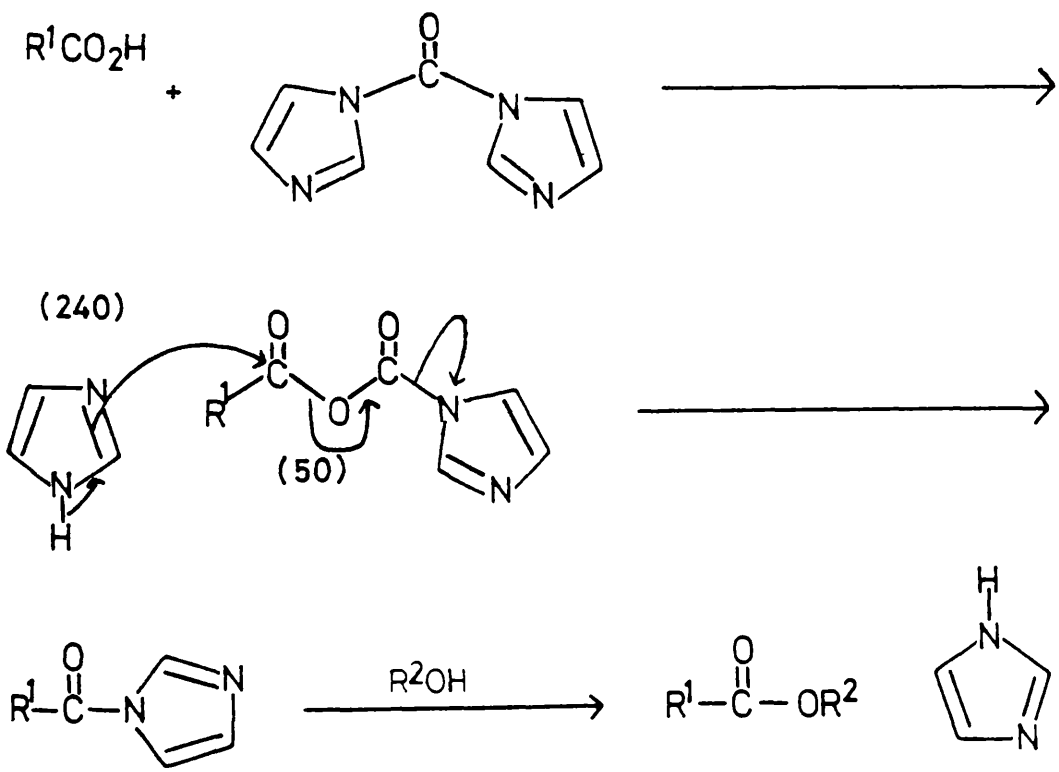
The alkoxide (236) immediately reacted with more 1-(trimethylsilyl)imidazole (233) and the catalytic cycle continued until the ester (234) was completely transformed into the N-acylimidazolide (235). The reverse reaction, conversion of the N-acylimidazolide (235) back into the ester (234) was suppressed by the removal of the alkoxide (236) as the TMS ether (237). Since alkyl esters are inert to the silyl reagent (237) this method permits the selective activation of one ester in the presence of another.

The activation process described above appeared to be suitable for the penultimate step of the synthetic route shown in Scheme 42. Therefore, it was decided to explore further this route for preparing larger ring macrocyclic diesters (Scheme 42), using a phenyl or a 2,2,2-trifluoroethyl ester as the protecting group. In our initial investigations adipic acid (223) was chosen as the diacid component since 12-membered macrocyclic pyrrolizidine alkaloids are commonly observed. Phenyl esters were employed instead of 2,2,2-trifluoroethyl ester since phenol was more readily available than 2,2,2,-trifluoroethanol. The monophenyl ester (238) of adipic acid was prepared when molar equivalents of phenol and adipic acid were coupled using 1-chloro-2-methylpyridinium iodide (119) (see Scheme 44) (Also see Chapter 2.4, Mukaiyama method). However, complete separation of the half ester (238) from the pyridone by-product (144) proved to be difficult.



SCHEME 44

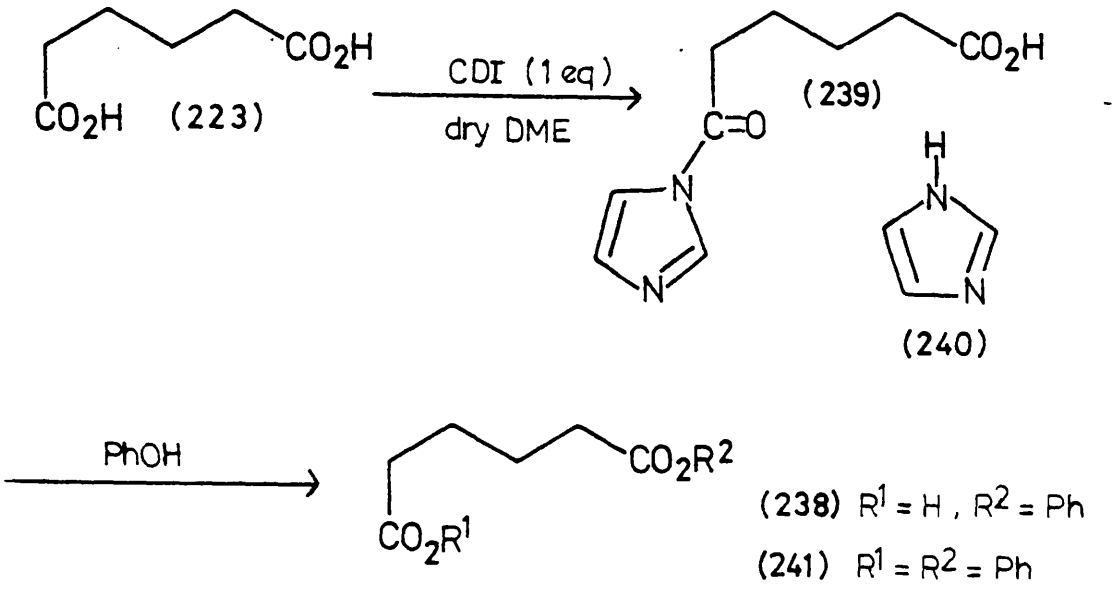
An alternative method for the activation of carboxylic acids for esterification is by their conversion into N-acyl-imidazolides by treatment with N,N' carbonyldiimidazole (CDI) (50)



SCHEME 45

(see Scheme 45). A comprehensive review on the preparation and properties of N-acylimidazolides has been published by Staab.[90]

Treatment of adipic acid (223) with a molar equivalent of CDI produced a white precipitate of an N-acylimidazolide (see Scheme 46). A ^{1}H n.m.r. spectrum of the product



SCHEME 46

obtained from this reaction displayed 3 singlets of equal intensity with chemical shifts of 7.10, 7.47 and 8.19 p.p.m. These signals correspond to the three protons on the imidazole ring of the activated acid (239). Also, the signals assigned to the methylene protons α to the imidazole carbonyl were shifted downfield by 0.5 p.p.m. relative to the signals for adipic acid. The occurrence of this downfield shift and the presence of the 3 singlets discussed above indicate the formation of an N-acylimidazolide. This ^{1}H n.m.r. spectrum also showed two singlets at 7.08

p.p.m. and 7.77 p.p.m. of relative intensity 2:1 which correspond to the three protons of imidazole (240) which is a by-product of this reaction. Imidazole (240) can normally be removed by washing a solution of the reaction mixture in CH_2Cl_2, with water. Similarly, to remove the imidazole peaks from an n.m.r. spectrum the organic solution in the n.m.r. tube is shaken with D_2O. When D_2O was added to the $CDCl_3$ solution of this product mixture the spectrum of the N-acylimidazolide (239) was not observed. Therefore, the N-acylimidazolide (239) is probably present as a zwitterion or an imidazolium salt.

When the precipitate of the N-acylimidazolide (239) was treated directly with phenol a mixture of the required monophenyl adipate (238), unreacted adipic acid (223) and diphenyl adipate (241) was obtained (see Scheme 46); however the monophenyl ester (238) was easily purified by an acid base recycle. Extraction of monophenyl adipate (238) was carried out at ca. pH 5 to ensure that adipic acid (223) remained dissolved in the acidic solution.

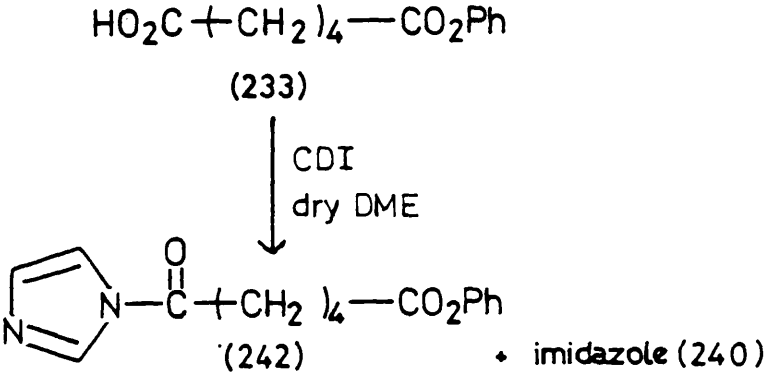
Finally, a mixture of monophenyl adipate (238) diphenyl adipate and adipic acid was also prepared by reacting equimolar amounts of adipic acid (223) and phenol under Dean Stark conditions, with concentrated sulphuric acid as the catalyst. Purification of this reaction mixture was accomplished by an acid base recycle as discussed above. This latter method was most convenient as it did not require a complex coupling reagent.

The monophenyl ester (238) of adipic acid was

characterised as the free acid. An accurate mass measurement of the half ester (238) gave a molecular formula of $C_{12}H_{14}O_4$ and the mass spectral fragmentation pattern displayed a peak at m/z 129 that corresponds to the loss of OPh. The i.r. spectrum showed a phenyl ester carbonyl absorption at 1755 cm^{-1} and a free acid carbonyl absorption at 1695 cm^{-1}. In the ^1H n.m.r. spectrum of the phenyl ester (238) of adipic acid, recorded in deuteriochloroform, the signals assigned to the methylene protons α to the ester carbonyl were shifted downfield by ca. 0.18 p.p.m. compared with the signals for adipic acid. A complex array of signals between 7.0 and 7.5 p.p.m. were assigned to the phenyl protons.

For the activation of the free acid, preparation of an N-acylimidazolide was chosen. N-Acylimidazolides are accessible in good yields by the method described by Staab[90] (see Scheme 45).

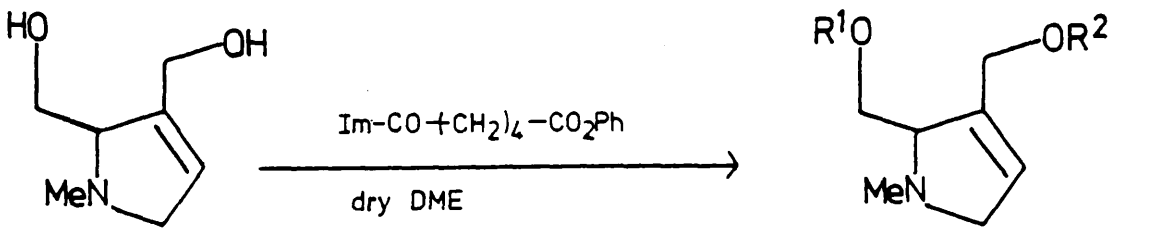
Reaction of monophenyl adipate (238) with CDI at room temperature afforded the desired phenyl imidazolyl adipate (242) (see Scheme 47). The N-acylimidazolide (243) was isolated in 85% yield. Formation of this activated acid



SCHEME 47

(242) was indicated by the presence of the three singlets of equal intensity at 7.11, 7.46 and 8.15 p.p.m. in its ^1H n.m.r. spectrum. These signals were assigned to the three protons of the imidazole ring. A downfield shift of 0.5 p.p.m. for the methylene protons α to the imidazolide carbonyl relative to the signals for adipic acid was also observed. The accurate mass spectrum of the product (242) did not display a molecular ion. However, a peak at m/z 205 corresponds to the molecular ion minus $C_3H_3N_2$. The i.r. spectrum showed a phenyl ester carbonyl absorption at 1760 cm^{-1}, and an imidazolide carbonyl absorption at 1730 cm^{-1}. No sign of an absorption at 1695 cm^{-1} corresponding to a free acid carbonyl stretch could be found. A ^{13}C n.m.r. spectrum on the product was also consistent with the formation of phenyl imidazolyl adipate (242).

Treatment of a solution of synthanecine A in dry DME with the imidazolide (242) at room temperature afforded the C-6 monoester (243) of synthanecine A selectively (see Scheme 48).



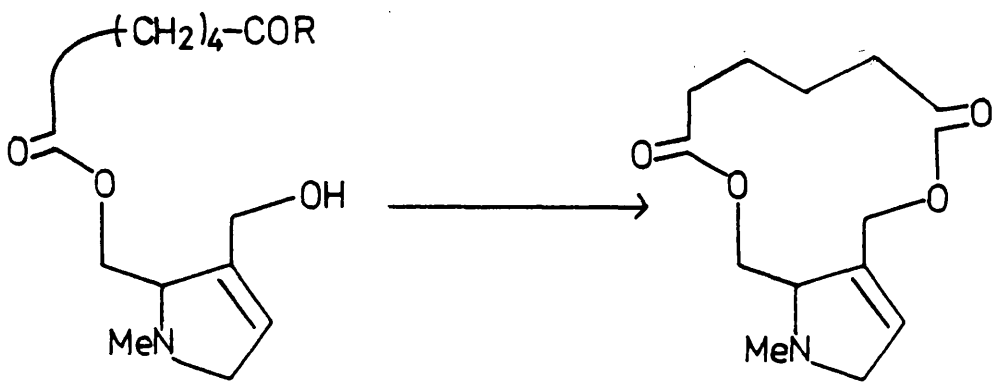
(243) $R^1 = CO+CH_2)_4-CO_2Ph$, $R^2 = H$

(244) $R^1 = R^2 = CO+CH_2)_4-CO_2Ph$

SCHEME 48

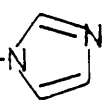
The presence of some unreacted synthanecine A and diester (244) of synthanecine A was indicated by t.l.c. The t.l.c. procedure used and method of detection was the same as that discussed in Chapter 3.4. Imidazole (240) and the unreacted synthanecine A were removed when a solution of the reaction mixture in CH_2Cl_2 was washed with water. The protected intermediate (243) was obtained in 49% yield after purification by column chromatography on basic alumina. The ^1H n.m.r. spectrum of the synthanecine A derivative (243) displayed a downfield shift of 0.4 p.p.m. for the C-6 protons and no change in the chemical shifts of the protons on C-7 when compared to the ^1H n.m.r. spectrum of synthanecine A. This indicated that the C-6 hydroxyl of synthanecine A had been selectively esterified. The signals corresponding to the methylene protons α to the carboxyl esterified with synthanecine A were shifted upfield by 0.53 p.p.m. relative to the imidazolide (242). An accurate mass measurement on the monoester (243) of synthanecine A gave a molecular ion of $C_{19}H_{25}NO_5$ and the mass spectral fragmentation pattern was typical for a molecule containing a synthanecine A moiety. An ion at $\underline{m}/\underline{z}$ 254 which corresponds to the molecular ion minus OPh showed that the product (243) also contained a phenyl ester. An i.r. spectrum of the compound (243) in chloroform showed two carbonyl absorptions. In addition to the phenyl ester carbonyl stretch at 1760 cm^{-1} there was another carbonyl stretch at 1740 cm^{-1} corresponding to the saturated ester attached to synthanecine A.

To complete this synthetic route the phenyl ester (243) had to be converted into the N-acylimidazolide (245), by the route illustrated in Scheme 43, followed by the intramolecular condensation of the allylic hydroxyl with this activated acid to give the cyclised product (226) (see Scheme 49).



(243) R = OPh

(226)

(245) R = 

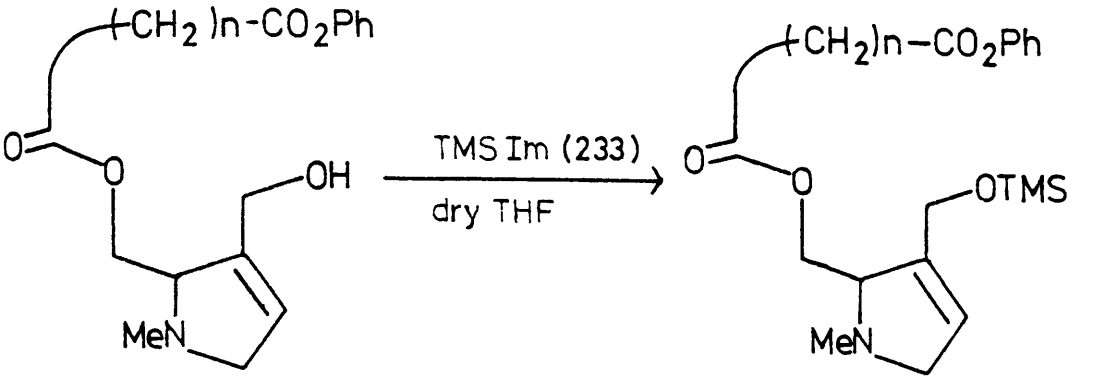
SCHEME 49

A solution of the synthanecine A derivative (243) in dry THF was treated with 1 equivalent of 1-(trimethylsilyl)imidazole (233) in the presence of a catalytic amount of sodium phenoxide. When a ^{1}H n.m.r. spectrum of the product from this reaction was initially analysed it appeared as if no reaction had occurred. However t.l.c. of the reaction mixture showed the presence of a number of pyrroline-containing compounds. After a closer examination of the ^{1}H n.m.r. spectrum a singlet at ca. 0.00 p.p.m.

suggested that the allylic hydroxyl might have reacted with the trimethylsilylimidazole to afford the trimethylsilyl-ether (246) (Scheme 50 shows possible products for the reaction discussed above, and the following series of reactions).

Treatment of the phenyl ester (243) with 2.2 equivalents of 1-(TMS)imidazole (233) and a catalytic amount of sodium phenoxide also yielded a complex product mixture (t.l.c.). A ^{1}H n.m.r. spectrum of the product from this reaction did not display any signals corresponding to methylene protons α to a phenyl ester carbonyl. A small signal at 8.1 p.p.m. indicated the presence of a very small quantity of an N-acylimidazolide, which may be compound (247). Although the phenyl ester (243) may have been transformed into an N-acylimidazolide, the lactonisation could not occur if the allylic hydroxyl was protected as the TMS ether. To permit the cyclisation, the TMS ether would have to be removed after the formation of the N-acylimidazolide (247).

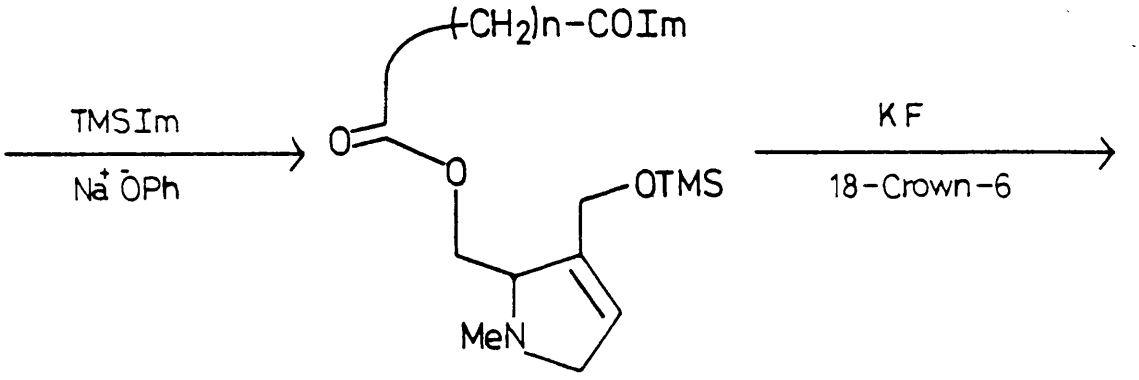
A convenient procedure for the cleavage of silyl ethers under anhydrous conditions was reported by Corey and Snider.[91] Fluoride ion, in the form of tetra n-butylammonium fluoride was used in this procedure making use of the strength of the Si-F bond. Potassium fluoride in the presence of 1,4,7,10,13,16-hexaoxocyclooctadecane (18-Crown-6) (248) has also been used as a source of 'naked' fluoride ion.[92] The crown ether (248) has the ability to complex metal salts and dissolve them in polar, non polar and aprotic solvents.



(243) n = 4

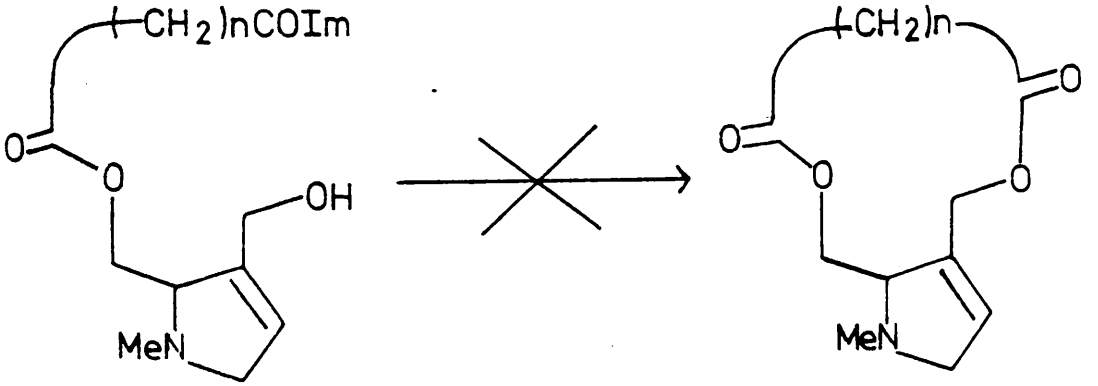
(251) n = 5

(246) n = 4



(247) n = 4

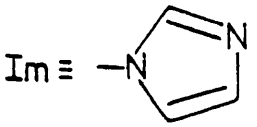
(252) n = 5



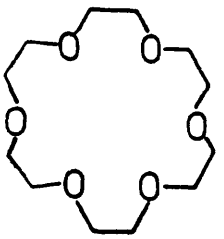
(245) n = 4

(226) n = 4

(227) n = 5



SCHEME 50



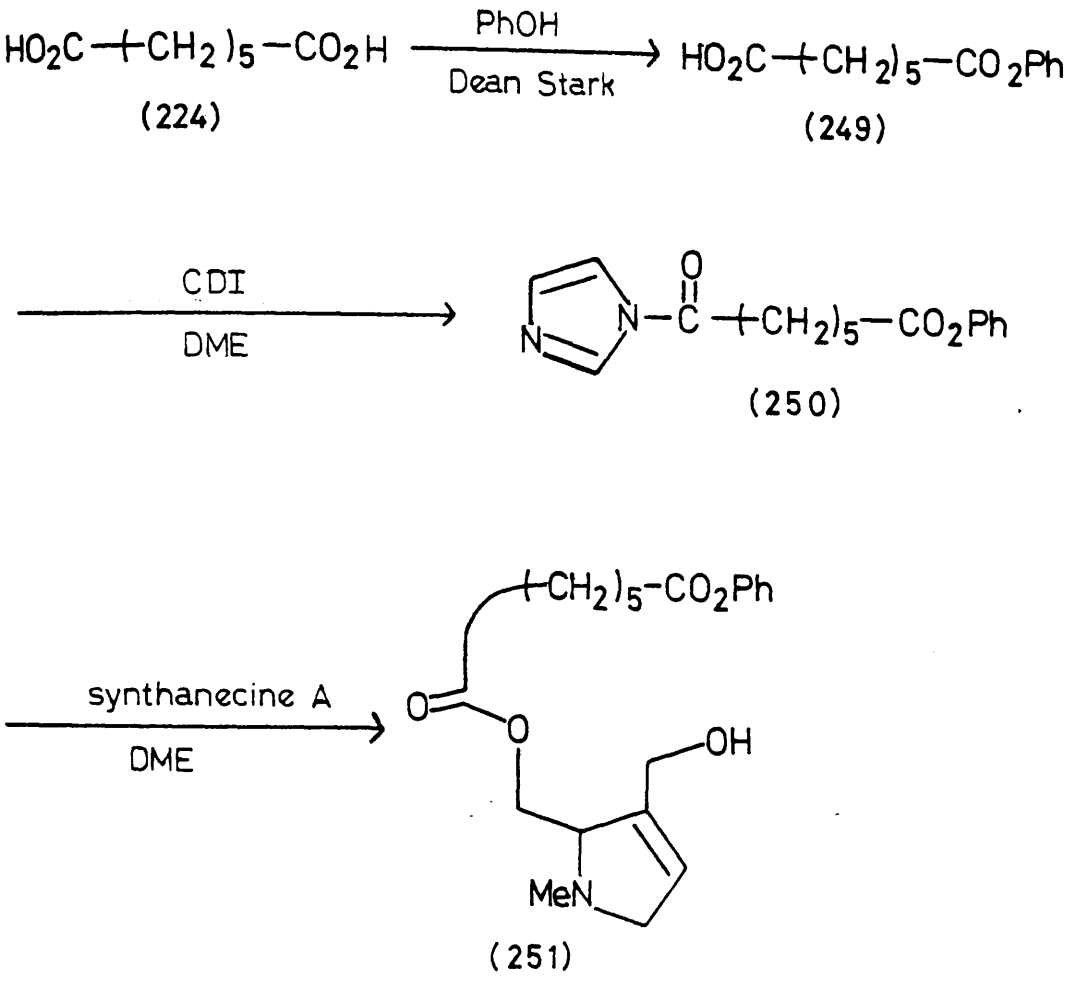
(248)

Cleavage of the TMS ether (247) by fluoride ion under anhydrous conditions may yield the alkoxide of the allylic hydroxyl (245). Spontaneous lactonisation of this alkoxide would afford the required macrocyclic diester (226) (see Scheme 50).

The phenyl ester (243) was reacted with 2.2 equivalents of TMS imidazole (233) and a catalytic quantity of sodium phenoxide at room temperature. After 2 h ca. 2 equivalents of anhydrous potassium fluoride and a catalytic amount of 18-Crown-6 (248) were added to the reaction mixture. The reaction mixture was stirred at room temperature for a further 5 h. A solution of the reaction mixture in the CH_2Cl_2 was washed with aqueous KCl to remove the unreacted KF, the 18-Crown-6 and imidazole. The only other by-product, phenol, was removed by washing the CH_2Cl_2 solution of the reaction mixture with 1M NaOH. A ^1H n.m.r. spectrum of the purified product displayed a downfield shift of 0.4 p.p.m. for the C-7 protons when compared to the ^1H n.m.r. spectrum of the starting material (243). The signal assigned to the

protons on C-7 appeared as a broad singlet and no chemical shift difference ($\Delta\delta$H-6) was observed for the C-6 protons. It may be that this larger macrocycle adopts a conformation in which the C-6 and C-7 diastereotopic protons are magnetically equivalent. Also, the signals at 2.58 p.p.m. in the ^{1}H n.m.r. spectrum of the starting material (243) which correspond to the methylene protons α to the phenyl ester carbonyl have shifted upfield to 2.30 p.p.m., as would be expected for the 12-membered cyclic diester (226). However, an accurate mass measurement of the isolated product gave molecular ions plus 1 and minus 1 from the expected value of m/z 253. T.l.c. of the reaction product showed a complex mixture of pyrroline-containing compounds and no crystalline derivatives could be prepared. One possible explanation for this result is that a mixture of oligomers has been formed.

This previous sequence of reactions was repeated except that pimelic acid (224) was used instead of adipic acid (223) (see Scheme 51). Monophenyl pimelate (249), phenyl-imidazolyl pimelate (250) and the synthanecine A derivative (251) were prepared and characterised as for the adipate homologues [(238), p.126], [(242), Scheme 47] and [(243), Scheme 49] respectively. Lactonisation of the phenyl pimelate derivative (251) was attempted using 1-(trimethylsilyl)imidazole (233) and a catalytic amount of sodium phenoxide as described previously for the adipate homologue. The phenyl ester (251) was treated with 2.2 equivalents of 1-(TMS)imidazole (233) and sodium phenoxide for 30 min. at



SCHEME 51

room temperature. A ^{1}H n.m.r. spectrum of the purified product of this reaction displayed 3 singlets with chemical shifts of 7.11, 7.48 and 8.15 p.p.m. which is characteristic of an N-acylimidazolide [Probably compound (252) in Scheme 50]. These three singlets of equal intensity were also of equal intensity to the signal corresponding to the C-4 olefinic proton. A broad triplet at 2.85 p.p.m. was assigned to the methylene protons α to the imidazolide carbonyl and a broad triplet of equal intensity was observed

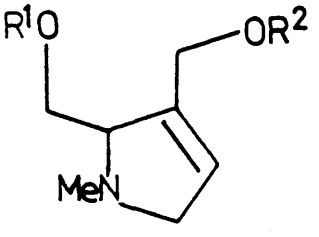
at 2.30 p.p.m. that corresponds to the methylene protons α to the carboxyl esterified with synthanecine A. The signals for the protons of the synthanecine A moiety remained unchanged relative to the starting phenyl ester (251). The product obtained above was dissolved in dry THF and treated with KF and 18-Crown-6 (248). The product mixture obtained was purified as described earlier (p.133). A ^{1}H n.m.r. spectrum of the mixture after purification, was analogous to that of the mixture of compounds obtained previously (p.134) for the adipate homologue. Again the signals corresponding to the C-7 protons were shifted downfield by 0.5 p.p.m. relative to the phenyl ester (251), and appeared as a broad singlet. The C-6 protons were also magnetically equivalent. The high resolution mass spectrum of the mixture displayed the correct accurate mass measurement but a crystalline derivative could not be prepared. T.l.c. of the product indicated the presence of a complex mixture of compounds. It may be that during the lactonisation step the reaction solution was too concentrated and intermolecular reactions occurred to yield a mixture of oligomers.

Accordingly, the synthanecine A derivative (231) was treated with TMS imidazole (233) and sodium phenoxide and then this product mixture was added dropwise to a solution of KF and 18-Crown-6 in THF at reflux. A complex mixture of products was still obtained. T.l.c. and spectroscopic data obtained were similar to those obtained above.

At this point it was decided that a fresh approach to the synthesis of dilactones (226) and (227) was necessary.

5.3 Synthesis of Macrocyclic Diesters With 12-Membered and Larger Rings

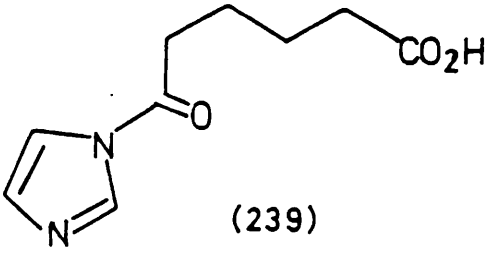
The Corey-Nicolaou lactonisation method has been successfully employed for the synthesis of 10- (193) and 11-membered (183 a) macrocyclic diesters of synthanecine A (see Chapter 3). Therefore, a possible route to the 12-membered dilactone (226) is by Corey-Nicolaou lactonisation of the 6- (253) or 7- (254) monoester hydroxy acids.



(253) $R^1 = CO(CH_2)_4-CO_2H$, $R^2 = H$

(254) $R^1 = H$, $R^2 = CO(CH_2)_4-CO_2H$

6-Monoesters, such as (253), are formed selectively when synthanecine A is esterified with an activated acid, since the C-6 hydroxyl is a stronger nucleophile than the primary allylic hydroxyl. Therefore, the 6-monoester (253) might be prepared by the selective monoesterification of synthanecine A using an N-acylimidazolide such as (239) (see p.125).

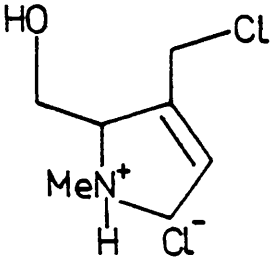


(239)

The N-acylimidazolide (239), produced by the reaction of adipic acid and 1 equivalent of CDI (50), was treated directly with a molar equivalent of synthanecine A at room temperature. A highly polar product was formed (t.l.c.). When lactonisation of this presumed monoester intermediate was attempted by the Corey-Nicolaou method none of the desired product (226) was isolated.

The work presented in Chapter 4 showed that the C-7 hydroxyl of synthanecine A can be readily esterified via the allylic chloride (200). It was considered that the chloromethyl derivative (200) might be used to prepare the desired 7-monoester (254) which could then be cyclised to give the required dilactone (226) by the Corey-Nicolaou procedure.

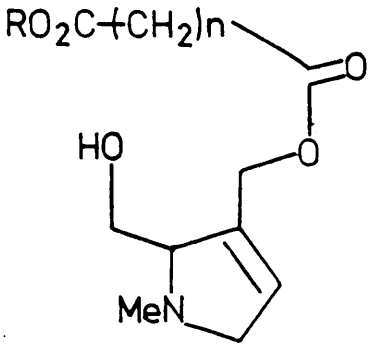
The allylic chloride (200) in dry DMF was treated with adipic acid and 2 equivalents of DBU (211) at room temperature. After 48 h a highly polar product was observed (t.l.c.). (This reaction was monitored by t.l.c. using the same solvent system and method of detection as reported in Chapter 3.4). This product was considered to be the required 7-monoester (254). The solvent (DMF) was removed under reduced pressure and dry DME was added to the reaction mixture since our successful lactonisations, by the Corey-Nicolaou method, were effected in this solvent. When 2.2 equivalents of 2,2'-dithiodipyridine (85) and triphenylphosphine (84) were added to the vigorously stirred reaction mixture the zwitterionic monoester (254) gradually dissolved, leaving a white solid. Formation of



DBU , DMF ,

$HO_2C(CH_2)n-CO_2H$

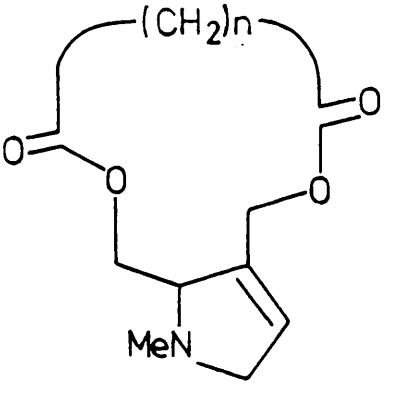
(223) n = 4
(224) n = 5
(256) n = 6
(257) n = 7
(258) n = 8



(254) n = 4 R = H
(255) n = 4 R = SPy
(259) n = 6 R = H
(260) n = 7 R = H
(261) n = 3 R = H

$(PyS)_2$, Ph_3P

DME



(226) n = 4
(227) n = 5
(262) n = 6
(263) n = 7
(264) n = 8

SCHEME 52

the thiol ester (255) was indicated by the presence of a distinctive u.v. active product of Rf ca. 0.3 (t.l.c.). The white solid, probably the hydrochloride salt of DBU, was allowed to settle out before the yellow solution of the thiol ester (255) was removed, by syringe, and added dropwise to DME heated at reflux. The formation of lactonised material was indicated by the appearance of a product of Rf 0.6 (t.l.c.). A pure product was obtained (12% yield) after the reaction mixture had been subjected to an acid base recycle followed by column chromatography on basic alumina. Again, the initial acid base recycle was required to remove the bulk of the oxide (186) and 2-pyridthione (185) by-products.

Correct microanalytical data was obtained for the picrolonate salt of the 12-membered macrocyclic diester (226). An accurate mass spectrum of the free base (226) showed a molecular ion at m/z 253 corresponding to $C_{13}H_{19}NO_4$. The mass spectral fragmentation pattern displayed main peaks at m/z 123, 111, 107, 94, 82 and 80 which is also consistent with the formation of the 12-membered dilactone (226). A characteristic carbonyl absorption at 1735 cm^{-1} was observed from the i.r. spectrum of the pure product (226). In the ^1H n.m.r. spectrum of the dilactone (226) the C-6 and C-7 diastereotopic protons appeared distinctly non-equivalent ($\Delta\delta H\text{-}6 = 0.76$ p.p.m. and $\Delta\delta H\text{-}7 = 0.72$ p.p.m.). The other signals in this ^1H n.m.r. spectrum were approximately identical to those observed in the ^1H n.m.r. spectrum of the adipate product prepared via an N-acylimidazolide (see previous section). The ^{13}C n.m.r. spectra of these products were also nearly identical.

The 13-membered macrocyclic diester (227) was prepared in a 16% yield from the allylic chloride (200) and pimelic acid (224) using the procedure described above. This dilactone (227) was characterised as the picrolonate salt and consistent chromatographic and spectroscopic data were obtained on the free base (227). The ^{1}H n.m.r. spectrum of the pimelate cyclic diester (227) showed that the C-6 ($\Delta\delta H = 0.47$ p.p.m.) and C-7 ($\Delta\delta H\text{-}7 = 0.16$ p.p.m.) diastereotopic protons are magnetically non equivalent. High resolution mass spectral data displayed a molecular ion corresponding to $C_{14}H_{21}NO_4$ and a fragmentation pattern indicative of a synthanecine A dilactone. The i.r. spectrum of compound (227) showed a saturated ester carbonyl at 1735 cm^{-1}.

It is now apparent that the two mixtures of products, obtained from adipic (223) and pimelic acids (224), discussed in the previous section were not the required dilactones [(226) and (227)] of synthanecine A. The spectroscopic and chromatographic data obtained for these mixtures suggest that they are oligomeric.

Novel pyrrolizidine alkaloid analogues with 14- (262), 15- (263) and 16-membered (264) macrocycles were prepared by treating the allylic chloride (200) with suberic (256), azelaic (257) and sebacic acids (258) respectively. The intermediate monoesters (259), (260) and (261) were lactonised by the Corey-Nicolàou procedure as described previously for the adipate derivative (226) (see Scheme 52). The dilactone (262) was characterised as its picrolonate

salt; however crystalline derivatives could not be prepared from the 15- (263) and 16-membered (264) dilactones. Correct accurate mass measurements were obtained for the picrolonate of compound (262) and for the free bases (263) and (264). The mass spectral fragmentation patterns exhibited by these macrocycles were indicative of macrocyclic diesters of synthanecine A (see Chapter 3.4). Saturated ester carbonyl absorptions at 1735 cm^{-1} were observed in the i.r. spectra of the 14- (262) 15- (263) and 16-membered (264) dilactones. The ^{1}H n.m.r. spectra of the 12- (226), 13- (227), 14- (262), 15- (263) and 16-membered (264) macrocyclic diesters of synthanecine A showed varying degrees of non equivalence for the C-6 and C-7 diastereotopic protons (see Table 7).

Synthanecine A dilactones		Δδ H-6 (p.p.m.)	Δδ H-7 (p.p.m.)	Yields (%)
adipate	(226)	0.76	0.72	12
pimelate	(227)	0.47	0.16	16
suberate	(262)	0.73	0.21	10
azelate	(263)	0.54	0.14	15
sebacate	(264)	0.30	0.08	15

TABLE 7.

The isolated yields of the macrocyclic diesters (226), (227), (262 - 264) are poor, but it should be noted that uncrystallised allylic chloride (200) was used in these

syntheses. Crystalline material was prepared later in this project. Also, due to time restrictions the optimum conditions for this route were not established.

Some areas for further research are:

1. The use of other solvents for both the esterification and lactonisation steps. e.g. Yamada and co-workers[93] used benzene for the esterification of carboxylic acids using DBU and alkyl halides. DMF was never used for the lactonisation step.

2. Use of other bases could be investigated.

3. Lactonisation of the monoester hydroxy acids by another method, e.g. the Keck[79] procedure, could be attempted.

5.4 Conclusions.

Using the Masamune procedure [1-(TMS) imidazole/NaOPh] to convert the phenyl ester (243) of 6-O-(adipyl) synthenecine A into its imidazolyl derivative (245), for subsequent lactonisation afforded a complex mixture of products. These products are probably oligomers, formed by the non specific attack of the nucleophilic reagents.

Synthanecine A dilactones with 12 - 16 membered macrocycles have been prepared. The initial esterification step was achieved by the carboxylate displacement of the C-7 allylic chloride of compound (200), and the lactonisation was effected by the Corey-Nicolaou procedure. Although only low yields of the macrocyclic diesters were obtained, it should be noted that the reaction conditions for this sequence have not been optimised.

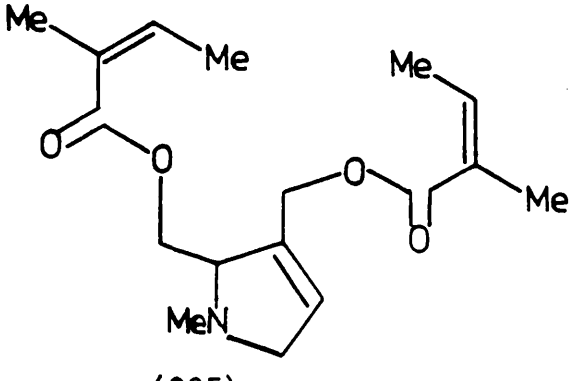
CHAPTER 6

BIOLOGICAL ACTIVITY OF MACROCYCLIC DIESTERS OF SYNTHANECINE A.

6.1 Introduction.

Pyrrolizidine alkaloids which are esters of unsaturated amino alcohols, such as retronecine (3), are hepatotoxic.[4] These alkaloids are transformed, in the liver, into reactive pyrrolic metabolites which are potent alkylating agents. Other organs, such as the lungs and kidneys, can be damaged if these pyrrolic derivatives are sufficiently stable to be transported in the bloodstream. Detoxification of hepatotoxic ester alkaloids occurs mainly by esterase hydrolysis or N-oxidation. Both processes form water soluble products which are readily excreted. For further details on the metabolism of pyrrolizidine alkaloids see Chapter 1.4.

Previous investigations, by Mattocks, have shown that diesters of synthanecine A, such as (265), are metabolised in a similar fashion and can exhibit an analogous toxic action.[94] However, they have relatively low toxicity as their structures are open to hydrolysis by esterase enzymes.



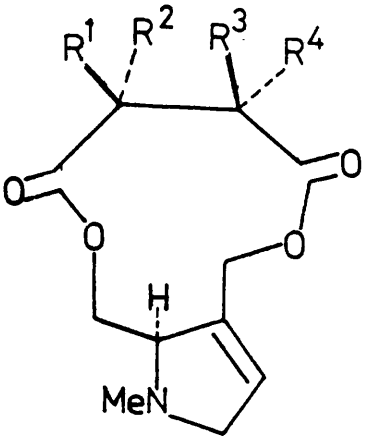
(265)

The aim of this work was to investigate the biological activity of a series of macrocyclic diesters of synthanecine A, shown in Figure 18, as they are analogues of the most toxic pyrrolizidine alkaloids. The experimental work for this Chapter was carried out at the M.R.C. Toxicology Unit, Carshalton by Dr A.R. Mattocks, Miss J. Brown, and myself, during my 3 month visit to this establishment. From the results obtained an attempt was made to relate the metabolism and likely toxicity of the pyrrolizidine alkaloid analogues to their structure.

6.2 Experimental Discussion.

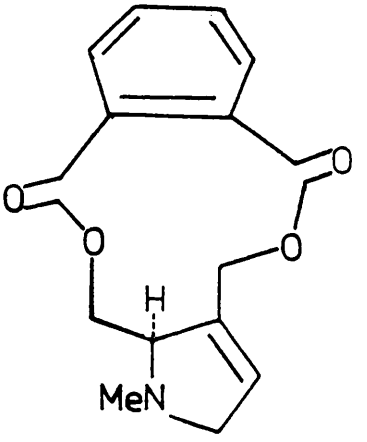
Similar doses of a series of synthanecine A dilactones (see Figure 18) were given, as single intraperitoneal injections, to rats, some of which had been pretreated with triorthocresyl phosphate (TOCP). TOCP is an esterase inhibitor. Two hours after dosing, the levels of pyrrolic metabolites in the livers and lungs of the rats were measured by the procedure reported by Mattocks and White.[95] (Previous work[96] had shown that the levels of pyrrolic metabolites in the livers of rats, 2 h after being dosed with synthanecine A diesters, had passed a transient peak and reached a comparatively steady level). The pyrrole measurements are shown in Table 8. Each result shown is an average value, obtained from a series of experiments differing only in the size of dose, in the range 20 - 150 mg/Kg (error limits are also shown). For comparison, pyrrole levels from monocrotaline (19) and ditiglyl synthanecine A (265) are also shown.

Figure 18

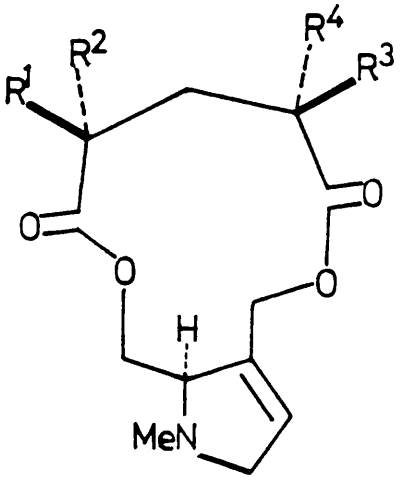


(193) R = R = H

(194) $R^1 = R^4 = Me$, $R^2 = R^3 = H$
$R^1 = R^4 = H$ $R^2 = R^3 = Me$



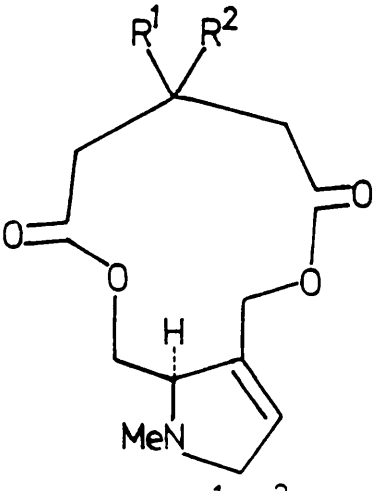
(196)



(183a) $R^1 = R^2 = R^3 = R^4 = H$

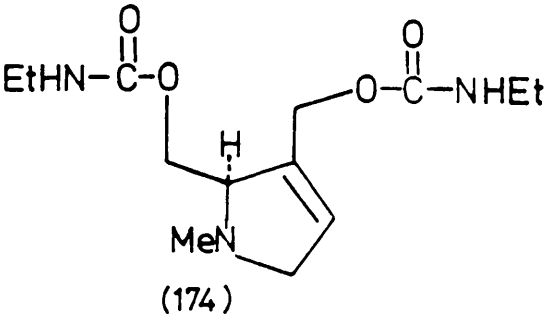
(189) $R^1 = R^2 = H$, $R^3 = R^4 = Me$

(190) $R^1 = R^3 = Me$, $R^2 = R^4 = H$



(183b) $R^1 = R^2 = Me$

(183d) $R^1, R^2 = (CH_2)_5$



(174)

(only one enantiomer shown in each case)

TABLE 8 PYRROLIC METABOLITE LEVELS IN LIVERS AND LUNGS

	Compound	TOCP predose		Pyrr. per 100 Liver
(193)	succinate	0	(12)[d]	0.07 ± 0.004
		+	(12)	0.55 ± 0.04
(194)	(±)-2,3-dimethyl-succinate	0	(12)	1.05 ± 0.05
		+	(12)	2.47 ± 0.11
(196)	phthalate	0	(12)	0.34 ± 0.03
		+	(12)	2.57 ± 0.25
(183a)	glutarate	0	(9)	0.16 ± 0.008
		+	(9)	2.68 ± 0.07
[(189)+(190)]	meso-2,4-dimethyl-glutarate	0	(18)	0.145± 0.014
		+	(12)	2.54 ± 0.11
(183b)	3,3-dimethyl-glutarate	0	(21)	2.07 ± 0.15
		+	(21)	1.77 ± 0.14
(183d)	3,3-pentamethylene-glutarate	0	(9)	1.22 ± 0.19
		+	(9)	1.48 ± 0.18
(19)	monocrotaline			0.64[e]
(265)	bistiglylsynthanecine A			0.0024[e]

a Expressed as absorbance of Ehrlich colour from 0.5g tissue

b (Liver pyrrole level after TOCP predosing)/(liver pyrrole

c The dose calculated to give the same 2h liver pyrrole level bis-N-ethylcarbamate (174).

d Number of results in parentheses.

e For reference.

OF RATS GIVEN SYNTHANECINE A DILACTONES

mg/kg ± SE[a] Lung	TOCP Enhancement[b]	Potential toxic dose[c] (mg/kg)
Negligible		770
(4) 0.27 ± 0.07	7.8	100
(4) 0.425±0.07		50
(4) 0.53 ± 0.15	2.4	22
(3) 0.04 ± 0.009		160
(4) 0.18 ± 0.055	7.6	21
(3) 0.07 ± 0.015		340
(3) 0.61 ± 0.05	16.8	20
Negligible		370
(4) 1.27 ± 0.07	17.5	22
(6) 1.32 ± 0.11		26
(6) 0.95 ± 0.19	0.9	31
(3) 0.29 ± 0.11		44
(3) 0.44 ± 0.14	1.2	36

samples.

without TOCP).

(0.54) as an LD_{50} dose (44 mg/kg) of synthanecine A

6.3 Results and Discussion.

The metabolic fates and potential hepatotoxicities of the synthanecine A dilactones shown in Figure 18, were revealed upon examination of the pyrrole measurements shown in Table 8. Furthermore, the susceptibilities of these dilactones to esterase hydrolysis were also assessed.

The levels of pyrrolic metabolites found in the livers of rats, which had not received TOCP, represent the metabolic fates of the compounds administered. Thus, the 3,3-dimethylglutarate (183 b) and 3,3-pentamethylene glutarate dilactones (183 d), which have large liver pyrrole measurements, are readily metabolised by hepatic microsomal oxidases to pyrrolic metabolites. Conversely, the succinate (193), glutarate (183 a) and 2,4-dimethyl-glutarate [(189) + (190)](mixture of diastereomers used) dilactones, which gave low levels of pyrrolic metabolites, appear to be metabolised primarily by the other available routes (i.e. hydrolysis or N-oxidation).

These measurements also give an indication of the potential hepatotoxicities of the compounds.[97] Therefore, the succinate (193) and glutarate dilactones (183 a) and [(189) + (190)] should have low toxicity whereas the 3,3-dimethylglutarate dilactone (183 b) should have exceptionally high toxicity; in fact on a par with the most toxic natural pyrrolizidine alkaloids, c.f. monocrotaline (19). Finally, from the results shown, macrocyclic diester analogues should be more hepatotoxic than simple diesters, c.f. compound (265).

Hydrolysis of the synthanecine A dilactones (Figure 18), by esterase enzymes, was also examined. The extent of esterase hydrolysis was determined from the increase in the level of pyrrolic metabolites produced by rats which had received TOCP (TOCP enhancement), the esterase inhibitor. [Enzymic hydrolysis limits pyrrole production because the hydrolysis products are less lipophilic and microsomal oxidation occurs more readily with lipophilic molecules. The hydrolysis products are also readily excreted.] From Table 8 it can be seen that large TOCP enhancements are observed for dilactones which gave low levels of pyrrolic metabolites when untreated rats were used, and vice versa. Therefore, esterase hydrolysis is an important detoxification route as it has a significant effect on the amounts of pyrrolic metabolites formed from many of these compounds.

TOCP enhancement values are a measure of the susceptibilities of these dilactones to esterase hydrolysis. Thus, dilactones (183 a) and [(189) + (190)] which display the largest TOCP enhancements are the most susceptible to hydrolysis in vivo. Dilactones (193), (196), (194) and (183 d) are progressively less vulnerable to enzymic hydrolysis and dilactone (183 b) appears to be resistant to the esterase action.

The succinate (193) and glutarate (183 a) dilactones are extremely susceptible to hydrolysis, as expected, since their ester groups are relatively unhindered. The 2,3-dimethyl succinate analogue (194) is less susceptible

to hydrolysis due to the presence of the methyl substituents α to the carbonyl groups of its necic acid. These substituents protect the dilactone either due to steric hindrance or because steric interactions restrict the dilactone from attaining a conformation that allows the esterase enzyme access to either of its ester groups. Surprisingly, the 2,4-dimethyl glutarate dilactone mixture [(189) + (190)] showed a significantly large TOCP enhancement. In fact this compound was no more protected from hydrolysis than the unsubstituted glutarate dilactone (183 a). In contrast to this, the 3,3-dimethyl glutarate dilactone (183 b) has a TOCP enhancement of 0.9. This shows that disubstitution β to the ester carbonyls renders this 11-membered dilactone resistant to esterase hydrolysis. Similarly, the 3,3-disubstituted glutarate dilactone (183 d) is virtually unaffected by the esterase action. This suggests that the geminal disubstitution of dilactones (183 b) and (183 d) limits the extent to which their esters are capable of being forced into contact with an esterase. In support of this, molecular models can be used to illustrate the considerable limitations that the gem-dimethyl substituents impose on the flexibility of the glutarate moiety. Finally, the fused aromatic ring of dilactone (196) does not appear to have much influence on the esterase activity.

As TOCP pretreatment eliminates the effects of esterase hydrolysis, the residual differences in the levels of pyrrolic metabolites must be due to the influence of other

metabolic processes, such as N-oxidation. To observe these differences more readily a potential toxic dose, relative to synthanecine A bis-N-ethylcarbamate (174), was calculated for each dilactone (see Table 8). [This is the dose required to give the same 2 h pyrrole level (1.23) as an LD_{50} dose (44 mg/Kg) of the dicarbamate (174)]. From these results it can be seen that dilactones (194), (196), (183 a) and [(189) + (190)] afford slightly higher levels of pyrrolic metabolites than dilactones (183 b) and (183 d), when esterase hydrolysis influences are eliminated. Therefore, the functional requirements for esterase resistance are not necessarily the same as those for pyrroline ring oxidation.

Pyrrolizidine alkaloids are not significantly converted into pyrrolic metabolites in lung tissue. As this is probably also true of synthanecine A dilactones, the metabolites found in the lungs of most rats were presumably formed in the liver. These metabolites must be sufficiently stable to be transported.

6.4 Toxicity Tests.

Investigations into the toxicity of the synthanecine A dilactones (Fig. A) have also been carried out at the MRC Toxicology Unit, Cashalton, by H.E. Driver. The results obtained, which will be presented elsewhere, confirm that, for this series of dilactones, hepatotoxicity is directly related to the liver levels of pyrrolic metabolites. Thus, the 3,3-dimethylglutarate dilactone (183 b) is highly hepatotoxic whereas the 2,4-dimethyl analogue mixture

[(189) + (190)] has low toxicity.

6.5 Hydrolysis of 11-membered Synthanecine A Dilactones under aqueous alkaline conditions.

6.5.1 Introduction.

Hydrolysis, by esterase enzymes, is an important detoxification route for synthanecine A dilactones, in vivo. Steric interactions, caused by acid branching, can have a significant bearing on the extent of the esterase activity i.e. 3,3-Disubstituted glutarate dilactones are virtually resistant to esterase hydrolysis whereas the 2,4-dimethyl [(189) + (190)] and glutarate (183 a) dilactones are readily hydrolysed (see Chapter 6.4). As this observation was unexpected we decided to study the effects of acid branching on the rates of hydrolysis of synthanecine A dilactones under aqueous methanolic alkaline conditions.

The rates of alkaline hydrolysis of esters can be determined using the procedure reported by Mattocks.[98] This method involves measuring the decrease in pH against time as the ester is hydrolysed. i.e. When an ester is hydrolysed by an equivalent of OH^-, the latter is expended and the pH falls. If excess ester is present, the disappearance of OH^- will approximate to first order kinetics, and

$$Kt \log e = p$$

t = time
p = change in pH

Therefore, a plot of pH change against time should be linear through the origin, and the reaction half time with

Rates of alkaline hydrolysis of synthanecine A dilactones (183a), (183b) and [(189)+(190)].

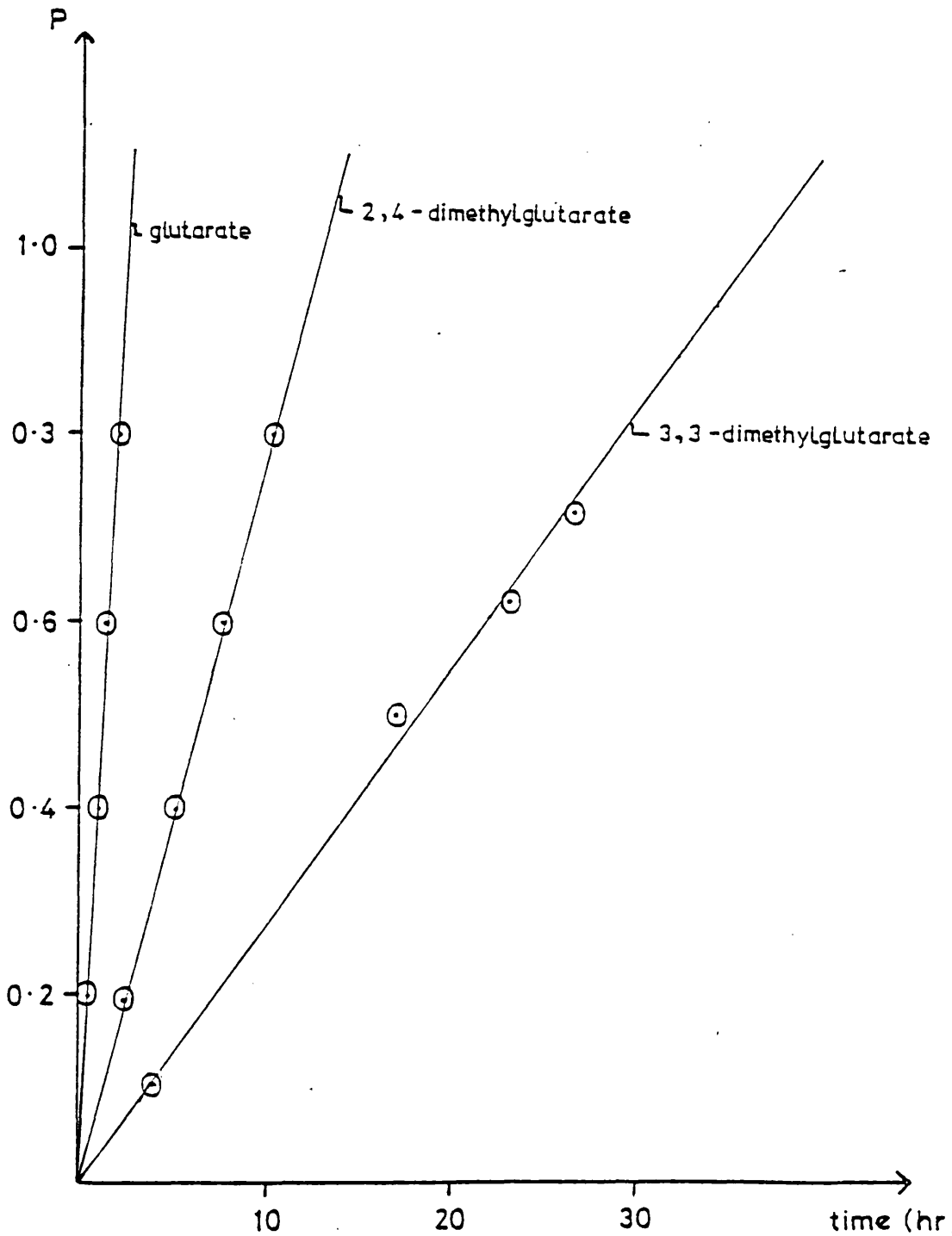


Figure 19

respect to OH^- is log 2/slope. When 1 equivalent of OH^- was used per diester the fall in pH was almost linear with time over at least 1 pH unit, and reaction half times could be calculated from curves representing a pseudo first-order disappearance of OH^-.

6.5.2 Results and Discussion.

The reaction half-times (see Table 9) for the alkaline hydrolysis of the synthanecine A dilactones (183 a), (183 b) and [(189) + (190)] (a mixture of diastereomers was used) were determined as described above. A plot of pH change against time is shown on Figure 19. As one equivalent of OH^- was used per dilactone, these plots represent the pseudo first-order disappearance of OH^-.

Table 9

Dilactone	$t_{\frac{1}{2}}$ (h)
(183 a) glutarate	0.8
[(189) + (190)] 2,4-dimethylglurate	4.9
(183 b) 3,3-dimethylglutarate	10.3

From the result, it can be seen that the dilactones (183 a), (183 B) and [(198) + (190)] are hydrolysed at different rates under the reported conditions. Again, the 3,3-di-substituted glutarate dilactone (183 b) appears to be less vulnerable to hydrolysis than its structural isomers [(189) + (190)]. This gives further support to our previous findings, that substitution β to the ester carbonyls of 11-membered

dilactones provides more protection against hydrolysis than α substitution. As expected, the glutarate dilactone (183 a) was the most susceptible to alkaline hydrolysis as its ester groups are relatively unhindered.

Attempts to repeat this analysis for the pentamethylene glutarate (183 d) and phthalate (196) dilactones were unsuccessful, as these dilactones were not soluble in the specified conditions.

6.6 Conclusions.

The metabolic fates of a series of 10- and 11-membered macrocyclic diesters of synthanecine A were determined. As expected, these alkaloid analogues were metabolised similarly to pyrrolizidine alkaloids. The levels of pyrrolic metabolites formed in the livers of rats varied considerably, and gave a good indication of the potential hepatotoxicity of the compound dosed. Detoxification of the synthanecine A dilactones occurred mainly by esterase hydrolysis, and *in vivo* and *in vitro* studies showed that acid branching had a significant influence on the esterase activity. 3,3-Disubstituted glutarate dilactones (183 b) and (183 d) were virtually resistant to esterase hydrolysis and therefore are good analogues of the most toxic pyrrolizidine alkaloids. Surprisingly, the 2,4-dimethyl glutarate mixture [(189) + (190)] was readily hydrolysed. This result is inconsistent with previous findings,[9] that dilactones of α substituted necic acids are less susceptible to esterase hydrolysis.

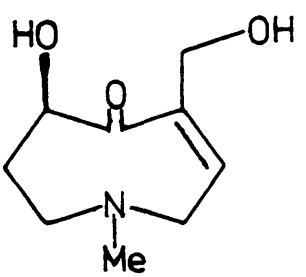
CHAPTER 7

EMILINE

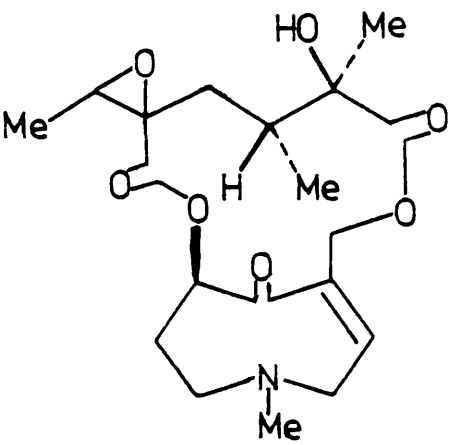
7.1 Introduction.

Esters of otonecine (266) constitute a group of unusual pyrrolizidine alkaloids because they do not contain the 1-azabicyclo[3,3,0]octane (pyrrolizidine) nucleus (1). About 23 have been discovered, all of which are macrocyclic diesters. The majority of these [20] are 12-membered [such as (267)] and are mainly found in plants belonging to the family Compositae. The rest [3] are 11-membered otonecine dilactones. Two of these were isolated from plants belonging to the family Leguminosae, whereas the third, emiline was isolated from Emilia flammea Cass., a plant also belonging to the family Compositae.

Emiline was first discovered in 1969,[99] and in 1970[100]



(266)



(267)

its structure (268) was assigned. This structure was formulated from spectroscopic (n.m.r., i.r., m.s.) and analytical data. When the ^1H n.m.r. spectrum used in this analysis was examined, it did not appear to be consistent with the reported structure. e.g. The singlets at δ1.54 and 2.08 p.p.m. appeared to be of equal intensity rather than 2:1, as reported. Consequently, we decided to reinvestigate the structure of emiline, using high resolution n.m.r. spectroscopy and modern n.m.r. spectroscopic techniques, such as distortionless enhancement by polarisation transfer (DEPT) and two-dimensional ^1H n.m.r. correlation spectroscopy (COSY), which are readily available today.

7.2 Results and Discussion.

Emilia flammea, grown at the Botanical Gardens in Glasgow, was extracted by a standard method (see Experimental). The crude alkaloidal mixture was composed of five compounds (t.l.c.). However, 90 and 200 MHz ^1H n.m.r. spectra of the mixture showed that it was primarily emiline. Upon trituration and crystallisation of the crude mixture, using hexane, a pure white solid was obtained. This product was spectroscopically identical to emiline. An accurate mass spectrum of the white solid displayed a molecular ion at m/z 365.1825, which is consistent with the proposed molecular formula of emiline, $C_{19}H_{27}NO_6$. The ^1H n.m.r. spectrum of this product (Figure 20) is identical to the published spectrum[100] and the white solid has a m.pt. of 103 - 105 °C (Lit. 105 - 107 °C).

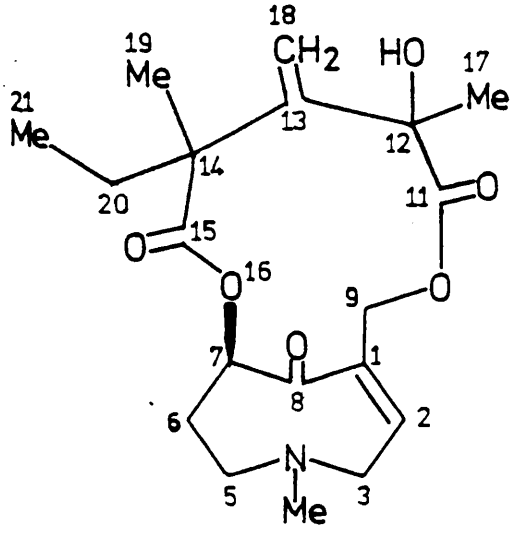
Further analysis of this product confirmed our belief, that emiline had been assigned the wrong structure. ^{13}C N.m.r. spectroscopy, involving a DEPT pulse sequence, was employed to determine the number of each type of carbon atom (CH_3, CH_2, etc.) in this alkaloid. The results obtained are shown in Table 10. This Table also includes the expected values for structure (268).

Table 10

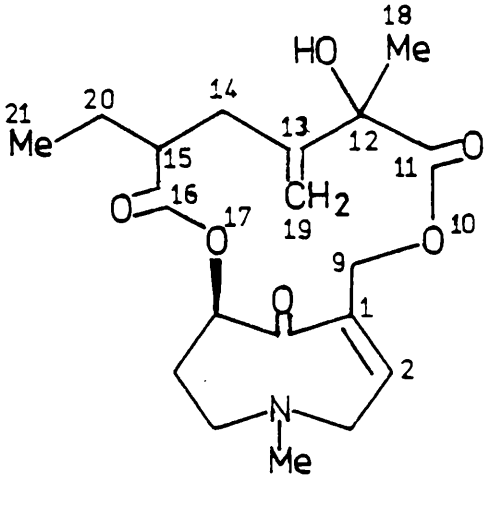
Carbon Type	Found	Expected
C	6	7
CH	3	2
CH_2	7	6
CH_3	3	4

As expected emiline has one less methyl group than initially reported.

A revised structure (269) is proposed for emiline.



(268)



(269)

This structure, which is consistent with the published data,[100] is based on the results obtained from an in depth spectroscopic analysis, involving ^{13}C DEPT n.m.r. spectroscopy, high resolution (200 MHz) ^{1}H n.m.r. spectroscopy and ^{1}H n.m.r. two-dimensional correlation spectroscopy (COSY), of our sample of emiline.

The ^{13}C (C=O at 191.5 p.p.m.) and ^{1}H (N-Me at 2.06 p.p.m.) n.m.r. spectroscopic data obtained are consistent with the presence of the otonecine moiety; therefore it was the necic acid moiety, of (268), that required the structural alteration. As mentioned previously, this structural reassignment should involve the loss of a methyl group and the introduction of a methylene group (see Table 10), which would increase the size of the macrocycle to 12. A simultaneous change in the number of quaternary and tertiary carbons, by -1 and +1 respectively, should follow.

Of the four methyl groups, in structure (268) only two, C-17 and C-19 were candidates for exclusion. Loss of the C-17 methyl would introduce a CHOH proton. As this type of proton is not observed in ^{1}H n.m.r. spectrum (see Figure 20), removal of the C-19 methyl was favoured.

The position for insertion of the methylene was chosen to accommodate the coupling pattern observed in the two-dimensional ^{1}H n.m.r. COSY spectrum (see Figures 21 and 22).

In a 2-D ^{1}H n.m.r. COSY spectrum the diagonal represents the one dimensional ^{1}H n.m.r. spectrum and the off-diagonal shaded areas, which are symmetrically distributed about the

diagonal, are indicative of coupling between protons. For example, the boxed area at ca. 3.3 p.p.m. is a result of coupling between the C-2 proton and both of the C-3 protons.

Those couplings (connectivities) associated with the necic acid are outlined in Figures 21 and 22 and are listed in Table 11. The following carbon numbers are from our proposed structure (269). Examination of the 2-D ^{1}H n.m.r. spectrum (Figure 21) showed that the olefinic (C-19) protons ($C=C\underline{H}_2$) are coupled to the C-18 methyl protons (A) (homo-allylic coupling) and two other proton sources (B and C), now known to be the 14-H_a and 14-H_b protons. Furthermore, it can be seen that the C-21 methyl protons are coupled to the neighbouring methylene protons (D) which in turn are coupled to another proton source (E), the chemical shift of which was determined by decoupling at 1.5 p.p.m. This proton source is the C-15 proton. Finally, upon examination of the expanded spectrum, (Figure 22), coupling between the C-15 proton and both of the C-14 protons is observed (F and G).

Table 11

A	19-H_2 ↔ 18-H_3	E	20-H_2 ↔ 15-H
B	19-H_2 ↔ 14-H_a	F	15-H ↔ 14-H_a
C	19-H_2 ↔ 14-H_b	G	15-H ↔ 14-H_b
D	21-H_3 ↔ 20-H_2		

As well as being consistent with the spectroscopic data presented, structure (269) is also more suited to the plant

origin of the alkaloid (i.e. otonecine alkaloids isolated from plants belonging to the family Compositae are generally 12-membered). Also, the substitution pattern of the necic acid of structure (269) is similar to that of known otonecine alkaloids, such as (267).

Confirmation of this structure and determination of the stereochemistry could be achieved from X-ray crystallographic data, but unfortunately a suitable crystal could not be grown.

7.3 Conclusions.

Detailed n.m.r. spectroscopic analysis of emiline, an alkaloid from Emilia flammea, showed that the published structure (268) is incorrect. On the basis of our spectroscopic data a revised structure (269) is proposed.

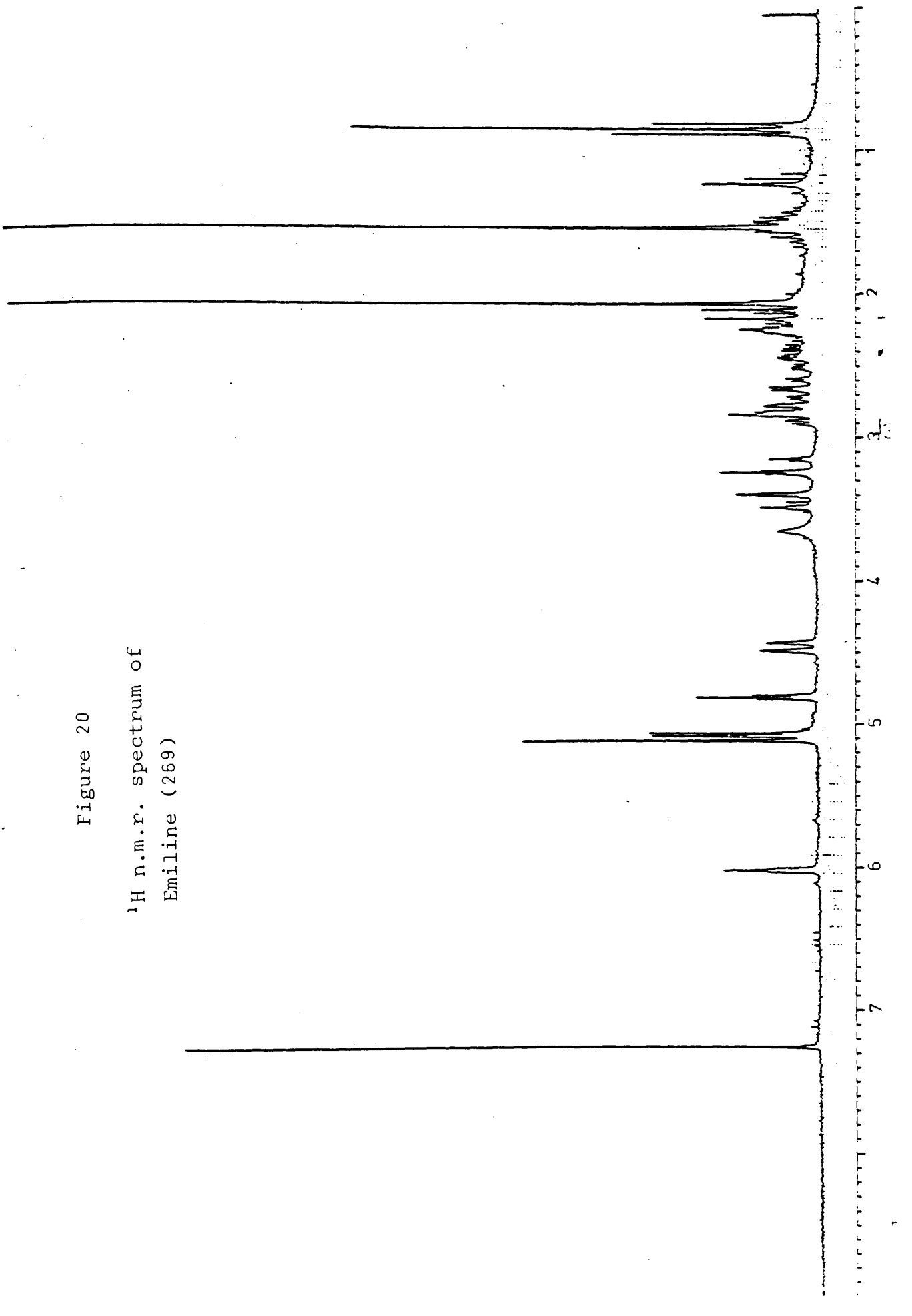


Figure 20

^{1}H n.m.r. spectrum of Emiline (269)

Figure 21

2-D ^{1}H n.m.r. correlation spectrum of Emiline (269)

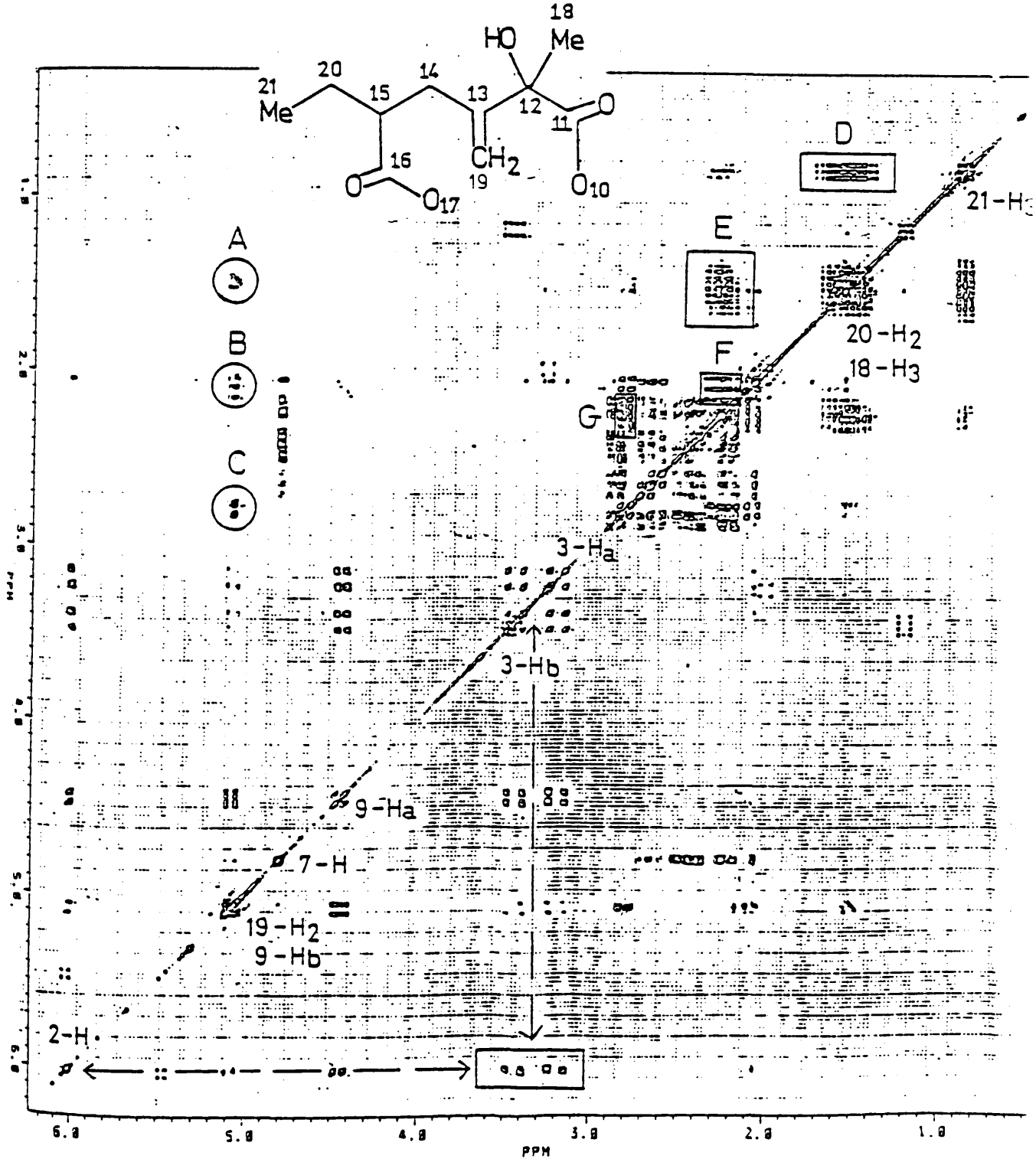
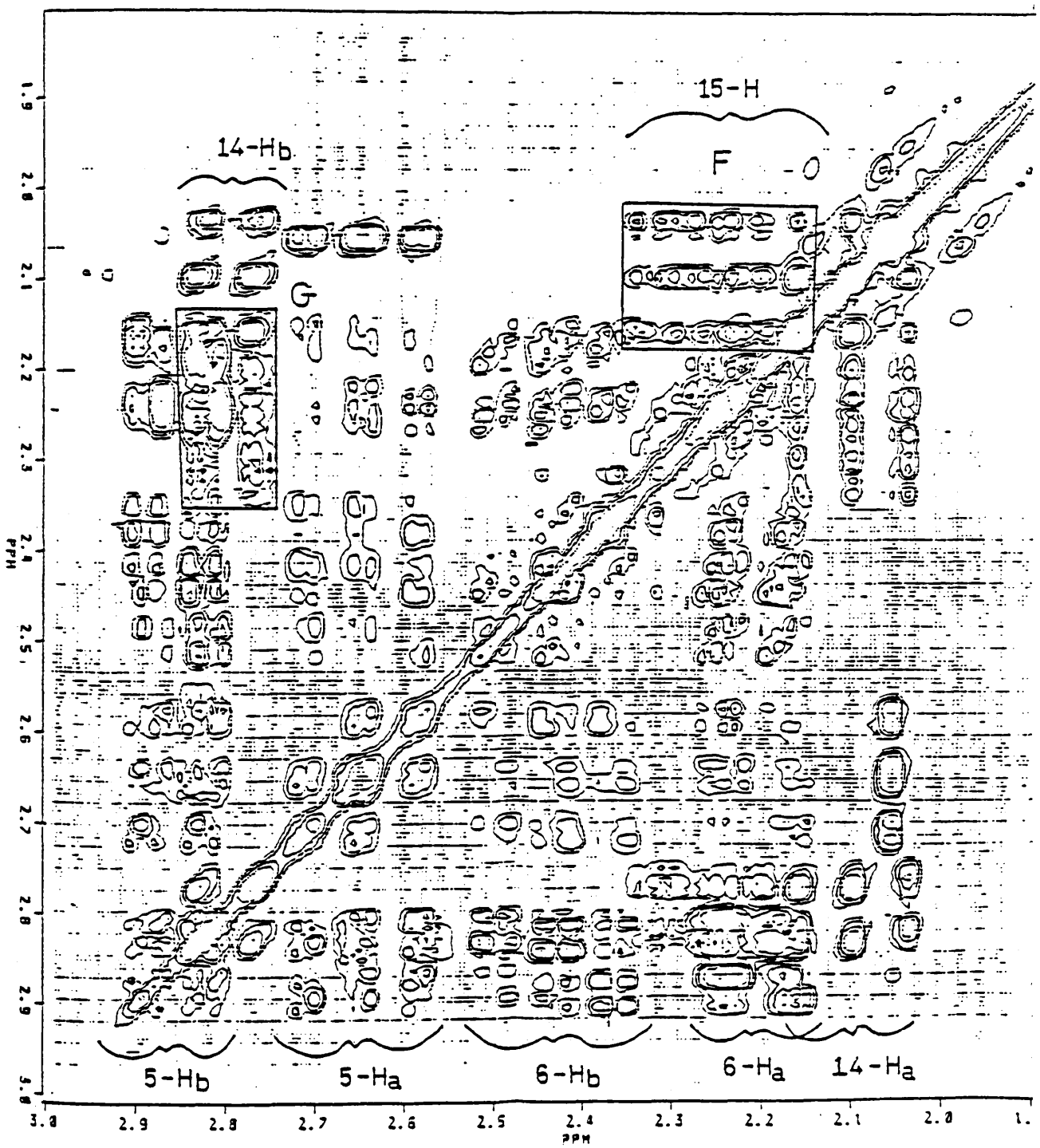


Figure 22

Expanded section of Figure 21



CHAPTER 8

EXPERIMENTAL.

8.1 General Notes.

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

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

1,2-Dimethoxyethane (DME) and tetrahydrofuran (THF) were dried by distillation from potassium hydroxide and then from sodium-benzophenone under argon prior to use. N,N-Dimethylformamide (DMF) was dried utilising 3A molecular sieves as detailed by Burfield and Smithers.[101] Organic solutions were dried with anhydrous magnesium sulphate and solvents were evaporated off under reduced pressure below 50 °C.

8.2 EXPERIMENTAL TO CHAPTER 3

8.2.1 Preparation of Synthanecine A (9)

Diethyl 2-methylaminosuccinate (164)[102] - Methylamine in ethanol (33%, 120 ml, 1.15 mol) was added, in portions at 0 - 5 °C, to diethyl maleate (172 g, 1.0 mol) with occasional stirring and ice-bath cooling (in order to maintain the exothermic reaction below 10 °C). After addition was complete, the flask was stoppered and kept in the ice-bath for a further 30 min, then allowed to stand at room temperature for 48 h. The ethanol was removed under reduced pressure and the colourless residue was dissolved in sufficient 4M hydrochloric acid to give an acidic solution. The acidic solution was washed with methylene chloride (3 x 500 ml), basified with conc. ammonia, and extracted with methylene chloride (3 x 500 ml). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give diethyl 2-methylaminosuccinate (164) as a colourless oil (184.8 g, 90%); $\nu_{max.}$($CHCl_3$) 3 350 (N-H), 2 980 (C-H), 2 810 (N-Me), 1 730 (C=O), and 1 180 cm^{-1} (C-O); δ_H 1.25 (3 H, t, J 8 Hz, $CO_2CH_2CH_3$), 1.27 (3 H, t, J 8 Hz, $CO_2CH_2CH_3$), 1.78 (1 H, s, NH), 2.40 (3 H, s, NMe), 2.60 (1 H, dd, J_{gem} 16, J_{vic} 7 Hz, 3-H), 2.75 (1 H, dd, J_{gem} 16, J_{vic} 5 Hz, 3-H), 3.57 (1 H, dd, J_{vic} 5, J_{vic} 7 Hz, 2-H), 4.04 - 4.33 p.p.m. (4 H, complex, 2 x CO_2CH_2). (These are J obs values)

Diethyl(N-ethoxycarbonylmethyl)-2-methylaminosuccinate (166) - Ethyl bromoacetate (34 ml, 0.31 mol) was added to a mixture of diethyl 2-methylaminosuccinate (60 g, 0.30 mol)

and anhydrous potassium carbonate (62 g, 0.45 mol) in aqueous acetone (7% H_2O) (220 ml). The mixture was heated at reflux for 24 h, cooled to room temperature, filtered and the filtrate concentrated in vacuo. The oily residue was dissolved in sufficient 2M hydrochloric acid to give an acidic solution. This acidic solution was washed with ether (3 × 50 ml), basified with conc. ammonia solution and extracted with ether (3 × 70 ml). The combined extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the title compound (166) (61 g, 70%); $\nu_{max.}$ ($CHCl_3$) 2 980 (C-H), 2 800 (NMe), 1 730 (C=O) and 1 180 cm^{-1} (C-O); δ_H 1.12 - 1.40 (9 H, complex 3 × $CO_2CH_2\underline{CH}_3$), 2.44 (3 H, s, NMe), 2.67 (1 H, dd, J_{gem} 16, J_{vic} 8 Hz, 3-H), 2.84 (1 H, dd, J_{gem} 16, J_{vic} 7 Hz, 3-H), 3.43 (2 H, s, $NC\underline{H}_2$), 3.88 (1 H, dd, J_{vic} 7, J_{vic} 8 Hz, 2-H), 4.04 - 4.34 p.p.m. (6 H, complex, 3 × $CO_2C\underline{H}_2$).

Diethyl 1-methyl-4-oxopyrrolidine-2,3-dicarboxylate (167) - The triester (166) (20 g, 70 mmol) in dry benzene (130 ml) was stirred with sodium hydride (oil free) (1.74 g, 73 mmol) at room temperature, under dry argon. After 5 h the reaction mixture was extracted with water (3 × 80 ml). The aqueous extracts were combined, washed with diethyl ether (2 × 200 ml) and acidified with concentrated hydrochloric acid, with ice cooling, to pH 1. The acidic solution was washed with ether (3 × 250 ml), and then the pH was adjusted to 6 with conc. ammonia solution. The aqueous solution was extracted with chloroform (3 × 300 ml) and the combined chloroform extracts were dried ($MgSO_4$), filtered,

and concentrated *in vacuo* to give the pyrrolidone (167) as an oil (15.8 g, 94%); $\nu_{max.}$($CHCl_3$) 2 980 (C-H), 2 800 (N-Me), 1 770 (C=O, ketone), 1 730 (C=O, ester), and 1 180 cm^{-1} (C-O); δ_H 1.23 (6 H, t, *J* 7.5 Hz, 2 × $CO_2CH_2C\underline{H}_3$), 2.50 (3 H, s, NMe), 2.95 (1 H, d, J_{gem} 18 Hz, 5-H), 3.56 (1 H, d, J_{gem} 18 Hz, 5-H), 3.58 (1 H, d, J_{vic} 8 Hz, 2-H), 3.83 (1 H, d, J_{vic} 8 Hz, 3-H) and 4.21 p.p.m. (4 H, m, 2 × $CO_2C\underline{H}_2$).

Diethyl 4-hydroxy-1-methylpyrrolidine-2,3-dicarboxylate (178) A solution of sodium borohydride (1.77 g, 20.5 mmol) in water (10 ml) was added to an ice-cold solution of the crude pyrrolidone (167) (10 g, 41 mmol) in aqueous sodium hydroxide (2%) (75 ml). The mixture was stirred at 0 °C for 1.5 h, and then carefully acidified with 2M hydrochloric acid. The acidic solution was washed with ether (2 × 100 ml), basified with conc. ammonia solution, and extracted with ether (3 × 120 ml). The combined extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give the title compound (178) as an oil (5.2 g, 52%); $\nu_{max.}$($CHCl_3$) 3 500 (O-H), 2 980 (C-H), 2 800 (NMe), 1 730 (C=O), 1 180 cm^{-1} (C-O); δ_H 1.16 - 1.40 (6 H, complex, 2 × $CO_2CH_2C\underline{H}_3$), 2.45 (3 H, s, NMe), 2.5 - 3.7 (6 H, complex, 2-H, 3-H, 4-H, 5-H_2 and OH), and 4.08 - 4.50 p.p.m. (4 H, complex, 2 × $CO_2C\underline{H}_2$).

Diethyl 1-methyl-3-pyrroline-2,3-dicarboxylate (168) - A solution of toluene-*p*-sulphonyl chloride (45 g, 2.4 mol) in pyridine (70 ml) was added to a solution of the hydroxy-pyrrolidine (178) (15 g, 0.6 mol) in pyridine (30 ml). The

mixture was heated on a steam-bath in a stoppered flask for 2 h, after which most of the solvent was removed under reduced pressure. The residue was dissolved in sufficient ice-cold dilute hydrochloric acid to give an acidic suspension. The acidic suspension was washed with ether (4 × 150 ml), basified with conc. ammonia solution, and extracted with ether (3 × 200 ml). The combined ether extracts were dried, filtered, and concentrated by rotary evaporation to give a dark red oil. To remove the pyridine residues, the oil was azeotroped with water (3 × 10 ml), and then benzene (3 × 15 ml). The product was redissolved in ether, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give the title compound (168) as a dark red oil (12.2 g, 88%); $\nu_{max.}$($CHCl_3$) 2 980 (C-H), 2 800 (NMe), 1 725 (C=O), 1 645 (C=C) and 1 180 cm^{-1} (C-O); δ_H 1.23 (6 H, t, *J* 8 Hz, 2 × $CO_2CH_2C\underline{H}_3$), 2.50 (3 H, s, NMe), 3.52 (1 H, br d, J_{gem} 17 Hz, 5-H), 4.00 (1 H, br d, J_{gem} 17 Hz, 5-H), 4.20 (1 H, s, 2-H), 4.05-4.35 (4 H, complex, 2 × $CO_2C\underline{H}_2$) and 6.85 p.p.m. (1 H, br s, 4-H).

2,3-Bishydroxymethyl-1-methyl-3-pyrroline (Synthanecine A) (9) A 1M solution of di-isobutylaluminium hydride (DIBAL) in toluene (137 ml, 0.13 mol) was added, with stirring and cooling to 20 - 25 °C over 30 min., to a solution of the diester (168) (5.1 g, 0.02 mol) in dry toluene (37 ml), under argon. The reaction mixture was kept at room temperature for 1 h. Ethyl acetate (9 ml) was added to consume the excess DIBAL, followed after 5 min by acetone (80 ml) and celite (16 g). Then methanol (16 ml) was added

very slowly with cooling to 30 - 35 °C. The mixture was shaken vigorously until gelling occurred (5 min), then water (200 ml) was added. The mixture was shaken again, to break up the gel, then stirred at room temperature for 1.5 h. The resulting suspension was filtered (water pump) and the solid residue was washed with hot water (2 × 50 ml) and hot methanol (2 × 50 ml). The combined filtrates were concentrated under reduced pressure, and then azeotroped with benzene (4 × 20 ml). Finally, a solution of the product in DME was dried ($MgSO_4$), filtered, and concentrated to give a brown oil (3.02 g, 94%) which contained the title compound (9) and the pyrrole derivative (179) in a ratio of 85:15.

Purification of the product mixture was attempted using a citrate-phosphate buffer as reported.[13] A solution of the product mixture (231 mg) in chloroform (5 ml) was extracted with the pH 4.5 buffer (3 × 5 ml) (The buffer solution was prepared from 61.5 ml of 0.1$\underline{\underline{M}}$ citric acid and 38.5 ml of 0.2$\underline{\underline{M}}$ sodium hydrogen phosphate). The combined buffer extracts were washed with chloroform (5 × 15 ml), basified with 1$\underline{\underline{M}}$ potassium hydroxide, and saturated with potassium carbonate. This alkaline solution was extracted with chloroform (5 × 20 ml) and the combined extracts were dried ($MgSO_4$). Filtration and concentration of the organic solution gave a brown oil (125 mg, 54%) which contained synthanecine A (9), but was still contaminated with the pyrrolic byproduct (179).

Synthanecine A (9) was successfully purified, by

column chromatography, as follows. The mixture (500 mg, 3.5 mmol) was chromatographed on silica gel (×40). Elution with chloroform - methanol - TEA (85:14:1) gave first the pyrrole diol (179); δ_H (90 MHz) 3.55 (2 H, br s, 2 × OH), 3.62 (3 H, s, NMe), 4.49 (2 H, s, 7-H_2), 4.56 (2 H, s, 6-H_2), 6.05 (1 H, d, J 3 Hz, 4-H) and 6.53 p.p.m. (1 H, d, J 3 Hz, 5-H). Further elution afforded pure synthanecine A (9) (280 mg, 56%); $\nu_{max.}$($CHCl_3$) 3 400 (O-H), 2 980 (C-H), 2 800 (NMe), and 1 650 cm^{-1} (C=C); δ_H (90 MHz) 2.50 (3 H, s, NMe), 3.25 (1 H, m, 5-H), 3.53 (1 H, m, 2-H), 3.67 (2 H, br d, J 3 Hz, 6-H_2), 3.88 (1 H, m, 5-H), 4.16 (2 H, br s, 7-H_2), 4.79 (2 H, br s, 2 × OH) and 5.73 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 40.9 (NMe), 59.2 (C-6), 61.0 (C-5 and -7), 73.8 (C-2), 124.2 (C-4) and 141.7 p.p.m. (C-3); m/z 143 (M^+), 112, 94, 82, 67, 53 and 42. The picrolonate of synthanecine A had m.p. 175 - 176 °C. (Lit.[12] 176 °C).

8.2.2 Synthesis of 11-Membered Macrocyclic Diesters of Synthanecine A.

1. General Procedure - Glutaric anhydride or a derivative (1 mmol) was added to a solution of (±)-synthanecine A (9) (1 mmol) in dry DME (20 ml) under argon. The reaction mixture was stirred at room temperature until all the synthanecine A had reacted to form a zwitterionic monoester (t.l.c., R_F 0.0, 12 - 18 h). 2,2'-Dithiodipyridine (85) (1.2 mmol) and triphenylphosphine (84) (1.2 mmol) were added and the mixture was stirred vigorously until thiol ester formation was complete (t.l.c., R_F ca. 0.3, 12 - 18 h).

The reaction mixture, a clear yellow solution, was diluted with DME (20 ml) and then heated at reflux under argon until lactonisation was complete (t.l.c., R_F 0.5 - 0.6, ca. 6 h). The solution was cooled and then concentrated under reduced pressure to afford a yellow oil; this oil was extracted with 1M citric acid (3 × 4 ml). The combined acid extracts were washed with chloroform (6 × 12 ml), then basified with conc. ammonia (pH > 10), and extracted with chloroform (4 × 15 ml). The chloroform extracts were dried, filtered, and concentrated to give crude cyclised products as yellow oils. Purification was achieved by column chromatography on basic alumina eluting with increasing proportions of chloroform in dichloromethane (starting with 10% chloroform).

(±)-6,7-O,O-(Glutaryl)synthanecine A (183 a) - By using glutaric anhydride (180 a) the glutarate dilactone (183 a) was obtained as an oil (21%), R_F 0.55; $\nu_{max.}$(CCl_4) 2 950, 2 850, 2 790, 1 745, 1 455, 1 275, 1 245 and 1 025 cm^{-1}; δ_H (200 MHz) 1.98 (2 H, m, 11-H_2), 2.33 (4 H, m, 10- and 12-H_2), 2.42 (3 H, s, NMe), 3.16 (1 H, m, 5-H), 3.45 (1 H, m, 2-H), 3.77 (1 H, m, 5-H), 4.05 (1 H, dd, J_{gem} 12, J_{vic} 5 Hz, 6-H), 4.12 (1 H, dd, J_{gem} 12, J_{vic} 3 Hz, 6-H), 4.63 (2 H, br s, 7-H_2) and 5.86 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 20.7 (C-11), 33.7 and 34.1 (C-10 and -12), 40.8 (NMe), 59.8 and 60.7 (C-5 and -6), 63.6 (C-7), 71.0 (C-2), 130.0 (C-4), 137.0 (C-3), and 172.3 p.p.m. (C-9 and -13); m/z 239 (M^+), 123, 107, 94, 82, 67, 53 and 42 (Found: M^+, 239.1154. $C_{12}H_{17}NO_4$ requires M, 239.1158). The picrolonate had m.p. 192 - 194 °C (decomp.), (from ethanol), (Found: C, 52.45;

H, 4.9; N, 13.6. $C_{22}H_{25}N_5O_9$ requires C, 52.48; H, 5.01; N, 13.91%).

(±)-6,7-O,O-(3,3-Dimethylglutaryl)synthanecine A (183 b) - The general procedure was repeated using 3,3-dimethylglutaric anhydride to give the title compound (183 b) (25% yield) as white prisms, m.p. 93 - 94 °C [from benzene-light petroleum (b.p. 60 - 80 °C)], R_F 0.6; $\nu_{max.}$ (CCl_4) 2 960, 2 950, 2 880, 2 790, 1 740, 1 330, 1 180, and 1 150 cm^{-1}; δ_H (90 MHz) 1.20 (6 H, s, 15- and 16-H_3), 2.21 and 2.25 (both 2 H, s, together 10- and 12-H_2), 2.48 (3 H, s, NMe), 3.22 (1 H, m, 5-H), 3.59 (1 H, m, 2-H), 3.84 (1 H, m, 5-H), 3.96 (1 H, dd, J_{gem} 11, J_{vic} 7 Hz, 6-H), 4.13 (1 H, dd, J_{gem} 11, J_{vic} 3 Hz, 6-H), 4.58 (1 H, d, J_{gem} 12 Hz, 7-H), 4.74 (1 H, d, J_{gem} 12 Hz, 7-H) and 5.87 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 30.1 (C-15 and -16), 34.0 (C-11), 41.4 (NMe), 44.2 and 44.3 (C-10 and -12), 60.9 and 61.1 (C-5 and -6), 65.4 (C-7), 71.3 (C-2), 129.9 (C-4), 137.0 (C-3), and 171.3 and 171.8 p.p.m. (C-9 and -13); m/z 267 (M^+), 153, 123, 107, 94, 80, 55, and 41 (Found: M^+, 267.1474; C, 62.9; H, 7.78; N, 5.2. $C_{14}H_{21}NO_4$ requires M, 267.1471; C, 62.90; H, 7.92; N, 5.24%). The picrolonate had m.p. 207 - 208 °C (decomp.) (from ethanol). (Found: C, 54.2; H, 5.4; N, 13.1. $C_{24}H_{29}N_5O_9$ requires C, 54.23; H, 5.50; N, 13.18%).

(±)-6,7-O,O-(3,3-Tetramethyleneglutaryl)synthanecine A (183 c) - The title compound (183 c) was afforded (26% yield) as white prisms when 3,3-tetramethyleneglutaric anhydride (180 c) and synthanecine A (9) were treated as described in

the general procedure; m.p. 93 - 96 °C [from benzene-light petroleum (b.p. 60 - 80 °C)]; R_F 0.6; $\nu_{max.}$($CHCl_3$) 2 950, 2 880, 2 790, 1 733, 1 460, 1 328, and 1 160 cm^{-1}; δ_H (360 MHz) 1.66 (8 H, m, 15-, 16-, 17- and 18-H_2), 2.31 (4 H, m, 10- and 12-H_2), 2.49 (3 H, s, NMe), 3.22 (1 H, m, 5-H), 3.55 (1 H, m, 2-H), 3.86 (1 H, m, 5-H), 4.03 (1 H, dd, J_{gem} 11, J_{vic} 7 Hz, 6-H), 4.10 (1 H, dd, J_{gem} 11, J_{vic} 3 Hz, 6-H), 4.62 (1 H, br d, J_{gem} 12 Hz, 7-H), 4.72 (1 H, br d, J_{gem} 12 Hz, 7-H), and 5.87 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 23.4 (C-16 and -17), 39.3 and 39.5 (C-15 and -18), 41.4 (NMe), 42.7 (C-10 and -12), 44.7 (C-11), 60.9 and 61.1 (C-5 and -6), 65.4 (C-7), 71.4 (C-2), 129.8 (C-4), 137.2 (C-3), and 171.8 and 172.2 p.p.m. (C-9 and -13); m/z 293 (M^+), 123, 107, 94, 82, 67, 53 and 42 (Found: M^+, 293.1633; C, 65.55; H, 8.0; N, 4.65. $C_{16}H_{23}NO_4$ requires M, 293.1627; C, 65.51; H, 7.90; N, 4.75%). The picrolonate had m.p. 217 - 220 °C (decomp.) (from ethanol) (Found: C, 56.1; H, 5.7; N, 12.6. $C_{26}H_{31}N_5O_9$ requires C, 56.01; H, 5.60; N, 12.56%).

(±)-6,7-O,O-(3,3-Pentamethyleneglutaryl)synthanecine A (183 d) - Treatment of 3,3-pentamethyleneglutaric anhydride as described in the general procedure afforded the title compound (183 d) (26%) as prisms; m.p. 101 - 102 °C [from benzene - light petroleum (b.p. 60 - 80 °C)]; R_F 0.6; $\nu_{max.}$($CHCl_3$) 2 930, 2 860, 2 790, 1 730, 1 455, 1 330, and 1 165 cm^{-1}; δ_H (90 MHz) 1.55 (10 H, m, 15-, 16-, 17-, 18- and 19-H_2), 2.28 (2 H, s, 10- or 12-H_2), 2.30 (2 H, s, 12- or 10-H_2), 2.46 (3 H, s, NMe), 3.20 (1 H, m, 5-H), 3.58

(1 H, m, 2-H), 3.85 (1 H, m, 5-H), 3.98 (1 H, dd, J_{gem} 13, J_{vic} 7 Hz, 6-H), 4.11 (1 H, dd, J_{gem} 13, J_{vic} 4 Hz, 6-H), 4.58 (1 H, d, J_{gem} 13 Hz, 7-H), 4.71 (1 H, d, J_{gem} 13 Hz, 7-H), and 5.86 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 21.6 (C-16 and -18), 25.8 (C-17), 36.7 (C-11), 37.4 and 37.6 (C-15 and -19), 41.4 (NMe), 41.8 (C-10 and -12), 60.9 and 61.1 (C-5 and -6), 65.5 (C-7), 71.2 (C-2), 129.8 (C-4), 137.0 (C-3), and 171.5 and 171.9 p.p.m. (C-9 and -13); m/z 307 (M^+), 167, 123, 108, 107, 94, 81, 67, 53 and 42 (Found: M^+, 307.1795; C, 66.6; H, 8.3; N, 4.6. $C_{17}H_{25}NO_4$ requires M, 307.1784; C, 66.42; H, 8.20; N, 4.57%). The picrolonate had m.p. 210 - 214 °C (decomp.) (from ethanol) (Found: C, 56.8; H, 5.75; N, 12.0. $C_{27}H_{33}N_5O_9$ requires C, 56.73; H, 5.82; N, 12.25%).

6,7-O,O-(3-Methylglutaryl)synthanecine A (183 e) - The title compound (183 e) was obtained (25%) as a mixture of two diastereomeric racemates, when 3-methylglutaric anhydride was treated with synthanecine A as described in the general procedure, R_F 0.6; $\nu_{max.}$ ($CHCl_3$) 2 970, 2 950, 2 880, 2 785, 1 740, 1 460, 1 379, 1 300, 1 257, and 1 185 cm^{-1}, δ_H (90 MHz) 0.98 and 1.05 (3 H, d, 15-H_3), 2.25 (5 H, m, 10-H_2, 11-H and 12-H_2), 2.50 (3 H, s, NMe), 3.14 (1 H, m, 5-H), 3.45 (1 H, m, 2-H), 3.80 (1 H, m, 5-H), 4.07 (2 H, m, 6-H_2), 4.62 (2 H, m, 7-H_2) and 5.85 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 22.6 (C-15), 28.6 and 29.0 (C-11), 40.8, 41.0, 41.2, 41.8 and 42.5 (C-10, C-12 and NMe), 59.6, 60.0 and 60.8 (C-5 and -6), 63.4 and 63.8 (C-7), 71.3 and 71.5 (C-2), 129.6 and 130.5 (C-4), 137.0 and 137.4

(C-3), and 171.7, 172.0 and 172.5 p.p.m. (C-9 and -13); m/z 253 (M^+), 123, 107, 94, 82, 69, 53 and 41 (Found: 253.1304. $C_{13}H_{19}NO_4$ requires M, 253.1314). The picrolonate of the mixture (183 e) had m.p. 172 - 174 °C (from ethanol) (Found: C, 53.4; H, 5.3; N, 13.4. $C_{23}H_{27}N_5O_9$ requires C, 53.38; H, 5.26; N, 13.54%).

6,7-O,O-(3-Hydroxy-3-methylglutaryl)synthanecine A (183 f) - 3-Hydroxy-3-methylglutaric anhydride (180 f) was treated as described above to give the title compound (183 f) (as a 1:1 mixture of two diastereomeric racemates) as an oil (22% yield), R_F 0.5; $\nu_{max.}$ (CCl_4) 3 530, 2 980, 2 950, 2 850, 2 790, 1 740, 1 725, 1 455, 1 375, 1 325, 1 260 and 1 165 cm^{-1}; δ_H (90 MHz) 1.39 (3 H, s, 15-H_3), 2.47 (3 H, s, NMe), 2.50 (2 H, s, 10- or 12-H_2), 2.56 (2 H, s, 12- or 10-H_2), 3.23 (1 H, m, 5-H), 3.58 (1 H, m, 2-H), 3.85 (1 H, m, 5-H), 3.92 (1 H, br s, OH), 4.37 (2 H, m, 6-H_2), 4.55 (1 H, d, J_{gem} 12 Hz, 7-H), 4.72 (1 H, d, J_{gem} 12 Hz, 7-H) and 4.91 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 29.5 (C-15), 41.2 (NMe), 45.5, 45.6 and 45.8 (C-10, -11, and -12), 60.7, 60.9 and 61.6 (C-5 and -6), 65.4 and 65.5 (C-7), 70.9 and 71.1 (C-2), 130.6 and 131.2 (C-4), 136.2 and 136.4 (C-3), and 171.1, 171.4 and 171.8 p.p.m. (C-9 and -13); m/z 269 (M^+), 107, 94, 82, 60, 51 and 43 (Found: M^+, 269.1261. $C_{13}H_{19}NO_5$ requires M, 269.1263).

6,7-O,O-(Meso-2,4-Dimethylglutaryl)synthanecine A [(189) + (190)] - A mixture of two diastereomeric racemates [(189) + (190)] (R_F 0.6 and 0.55 respectively) was obtained when

synthanecine A (9) was treated with meso-2,4-dimethylglutaric anhydride as described in the general procedure (30% yield). The picrolonate salt of this mixture had m.p. 192 - 196 °C (decomp.) (Found: C, 54.35; H, 5.3; N, 13.2. $C_{24}H_{29}N_5O_9$ requires C, 54.23; H, 5.50; N, 13.14%).

Pure samples of each diastereomeric racemate were obtained by preparative layer chromatography when 2 mm silica plates were eluted with chloroform - methanol - conc. ammonia (85:14:1).

(2R,10S,12R)- and (2S,10R,12S)-6,7-O,O-(Meso-2,4-dimethylglutaryl)synthanecine A (189) was obtained as an oil; R_F 0.6; $\nu_{max.}$ (CCl_4) 2 975, 2 940, 2 880, 2 790, 1 745, 1 463, 1 380 and 1 255 cm^{-1}; δ_H (90 MHz) 1.12 (6H, d, J_{vic} 7 Hz, 15- and 16-H_3), 1.16 - 2.50 (4 H, m, 10-H, 11-H_2, and 12-H), 2.48 (3 H, s, NMe), 3.24 (1 H, m, 5-H), 3.50 (1 H, m, 2-H), 3.82 (1 H, m, 5-H), 4.00 (1 H, dd, J_{gem} 12, J_{vic} 4 Hz, 6-H), 4.48 (1 H, dd, J_{gem} 12, J_{vic} 2 Hz, 6-H), 4.56 (1 H, d, J_{gem} 12 Hz, 7-H), 4.76 (1 H, d, J_{gem} 12 Hz, 7-H), and 5.94 p.p.m. (1 H, br s, 4-H); δ_C (50 MHz) 18.7 (C-15 and -16), 38.8 (C-10 or -12), 40.0 (C-11), 40.6 (NMe and C-12 or -10), 58.8 and 60.8 (C-5 and -6), 62.4 (C-7), 71.1 (C-2), 130.7 (C-4), 137.2 (C-3), and 175.3 and 176.8 p.p.m. (C-9 and -13); m/z 267 (M^+), 123, 107, 94, 82, 67, 55, and 42. (Found: M^+, 267.1471. $C_{14}H_{21}NO_4$ requires M, 267.1470).

(2R,10R,12S)- and (2S,10S,12R)-6,7-O,O-(Meso-2,4-dimethylglutaryl)synthanecine A (190) was obtained as prisms [from

benzene - light petroleum (b.p. 60 - 80 °C)], m.p. 53 °C; R_F 0.55; $\nu_{max.}(CCl_4)$ 2 980, 2 940, 2 880, 2 785, 1 750, 1 462, 1 258, 1 180, and 1 155 cm^{-1}; δ_H (90 MHz) 1.13 (3 H, d, J_{vic} 7 Hz, 15- or 16-H_3), 1.14 (3 H, d, J_{vic} 7 Hz, 16- or 15-H_3), 1.17 - 2.50 (4 H, m, 10-H, 11-H_2, and 12-H), 3.24 (1 H, m, 5-H), 3.52 (1 H, m, 2-H), 3.77 (1 H, m, 5-H), 3.90 (1 H, dd, J_{gem} 12, J_{vic} 6 Hz, 6-H), 4.41 (1 H, dd, J_{gem} 12, J_{vic} 6 Hz, 6-H), 4.53 (1 H, d, J_{gem} 14 Hz, 7-H), 4.93 (1 H, d, J_{gem} 14 Hz, 7-H), and 5.82 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz), 18.9 (C-15 and -16), 39.2 (C-10 or -12), 39.7 (C-11), 40.5 (C-12 or -10), 40.9 (NMe), 60.3 and 60.7 (C-5 and -6), 63.3 (C-7), 71.8 (C-2), 127.4 (C-4), 138.0 (C-3), and 175.5 and 176.1 p.p.m. (C-9 and -13); m/z 267 (M^+), 181, 123, 107, 94, 82, 56, and 42 (Found: M^+, 267.1470. $C_{14}H_{21}NO_4$ requires M, 267.1470).

Attempted Synthesis of (±)-6,7-O,O-(2,2,4,4-Tetramethylglutaryl)synthanecine A (191) - When synthanecine A (9) was treated with 2,2,4,4-tetramethylglutaric anhydride as described in the general procedure (p. 169) none of the desired cyclised product (191) was isolated.

2. Modifications of the General Procedure

A) Use of 2,2'-Dithiobis(4-tert-butyl-1-isopropylimidazole)[71] Synthanecine A (200 mg, 1.40 mmol) was treated with 3,3-dimethylglutaric anhydride (199 mg, 1.40 mmol) as described in the general procedure (p. 169), except that 2,2'-dithiobis-(4-t-butyl-1-isopropylimidazole)(140) was added instead of 2,2'-dithiodipyridine (85). The solution of the

intermediate thiol ester in DME was heated at reflux for 3 h only. The cyclised product obtained after the work-up (65 mg, 17%), was identical in all respect to (±)-6,7-O,O-(3,3-dimethylglutaryl)synthanecine A (183 b) obtained previously (p. 171).

B) Use of Silver Perchlorate[72] - Treatment of synthanecine A (190 mg, 1.33 mmol) with 3,3-dimethylglutaric anhydride (189 mg, 1.33 mmol) was carried out as described in the general procedure, except that the solution of the intermediate thiol ester (182 b) in DME was added dropwise, over 1 h, to a suspension of silver perchlorate (566 mg, 2.74 mmol) in DME heated at reflux under argon. During this addition the refluxing DME gradually discoloured until a brown gum was deposited. The resulting clear yellow solution was heated at reflux for a further 6 h, and it was then cooled and decanted from the brown deposit. Purification of the supernatant layer afforded a cyclised product (26 mg, 7%) that was spectroscopically and chromatographically identical to (±)-6,7-O,O-(3,3-dimethylglutaryl)synthanecine A (183 b).

3. Acid-Base Recycling of (±)-6,7-O,O-(3,3-dimethylglutaryl)synthanecine A (183 b) - A solution of the title compound (183 b) (55 mg) in dichloromethane (4 ml) was extracted with 1M citric acid (3 × 3 ml). The combined acidic extracts were basified with conc. ammonia, added to the initial solution of dichloromethane, and extracted further (4 × 12 ml) with the same solvent. The combined

organic extracts were dried ($MgSO_4$), filtered and concentrated to give the title compound (183 b) (22 mg, 40%).

When this procedure was repeated, keeping all apparatus and solutions as cool as possible, (±)-6,7-O,O-(3,3-dimethylglutaryl)synthanecine A was recovered in 51% yield.

8.2.3 Synthesis of 10-Membered Macrocyclic Diesters of Synthanecine A.

General Procedure. - The general procedure for the preparation of 11-membered macrocyclic diesters of synthanecine A, described on p. 169, was repeated using succinic anhydride and derivatives.

(±)-6,7-O,O-(Succinyl)synthanecine A (193) - By use of succinic anhydride, the succinate dilactone (193) was obtained as white needles (30%), m.p. 106 - 108 °C [from benzene - light petroleum (b.p. 60 - 80 °C)]; R_F 0.67; $\nu_{max.}$ ($CHCl_3$) 2 950, 2 795, 1 735, 1 600, 1 575, 1 560, 1 419, 1 165, and 1 040 cm^{-1}; δ_H (200 MHz) 2.50 (3 H, s, NMe), 2.41 - 2.72 (4 H, m, 10-H_2 and 11-H_2), 3.22 (1 H, m, 5-H), 3.59 (1 H, m, 2-H), 3.85 (1 H, m, 5-H), 3.98 (1 H, dd, J_{gem} 12, J_{vic} 5 Hz, 6-H), 4.40 (1 H, dd, J_{gem} 12, J_{vic} 2 Hz, 6-H), 4.40 (1 H, d, J_{gem} 13 Hz, 7-H), 5.08 (1 H, d, J_{gem} 13 Hz, 7-H) and 5.94 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 33.2 (C-10 and C-11), 41.4 (NMe), 60.9 and 61.6 (C-5 and -6), 65.7 (C-7), 71.1 (C-2), 131.0 (C-4), 136.1 (C-3), 171.9 and 172.8 p.p.m. (C-9 and -12); m/z 225 (M^+), 123, 111, 108, 107, 94, 82 and 80. (Found: M^+ 225.1005; C, 58.8; H, 6.9; N, 5.9. $C_{11}H_{15}NO_4$ requires M, 225.1001; C, 58.65; H, 6.71; N, 6.22%).

6,7-O,O-(Trans-2,3-dimethylsuccinyl)synthanecine A (194) - Treatment of (±)-trans-2,3-dimethylsuccinic anhydride as described in the general procedure gave the title compound (194) (32%) as a mixture of the two diastereomeric racemates, R_F 0.67; $\nu_{max.}$($CHCl_3$) 2 980, 2 945, 2 790, 1 735, 1 452, 1 335, 1 185, and 1 096 cm^{-1}; δ_H (360 MHz) 1.15 - 1.22 (6 H, m, 14- and 15-H_3), 2.34 - 2.53 (2 H, m, 10- and 11-H), 2.48 and 2.50 (3 H, s, NMe), 3.22 (1 H, m, 5-H), 3.62 (1 H, m, 2-H), 3.88 (1 H, m, 5-H), 3.89 - 3.99 (1 H, m, 6-H), 4.16 - 4.30 (1 H, m, 7-H), 4.52 - 4.57 (1 H, m, 6-H), 5.11 and 5.21 (1 H, d, J ca. 13 Hz, 7-H), 5.86 and 5.93 p.p.m. (1 H, br s, 4-H); m/z 253 (M^+), 111, 108, 107, 94, 82, 69, and 55. (Found: M^+, 253.1313. $C_{13}H_{19}NO_4$ requires M, 253.1313).

6,7-O,O-(Trans-cyclohexane-1,2-dicarboxyl)synthanecine A (195) - The title compound (195) was prepared, as a mixture of two diastereomeric racemates, when synthanecine A was treated with (±)-trans-cyclohexane-1,2-dicarboxylic anhydride in a similar fashion (23%), R_F 0.75; $\nu_{max.}$($CHCl_3$) 2 945, 2 865, 2 790, 1 733, 1 578, 1 450, 1 420, 1 328, 1 255, 1 183 and 1 120 cm^{-1}; δ_H (200 MHz) 1.07 - 1.86 (8 H, m, 14-, 15-, 16- and 17-H_2), 2.23 - 2.50 (2 H, m, 10- and 11-H), 2.47 and 2.49 (3 H, s, NMe), 3.21 (1 H, m, 5-H), 3.58 (1 H, m, 2-H), 3.60 - 3.91 (1 H, m, 6-H), 3.87 (1 H, m, 5-H), 4.08 - 4.78 (2 H, m, 6- and 7-H), 5.20 - 5.52 (1 H, m, 7-H) and 5.85 and 5.92 p.p.m. (1 H, br s, 4-H); m/z 279 (M^+) 123, 111, 108, 107, 94, 82 and 67 (Found: M^+, 279.1465. $C_{15}H_{21}NO_4$ requires M, 279.1471).

(±)-6,7-O,O-(Phthalyl)synthanecine A (196) - When synthanecine A (9) was treated with phthalic anhydride by the standard method, the title compound (196) was afforded (16%) as white prisms, m.p. 153 - 155 °C [benzene - light petroleum (b.p. 60 - 80 °C)]; R_F 0.72; $\nu_{max.}$ ($CHCl_3$) 2 950, 2 785, 1720, 1 602, 1 450, 1 283, 1 130, and 1 035 cm^{-1}; δ_H (200 MHz) 2.55 (3 H, s, NMe), 3.26 (1 H, m, 5-H), 3.80 (1 H, m, 2-H), 3.92 (1 H, m, 5-H), 4.30 (1 H, dd, J_{gem} 12, J_{vic} 7 Hz, 6-H), 4.66 (1 H, dd, J_{gem} 12, J_{vic} 4 Hz, 6-H), 4.80 (1 H, d, J_{gem} 12 Hz, 7-H), 5.28 (1 H, d, J_{gem} 12 Hz, 7-H), 6.00 (1 H, br s, 4-H) and 7.50 - 7.85 (4 H, m, 14-, 15-, 16- and 17-H); δ_C 41.6 (NMe), 60.5 and 61.9 (C-5 and -6), 65.4 (C-7), 71.3 (C-2), 129.3, 129.5, 131.7, and 131.8 (C-14, -15, -16, and -17), 130.6 (C-4), 132.1 and 132.4 (C-10 and -11), 136.8 (C-3), 168.1 and 168.6 p.p.m. (C-9 and -13); m/z 273 (M^+), 197, 124, 110, 94, 82, and 42 (Found: M^+, 273.1004; C, 66.1; H, 5.7; N, 5.1. $C_{15}H_{15}NO_4$ requires M, 273.1001; C, 65.92; H, 5.52; N, 5.13%).

Attempted Synthesis of (±)-6,7-O,O-(Maleyl)synthanecine A (214) - Maleic anhydride (1 mmol) was added to a stirred solution of synthanecine A (9) (1 mmol) in dry DME (20 ml) under argon; after a few seconds a white gum was deposited. When 2,2'-dithiodipyridine (1.2 mmol) and triphenylphosphine (1.2 mmol) were added to the reaction mixture the solution gradually turned dark red and no thiol ester formation was observed.

Attempted synthesis of (±)-6,7-O,O-(4,5-dichlorophthalyl) synthanecine A (218) - When synthanecine A (9) was treated with 4,5-dichlorophthalic anhydride as described in the general procedure, thiol ester formation appeared to occur (t.l.c.). However, none of the chlorinated phthalate derivative (218) was isolated after the reaction mixture was heated at reflux for 8 h and then worked-up by the usual procedure.

8.3 EXPERIMENTAL TO CHAPTER 4

8.3.1 Synthesis of (±)-3-Chloromethyl-2-hydroxymethyl-3-pyrrolinium chloride (200)

Method (a) - Thionyl chloride (2 ml, 2.8 mmol), cooled to 0 °C, was added to synthanecine A (9) (0.5 g, 3.5 mmol) at -5 °C. The mixture was stirred, to facilitate dissolution of the synthanecine A, for 45 min at 0 °C after which the excess thionyl chloride was removed under reduced pressure (0 °C, ca. 2 mm Hg). The resulting purple residue was dissolved in ethanol and treated with activated charcoal until the purple colouration was removed (ca. ×2). The solution was then filtered through celite, to remove the charcoal, and concentrated in vacuo to give a pale brown oil (0.59 g, 85%). Crystallisation using ethanol/acetone afforded the title compound (200) (0.38 g, 55%) as white prisms, m. pt. 135 °C; R_F 0.28; $\nu_{max.}$(KBr) 3 340 (O-H), 2 920 (C-H), 2 640 (R_3N^+-H), 1 090 (C-O), 835 ($R_2C=C(R)$-H) and 695 cm^{-1} (C-Cl); $\delta_H(D_2O)$ (90 MHz) 3.12 (3 H, s, NMe), 3.85 - 4.65 (3 H, complex 5-H_2 and 2-H), 4.09 (2 H, br s, 6-H_2), 4.35 (2 H, br s, 7-H_2), and 6.07 p.p.m. (1 H, br s, 4-H); $\delta_C(D_2O)$ (25 MHz) 41.0 (C-7), 44.7 (NMe), 59.9 and 64.0 (C-5 and -6), 78.4 (C-2), 127.5 (C-4) and 136.8 p.p.m. (C-3), m/z 132 and 130 (M^+-CH_3OH), 112, 94, 81, 67 and 53. (Found: C, 42.3; H, 6.6; N, 7.0. $C_7H_{13}NOCl_2$ requires C, 42.44,; H, 6.62; N, 7.07%).

Method (b) - Thionyl chloride (20 ml, 28 mmol) was added to a solution of synthanecine A (1.0 g, 7.0 mmol) in dry DME (10 ml), under argon, at -5 °C and the mixture was treated as described in Method (a). The product (0.8 g,

58%), obtained as white prisms, was identical in all respects to the foregoing hydrochloride salt (200).

8.3.2 Synthesis of Synthanecine A Dilactones

General Procedure - The anhydride (1 mmol) and base (2.1 mmol) were added to a solution of (±)-3-chloromethyl-2-hydroxymethyl-1-methyl-3-pyrrolinium chloride (200) (1 mmol) in dry DMF (18 ml) under argon, and the mixture was stirred at room temperature, generally for 24 h. The solvent (DMF) was removed in vacuo (ca. 40 °C, 3 mm Hg) to give the crude product as an oily or crystalline residue, which was purified by column chromatography on basic alumina (5% v/v chloroform in dichloromethane as eluent). The polarity of the eluant was steadily increased by increasing the proportion of chloroform.

Attempted synthesis of (±)-6,7-O,O-(3,3-Dimethylglutaryl)-synthanecine A (183 b) - When 3,3-dimethylglutaric anhydride (180 b) was treated with synthanecine A (9), as described in the above general procedure, using Hunig's base as base, none of the desired cyclised product was observed (t.l.c.).

(±)-6,7-O,O-(3,3-Dimethylglutaryl)synthanecine A - When the reaction above was repeated using DBU (211) instead of Hunig's base (210) the white crystalline product afforded (53%) was identical in all respects to the title compound (183 b), prepared previously (p. 171).

(±)-6,7-O,O-(3,3-Dimethylglutaryl)synthanecine A (183 b) - The above reaction was repeated, using DBN (212) instead of DBU (211). The pure product (37%) was chromatographically and spectroscopically identical to the foregoing dilactone (183 b).

(±)-6,7-O,O-(Glutaryl)synthanecine A (183 a) - When glutaric anhydride (180 a) was used in the presence of DBU (211) the glutarate dilactone (183 a) was afforded in 37% yield. This product was spectroscopically and chromatographically identical with the product obtained by the Corey-Nicolaou method (p. 170).

(±)-6,7-O,O-(3,3-Pentamethyleneglutaryl)synthanecine A (183 d) - Using 3,3-pentamethyleneglutaric anhydride (180 d) in the presence of DBU, the title compound (183 d) was obtained in 66% yield. This product was identical in all respects to the product prepared by the Corey-Nicolaou method (p. 172).

6,7-O,O-(3-Hydroxy-3-methylglutaryl)synthanecine A (183 f) - When 3-hydroxy-3-methylglutaric anhydride (183 f) was treated as described in the general procedure, using DBU (211) as base, the title compound (183 f) was obtained (52%) as a mixture of two diastereomeric racemates. This product was spectroscopically and chromatographically identical to the dilactone (183 f) obtained previously (p. 174).

6,7-O,O-(Meso-2,4-dimethylglutaryl)synthanecine A [(189) + (190)] - Treatment of meso-2,4-dimethylglutaric anhydride (188) as described in the general procedure, using DBU (211) as base, afforded the title compounds (77% yield) as a mixture of two diastereomeric racemates. This product was identical in all respects to the mixture obtained previously (p. 174).

(±)-6,7-O,O-(2,2,4,4-Tetramethylglutaryl)synthanecine A (191) - Treatment of 2,2,4,4-tetramethylglutaric anhydride (213) with synthanecine A as described in the general procedure, using DBU as base, gave the title compound (191) (44%) as a colourless oil which crystallised upon standing; m.p. 83 - 84 °C; R_F 0.68; $\nu_{max.}$ (CCl_4) 2 975, 2 780, 1 740, 1 280 and 1 160 cm^{-1}; δ_H (200 MHz) 2 × 1.19, 1.20 and 1.21 (3 H each, s, 15-, 16-, 17- and 18-H_3), 1.73 (1 H, d, J_{gem} 14.5 Hz, 11-H), 2.05 (1 H, d, J_{gem} 14.5 Hz, 11-H), 2.48 (3 H, s, NMe), 3.23 (1 H, m, 5-H), 3.53 (1 H, m, 2-H), 3.76 (1 H, m, 5-H), 3.81 (1 H, dd, J_{gem} 12 Hz, J_{vic} 2.5 Hz, 6-H), 3.86 (1 H, dd, J_{gem} 12 Hz, J_{vic} 7.5 Hz, 6-H), 4.63 (2 H, br s, 7-H_2) and 5.77 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 26.0, 27.4, 28.3 and 28.9 (C-15, -16, -17 and -18), 41.0 (NMe), 41.7 and 42.0 (C-10 and -12), 51.7 (C-11), 60.3 and 60.9 (C-5 and -6), 63.7 (C-7), 71.3 (C-2), 127.3 (C-4), 137.9 (C-3) and 176.2 and 176.4 p.p.m. (C-9 and -13); m/z 295 (M^+), 123, 108, 107, 94, 83 and 70 (Found: M^+, 295.1767. $C_{16}H_{25}NO_4$ requires M, 295.1783). The picrolonate salt had m.p. 191 - 192 °C (from ethanol). (Found: C, 55.8, H, 6.0; N, 12.5. $C_{26}H_{33}N_5O_9$ requires C, 55.80; H, 5.94; N, 12.52%).

(±)-6,7-O,O-(Succinyl)synthanecine A (193) - Use of succinic anhydride with DBU (211) as base gave a product (10% yield) after 72 h, which was identical in all respects to the title compound (193), prepared previously (p. 178). When the above procedure was repeated with heating at 70 °C for 6 h, the title compound (193) was obtained in 18% yield.

6,7-O,O-(Trans-2,3-dimethylsuccinyl)synthanecine A (194) - Treatment of (±)-trans-2,3-dimethylsuccinic anhydride with synthanecine A, using DBU as base, for 72 h at room temperature gave the title compound (194) (17% yield), as a mixture of two diastereomeric racemates. This product was spectrally and chromatographically identical to the mixture prepared by the Corey-Nicolaou procedure (p. 179).

6,7-O,O-(Trans-cyclohexane-1,2-dicarboxyl)synthanecine A (195) - Use of (±)-trans-cyclohexane-1,2-dicarboxylic anhydride in the presence of DBU (211), as described in the general procedure (p. 183), gave the title compound (195) as a mixture of two diastereomeric racemates (51% yield). Characterisation data on this mixture was identical to that obtained for the product prepared previously (p. 179).

(±)-6,7-O,O-(Maleyl)synthanecine A (214) - When maleic anhydride was treated with synthanecine A (9), as described in the general procedure, with Hunig's base (210) or DBN (212) as base none of the desired cyclised product (214) was observed (t.l.c.). Using DBU (211) as base, the title compound (214) was obtained (49% yield) as prisms, m.p. 82

-83 °C (ethyl acetate - hexane); R_F 0.69; $\nu_{max.}$(CCl_4) 2 955, 2 790, 1 735, 1 290 and 1 151 cm^{-1}; $\lambda_{max.}$(EtOH) 248 nm (ε 924); δ_H (200 MHz) 2.47 (3 H, s, NMe), 3.18 (1 H, m, 5-H), 3.62 (1 H, m, 2-H), 3.83 (1 H, m, 5-H), 4.10 (1 H, dd, J_{gem} 12 Hz, J_{vic} 6 Hz, 6-H), 4.46 (1 H, dd, J_{gem} 12 Hz, J_{vic} 3.5 Hz, 6-H), 4.60 (1 H, d, J_{gem} 13 Hz, 7-H), 5.13 (1 H, d, J_{gem} 13 Hz, 7-H), 5.95 (1 H, br s, 4-H), 6.27 and 6.35 p.p.m. (2 H, ABq, J 12 Hz, 10- and 11-H); δ_C (25 MHz) 41.4 (NMe), 60.5 and 61.8 (C-5 and -6), 65.5 (C-7), 71.4 (C-2), 130.6 131.1 and 131.6 (C-4, -10, and -11) 136.2 (C-3), 166.2 and 165.4 p.p.m. (C-9 and -12); m/z 223 (M^+) 110, 94, 82 and 67 (Found: M^+, 223.0842; C, 59.5; H, 5.9; N, 6.0. $C_{11}H_{13}NO_4$ requires M, 223.0844; C, 59.18; H, 5.87; N, 6.27%).

(±)-6,7-O,O-(2,3-Dimethylmaleyl)synthanecine A (215) - Treatment of synthanecine A with 2,3-dimethylmaleic anhydride, using DBU (211) as base, gave the title compound (215) (33% yield) as thread-like needles, m.p. 96 - 97 °C (hexane); R_F 0.72; $\nu_{max.}$(CCl_4) 2 940, 2 850, 2 785, 1 720, 1 650, 1 267 and 1 105 cm^{-1}; $\lambda_{max.}$(EtOH) 238 nm (ε 2 854); δ_H (200 MHz), 1.89 (6 H, m, 14- and 15-H_3), 2.41 (3 H, s, NMe), 3.11 (1 H, m, 5-H), 3.55 (1 H, m, 2-H), 3.77 (1 H, m, 5-H), 4.00 (1 H, dd, J_{gem} 12 Hz, J_{vic} 6 Hz, 6-H), 4.44 (1 H, dd, J_{gem} 12 Hz, J_{vic} 3.5 Hz, 6-H), 4.53 (1 H, d, J_{gem} 13 Hz, 7-H), 5.06 (1 H, d, J_{gem} 13 Hz, 7-H) and 5.88 p.p.m. (1 H, br s, 4-H); δ_C (50 MHz) 15.3 and 15.7 (C-14 and -15), 41.3 (NMe), 60.3 and 61.2 (C-5 and -6), 64.7 (C-7), 71.4 (C-2), 131.2 (C-4), 133.7 and 135.0 (C-10 and -11), 136.36 (C-3), 168.6 and 169.8 p.p.m. (C-9 and -12); m/z 251 (M^+), 148, 124, 107,94,

82 and 70 (Found: $\underline{M}^+$, 251.1169; C, 62.0; H, 6.9; N, 5.3. $C_{13}H_{17}NO_4$ requires $\underline{M}$, 251.1158; C, 62.13; H, 6.82; N, 5.57%).

(±)-6,7-O,O-(3,4,5,6-Tetrahydrophthalyl)synthanecine A (216)

By use of 3,4,5,6-tetrahydrophthalic anhydride and DBU (211) as base the title compound (216) (71%) was afforded as thread-like needles, m.p. 112 - 113 °C (Hexane); R_F 0.74; $\nu_{max.}$(CCl_4) 2 950, 2 870, 2 785, 1 725, 1 450, 1 270, 1 140, 1 095 and 1 035 cm^{-1}; $\lambda_{max.}$(EtOH) 254 nm (ε 2 013); δ_H (200 MHz) 1.65 (4 H, m, 15- and 16-H_2), 2.40 (4 H, m, 14- and 17-H_2), 2.47 (3 H, s, NMe), 3.17 (1 H, m, 5-H), 3.60 (1 H, m, 2-H), 3.83 (1 H, m, 5-H), 4.06 (1 H, dd, $\underline{J}_{gem}$ 12 Hz, $\underline{J}_{vic}$ 6 Hz, 6-H), 4.52 (1 H, dd, $\underline{J}_{gem}$ 12 Hz, $\underline{J}_{vic}$ 3.5 Hz, 6-H), 4.55 (1 H, d, $\underline{J}_{gem}$ 13 Hz, 7-H), 5.15 (1 H, d, $\underline{J}_{gem}$ 13 Hz, 7-H) and 5.94 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 21.1 and 21.2 (C-15 and -16) 25.7 and 26.1 (C-14 and -17) 41.3 (NMe), 60.4 and 61.2 (C-5 and -6), 64.7 (C-7), 71.6 (C-2), 131.5 (C-4), 135.8 and 136.4 (C-9 and -10), 137.6 (C-3), 168.3 and 169.7 p.p.m. (C-9 and -12); $\underline{m/z}$ 277 ($\underline{M}^+$), 123, 110, 107, 94, 82, 53 and 42 (Found: $\underline{M}^+$, 277.1315; C, 64.8; H, 7.0; N, 5.0. $C_{15}H_{19}NO_4$ requires M, 277.1315; C, 64.96; H, 6.91; N, 5.05%).

(±)-6,7-O,O-(Phthalyl)synthanecine A (196) - Using phthalic anhydride in the presence of DBU (211), the title compound (196) was afforded in 86% yield. This product was identical in all respects to the phthalate dilactone (196) prepared previously (p. 180).

(±)-6,7-O,O-(4,5-Dichlorophthalyl)synthanecine A (218) - Treatment of synthanecine A (9) with 4,5-dichlorophthalic anhydride, using Hunig's base (210) as base, gave the title compound (218) in 40% yield, m.p. 177 - 178 °C (ethanol); R_F 0.76; $\nu_{max.}$(KBr) 2 960, 2 855, 2 790, 1 730, 1 590, 1 550, 1 375, 1 300, 1 127, 833 and 775 cm^{-1}; $\lambda_{max.}$(EtOH) 252 (ε 5 550), 286 (ε 843), and 269 nm (ε 630); δ_H (200 MHz), 2.49 (3 H, s, NMe), 3.20 (1 H, m, 5-H), 3.71 (1 H, m, 2-H), 3.85 (1 H, m, 5-H), 4.23 (1 H, dd, J_{gem} 12 Hz, J_{vic} 6 Hz, 6-H), 4.57 (1 H, dd, J_{gem} 12 Hz, J_{vic} 4 Hz, 6-H), 4.77 (1 H, d, J_{gem} 13 Hz, 7-H), 5.24 (1 H, d, J_{gem} 13 Hz, 7-H), 6.02 (1 H, m, 4-H), 7.61 (1 H, s, 14- or 17-H) and 7.64 p.p.m. (1 H, s, 17- or 14-H); δ_C (50 MHz) 41.6 (NMe), 60.5 and 62.6 (C-5 and -6), 66.3 (C-7), 71.3 (C-2), 131.3, 131.5, 131.9 and 132.0 (C-4, -10, -11, -14 and -17), 136.2, 136.3 and 136.5 (C-3, -15 and -16), 166.0 and 166.8 p.p.m. (C-9 and -12); m/z 343 and 341 (M^+), 265, 144, 124, 110, 94, 82 and 74 (Found: M^+, 341.0199; C, 52.5; H, 3.7; N, 4.0. $C_{15}H_{13}{}^{35}Cl_2NO_4$ requires M, 341.0222; C, 52.76; H, 3.84; N, 4.10%).

When DBN (212) or DBU (211) was used as base the title compound (218) was obtained in yields of 24 and 91% respectively.

(±)-6,7-O,O-(3,4,5,6-Tetrachlorophthalyl)synthanecine A (217) - Treatment of 3,4,5,6-tetrachlorophthalic anhydride with synthanecine A (9), as described in the general procedure (p. 183), using DBU as base give the title compound (217) (67%) as needles, m.p. 139 - 141 °C (ethyl acetate);

R_F 0.77; $\nu_{max.}$(KBr) 2 955, 2 890, 2 845, 2 770, 1 735, 1 525, 1 452, 1 232, 1 169, 1 095, 1 082, 689 and 578 cm^{-1}; $\nu_{max.}$($CHCl_3$) 2 945, 2 850, 2 785, 1 740, 1 450, 1 355, 1 270, 1 175, and 1 100 cm^{-1}; $\lambda_{max.}$(EtOH) 225 (ε 39 810), 298 (ε 1 053) and 308 nm (ε 1 100); δ_H (200 MHz) 2.49 (3 H, s, NMe), 3.19 (1 H, m, 5-H), 3.77 (1 H, m, 2-H), 3.87 (1 H, m, 5-H), 4.31 (1 H, dd, J_{gem} 11.5 Hz, J_{vic} 7 Hz, 6-H), 4.54 (1 H, dd, J_{gem} 11.5 Hz, J_{vic} 4 Hz, 6-H), 4.95 (1 H, d, J_{gem} 13 Hz, 7-H), 5.18 (1 H, d, J_{gem} 13 Hz, 7-H), and 6.06 (1 H, br s, 4-H); δ_C (50 MHz) 42.2 (NMe), 60.3 and 62.6 (C-5 and -6), 65.9 (C-7), 71.0 (C-2), 131.2, 131.4, 131.5, 131.8, 136.9 and 137.0 (C-10, -11, -14, -15, -16, -17), 132.5 (C-4), 136.1 (C-3), 162.8 and 163.2 p.p.m. (C-9 and -12); m/z 414, 412, 410 and 408 (M^+), 241, 213, 142, 110, 94 and 82 (Found: M^+, 410.9404; C, 52.5; H, 3.7; N, 4.0. $C_{15}H_{11}{}^{35}Cl_3{}^{37}ClNO_4$ requires M, 410.9413; C, 52.76; H, 3.84; N, 4.10%).

(±)-6,7-O,O-(3,4,5,6-Tetrabromophthalyl)synthanecine A (219)

Treatment of synthanecine A (9) with 3,4,5,6-tetrabromophthalic anhydride as described in the general procedure, using DBU (211) as base, gave the title compound (219) in an 81% yield, m.p. 160 - 161 °C (ethanol); R_F 0.77; $\nu_{max.}$ (KBr) 2 955, 2 850, 2 785, 1 738, 1 385, 1 245, 1 220, 1 150, 1 070, and 989 cm^{-1}; $\lambda_{max.}$(EtOH) 230 (ε 4 326) and 310 nm (ε 127); δ_H (200 MHz) 2.48 (3 H, s, NMe), 3.19 (1 H, m, 5-H), 3.78 (1 H, m, 2-H), 3.87 (1 H, m, 5-H), 4.35 (1 H, dd, J_{gem} 11 Hz, J_{vic} 8 Hz, 6-H), 4.48 (1 H, dd, J_{gem} 11 Hz, J_{vic} 4 Hz, 6-H), 4.98 (1 H, d, J_{gem} 13 Hz, 7-H), 5.13 (1 H, d,

J_{gem} 13 Hz, 7-H), and 6.05 p.p.m. (1 H, br s, 4-H); δ_C (50 MHz) 42.3 (NMe), 60.2 and 62.7 (C-5 and -6), 65.8 (C-7), 70.9 (C-2), 123.7, 123.8, 2 × 133.7, 134.4, and 134.7 (C-10, -11, -14, -15, -16 and -17), 132.2 (C-4), 136.4 (C-3), 163.9 and 164.1 p.p.m. (C-9 and -12); m/z 588 (M^+), with a cluster of isotope peaks, 419, 231, 124, 110, 107, 94 and 82 (Found: M^+, 588.7364; C, 30.8; H, 2.0; N, 2.4. $C_{15}H_{11}{}^{79}Br_2{}^{81}Br_2NO_4$ requires M, 588.7380; C, 30.59; H, 1.88; N, 2.38%).

(±)-6,7-O,O-(Naphthalene-1,8-dicarboxyl)synthanecine A (220)

The title compound (220) was afforded in 65% yield when synthanecine A (9) was treated with naphthalene-1,8-dicarboxylic anhydride, in the presence of DBU (211) as base; m.p. 178 - 179 °C (ethanol); R_F 0.70; $\nu_{max.}$(KBr) 3 300, 2 950, 2 790, 1 715, 1 578, 1 505, 1465, 1 390, 1 285, 1 200, 1 150 and 770 cm^{-1}; $\lambda_{max.}$(EtOH) 226 (ε 4 307) and 296 nm (ε 875); δ_H(200 MHz) 2.50 (3 H, s, NMe), 3.22 (1 H, m, 5-H), 3.73 (1 H, m, 2-H), 3.81 (1 H, m, 5-H), 4.23 (1 H, dd, J_{gem} 12 Hz, J_{vic} 6 Hz, 6-H), 4.79 (1 H, dd, J_{gem} 12 Hz, J_{vic} 3 Hz, 6-H), 4.85 (1 H, d, J_{gem} 13 Hz, 7-H), 5.19 (1 H, d, J_{gem} 13 Hz, 7-H), 5.92 (1 H, br s, 4-H), 7.52 (2 H, t, J 8 Hz, 15- and 21-H), 7.89 - 8.01 p.p.m. (4 H, m, 16-, 17-, 19- and 20-H); δ_C(50 MHz) 41.2 (NMe), 60.4 and 60.7 (C-5 and -6), 64.1 (C-7), 71.7 (C-2), 125.2, 125.3, 127.2, 129.4, 2 × 129.6, 2 × 129.9, 132.2, 132.3, 134.1 and 137.5 (C-3, -4, -10, -11, -12, -16, -17, -18, -19, -20 and -21) and 2 × 169.0 p.p.m. (C-9 and -13); m/z 323 (M^+) 220, 198, 154, 126, 107, 94 and 82 (Found: M^+, 323.1169; C, 70.5; H, 5.3; N, 4.3. $C_{19}H_{17}NO_4$ requires M, 323.1157; C, 70.58; H, 5.26; N, 9.33%).

8.3 EXPERIMENTAL TO CHAPTER 5

8.3.1 Attempted synthesis of 12-membered and larger macrocyclic diesters via N-acylimidazolides.

Monophenyl adipate (238) - [Method (a)] 2-Chloro-1-methylpyridinium iodide (9.6 g, 38 mmol) was added to a stirred solution of adipic acid (5 g, 34 mmol) and TEA (9.8 ml, 71 mmol) in dry dichloromethane (50 ml) under argon. After 15 min phenol (3.2 g, 34 mmol) was added and the reaction mixture was stirred at room temperature for a further 10 h. Most of the dichloromethane was removed in vacuo and the resulting solution was extracted with 1M sodium hydroxide (3 × 15 ml). The combined basic extracts were washed with dichloromethane (3 × 40 ml), acidified with 2M hydrochloric acid, and extracted with dichloromethane (4 × 70 ml). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated at reduced pressure to give the crude phenyl ester (238) (2.2 g, 29%). The only impurity was a small amount of the pyridone byproduct (144). Multiple recrystallisations were required to obtain a pure sample of monophenyl adipate (238); m.p. 96 - 97 °C [from benzene - light petroleum (b.p. 60 - 80 °C)]; R_F 0.5 [silica, ethyl acetate - light petroleum (b.p. 60 - 80 °C) (1:1)]; $\nu_{max.}$(KBr) 3 040 (OH), 1 758 (C=O, phenyl ester), 1 695 (C=O, acid) and 1 130 cm^{-1} (C-O); δ_H (90 MHz) 1.80 (4 H, m, 3- and 4-H_2), 2.44 (2 H, t, J 6 Hz, 5-H_2) and 2.59 (2 H, t, J 6 Hz, 2-H_2), and 7.00 - 7.50 p.p.m. (5 H, m, Ph); δ_C (25 MHz) 24.0 and 24.2 (C-3 and -4), 33.6 and 33.9 (C-2 and -5), 121.6 (C-3' and -5'), 125.8 (C-4'), 129.4 (C-2' and -6'), 150.7 (C-1'), 171.8

(C-1) and 179.7 p.p.m. (C-6); m/z 222 (M^+) 129, 111, 101, 94, 83 and 77. (Found: M^+, 222.0899; C, 64.76; H, 6.33. $C_{12}H_{14}O_4$ requires M, 222.0893; C, 64.85; H, 6.35%).

Monophenyl adipate (238) - [Method (b)] CDI (50) (2.2 g, 13.7 mmol) was added to a solution of adipic acid (2.0 g, 13.7 mmol) in dry DME (35 ml) under argon. The reaction mixture was stirred until effervescence had subsided (ca. 20 min); a white suspension was observed. The solvent was removed in vacuo to give the N-acylimidazolide (239) as a white crystalline solid. δ_H (90 MHz) 1.87 (4 H, m, 2 × $CH_2CH_2CO_2$), 2.41 (2 H, t, J 6 Hz, CH_2CO_2H), 2.90 (2 H, t, J 6 Hz, CH_2CO_2Im), 7.10 (1 H, s, Im), 7.47 (1 H, s, Im) and 8.19 p.p.m. (1 H, s, Im). Singlets at 7.10 and 7.68 p.p.m. correspond to imidazole (240). $\nu_{max.}$ (KBr) 3 300 (OH), 1 730 (C=O, imidazolide) 1 700 (C=O, acid). A suspension of the N-acylimidazolide (239), prepared as described above, in dry DME (35 ml) under argon, was treated with phenol (1.3 g, 13.7 mmol) at room temperature. After 16 h the solvent was removed by rotary evaporation and the white solid afforded was treated with saturated aqueous sodium hydrogen carbonate (50 ml) until effervescence ceased. The aqueous solution was washed with ether (3 × 50 ml), acidified with 2M hydrochloric acid to pH 5 and extracted with dichloromethane (3 × 60 ml). The combined organic extractions were dried ($MgSO_4$), filtered and concentrated in vacuo to give monophenyl adipate (238) (1.1 g, 36%) which was identical in all respects to the sample obtained as described in method (a).

Monophenyl adipate (238) - [Method (c)] Adipic acid (50 g, 0.34 mol), phenol (32 g, 0.34 mol) and conc. sulphuric acid (1 ml) were added to toluene (350 ml). The solution was heated at reflux until the correct amount of water (ca. 6 ml) was collected in a Dean-Stark apparatus (ca. 30 h). Some of the toluene (150 ml) was removed by distillation and the resulting solution was allowed to cool before it was added, slowly with stirring, to a flask containing saturated aqueous sodium hydrogen carbonate (350 ml). When effervescence had subsided the aqueous solution was washed with diethyl ether (3 × 350 ml) and acidified with 2M hydrochloric acid to pH 5. The acidic solution was extracted with chloroform (3 × 400 ml) and the combined organic extracts dried ($MgSO_4$). Removal of the solvent gave a white crystalline solid (33 g, 44%) which was identical in all respects to the phenyl ester (238).

(N-imidazolyl)phenyl adipate (242) - CDI (50) (1.75 g, 11 mmol) was added to a solution of monophenyl adipate (238) (2 g, 9 mmol) in dry DME (45 ml) under argon. When effervescence had subsided (ca. 20 min) the solvent was removed in vacuo and the resulting white solid was dissolved in dichloromethane (40 ml). The organic solution was washed with water (3 × 40 ml), to remove imidazole (240), dried ($MgSO_4$), and concentrated at reduced pressure to give the title compound (242) (2.45 g, 85%) as a white solid; $\nu_{max.}$ (KBr) 3 122 (C=C-H), 1 760 (C=O, phenyl ester), 1 730 (C=O imidazolide) and 1 130 cm^{-1} (C-O); δ_H (90 MHz) 1.90 (4 H, m, 3- and 4-H_2), 2.63 (2 H, t, J 6 Hz, 2-H_2), 2.65 (2 H, t, J 6 Hz, 5-H_2), 7.00 - 7.50 (5 H, m, Ph), 7.19 (1 H, s, Im),

7.48 (1 H, s, Im) and 8.16 p.p.m. (1 H, s, Im); δ_C (25 MHz) 23.3 and 24.1 (C-3 and -4), 33.9 and 34.8 (C-2 and -5), 116.0 (Im), 121.5 (C-3' and -5'), 125.9 (C-4'), 129.4 (C-2' and -6'), 131.1 (Im), 136.1 (Im), 150.7 (C-1'), 169.0 (C=O, imidazolide) and 171.6 p.p.m. (C=O, phenyl ester); m/z 205 (M^+-Im), 177, 159, 135, 111, 94, 87 and 77.

Phenyl ester (243) of 6-O-(adipyl)synthanecine A - Synthanecine A (9) (300 mg, 2.1 mmol) was dissolved in dry DME (20 ml) under argon and the N-acylimidazolide (242) (510 mg, 2.1 mmol) was added. The reaction mixture was stirred at room temperature. After 48 h the solvent was removed in vacuo and the resulting brown oil was dissolved in dichloromethane (20 ml). The organic solution was washed with water (3 × 20 ml) to remove imidazole (240), dried ($MgSO_4$) and filtered. After removal of the solvent under reduced pressure the residual oil was purified by column chromatography [basic alumina, chloroform - dichloromethane (1:5)]. This afforded pure phenyl ester (243) of 6-O-(adipyl)-synthanecine A (360 mg, 49%); R_F 0.47; $\nu_{max.}$(CCl_4) 3 460 (OH), 2 780 (N-Me), 1 760 (C=O, phenyl ester), 1 740 (C=O, saturated ester) and 1 130 cm^{-1} (C-O); δ_H (90 MHz) 1.75 (4 H, m, 2 × $CH_2CH_2CO_2$), 2.39 (2 H, m, CH_2CO_2), 2.48 (3 H, s, N-Me), 2.56 (2 H, m, CH_2CO_2Ph), 2.83 (1 H, br s, OH), 3.25 (1 H, m, 5-H), 3.59 (1 H, m, 2-H), 3.81 (1 H, m, 5-H), 4.16 (1 H, s, 7-H_2), 4.18 (1 H, d, J 5 Hz, 6-H_2), 5.71 (1 H, br s, 4-H) and 7.00 - 7.50 p.p.m. (5 H, m, Ph); δ_C (25 MHz) 24.2 (2 × $CH_2CH_2CO_2$), 33.8 (2 × CH_2CO_2) 41.8 (N-Me), 59.5

and 61.4 (C-5 and -7), 65.2 (C-8), 70.7 (C-2), 2 × 121.5 (Ph), 124.0 (C-4), 125.7 (Ph), 2 × 129.4 (Ph), 141.2 (C-3), 150.7 (Ph), 171.8 (C=O phenyl ester), 173.2 (C=O, saturated ester); m/z 347 (M^+) 254, 222, 124, 111, 108, 94 and 82. (Found: M^+, 347.1732. $C_{19}H_{25}NO_5$ requires M, 347.1732.

Attempted lactonisation of the phenyl ester (243) of 6-O-(adipyl)synthanecine A - First attempt - 1-(Trimethylsilyl)-imidazole (42 µl, 0.29 mmol) and sodium phenoxide (catalytic) were added to a stirred solution of the phenyl ester (243) (100 mg, 0.29 mmol) in dry THF (1.4 ml) under argon. After 1 h the solvent was removed in vacuo to leave an oil; δ_H (90 MHz) 0.02 (9 H, s, TMS), 1.76 (4 H, m, 2 × $CH_2CH_2CO_2$), 2.39 (2 H, m, CH_2CO_2), 2.50 (3 H, s, N-Me), 2.56 (2 H, m, CH_2CO_2Ph), 3.25 (1 H, m, 5-H), 3.60 (1 H, m, 2-H), 3.80 (1 H, m, 5-H), 4.20 (2 H, s, 7-H_2), 4.21 (2 H, d, J 5 Hz, 6-H_2), 5.71 (1 H, br s, 4-H) and 7.00 - 7.50 p.p.m. (5 H, m, Ph). Singlets at 7.10 and 7.68 p.p.m. correspond to imidazole (240); R_F 0.7, 0.6 and 0.2 (imidazole).

Second attempt - A solution of the phenyl ester (243) (100 mg, 0.29 mmol) in dry THF (1.4 ml) under argon was treated with 1-(trimethylsilyl)imidazole (93 µl, 0.64 mmol) and sodium phenoxide (catalytic) as described above. A clear oil was formed; δ_H (90 MHz) 1.75 (4 H, m, 2 × $CH_2CH_2CO_2$), 2.35 (4 H, m, CH_2CO_2), 2.52 (3 H, s, N-Me), 3.27 (1 H, m, 5-H), 3.65 (1 H, m, 2-H), 3.83 (1 H, m, 5-H), 4.23 (2 H, s, 7-H_2), 4.23 (2 H, d, J 5 Hz, 6-H_2) and 5.78 (1 H, br s, 4-H). Small signals at 2.88 (CH_2COIm) and 8.17 p.p.m. (Im) are probably

due to an N-acylimidazolide. Signals at 7.10 and 7.67 p.p.m. correspond to imidazole (240); R_F 0.6, 0.5, 0.4 and 0.3.

Third attempt - To a stirred solution of the phenyl ester (243) (100 mg, 0.29 mmol) in dry THF (1.4 ml) under argon was added 1-(trimethylsilyl)imidazole (233) (93 µl, 0.64 mmol). After 5 min at room temperature sodium phenoxide (catalytic) was added. The mixture was stirred for 2 h and was then treated with anhydrous potassium fluoride (37 mg, 0.64 mmol) and 18 - Crown - 6 (248) (catalytic). After a further 5 h at room temperature the solvent was removed by rotary evaporation. The oily residue was dissolved in dichloromethane (10 ml) and the organic solution was washed successively with aqueous potassium chloride (1M) (3 × 8 ml) to remove imidazole (240) and 18 - Crown - 6 (248), and then with dilute sodium hydroxide (8 ml) and water (2 × 8 ml) to remove phenol. The organic solution was dried ($MgSO_4$), filtered and concentrated to give an oil (69 mg); R_F 0.4, 0.5 and 0.6; $\nu_{max.}$ ($CHCl_3$) 2 950 (C-H), 2 870 (N-Me) and 1 730 cm^{-1} (C=O, saturated ester); δ_H (90 MHz) 1.65 (4 H, m, 2 × $C\underline{H}_2CH_2CO_2$), 2.34 (4 H, m, 2 × $C\underline{H}_2CO_2$), 2.50 (3 H, s, N-Me), 3.27 (1 H, m, 5-H), 3.60 (1 H, m, 2-H), 3.84 (1 H, m, 5-H), 4.16 (2 H, d, J 5 Hz, 6-H_2), 4.65 (2 H, s, 7-H_2) and 5.81 p.p.m. (1 H, br s, 4-H); δ_C (50 MHz) 24.3 (2 × $\underline{C}H_2CH_2CO_2$), 33.7 (2 × $\underline{C}H_2CO_2$), 41.8 (N-Me), 60.5 and 61.4 (C-5 and -6), 65.0 (C-7), 70.6 (C-2), 127.2 (C-4), 136.0 (C-3), 172.8 and 173.0 (2 × C=O); m/z 254, 252, 199, 123, 107 and 94 [expected M^+ for (±)-6,7-O,O-(adipyl)synthanecine A (226) is m/z 253].

Monophenyl pimelate (249) - A mixture of pimelic acid (50.0 g, 0.31 mol), phenol (29.4 g, 0.31 mol) and conc. sulphuric acid (1 ml) in toluene (400 ml) was heated at reflux for ca. 35 h with azeotropic removal of water (5.6 ml) using a Dean-Stark apparatus. Some of the toluene (200 ml) was removed by distillation and the resulting solution was allowed to cool before it was added slowly to a stirred solution of saturated aqueous sodium hydrogen carbonate (350 ml). When effervescence had subsided the aqueous solution was washed with diethyl ether (3 × 300 ml), and then carefully neutralised with 2M hydrochloric acid until a white suspension was observed (pH 7). The suspended white solid was extracted with diethyl ether (3 × 400 ml). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo to give the title compound (249) (30.3 g, 4.1%); m.p. 51 - 53 °C [from benzene - light petroleum (b.p. 60 - 80 °C)]; R_F 0.5 [silica, ethyl acetate - light petroleum (b.p. 60 - 80 °C) (1:1)]; $\nu_{max.}$ (KBr) 3 050 (OH), 2 970 (C-H), 1 755 (C=O, phenyl ester), 1 700 (C=O, acid), 1 205 (C-O), 690 and 762 cm^{-1} (monosubstituted phenyl); δ_H (90 MHz) 1.20 - 2.00 (6 H, m, 3-H_2, 4-H_2 and 5-H_2), 2.36 (2 H, t, J 6 Hz, 6-H_2) and 2.54 (2 H, t, J 6 Hz, 2-H_2), and 7.00 - 7.50 p.p.m. (5 H, m, Ph); δ_C (25 MHz) 24.2 and 24.5 (C-3 and -5), 28.4 (C-4), 33.8 and 34.0 (C-2 and -6), 121.6 (C-3' and -5'), 125.7 (C-4'), 129.4 (C-2' and -6'), 150.8 (C-1'), 172.0 (C=O, phenyl ester) and 179 p.p.m. (C=O, acid); m/z 236 (M^+) 143, 125, 94 and 69. (Found: M^+, 236.1049; C, 66.12; H, 6.81. $C_{13}H_{16}O_4$ requires M, 236.1049; C, 66.08; H, 6.83%).

(N-imidazolyl)phenyl adipate (250) - Treatment of monophenyl pimelate (249) (2.0 g, 8.47 mmol) with CDI (50) (1.65 g, 10.2 mmol) as described for compound (242), gave the title compound (250) (2.30 g, 95%); $\nu_{max.}$ (KBr) 3 060 (C=C-H), 2 970 (C-H), 1 760 (C=O, phenyl ester) and 1 730 cm^{-1} (C=O, imidazolide); δ_H (90 MHz) 1.20 - 2.00 (6 H, m, 3-H_2, 4-H_2 and 5-H_2), 2.55 (2 H, t, J 6 Hz, 2-H_2), 2.83 (2 H, t, J 6 Hz, 6-H_2), 7.00 - 7.50 (5 H, m, Ph), 7.08 (1 H, s, Im), 7.45 (1 H, s, Im) and 8.12 p.p.m. (1 H, s, Im); m/z 219 (M^+-Im), 173, 143, 125, 114, 94 and 83.

Phenyl ester (251) of 6-O-(pimelyl)synthanecine A - Synthanecine A (310 mg, 2.2 mmol), dissolved in dry DME (20 ml) under argon, was treated with the N-acylimidazolide (250) (620 mg, 2.2 mmol) as described for the preparation of compound (243). The title compound (251) was afforded as an oil (329 mg, 41%). $\nu_{max.}$ ($CHCl_3$) 3 610 (OH), 2 970 (C-H), 2 790 (N-Me), 1 755 (C=O, phenyl ester) and, 1 730 cm^{-1} (C=O, saturated ester); δ_H (90 MHz) 1.20 - 2.00 (6 H, m, $CH_2CH_2CH_2CO_2$ and 2 × $CH_2CH_2CO_2$), 2.37 (2 H, t, J 6 Hz, CH_2CO_2), 2.51 (3 H, s, N-Me), 2.57 (2 H, t, J 6 Hz, CH_2CO_2Ph), 2.71 (1 H, s, OH), 3.26 (1 H, m, 5-H), 3.63 (1 H, m, 2-H), 3.75 (1 H, s, 5-H), 4.18 (2 H, s, 7-H_2), 4.20 (2 H, d, J 5 Hz, 6-H_2), 5.75 (1 H, br s, 4-H), 7.00 - 7.50 (5H, m, Ph). δ_C (25 MHz) 24.5 (2 × $CH_2CH_2CO_2$), 28.5 ($CH_2CH_2CH_2CO_2$), 34.1 (2 × CH_2CO_2), 41.8 (N-Me), 59.6 and 61.5 (C-5 and -6), 65.3 (C-7), 70.6 (C-2), 2 × 121.6 (Ph), 124.1 (C-4), 125.8 (Ph), 2 × 129.4 (Ph), 141.3 (C-3), 150.8 (Ph), 172.1 (C=O, phenyl ester) and 173.5 p.p.m. (C=O, saturated ester); m/z 361

($\underline{M}^+$) 143, 112, 124, 108, 94 and 82. (Found: $\underline{M}^+$, 361.1861. $C_{20}H_{27}NO_5$ requires $\underline{M}$, 361.1889).

Attempted lactonisation of the phenyl ester (251) of 6-O-(pimelyl)synthanecine A. - When a stirred solution of the phenyl ester (251) (85 mg, 0.24 mmol) in dry THF (2.3 ml) under argon, was treated with 1-(trimethylsilyl)-imidazole (75 µl, 0.52 mmol) and sodium phenoxide (catalytic) in a manner similar to that described for the attempted lactonisation of the adipic homologue (243) (first attempt) a colourless oil was afforded; δ_H (90 MHz) 1.20 - 2.00 (6 H, m, $C\underline{H}_2CH_2CH_2CO_2$ and 2 × $C\underline{H}_2CH_2CO_2$), 2.30 (2 H, t, $\underline{J}$ 6 Hz, $C\underline{H}_2CO_2$), 2.51 (3 H, s, N-Me), 2.85 (2 H, t, $\underline{J}$ 6 Hz, $C\underline{H}_2COIm$), 3.25 (1 H, m, 5-H), 3.62 (1 H, m, 2-H), 3.74 (1 H, m, 5-H), 4.20 (2 H, s, 7-H_2), 4.21 (2 H, d, $\underline{J}$ 5 Hz, 6-H_2), 5.72 (1 H, br s, 4-H), 7.11 (1 H, s, Im), 7.48 (1 H, s, Im) and 8.17 p.p.m. (1 H, s, Im). This ^{1}H n.m.r. spectrum is consistent for the $\underline{N}$-acylimidazolide (252). Singlets at 7.10 and 7.69 p.p.m. correspond to imidazole (240) and signals between 6.78 and 7.30 p.p.m. were assigned to the trimethylsilyl ether of phenol.

The colourless oil, prepared above, was dissolved in dry THF (2.5 ml) under argon and anhydrous potassium fluoride (30 mg, 0.52 mmol) and 18 - Crown - 6 (catalytic) were added. The reaction mixture was stirred at room temperature for 5 h then purified by a series of washings, as described previously (p. 197, third attempt), to yield a mixture of pyrroline containing compounds (t.l.c.); R_F 0.35, 0.45,

0.50, 0.60; $\nu_{max.}$($CHCl_3$) 2 970 (C-H) and 1 735 cm^{-1} (C=O); δ_H (90 MHz) 1.55 (6 H, m, $C\underline{H}_2CH_2CH_2CO_2$ and 2 × $C\underline{H}_2CH_2CO_2$), 2.31 (4 H, m, 2 × $C\underline{H}_2CO_2$), 2.51 (3 H, s, N-Me), 3.29 (1 H, m, 5-H), 3.65 (1 H, m, 2-H), 3.85 (1 H, m, 5-H), 4.18 (2 H, d, $\underline{J}$ 5 Hz, 6-H_2), 4.67 (2 H, s, 7-H_2) and 5.83 p.p.m. (1 H, br s, 4-H); δ_C (25 MHz) 24.5 (2 × $\underline{C}H_2CH_2CO_2$), 28.6 ($\underline{C}H_2CH_2CH_2CO_2$), 41.7 (N-Me), 60.7 and 61.4 (C-5 and -6), 65.4 (C-7), 70.7 (C-2), 127.7 (C-4), 136.7 (C-3), 173.1 and 173.3 p.p.m. (2 × C=O); $\underline{m}/\underline{z}$ 267 ($\underline{M}^+$) 167, 149, 107, 94, 83 and 71. (Found: $\underline{M}$, 267.1485. $C_{14}H_{21}NO_4$ requires $\underline{M}$, 267.1470.

Second attempt. - The phenyl ester (251) (100 mg, 0.28 mmol) was treated with 1-(trimethylsilyl)imidazole (89 µl, 0.61 mmol) and sodium phenoxide (catalytic) as described above. The resulting solution was added (via syringe) dropwise over a period of 6 h to a stirred suspension of anhydrous potassium fluoride (35 mg, 0.61 mmol) and 18 - Crown - 6 (248) (catalytic) in dry THF (10 ml) heated at reflux under argon. Heating at reflux was continued for a further 1 h, and the reaction mixture was worked up, as reported previously (p. 197), to yield a colourless oil that was identical in all respects to the foregoing mixture.

8.3.2 Synthesis of 12-membered and larger macrocyclic diesters of synthanecine A.

General procedure - The diacid (0.5 mmol) and DBU (211) (1.05 mmol) were added to a solution of the allylic chloride (200) (0.5 mmol) in dry DMF (3 ml) under argon. The mixture

was stirred at room temperature until the starting material (200) had reacted (t.l.c.) (ca. 48 h). The DMF was removed under reduced pressure (3 mmHg) and dry DME (35 ml) was added to the resulting oily residue. 2,2'-Dithiodipyridine (1.06 mmol) and triphenylphosphine (1.06 mmol) were added and the reaction mixture was stirred vigorously at room temperature, under argon, until thiol ester formation was complete (t.l.c., R_F ca. 0.3) (ca. 48 h); a yellow solution containing a suspended white solid was observed. The white solid was allowed to settle out before the yellow solution was taken up in a syringe. This solution was added dropwise over 2 h to dry DME (70 ml) heated at reflux, under argon. Refluxing was continued until lactonisation was complete (t.l.c., R_F 0.6 - 0.7)(ca. 10 h). The reaction mixture was worked up as described in the general procedure on page 169.

(±)-6,7-O,O-(Adipyl)synthanecine A (226) - The reaction of adipic acid (73 mg, 0.5 mmol) with the allylic chloride (200) (100 mg, 0.5 mmol), was carried out as described in the general procedure to yield the title compound (226) (15 mg, 12%) as an oil, R_F 0.68; $\nu_{max.}(CCl_4)$ 2 940 (C-H), 2 780 (N-Me), 1 735 (C=O), 1 260 and 1 170 (C-O), 994 and 908 cm^{-1}; δ_H (200 MHz) 1.70 (4 H, m, 11- and 12-H_2), 2.30 (4 H, m, 10- and 13-H_2), 2.50 (3 H, s, N-Me), 3.25 (1 H, m, 5-H), 3.61 (1 H, m, 2-H), 3.76 (1 H, dd, J_{gem} 11, J_{vic} 7 Hz, 6-H), 3.81 (1 H, m, 5-H), 4.52 (1 H, dd, J_{gem} 11, J_{vic} 2.6 Hz, 6-H), 4.31 (1 H, d, J_{gem} 12 Hz, 7-H), 5.03 (1 H, d, J_{gem} 12 Hz, 7-H), 5.86 (1 H, br s, 4-H); δ_C (50 MHz) 23.7 and 23.8 (C-11 and -12), 33.7 and 33.8 (C-10 and -13), 41.25 (N-Me),

61.0 and 61.2 (C-5 and -6), 65.2 (C-7), 70.3 (C-2), 129.6 (C-4), 136.5 (C-3), 173.0 and 173.4 p.p.m. (C-9 and -14); m/z 253 (M^+), 251, 123, 107, 94 and 55 (Found: M^+, 253.1316. $C_{13}H_{19}NO_4$ requires M, 253.1314). The picrolonate had m.p. 207 - 209 °C (from ethanol); m/z 264 (picrolonic acid), 253 (M^+), 111, 108, 107, 94 and 80 (Found: M^+, 253.1320. $C_{13}H_{19}NO_4$ requires M, 253.1314). (Found: C, 53.6; H, 5.4; N, 13.4. $C_{23}H_{27}N_5O_9$ requires C, 53.38; H, 5.26; N, 13.53%).

(±)-6,7-O,O-(Pimelyl)synthanecine A (227) - Treatment of the allylic chloride (200) (166 mg, 0.84 mmol) with pimelic acid (134 mg, 0.84 mmol) was performed as described in the general procedure to afford the title compound (227) (35 mg, 16%) as an oil; R_F 0.69; $\nu_{max.}$(CCl_4) 2 940 (C-H), 2 780 (N-Me), 1 735 (C=O), 1 260, 1 160 (C-O) and 1060 cm^{-1}. δ_H (200 MHz), 1.20 - 1.90 (6 H, m, 11-, 12- and 13-H_2), 2.35 (4 H, m, 10- and 14-H_2), 2.50 (3 H, s, N-Me), 3.25 (1 H, m, 5-H), 3.50 (1 H, m, 2-H), 3.81 (1 H, m, 5-H), 3.88 (1 H, dd, J_{gem} 11.5 Hz, J_{vic} 7.5 Hz, 6-H), 4.35 (1 H, dd, J_{gem} 11.5 Hz, J_{vic} 2 Hz, 6-H), 4.54 (1 H, d, J_{gem} 12 Hz, 7-H), 4.70 (1 H, d, J_{gem} 12 Hz, 7-H), and 5.88 (1 H, br s, 4-H); δ_C (50 MHz) 23.8 and 24.1 (C-11 and -13), 27.1 (C-12), 34.2 and 36.0 (C-10 and -14), 40.9 (N-Me), 60.6 and 60.8 (C-5 and -6), 64.9 (C-7), 70.6 (C-2), 130.3 (C-4), 136.9 (C-3), 173.7 and 173.8 (C-9 and -15). The picrolonate salt had m.p. 186 - 187 °C (from ethanol); m/z 267 (M^+) 264 (picrolonic acid) 234, 156, 124, 123, 111, 108, 107 and 94. (Found: M^+, 267.1468. $C_{14}H_{21}NO_4$ requires M, 267.1470). (Found: C, 54.1; H, 5.4; N, 13.1. $C_{24}H_{29}N_5O_9$ requires C, 54.23; H, 5.50; N, 13.8%).

(±)-6,7-O,O-(Suberyl)synthanecine A (262) - In a similar fashion, suberic acid (87 mg, 0.5 mmol) was reacted with the allylic chloride (200) (100 mg, 0.5 mmol) to afford the title compound (262) (14 mg, 10%) as an oil; R_F 0.73; $\nu_{max.}$(CCl_4) 2 940 (C-H), 2 785 (N-Me), 1 735 (C=O), 1 250 and 1 168 (C-O) and 910 cm^{-1}; δ_H (200 MHz) 1.30 (4 H, m, 12- and 13-H_2), 1.70 (4 H, m, 11- and 14-H_2), 2.33 (4 H, m, 10- and 15-H_2), 2.54 (3 H, s, N-Me), 3.29 (1 H, m, 5-H), 3.60 (1 H, m, 2-H), 3.74 (1 H, dd, J_{gem} 11 Hz, J_{vic} 3.6 Hz, 6-H), 3.80 (1 H, m, 5-H), 4.47 (1 H, dd, J_{gem} 11 Hz, J_{vic} 2 Hz, 6-H), 4.54 (1 H, d, J_{gem} 12 Hz, 7-H), 4.75 (1 H, d, J_{gem} 12 Hz, 7-H), and 5.88 p.p.m. (1 H, br s, 4-H); δ_C (50 MHz) 24.4 and 24.5 (C-12 and -13), 25.7 and 25.8 (C-11 and -14), 32.6 and 32.9 (C-10 and -15), 41.2 (N-Me), 60.8 and 61.1 (C-5 and -6), 66.3 (C-7), 70.2 (C-2), 130.6 (C-4), 137.2 (C-3), 173.8 and 174.0 p.p.m. (C-9 and -16). The picrolonate salt had m.p. 191 - 193 °C (from ethanol); m/z 281 (M^+) 264 (picrolonic acid), 248, 111, 108, 107 and 94. (Found: M^+, 281.1632. $C_{15}H_{23}NO_4$ requires M, 281.1627). (Found: C, 55.1; H, 5.5; N, 12.6. $C_{25}H_{31}N_5O_9$ requires C, 55.04; H, 5.73; N, 12.84%).

(±)-6,7-O,O-(Azelayl)synthanecine A (263) - Treatment of the allylic chloride (200) (250 mg, 1.26 mmol) with azelaic acid (237 mg, 1.26 mmol) as described above afforded the title compound (263) (56 mg, 15%) as a colourless oil; R_F 0.68; $\nu_{max.}$(CCl_4) 2 940 (C-H), 2 785 (N-Me), 1 735 (C=O), 1 242 and 1 162 (C-O); δ_H (200 MHz) 1.30 (6 H, m, 12-, 13- and 14-H_2), 1.60 (4 H, m, 11- and 15-H_2), 2.35 (4 H, m, 10-

and 16-H_2), 2.51 (3 H, s, N-Me), 3.28 (1 H, m, 5-H), 3.52 (1 H, m, 2-H), 3.80 (1 H, m, 5-H), 3.88 (1 H, dd, J_{gem} 11 Hz, J_{vic} 7.5 Hz, 6-H), 4.42 (1 H, dd, J_{gem} 11 Hz, J_{vic} 3 Hz, 6-H), 4.59 (1 H, d, J_{gem} 12 Hz, 7-H), 4.73 (1 H, d, J_{gem} 12 Hz, 7-H), and 5.88 p.p.m. (1 H, br s, 4-H); δ_C (50 MHz) 23.5 and 23.6 (C-12 and -14), 26.0 (C-13), 26.9 and 27.1 (C-11 and -15), 33.9 and 34.1 (C-10 and -16), 41.1 (N-Me), 60.6 and 61.1 (C-5 and -6), 65.5 (C-7), 70.6 (C-2), 130.0 (C-4), 137.1 (C-3), 173.6 and 173.7 p.p.m. (C-9 and -17); m/z 295 (M^+), 217, 123, 107, 94, 82 and 67. (Found: M^+, 295.1785. $C_{16}H_{25}NO_4$ requires M, 295.1784). The picrate and picrolonate salts could not be prepared.

(±)-6,7-O,O-(Sebacyl)synthanecine A (264) - The allylic chloride (200) (200 mg, 1 mmol) and sebacic acid (204 mg, 1 mmol) were reacted as described in the general procedure, to yield the title compound (264) (47 mg, 15%) as a colourless oil; R_F 0.68; $\nu_{max.}$(CCl_4) 2 940 (C-H), 2 780 (N-Me), 1 735 (C=O), 1 260 and 1 168 cm^{-1} (C-O); δ_H (200 MHz) 1.31 (8H, m, 12-, 13-, 14- and 15-H_2), 1.65 (4 H, m, 11- and 16-H_2), 2.33 (4 H, m, 10- and 17-H_2), 2.53 (N-Me), 3.32 (1 H, m, 5-H), 3.60 (1 H, m, 2-H), 3.83 (1 H, m, 5-H), 4.00 (1 H, dd, J_{gem} 11 Hz, J_{vic} 5 Hz, 6-H), 4.30 (1 H, dd, J_{gem} 11 Hz, J_{vic} 4 Hz, 6-H), 4.60 (1 H, d, J_{gem} 13 Hz, 7-H), 4.68 (1 H, d, J_{gem} 13 Hz, 7-H) and 5.86 p.p.m. (1 H, br s, 4-H); δ_C (50 MHz) 23.9, 24.0, 26.1, 26.4, 2 × 26.8 (C-11, -12, -13, -14, -15 and -16), 33.9 and 34.3 (C-10 and -17), 41.8 (N-Me), 60.7 and 61.5 (C-5 and -6), 65.4 (C-7), 70.5 (C-2), 128.0 (C-4), 136.0 (C-3), 173.5 and 173.8 p.p.m. (C-9 and -18); m/z 309

(M^+), 218, 124, 108, 107, 94, 78 and 67. (Found: $\underline{M}^+$, 309.1965. $C_{17}H_{27}NO_4$ requires $\underline{M}$, 309.1940). The picrolonate salt could not be prepared.

8.5 EXPERIMENTAL TO CHAPTER 6

8.5.1 Synthesis of synthanecine A dilactones - This was achieved as described in Chapter 3.

8.5.2 Animal and Dosing - Male albino Wistar rats (Porton strain), bred at the Carshalton Laboratories, were used for all experiments. They were weanlings, aged 22 - 23 days, and had access to food (MRC diet) and water at all times.

The basic synthanecine A dilactones were dissolved in 0.9% saline with an equivalent of HCl to give neutral solutions with conc. 15 or 30 mg/ml; they were given as a single intra peritoneal (i.p.) injection. Some rats were predosed via a stomach tube, 4 h before the synthanecine A dilactone, with undiluted TOCP (0.5 ml/Kg body weight). These animals were always caged separately from rats not so treated.

8.5.3 Measurement of pyrrolic metabolites - Rats were given a single dose of a synthanecine A dilactone, and killed (with CO_2) 2 h later. Some were given TOCP 4 h before the synthanecine A derivative. Levels of pyrrolic metabolites in 3 portons (each 0.5 g) of liver and 1 portion of lung tissue were measured as follows. The portions of tissue, liver or lung, were homogenised in ethanolic (5%) mercuric chloride (5 ml) for 15 - 30 sec. using a Nelco 10 blender. The solids were separated by certrifugation (1 min.), washed once with ethanol (10 ml) and again centrifuged. The residues were resuspended in ethanol (2 ml) and Ehrlich's reagent (2 ml), heated in a water bath (90 - 100 ^{o}C) with

continuous agitation for 1 min., and then cooled to room temperature. The suspensions were diluted to 5 ml with ethanol and centrifuged for 10 min., and the absorbances at 565 and 625 nm were measured against an ethanol blank. The corrected absorance value was calculated using the formula shown below. Levels of pyrrolic metabolites are generally quoted as absorbances

$$\text{Corrected Absorbance} = 1.1\ (Abs^{565} - Abs^{625})$$

8.5.4 Alkaline Hydrolysis - To the dilactone (0.1 mmol) dissolved in methanol (2.0 ml) and water (2.0 ml) was added 0.1M sodium hydroxide (1.0 ml) with thorough mixing. The initial pH (approx. 12) was measured (meter), then the mixture was kept at 23 ^{o}C in a closed flask and the time was noted for each decrease of 0.1 unit in the pH. This method was tested using ethyl acetate and then used for the synthanecine A dilactones (183 a), (183 b) and [(189) + (190)].

8.6 EXPERIMENTAL FOR CHAPTER 7

Emilia flammea Cass. (Syn. Cacalia coccinea) was grown at the Botanical gardens in Glasgow, and cropped when flowering. The fresh plant material (300 g) was finely chopped and blended with methanol in portions. The blended extracts were filtered and the methanolic filtrates were concentrated under reduced pressure. The resulting green residue was extracted with 1M sulphuric acid (3 × 100 ml) and the combined acidic extracts were washed with dichloromethane (10 × 200 ml) until the washings became colourless. Zinc powder (5 g) was added to the aqueous solution and the mixture was stirred at room temperature for 2 h. The acidic suspension was then filtered through celite to remove the zinc powder. The filtrate was basified with concentrated ammonia solution, and extracted with dichloromethane (4 × 400 ml). The combined organic extracts were dried, filtered and concentrated in vacuo affording a crude alkaloidal mixture (50 mg). The crude product was triturated with hexane (3 × 1 ml) and the combined extracts were left at 0 °C overnight. A white amorphous solid, which had crystallised, was collected and further purified by trituration and recrystallisation from hexane. The resulting white solid (25 mg) had m. pt. 103 - 105 °C (Lit. 105 - 107 °C); ^{100} R_F 0.34; $[\alpha]_D^{22.5}$ = -17.5 ° ($CHCl_3$); δ_H (200 MHz) 0.85 (3 H, t, J 7 Hz, 21-H_3), 1.53 (2 H, m, 20-H_2), 1.53 (3 H, s, 18-H_3), 2.06 (3 H, s, NMe), 2.10 (1 H, m, 14-H_a), 2.20 (1 H, m, 6-H), 2.25 (1 H, m, 15-H), 2.43 (1 H, m, 6-H), 2.65 (1 H, m, 5-H), 2.80 (1 H, m, 14-H_b), 2.85 (1 H, m, 5-H), 3.20 (1 H, br d, J_{gem} 18 Hz,

3-H), 3.44 (1 H, br d, $\underline{J}_{gem}$ 18 Hz, 3-H), 3.66 (1 H, br s, O-H), 4.46 (1 H, br d, $\underline{J}_{gem}$ 11 Hz, 9-H_a), 4.81 (1 H, t, $\underline{J}$ 3 Hz, 7-H), 5.09 (1 H, d, $\underline{J}_{gem}$ 11 Hz, 9-H_b), 5.10 (2 H, d, $\underline{J}$ 6 Hz, 19-H_2), and 6.02 p.p.m. (1 H, br s, 2-H). δ_C (50 MHz) 12.0 (C-21), 26.5 (C-20), 28.5 (C-18), 36.1 (C-14), 37.5 (C-6), 40.2 (NMe), 46.9 (C-15), 53.2 (C-5), 58.6 (C-9), 66.6 (C-3), 75.2 (C-12), 77.2 (C-7), 117.9 (C-19), 131.8 (C-2), 135.7 (C-1), 146.5 (C-13), 174.7 and 177.6 (C-11 and -16) and 191.5 p.p.m. (C-8); $\underline{m}/\underline{z}$ 365 (M^+) 337, 321, 306, 168, 151, 125, 110, 96, 53 and 43. (Found: $\underline{M}^+$, 365.1825. $C_{19}H_{27}NO_6$ requires $\underline{M}$, 365.1838).

REFERENCES

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

2. D.J. Robins, The Pyrrolizidine Alkaloids, iii. Fortschr. Chem. Org. Naturstoffe, 1982, 41, 115.

3. C.C.J. Culvenor, Alkaloids and human disease, in "Toxicology in the Tropics" (Eds. R.L. Smith and E.A. Bababunmi), Taylor and Francis, London, 1980.

4. A.R. Mattocks in 'Phytochemical Ecology,' ed. J.B. Harborne, Academic Press, New York, 1972, p.179.

5. E. Roder, and H. Wiedenfeld, Phytochemistry, 1980, 19, 1275.

6. J.A. Edgar, N.J. Eggers, A.J. Jones, and G.B. Russell, Tet. Letts., 1980, 2657.

7. L.W. Smith, and C.C.J. Culvenor, J. Nat. Products, 1981, 44, 129.

8. D.J. Robins, Advances in Pyrrolizidine Chemistry, Adv. Heterocyclic Chem., 1969, 24, 247.

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

10. J.E. Saxton in "The Alkaloids" (J.E. Saxton, Ed.), Specialist Periodical Reports, The Chemical Society, London, 1971-5, Vols. 1-5; D.H.G. Crout in "The Alkaloids" (M.F. Grundon, Ed.), Specialist Periodical Reports, The Chemical Society, London, 1976, 2976-7, Vols. 6-7; D.J. Robins in "The Alkaloids" (M.F. Grundon, Ed.) Specialist Periodical Reports, The Chemical Society, London, 1978-83, Vols. 8-13.

11. D.J. Robins, Nat. Prod. Rep., 1984, 1, 235; 1985, 2, 294.

12. A.R. Mattocks, J. Chem. Soc. Perkin Trans. 1, 1974, 707.

13. A.R. Mattocks, J. Chem. Soc. Perkin Trans. 1, 1978, 896.

14. A.R. Mattocks, Nature, 1971, 232, 476.

15. H.E. Watt, J. Chem. Soc., 1909, 95, 466.

16. A.R. Cushny, J. Pharm. Exp. Ther., 1911, 2, 531.

17. R. Schoental, Cancer Res., 1968, 28, 2237.
R. Schoental, ibid., 1975, 35, 2020.

18. K. Hill et al., West Indian Med. J., 1951, 1, 49.
G. Bras et al., Archs. Path., 1954, 57, 285.

19. G. Bras et al., Lancet, 1957, 272, (1), 960.

20. O. Mohabbat et al., Lancet, 1976, 269.

21. B.N. Tandon et al., Lancet, 1976, 271.

22. J.O. Dickson and R.R. King, Effects of Poisonous Plants on Livestock, (Ed.) R.F. Keeler, K.R. Van kampen, L.F. James, Academic Press, New York, 1978, 201.

23. C.C.J. Culvenor, Trends. Pharmacol. Sci., 1985, 18.

24. R. Schoental, Nature, 1957, 179, 361.

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

26. A.R. Mattocks, Nature, 1968, 217, 723.

27. A.R. Mattocks, J. Chem. Soc. (C)., 1969, 1155.

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

29. A.R. Mattocks, Nature, 1970, 228, 174.

30. A.R. Mattocks, Toxicol. Letts., 1982, 14, 111.

31. M. Kugelman, W.C. Liu, M. Axelrod, T.J. McBride and K.V. Rao, Lloydia, 1976, 39, 125.

32. J.S. Kovach et al., Cancer Res., 1979, 39, 4540.

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

34. C.K. Atal, J. Nat. Prod. (Lloydia), 1978, 41, 312.

35. C.C.J. Culvenor, Tet. Letts., 1966, 10, 1091.

36. G. Barger, T.R. Seshadri, H.E. Watt, and T. Yabuta, J. Chem. Soc., 1935, 1, 11.

37. R. Konovalova and A.P. Orekhov, Ber., 1935, 68B, 1886.

38. E.L. Gurevich and G.P. Menshikov, Zh. Obshch. Khim., 1947, 17, 1714. (Chem. Abs., 1948, 42, 25981).

39. N.K. Hart and J.A. Lamberton, Austral. J. Chem., 1966, 19, 1259.

40. H. Tanino, S. Inoue, K. Nishikawa, and Y. Hirata, Tetrahedron, 1969, 25, 3033.

41. B. Lundstron and B. Luning, Acta. Chem. Scand., 1969, 23, 3352.

42. A.R. Mattocks, J. Chem. Soc.(C)., 1969, 2698.

43. C.C.J. Culvenor, J.A. Edgar, M.V. Jago, A. Outteridge, J.E. Peterson and L.W. Smith, Chem.-Biol. Interactions, 1976, 12, 299.

44. C.C.J. Culvenor, A.T. Dann, and L.W. Smith, Chem. Ind., 1959, 20.

45. C.C.J. Culvenor and L.W. Smith, Austral. J. Chem., 1966, 19, 1955.

46. C.C.J. Culvenor, S.R. Johns, J.A. Lamberton, and L.W. Smith, Austral. J. Chem., 1970, 23, 1279.

47. J.A. Glinski and L.H. Zalkow, Tet. Letts., 1985, 26, 2857.

48. W.H. Kruizinga, B. Strijtveen and R.M. Kellog, J. Org. Chem., 1981, 46, 4321.

49. N.K. Kochetkov, A.M. Likhosherstov, and V.N. Kulakov, Tetrahedron, 1969, 25, 2313.

50. W.M. Hoskins and D.H.G. Crout, J. Chem. Soc. Perkin Trans. 1, 1977, 538.

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

52. E.J. Corey and K.C. Nicolaou, J. Amer. Chem. Soc., 1974, 96, 5614.

53. T. Mukaiyama, Angew. Chem. Internat. Edit., 1976, 15, 94.

54. J.A. Devlin, D.J. Robins, S. Sakdarat, J. Chem. Soc. Perkin Trans. 1, 1982, 1117.

55. K. Brown, J.A. Devlin, D.J. Robins, J. Chem. Soc., Perkin Trans. 1, 1983, 1819.

56. E.J. Corey, K.C. Nicolaou and L.S. Melvin, Jr., J. Amer. Chem. Soc., 1975, 97, 654.

57. S. Masamune, H. Yamamoto, S. Kamata, and A. Fukuzawa, J. Amer. Chem. Soc., 1975, 97, 3513. S. Masamune, S. Kamata, and W. Schilling, ibid., 1975, 97, 3515.

58. J. Huang and J. Meinwald, J. Amer. Chem. Soc., 1981, 103, 861.

59. E. Vedejs, Kagaku Zokan (Kyoto), 1983, 99, 59.

60. E. Vedejs and S.D. Larsen, J. Amer. Chem. Soc., 1984, 106, 3030.

61. K. Narasaka, T. Sakakura, T. Uchimaru, and D. Guédin-Vuong, J. Amer. Chem. Soc., 1984, 106, 2954.

62. S.E. Drewes and A.T. Pitchford, J. Chem. Soc. Perkin Trans. 1, 1981, 408.

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

64. K.C. Nicolaou, Tetrahedron, 1977, 33, 683.

65. T.G. Back, Tetrahedron, 1977, 33, 3041.

66. E. Haslam, Tetrahedron, 1980, 36, 2409.

67. S. Masamune, Aldrichimica Acta, 1978, 11, 23.

68. P. Deslongchamps, S. Lamothe and H. Lin, Can. J. Chem. 1984, 62, 2395.

69. G. Illuminati et al., J. Amer. Chem. Soc., 1977, 99, 2591.

70. A.C. Oehlschlager, J.W. Wong, V.G. Verigin and H.D. Pierce Jr., J. Org. Chem., 1983, 48, 5009.

71. E.J. Corey and D.J. Brunelle, Tet. Letts., 1976, 3409.

72. H. Gerlach and A. Thalmann, Helv. Chim. Acta., 1974, 57, 2661.

73. T. Mukaiyama, M. Usui and K. Saigo, Chem. Letts., 1976, 49.

74. J.G. Millar, A.C. Oehlschlager and J.W. Wong, J. Org. Chem., 1983, 48, 4404.

75. T. Kurihara, Y. Nakajima and O. Mitsunobu, Tet. Letts., 1976, 28, 2455. O. Mitsunobu, Synthesis, 1981, 1.

76. H. Tsutsui and O. Mitsunobu, Tet. Letts., 1984, 25, 2163.

77. A. Ahmed, N. Taniguchi, H. Fukuda, H. Kinoshita, K. Inomata and H. Kotake, Bull. Chem. Soc., Jpn., 1984, 57, 781.

78. N. Taniguchi, H. Kinoshita, K. Inomata and H. Kotake, Chem. Letts., 1984, 1347.

79. E.P. Boden and G.E. Keck, J. Org. Chem., 1985, 50, 2394.

80. S. Mohanraj and W.T. Ford, J. Org. Chem., 1985, 50, 1616.

81. R.A. Amos, R.W. Emblidge, N. Havens, J. Org. Chem., 1983, 48, 3598.

82. S.L. Regen, and Y.J. Kimura, J. Amer. Chem. Soc., 1982, 104, 2064.

83. W.H. Kruizinga and R.M. Kellog, J. Amer. Chem. Soc., 1981, 103, 5183.

84. Professor G.A. Sim, unpublished results.

85. R.J. Molyneux and J.N. Roitman, J. Chromatogr., 1980, 195, 407.

86. L.M. Jackman, "Application of Nuclear Magnetic Resonance Spectroscopy to Organic Chemistry," Pergamon Press, 1959; p.55.

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

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

89. G.S. Bates, J. Diakur, and S. Masamune, Tet. Letts., 1976, 49, 4423.

90. H.A. Staab, Angew. Chem. Internat. Edit., 1962, 7, 351.

91. E.J. Corey and B.B. Snider, J. Amer. Chem. Soc., 1972, 94, 2549.

92. C.L. Liotta and H.P. Harris, J. Amer. Chem. Soc., 1974, 96, 2250.

93. N. Ono, T. Yamada, T. Saito, K. Tanaka and A. Kaji, Bull. Chem. Soc. Jpn., 1978, 51, 2401.

94. A.R. Mattocks, Nature, 1971, 232, 476.

95. A.R. Mattocks and I.N.H. White, Anal. Biochem., 1970, 38, 529.

96. H.E. Driver and A.R. Mattocks, Chem.-Biol. Interact. 1984, 51, 201.

97. A.R. Mattocks, Chem.-Biol. Interact., 1981, 35, 301.

98. A.R. Mattocks, Toxicology Letters, 1982, 14, 111.

99. S. Kohlmünzer and H. Tomczyk, Diss. Pharm. Pharmacol., 1969, 21, 433.

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

101. D.R. Burfield and R.H. Smithers, J. Org. Chem., 1978, 43, 3966.

102. R.L. Augustine, Z.S. Zelawski and D.H. Malarek, J. Org. Chem., 1967, 32, 2257.