THE UNIVERSITY OF CALGARY

ISOLATION AND CHEMICAL CHARACTERISATION OF PYRROLIZIDINE ALKALOIDS FROM SOME *SENECIO*

by

OBUYA WERE STEVENS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Isolation and Chemical Characterisation of Pyrrolizidine Alkaloids from some *Senecio*", submitted by Obuya Were Stevens in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

A study of the alkaloids of three East African and two Canadian *Senecio* was undertaken. This resulted in the isolation of 12 known and 12 new alkaloids. The known alkaloids were rosmarinine, rosmarinine N-oxide, angularine, senecionine, retrorsine, 7-O-senecioyl-9-O-sarracinylretronecine, 7-O-angeloylplatynecine, 9-O-angeloylplatynecine, 7-O-angeloylretronecine, sarracine, 7-O-senecioylretronecine and cinchonidine; while the alkaloids hadiensine, neorosmarinine, 12-O-acetyhadiensine, 2-hydroxyhadiensine, 12-O-acetylneohadiensine, 12-O-acetylrosmarinine, petitianine, 12-O-acetylhadiensine N-oxide, hadiensine N-oxide, angularine N-oxide, 12-O-acetylrosmarinine N-oxide, and 7-O-senecioyl-9-O-neosarracinylretronecine (foetidine) appeared to be new. The alkaloids were characterised by spectrometric methods (MS, NMR, IR) and chemical transformations.

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To Naburi

TABLE OF CONTENTS

	Page
The approval page	ii
Abstract	iii
Acknowledgement	iv
Dedication	v
Table of Contents	vi
List of Tables	x
List of Figures	xiv
List of Schemes	xv
Abbreviations	xvi
Chapter 1 INTRODUCTION	
1.1 General Objectives	1
1.2 Structures and Biosynthesis of the PAs Encountered in *Senecio*	2
1.2.1 Structures of Pyrrolizidine Alkaloids	2
1.2.2 The Biosynthesis of Pyrrolizidine Alkaloids	2
1.2.2.1 Biosynthesis of PA Necines	2
1.2.2.2 Biosynthesis of PA Necic Acids	5
1.3 Botanical Ditribution of Pyrrolizidine Alkaloids	7
1.3.1 General Distribution	7
1.3.2 The Genus *Senecio*	9
1.4 Pharmacological and Biological Properties of Pyrrolizidine Alkaloids	10
1.5 The Role of Pyrrolizidine Alkaloids in Chemical Ecology	14
Chapter 2 RESULTS AND DISCUSSION	
2.1 The Alkaloids of *Senecio hadiensis* Forsk.	16
2.1.1 Alkaloid **A**	17
2.1.2 Alkaloid **B**	21
2.1.3 Alkaloid **C**	28

Page

2.1.4 Alkaloid **D** 32

2.1.5 Alkaloid **E** 33

2.1.6 Alkaloid **F** 40

2.1.7 Alkaloid **G** 40

2.1.8 Alkaloid **H** 43

2.1.9 Alkaloid **I** 48

2.1.10 Alkaloid **J** 54

2.1.11 Alkaloid **K** 58

2.2 The Alkaloids of *Senecio syringifolius* O. Hoff. 60

2.2.1 Alkaloid **L** 60

2.2.2 Alkaloid **M** 66

2.2.3 Alkaloid **N** 67

2.3 The Alkaloids of *Senecio canus* Hook. 70

2.3.1 Alkaloid **O** 71

2.3.2 Alkaloid **P** 76

2.4 The Alkaloids of *Senecio foetidus* Howell (syn. *S. hydrophiloides* Rydb.) 81

2.4.1 Alkaloid **Q** 82

2.4.2 Alkaloid **R** 88

2.4.3 Alkaloid **S** 89

2.4.4 Alkaloid **T** 95

2.4.5 Alkaloid **U** 96

2.4.6 Alkaloid **V** 102

2.4.7 Alkaloid **W** 104

2.5 The Alkaloids of *Dendrosenecio cottonii* J. Hutch. and G. Tayl. 108

2.5.1 Alkaloid **X** 111

Page

2.5.2 Alkaloid Y 116

2.6 Conclusion

2.6.1 The Alkaloids of *Senecio hadiensis* 121

2.6.2 The Alkaloids of *Senecio syringifolius* 122

2.6.3 The Alkaloids *Senecio canus* 123

2.6.4 The Alkaloids of *Senecio foetidus* 123

2.6.5 The Alkaloids of *Dendrosenecio cottonii* 124

2.6.6 Overall 124

Chapter 3 EXPERIMENTAL

3.1 General Experimental Procedures 125

3.2 Plant Materials 129

3.2.1 *S. hadiensis* Forsk. (syn. *S. petitianus* A. Rich) 129

3.2.2 *S. syringifolius* O. Hoff. 129

3.2.3 *S. canus* Hook. 129

3.2.4 *S. foetidus* Howell (syn. *S. hydrophiloides* Rydb.) 129

3.2.5 *D. cottonii* J. Hutch. and G. Tayl. 129

3.3 Extraction and Separation Procedures 130

3.3.1 Isolation of the Alkaloids of *S. hadiensis* Forsk. (syn. *S. petitianus* A. Rich. 130

3.3.1.1 With Reductive Processing 130

3.3.1.2 Without Reductive Processing 130

3.3.2 Separation of the Individual Aklaloids 131

3.3.2.1 Separation of the Reduced Bases 131

3.3.2.1.1 Separation of the Alkaloids mixture by RCTLC 131

3.3.2.1.2 Separation of the Alkaloid mixture by Vacuum Liquid Chromatography (VLC) 138

Page

3.3.2.1.3 Fractionation of the remaining "reduced" alkaloid mixture from *S. hadiensis* 147

3.3.2.2 Separation of the "unreduced" Bases of *S. hadiensis* 148

3.3.2.2.1 Chloroform Extract 148

3.3.2.2.2 The n-Butanol Extract 149

3.4 Chemical transformation studies with the *S. hadiensis alkaloids* 150

3.5 Isolation of the alkaloids of *S. syringifolius* O. Hoff. 158

3.5.1 With reductive processing 158

3.5.1.1 Separation of the individual alkaloids 159

3.5.2 Without reductive processing 161

3.5.2.1 Separation of the "unreduced" bases of *S. syringifolius* (chloroform extract) 161

3.6 Chemical transformation studies with *S. syringifolius* alkaloids 163

3.7 Isolation of the alkaloids of *S. canus* Hook. 164

3.8 Isolation of the alkaloids of *S. foetidus* Howell (*S. hydrophiloides* Rydb.) 165

3.8.1 Separation of the Reduced bases of *S. foetidus* 166

3.8.2 Separation of the alkaloid mixture by vacuum liquid chromatography 167

3.9 Isolation of the alkaloids of *D. cottonii* J. Hutch. and G. Tayl. 171

3.9.1 Separation of the reduced bases of *D. cottonii* 171

3.9.2 Separation of the unreduced bases of *D. cottonii* 172

3.10 Conversion of cinchonidine to deoxycinchonidine using zinc powder 173

References 174

LIST OF TABLES

Table		Page
1	Some structural groups of the necic acids	8
2	Necine stereochemistry and ΣJ_7	20
3	^{1}H NMR Chemical Shifts and assignments for alkaloid **A** and rosmarinine	22
4	^{13}C NMR Chemical Shifts and assignments for alkaloid **A** and rosmarinine	23
5	^{1}H and ^{13}C NMR data for alkaloid **B**	25
6	^{1}H and ^{13}C NMR data for hadienecine	29
7	^{1}H and ^{13}C NMR data for alkaloid **C**	31
8	^{1}H and ^{13}C NMR data for alkaloid **79**	34
9	^{1}H and ^{13}C NMR data for aklaloid **D**	35
10	^{1}H and ^{13}C NMR data for 1,12-di-O-acetylhadiensine (**80**)	36
11	^{1}H and ^{13}C NMR data for alkaloid **E**	39
12	^{1}H and ^{13}C NMR data for alkaloid **F**	41
13	^{1}H and ^{13}C NMR data for 2-O-acetylrosmarinine	44
14	^{1}H and ^{13}C NMR data for 12-O-acetylrosmarinine	45
15	^{1}H and ^{13}C NMR data for 2, 12-di-O-acetylrosmarinine	46
16	^{1}H and ^{13}C NMR data for alkaloid **H**	49
17	^{1}H NMR Chemical Shifts and Assignments for alkaloid **I** and 12-O-acetylhadiensine	52
18	^{13}C NMR Chemical Shifts and Assignments for alkaloid **I** and 12-O-acetylhadiensine	53
19	^{1}H NMR Chemical Shifts and Assignments for alkaloid **J** and hadiensine (**70**)	56

LIST OF TABLES

Table		Page
20	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **J** and hadiensine	57
21	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **K** and rosmarinine (**68**)	61
22	^{13}C NMR Chemical Shifts and Asignments for Alkaloid **K** and rosmarinine (**68**)	62
23	^{1}H and ^{13}C NMR data for Alkaloid **L**	65
24	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **M** and angularine (**92**)	68
25	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **M** and angularine (**92**)	69
26	^{1}H NMR Chemical Shift and Assignments for Alkaloid **N** and 12-O-acetylrosmarinine	72
27	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **N** and 12-O-acetylrosmarinine (**85**)	73
28	Chemical shift difference of H-9 protons in 11 and 12 membered dilactones of retronecine	76
29	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **O** and senecionine (**4**)	77
30	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **O** and senecionine (**4**)	78.
31	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **P** and retrorsine (**76**)	79
32	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **P** and retrorsine	80
33	^{1}H NMR Chemical shifts and Assignments for Alkaloid **Q** and 7-O-senecioyl-9-O-sarracinylretronecine	86
34	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **Q** and 7-O-senecioyl-9-O-sarracinylretronecine	87

LIST OF TABLES

Table		Page
35	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **R** and 7-O-angeloylplatynecine (**100**)	90
36	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **R** and 7-O-angeloylplatynecine (**100**)	91
37	^{1}H NMR Chemical Shifts and Asignments for Alkaloid **S** and 9-O-angeloylplatynecine (**101**)	93
38	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **S**	94
39	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **T**, **103** and **102**	97
40	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **T**, **103** and **102**	98
41	^{1}H NMR Chemical Shift and Assignments for Alkaloid **U**, and sarracine (**67**)	100
42	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **U** and sarracine (**67**)	101
43	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **V** and 7-O-senecioylretronecine (**105**)	105
44	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **V** and **105**	106
45	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **W** and 7-O-senecioyl-9-O-sarracinylretronecine (**99**)	109
46	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **W** and 7-O-senecioyl-9-O-sarracinylretronecine (**99**)	110
47	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **X** and **111**	114
48	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **X**	115
49	^{1}H NMR Chemical Shifts and Assignments for Alkaloid **Y** and **111**	118

LIST OF TABLES

Table		Page
50	^{13}C NMR Chemical Shifts and Assignments for Alkaloid **Y** and cinchonidine (**110**)	119
51	Radial centifugal thin layer chromatography of crude alkaloid mixture (0.85 g). The eluant was $CHCl_3$-MeOH-NH_4OH (100:20:1)	132
52	Vacuum column chromatography for the fractionation of crude alkaloid mixture (3.5 g)	140
53	PA profile for fractionation of crude alkaloid mixture (3.49 g) of *S. hadiensis* by vacuum colunn chromatography	141
54	PA profile for *S. syringifolius* as determined by GLC analysis	159
55	PA profile for *S. canus* as determined by GLC analysis	164
56	PA profile for *S. foetidus* determined by GLC analysis	166
57	Vacuum column chromatography for the fractionation of crude alkaloid mixture (3.5 g) from *S. foetidus*	168
58	PA profile for *D. cottonii* as determined by GLC analysis	172

LIST OF FIGURES

Figure		Page
1	Some necines	4
2	Some necic acids	5
3	A proposed mechanism for the formation of Biological Alkylating Agents from PAs	12

LIST OF SCHEMES

Scheme		Page
1	The Biosynthesis of pyrrolizidine necine systems	6
2	Biosynthesis of pyrrolizidine necic acids	7
3	Mass spectral fragmentation of non-macrocyclic unsaturated diesters	85
4	An alternative mass spectral fragmentation at C-9 of non-macrocyclic unsaturated diesters	85
5	Mass spectral fragmentation of non-macrocyclic saturated diesters	102

ABBREVIATIONS

The abbreviations used in this thesis are those employed by the Journal of Organic Chemistry, and most of them are identified in the text at their first appearance.

CHAPTER 1. INTRODUCTION

1.1 General Objectives

The work described in this thesis began as an exercise in comparative chemotaxonomy and a search for toxic alkaloids in three East African species of *Senecio*. The scope of this project was ultimately expanded to include work on two Albertan *Senecio*.

The starting point of these studies was an examination of the pyrrolizidine alkaloid (PA) content of *S. hadiensis* Forsk.(syn. *S. petitianus* A. Rich), *S. syringifolius* O. Hoff. and *Dendrosenecio cottonii* J. Hutch. and G. Tayl. The first two of these plants are trailing climbers common along the edges of the forests in the interior of Kenya. Both have enjoyed some use as herbal medicine [1], and the ingestion of *S. hadiensis* has been associated with liver-fluke in cattle [2]. The third is an example of the giant tree-*Senecio* of the Afro-alpine regions. We focussed upon their PA contents because a very considerable body of information has been accumulated on the appearances of these alkaloids in *Senecio* species outside East Africa and because they are known to be associated with a variety of biological effects. Thus, by examining the PA content of our East African species we planned to gain data which could be compared with those of other members of this genus, and as well obtain some insight into their alleged medicinal properties. The chemotaxonomy of *Senecio* and the physiological properties of the PAs, as well as the chemistry of these alkaloids receive further attention in the sections which follow.

1.2 Structures and Biosyntheses of the PAs Encountered in Senecio

1.2.1 Structure of Pyrrolizidine Alkaloids

Pyrrolizidine alkaloids (PAs) are characterised by the presence of the 1-azabicyclo[3.3.0]octane system (**1**), indexed in Chemical Abstracts as hexahydro-1H-pyrrolizine. Usually this system is elaborated by the addition of a single carbon at the C-1 (pyrrolizidine ring numbering), oxygenation and esterification as illustrated by anagyroidine (**2**), europine (**3**) and senecionine (**4**). They often co-occur with the corresponding N-oxides, and occasionally as N,8-*seco*-derivatives such as senkirkine (**5**). The occurrence of pyrrolic tetradehydropyrrolizidines (dihydro-1-H-pyrrolizine), as for example **6** has been observed in some *Senecio* species [3-5] and may be more widespread. In the case of the ester PAs, the esterifying acids are called necic acids, while the pyrrolizidinol units are known as necines. Some examples of these constituent necines and necic acids are given in Figures 1 and 2.

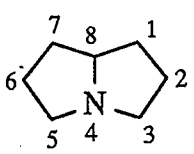
For the purposes of this thesis, certain other types of PAs, such as loline (**7**) and thelepogine (**8**), will be excluded, since these are not PAs of the kinds associated with *Senecio*.

1.2.2 The Biosynthesis of Pyrrolizidine Alkaloids

Although some PAs occur unesterified [6, 7], they usually occur as esters. The biosyntheses of the latter are conveniently viewed as involving the construction of the necines and acids separately.

1.2.2.1 Biosynthesis of PA Necines

Studies of the biosyntheses of PA necines have mainly concentrated on retronecine (**15**). It has been shown that this is constructed from putrescine (**33**) via homospermidine (**34**). Putrescine (**33**) is derived from ornithine (**31**) [8-14] or arginine (**32**) [11, 12] (only



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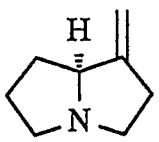

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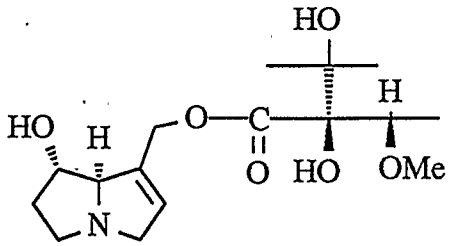

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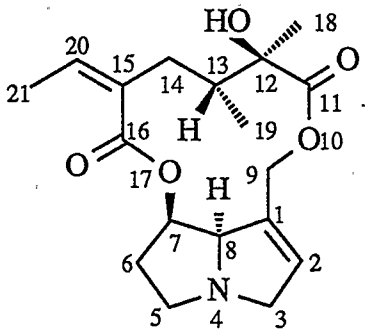

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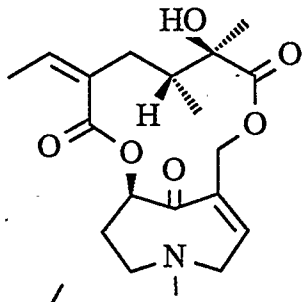

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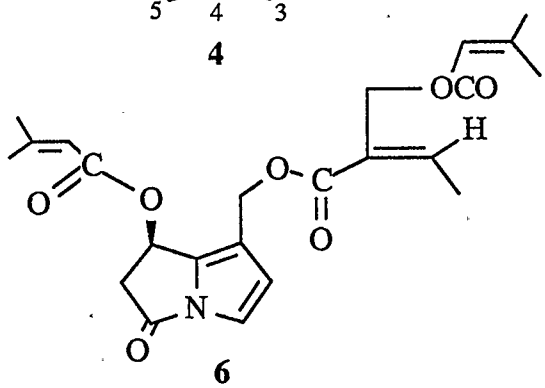

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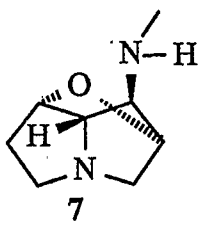

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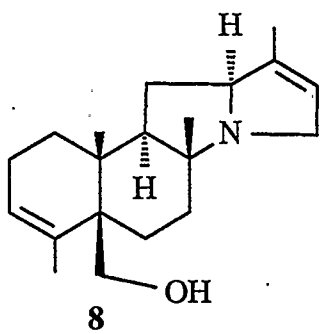

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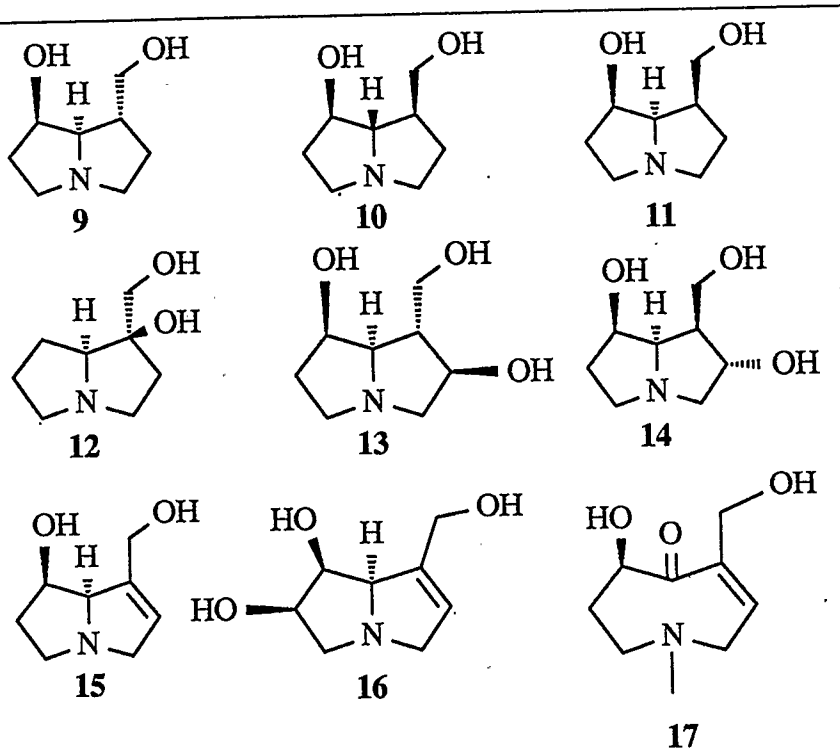
the L-enantiomer being incorporated [15]). The involvement of homospermidine (**34**) was established by its conversion into trachelanthamidine (**37**) [16] which was shown to be a precursor for retronecine (**15**) [17, 18]. Similarly it has been proved in *Senecio*



(**9**) (-)- Turneforcidine (**10**) (-)- Hastanecine

(**11**) (-)- Platynecine (**12**) Curassanecine

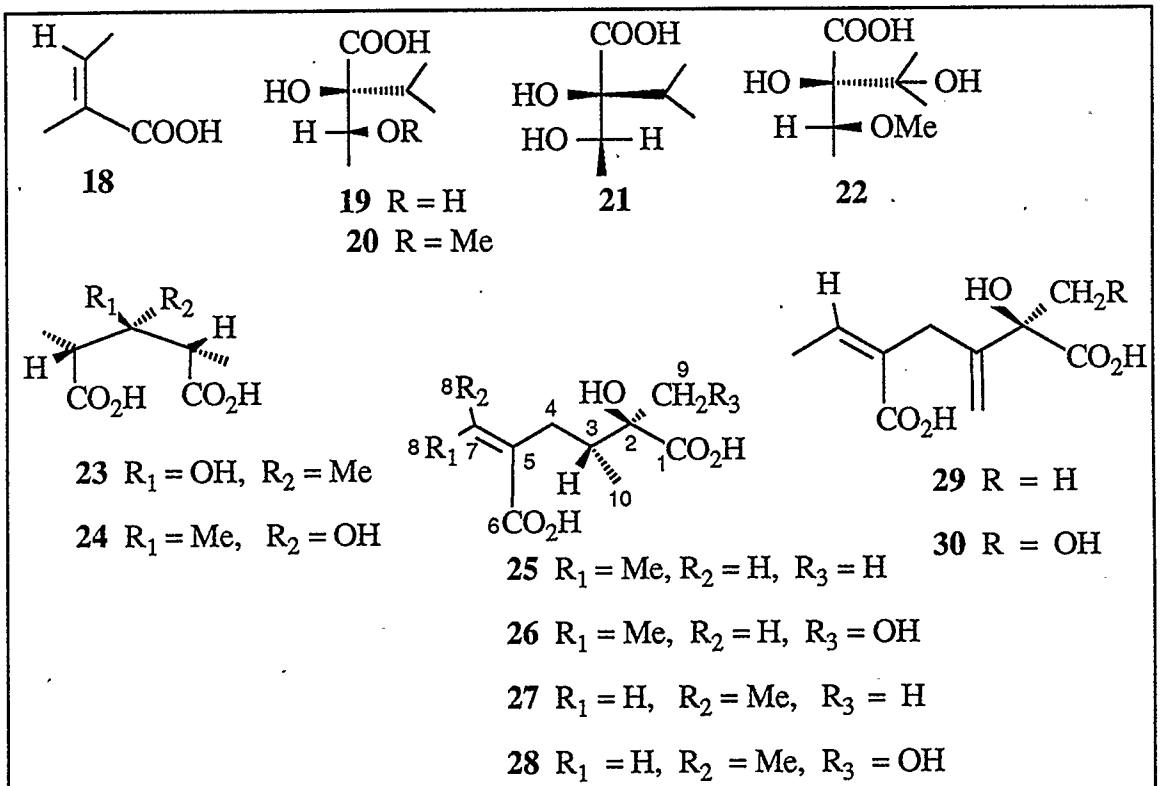
(**13**) Croalbinecine (**14**) (-)- Rosmarinecine

(**15**) (+)- Retronecine (**16**) (+)- Crotanecine

(**17**) Otonecine

Figure 1: Some necines

pleistocephalus plants that the biosynthesis of rosmarinecine (**14**) takes place from putrescine (**33**) via homospermidine (**34**) [19]. However, unlike the formation of retronecine (**15**), isoretronecanol (**38**) is much better incorporated into rosmarinecine (**14**) than trachelanthamidine (**37**) [18]. It is now thought that homospermidine (**34**) is oxidized by a diamine oxidase to yield a dialdehyde (**35**), which then undergoes intramolecular cyclisation to give 1-formylpyrrolizidine (**36**) [18]. This aldehyde undergoes reduction to yield trachelanthamidine (**37**) and isoretronecanol (**38**) which are then converted to retronecine (**15**) and rosmarinecine (**14**) respectively. This biosynthesis of the



18 Angelic acid

19 (+)- Trachelanthic acid

20 Heliotric (Heliotrinic acid)

21 (-) - Viridifloric acid

22 Lasiocarpic acid

23 Fulvinic acid

24 Crispatic acid

25 Senecic acid

26 Isatinecic acid

27 Integerrinecic acid

28 Retronecic acid

29 Seneciphyllic acid

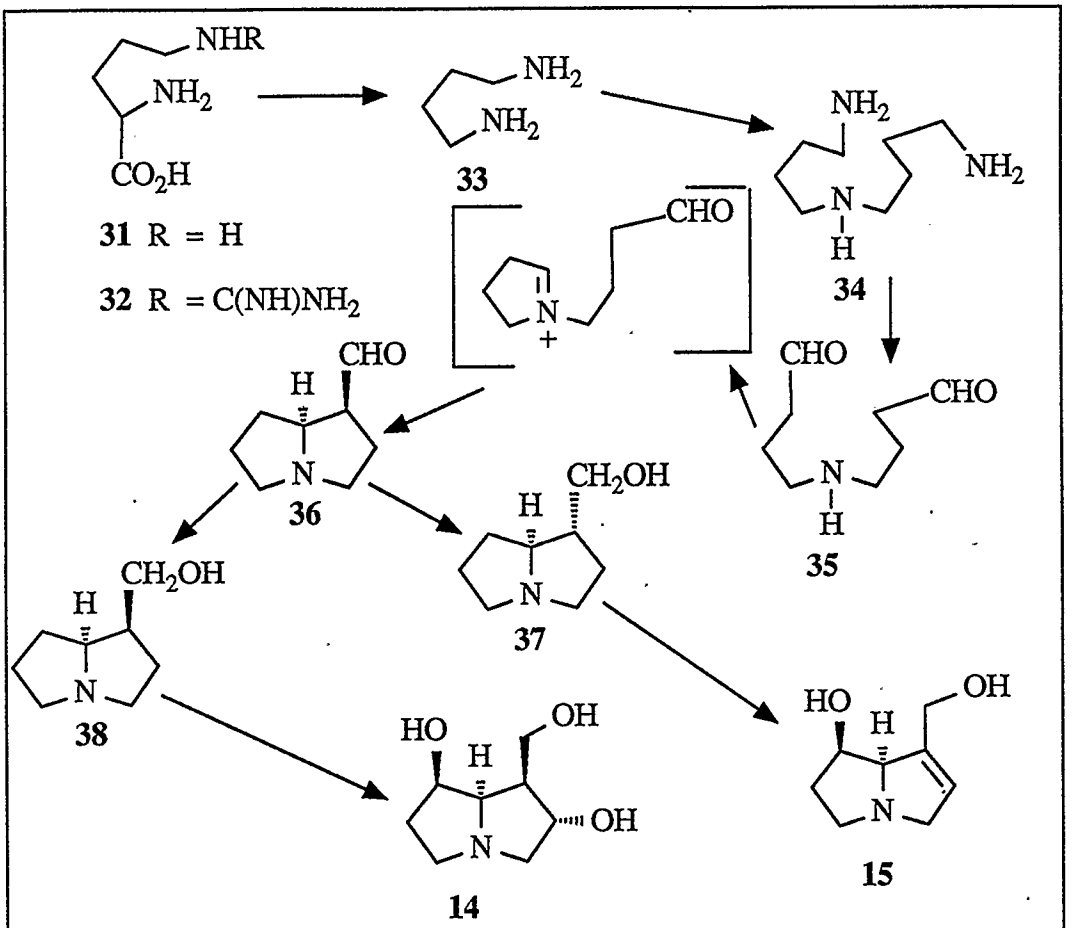
30 Riddellic acid

Figure 2: Some necic acids

pyrrolizidine system is summarized in Scheme 1.

1.2.2.2 Biosynthesis of PA Necic Acids

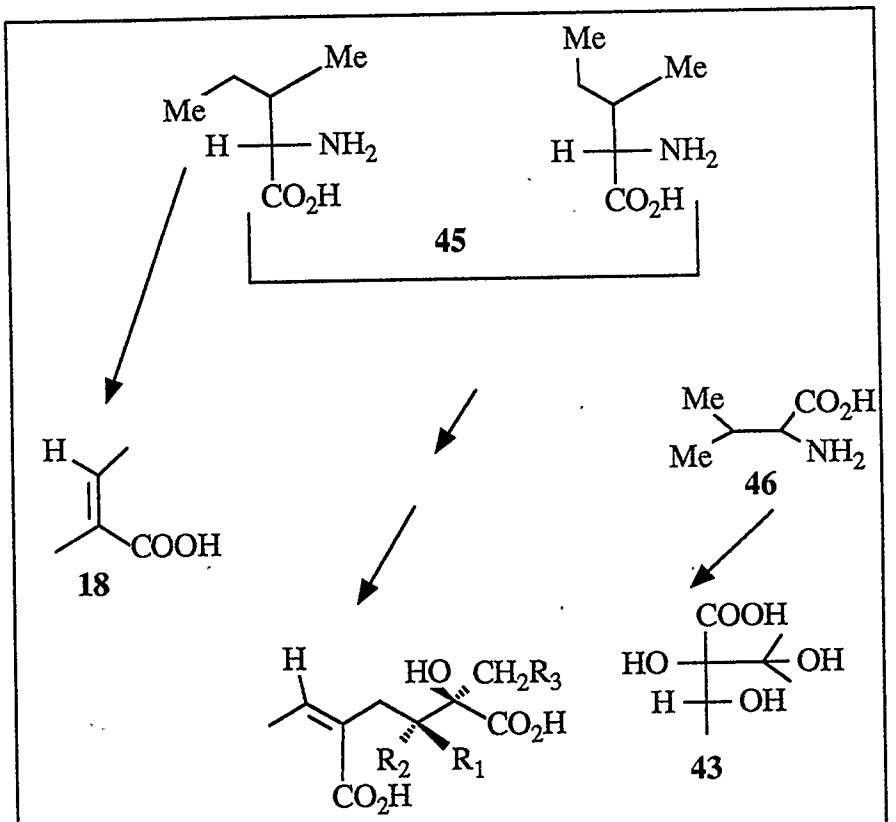
Most PA necine diols are esterified with one or two acids, often with five carbon



Scheme 1: The Biosynthesis of Pyrrolizidine necine systems [18-21].

atoms, or dilactonised with a dibasic acid often containing 10 carbon atoms. Other acids with six, seven, eight and nine carbon atoms have also been isolated from esters of the necines especially from plants of the families Boraginaceae and Fabaceae [22] . Some of the commonly encountered necic acids are shown in Figure 2 and Table 1. Of these necic acids some appear to have isoprene skeletons, however investigations have revealed that they are not derived via the acetate-mevalonate pathway but instead come from the common branched-chain amino acids [23]. Thus, angelic acid (**18**) is biosynthesized from isoleucine (**45**) [24] and the C_{10}-necic acids such as senecic (**25**), isatinecic (**26**) and seneciphyllic (**29**) are formed from two molecules of L-isoleucine (**45**) [25-27] although the means by which the two C_5-units are coupled has yet to be established. Echimidic acid (**43**), a C_7 acid, on the other hand, is biosynthesized from valine (**46**) [22]. The

biosynthesis of the necic acids is summarized in Scheme 2.



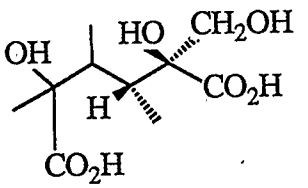
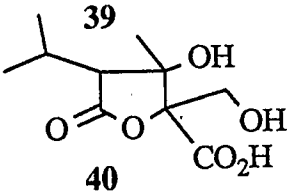
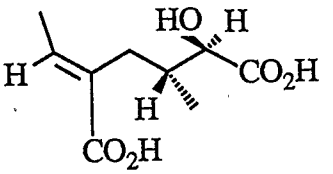
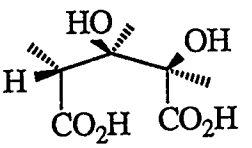
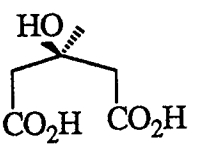
Scheme 2: Biosynthesis of Pyrrolizidine necic acids.

1.3 Botanical Distribution of Pyrrolizidine Alkaloids

1.3.1 General Distribution

Unlike many other alkaloids, PAs are not restricted within the plant kingdom to a single family. Indeed they may be the most widely distributed structural type of alkaloids. About 200 PAs are known, distributed through 300 plant species in more than 60 genera in

Table 1: Some structural groups of the necic acids.

Carbon atom	Structure	Name
C_{10}	**25**	Senecic acid
C_{10}	 **39**	Sceleranecic acid
C_{10}	 **40**	Junceic acid
C_9	 **41**	Nilgiric acid
C_8	 **42**	Monocrotalic acid
C_7	**43**	Echimidinic acid
C_6	 **44**	Dicrotalic acid
C_5	**18**	Angelic acid

at least 13 families [28-31]. Most of these alkaloids have been isolated from the families Boraginaceae (all genera), Asteraceae [synonym Compositae] (tribes Senecioneae and Eupatorieae), Fabaceae [synonym Leguminosae, or Papilionaceae] (genus *Crotalaria*) and Apocynaceae [28, 29].

1.3.2 The Genus *Senecio*

Prominent among the genera of plants which produce PAs is *Senecio*. Indeed the old generic name for the PAs was Senecio-alkaloids, reflecting an association which pre-dated the recognition that this class of alkaloids had a much wider distribution in Nature.

The genus *Senecio* belongs to the family Asteraceae [Compositae], which is thought to be the largest of the families of vascular plants, encompassing about a tenth of all species of flowering plants [32]. In the usual taxonomic treatment , the family is sub-divided into the Asteroideae (old name Tubuliflorae) and the Cichorioideae (old name Liguliflorae) [32-34]. A further subdivision views the Cichorioideae as comprised of a single tribe, the Lactuceae, but breaks the Asteroideae into twelve tribes, namely, Vernonieae, Eupatorieae, Astereae, Inuleae, Heliantheae, Helenieae, Anthemideae, Senecioneae, Calenduleae, Arctotideae, Cynareae and Mutisieae, with the Senecioneae containing and being named after the genus *Senecio* [32].

There have been two phyletic interpretations of the family Asteraceae [35]. The first one placed the Senecioneae at the base and suggested a later derivation of the Eupatorieae from the Astereae. The second one derived all tribes from the Heliantheae and suggested the Eupatorieae were closest to the Vernonieae. However, so far as is presently known PAs are restricted to just two of the tribes of the Asteraceae, the Eupatorieae and the Senecioneae. Thus, it is tempting to speculate that the Eupatorieae are in fact closest to the Senecioneae i.e. to revise the phylogenetic relationships on the basis of chemical data.

The Senecioneae is an extremely large collection of species and attempts have been made to split it into smaller groups. Originally this treatment led to four sub-tribes; the Senecioninae, the Liabinae, the Tussilagininae and the Othonninae. In a further reorganisation, the subtribes Tussilagininae and Othonninae were merged with the Senecioneae, to form one large and natural subtribe Senecioninae, and a new subtribe, the Blennospermatinae was created, with the Liabinae being raised to a tribal rank, Liabeae [35]. The subtribe Blennospematinae is more of a gathering of problematic genera than a natural assemblage [35].

As thus constructed, the sub-tribe Senecioninae has about 100 genera (for a listing of these see [35]) and close to 3000 species [35, 36]. Some of the taxonomic features used to characterise the genus *Senecio,* in particular details of the morphology of the styles and stamen are absent in the giant Afro-alpine tree-*Senecio:* for which reason, as well as their extraordinary megaphtic rosette-tree form, they have been separated into their own genus, *Dendrosenecio* [36].

The investigations described in this thesis involved four traditional, "uncontroversial", *Senecio*; and one *Dendrosenecio.*

1.4 Pharmacological and Biological Properties Of Pyrrolizidine Alkaloids.

Current interest in the PAs stems in large part from their biological properties. Although some of the plants now known to be responsible for hepatotoxicity were suspected for many years as being harmful to livestock, it was not until 1911 that Cushny showed that the alkaloids isolated from *Senecio latifolius* were toxic to frogs, cats, rats and rabbits [37].

The plants that have attracted most attention in recent years are those responsible for heavy loss of livestock in many parts of the world [29, 37]. Most of the alkaloids isolated from these plants have hepatotoxic properties. All the hepatotoxic alkaloids are

esters of unsaturated necines such as retronecine (**15**), crotanecine (**16**) and otonecine (**17**). The structural requirement for hepatotoxicity in the pyrrolizidine alkaloids has been suggested to be the presence of a 1,2-double bond and of ester functions at C-7 and C-9, or C-9 alone [29,37]. The toxicity of the alkaloids is attributed to the formation in the liver of reactive pyrrolic metabolites, which are highly active alkylating agents and therefore considerably more toxic than the parent alkaloids [38]. It has been suggested that the formation of these pyrrolic metabolites involves alkyl-oxygen fission of both the C(7)-O and C(9)-O bonds in the macrocyclic alkaloids [39, 40] (see Figure 3.). If the substituent at position 7 is hydroxyl, a poorer leaving group than carboxylate, then the pyrrolic metabolite becomes a weaker alkylating agent.

A number of PAs were tested for mutagenicity on *Drosophila melanogaster* [29, 41]. Also the genotoxicity of heliotrine (**53**), monocrotaline (**54**), seneciphylline (**55**), senkirkine (**5**) and jacobine (**56**) in chick embryo has been established [42]. All the PAs that were mutagenic or had the ability to damage DNA were esters of unsaturated necines i.e. corresponded to hepatotoxic alkaloids. Pyrrole itself is inactive towards DNA. Thus the ability to damage DNA is not a property of the pyrrole ring but of esters rendered chemically reactive by their attachment to a pyrrolic nucleus [43].

Mattocks [44] suggested that the PA N-oxides *per se* are not hepatotoxic. He observed that intraperitoneal or intravenous dosing of these compounds leads to a very much reduced toxicity as compared to the figures obtained for doses made by oral administration. It was hypothesised that the relatively non-toxic N-oxides are converted to free bases in the gastro-intestinal tract [45]. Some support for this proposal comes from studies of the toxicology of heliotrine (**53**) and its N-oxide in rats. The acute LD_{50} for heliotrine (**53**) was 300 mg/Kg while that for its N-oxide was 5000 mg/Kg [44].

A number of the PA N-oxides have been observed to express anti-tumor activity, but indicine N-oxide (INO) (**57**) is the only one that has been subjected to clinical trials as

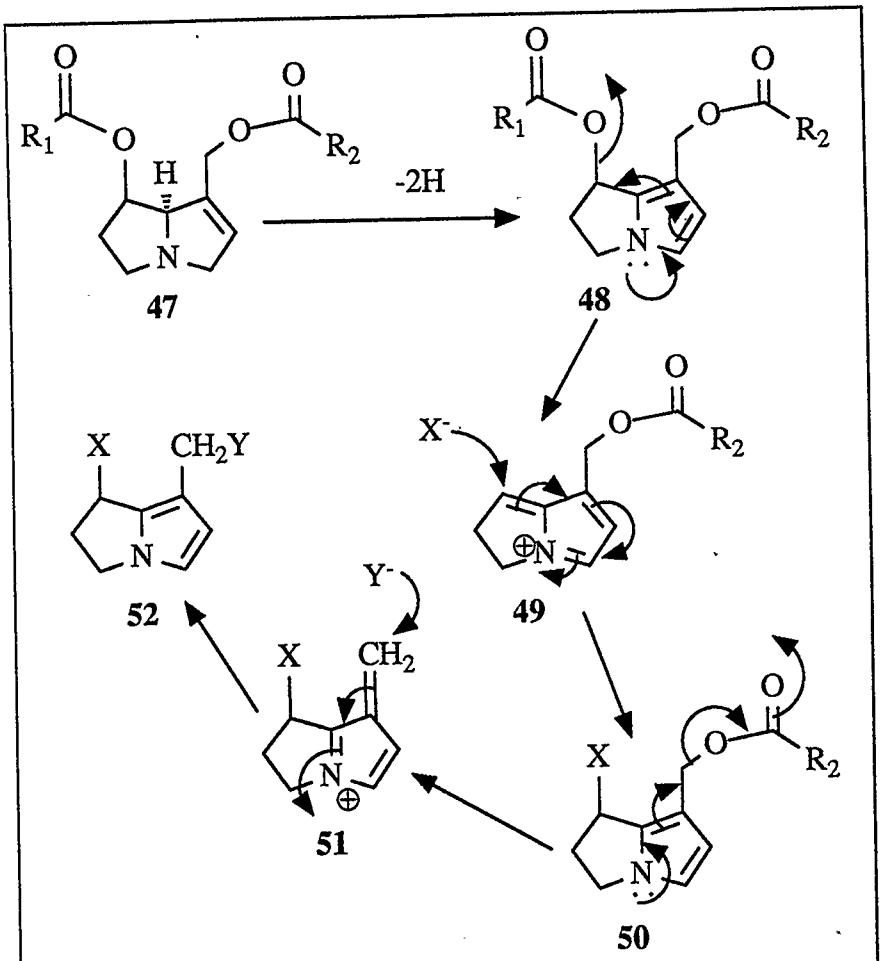
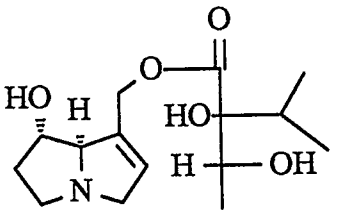


Figure 3: A proposed mechanism for the formation of Biological Alkylating Agents from PAs [39].

an anticancer drug. Unfortunately, there was no significant therapeutic response [46]. Other PAs tested include senecionine (**4**) and its N-oxide [47], monocrotaline (**54**) and its semi-synthetic derivatives, retusine (**58**), and usaramine (**59**) [48, 49]. All were active against the tumors tested except for usaramine (**59**), and the methiodide of monocrotaline. The pyrrolic alkylating catabolites also possess antitumor activity [50]. The mechanism by which the PAs inhibit tumors still requires further assessment, although one may predict conversion *in vivo* of the PAs to the pyrrolic alkylating agents.

A number of individual PAs and some plants known to contain cytotoxic PAs have been tested for carcinogenicity [29]. The results have suggested that the PAs are not



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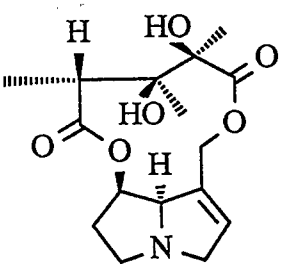

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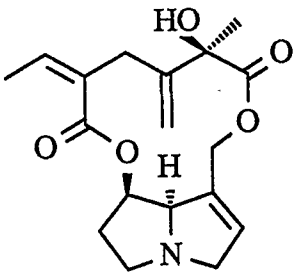

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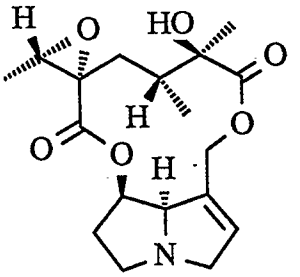

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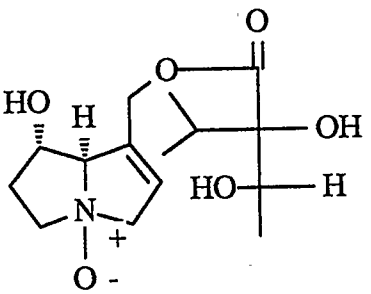

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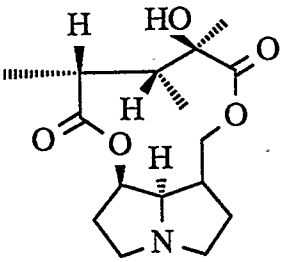

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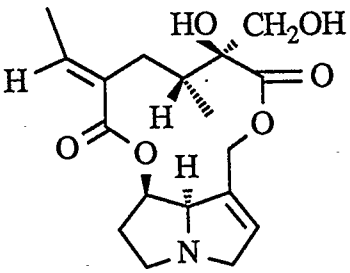
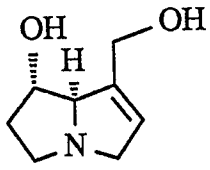


59

powerful carcinogens although it is generally accepted that some PAs and the plants containing them are carcinogenic to experimental animals. In general carcinogenic activity was demonstrated in macrocyclic or open diester PAs in which the necine is retronecine (**15**), heliotridine (**60**) or otonecine (**17**).

The non-hepatotoxic pyrolizidine alkaloid platyphylline (**61**) has been used



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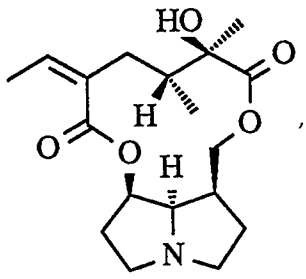
clinically for its atropine-like activity [28]. Besides this, investigations of a series of semi-synthetic derivatives of PAs have revealed a range of pharmacological activities including hypotensive, local anaesthetic, ganglion blocking, neuromuscular blocking and antispasmodic activity [51].



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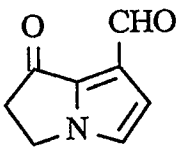
1.5 The Role of Pyrrolizidine Alkaloids in Chemical Ecology.

Pyrrolizidine alkaloids have been shown to exert anti-feeding effects on some herbivorous insects [52, 53]. However, in line with Fraenkel's prediction [54], some oligophageous insects are associated with PA producing plants. In a number of these latter cases it has been shown that not only do these insects not suffer deleterious consequences, but they actually sequester PAs: apparently using them as defensive substances, or for the formation of pheromonal compounds.

The insect-PA relationship has been studied mainly with a number of lepidopteran species within the families Daninae, Ithomiinae, Arctiidae and Ctenuchiidae [55-59]. Withering and dead plants of several PA-containing taxa, including *Heliotropium* (Boraginaceae) attract male danaine butterflies of several genera [57]. From the androconial organs and hairpencils of these butterflies have been isolated the pyrrolic PAs, danaidone (**62**), **R**-(-)-hydroxydanaidal (**63**) and danaidal (**64**). Several species of Arctiid

were attracted on exposure to hydroxydanaidal (**63**) [60]. Experimental results have shown that the ingested plant alkaloids are utilized for the production of the pheromones **62-64** that males employ in courtship. The insects must have PAs in their diet in order to produce the pheromones [61], i.e.these sex-pheromones are plant-derived, rather than the more usual products of *de novo* synthesis.

Many lepidopteran herbivores of PA containing plants have warning colours (aposematic) and store PAs, probably for protection from predators. Thus the arctiid moth, *Utetheisa ornatrix* is rejected by spiders and birds after sequestering the PA monocrotaline (**54**) from its leguminous host plants (*Crotalaria* spp.) [62]. The circumstantial link between PA content and unpalatability is supported by the observations that *U. ornatrix* reared on a PA-free diet, are eaten by spiders; and that when the alkaloids are applied to normally palatable insects such as mealworms, predatory birds and spiders reject them [62].



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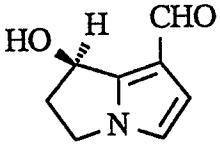

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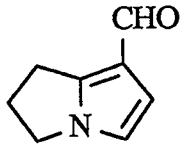


64

2. RESULTS AND DISCUSSION

2.1 The Alkaloids of *Senecio hadiensis* Forsk.

Senecio hadiensis Forsk. (syn. *S. petitianus* A. Rich.) is found along forest edges at an altitude range of 800-2700 m. In Kenya the plant has been recorded in Tinderet, Mau, Aberdares, Narok, Rift Valley including Ngong Hills, Nanyuki and Machakos district. Outside Kenya the plant has also been reported from Tanzania, Ethiopia, Somalia, Rwanda and Zaire [2].

PAs commonly occur as mixtures of tertiary bases and the corresponding N-oxides [29, 63, 64]. Since the latter are polar substances, not readily extracted from aqueous solution, it has been common practice to reduce them to the parent tertiary amine prior to isolation of the bases. Although this is convenient, since it simplifies the extraction procedures and reduces the number of compounds to be isolated, it is objectionable in as much as it destroys the natural products and changes, at least quantitatively, the pattern of PAs present in the plant. Our approach was to carry out a traditional reductive workup for our initial studies and then, once the identity of the PA tertiary amines had been established, in at least some cases, to repeat the isolation of the alkaloids without reductive processing i.e. to first identify the tertiary bases and then to establish the base/N-oxide pattern present in the plant.

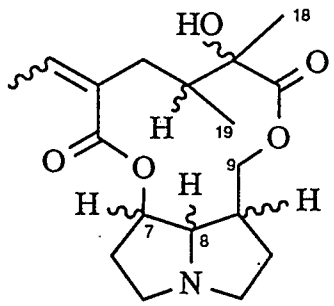
Accordingly, an exhaustive ethanol extract of air-dried epigeal parts of *S. hadiensis* was partitioned between aqueous acid and $CHCl_3$, and the aqueous phase was then stirred with zinc dust overnight. Basification and extraction with $CHCl_3$ then provided the PA tertiary bases (*ca.* 1.1% dry weight plant). GLC and TLC analyses of this material revealed the presence of at least nine compounds. Another exhaustive ethanol extract of the plant was subjected to a similar processing but

without the treatment with zinc dust. In this case the alkaloids were extracted with $CHCl_3$ and then n-butanol. Analyses (GLC and TLC) of the $CHCl_3$ extract amounting to 0.2% again revealed nine components indistinguishable as shown by GLC analysis results, but with some quantitative variations. These mixtures were then subjected to fractionation by various chromatographic procedures as described in the Experimental section, which resulted in the isolation of eleven alkaloids, **A-K**, which were homogenous or very nearly so by the TLC and/or GLC analyses. These were then characterised, largely by spectrometric methods, with the following results.

2.1.1. Alkaloid **A**

This alkaloid comprised *ca.* 34% of the total reduced bases. It was isolated as an amorphous white solid which readily crystallized from acetone (Me_2CO). The low resolution electron impact mass spectrum (EIMS) of **A** contained an apparent molecular ion at m/z 353, which was seen in the chemical ionisation (NH_3) mass spectrum (CIMS) at m/z 354 $[M+1]^+$. As its ^{13}C NMR spectrum contained 18 discrete resonances, and none appeared to correspond in intensity to coincident signals it seemed likely that **A** contained 18 carbons. A set of ^{13}C NMR DEPT experiments revealed 25 hydrogen atoms attached to carbon. Of the ^{13}C resonances, six had chemical shifts which corresponded to carbons carrying oxygen substituents: 2 ester carbonyls, one of which appeared to be saturated and the other αβ-unsaturated (δ 180.6 and 167.5 ppm); and four C-O (δ 62.1, 69.1, 75.1 and 77.5 ppm). The IR spectrum revealed the presence of hydroxyl (3408 cm^{-1}), carbonyl groups (1719, 1743 cm^{-1}), and a carbon-carbon double bond (1655 cm^{-1}). The only unsaturation appeared to be a trisubstituted alkene, seen in the ^{13}C NMR spectrum as resonances at δ 132.7 and 134.7 ppm (the single attached hydrogen

being revealed by DEPT spectroscopy as attached to the signal at δ 134.7 ppm, and appearing in the ^{1}H NMR spectrum as a single resonance in the vinylic region (δ 5.78, q, J = 7.1 Hz)). Thus, from the foregoing discussion , a molecular composition of $C_{18}H_{27}NO_6$ was formulated. This requires an index of hydrogen deficiency (IHD) of six and it seemed likely that **A** was a tricyclic compound containing two esters and one alkene functionalities. Given the traditional association of *Senecio* with pyrrolizidine alkaloids [29], a plausible possibility for **A** was that it belonged to the macrocyclic dilactonic group of PAs with the general skeleton shown in (**65**) in which the pyrrolizidine unit accounts for two of these rings, and the macro-dilactone for the third.



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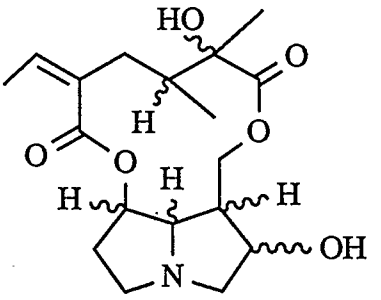
The ^{1}H-spectrum of **A** was consistent with this idea, and provided additional evidence for the location of the sixth oxygen in a secondary alcohol functionality. Of particular importance, the vinylic hydrogen resonance (δ 5.78 ppm) already noted was consistent with the presence of the ethylidene fragment, and the shift agrees well with the expectation for (Z)- but not (E)-stereochemistry: the chemical shift for the vinylic proton of the (Z)-system was calculated to be 5.99 ppm versus 6.55 ppm for the E-system [65], and the shifts observed for such isomers in the macrocyclic system of **A** are typically δ 5.68-5.92 and 6.51-7.08 ppm respectively [37]. As well, a resonance at δ 1.34 (3H, s) corresponded to a methyl group attached to a quaternary carbon carrying an oxygen i.e C-18 of (**65**), while another at δ 0.97 (3H, d, J = 6.7 Hz) was as required for C-19. Likewise, an

ABX spin system in which the AB pair were seen at δ 4.90 (1H, dd, J = 5.2 and 12.6) and 4.12 (1H, d, J = 12.6 Hz) was in accord with (**65**), in which only one of the H-9 diastereotopic protons is strongly coupled to an adjacent proton at H-1, presumably as a result of a preferred conformation of the dilactone resembling that demonstrated for senecionine (**4**) [37]. Other resonances at δ 5.05 (1H, m) and 3.55 (1H, dd, J = 3.2, 2.9 Hz) were as expected for H-7 and H-8 [37]. The remaining low-field methine resonance δ 4.23 (1H, m) suggested the presence of a secondary alcohol, and ^{1}H-^{1}H COSY and decoupling experiments showed this to be most likely vicinal to the proton assigned to position 1. At this point **A** had been deduced to have the structure **66**.



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The ^{13}C NMR spectrum of **A** was also consistent with this structure and ^{1}H-^{1}H COSY and ^{1}H- ^{13}C HXCORR spectroscopy resulted in the complete assignment of all the resonances in the ^{1}H and ^{13}C NMR spectra of **A**.

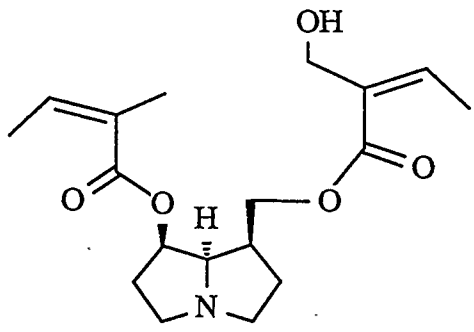
The sum of the coupling constants in H-7 as measured by width at half height (ΣJ_7) has been used previously for establishing configuration in saturated pyrrolizidines [66-69] (see Table 2). For alkaloid **A** this was 6.7 Hz, very close to the values found in platynecine (**11**) and sarracine (**67**) i.e. suggested an all-*syn* arrangement for H-1, -7, and -8. The pyrrolizidine ester macrocyclic alkaloids previously reported derived from 1-hydroxymethyl-2,7-dihydroxypyrrolizidines are croalbidine [70] and rosmarinine [71] i.e. alkaloid **A** could be a macrocyclic

ester of croalbinecine (**13**)[=helifolinecine [72]], rosmarinecine (**14**), or one of their as yet unknown stereoisomers. If the hydroxylation at C-2 was α-, the necine base would correspond to rosmarinecine and alkaloid **A** would then be rosmarinine (**68**). Comparison of the physical properties of this alkaloid and **A**, namely their ^{1}H and ^{13}C NMR (see Tables 3 and 4), mp and $[\alpha]_D$ (see Experimental section), revealed the two to be identical [71,73]. Confirmation of this conclusion was provided by saponification of alkaloid **A**, which resulted in senecic acid (**25**), identified by its mp, $[\alpha]_D$ and spectral properties (^{1}H and ^{13}C NMR, IR and MS) [74-76], and rosmarinecine (**14**), similarly identified by comparison of the $[\alpha]_D$ value and the mp with those reported for this necine [75, 76], whose characterisation was extended by ^{1}H and ^{13}C NMR spectroscopy (see page 154).

Table 2: Necine stereochemistry and ΣJ_7

Alkaloid	Relative Configuration	ΣJ_7 (Hz)
Platynecine (**11**)	H-1α, H-7α and H-8α	8.4
Hastanecine (**10**)	H-1α, H-7α and H-8β	12.0
Turneforcidine (**9**)	H-1β, H-7α and H-8α	14.1
Sarracine (**67**)	H-1α, H-7α and H-8α	6.4
Alkaloid **A**	?	6.7

Rosmarinine was first isolated from a South African plant, *Senecio rosmarinifolius* L. [77], but has since been found in other *Senecio* e.g. *S. brachypodus* DC [75], *S. hygrophyllus* R.A Dyer [75], *S. angulatus* L. [76], *S.*



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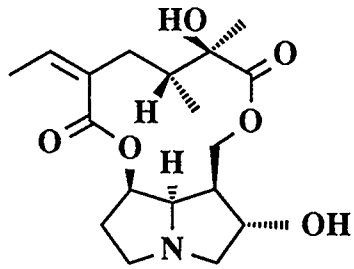


68

taiwanensis Hayata [73], *S. triangularis* Hook [71], and *S. pleistocephalus* S. Moore [19].

2.1.2 Alkaloid **B**

This was the major alkaloid (*ca.* 52 % of the reduced bases). It was obtained as a gum which gave nicely crystalline perchlorate and methiodide salts.

The EIMS of **B** revealed an apparent molecular ion at m/z 353 whose composition was established by high resolution mass measurements as $C_{18}H_{27}NO_6$. Support for this conclusion, which required **B** to be isomeric with **A** (rosmarinine) and have an IHD of 6, came from the ^{13}C NMR spectrum of **B**, which contained resonances corresponding to 18 magnetically non-equivalent nuclei. Two of these resonances corresponded to ester or lactone carbonyls, one being αβ-unsaturated (δ 167.5 ppm) and the other unconjugated (δ 178.4 ppm) [78], while two others corresponded to olefinic carbons (δ 134.7 and 131.6 ppm). With 3 IHD units thus accounted for, **B** was deduced to be tricyclic. As a working hypothesis it therefore seemed very reasonable to suspect that **B** was a macrocyclic dilactone, probably closely related to **A**.

Consistent with this idea, the ^1H and ^{13}C NMR spectra of **B** (see Table 5) exhibited resonances similar to those seen for **A** and ascribed to a C_{10}-acid moiety such as senecic acid (**25**). In particular, the (Z)-αβ-disubstituted αβ-unsaturated

Table 3: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid A and rosmarinine (**68**)

Proton	Compound	
	Alkaloid A	Rosmarinine [71]
H-1	2.50, m	2.48, m
H-2	4.23, m	4.25, ddd, J = 9.4, 8.0, 7.3 Hz
H-3a	3.07, dd, J = 7.6, 11.4 Hz	3.06,m
H-3b	2.92, dd, J = 8.1, 11.1 Hz	2.90, m
H-5a	3.27, m	3.24, ddd, J = 9.0, 7.0, 2.0 Hz
H-5b	2.58, m	2.59, m
H-6a	2.00, m	2.00, m
H-6b	2.26, m	
H-7	5.05, m	5.05, ddd, J = 3.5, 3.5, 1.5 Hz
H-8	3.55, dd, J = 3.2, 7.9 Hz	3.55, dd, J = 7.7, 3.5 Hz
H-9a	4.90, dd, J = 5.2, 12.6 Hz	4.88, dd, J = 5.5, 12.5 Hz
H-9b	4.12, d, J = 12.6 Hz	4.09, dd, J = 1.2, 12.5 Hz
H-13	1.8, m	1.77, m
H-14a	1.9, dd, J = 9.6, 13.2 Hz	
H-14b	2.26, m	2.25, m
H-18	1.34, s	1.33, s
H-19	0.97, d, J = 6.7 Hz	0.96, d, J = 6.5 Hz
H-20	5.78, q, J = 7.1 Hz	5.77, qm, J = 7.0 Hz
H-21	1.84, d, J = 7.1 Hz	1.84, dd, J = 7.0, 1.5 Hz

Table 4: ^{13}C NMR Chemical Shifts and assignments for Alkaloid **A** and rosmarinine (**68**)

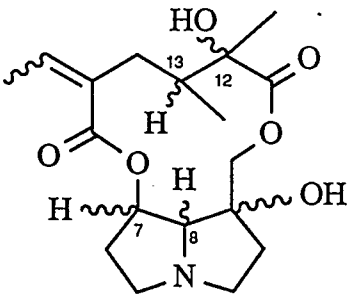
	Compound		
Carbon	Alkaloid **A**	Rosmarinine (**68**)	
		[19]	[71]
C-1	49.1	49.1	49.1
C-2	69.1	69.1	69.3
C-3	61.3	61.3	61.4
C-5	53.4	53.5	53.5
C-6	34.4	34.4	34.5
C-7	75.1	75.3	75.2
C-8	69.3	69.3	69.3
C-9	62.1	62.2	62.3
C-11	180.6	180.6	180.0
C-12	77.5	77.5	77.4
C-13	37.9	37.8	37.8
C-14	39.6	39.5	39.5
C-15	132.7	132.7	132.6
C-16	167.5	167.5	167.5
C-18	25.7	25.6	25.7
C-19	11.7	11.6	11.7
C-20	134.7	134.4	134.4
C-21	15.1	15.1	15.1

ester was recognised by the appearence of the vinylic proton resonance at δ 5.88 (q, J = 7.1 Hz) coupled to a methyl group resonating at δ 1.83 (d, J = 7.1 Hz), while other ^{1}H NMR resonances at δ 0.97 (d, J = 6.8 Hz) and 1.34 (s) could be assigned to $-\overset{|}{C}H\text{-}CH_3$ and $-\overset{|}{C}(OH)CH_3$ fragments. Resonances were observed for the corresponding carbon atoms and in the case of protonated nuclei, correlated (XHCORR) with ^{1}H NMR signals. There were however, some striking differences in the ^{1}H and ^{13}C NMR spectra of alkaloids **A** and **B**: in the ^{1}H spectrum of **B** the resonances attributed to the diastereotopic H-9 protons appeared as a simple AB system (δ 4.04, d and 4.57, d, J = 11.6 Hz), and no resonance was observed corresponding to the H-2 carbinyl one seen in the spectrum of rosmarinine; and in the ^{13}C NMR spectrum of **B** the methine resonance attributed to C-1 in **A** was replaced by a signal (δ 79.0 ppm) corresponding to another oxygenated quaternary carbon. These differences suggested that alkaloid **B** was hydroxylated at C-1 i.e. was one of the 64 stereoisomers represented by the structural formula **69**.

Two experiments served to establish the stereochemistry at C-7, 8, 12 and 13 (as well as confirming the Z-geometry of the alkene unit). The first of these involved the saponification of **B** and the isolation of the constituent necic acid and necine. The acid proved to be the same as that in rosmarinine, senecic acid (**25**), and so fixed the stereochemistry at C-12 and -13, and of the ethylidene unit. The second experiment involved the selective dehydration of **B**, an approach similar to that used on rosmarinine by Koekemer and Warren [79]. This was guided by the thoughts that if the necine unit of **B** also had the same stereochemistry at C-7 and 8 as rosmarinine, and if 1,2-elimination of the 1-hydroxy function could be achieved the product would be senecionine (**4**): an alkaloid whose absolute stereochemistry was known [80]. Acetylation of **B** was found to proceed much more readily at the C-1 tertiary hydroxyl than that at C-12 (see pages 26 and 33, and Table 8), so there

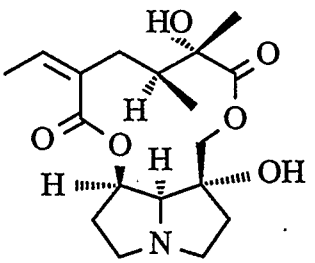
Table 5: ^{1}H and ^{13}C NMR data for Alkaloid **B**

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	-	C-1	79.0
H-2a	2.21, m	C-2	39.2
H-2b	2.19, m	C-3	53.6
H-3a	3.27, m	C-5	52.1
H-3b	2.78, m	C-6	34.5
H-5a	3.11, m	C-7	73.3
H-5b	2.72, m	C-8	77.0
H-6a	2.19, m	C-9	67.7
H-6b		C-11	178.4
H-7	5.30, q, J = 4.9 Hz	C-12	76.2
H-8	3.41, d, J = 4.9 Hz	C-13	37.2
H-9a	4.57, d, J = 11.6 Hz	C-14	38.4
H-9b	4.04, d, J = 11.6 Hz	C-15	131.6
H-13	*ca.* 1.95, m	C-16	167.5
H-14a	*ca.* 1.97, m	C-18	26.2
H-14b		C-19	13.0
H-18	1.32, s	C-20	136.2
H-19	0.97, d, J = 6.8 Hz	C-21	15.5
H-20	5.88, q, J =7.1 Hz		
H-21	1.83, d, J =7.1 Hz		



69

was some basis for attempting the selective dehydration of **B** via preferential activation of the 1-hydroxy group. In the event, when **B** was heated with mesyl chloride (MsCl) in pyridine (Py) it afforded in 44% yield a product indistinguishable from an authentic sample of senecionine. This transformation established the stereochemistry of **B** at C-7 and 8, confirmed that at C-12 and 13, and proved the regiochemistry of the dilactonisation of senecic acid onto the necine. The structure of alkaloid **B** was thus revealed to be either **70** or **71**.



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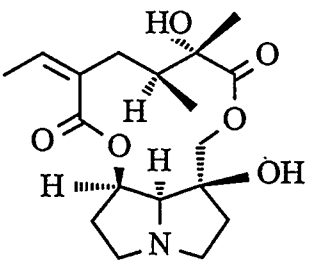
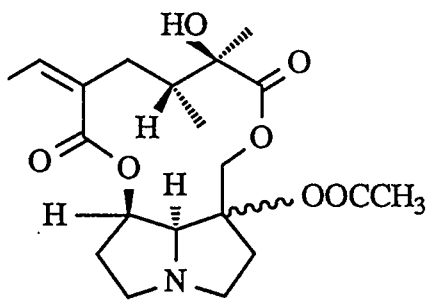


71

Of these two alternatives, the former results in a macrocyclic dilactone being attached to the necine in a *cis* fashion, but *trans* in the latter. Although both geometries are encountered in Nature, the *cis*-system, exemplified by rosmarinine (**68**) and platyphylline (**61**), is more usual. In the *cis*-case **70** the 1α-hydroxy group is *exo*-oriented with respect to the bicyclic pyrrolizidine system, but *endo* in the *trans* isomer **71**. This suggested that nOe measurements might differentiate between them.

Acetylation of alkaloid **B** with acetic anhydride (Ac_2O) in pyridine readily afforded the 1-O-acetate **72** (proof of its structure initially rested on ^1H and ^{13}C NMR data but was later corroborated by a study involving the isomeric 12-O-acetate, see page 33). This seemed a suitable substance for nOe measurement, but when the methyl protons of the acetate function were irradiated (at 200 or 400 MHz) no nOe were observed in the resonances due to H-7 or 8: results which would be consistent with an *endo*-arrangement of the 1-O-acetate, or an *exo*-one in which **72** had a preference for adopting conformations with the acetate methyl directed away from H-7 and 8. If the latter, but not the former, were the case the acetate carbonyl should then approach these two ring protons and might be expected to result in diamagnetic changes in their chemical shifts. In fact, in the ^1H NMR spectrum of **72** the resonance for H-7 was shifted downfield by 0.25 ppm as compared to the position of this signal in the spectrum of **B**. The conclusion that the stereochemical relationship between the acetate and H-7 was *syn* requires alkaloid **B** to have the structure **70**, which corresponds to 1α-hydroxyplatyphylline.

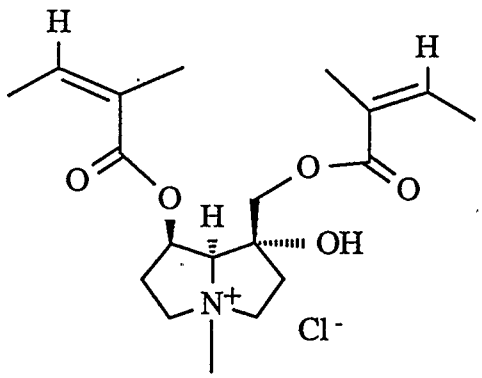


72

Among the multitude of pyrrolizidine alkaloids described to date [29, 31, 81] the only other 1-hydroxypyrrolizidine alkaloids which appear to have been reported before are curassanecine (**12**) [82], and N-methyl-O^7,O^9-diangeloyl-1-hydroxyplatynecinium chloride (**73**) [69] with the suggested but unproven

stereochemistry shown. Given this rarity, we were concerned that **B** might be an artifact produced by a reductive ring opening of a 1,2-α epoxy precusor during the isolation of the alkaloids. But, as will be recounted later, when we repeated the isolation of the alkaloids from *S. hadiensis* omitting the reductive step, alkaloid **B** was again obtained as a major component of the mixed bases. Accordingly we conclude that it is a genuine natural product, which we have named hadiensine.

Saponification of hadiensine yielded besides senecic acid (**25**) the novel necine **74,** which we have called hadienecine. This was obtained crystalline and characterised by MS, ^{1}H and ^{13}C NMR measurements (see Table 6 and also Experimental section, p. 150). The properties reported here differ significantly from those of the material which we first isolated [83] and now recognised to have been a salt of hadienecine (**74**).



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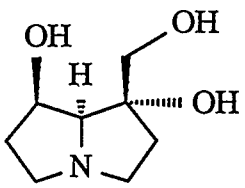


74

2.1.3 Alkaloid C

This alkaloid accounted for *ca.* 5% of the total reduced bases and was isolated as a gum from which a crystalline perchlorate salt was prepared. The molecular formula of the base was $C_{18}H_{27}NO_6$ as determined by high-resolution electron impact mass spectroscopy (HRMS), and thus isomeric with rosmarinine

Table 6: ^{1}H and ^{13}C NMR data for hadienecine*

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	-	C-1	82.4
H-2a	2.65, ddd, J = 9.6, 9.6, 12.0 Hz	C-2	36.9
H-2b	1.99, ddd, J = 2.3, 6.8, 12.0 Hz	C-3	55.5
H-3a	3.77, ddd, J =9.8, 9.8, 6.8 Hz	C-5	54.7
H-3b	2.95, ddd, J = 9.6, 9.6, 2.3 Hz	C-6	37.8
H-5a	3.36, dd, J = 8.0, 8.3 Hz	C-7	70.9
H-5b	3.12, ddd, J = 6.3, 8.3, 11.4 Hz	C-8	80.5
H-6a	2.04, dd, J = 6.3, 11.4 Hz	C-9	66.4
H-6b	1.90, dddd, J = 3.4, 8.3, 11.4, 11.9 Hz		
H-7	4.59, dd, J = 3.0, 3.0 Hz		
H-8	3.69, d, J = 3.0 Hz		
H-9a	4.49, d, J = 11.0 Hz		
H-9b	4.46, d, J = 11.0 Hz		

* In pyridine - d_5 reference δ 123.5 for carbon spectrum, and δ 7.19 for proton spectrum.

and hadiensine. The ^{1}H NMR spectrum (Table 7) contained resonances at δ 4.09 (1H, dd, J = 1.1, 12.3 Hz), 4.75 (1H, dd, J = 6.3, 12.3 Hz) and 2.47 (1H, dq, J = 1.1, *ca.* 7.5 Hz) representing an ABX system which characterises saturated macrocyclic PAs such as platyphylline (**61**), with the AB portion being assigned to H-9 and the X part to H-1. The only striking difference between this alkaloid and rosmarinine was that in the ^{1}H NMR spectrum of alkaloid C, the resonance for the vinylic C-20 proton was at δ 6.51 ppm, as compared to δ 5.78 ppm in the spectrum of rosmarinine (**68**). This suggested that this alkaloid was the (E) isomer of rosmarinine [37]. This characteristic chemical shift difference arises because of the influence of the carbonyl group on the C-20 hydrogen : there is greater deshielding of an olefinic proton when *cis* to a carbonyl than there is when it is *trans* because of the anisotropy effects of the carbonyl group [37, 84].

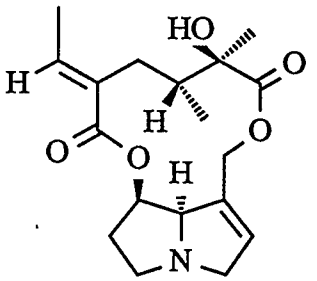
Consistent with this geometry, in alkaloid **C** the ^{13}C NMR resonance for C-14 (δ 30.8 ppm) was shifted upfield by 8.8 ppm as compared to its position in the ^{13}C NMR spectrum of rosmarinine (δ 39.6 ppm). The corresponding position of this signal in senecionine (**4**) is δ 38.3 ppm, and in its (E) isomer, integerrimine (**75**) 29.6 ppm [85, 86]. In this case the shielding effects in the two isomers are induced by the proximity of the C-21 methyl group to the C-14 methylene [78]. Complete ^{1}H and ^{13}C NMR assignment for **C** are provided in Table 7.

To confirm the stereochemistry of the ethylidene unit, and establish the stereochemistry at various chiral centres, the conversion of rosmarinine (**68**) into alkaloid **C** by ultraviolet irradiation was undertaken. Similar *cis/trans* transformations had been achieved in converting usaramine (**59**) into retrorsine (**76**) [87], and of integerrinecic acid (**27**) and its lactone into senecic acid (**25**) and its lactone [88]. The photoisomerisation of αβ-unsaturated esters and acids is documented and involves rapid interconversions of the *cis/trans* isomers, followed

Table 7: ^{1}H and ^{13}C NMR data for Alkaloid C

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	2.47, dq, J = 1.1, *ca.* 7.5 Hz	C-1	49.0.
H-2a	4.20, q, J = 7.3 Hz	C-2	68.8
H-2b	-	C-3	60.6
H-3a	3.01, dd, J = 7.2, 11.2 Hz	C-5	53.1
H-3b	2.92, dd, J = 7.3, 11.2 Hz	C-6	34.1
H-5a	3.20, ddd, J = 2.5, 8.0, 10.0 Hz	C-7	75.0
H-5b	2.59, ddd, J=6.6, 10.0, 10.0 Hz	C-8	71.5
H-6a	2.09, m	C-9	63.2
H-6b	2.26, m	C-11	180.2
H-7	5.10, m	C-12	76.8
H-8	3.65, dd, J =3.9, 7.6 Hz	C-13	38.3
H-9a	4.75, dd, J = 6.3, 12.3 Hz	C-14	30.8
H-9b	4.09, dd, J = 1.1, 12.3 Hz	C-15	133.3
H-13	*ca.* 2.00, m	C-16	168.5
H-14a	2.32, dd, J = 8.4, 13.9 Hz	C-18	26.1
H-14b	2.18, dd, J = 4.2, 13.9 Hz	C-19	12.9
H-18	1.33, s	C-20	135.9
H-19	0.99, d, J = 6.8 Hz	C-21	14.2
H-20	6.51, q, J =7.1 Hz		
H-21	1.78, d, J =7.1 Hz		

by the production of their βγ-unsaturated isomers [89-92]. In the event, irradiation of a solution of rosmarinine in 95% ethanol contained in a quartz tube with 254 nm light generated a mixture of rosmarinine, alkaloid C and a third substance which appeared to be the other expected intramolecular rearrangement product **77**. In accord with the terminology used for other pairs of geometrical isomeric pyrrolizidine alkaloids [37], alkaloid C has been named neorosmarinine (**78**).



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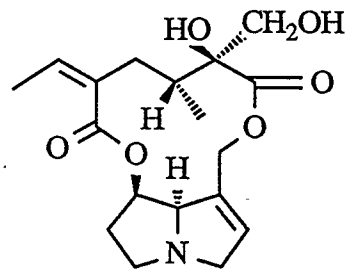

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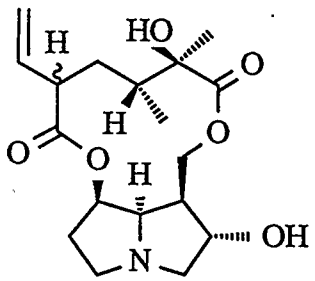

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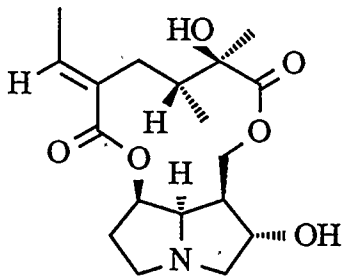


78

2.1.4 Alkaloid D

This alkaloid accounted for *ca.* 3% of the total reduced bases. It was obtained as a gum. The apparent molecular ion seen in the EIMS at m/z 395 was established by HRMS to have the composition $C_{20}H_{29}NO_7$. The ^{13}C NMR spectrum of this alkaloid contained 20 signals corresponding to 20 magnetically non-equivalent carbon atoms. DEPT spectroscopy showed the presence of six quaternary carbons, four methine, six methylene and four methyl groups. This

contrasts with the five quaternary, four methine, six methylene and three methyl groups that characterized hadiensine (**70**). The ^{1}H NMR spectrum of **D** like that of **B** displayed a simple AB system (δ 4.41, d, J = 11.6 Hz and 4.17, d, J = 11.6 Hz) corresponding to the H-9 protons of a PA, thus requiring a substituent at C-1. The presence of an acetate functionality in **D** was deduced from the presence of a methyl resonance at δ 2.10 ppm in its ^{1}H NMR spectrum, which correlated (XHCORR) with a ^{13}C NMR resonance at δ 21.5 ppm; as well as the appearance of a third carbonyl resonance (169.8 ppm). It seemed very likely that **D** was an acetate of hadiensine (**70**). However, acetylation of hadiensine gave rise to 1-O-acetylhadiensine (**79**) (see Table 8 for its ^{1}H and ^{13}C NMR data) which was not the same as alkaloid **D**. This required attachment of the acetoxy function to C-12 in **D**, as supported by the shifts for C-12 and -13 compared with that for hadiensine (**70**) (see Table 9). Proof of this was provided by acetylation of alkaloid **D** which gave the same diacetate as that obtained from hadiensine i.e. 1,12-di-O-acetylhadiensine (**80**) (see Table 10 for ^{1}H and ^{13}C NMR data). Thus alkaloid **D** was 12-O-acetylhadiensine (**81**).

2.1.5 Alkaloid **E**

This compound constituted *ca.* 3% of the total reduced bases, and was isolated as a gum.

As no satisfactory EIMS of this alkaloid was obtained, probably due to lack of volatility, we resorted to CIMS (NH_3) analysis. This showed an apparent (M+1) ion with m/z 370. The ^{13}C NMR spectrum showed 18 signals, apparently corresponding to 18 carbon atoms, of which four were observed in the sp^2 region (with two signals at δ 180.9 and 167.3 ppm corresponding to ester or lactonic carbons, and two others at δ 134.8 and 132.5 ppm seemingly olefinic) and the

Table 8: ^{1}H and ^{13}C NMR data for Alkaloid **79**

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	-	C-1	83.8
H-2a	2.49, m	C-2	40.0
H-2b	2.35, m	C-3	50.4
H-3a	*ca.* 2.80, m	C-5	50.4
H-3b	*ca.* 2.46, m	C-6	36.4
H-5a	*ca.* 2.80, m	C-7	71.5
H-5b	*ca.* 2.46, m	C-8	76.2
H-6a	*ca.* 2.47, m	C-9	66.2
H-6b	2.13, m	C-11	177.9
H-7	5.55, ddd, J = 3.3, 4.9, 4.9 Hz	C-12	75.2
H-8	3.38, d, J = 4.9 Hz	C-13	37.3
H-9a	4.42, d, J = 11.7 Hz	C-14	39.0
H-9b	4.63, d, J = 11.7 Hz	C-15	131.6
H-13	*ca.* 2.00, m	C-16	167.6
H-14a	*ca.* 2.30, m	C-18	26.0
H-14b	*ca.* 2.26, m	C-19	14.2
H-18	1.25, s	C-20	135.7
H-19	0.98, d, J = 6.8 Hz	C-21	15.6
H-20	5.85, q, J =7.2 Hz	CO	169.6
H-21	1.83, d, J =7.2 Hz	Me	21.4
Ac	2.01, s		

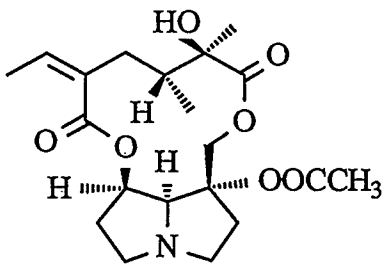
Table 9: ^{1}H and ^{13}C NMR data for Alkaloid **D**

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	-	C-1	78.7
H-2a	*ca.* 2.08, m	C-2	39.5
H-2b		C-3	52.7
H-3a	3.15, m	C-5	51.5
H-3b	2.84, m	C-6	35.0
H-5a	3.02, m	C-7	73.3
H-5b	2.78, m	C-8	77.7
H-6a	2.26, m	C-9	67.9
H-6b	2.08, m	C-11	170.7
H-7	5.30, q, J = 5.0 Hz	C-12	83.2
H-8	3.31, d, J = 4.9 Hz	C-13	40.6
H-9a	4.41, d, J = 11.6 Hz	C-14	38.1
H-9b	4.17, d, J = 11.6 Hz	C-15	131.6
H-13	1.83, m	C-16	167.5
H-14a	2.53, dd, J = 4.5, 14.3 Hz	C-18	22.7
H-14b	*ca.* 2.05, m	C-19	13.5
H-18	1.72, s	C-20	136.9
H-19	1.01, d, J = 6.8 Hz	C-21	15.5
H-20	5.89, q, J =7.1 Hz	CO	169.8
H-21	1.89, d, J =7.1 Hz	Me	21.5
Ac	2.10, s		

Table 10: ^{1}H and ^{13}C NMR data for 1, 12-di-O-acetylhadiensine (**80**)

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	-	C-1	84.6
H-2a	*ca.* 2.40, m	C-2	36.1
H-2b		C-3	50.9*
H-3a	*ca.* 2.83, m	C-5	51.0*
H-3b		C-6	38.9
H-5a	*ca.* 2.83, m	C-7	73.0
H-5b		C-8	75.9
H-6a	*ca.* 2.39, m	C-9	66.4
H-6b	*ca.* 2.08, m	C-11	170.5
H-7	5.45, m	C-12	82.5
H-8	3.51, d, J = 4.1 Hz	C-13	40.7
H-9a	4.63, d, J = 12.2 Hz	C-14	37.4
H-9b	4.35, d, J = 12.2 Hz	C-15	131.8
H-13	1.84, m	C-16	167.3
H-14a	2.63, dd, J = 5.2, 14.4 Hz	C-18	22.3
H-14b	*ca.* 2.08, m	C-19	14.4
H-18	1.65, s	C-20	136.2
H-19	1.03, d, J = 6.8 Hz	C-21	15.6
H-20	5.87, q, J =7.1 Hz	CO |	169.8 x 2
H-21	1.88, d, J =7.1 Hz	Me	21.5, 21.4
Ac	2.09, s, 2.02, s		

* May be interchanged.



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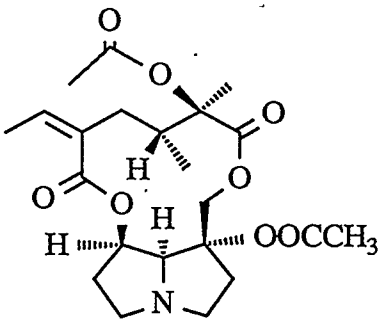

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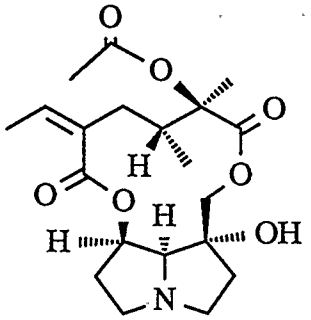


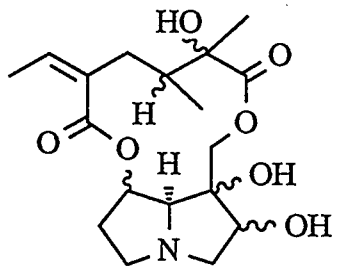
81

remainder in the sp^3 region. The chemical shifts of five of the latter appeared to correspond to oxygenated carbons (δ 65.2, 70.1, 74.1, 77.7 and 78.1 ppm). The ^1H NMR integrated for *ca.* 24 hydrogens i.e. hydrogens not attached to heteroatoms. Of the various possibilities for $C_{18}N$, that with $H_{27}O_7$ seemed most reasonable, thus the molecular formular $C_{18}H_{27}NO_7$ and a corresponding IHD 6 appeared probable. This molecular formula corresponded to one oxygen more than that in alkaloids **A-C**.

The two carbonyls and one alkene functionalities accounted for 3 units of unsaturation, thus **E** was judged to be tricyclic. As such it seemed likely to be a member of the macrocyclic dilactonic group of PAs already encountered in **A-D.** Consistent with this idea, the ^1H NMR spectrum of **E** (Table 11) revealed the presence of diastereotopic methylene protons, ascribed to those at C-9 which appeared as an AB system at δ 4.09 (1H, d, J = 12.6 Hz) and 4.46 (1H, d, J = 12.6 Hz). These signals were correlated (XHCORR) to a ^{13}C NMR resonance at δ 65.2 ppm. Comparison of the ^1H NMR spectra of **E** and **B** revealed in the spectrum of **E**

a lowfield signal absent in that of **B.** This was a carbinyl resonance at δ 4.30 (t, J = 8.4 Hz) corresponding (XHCORR) to a ^{13}C NMR methine (DEPT) resonance at δ 70.1 ppm, with the chemical shift expected for a carbon attached to oxygen. It was therefore suspected that **E** differed from **B** by an additional secondary hydroxyl group. The location of this function was deduced as follows:

A COSY spectrum of **E** showed that the proton of the additional carbinyl unit was coupled to the protons of a methylene group which appeared at δ 2.94 (1H, dd, J = 8.7, 11.1 Hz) and 3.21 (1H, dd, J = 8.3, 11.1 Hz). The carbon of this methylene was shown (XHCORR spectrum) to correspond to a signal at δ 58.6 ppm in the ^{13}C NMR spectrum. Both this chemical shift and the fact that the methylene protons were not further vicinally coupled suggested that the methylene was adjacent to nitrogen. Thus the most likely placement of the hydroxyl was at C-2. This led to a tentative structure **82**, which might well correspond to a C-2 hydroxylated **B** (hadiensine). Unfortunately, **E** was isolated in very small amount and was exhausted before saponification (to establish the identity of the necic acid) and derivatisation (if the 1,2-diol were *cis*, ketalisation with acetone might have occurred) could be attempted. Thus, its complete structure remains unestablished. Synopses of its ^{1}H and ^{13}C NMR data are provided in Table 11.



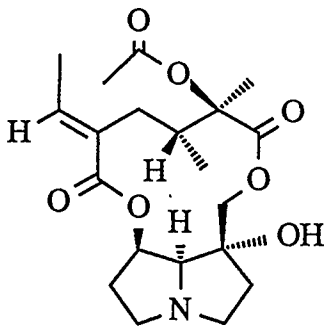
82

Table 11: ^{1}H and ^{13}C NMR data for Alkaloid **E**

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	-	C-1	78.1
H-2a	4.30, t, J = 8.4 Hz	C-2	70.1
H-2b	-	C-3	58.6
H-3a	3.21, dd, J = 8.3, 11.1 Hz	C-5	53.2
H-3b	2.94, dd, J = 8.7, 11.1 Hz	C-6	34.5
H-5a	3.33, t, J = 8.6, 8.7 Hz	C-7	74.1
H-5b	2.60, m	C-8	75.3
H-6a	2.26, m	C-9	65.2
H-6b	2.01, m	C-11	180.9
H-7	5.07, br s	C-12	77.7
H-8	3.51, d, J = 3.0 Hz	C-13	37.8
H-9a	4.46, d, J = 12.6 Hz	C-14	39.6
H-9b	4.09, d, J = 12.6 Hz	C-15	132.5
H-13	1.79, m	C-16	167.3
H-14a	1.97, dd, J = 4.2, 9.5 Hz	C-18	25.7
H-14b	2.26, m	C-19	11.6
H-18	1.35, s	C-20	134.8
H-19	0.99, d, J = 6.7 Hz	C-21	15.1
H-20	5.81, q, J =7.1 Hz		
H-21	1.87, d, J =7.1 Hz		

2.1.6 Alkaloid **F**

This alkaloid amounted to *ca.* 1% of the total reduced bases. It was obtained as a gum. The EIMS of the base revealed an apparent molecular ion m/z 395 with composition $C_{20}H_{29}NO_7$, as established by HRMS, and thus isomeric with 12-O-acetylhadiensine (**81**). In the ^1H NMR spectrum of **F** (see Table 12) the resonances corresponding to the H-9 protons were observed as a simple AB system (δ 4.15 and 4.41 ppm, each d, J = 11.4 Hz), once again indicating a substituent at the C-1 position. The presence of an acetyl group was revealed by a ^1H-NMR absorption at δ 2.13 ppm corresponding (XHCORR spectrum) to a resonance at δ 21.6 ppm in the ^{13}C NMR spectrum. Notably, the vinylic C-20 proton resonance appeared at δ 6.78 ppm as compared to the δ 5.89 ppm in 12-O-acetylhadiensine (**81**); while in the ^{13}C NMR spectrum of **F** the C-14 resonance appeared at δ 29.6 ppm as compared to δ 38.1 ppm in **81**. As discussed earlier (page 30) this suggested that alkaloid **F** was the E-isomer of 12-O-acetylhadiensine (**81**). This deduction was confirmed by photolysis of 12-O-acetylhadiensine to give roughly a 1:1 mixture of 12-O-acetylhadiensine and alkaloid **F**. This alkaloid was given the name 12-O-acetylneohadiensine (**83**).



83

2.1.7 Alkaloid **G**

This alkaloid was *ca.* 0.5% of the total reduced bases. It was obtained as a

Table 12: ^{1}H and ^{13}C NMR data for Alkaloid **F**

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	-	C-1	79.1
H-2a	1.95, m	C-2	39.1
H-2b	1.85, m	C-3	53.0
H-3a	3.15, ddd, J = 6.5, 6.5, 10.6 Hz	C-5	51.8
H-3b	2.87, ddd, J = 2.6, 7.2, 10.6 Hz	C-6	33.4
H-5a	3.01, m	C-7	73.3
H-5b	2.77, ddd, J = 4.1, 10.0, 11.5 Hz	C-8	76.9
H-6a	2.24, m	C-9	67.5
H-6b	*ca.* 1.90, m	C-11	170.5
H-7	5.45, q, J = 6.3 Hz	C-12	83.1
H-8	3.38, d, J = 6.1 Hz	C-13	41.0
H-9a	4.41, d, J = 11.4 Hz	C-14	29.6
H-9b	4.15, d, J = 11.4 Hz	C-15	131.4
H-13	*ca.* 2.10, m	C-16	167.7
H-14a	2.41, dd, J = 6.2, 14.2 Hz	C-18	22.6
H-14b	2.28, dd, J = 7.2, 14.2 Hz	C-19	14.0
H-18	1.76, s	C-20	139.1
H-19	1.05, d, J = 6.8 Hz	C-21	14.5
H-20	6.78, q, J =7.1 Hz	CO	169.8
H-21	1.80, d, J =7.1 Hz	Me	21.6
Ac	2.13, s		

gum. Attempts to prepare crystalline perchlorate and picrate salts failed. This alkaloid gave an MS which contained an apparent molecular ion at m/z 395, with composition $C_{20}H_{29}NO_7$ as established by HRMS, isomeric with 12-O-acetylhadiensine and 12-O-acetylneohadiensine. However, the ^1H NMR spectrum of **G** did not show the H-9 simple AB system which characterizes those compounds, but instead displayed an ABX multiplet consistent with a saturated pyrrolizidine alkaloid of the platyphylline (**61**) type, i.e. with hydrogen at C-1 not OH. Thus a one proton double-doublet at δ 4.10 (J = 1.0, 12.0 Hz) and another one proton double doublet at δ 4.88 (J = 5.0, 12.0 Hz), were assigned to the C-9 methylene protons and constituted the AB system, with the X portion appearing as a multiplet at δ 2.45 ppm. This was substantiated by decoupling and ^1H-^1H COSY experiments. Irradiation at the frequency of H-1 (δ 2.45 ppm) collapsed not only the H-9 double-doublets to simple AB doublets, but also reduced a one-proton double-doublet at δ 3.56 ppm to a doublet (J = 3.1 Hz) and effected simplification of a one proton quartet centred at δ 4.31 ppm (which correlated to an oxygenated carbon at δ 69.1 ppm in the ^{13}C NMR spectrum.). The signal at δ 3.56 ppm corresponded to a resonance at δ 69.4 ppm (XHCORR spectroscopy) and was assigned to H-8 as the ^{13}C chemical shift was in agreement with that found for this carbon in other pyrrolizidine alkaloids [31]. The resonance at δ 4.31 ppm was assigned (from ^1H-^1H COSY and decoupling experiments) to H-2 and so indicated attachment of the extra OH to C-2. Irradiation at the frequency of H-8 simplified, besides the H-1 multiplet at δ 2.45 ppm, a one proton multiplet at δ 4.98 ppm: which should therefore be H-7. An XHCORR spectrum then correlated that resonance to one at δ 75.6 ppm in the ^{13}C NMR spectrum.

The IR spectrum of **G** exhibited absorption bands at 1708, 1729 and 1750 cm^{-1} suggesting the presence of three carbonyl groups with one being

αβ-unsaturated and the other two being saturated esters. This was supported by the presence of ester carbonyl absorptions at δ 167.4, 169.6 and 173.4 ppm in the ^{13}C NMR spectrum. As in the previous compounds containing an acetyl group, its presence in **G** was also indicated by an absorption for the methyl group at δ 2.11 ppm in the ^{1}H NMR spectrum, with a corresponding resonance at δ 21.6 ppm in the ^{13}C NMR spectrum.

It seemed probable that **G** was an acetate of rosmarinine (**68**). Comparison of the properties (^{1}H and ^{13}C NMR) of **G** with those of 2-O-acetylrosmarinine (**84**) (see Tables 13 and 14) revealed that these were different and thus suggested that alkaloid **G** was the 12-O-acetyl compound. This conclusion was supported by a comparison of the ^{13}C NMR spectra of **G** and rosmarinine (see Tables 4 and 14). The shift for the resonances attributed to C-12 and -13 were 77.5 and 37.9 ppm in rosmarinine (**68**), and 84.5 and 40.7 ppm in **G** i.e. the δ values for C-12 and -13 were consistent with **G** being 12-O-acetylrosmarinine.

When acetylated, rosmarinine and alkaloid **G** gave the same diacetate (**85**) (see Table 15). Thus alkaloid G was 12-O-acetylrosmarinine (**86**).

2.1.8 Alkaloid **H**

Like alkaloid **G**, this alkaloid was a minor component, *ca.* 0.5%, of the total bases. It was obtained as a white powder from methanol, mp 207-209° C, and its structure established as follows:

The EIMS of **H** showed an apparent molecular ion m/z 369 with a composition $C_{18}H_{27}NO_7$, as established by HRMS. This formula implies an IHD of six. As in the previous cases there was evidence for the presence of an αβ-unsaturated ester and another nonconjugated one (^{13}C NMR resonances at δ 169.0 and 177.2 ppm; IR absorptions at υ_{max} 1717 and 1745 cm^{-1}), with the former

Table 13: ^{1}H and ^{13}C NMR data for 2-O-acetylrosmarinine (**84**)

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	2.68, m	C-1	45.0
H-2a	5.06, ddd, J = 5.0, 6.8, 6.8 Hz	C-2	74.3
H-2b	-	C-3	59.5
H-3a	3.08, dd, J = 5.1, 11.3 Hz	C-5	52.3
H-3b	2.91, dd, J = 6.8, 11.3 Hz	C-6	35.2
H-5a	*ca.* 3.14, m	C-7	75.6
H-5b	*ca.* 2.69, m	C-8	68.3
H-6a	*ca.* 2.23, m	C-9	63.1
H-6b		C-11	178.5
H-7	5.19, q, J *ca.* 4.0 Hz	C-12	76.2
H-8	3.60, dd, J = 4.2, 7.2 Hz	C-13	37.3
H-9a	4.69, dd, J = 7.6, 11.8 Hz	C-14	39.2
H-9b	4.00, dd, J = 1.7, 11.8 Hz	C-15	132.1
H-13	1.90, m	C-16	167.6
H-14a	2.26, m	C-18	26.0
H-14b	2.12, dd, J = 7.1, 13.9 Hz	C-19	13.0
H-18	1.29, s	C-20	135.2
H-19	0.96, d, J = 6.8 Hz	C-21	15.3
H-20	5.83, q, J =7.1 Hz	CO	170.7
H-21	1.82, d, J =7.1 Hz	Me	20.9
Ac	2.08, s		

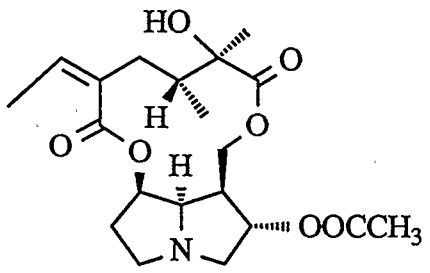
Table 14: ^{1}H and ^{13}C NMR data for 12-O-acetylrosmarinine (**86**)

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	2.45, m	C-1	49.3
H-2a	4.31, q, *ca.* 8.0 Hz	C-2	69.1
H-2b	-	C-3	61.2
H-3a	3.09, dd, J = 7.7, 11.1 Hz	C-5	53.5
H-3b	2.91, dd, J = 8.0, 11.1 Hz	C-6	34.3
H-5a	3.30, t, J = 8.7 Hz	C-7	75.6
H-5b	*ca.* 2.50, m	C-8	69.4
H-6a	2.30, dd, J = 6.4, 14.0 Hz	C-9	61.7
H-6b	2.05, m	C-11	173.4
H-7	4.98, m	C-12	84.5
H-8	3.56, dd, J = 3.1, 7.6 Hz	C-13	40.7
H-9a	4.88, dd, J = 5.0, 12.0 Hz	C-14	38.7
H-9b	4.10, dd, *ca.* 1.0, 12.0 Hz	C-15	132.7
H-13	1.72, m	C-16	167.4
H-14a	*ca.*2.5, m	C-18	22.1
H-14b	1.91, dd, J = 9.8, 14.0 Hz	C-19	12.0
H-18	1.72, s	C-20	134.7
H-19	0.99, d, J = 6.8 Hz	C-21	15.1
H-20	5.79, dq, J = 1.1, 7.1 Hz	CO	170.0
H-21	1.86, dd, J = 1.5, 7.1 Hz	Me	21.6
Ac	2.11, s		

Table 15: ^{1}H and ^{13}C NMR data for 2,12-di-O-acetylrosmarinine (**85**)

Proton	δ (ppm)	Carbon	δ (ppm)
H-1	*ca.* 2.7, m	C-1	44.0
H-2a	5.19, m	C-2	74.8*
H-2b	-	C-3	60.0
H-3a	3.16, m	C-5	52.4
H-3b	2.92, dd, J = 5.3, 11.2 Hz	C-6	34.6
H-5a	*ca.* 3.18, m	C-7	74.5*
H-5b	2.94, m	C-8	68.2
H-6a	*ca.* 2.22, m	C-9	61.6
H-6b		C-11	171.4
H-7	5.19, m	C-12	83.0
H-8	3.59, dd, J = 4.2, 7.9 Hz	C-13	40.5
H-9a	4.49, dd, J = 7.8, 12.0 Hz	C-14	37.8
H-9b	4.21, dd, J = 1.5, 12.0 Hz	C-15	132.2
H-13	*ca.* 1.70, m	C-16	167.5
H-14a	*ca.* 2.7, m	C-18	22.2
H-14b	2.01, dd, J = 7.3, 14.4 Hz	C-19	13.5
H-18	1.66, s	C-20	135.7
H-19	1.01, d, J = 6.8 Hz	C-21	15.4
H-20	5.85, q, J = 7.1 Hz	CO	170.8, 170.0
H-21	1.87, d, J = 7.1 Hz	Me	21.4, 20.9
Ac	2.08, s x 2		

* May be interchanged.



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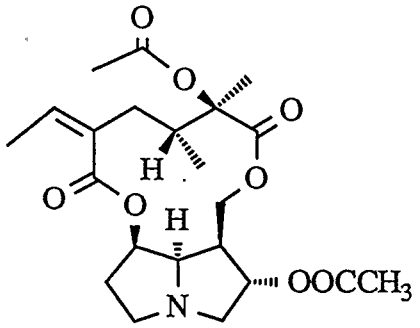

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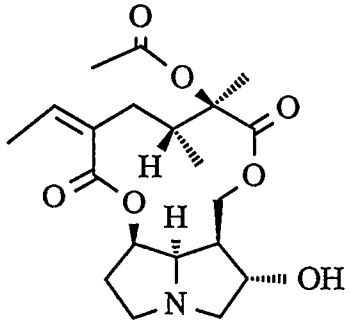


86

attached to a (Z)-ethylidene unit (^{1}H NMR δ 5.91 (1H, q, J = 7.2 Hz), 1.83 (3H, d, J = 7.2 Hz); ^{13}C NMR 136.7, 15.5 ppm). In the absence of any other indication of multiple bonds if was therefore concluded that alkaloid **H**, like **A-G**, was a tricyclic macrodilactonic PA.

A striking difference in the ^{1}H NMR spectrum of **H** as compared to that of those other alkaloids was that there were resonances for only two methyl groups: one corresponding to that in the ethylidene unit and the other, a doublet at δ 0.89 (J = 6.6 Hz), to a $\overset{|}{C}H\text{-}CH_3$ unit. In contrast with the other alkaloids isolated from *S. hadiensis* and so far discussed, there was no signal in the ^{1}H NMR spectrum of **H** that corresponded to a methyl group attached to a quaternary carbon carrying an oxygen substituent i.e. the C_{12}-C_{18} system seen in **A-G**.

Of the seven oxygen atoms in **H**, four could be attributed to a dilactone and one to a C-12 tertiary alcohol. This left two oxygens to be placed in alcohol or ether functionalities. Coupled with the absence of a C-18 methyl, this prompted the

thought that one might be at C-18. This idea was supported by the presence of signals in the ^1H NMR spectrum of **H** for a pair of magnetically non-equivalent methylene protons at *ca.* δ 3.62 ppm, appearing as an AB quartet, with the corresponding methylene carbon seen as a resonance at δ 68.5 ppm, as revealed by an XHCORR spectrum.

The placement of the remaining oxygen might be in either the necine or necic acid portion of the alkaloid. However, there was no other major modification of the ^1H and ^{13}C resonances attributable to the necic acid portion of **H** (see Table 16) and as hydroxylation at C-1 or 2 of the necine had been encountered in the other alkaloids, it seemed most probable that the additional oxygen would appear as a hydroxyl at one of these positions. It was immediately obvious that it was not at C-1 since the ^1H NMR resonance for H-9 (δ 4.10 (1H, dd, J = 1.9, 12.0 Hz and 4.78 ppm (1H, dd, J = 7.5, 12.0 Hz) appeared as an ABX system i.e. revealed coupling to H-1 at δ 2.43 (1H, m) (^1H-^1H COSY, and decoupling experiments). Thus it was concluded that **H** might well be 18-hydroxyrosmarinine (**87**), an alkaloid which does not appear to have been described before. This tentative identification of the alkaloid **H** was finally confirmed by its saponification, which yielded isatinecic acid (**26**) [93] and rosmarinecine (**14**). Alkaloid **H** was therefore 18-hydroxyrosmarinine (2α-hydroxy-1,2-dihydroretrorsine) (**87**) which we have named petitianine . This alkaloid represents the first example of a saturated 12-membered macrocyclic PA based on rosmarinecine having a hydroxyl group at position C-18.

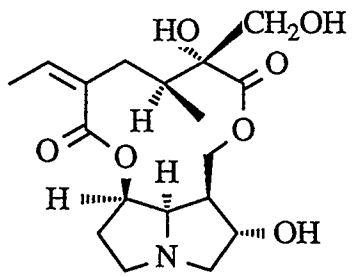
2.1.9 Alkaloid I

This alkaloid was isolated as a pale yellow oil from the chloroform extract

Table 16: ^{1}H and ^{13}C NMR data for alkaloid **H***

Proton		Carbon	δ (ppm)
H-1	2.43, m	C-1	50.0
H-2a	4.23, m	C-2	72.8
H-2b	-	C-3	63.0
H-3a	*ca.* 2.94, m	C-5	53.6
H-3b		C-6	34.9
H-5a	3.19, m	C-7	76.4
H-5b	2.63, m	C-8	69.1
H-6a	2.68, m	C-9	63.6
H-6b	2.21, m	C-11	177.2
H-7	5.23, m,	C-12	82.2
H-8	3.72, dd, J = 4.7, 7.8 Hz	C-13	36.2
H-9a	4.78, dd, J = 7.5, 12.0 Hz	C-14	40.2
H-9b	4.10, dd, J = 1.9, 12.0 Hz	C-15	133.2
H-13	2.02, m	C-16	169.0
H-14a	2.34, dd, J = 4.1, 13.0 Hz	C-18	68.5
H-14b	2.07, dd, J = 7.7, 13.0 Hz	C-19	13.2
H-18	3.60, d, J = 11.2 Hz 3.64, d, J = 11.2 Hz	C-20	136.7
H-19	0.89, d, J = 6.6 Hz	C-21	15.5
H-20	5.91, q, J = 7.2 Hz		
H-21	1.83, d, J = 7.2 Hz		

* In CD_3OD (reference CHD_2OD δ 3.31



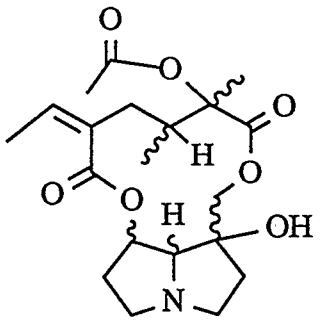
87

of the unreduced bases of *S. hadiensis*.

The CIMS (NH_3) of this compound failed to show a molecular ion. This failure might to due to the labile nature of this alkaloid and/or its lack of volatility. For this reason a fast atom bombardment (FAB) mass spectrum was obtained. This revealed an apparent pseudo-molecular ion at m/z 412. As the ^{13}C NMR spectrum showed 20 signals, apparently corresponding to 20 carbon atoms, and careful integration of the ^{1}H NMR spectrum revealed about 29 protons, it was concluded that the m/z 412 ion corresponded to an $[M+H]^+$ species, with **I** having the composition $C_{20}H_{29}NO_8$.

In the ^{1}H NMR spectrum of **I**, a signal at δ 2.05 (3H, s) corresponding to a resonance at δ 22.7 ppm in the ^{13}C NMR (XHCORR spectroscopy) together with another ^{13}C NMR resonance at δ 169.6 ppm, revealed the presence of an acetoxyl group. In the ^{13}C NMR spectrum, besides the acetate carbonyl, two other ester carbonyl resonances were observed at δ 166.3 and 171.4 ppm. Three methyl resonances seen in the ^{1}H NMR spectrum at δ 0.96 (d, J = 6.7 Hz), 1.61 (s) and 1.85 ppm (d, J = 7.I Hz) were reminiscent of a similar set seen in the spectrum of **D** and **F**, with the signal at δ 1.61 suggesting attachment of the acetoxy at C-12 of a senecic acid unit. Further, in the ^{1}H NMR spectrum of **I**, resonances at δ 4.26 and 4.41 ppm appeared as a simple AB system with geminal coupling (J = 12.6 Hz) to one another which were assigned to the C-9 methylene protons of a pyrrolizidine necine system with the corresponding carbon resonance appearing at δ 65.3 ppm

(XHCORR spectrum). These data were consistent with the structural units shown in **88**.



88

All of these data were consistent with **I** being an oxygenated derivative of 12-O-acetyhadiensine (**81**). Extensive ^{1}H and ^{13}C NMR measurements, including $^{1}H,^{1}H$-COSY and $^{13}C,^{1}H$-XHCORR spectroscopy resulted in the assignments shown in Tables 17 and 18 where they are compared with those for **81.** The ^{13}C NMR chemical shifts for C-12 and -13 appeared at δ 83.2 and 40.7 ppm and these too were consistent with acetoxylation at C-12 of a senecic unit.

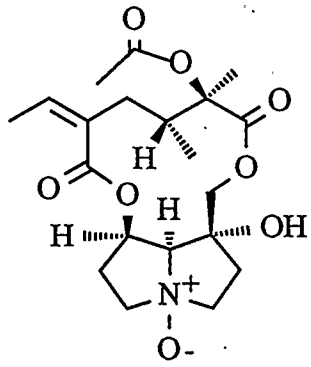
Examining the ^{1}H NMR data, it was seen that the H-3, -5 and -8 resonances of **I** were shifted downfield from their positions in **81** (see Table 17), with H-3 and -5 being the most strongly deshielded. The ^{13}C NMR spectrum revealed a similar trend (Table 18), with C-3 exhibiting the largest downfield shift (Δδ 18.1 ppm). Such deshielding effects have been observed in macrocyclic PAs as one passes from amines to the corresponding N-oxides and methiodides [94-97]. Accordingly it was concluded that alkaloid **I** was 12-O-acetylhadiensine N-oxide (**89**). This conclusion was verified by reduction of **I**, using zinc dust in aqueous H_2SO_4, which generated a product spectroscopically identical to 12-O-acetylhadiensine (**81**).

Table 17: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **I** and 12-O-acetylhadiensine.

Proton	Alkaloid **I**	12-O-Acetylhadiensine
H-1	-	-
H-2	2.48, m	*ca.* 2.08, m
H-3a	4.14, m	3.15, m
H-3b	3.73, m	2.84, m
H-5a	3.88, m	3.02, m
H-5b	3.73, m	2.78, m
H-6a	2.86, m	2.26, m
H-6b	2.16, m	2.08, m
H-7	5.45, m	5.30, q, J = 5.0 Hz
H-8	3.99, d, J = 5.5 Hz	3.31, d, J = 4.9 Hz
H-9a	4.41, d, J = 12.6 Hz	4.41, d, J = 11.6 Hz
H-9b	4.26, d, J = 12.6 Hz	4.17, d, J = 11.6 Hz
H-13	1.64, m	1.83, m
H-14a	2.79, d, J = 14.2 Hz	2.53, dd, J = 4.5, 14.3 Hz
H-14b	1.80, dd, J = 9.2, 14.2 Hz	*ca.* 2.05, m
H-18	1.61, s	1.78, s
H-19	0.96, d, J = 6.7 Hz	1.01, d, J = 6.8 Hz
H-20	5.86, q, J = 7.1 Hz	5.89, d, J = 7.1 Hz
H-21	1.85, d, J = 7.1 Hz	1.89, d, J = 7.1 Hz
Ac	2.05, s	2.10, s

Table 18: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **I** and 12-O-acetylhadiensine.

Carbon	Compound	
	Alkaloid **I**	12-O-Acetylhadiensine
C-1	79.1	78.7
C-2	36.2	39.5
C-3	70.8	52.7
C-5	67.7	51.5
C-6	32.0	35.0
C-7	73.3	73.3
C-8	90.1	77.7
C-9	65.3	67.9
C-11	171.4	170.7
C-12	83.2	83.2
C-13	40.6	40.6
C-14	37.7	38.1
C-15	131.8	131.6
C-16	166.3	167.5
C-18	22.7	22.7
C-19	13.0	13.5
C-20	136.9	136.9
C-21	15.3	15.5
CO	169.6	169.8
|		
Me	22.7	21.5



89

2.1.10 Alkaloid J

This base was isolated from the unreduced *S. hadiensis* (both $CHCl_3$ and BuOH extracts) as a pale yellow gum, with R_f 0.17 ($CHCl_3$-MeOH-NH_4OH (50:10:1)), from which a crystalline perchlorate derivative was prepared, mp 160-1° C. As with alkaloid **I**, the CIMS failed to show a molecular ion, but FAB-MS revealed an $[M+1]^+$ pseudo-molecular ion as the base peak at m/z 370, with other intense fragment ions at 354 ((M+H)-16), 156 and 138. The ^{13}C NMR spectrum revealed 18 signals, apparently corresponding to 18 carbon atoms; with two being carbonyl carbons (δ 166.7 and 178.4 ppm) and two olefinic carbons (δ 131.9 and 136.2 ppm). With the ^1H NMR of **J** integrating for 27(±2) hydrogens, the molecular formula of **J** was inferred to be $C_{18}H_{27}NO_7$.

Its ^1H NMR spectrum revealed the presence of a set of AB-type doublets δ 4.00 (1H, J = 12.5 Hz) and 4.83 ppm (1H, J = 12.5 Hz) corresponding to a methylene group, whose carbon was seen (XHCORR) as a resonance at δ 64.7 ppm in the ^{13}C NMR spectrum. These signals were assignable to the C-9 methylene of a pyrrolizidine unit, with no hydrogen at the adjacent C-1 position. An olefinic signal at δ 5.86 (1H, dq, J = 1.0, 7.1 Hz) was coupled to the signal at δ 1.86 (3H, dd, J = 1.5, 7.1 Hz), as required for a (Z)-ethylidene moiety. Two

additional methyl signals at δ 0.94 (d, J = 6.8 Hz) and 1.33 (s) were consistent with the presence of a senecic acid (or stereoisomeric) fragment, therefore requiring alkaloid **J** to have the basic skeleton **69**. All of the protons of **J** were unambiguously assigned by ^{1}H NMR COSY experiments, and these assignments confirmed by decoupling experiments. The ^{1}H-^{13}C correlated spectroscopy then revealed the corresponding carbons (see Tables 19 and 20 for the specific assignments.)

Comparison of the ^{1}H and ^{13}C NMR data of hadiensine (**70**) and **J** showed considerable similiarities between these alkaloids (see Tables 19 and 20). However, there were also some significant differences which indicated the presence of a new functional group in **J**. In particular, carbon resonances at δ 53.6, 52.1 and 77.0 ppm which corresponded to the C-3, -5 and -8 of hadiensine (**70**), were shifted to δ 71.8, 67.7 and 89.2 in **J** respectively (see Table 20). Similiarly, in the ^{1}H NMR spectrum of **J** (see table 19), the two methylene protons at δ 2.78 (1H, m), 3.27 (1H, m) and 2.72 (1H, m) and 3.11 (1H, m); and the methine proton at δ 3.41 (1H, d, J = 4.9Hz) corresponding to H-3, -5 and -8 in **B** were shifted to δ 3.80 (1H, m), 4.19 (1H, m) and 3.80 (1H, m), 3.98 (1H, m) and 3.94, (1H, d, J = 5.4 Hz) in **J**. Since the NMR spectra showed all the carbons one bond removed from the nitrogen were most deshielded in comparison with those in hadiensine (**70**), and alkaloid **J** had one more oxygen than hadiensine, these findings suggested the new base was hadiensine N-oxide (**90**). The intense ([M+H]-16) ion in the FAB-MS was also consistent with these findings [98].

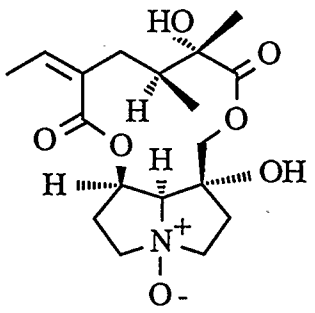
This assignment was confirmed by oxidation of hadiensine (**70**) with MCPBA in $CHCl_3$ to give its N-oxide, whose spectroscopic properties (^{1}H, ^{13}C NMR, IR, mp) were identical to those of alkaloid **J.**

Table 19: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **J** and hadiensine (**70**).

Proton	Alkaloid **J**	hadiensine (**70**)
H-1	-	-
H-2	2.49, m; 2.40, m.	2.21, m; 2.19, m
H-3a	4.19, m	3.27, m
H-3b	3.80, m	2.78, m
H-5a	3.98, m	3.11, m
H-5b	3.80, m	2.72, m
H-6a	2.91, m	2.19, m
H-6b	2.27, m	
H-7	5.43, m	5.30, q, J = 4.9 Hz
H-8	3.94, d, J = 5.4 Hz	3.41, d, J = 4.9 Hz
H-9a	4.83, d, J = 12.5 Hz	4.57, d, J = 11.6 Hz
H-9b	4.00, d, J = 12.5 Hz	4.04, d, J = 11.6 Hz
H-13	1.75, m	*ca.* 1.95, m
H-14a	2.24, d, J = 13.7 Hz	*ca.* 1.97, m
H-14b	1.92, dd, J = 10.0, 13.7 Hz	
H-18	1.33, s	1.32, s
H-19	0.94, d, J = 6.8 Hz	0.97, d, J = 6.8 Hz
H-20	5.86, dq, J = 1.0, 7.1 Hz	5.88, d, J = 7.1 Hz
H-21	1.86, dd, J = 1.5, 7.1 Hz	1.83, d, J = 7.1 Hz

Table 20: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **J** and hadiensine(**70**).

	Compound	
Carbon	Alkaloid **J**	hadiensine (**70**)
C-1	79.4	79.0
C-2	36.4	39.2
C-3	71.8	53.6
C-5	67.7	52.1
C-6	32.2	34.5
C-7	72.5	73.3
C-8	89.2	77.0
C-9	64.7	67.9
C-11	178.4	178.4
C-12	77.4	76.2
C-13	37.8	37.2
C-14	39.2	38.4
C-15	131.9	131.6
C-16	166.7	167.5
C-18	25.8	26.2
C-19	11.3	13.0
C-20	136.2	136.2
C-21	15.2	15.5



90

2.1.11 Alkaloid **K**

This alkaloid was isolated as a colourless gum from the unreduced BuOH extract of *S. hadiensis* which upon addition of H_2O followed by evaporation resulted in white crystals, mp 152 °C with decomposition (dec.).

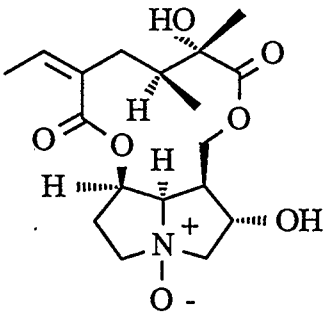
The EIMS of **K** exhibited an apparent molecular ion at m/z 353 (6), but the CIMS (NH_3) showed a higher mass ion at m/z 370 (8) with abundant fragment ions at m/z 354 (100), 199 (74), 153 (36) and 138 (43). If **K** was an N-oxide, like **I** and **J**, these findings could be rationalised as the ion with m/z 370 corresponding to an $[M+H]^+$ ion, with the EIMS ion at m/z 353 and the CIMS ion at m/z 354 corresponding to the loss of O from M^+ and $[M+H]^+$ respectively. Such MS losses of O from N-oxides are precedented [29, 31, 99, 100]. As 18 magnetically non-equivalent carbon resonances were observed in the ^{13}C NMR spectrum of **K**, with two being carbonyl (δ166.6 and 178.5 ppm), its molecular formula was deduced to be $C_{18}H_{27}NO_7$.

The IR spectrum of **K** revealed the presence of hydroxyl (3422 cm^{-1}, broad) and ester carbonyl (1720 cm^{-1}, broad) groups. The ^1H NMR spectrum showed the presence of three methyl signals: one appearing at δ 1.28 ppm as a singlet, and the other two appearing as doublets at δ 0.90 (J = 6.8 Hz) and 1.82 ppm (J = 7.1 Hz)

with the latter being coupled to the olefinic proton at δ 5.89 (J = 7.1 Hz). As well, there were signals which corresponded to an H-1, 9A, 9B ABX system in which the H-9A, 9B AB part appeared at δ 4.05 (1H, d, J = 12.4 Hz) and 4.95 (1H, dd, J = 6.9, 12.4 Hz) while the H-1 X part appeared at δ 3.28 (m). There were also resonances which could be attributed to three methine proton signals, two of which were overlapped at δ 4.29 and the other at δ 5.52 ppm, apparently corresponding to H-7, H-8 and a carbinyl group.

The ^{13}C NMR revealed two olefinic carbons at δ 131.3 and 137.2 ppm with one at δ 137.2 ppm correlated (XHCORR) to the vinylic proton at δ 5.89 ppm, and thus suggested a trisubstituted alkene. The foregoing discussion pointed towards the structure **66** and therefore rosmarinine (**68**). However, the chemical shifts for the carbon atoms directly attached to nitrogen were shifted downfield as compared to rosmarinine (**68**) (see Tables 21 and 22) and therefore required that **K** be an N-oxide, as earlier deduced from the mass spectra. Thus **K** was identified as rosmarinine N-oxide (**91**).

This assignment was confirmed by the oxidation of rosmarinine with MCPBA to the N-oxide. The oxidation product, mp 152°C (dec.) was found to be identical with the natural product by comparison of their spectral data (IR, ^{1}H and ^{13}C NMR). This is the first spectroscopic characterisation of rosmarinine N-oxide, a compound which was first prepared by the oxidation of rosmarinine with hydrogen peroxide to give a hygroscopic, amorphous powder that could not be recrystallized and which decomposed *in vacuo* at 169 °C [101].



91

2.2 The Alkaloids of Senecio syringifolius O. Hoff.

Like *S. hadiensis*, this plant is a climber, common in montane rain forest and bamboo zones, especially Elgon, Mau, Aberdares, Mt. Kenya, Kajiado district, Machakos and the Chyulu Hills of Kenya at an altitude range of 2100-3000 m, extending to 1600 m in the Chyulus. The plant is also native to Tanzania, Uganda and Malawi [2].

Both the wet and air-dried epigeal part of this plant were processed as for *S. hadiensis* resulting in a mixture of alkaloids. As detailed in the Experimental section, fractionation of the reduced and unreduced alkaloids solely by preparative TLC resulted in the isolation of the alkaloids rosmarinine, neorosmarinine, 12-acetylrosmarinine and rosmarinine N-oxide which had been identified earlier from *S. hadiensis*, plus three other alkaloids whose identification is discussed below.

2.2.1 Alkaloid **L**

This alkaloid constituted about 32 % of the reduced bases, and was isolated as a white powder easily crystallized from ethyl acetate (EtOAc) to afford prism-like crystals, mp 197-199° C.

The EIMS of **L** showed an apparent molecular ion at m/z 351 with intense

Table 21: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **K** and rosmarinine (**68**).

Proton	Alkaloid **K**	Rosmarinine (**68**)
H-1	3.28, m	2.50, m
H-2	4.29, m	4.23, m
H-3a	3.78, m	3.07, dd, J = 7.6, 11.4 Hz
H-3b		2.92, dd, J = 8.1, 11.I Hz
H-5a	3.78, m	3.27, m
H-5b		2.58, m
H-6a	2.84, m	2.00, m
H-6b		2.26, m
H-7	5.53, m	5.05, m,
H-8	4.29, m	3.55, dd, J = 3.2, 7.9 Hz
H-9a	4.95, dd, J = 6.9, 12.4 Hz	4.90, dd, J = 5.2, 12.6 Hz
H-9b	4.05, d, J = 12.4 Hz	4.12, d, J = 12.6 Hz
H-13	1.82, m	1.80, m
H-14a	2.24, d, J = 13.7 Hz	1.90, dd, J = 9.6, 13.2 Hz
H-14b	2.01, dd, J = 8.2, 13.7 Hz	2.26, m
H-18	1.28, s	1.34, s
H-19	0.90, d, J = 6.8 Hz	0.97, d, J = 6.7 Hz
H-20	5.89, q, J = 7.1 Hz	5.78, d, J = 7.1 Hz
H-21	1.82, d, J = 7.1 Hz	1.84, d, J = 7.1 Hz

Table 22: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **K** and rosmarinine (**68**).

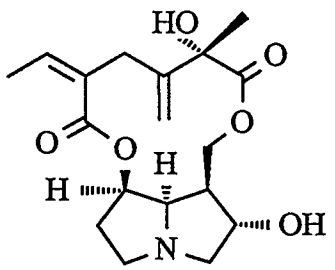
	Compound	
Carbon	Alkaloid **K**	Rosmarinine (**68**)
C-1	47.2	49.1
C-2	70.9	69.1
C-3	75.5	61.3
C-5	68.3	53.4
C-6	31.7	34.4
C-7	72.9	75.1
C-8	85.6	69.3
C-9	61.5	62.1
C-11	178.5	180.6
C-12	76.7	77.5
C-13	37.8	37.9
C-14	39.0	39.6
C-15	131.3	132.7
C-16	166.6	167.5
C-18	25.8	25.7
C-19	12.3	11.7
C-20	137.2	134.7
C-21	15.4	15.1

fragment ions at m/z 154, 151, 138, 98, 92 and 43. This apparent molecular ion was seen in the CIMS (NH_3) at m/z 352 $[M+1]^+$. As the ^{13}C NMR spectrum showed 18 signals, apparently corresponding to 18 carbon atoms, the molecular formula of **L** was deduced to be $C_{18}H_{25}NO_6$.

Two of the seven units of unsaturation required by this formula were assigned to a trisubstituted double bond (seen in the ^{13}C NMR at δ 131.7 and 135.4 ppm, with the signal at δ 135.4 ppm correlated (XHCORR) with an olefinic proton at δ 5.88 ppm in the ^1H NMR spectrum), and a terminal methylene unit (seen at δ 115.8 and 145.0 ppm in the ^{13}C NMR, with the former correlated with two broad singlet vinylic ^1H NMR resonances at δ 5.16 and 5.31 ppm). As earlier noted in alkaloids **A-K**, the presence in **L** of two lactonic functionalities, one αβ-unsaturated and the other saturated, was suggested by the ^{13}C NMR resonances at δ 166.9 and 179.3 ppm, and IR absorptions at 1723 and 1741 cm^{-1}. With a total of four units of IHD thus accounted for in multiple bonds, **L** should be tricyclic, and it was suspected that like the *S. hadiensis* alkaloids it too belonged to the macrocyclic dilactonic group of PAs. Four other ^{13}C-resonances could be ascribed to oxygenated carbons. Of these, one was a methine (δ 75.2 ppm, with the hydrogen observed at 4.95 ppm in the ^1H NMR). Another (δ 62.6 ppm) corresponded to a methylene unit whose protons appeared in the ^1H NMR at δ 4.08 (1H, d, J = 12.4 Hz), and 4.85 (1H, dd, J = 5.3, 12.4 Hz). By homonuclear decoupling experiments it was shown that one of the methylene protons was strongly coupled (J = 12.4 Hz) to a methine proton at δ 2.49 ppm which itself appeared as a complex multiplet. The remaining two oxygen-bearing carbon atoms (δ 69.4 and 76.8 ppm) corresponded (DEPT spectra) to methine and quaternary carbons, and were inferred to correspond to a secondary and a tertiary alcohol group respectively.

Comparison of the ^{1}H and ^{13}C NMR spectral data of **L** with those of rosmarinine (see Tables 3 and 4, and 23) indicated that a methyl group of rosmarinine (**68**), seen at δ 0.97 (d, J = 6.7 Hz) and corresponding to C-19, was replaced in **L** by the methylene unit noted earlier (^{1}H NMR δ 5.16 and 5.31, br s) [at high resolution seen as having *ca.* 2.0 Hz couplings]. This was supported by the appearance in the ^{13}C NMR spectrum of **L** of the olefinic resonances at δ 115.8 and 145.0 ppm and the absence of signals attributable to the $CH_3\overset{|}{C}H$ unit of rosmarinine (δ 11.7 and 37.9 ppm). On the basis of the above spectral evidence, alkaloid L was assigned structure **92**, which corresponds to angularine, a compound which had been described once before [76]. The relative downfield shift of the H-14, H-18 and H-19 signals in the ^{1}H NMR spectrum in alkaloid **L**, compared to those of rosmarinine, may be attributed to the introduction of anisotropic deshielding effects due to the vinylidene group, a phenomena observed in moving from senecionine (**4**) to seneciphylline (**56**) [94].

No ^{1}H and ^{13}C NMR data have been reported for angularine, but there was an excellent concordance of the mp and $[\alpha]_D$ values of **L** with those reported for angularine by Porter and Geissman [76]. They isolated this compound from *Senecio angulatus* L., a plant originally native to South Africa and now cultivated in Southern California as an ornamental.



92

Table 23: ^{1}H and ^{13}C NMR data for Alkaloid **L**

Proton		Carbon	δ (ppm)
H-1	2.49, m	C-1	49.2
H-2a	4.20, q, J = 7.8 Hz	C-2	69.4
H-2b	-	C-3	61.3
H-3a	3.09, m	C-5	53.5
H-3b	2.92, m	C-6	34.5
H-5a	3.27, m	C-7	75.2
H-5b	2.57, m	C-8	69.4
H-6a	2.23, dd, J = 6.4, 14.0 Hz	C-9	62.6
H-6b	2.02, m	C-11	179.3
H-7	4.95, br t	C-12	76.8
H-8	3.53, dd, J = 3.2, 7.6 Hz	C-13	145.0
H-9a	4.85, dd, J = 5.3, 12.4 Hz	C-14	38.3
H-9b	4.08, d, J = 12.4 Hz	C-15	131.7
H-13	-	C-16	169.9
H-14a	3.09, d, J = *ca.* 12.0 Hz	C-18	25.3
H-14b	2.92, d, J = *ca.* 12.0 Hz	C-19	115.8
H-18	1.57, s	C-20	135.4
H-19	5.31, d, J = 2.1 Hz 5.16, d, J = 1.7 Hz	C-21	15.1
H-20	5.88, q, J = 7.1 Hz		
H-21	1.89, d, J = 7.1 Hz		

2.2.2 Alkaloid **M**

This compound was isolated as a colourless oil from the unreduced $CHCl_3$ extract of *S. syringifolius*, with R_f 0.09 ($CHCl_3$: MeOH: NH_4OH (95:4:1)). Addition of H_2O followed by evaporation resulted in white crystals, mp 138 ^oC (dec.).

The EIMS revealed an apparent molecular ion at m/z 367 and a CIMS (NH_3) showed a corresponding ion at m/z 368 $[M+1]^+$. Both the EI and CI mass spectra showed ions corresponding to the loss of O from the presumed fragment M^+ and $[M+1]^+$ species, suggesting that **M** was an N-oxide [99,100], and the high polarity demonstrated by **M** on TLC was consistent with the presence of this functionality. The ^{13}C NMR of **M** revealed 18 signals corresponding to 18 carbon atoms, while the ^1H NMR spectrum integrated for about 25 hydrogens. Thus a molecular composition $C_{18}H_{25}NO_7$ appeared possible, with an IHD of seven.

Two resonances at δ 166.2 and 174.8 ppm in the ^{13}C NMR spectrum of **M** were assigned to two ester or lactone carbonyl carbons. An intense IR band at 1728 cm^{-1} was assigned to overlapping ester carbonyl absorptions. Evidence for the presence of olefinic functionalities came from the presence of four ^{13}C NMR resonances at δ 113.9, 131.1, 137.4 and 148.2 ppm. Of these four signals, that at δ 137.4 ppm was correlated (XHCORR spectrum) to a ^1H NMR olefinic methine resonance at δ 5.93 (q, J = 7.1 Hz) that was itself coupled to a deshielded methyl resonance at δ 1.87 (d, J = 7.1 Hz). This indicated the presence of the (Z)-ethylidene functionality in **M**. The ^{13}C NMR resonance at δ 113.9 ppm was similarly correlated (XHCORR spectrum) to ^1H NMR resonances at δ 4.94 and 5.14 ppm, both of which appeared as broad singlets. These signals were assigned to an exocyclic vinyl methylene functionality. There was no ^1H and ^{13}C NMR

evidence for additional unsaturation functionalities so, with four units accounted for in the carbonyl and olefinic groups, M was judged be tricyclic.

Comparison of the ^{1}H and ^{13}C chemical shifts with those reported for angularine (see alkaloid **L**) revealed downfield shifts in **M** for the proton and carbon absorption peaks at 3, 5, and 8 relative to those for angularine (see Tables 24 and 25). Based on the above data, the structure of alkaloid **M** was proposed to be **93**, a previously undescribed alkaloid now named angularine N-oxide.

This structure was confirmed by oxidation of angularine with MCPBA to the N-oxide, identical (^{1}H, ^{13}C NMR, mp and $[\alpha]_D$) with the natural product.

2.2.3 Alkaloid **N**

This alkaloid was obtained as an oil, from which white crystals were obtained, mp 135-7^{o} C on addition of water followed by evaporation. The FAB-MS of this alkaloid, which was measured after EIMS and CIMS (NH_3) failed to show an apparent molecular ion, showed a quasi-moleclular $[M+1]^+$ ion at m/z 412. The ^{13}C NMR spectra (including DEPT experiments) showed the presence of 20 carbons with 28 attached hydrogens. The IR spectrum of **N** showed the presence of hydroxyl (3389 cm^{-1}) as well as ester absorption. On the basis of these data, the molecular composition of **N** was deduced to be $C_{20}H_{29}NO_8$, with an IHD of seven.

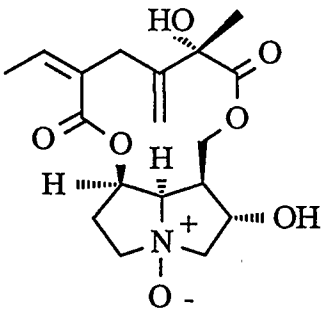
Resonances at δ 166.2, 169.8, and 171.7 ppm in the ^{13}C NMR spectrum of **N** were assigned to three ester carbonyl carbons, one of which was an acetate, as judged by a resonance at δ 2.08 (3H, s) in the ^{1}H NMR spectrum of **N** which correlated (XHCORR) to a ^{13}C NMR resonance at δ 21.4 ppm. Apart from these three units of unsaturation, a (Z)-ethylidene unit was present: as shown by

Table 24: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **M** and Angularine (**92**).

Proton	Alkaloid **M**	Angularine (**92**)
H-1	3.19, m	2.49, m
H-2	4.57, ddd, J = 7.3, 7.3, 8.4 Hz	4.20, q, J = 7.8 Hz
H-3a	3.93, dd, J = 7.3, 12.0 Hz	3.09, m
H-3b	3.61, dd, J = 7.2, 12.0 Hz	2.92, m
H-5a	3.77, m	3.27, m
H-5b	3.70, m	2.57, m
H-6a	2.78, m	2.02, m
H-6b	2.14, m	
H-7	5.39, m	4.95, br t
H-8	4.14, dd, J = 5.5, *ca.* 8.3 Hz	3.55, dd, J = 3.2, 7.6 Hz
H-9a	4.73, dd, J = 6.5, 12.5 Hz	4.85, dd, J = 5.3, 12.4 Hz
H-9b	4.02, d, J = 12.5 Hz	4.08, d, J = 12.4 Hz
H-13	-	-
H-14a	3.36, d, J = 16.4 Hz	3.09, d, J = 12.0 Hz
H-14b	2.92, d, J = 16.4 Hz	2.92, d, J = 12.0 Hz
H-18	1.52, s	1.57, s
H-19	5.14, br s 4.94, br s	5.31, d, J = 2.1 Hz 5.16, d, J = 1.7 Hz
H-20	5.93, q, J = 7.1 Hz	5.88, q, J = 7.1 Hz
H-21	1.87, d, J = 7.1 Hz	1.89, d, J = 7.1 Hz

Table 25: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **M** and angularine (**92**).

	Compound	
Carbon	Alkaloid M	Angularine (**92**)
C-1	45.3	49.2
C-2	69.6	69.4
C-3	75.7	61.3
C-5	69.0	53.5
C-6	31.9	34.5
C-7	73.3	75.2
C-8	87.4	69.4
C-9	59.8	62.6
C-11	174.8	179.3
C-12	76.2	76.8
C-13	148.2	145.0
C-14	37.4	38.3
C-15	131.1	131.7
C-16	166.2	166.9
C-18	25.4	25.3
C-19	113.9	115.8
C-20	137.4	135.4
C-21	15.3	15.1



93

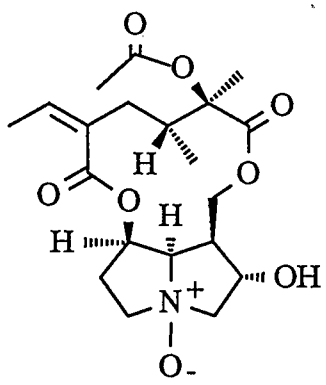
resonances at δ 131.2 and 138.4 ppm in the ^{13}C NMR spectrum of **N**, with the resonance at δ 138.4 ppm correlated (XHCORR) to a ^{1}H NMR resonance at δ 5.98 (1H, q, J = 7.2 Hz) which was in turn coupled to a deshielded methyl resonance at δ 1.90 (J = 7.2 Hz). Thus, the remaining units of unsaturation represent a tricyclic skeleton, which was once more most likely that of a macrocyclic dilactonic PA.

Comparison of the ^{1}H and ^{13}C NMR data for **N** with those for 12-O-acetylrosmarinine (**85**) showed that the two molecules were closely related (see Tables 26 and 27) except for the downfield shifts for the resonances corresponding to C-3, 5 and 8 adjacent to nitrogen. This could be explained as before by a through-bond deshielding effect of an N-oxide. Thus the structure of alkaloid **N** was deduced to be **94**, i.e. 12-O-acetylrosmarinine N-Oxide.

Indeed, the N-oxide **94** was obtained on oxidation of 12-O-acetylrosmarinine with MCPBA in $CHCl_3$. Comparison of spectal data (^{1}H,^{13}C NMR) with those of alkaloid **N** revealed the two alkaloids to be identical.

2.3 The alkaloids of *Senecio canus* Hook.

This plant occurs in dry, often rocky places from the plains and foothills to fairly high elevation in the mountains from British Columbia eastwards to



94

Manitoba and southwards to California, Colorado and Nebraska [102].

A collection of fresh Albertan plant material was processed as in the previous cases and the resulting mixture of alkaloids separated as detailed in the Experimental section to give the following alkaloids:

2.3.1 Alkaloid **O**

The alkaloid amounted to *ca.* 76% of the total PAs in the plant and was isolated as a white amorphous solid which crystallized from EtOH to give colourless crystals, mp 229-230 °C.

The EIMS revealed an apparent molecular mass ion peak at m/z 335, with high mass fragment ions at m/z 138 (28), 137 (20), 136 (58), 121 (25), 120 (53), 119 (49), 117 (70), 95 (28), 94 (34), 93 (52). The ^{13}C NMR spectrum showed 18 signals corresponding to 18 carbon atoms, so a molecular formula $C_{18}H_{25}NO_5$ was deduced.

The IR spectrum revealed the presence of a carbon-carbon double bond (1659 cm^{-1}), two carbonyl groups, of which one was non-conjugated (1739 cm^{-1}) and the other conjugated (1715 cm^{-1}), and a hydroxyl group (3457 cm^{-1}).

The ^{13}C NMR data revealed the presence of two ester carbonyl signals at δ

Table 26: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **N** and 12-O-acetylrosmarinine (**85**)

Proton	Alkaloid **N**	12-O-Acetylrosmarinine (**85**)
H-1	3.29, m	2.45, m
H-2	4.41, m	4.32, q, *ca.* 8.0 Hz
H-3a	3.78, m	3.09, dd, J = 7.4, 11.1 Hz
H-3b	3.65, m	2.91, dd, J = 8.0, 11.1 Hz
H-5a	3.78, m	3.30, t, J = 8.7 Hz
H-5b		*ca.* 2.50, m
H-6a	2.94, m	2.30, dd, J = 6.4, 14.0 Hz
H-6b	2.16, m	2.05, m
H-7	5.60, m	4.98, m
H-8	4.45, m	3.56, dd, J = 3.1, 7.6 Hz
H-9a	4.55, dd, J = 7.6, 12.3 Hz	4.88, dd, J = 5.0, 12.0 Hz
H-9b	4.14, d, J = 12.3 Hz	4.10, d, J = 12.0 Hz
H-13	1.76, m	1.72, m
H-14a	2.64, dd, J = 4.7, 14.5 Hz	*ca.* 2.5, m
H-14b	2.00, dd, J = 6.3, 14.5 Hz	1.91, dd, J = 9.8, 14.0 Hz
H-18	1.66, s	1.72, s
H-19	1.04, d, J = 6.9 Hz	0.99, d, J = 6.8 Hz
H-20	5.98, q, J = 7.2 Hz	5.79, q, J = 7.1 Hz
H-21	1.90, d, J = 7.2 Hz	1.86, d, J = 7.1 Hz
Ac	2.08, s	2.11, s

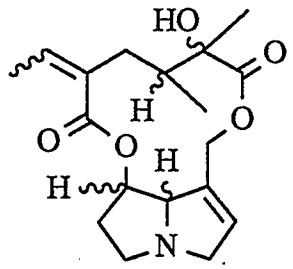
Table 27: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid N and 12-O-acetylrosmarinine (**85**).

Carbon	Compound	
	Alkaloid **N**	12-O-Acetylrosmarinine (**85**)
C-1	47.4	49.3
C-2	74.1	69.1
C-3	74.4	61.2
C-5	68.2	53.5
C-6	33.0	34.3
C-7	72.9	75.6
C-8	88.1	69.4
C-9	62.0	61.7
C-11	171.7	173.4
C-12	82.7	84.5
C-13	41.4	40.7
C-14	36.8	38.7
C-15	131.2	132.7
C-16	166.2	167.4
C-18	22.5	22.1
C-19	14.2	12.0
C-20	138.4	134.74
C-21	15.6	15.1
C=O	169.8	170.0
Me	21.4	21.6

167.6 and 178.2 ppm, and two olefinic groups as represented by four resonances at δ 131.6, 133.2, 134.1 and 136.6 ppm. Both double bonds were trisubstituted, as revealed by the ^{1}H NMR spectrum of **O** which displayed an olefinic resonance at δ 5.73 ppm (1H, dq, J = 1.3, 7.2 Hz) correlated (COSY) to the resonance at δ 1.83 (3H, dd, J = 1.3, 7.3 Hz), with the former being correlated via an XHCORR spectrum to the signal at 134.4 ppm; and a broad singlet at δ 6.20 ppm (1H) which was similarly correlated to the resonance at δ 136.6 ppm in the ^{13}C NMR spectrum. As no further units of unsaturation were observed from the ^{1}H and ^{13}C NMR spectra, the remaining three units of unsaturation must therefore result from a tricyclic carbon framework, probably the PA necine elaborated into a macrocyclic dilactone.

As in the previous cases (alkaloids **A**-**N**) one of the trisubstituted double bonds appeared to be present in a (Z)-ethylidene functionality. However, the second olefinic unit appeared to be of a kind not encountered before in these investigations. The presence of three methyl groups, one belonging to the ethylidene unit, and two appearing at δ 0.93 (d, J = 6.5 Hz) and 1.33 (s) and being assignable to CH_3-18 and -19 respectively, revealed the necic moiety as being senecic acid or one of the as yet unknown stereoisomers. This therefore requires the second double bond to be part of the necine. There would be two possibilities for accommodating this into the usual necine framework: as a 1- or 6-ene. The latter is unprecedented, and would require an enol ester functionality, with characteristic ^{13}C resonances for C-6 and C-7 at δ 147-151 and 112-116 ppm respectively [103], not observed in **O**. The former is, however, quite commonly encountered in PAs [29] and both the chemical shifts of the vinylic proton and the H-9 methylene was consistent with placement of the double bond between C-1 and 2. Accordingly the vinylic proton at δ 6.20 ppm was assigned to H-2, and this led

to the proposal that alkaloid **O** was a 1,2-unsaturated macrocyclic diester pyrrolizidine alkaloid, incorporating a retronecine unit. Thus the skeleton **95** was assigned to this alkaloid.



95

Supporting evidence for this skeleton came from the mass spectrum, which showed three prominent triads of ions at m/z 138, 137 and 136; 121, 120 and 119; and 95, 94 and 93 as earlier reported by Atal and Kapur [104] as characteristic of this system.

As previously noted, the ^{1}H NMR spectrum of **O** revealed a pair of AB signals at δ 4.06 (1H, d, J = 11.6 Hz) and 5.51 (1H, d, J = 11.6 Hz) which were assigned to C-9 methylene protons. Culvenor *et al.* [66,105] have shown that the non-equivalent protons at C-9 in macrocyclic diesters of retronecine provide a recognisable AB signal and the chemical shift difference between the signals due to these protons give a criterion for differentiating between 11 and 12-membered diester ring systems (see Table 28 below). The chemical shift difference of 1.45 ppm for alkaloid **O** is in the range expected for an alkaloid with a twelve-membered diester ring: a result again consistent with **95.**

The ^{13}C NMR spectrum of alkaloid **O** was also in accord with expectation for **95** and, most importantly, was in excellent agreement with that reported for senecionine (**4**) [71, 85, 86, 106] (see Table 30). The ^{1}H NMR spectrum of **O** was similarly in accord with that reported for senecionine (**4**) [94, 107] (see Table 29).

Table 28: Chemical shift difference of H-9 protons in 11 and 12 membered dilactones of retronecine [66, 105].	
Size of diester ring system	Chemical shift difference range $\Delta\delta$H9 (ppm)
11	0.0-0.73
12	1.25-1.53

Further confirmation of identity was made by comparison of the IR spectrum of **O** with that of an authentic sample of senecionine: the two spectra were found to be superimposible upon one another.

Senecionine is a commonly encountered PA, albeit as yet only known from members of the Compositae (Asteraceae) and Papilionaceae (Leguminosae) [31].

2.3.2 Alkaloid **P**

This alkaloid constituted *ca.* 17% of the total PA in the plant. It was isolated as a white amorphous solid and crystallized from Me_2CO to afford colourless crystals, mp 208-9° C.

The EI-MS of **P** exhibited a molecular ion peak at m/z 351, with high mass fragment ions at 138 (66), 137 (42), 136 (89), 122 (59), 120 (95), 119 (87), 95 (74), 94 (88) and 93 (100). The ^{13}C NMR showed 18 signals corresponding to 18 carbon atoms. Thus, the molecular formula $C_{18}H_{25}NO_6$ was inferred, with the same degree of unsaturation, but one more oxygen than senecionine (**4**). As the mass spectrum showed the three prominent triads shown by the high mass fragment ions above, it was concluded that **P** belongs to the macrocyclic groups of PAs with retronecine as the base.

Table 29: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **O** and Senecionine (**4**).

Proton	Alkaloid **O**	Senecionine (**4**) [107]
H-1	-	-
H-2	6.20, br s	6.19, br s
H-3a	3.91, m	3.90, d, J = 16.0 Hz
H-3b	3.40, m	3.38, dd, J = 1.0, 6.0 Hz
H-5a	3.27, t, J = 8.3 Hz	3.26, t, J = 8.3 Hz
H-5b	2.53, m	2.53, m
H-6a	2.39, dd, J = 5.7, 14.2 Hz	2.37, dd J = 5.8, 14.1 Hz
H-6b	2.13, m	2.13, m
H-7	5.02, dd, J = 3.5, 5.7 Hz	5.02, dd, J = 3.5, 5.8 Hz
H-8	4.28, m	4.27, m
H-9a	5.51, d, J = 11.6 Hz	5.50, d, J = 11.7 Hz
H-9b	4.06, d, J = 11.6 Hz	4.04, d, J = 11.7 Hz
H-13	1.69, m	1.69, m
H-14a	2.16, m	2.16, m
H-14b	1.75, m	1.75, m
H-18	1.33, s	1.32, s
H-19	0.93, d, J = 6.5 Hz	0.93, d, J = 6.5 Hz
H-20	5.73, dq, J = 1.3, 7.2 Hz	5.72, dq, J = 1.3, 7.2 Hz
H-21	1.83, dd, J = 1.3, 7.2 Hz	1.84, dd, J = 1.6, 7.2 Hz

Table 30: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **O** and Senecionine (**4**).

Carbon	Compound	
	Alkaloid **O**	Senecionine (**4**) [85]
C-1	131.6	131.5
C-2	136.6	136.3
C-3	62.9	62.8
C-5	53.1	53.1
C-6	34.8	34.8
C-7	75.0	74.9
C-8	77.1	77.1
C-9	60.7	60.6
C-11	178.2	178.2
C-12	76.7	76.8
C-13	38.4	38.5
C-14	38.3	38.4
C-15	133.2	133.1
C-16	167.6	167.5
C-18	25.0	25.0
C-19	11.1	11.1
C-20	134.1	134.3
C-21	15.0	15.0

Table 31: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **P** and Retrorsine (**76**)

Proton	Alkaloid **P**	Retrorsine (**76**) [94]
H-1	-	-
H-2	6.21, br d, J = 1.5 Hz	6.21, d, J = 1.6 Hz
H-3a	3.94, dd, J = 1.7, 15.7 Hz	3.94, d, J = 15.9 Hz
H-3b	3.39, ddd, J = 1.6, 6.1, 15.7 Hz	3.39,ddd, J = 1.8, 6.3, 15.9 Hz
H-5a	3.26, t, J = 8.4 Hz	3.26, t, J = 8.7 Hz
H-5b	2.54, m	2.53, m
H-6a	2.39, dd, J = 5.7, 13.9 Hz	2.38, dd J = 5.8, 14.0 Hz
H-6b	2.12, m	2.15, m
H-7	5.01, t, J = 3.4 Hz	5.00, t, J = 3.3 Hz
H-8	4.28, br d, J = 2.3 Hz	4.27, m
H-9a	5.51, d, J = 11.8 Hz	5.49, d, J = 11.8 Hz
H-9b	4.10, d, J = 11.8 Hz	4.09, d, J = 11.8 Hz
H-13	1.64, m	1.64, m
H-14a	2.21, d, J = 12.9 Hz	2.19, d, J = 13.1 Hz
H-14b	1.73, m	1.73, m
H-18	3.74, d, J = 11.2 Hz 3.63, d, J = 11.2 Hz	3.74, d, J = 11.2 Hz 3.62, d, J = 11.2 Hz
H-19	0.86, d, J = 6.4 Hz	0.85, d, J = 6.4 Hz
H-20	5.72, dq, J = 1.3, 7.1 Hz	5.71, dq, J = 1.1, 7.1 Hz
H-21	1.84, dd, J = 1.6, 7.1 Hz	1.83, dd, J = 1.6, 7.1 Hz

Table 32: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **P** and Retrorsine (**76**)

Carbon	Compound	
	Alkaloid **P**	Retrorsine (**76**) [85]
C-1	132.5	132.4
C-2	134.6	134.7
C-3	62.8	62.7
C-5	53.0	52.9
C-6	37.9	37.9
C-7	75.1	75.0
C-8	77.5	77.4
C-9	61.1	61.0
C-11	175.6	175.7
C-12	81.3	81.3
C-13	35.6	35.7
C-14	34.7	34.7
C-15	131.3	131.2
C-16	167.4	167.3
C-18	66.9	66.9
C-19	11.7	11.6
C-20	136.9	136.6
C-21	15.0	14.9

This was also evident from the ^{1}H NMR spectrum of **P** which contained a vinylic-proton resonance at δ 6.21 ppm that characterises the 1,2-unsaturated PAs, i.e. H-2. Also, an AB set of signals at δ 4.10 (H, d, J = 11.8 Hz) and 5.51 (1H, d, J = 11.8 Hz) could be assigned to the methylene protons at C-9; and the Δδ 1.41 ppm, was as expected for a PA with a 12-membered diester ring (see Table 28).

Compared with senecionine (**4**), the ^{1}H NMR spectrum of **P** showed only two methyl groups, one at δ 0.86 (d, J = 6.4 Hz), assignable to CH_3-19, and the other at δ 1.84 (dd, J = 1.6 and 7.1 Hz) which was assigned to CH_3-21 of a C_{10}-dilactone unit. There was no signal corresponding to a methyl group attached to a quaternary carbon carrying an oxygen substituent i.e. C-18. As the MS had already revealed that **P** contained one more oxygen than **O**, we therefore surmised that C-18 was hydroxylated. This deduction was supported by the presence of a methylene ^{13}C resonance at δ 66.9 ppm with the corresponding protons seen in the ^{1}H NMR of **P** as two AB doublets at δ 3.62 (J = 11.2 Hz) and 3.74 (J = 11.2 Hz) respectively.

On the basis of the foregoing discussion, alkaloid **P** was suspected to be retrorsine (**76**). Indeed, the ^{13}C NMR spectrum of **P** showed a perfect correspondence with that reported for retrorsine [71, 85, 86, 106] (see Table 32), and the ^{1}H NMR spectrum was also in full agreement with that previously reported for this alkaloid [94] (see Table 31). Finally the IR spectrum of alkaloid **P** was superimposable on that of the authentic sample of retrorsine.

Like senecionine, retrorsine is a common PA of the plant families Asteraceae and Papilionaceae [31].

2.4 The alkaloids of *Senecio foetidus* Howell (syn. *S.hydrophiloides* Rydb.).

The plant occurs in wet meadows in the foothills and mountains, from

southern British Columbia and South West Alberta to Oregon, Idaho, and Montana [102].

The fresh flowering tops of this plant were processed as described in the Experimental section to yield the following alkaloids:

2.4.1 Alkaloid **Q**

This alkaloid constituted *ca.* 27% of the total alkaloidal tertiary bases in the plant, and was isolated as an oil.

The EIMS revealed an apparent molecular mass ion at m/z 335, while the ^{13}C NMR revealed 18 signals corresponding to 18 carbon atoms, thus the molecular formular $C_{18}H_{25}NO_5$ was derived, with the equivalent of 6 rings/double bonds.

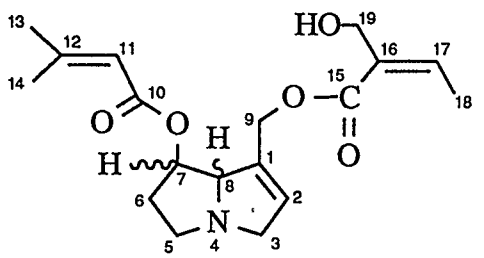
Examination of the ^{13}C NMR spectrum of **Q** revealed two ester or lactone carbonyls [108], of which both were αβ-unsaturated (δ 165.8 and 166.6 ppm); and six olefinic resonances at δ 115.9, 127.4, 131.7, 133.8, 140.8 and 157.5 ppm. The resonance at δ 140.8 ppm was correlated by an XHCORR spectrum to a vinylic ^1H NMR resonance at δ 6.35 (J = 7.2 Hz) that was in turn coupled to a deshielded methyl resonance at δ 2.04 (d, J = 7.2 Hz). There was also a resonance at δ 64.7 ppm in the ^{13}C NMR spectrum correlated via the XHCORR spectrum to a two proton ^1H NMR resonance at δ 4.20 ppm (m), which corresponded to a pair of almost magnetically equivalent methylene protons. The ^1H-^1H COSY spectrum connected this methylene resonance to the vinylic resonance at δ 6.35 ppm. Thus a group such as $CH_3CH=C(CH_2Y)CO_2$- was formulated. The molecular formula, and the position of absorption of CH_2Y both in the ^1H and ^{13}C NMR spectra strongly indicated Y to be OH. The chemical shift of the olefinic proton resonance at δ 6.35 ppm indicated that the configuration at the double bond was (Z) (the

calculated chemical shift value δ 6.36 ppm [65] for Y = OH was in excellent agreement with that observed).

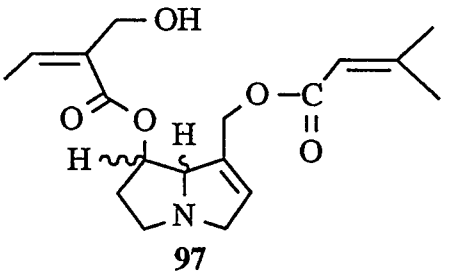
The second olefinic ^{13}C NMR resonance at δ 115.9 ppm was correlated (XHCORR spectrum) to another vinylic ^{1}H NMR resonance at δ 5.58 ppm, which was in turn coupled (COSY spectrum) to the deshielded methyl resonances at δ 1.87 (d, J = 1.1 Hz) and 2.12 (d, J = 1.1 Hz). These small coupling constants were attributed to long range allylic couplings. Thus, the presence of a $(CH_3)_2C{=}CHCO_2$- moiety was suggested as another part of the molecule.

A third olefinic ^{13}C NMR resonance at δ 127.4 ppm was correlated in the XHCORR spectrum to a vinylic proton at δ 5.79 ppm (d, J = 1.5 Hz). This methine resonance was coupled (COSY spectrum) to methylene resonances at δ 3.38 (1H, m) and 3.91 (1H, d, J = 15.2 Hz) i.e. suggested a structural fragment $\overset{|}{C}H_2\text{-}CH{=}\overset{|}{C}$. The remaining two units of unsaturation therefore corresponds to two rings, and were assigned to a pyrrolizidine system. This requires **Q** to be a non-macrocyclic diester. Putting these fragments together, the regioisomeric structures **96** and **97** were proposed for **Q**. The vinyl proton at δ 5.79 ppm was assigned to H-2, the chemical shift being characteristic for this proton in the noncyclic unsaturated diester PAs [37, 66, 71]. The resonances at δ 4.68 and 4.80 ppm (J = 13.5 Hz), Δδ 0.12 ppm, were assigned to the geminal H-9 methylene protons, these also being consistent with values reported for non-macrocyclic PAs [37, 66, 71]. Similarly, the resonance at δ 5.34 ppm in the ^{1}H NMR, which appeared as a multiplet assignable to H-7 (as revealed by analysis of COSY and decoupling experiments), was consistent with the presence of an acyloxy function at C-7 [37, 66].

In an attempt to decide between the two ways of attachment of the acids to C-7 and C-9, the fragmentation pattern of the EIMS was taken into consideration.



96

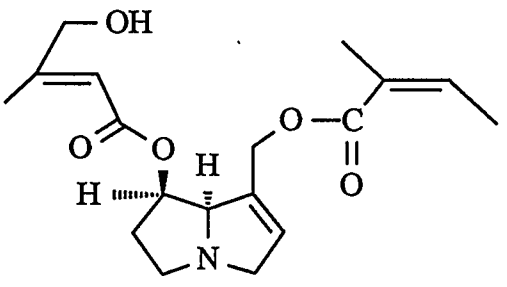


97

Fragmentation of PAs which have a 1,2-double bond involves loss of the allylic substituent to give the allylic cation [71, 109-111] (see Scheme 3) and an abundant ion at m/z 220 in the EIMS of **Q** corresponds to ester cleavage at C-9 of a PA bearing a C-7 3-methyl-2-butenoyloxy unit or an isomer thereof.

Attachment of a sarracinyl unit to C-9 has been observed to correlate with the appearence of an ion m/z 237, as seem in the EIMS of **Q**, which has been suggested to be formed via an eight membered cyclic system as shown in Scheme 4 [71,111].

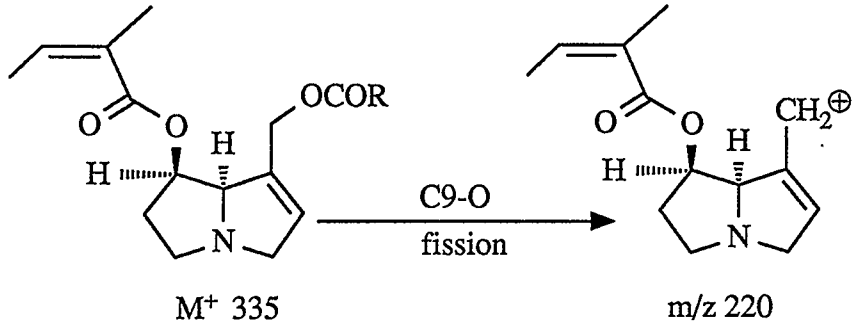
If **Q** had the alternative arrangement of the ester groups (structure **97**) it should give rise to an intense ion at m/z 236 resulting from the C9-O cleavage [37, 71]. This was not observed in the EIMS of **Q**. These results contrast with the EIMS of sencalenine (**98**) in which a fairly intense ion at m/z 236 was observed, attributed to loss of the C-9 substituents, while no ion was observed at m/z 220 [109].



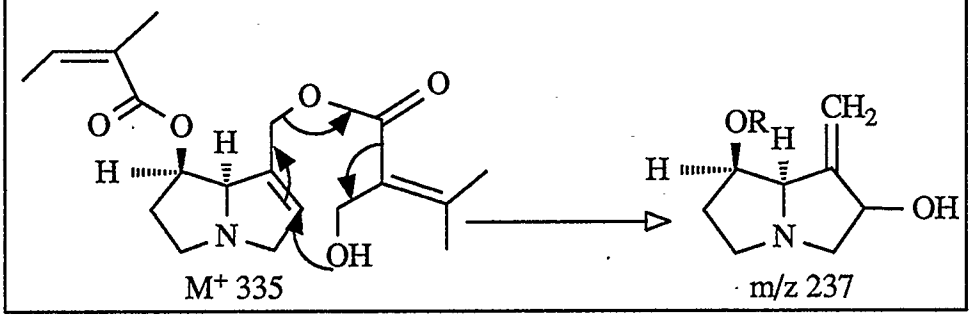
98

Hence, structure **96** was adopted for **Q**. The ^{1}H and ^{13}C NMR spectra were

Scheme 3: Mass spectral fragmentation of non-macrocyclic unsaturated diester [71].

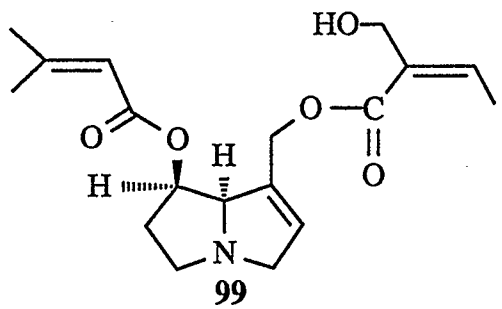


Scheme 4: An alternative mass spectral fragmentation at C-9 of non-macrocyclic unsaturated diester [71]



consistent with those reported for 7-O-senecioyl-9-O-sarracinylretronecine (**99**) [109] (see Tables 33 and 34).

PAs esterified with senecioic acid have been rarely reported, but this particular alkaloid has been previously reported as a constituent of *S. triangularis* [110] and *S. cacaliaster* [109].



99

Table 33: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **Q** and 7-O-Senecioyl-9-O-sarracinylretronecine (**99**)

Proton	Alkaloid Q	Alkaloid **99** [109]
H-1	-	-
H-2	5.79, d, J = 1.5 Hz	5.78, m
H-3a	3.91, br d, J = 15.2 Hz	3.94, m
H-3b	3.38, m	3.49, m
H-5a	3.27, m	3.31, m
H-5b	2.64, m	2.67, m
H-6a H-6b	2.06, m	2.67, q, J = 9.0 Hz
H-7	5.34, m	5.34, m
H-8	4.34, m	4.51, m
H-9a	4.80, d, J = 13.5 Hz	4.78, m
H-9b	4.68, d, J = 13.5 Hz	
H-11	5.58, m	5.56, m
H-13	1.87, d, J = 1.1 Hz	1.87, d, J = 1.0 Hz
H-14	2.12, d, J = 1.1 Hz	2.05, d, J = 1.0 Hz
H-17	6.35, q, J = 7.2 Hz	6.38, q, J = 7.5 Hz
H-18	2.04, d, J = 7.2 Hz	2.02, d, J = 7.5 Hz
H-19	4.20, m	4.22, s

Table 34: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **Q** and 7-O-senecioyl-9-O-sarracinylretronecine (**99**)

	Compound	
Carbon	Alkaloid **Q**	Alkaloid **99** [109]
C-1	131.7	131.8
C-2	127.4	127.0
C-3	62.6	62.3
C-5	53.6	53.7
C-6	34.4	34.4
C-7	73.1	72.9
C-8	75.6	75.7
C-9	61.0	60.8
C-10	166.6	166.6
C-11	115.9	115.8
C-12	157.5	133.9
C-13	20.2	20.3
C-14	27.4	27.5
C-15	165.6	165.6
C-16	133.8	158.0
C-17	140.8	141.0
C-18	15.7	15.8
C-19	64.7	64.7

2.4.2 Alkaloid R

This alkaloid amounted to about 15% of the total alkaloid bases, and was obtained as an oil.

The EIMS exhibited an apparent molecular ion peak at m/z 239, with high mass fragment ions at 156 (32), 139 (40), 138 (12), 108 (10), 95 (17), 83 (16), 82 (100), and 55 (47). The ^{13}C NMR spectrum of **R** showed 13 signals corresponding to 13 carbon atoms, so a molecular formula $C_{13}H_{21}NO_3$ was deduced, corresponding to an IHD of 4.

In the ^{1}H NMR spectrum of **R**, there was a low-field vinylic methine resonance at δ 6.09 (qq, J = 1.4, 7.2 Hz) coupled to two methyl resonances at δ 2.03 (dq, J = 1.4, 7.2 Hz) and 1.90 (dq, J = 1.4, 1.5 Hz) as shown by a ^{1}H-^{1}H COSY spectrum, and correlated to a ^{13}C NMR resonance at δ 139.2 ppm by a XHCORR spectrum. Another ^{13}C NMR resonance at δ 167.0 ppm was assigned to an ester or lactone carbonyl. The presence of the coupled vinyl and methyl signals, together with the carbonyl signal, suggested the presence of a $CH_3CH=C(CH_3)CO_2$ moiety, such as found in pyrrolizidine esters of angelic (Z-2-methyl-2-butenoic) and tiglic (E-2-methyl-2-butenoic) acids. The observed value (6.08 ppm) for the vinyl proton is in accord with that calculated (6.02 ppm) for an angelate [65], and is in excellent agreement with those reported for other PAs bearing angeloyl groups [66, 72, 112-115], but not tigloyl, where this proton is normally observed at δ 6.50-7.30 ppm [114].

The remaining fragment of alkaloid **R** corresponds to an elemental composition $C_8H_{14}NO$. As there was no further unit of unsaturation, it had to be

bicyclic, and thus was most probably a saturated pyrrolizidine unit. Supporting evidence came from the mass spectrum which showed a base peak at m/z 82, a characteristic feature of saturated PAs [67, 82].

The point of esterification was located by an analysis of the ^{1}H NMR spectrum of the alkaloid: the chemical shift of the magnetically equivalent methylene protons at δ 3.77 (d, J = 7.5 Hz) assignable to the H-9 protons, as well as of a methine resonance at δ 5.35 (br t) assignable to the H-7 proton are consistent with the esterified hydroxyl function being at C-7, and not at C-9 [37, 66].

The assignment of the relative configuration of the necine portion was accomplished by comparison of the ΣJ_7 in the 400 MHz ^{1}H NMR spectrum of alkaloid **R** with those of the known saturated PAs [66-69] (see Table 2 p 20). The sum of the coupling constants in H-7 in alkaloid **R** as measured by the signal width at half-height ($W_{1/2}$) was 7.5 Hz which is close to that seen in derivatives of platynecine. Accordingly the alkaloid **R** was assigned structure **100** which corresponds to that of 7-O-angeloylplatynecine. This is a known alkaloid, and indeed the ^{1}H and ^{13}C NMR spectra of alkaloid **R** showed a perfect correspondence with those reported for angeloylplatynecine (Tables 35 and 36) [68].

2.4.3 Alkaloid S

This alkaloid comprised *ca.* 15% of the total bases and was obtained as an oil.

As its EIMS revealed an apparent molecular ion at m/z 239 and its ^{13}C NMR spectrum showed 13 resonances for 13 carbon atoms, **S** was deduced to have the molecular formula $C_{13}H_{21}NO_3$ i.e. was isomeric with **R**.

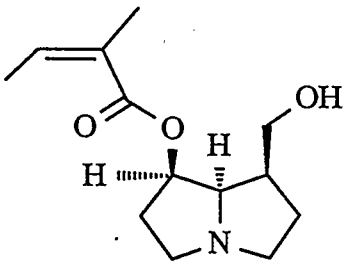
The ^{1}H NMR spectrum of **S**, like that of **R**, exhibited signals characteristic

Table 35: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **R** and 7-O-angeloylplatynecine (**100**)

Proton	Alkaloid **R**	**100** [68]
H-1	2.63, m	2.63, m
H-2	1.81, m	1.81, m
	1.76, m	1.76, m
H-3a	3.21, m	3.18, m
H-3b	2.78, m	2.78, m
H-5a	3.33, m	3.29, m
H-5b	2.72, m	2.72, m
H-6a	2.09, m	2.06, m
H-6b		
H-7	5.35, br t	5.34, br t
H-8	3.57, dd, J = 3.4, 8.0 Hz	3.54, dd, J = 3.4, 8.0 Hz
H-9a	3.77, d, J = 7.5 Hz	3.74, d, J = 7.5 Hz
H-9b		
H-12	6.09, qq, J = 1.4, 7.2 Hz	6.10, qq, J = 1.4, 7.2 Hz
H-13	2.03, dq, J = 1.4, 7.2 Hz	2.02, dq, J = 1.5, 7.2 Hz
H-14	1.90, dq, J = 1.5, 1.4 Hz	1.89, dq, J = 1.5, 1.4 Hz

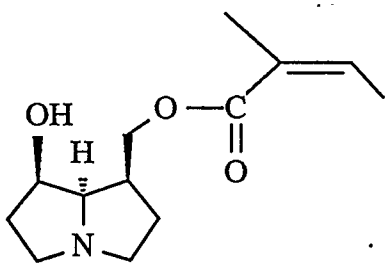
Table 36: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **R** and 7-O-angeloylplatynecine (**100**) [68].

Carbon	Compound	
	Alkaloid **R**	7-O-Angeloylplatynecine (**100**)
C-1	43.9	43.9
C-2	28.7	28.7
C-3	55.5	55.6
C-5	53.6	53.6
C-6	35.2	35.2
C-7	75.2	75.1
C-8	69.2	69.2
C-9	62.9	62.7
C-10	167.0	167.0
C-11	127.4	127.2
C-12	139.2	139.4
C-13	15.7	15.8
C-14	20.7	20.8



100

of an angelate moiety (methyl resonances at δ 1.91 and 2.00, with the latter showing a vicinal coupling to a vinylic proton at δ 6.09, which also showed long range coupling to the high-field methyl group). However, as compared to **R**, the ^{1}H NMR resonance for the proton ascribed to the H-7 methine was shifted upfield by 1.06 ppm to appear at δ 4.29 ppm, while the AB(X) system attributed to the H-9 protons was shifted downfield to 4.49 and 4.63 ppm. This suggested that **S** was 9-O-angeloylplatynecine (**101**).



101

Support for this conclusion was provided by the observation that the resonance for the proton ascribed to H-7 was broad, with a width at half-height of 7.6 Hz, corresponding to an all-*cis* arrangement of H-1, 7 and 8, as in platynecine (**11**) (see Table 2).

Like its regioisomer **R, S** corresponded to a known alkaloid and the ^{1}H NMR data previously reported for **101** were in agreement with our measurements [68] (see Table 37). Thus it was concluded that **S** was 9-O-angeloylplatynecine (**101**). No ^{13}C NMR data appeared to have been previously reported, so our data (Table 38) represents a further characterisation of this alkaloid. Both **100** and **101**

Table 37: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **S** and 9-O-angeloylplatynecine (**101**) [68].

Proton	Alkaloid **S**	**101**
H-1	2.73, m	2.73, m
H-2	1.82, m	
H-3a	3.19, ddd, J = 6.8, 10.4, 10.4	3.26, m*
H-3b	2.85, m	2.85-2.95, m*
H-5a	3.33, dd, J = 8.8, 8.8 Hz	3.44, m*
H-5b	2.85, m	
H-6a	1.82, m	1.70-2.2, m*
H-6b		
H-7	4.29, m	4.34, br t
H-8	3.40, dd, J = 3.0, 7.8 Hz	3.52, dd, J = 2.7, 8.0 Hz
H-9a	4.63, dd, J = 7.6, 11.0 Hz	4.62, dd, J = 7.6, 11.0 Hz
H-9b	4.49, dd, J = 7.1, 11.0 Hz	4.48, dd, J = 7.0, 11.0 Hz
H-12	6.09, qq, J = 1.5, 7.2 Hz	6.09, qq, J = 1.4, 7.2 Hz
H-13	2.00, dq, J = 1.5, 7.2 Hz	1.99, dq, J = 1.5, 7.2 Hz
H-14	1.91, dq, J = 1.5, 1.5 Hz	1.89, dq, 1.4, 1.5 Hz

* Not assigned to respective hydrogens.

Table 38: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **S** .

Carbon	Alkaloid **S**
C-1	40.7
C-2	29.7
C-3	55.4
C-5	53.7
C-6	37.6
C-7	72.7
C-8	70.5
C-9	64.3
C-10	168.1
C-11	127.9
C-12	137.8
C-13	15.7
C-14	20.8

were originally isolated from *Castilleja* aff. *miniata,* a hybrid of the Indian paintbrushes *C. rhexifolia* and *C. miniata* [68], and subsequently shown to have been obtained by this plant via root parasitism on *Senecio atratus* [116] i.e. actually biosynthesized by these *Senecio* and then sequestered by the paintbrush.

2.4.4 Alkaloid T

The amount of alkaloid **T** present in the plant accounted for *ca.* 15% of the bases. It was obtained as an oil.

The EIMS showed a molecular ion peak at m/z 237 with intense fragment ions at m/z 219 (5), 137 (22), 124 (29), 111 (41), 106 (45), 94 (27), and 80 (100). The ^{13}C NMR spectrum showed 13 signals, apparently corresponding to 13 carbon atoms. Thus, the formula $C_{13}H_{19}NO_3$ was inferred. This formula requires an IHD of 5.

Two units of unsaturation were accommodated in an angelate unit, which was detected by the appearance of characteristic ^1H NMR resonances (methyl groups at δ 1.82 and 1.97 ppm, the latter being coupled vicinally to a vinylic hydrogen at δ 6.08 ppm which was further long-range coupled to the high-field methyl). The other three units of the IHD were assigned to a pyrrolizidin-1-ene unit whose presence was signalled by a broad singlet ^1H NMR resonance at δ 5.65 ppm, which correlated with an olefinic methine ^{13}C resonance at 124.1 ppm.

These results suggested that **T** might be the angelate ester of one of the commonly encountered necines, namely, retronecine (**15**) or heliotridine (**60**).

To determine the location of the ester, the ^1H NMR spectrum of alkaloid **T** was examined. A signal at δ 4.19 ppm integrating for two protons was assigned to the nearly magnetically equivalent H-9 protons, while another at δ 5.41 ppm

integrating for one proton was assigned to H-7. This was consistent with esterification at C-7 and not at C-9 [37, 117].

To determine whether the C-7 functionality is *endo,* as in the retronecine (**15**) system, or *exo* as in heliotridine (**60**), the ^{1}H and ^{13}C NMR spectral data of alkaloid **T**, and those for the known diastereomers, 7-O-angeloylheliotridine (**102**) and 7-O-angeloylretronecine (**103**) were compared (see Tables 39 and 40). From these results, alkaloid **T** was found to be identical to 7-O-angeloylretronecine (**103**). Further support for this identification was made by comparison of the $[\alpha]_D$ value of alkaloid **T** ($[\alpha]_D$ +40°) with those reported for 7-O-angeloylretronecine (**103**) ($[\alpha]_D$ + 49° [37] and 7-O-angeloylheliotridine (**102**)) ($[\alpha]_D$ + 13.3 and 10.8°) [112]. The fragmentation pattern of alkaloid **T** was indistinguishable from those reported for 7-angeloylheliotridine (**102**) and 7-O-angeloylretronecine (**103**) [118].

This monoester, 7-O-angeloylretronecine (**103**), has previously been isolated from a variety of species of the families Boraginaceae, Celastraceae and Asteraceae [31].

2.4.5 Alkaloid **U**

This alkaloid accounted for *ca.* 6% of the total bases, and was obtained as an oil.

The EIMS of **U** indicated an apparent molecular ion at m/z 337. The ^{13}C NMR spectrum of **U** showed 18 signals corresponding to 18 carbon atoms. Thus a molecular formular of $C_{18}H_{27}NO_5$ was inferred, with an IHD of 6.

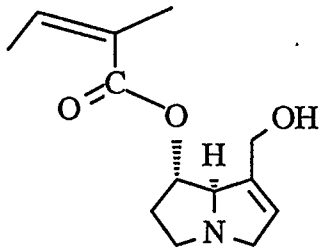
In the ^{1}H NMR, the methyl resonances at δ 1.91 (dq, J = 1.5, 1.6 Hz) and 2.01 (dq, J = 1.5, 7.2 Hz), both coupled to a methine resonance at δ 6.11 (qq, J = 1.5, 7.2 Hz) once more indicated the presence of an angelate moeity. As well a

Table 39 : ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **T**, **103** and **102**

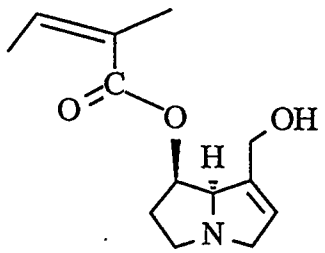
Proton	Alkaloid **T**	Alkaloid **103** [110]	Alkaloid **102** [112]
1	-	-	-
2 2b	5.65, m	5.63, s	5.63, m
3a	3.92, d, J = 14.7 Hz	3.96, d, J = 15.0 Hz	3.99, t, J = 16.0 Hz
3b	3.39, m	3.38, m	3.35, t, J = 64 Hz
5a	3.34, m	3.38, m	2.93, m
5b	2.69, q, J = 8.5 Hz	2.71, q, J = 8.0 Hz	
6a 6b	2.12, m	2.14, m	1.95, m
7	5.41, q, J = *ca.* 3.0 Hz	5.42, br s	5.11, 2t, J = 4.0 Hz
8	4.34, m	4.40 , br s	4.08, m
9a	4.19, d, J = 14.2 Hz	4.18, s	4.34, d, J = 1.0 Hz
9b	4.18, d, J = 14.2 Hz		
12	6.08, qq, J = 1.5, 7.3 Hz	6.08, q, J = 7.0 Hz	6.13, dq, J = 1.5, 7.0 Hz
13	1.97, dq, J = 1.5, 7.3 Hz	1.96, d, J = 7.0 Hz	1.98, dq, J = 1.5, 7.0 Hz
14	1.82, dq, J = 1.5, 1.5 Hz	1.81, s	1.90, q, J = 1.5 Hz

Table **40**: ^{13}C NMR Chemical Shifts and Assignments for Alkaloids **T, 103** and **102** .

Carbon	Alkaloid **T**	Alkaloid **102** [112]	Alkaloid **103** [110]
C-1	139.3	127.7	139.2
C-2	124.1	138.96	123.8
C-3	60.2	62.05	60.0
C-5	53.6	53.80	53.6
C-6	34.7	30.23	34.6
C-7	74.4	77.68	74.0
C-8	76.0	79.27	75.9
C-9	63.2	59.82	63.0
C-10	167.4	168.73	167.2
C-11	127.7	140.77	127.5
C-12	138.8	124.36	139.1
C-13	15.7	15.46	20.5
C-14	20.5	20.46	15.7



102



103

methine resonance at δ 6.38 (q, J = 7.2 Hz) coupled to a methyl resonance at δ 2.06 (d, J = 7.2 Hz) and showing long range coupling (COSY) to an oxygenated methylene at δ 4.20 (br s) suggested esterification by 5-hydroxyangelic acid (sarracinic acid). Consistent with the presence of these two C_5-acid units, the ^{13}C NMR spectrum of **U** contained resonances due to four olefinic and two ester/lactone carbonyl carbons. The acids contribute four units of the IHD and the remaining 2 units could be assigned to the pyrrolizidine system. This suggested that alkaloid **U** was an open-chain diester of a saturated necine. That the necine was saturated was also indicated by the absence of a vinylic hydrogen resonance at *ca.* 5.80 ppm which characterises non-cyclic unsaturated diester PAs [37, 66, 71].

As before, the relative configuration of the necine was identified as corresponding to platynecine since ΣJ_7 in the ^1H NMR spectrum of **U** was 6.4 Hz (see Table 2, p 20).

In the same way as for alkaloid **Q**, 7-O-angeloyl-9-O-sarracinylretronecine (**99**), the location of the esterifying acids was deduced from the mass spectrum. For PAs derived from pyrrolizidin-1-enes, the fragmentation of the allylic ester dominates the high-mass fragmentation processes, whereas in PAs derived from saturated necines, loss of the C-7 ester group by a McLafferty elimination is favoured [119-122]. In the EIMS of **U**, an abundant ion observed at m/z 237 (M-100, 17%) suggested that C-7 is esterified with angelic acid. The ion at m/z 222 (15%) presumably arises from the homolytic loss of the O-9 substituent as

Table 41 : ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **U** and sarracine (**67**).

Proton	Alkaloid **U**	Alkaloid **67** [127]
H-1	2.75, m	2.75, m
H-2	1.77-1.93, m 1.96-2.08, m	1.77-1.93, m 1.96-2.08, m
H-3a	2.77, m	2.73, m
H-3b	3.19, m	3.17, m
H-5a	2.70, m	2.70, m
H-5b	3.31, br t, J = *ca.* 8.8 Hz	3.31, br t,
H-6a	1.77-1.93, m	1.77-1.93, m
H-6b	1.96-2.08, m	1.96-2.08, m
H-7	5.33, br t, J = *ca.* 3.7 Hz	5.29, br t
H-8	3.59, dd, J = 3.7, 8.0 Hz	3.55, dd, J = 3.7, 8.0 Hz
H-9a	4.27, dd, J = 8.1, 11.0 Hz	4.22, dd, J = 7.0, 10.8 Hz
H-9b	4.43, dd, J = 7.7, 11.0 Hz	4.38, dd, J = 7.0, 11.0 Hz
H-11	-	-
H-12	6.11, qq, J = 1.5, 7.2 Hz	6.11, qq, J = 1.4, 7.2 Hz
H-13	2.01, dq, J = 1.5, 7.2 Hz	1.99, dq, J = 1.5, 7.4 Hz
H-14	1.91, dq, J = 1.5, 1.6 Hz	1.88, dq, J = 1.4, 1.6 Hz
H-17	6.38, q, J = 7.2 Hz	6.34, q, J = 7.2 Hz
H-18	2.06, d, J = 7.2 Hz	2.01, d, J = 7.4 Hz
H-19	4.20, s	4.20, s

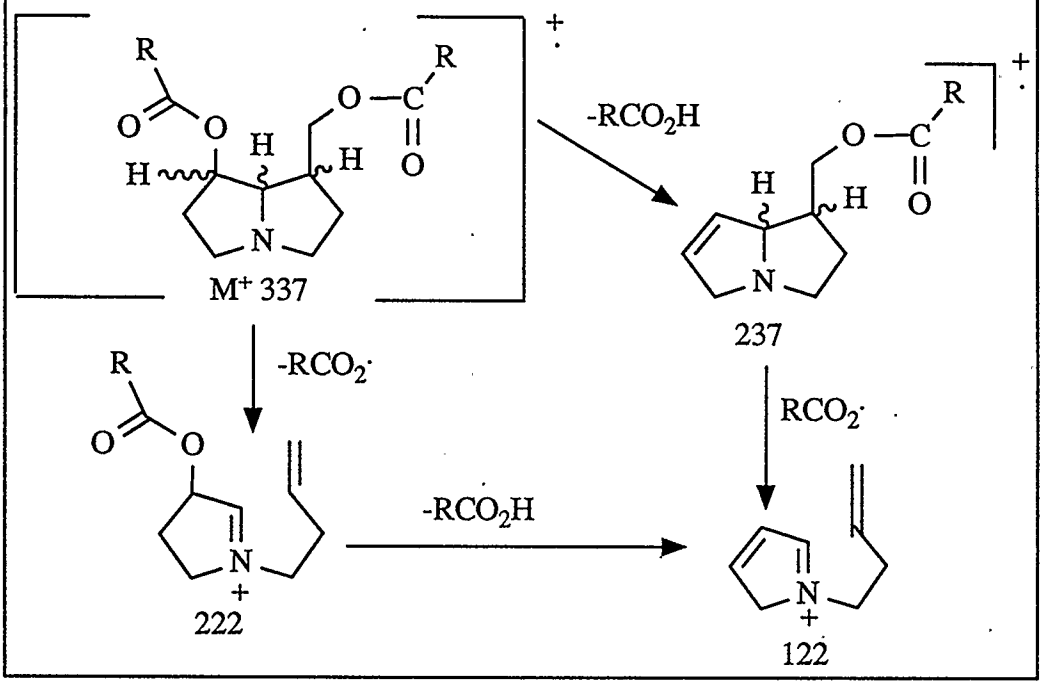
Table 42: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **U** and sarracine (**67**).

Carbon	Compound	
	Alkaloid **U**	Sarracine (**67**) [127]
C-1	40.3	40.2
C-2	28.9	28.8
C-3	55.2	55.2
C-5	53.6	53.6
C-6	35.1	35.0
C-7	75.2	68.8*
C-8	68.9	75.2*
C-9	64.2	64.0
C-10	166.9	166.8
C-11	131.8	131.9
C-12	140.9	140.4
C-13	15.7	15.7
C-14	65.2	64.9
C-16	127.2	127.1
C-17	139.6	139.6
C-18	20.7	20.8
C-19	65.0	15.8

* These are required to be interchanged.

precedented by the behaviour of other saturated PAs [121, 122] (see Scheme 5).

Scheme 5: Mass spectral fragmentation of non-macrocyclic saturated diesters [122].



Further fragmentations would give rise to the peak at m/z 122 as shown in the same Scheme. The alternative arrangement with sarracinic acid at O-7 and angelic acid at O-9 should result in the preferred loss of sarracinic acid to give an intense ion at m/z 221, with the alternative loss of the angeloyloxy radical to give m/z 238. Neither of these peaks was observed in the MS of alkaloid **U**, which was therefore assigned the structure **67** which corresponds to sarracine, an alkaloid previously isolated from other *Senecio* [116, 123-125]. Indeed, the ^{1}H and ^{13}C NMR data of **U** were in full agreement with those reported for sarracine (**67**) [68, 126, 127] (see Tables 41 and 42).

2.4.6 Alkaloid V

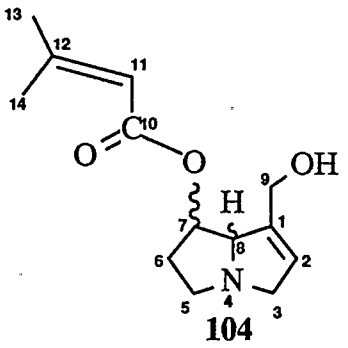
This alkaloid comprised *ca.* 4% of the total bases and was obtained as an

oil.

The EIMS revealed an apparent molecular ion at m/z 237, and its ^{13}C NMR spectrum showed 13 resonances for 13 carbon atoms. Thus **V** was deduced to have a molecular formula $C_{13}H_{19}NO_3$, isomeric with **T**.

In the ^{1}H NMR spectrum of **V** (see Table 43), the resonances at δ 1.90 (3H, d, J = 1.1 Hz), 2.15 (3H, d, J = 1.1 Hz) and 5.61 (1H, qq, J = 1.1 Hz) defined a senecioate (3-methyl-2-butenoate) unit [37, 128, 129]. There was also an olefinic resonance at δ 5.66 (1H, br s). The ^{13}C NMR spectrum of **V** contained signals due to two olefinic carbons, and the presence of a senecioate unit was indicated by the presence of signals at δ 116.0, 20.3 and 27.4 ppm, which may be compared with those for alkaloid **Q** (see page 87). The senecioate unit accounted for two olefinic carbons, and the other two appeared to represent a trisubstituted alkene. The remaining two units of unsaturation therefore correspond to two rings, as expected for a pyrrolizidine alkaloid.

The chemical shifts of the magnetically equivalent H-9 protons at δ 4.20, as well as of the H-7 proton at δ 5.35 (m) are consistent with the esterified hydroxyl function being at C-7 and not at C-9 [37, 66]. Thus the structure **104** was proposed for alkaloid **V**, pending determination of the stereochemistry of the necine system. One of the diastereomers (**105**) had been previously characterised, but a comparison of the ^{1}H and ^{13}C NMR data of alkaloid **V** and **105** (see Tables 43 and 44) revealed some differences as well as considerable congruity. Although saponification of **V** would have settled doubts, by yielding either retronecine (**15**) or heliotridine (**60**), supplies of the alkaloid (which was only isolated in very small amounts) were exhausted before this could be done. Thus the identity of **V** remains open, although given its co-occurrence with **99, 100, 101, 103** it seems probable that it is the retronecine derivative **105.**



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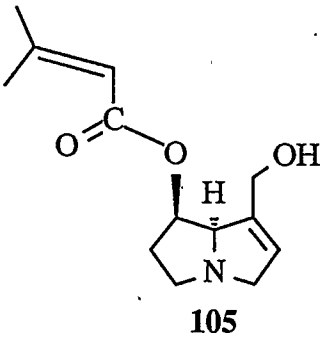


105

2.4.7 Alkaloid **W**

This alkaloid, *ca.* 3% of the total bases, was obtained as a gum.

Its EIMS revealed an apparent molecular ion at m/z 335 with a composition $C_{18}H_{25}NO_5$, isomeric with 7-O-senecioyl-9-O-sarracinylretronecine, as established by high resolution measurement.

In the ^1H NMR spectrum (see Table 45), a methine resonance at δ 5.59 ppm (m) was coupled to two methyl resonances at δ 1.89 (d, J = 1.2 Hz) and 2.14 (d, J = 1.2 Hz) as shown in ^1H-^1H COSY spectrum. An XHCORR spectrum revealed that the methine resonance corresponded to a ^{13}C NMR resonance at δ 116.0 while the methyl groups correlated with resonances at δ 20.6 and 27.5 ppm. The small coupling constants of J = 1.2 Hz were attributed to long range, allylic couplings, as previously noted for alkaloid **Q**. These observations suggested the presence of the senecioyl moiety in the molecule.

Another ^1H methine resonance at δ 6.96 (q, J = 7.2 Hz) coupled (^1H-^1H COSY) to a methyl resonance at δ 1.90 (d, J = 7.2 Hz) and a methylene resonance at δ 4.34 (m) defined a 2-(hydroxymethyl)-2-butenoic acid skeleton, as previously noted for alkaloids **Q** and **U**. Irradiation at δ 6.96 ppm collapsed the multiplet at δ 4.34 to a singlet, and the doublet at δ 1.90 to a singlet, thus confirming the above assignment. The striking differrence between sarracinic acid noted in alkaloids **Q**

Table 43: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **V** and 7-O-senecioylretronecine (**105**).

Proton	Alkaloid **V**	Alkaloid **105** [109]
H-1	-	-
H-2	5.66, br s	5.68, m
H-3a	3.26, m	3.28, m
H-3b	3.91, m	3.89
H-5a	2.70, m	2.71, m
H-5b	3.41, m	3.38, m
H-6a H-6b	2.07, m	2.06, m
H-7	5.35, m	5.45, m
H-8	4.33, m	4.7, m
H-9a H-9b	4.20, m	4.1, m
H-11	5.61, qq, J = 1.1 Hz	5.6, m
H-13	1.90, d, J = 1.1 Hz	1.89, d, J = 1.0 Hz
H-14	2.15, d, J = 1.1 Hz	2.16, d, J = 1.0 Hz

Table 44: ^{13}C NMR Chemical Shifts and Assignments for Alkaloids **V** and **105** .

Carbon	Alkaloid **V**	Alkaloid **105** [109]
C-1	-	138.79
C-2	124.1	121.8
C-3	63.1	61.8
C-5	53.4	53.47
C-6	34.5	34.31
C-7	74.0	72.44
C-8	76.4	76.32
C-9	60.2	59.43
C-10	-	168.0
C-11	116.0	115.0
C-12	-	138.75
C-13	20.3	20.46
C-14	27.4	27.58

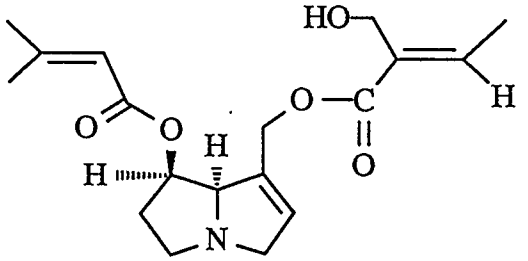
- stands for not observed.

and **U**, and the 2-(hydroxymethyl)-2-butenoic acid skeleton established in alkaloid **W** was that in the ^{1}H NMR spectra of alkaloid **W** the resonance for the vinylic proton was at δ 6.96 ppm , as compared to δ 6.37 and 6.38 ppm in the spectra of **Q** and **U** respectively. These findings were consistent with the vinylic proton in **W** being oriented *cis* to the carbonyl and the calculated value [65] of δ 6.80 ppm was close to that observed.

A ^{1}H NMR resonance at δ 5.80 ppm which appeared as a broad singlet with a small coupling constant (J = 1.6 Hz) was assigned to the vinylic H-2 proton of a pyrrolizidin-1-ene system, as previously observed for other such unsaturated non-macrocyclic pyrrolizidine alkaloids.

The EIMS of alkaloids **Q** and **W** differed only in relative abundance of the ions. This data suggested that **W** was esterified with 5-hydroxytiglic acid at C-9 and senecionic acid at C-7 i.e. **W** was a geometrical isomer of **Q**. Accordingly, the structure **106** was proposed for alkaloid **W**, which apparently has not been described before. This compound resembles neotriangularine (**107**) [71] except that the esterifying angelic acid at O-7 in neotriangularine is replaced by senecioic acid in **W**. As expected, the EIMS of neotriangularine (**107**) and triangularine (**108**) [71] and **106** were very similar.

Further support for the proposed structure was provided by a consideration of the ^{13}C NMR spectrum (see Table 46). As noted earlier p 30, the C-14 methylene carbon resonance in senecionine (**4**) occurred at δ 38.3 ppm while the corresponding resonance in the (E)-isomer, integerrimine (**75**), was observed at δ 29.6 ppm, an upfield shift of 8.7 ppm in the (E)-isomer [85]. A similar trend was observed in the geometrical isomers triangularine (**108**) and neotriangularine (**107**) in which the resonances at δ 64.7 and 56.7 ppm were assigned to the methylene carbons of the hydroxymethyl groups respectively. Again, an upfield shift of 8.0



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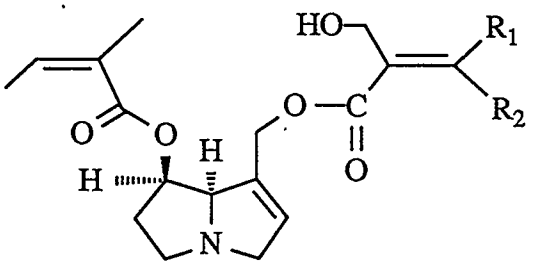


alkaloid	R_1	R_2	
107	Me	H	Neotriangularine
108	H	Me	Triangularine

ppm was noted in passing from the (Z) to (E) geometry. This concept was utilized in the assignment of the ethylidene moiety in the alkaloids **C** and **F** (see pages 30 and 40 respectively). The C-14 methylene carbon resonance in 7-O-senecioyl-9-O-sarracinylretronecine (**99**) occurred at δ 65.7 ppm while that of the supposed (E)-isomer, alkaloid **W**, occurred at δ 56.9 ppm: an upfield shift of 8.8 ppm consistent with expectation for **106**. Hence we concluded that alkaloid **W**, which we have named foetidine is indeed a geometrical isomer of alkaloid **Q**.

2.5 The alkaloids of *Dendrosenecio cottonii* J. Hutch. and G. Tayl.

This plant belongs to the genus *Dendrosenecio,* which are distributed in the mountains of tropical Africa. *D. cottonii* is endemic to Mt. Kilimanjaro at an altitude range of 3700-4500 m [130].

Table 45: ^{1}H NMR Chemical Shifts and Assignments for Alkaloid **W** and 7-O-Senecioyl-9-O-sarracinylretronecine (**99**)

Proton	Alkaloid **W**	Alkaloid **99** [109]
H-1	-	-
H-2	5.80, br s	5.78, m
H-3a	3.94, br d, J = 15.5 Hz	3.94, m
H-3b	3.43, m	3.49, m
H-5a	3.39, m	3.31, m
H-5b	2.65, q, J = *ca.* 8.6 Hz	2.67, m
H-6a H-6b	2.06, m	2.67, q, J = 9.0 Hz
H-7	5.33, m	5.34, m
H-8	4.34, m	4.51, m
H-9a	4.78, d, J = 13.2 Hz	4.78, m
H-9b	4.72, d, J = 13.2 Hz	
H-11	5.59, m	5.56, m
H-13	1.89, d, J = 1.2 Hz	1.87, d, J = 1.0 Hz
H-14	2.14, d, J = 1.2 Hz	2.05, d, J = 1.0 Hz
H-17	6.96, q, J = 7.2 Hz	6.38, q, J = 7.5 Hz
H-18	1.90, d, J = 7.2 Hz	2.02, d, J = 7.5 Hz
H-19	4.34, br s	4.22, s

Table 46: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **W** and 7-O-Senecioyl-9-O-sarracinylretronecine (**99**)

	Compound	
Carbon	Alkaloid **W**	Alkaloid **99** [109]
C-1	131.7	131.8
C-2	127.8	127.0
C-3	62.8	62.3
C-5	53.8	53.7
C-6	34.5	34.4
C-7	73.3	72.9
C-8	75.8	75.7
C-9	61.4	60.8
C-10	167.0	166.6
C-11	116.0	115.8
C-12	133.9	133.9
C-13	20.6	20.3
C-14	27.5	27.5
C-15	165.9	165.6
C-16	157.4	158.0
C-17	141.2	141.0
C-18	14.2	15.8
C-19	56.9	64.7

The dried ground leaves of *D.cottonii* were subjected to repeated extraction using ethanol. The extracts were concentrated to yield a residue which was partitioned between $CHCl_3$ and 0.5 M H_2SO_4. Part of the aq. phase was then subjected to a reductive workup to give a substantial yield (1.24%) of alkaloidal material. GLC analysis of this revealed one major and two minor components. These were separated by TLC to yield the following substances:

2.5.1 Alkaloid X

This alkaloid constituted *ca.* 83% of the total alkaloids, and was isolated as an oil, which could not be crystallized.

Its EIMS revealed a relatively abundant (20%) apparent molecular ion at m/z 278 with the presence of major fragment ions at m/z 154 (50), 143 (58), 137 (50) and 136 (100). This even-mass suggested the presence of an even number of nitrogen atoms.

As the broad-band ^{13}C NMR spectrum of **X** exhibited 19 resonances (see Table 48), none of which appeared doubled, and its ^1H NMR spectrum (see Table 47) integrated for a total of 22 hydrogens (which correponded to the results of DEPT spectroscopy which revealed 10 methine, 6 methylene and 3 quaternary carbons) a partial formula of $C_{19}H_{22}$ seemed likely. This would contribute 250 mass units to the molecular weight, and the balance of 28 was then accounted for by 2 nitrogens, thus the molecular formula $C_{19}H_{22}N_2$ was deduced, with an IHD of 10. The abundant molecular ion and high unsaturation number suggested that this alkaloid was aromatic. Indeed, its ^1H NMR spectrum contained resonances in the aromatic region corresponding to six protons, while another three appeared to be vinylic. Of the aromatic protons, one appeared as a doublet at remarkably low field (δ 8.81, d, J = 4.4 Hz): a chemical shift characteristic of the α-protons of pyridine

and quinoline [131]. As the proton coupled to this itself appeared as a doublet (δ 7.29, d, J = 4.4 Hz), it was concluded that if a pyrido-system was present it carried a γ-substituent. The remaining four aromatic protons formed a set (δ 8.10, 2H, each dd, J = 1.2, 8.2 Hz), 7.70, 1H, ddd, J = 1.2, 7.1, 8.2 Hz; 7.57, 1H, ddd, J = 1.2, 7.1, 8.2 Hz) corresponding to a 1,2-disubstituted benzene. These findings were very well accomodated by a γ-substituted quinoline.

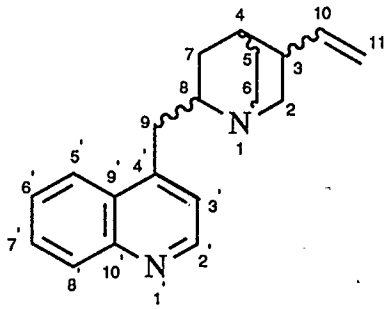
Consistent with this idea, the ^{13}C NMR spectrum of **X** contained 6 methine and 3 quaternary resonances with the chemical shifts expected for a γ-alkyl substituted quinoline [132], and the methine resonances correlated (XHCORR spectrum) with the aromatic protons.

With this C_9H_6N (IHD = 7) quinoline unit assigned, the identity of the $C_{10}H_{16}N$ (IHD = 3) substituent remained to be established.

The ^{13}C NMR data of **X** appeared to contain one mono-substituted ethylene unit as revealed by resonances at δ 141.4 and 114.0 ppm, the former was shown by DEPT spectra to be a methine and the latter a methylene carbon. The ^{1}H NMR data revealed resonances at δ 5.80 (1H, ddd, J = 17.6, 10.6, 7.6 Hz), 5.00 (1H, ddd, 17.6, 1.1, 1.1 Hz) and 4.96 (1H, ddd, J = 10.6, 1.1, 1.1 Hz) and XHCORR spectroscopy related the first of these resonances to the carbon with δ 141.4, and the other two to the carbon with δ 114.0 ppm. As there were no other indications of unsaturation, it was concluded that the system was bicyclic and since there appeared to be no hydrogens attached to heteroatoms, a bicyclic tertiary amine carrying a $CH{=}CH_2$ substituent was inferred.

The presence of a 4-substituted quinoline ring with a $C_{10}H_{16}N$ substituent containing a bicyclic tertiary amine (carrying a mono-substituted ethylenic unit) immediately brought to mind the *Cinchona* alkaloids, containing the bicyclic quinuclidine system [133]. Accordingly, the structure **109** was proposed for **X**,

which corresponds to a deoxyderivative of the commonly encountered *Cinchona* alkaloids such as cinchonidine (**110**).



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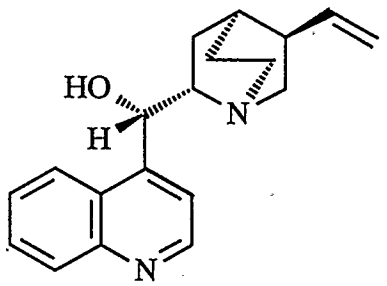
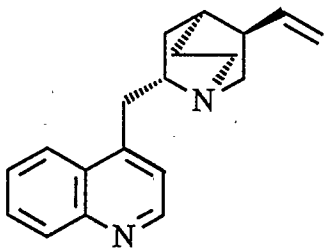


110

Supporting evidence for this structure came from the mass spectrum, in which the major fragments observed were m/z 136 corresponding to the quinuclidine moiety and m/z 143 corresponding to the quinoline plus benzylic centre [134, 135]. This alkaloid apparently had not been described as naturally occurring, but deoxycinchonidine (**111**) had been obtained from cinchonidine (**110**) by a photoreduction process [136, 137]. Determination of the $[\alpha]_D$ value of alkaloid **X** revealed this alkaloid to correspond to deoxycinchonidine (**111**), although ^{13}C data were not available for complete comparison (see Table 48 for our results). The ^{1}H NMR data reported appeared sketchy [137], but seemed to fall within the limits of our findings (see Table 47).



111

All the naturally occurring cinchona alkaloids characterised by the quinoline and quinuclidine ring systems bear a hydroxyl group on C-9 [133] as a consequence of the biosynthetic construction of these alkaloids [133, 138, 139].

Table 47: ^{1}H NMR Chemical Shifts and Assignments for Alkaloids **X** and **111**

Proton	Alkaloid **X**	**111** [137]
H-2	2.70, dd, J = 2.5, 5.0 Hz	
H-2	3.23, m	
H-3	2.29, m	
H-4	1.80, m	
H-5	*ca.* 1.65, m	2.20-0.85, 6H, m
H-6a	2.79, m	
H-6b	3.23, m	
H-7a	1.80, m	
H-7b	1.18, dd, J = 6.4, 6.7 Hz	3.52-2.33, 7H, m
H-8	3.23, m	
H-9a	3.09, dd, J = 8.5, 13.7 Hz	
H-9b	3.42, dd, J = 5.7, 13.7 Hz	
H-10	5.80, ddd, 7.6, 10.6, 17.6 Hz	6.12-5.50, 1H, m
H-11a	4.96, ddd, 1.1, 1.1, 10.6 Hz	5.17-4.77, 2H, m
H-11b	5.00, ddd, 1.1, 1.1, 17.6 Hz	
H-2′	8.81, d, J = 4.4 Hz	8.83, d, J = 4.5 Hz
H-3′	7.27, d, J = 4.4 Hz	7.26, d, J = 4.5 Hz
H-5′	8.10, dd, J = 1.2, 8.2 Hz	7.85-7.46, m
H-6′	7.57, ddd, J = 1.2, 7.1, 8.2 Hz	
H-7′	7.70, ddd, J = 1.2, 7.1, 8.2 Hz	
H-8′	8.10, dd, J = 0.5, 8.2 Hz	8.27-2.97, m

Table 48: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **X** .

Carbon	Alkaloid **X**
C-1	-
C-2	55.9
C-3	39.2
C-4	27.6
C-5	27.7
C-6	40.7
C-7	28.6
C-8	55.8
C-9	37.8
C-10	141.4
C-11	114.0
C-2′	149.7
C-3′	121.2
C-4′	148.1
C-5′	123.1
C-6′	126.1
C-7′	128.6
C-8′	130.0
C-9′	127.5
C-10′	145.1

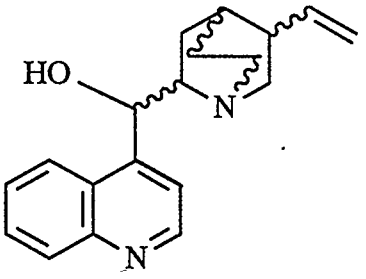
From our findings, it appeared to us that alkaloid **X**, now designated deoxycinchonidine, could be an artifact. Thus we resorted to examining the unreduced portion of the plant extract, which yielded a single metabolite which we referred to as alkaloid **Y**.

2.5.2 Alkaloid Y

The EIMS of **Y** showed an apparent molecular ion at m/z 294, 16 mass units higher than deoxycinchonidine (**111**). The ^{13}C NMR spectrum showed the presence of 19 carbons and 21 hydrogens, while the ^{1}H NMR integrated for 22 hydrogens. The IR spectrum revealed the presence of hydroxyl group (3200 cm^{-1}) and hydroxylic C-O stretching band (1098 cm^{-1}). Accordingly, the molecular formula $C_{19}H_{22}N_2O$ was formulated.

The ^{1}H NMR of alkaloid **Y** (see Table 49) showed signals at δ 7.55 (1H, d, J = 4.4 Hz), 8.72 (1H, d, J = 4.4 Hz), and 8.04 (1H, d, J = 8.5 Hz), 7.60 (dd, J = 7.0, 8.2 Hz), 7.31 (1H, dd, J = 7.0, 8.2 Hz) and 7.93 (1H, d, J = 8.5 Hz), which correspond to the signals due to the C-3′, C-2′ and C-5′-8′ protons in the quinoline moiety of *Cinchona* alkaloids [133]. The ^{13}C NMR spectrum of **Y** (see Table 50) resembles that of **X**, especially the signals due to C-2′ through C-10′ and C-2 through C-5. However, in the case of *Cinchona* alkaloids bearing a hydroxyl group at C-9, the C-9 resonance is observed in the region 69-73.5 ppm [133,140]. There occurred an absorption in the ^{13}C NMR spectrum of **Y** at δ 71.6 ppm, which was assigned to this functionality. This led to structure **112** for alkaloid **Y**, which corresponds to the stereoisomers cinchonidine (**110**) and cinchonine (**113**), alkaloids which have been previously isolated from many *Cinchona* and *Remijia* species, and also from *Olea europeae* (family Olaeceae) and

Ligustrum vulgare (family Rubiaceae) [133, 141].



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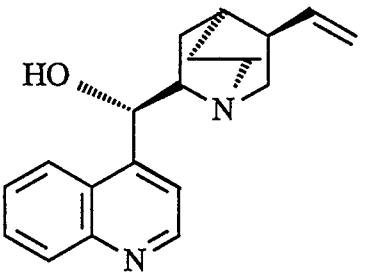

113

In support of these structures, the mass spectrometry revealed the major fragment ions at m/z 136 (quinuclidine moiety) and m/z 159 (quinoline plus benzylic centre) [134]. To differentiate between the two stereoisomers, a comparison of their $[\alpha]_D$ values was undertaken. The value for **Y** ($[\alpha]_D$ -102.5 (EtOH)) was in good agreement with that reported for cinchonidine (**110**) ($[\alpha]_D$ -110^0 (EtOH), but not cinchonine (**113**) ($[\alpha]_D$ +229 (EtOH) [141]. The ^{13}C NMR data was also in excellent agreement with that of authentic cinchonidine (**110**) (see Table 50). Finally, the mp and IR spectrum of alkaloid **Y** were identical to those of the authentic sample of cinchonidine.

To confirm if indeed alkaloid **X** (deoxycinchonidine) was derived from alkaloid **Y** (cinchonidine) during the reductive work-up, cinchonidine (**110**) was treated with zinc in aq. H_2SO_4 to afford an oil which was identical with deoxychinchonidine (**111**) obtained from the plant extract.

Since only a single metabolite was isolated from the unreduced portion of *D. cottonii,* it became clear that the minor components isolated from the reduced portion like the major one were artifacts, and no attempt was made to characterise them although it was anticipated that they would correspond to diastereomeric tetrahydroderivatives of deoxycinchonidine.

The discovery of cinchonidine in the extract of *Dendrosenecio cottonii* was strange and startling. Four possibilities came to mind: (1) the plant collected was

Table 49: ^{1}H NMR Chemical Shifts and Assignments for Alkaloids **Y** and **110**

Proton	Alkaloid **Y**	Alkaloid **110** [133]
H-2	3.01, m	n.a.
H-2	2.59, m	n.a.
H-3	2.43, m	3.41
H-4	1.78, m	3.05, q, J = 10.3, 13.6 Hz
H-5	1.78, m; 1.49, m	n.a.
H-6a	2.59, m	n.a.
H-6b	3.60, m	
H-7a	1.49, m	n.a.
H-7b	1.78, m	
H-8	3.04, m	3.11, oct, J = 4.6, 8.1, 8.1 Hz
H-9a	5.64, d, J = 2.6 Hz	5.62, d, J = 4.3 Hz
H-9b	-	-
H-10	5.67, ddd, m	5.71, oct, J = 7.4, 10.2, 17.3 Hz
H-11a	4.88, dd, 1.0, 10.5 Hz	4.89, d, J = 10.8 Hz
H-11b	4.92, ddd, 1.4, 1.0, 18.0 Hz	4.93, d, J = 15.9 Hz
H-2′	8.72, d, J = 4.4 Hz	8.82, d, J = 4.6 Hz
H-3′	7.55, d, J = 4.4 Hz	7.55, d, J = 4.6 Hz
H-5′	8.04, d, J = 8.5 Hz	8.09, d, J = 8.5 Hz
H-6′	7.60, dd, J = 7.0, 8.2 Hz	7.65, t, J = 7.6, 7.6 Hz
H-7′	7.31, dd, J = 7.0, 8.2 Hz	7.44, t, J = 7.6, 7.6 Hz
H-8′	7.93, d, J = 8.5 Hz	7.96, d, J = 8.5 Hz

n.a = not assigned, oct = octet

Table 50: ^{13}C NMR Chemical Shifts and Assignments for Alkaloid **Y** and Cinchonidine (**110**)

Carbon	Compound	
	Alkaloid **Y**	* Cinchonidine (**110**)
C-1	-	-
C-2	56.9	56.9
C-3	39.8	39.9
C-4	27.9	27.9
C-5	27.5	27.6
C-6	43.2	43.2
C-7	21.1	21.4
C-8	60.3	60.4
C-9	71.6	71.7
C-10	141.7	141.7
C-11	114.3	114.3
C-2′	149.9	149.9
C-3′	123.0	123.0
C-4′	149.7	149.7
C-5′	118.2	118.2
C-6′	126.6	126.5
C-7′	129.0	128.9
C-8′	130.0	130.0
C-9′	125.6	125.6
C-10′	147.9	148.0

* Values obtained for the Sigma Chem. Co. sample

not a *Dendrosenecio* but rather a *Cinchona*; (2) that the plant was a *Dendrosenecio* which had acquired the cinchonidine by root parasitism on some *Cinchona* (or other cinchonidine producing plant); (3) the extracts had in some way become adulterated with cinchonidine; or (4) that the result was genuine. Of these possibilities the first and last seem least likely. There are records of *Cinchona* having been introduced into Tanzania by colonial authorities who grew the plant on Mt. Kilimanjaro [142], but the collector is convinced that he collected a *Dendrosenecio,* which have very distinct form, and are unlike *Cinchona.* However, and most unfortunately, no herbarium specimen was retained. While similar extraordinary appearances of tightly restricted "characteristic" plant secondary metabolites have been authenticated (as for example that of *Cinchona* alkaloids in *Olea europeae* and *Ligustrum vulgare* [133, 141], and the *Delphinium/Aconitum [Ranunculaceae]* alkaloids in a single species (*Inula roylaena)* of the Asteraceae [143], the fact that the *Dendrosenecio* extract contained no detectable levels of any other alkaloid besides cinchonidine makes it seem improbable that this was biosynthesized in the plant. Plant alkaloid biosynthesis tends to produce several structurally related compounds, not a single product, the results of our investigations of species of *Senecio* being typical in this respect. Finally, the collector recalls no *Cinchona*-like shrub in the vicinity of the *Dendrosenecio* he collected or others that he saw, nor is there any report that these tree-like plants are parasitic on others. Thus it seems likely that the cinchonidine isolated from our collection was somehow introduced as an adulterant. However, if this is the correct answer we are at a loss to account for the way in which it occurred.

2.6 Conclusion

2.6.1 The Alkaloids of *Senecio hadiensis*.

Senecio hadiensis, like most *Senecio* yielded macrocyclic pyrrolizidine alkaloids, all of which were saturated. The unreduced extracts in addition to yielding the tertiary bases, also yielded their corresponding N-oxides. Thus the PA alkaloids occur in *S. hadiensis* as in other plants, as a mixture of tertiary bases and the corresponding N-oxides. For chemotaxonomic comments see the following section on *S. syringifolius*.

The most striking feature of the new alkaloids discovered in *S. hadiensis* was the presence of the 1-hydroxyl functionality as found in hadiensine, 12-O-acetylhadiensine, 12-O-acetylneohadiensine and the supposed 2-hydroxyhadiensine. Of the more than 200 PAs reported to date [28-31] only two others had previously been found to contain this system: curassanecine with the 1β, 8α- stereochemistry (**12**), and the 1α, 8α, platynecinium derivative (**73**).

As described in the Results and Discussion (p 26), our assignment of the α-configuration to the C-1 hydroxyl of hadiensine was based on a long-range chemical shift of H-7 induced by acetylation. We had hoped to put this on a firmer basis by nOe measurements, but these were fruitless. Also, although crystalline salts were prepared from hadiensine, itself a glass, none of these was suitable for X-ray crystallographic establishment of structure.

Circumstantial support for the 1α-configuration is provided by the fact that the other alkaloids isolated corresponded to rosmarinine and derivatives thereof i.e. if, as it seems most likely, the latter are obtained by P_{450}-catalysed 2α-hydroxylation of platynecine, the hadiensine system simply arises by alternate C-1α-hydroxylation. Consistent with this argument, which utilizes the fact that

P_{450} hydroxylations are known to proceed with retention of stereochemistry [144], curassanecine which has the 1β-hydroxylation was isolated from a plant whose main PAs were derivatives of trachelanthamidine (**37**) [82] i.e. **12** appears to have risen by similar P_{450} hydroxylation of that system.

However, unless some new derivatives of hadiensine provide crystals suitable for an X-ray structure determination, it seems that the stereochemistry at C-1 will have to be proven by synthesis, probably of hadienecine, as was done for curassanecine [145]. This should be a target for future work.

Since the structural requirement for hepatotoxicity in the PAs has been suggested to be the presence of a 1,2-double bond and an ester function at C-7 and C-9 or C-9 alone (see section 1.4), the PAs of *S. hadiensis* are unlikely to be responsible for liver damage in cattle.

2.6.2 The Alkaloids of *Senecio syringifolius.*

In Kenya, this plant and *S. hadiensis* are sympatric, and at our collection site they often grow intertwining with one another. Similarities between the plants extends to their alkaloids, for *S. syringifolius* proved to contain rosmarinine, and other PA dilactones of rosmarinecine (**14**) although there were no 1-hydroxy derivatives. Rosmarinine was first isolated in South Africa from *S. rosmarinifolius* and subsequently from several other *Senecio* species from that area, but is apparently rarer in North America, Australia, and Asia. This suggests that as evolution of the genus took place in Africa, the ability to hydroxylate platyphylline was retained. It will be interesting to see how the PA content of *Senecio* from Tanzania, Uganda, Ethiopia and elsewhere in Africa compare with those from Kenya.

Like the alkaloids of *S. hadiensis,* the PAs of *S. syringifolius* should be non-hepatotoxic and are unlikely to confer markedly poisonous properties.

2.6.3 The Alkaloids of *S. canus.*

Two macrocyclic PAs, namely senecionine (**4**) and retrorsine (**76**) were isolated from this plant. These PAs are commonly encountered in North American and European *Senecio*, and the PA content of *S. canus* is thus otherwise unremarkable.

Its a common plant of the dry prairies, hence its name of Prairie groundsel, but we know of no use having been made of it by the aboriginal population. Perhaps they were aware of the poisonous properties which we would predict from the presence of **4** and **76**, for these are hepatotoxic PAs.

2.6.4 The Alkaloids of *S. foetidus.*

The PAs of *S. foetidus* proved to be acyclic ester derivatives of the saturated base platynecine, and the unsaturated retronecine. The alkaloid composition was, qualitatively, in some ways similar to that of Albertan populations of *S. triangularis* whose range, in the moist foothills and mountain valleys of the Rockies overlaps with *S. foetidus*. Thus, although the two plants have obvious differences in morphology, the similarities in PAs may reflect a close common evolutionary ancestor. Alternatively, gene transfer may have occurred from one species to the other. In this regard it may be noted that *S. triangularis* collected in California [71] contained some different PAs to those found in the Albertan plant [110].

As with *S. canus,* we have been unable to find any record of aboriginal folk-lore concerning *S. foetidus*. However it should be a poisonous plant as a consequence of its content of 7,9 and 9-ester derivatives of retronecine, PAs whose structures satisify the requirements for hepatotoxicity.

2.6.5 The Alkaloids of *Dendrosenecio cottonii.*

About 26 species of *Dendrosenecio* have been described, all confined to the mountains of tropical East Africa [130]. No previous chemical studies of these giant tree-*Senecio* have previously been reported. However, our discovery of cinchonidine in a collection purported to be *D. cottonii*, from Mt. Kilimanjaro, is such an extraordinary result that it demands verification. Obtaining material from the original collection site may be difficult, and it may be more feasible to obtain other, authenticated, species of *Dendrosenecio* from Kenya for comparative analysis. Until this has been done the *D. cottonii* results must be regarded as highly suspect.

2.6.6. Overall

The results of the investigations described in this thesis added some useful information to our knowledge of the PAs of African and Canadian *Senecio,* including some new alkaloids, and suggests further experiments.

3. EXPERIMENTAL

3.1 General Experimental Procedures

Melting Points

Melting Points (mp) reported in $^{\circ}$ Celsius were determined on a Kofler apparatus and are uncorrected.

Infrared (IR) Spectra

Infrared Spectra were recorded with a Nicolet DX-I system or a Mattson FT-IR spectrophotometer model 4030, as thin films of neat liquid (film), as samples dispersed in KBr and pressed into pellets (KBr), or as chloroform solutions ($CHCl_3$). Absorption maxima (υ_{max}) are in cm^{-1} units and relative intensities are expressed as s (strong), m (medium) or w (weak).

Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectra

^{1}H NMR spectra were obtained at 200 or 400 MHz with Bruker ACE-200 and AM-400 spectrometers respectively. Unless otherwise specified, the residual chloroform signal (δ 7.27 ppm) was used as an internal reference from which chemical shifts (δ) are reported in parts per million (ppm), with multiplicities indicated by s (singlet), d (doublet), t (triplet), q (quartet), dq (double quartet), br d (broad doublet), br s (broad singlet), dd (doublet of a doublet) and m (complex multiplet). Spin-spin coupling constants (J) are given in Hertz (Hz) with a usual accuracy of $\pm$ 0.2 Hz.

Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) Spectra

^{13}C NMR data were collected on Bruker ACE-200 and AM-400 spectrometers at

50.4 and 100.7 MHz respectively. Unless otherwise specified, the samples were dissolved in $CDCl_3$ and the solvent resonance (δ 77.0 ppm) was used as an internal reference. The number of hydrogen atoms attached to individual carbons were determined using DEPT microprograms. The $^1H,^1H$ and $^1H,^{13}C$ 2D-correlation spectra were measured using COSY and XHCORR microprograms. In cases involving small amount of samples (less than 1 mg), the inverse detection technique [146] was used to correlate hydrogen and carbon resonances.

Mass Spectra (MS).

Mass spectra were obtained with a Kratos MS-80 or a VG-7070 instrument at an ionization potential of 70 eV and are given as mass to charge ratios (m/z) with relative ion intensities in parentheses. Fast atom bombardment (FAB) spectra were obtained at the University of Alberta using a matrix of a 5:1 mixture of dithiothreitol:dithioerythritol (DT's, "magic bullet") [147] xenon atoms being used at 6 kV accelerating potential.

Optical Rotations

Specific optical rotations, $[\alpha]_D$, were measured at the sodium D-line (589 nm) with a Rudolf Autopol III polarimeter using a 1 cm light-path cell. The solvents used were as specified.

Gas liquid chromatography (GLC).

The GLC analyses were performed with an HP-5890 chromatograph fitted with a flame ionisation detector (fid) and DB-1 megabore capillary column (30 m x 0.53 mm, i.d. x1.5 μ film thickness). A temperature program was used of 1 min at 200^0 C followed by a 10^0/min increase to 240^0 C, which temperature was then maintained for the duration of the analyses. Nitrogen was used as the carrier gas at a flow rate of 24 mL/min. The % values

given after retention time (R_T) are those obtained from the fid, i.e., from the detector uncalibrated for variations in sensitivity to individual alkaloids.

Thin Layer Chromatography (TLC)

Samples were spotted onto pre-coated silica gel TLC plates (Merck 60 F_{254}, 5 cm x 20 cm, 0.25 mm layer thickness) or aluminium oxide TLC plates (Merck type E F_{254}, 5 cm x 20 cm, 0.25 mm thickness). Where not otherwise specified TLC and PTLC were conducted on silica gel F_{254} plates. After elution, the plates were examined under UV light (254 nm, i.e short wavelength), and occasionally developed in an iodine chamber. The solvent systems used are specified in the individual experiments (see PTLC below). All solvent mixtures were prepared on a volume/volume basis.

Preparative TLC (PTLC)

A concentrated solution of the sample was applied to pre-coated silica gel plates (Merck, 60 F_{254}, 5 cm x 20 cm and 20 x 20 cm, 0.25 mm thickness and occasionally 1 mm thickness plates) or pre-coated aluminium oxide plates (Merck type E, F254, 5 x 20 cm and 20 x 20 cm, 0.25 mm thickness). The applied materials were developed with suitable solvent systems and the plates examined under UV light (254 nm short wavelength) to locate the zones containing individual components of the mixtures. Chloroform-methanol-ammonia (50:10:1) was used as the eluting solvent, unless specified otherwise. The R_f values are quoted in this solvent system using silica gel plates. The same applies to TLC.

Radial Centrifugal Thin Layer Chromatography (RCTLC) [148, 149]

Centrifugally accelerated radial chromatography was carried out with a Harrison Research Chromatotron model 7924T. The rotors were coated with silica gel 60 PF_{254}

(Merck 7749, 1 mm thickness) containing $CaSO_4$.

Vacuum Liquid Chromatography(VLC) [148]

The alkaloid mixture was subjected to column vacuum liquid chromatography as follows: a sintered glass Buchner filter funnel (150 mL) (protected with a disc of Whatman No 1 filter paper) was packed with TLC grade silica gel PF_{254}. The funnel was filled with silica gel and tapped on the bench until the silica gel was approximately 3/4 of its original volume. This was then followed by vacuum suction using a water aspirator. A small Erlenmeyer flask was used to tamp down the silica gel until a very hard smooth surface was produced. Then with the vacuum still on, $CHCl_3$ (225 mL) was applied onto the column. Just before the column surface was exposed the alkaloid sample dissolved in $CHCl_3$ (50 mL) was applied.

Photolysis

Photolyses were carried out using a Southern New England UV Rayonet RMR - 500 reactor, equiped with four 254 nm lamps. The samples were contained in quartz tubes.

Alkaloid Spot Test Reagents

The presence of alkaloids in the aqueous extracts was detected with Mayers reagent: an aqueous solution of a mixture of mercuric chloride (1.36 g) and potassium iodide (5.00 g) made up in water (1000 mL). A creamy white precipitate corresponded to a positive test for alkaloids.

3.2 Plant Materials

3.2.1 *S. hadiensis* Forsk. (syn. *S. petitianus* A.Rich.)

The epigeal parts of flowering *S. hadiensis* were collected at the forest margin along the top of the Ngong Hills, near Karen, Kajiado District, Kenya in June 1988 and July 1989. A voucher specimen is deposited in the Herbarium of the University of Calgary. The plant was air dried and then pulverised.

3.2.2 S. syringifolius O. Hoff.

The epigeal parts of flowering *S. syringifolius* were collected in the same area as *S. hadiensis* April 1990 and identified by Mr. S. Mathenge, curator of the Herbarium, University of Nairobi, Kenya.

3.2.3. *S. canus* Hook.

This was collected along the roadside edges of Highway 22 just North of Lundbreck Falls, Alberta, Canada, in June 1990.

3.2.4 *S. foetidus* Howell (syn. *S. hydrophloides* Rydb.)

The flowering heads of *S. foetidus* were collected along the forest road running south from Blairmore, into the Crowsnest forestry region, Alberta, Canada , in July 1990. A voucher specimen is deposited in the Herbarium of the University of Calgary.

3.2.5 *D. cottonii* J.Hutch. and G.Tayl.

A terminal rosette of this plant was collected from a gully on the track just below the Horombo Hut on Mt. Kilimanjaro, in July 1987, by M.H. Benn, and air-dried at the Department of Chemistry, University of Nairobi. No Herbarium specimen was retained.

3.3 Extraction and Separation Procedures

3.3.1 Isolation of the Alkaloids of *S. hadiensis* Forsk. (syn. *S. petitianus* A.Rich)

3.3.1.1 With Reductive processing.

The powdered plant material (1.24 kg) was first defatted by soaking in hexanes (4 L) then, after filtration and brief drying in air, transferred to a Waring blender (1 gal) and extracted by repeated maceration in 95% EtOH (10 x 4 L). The combined EtOH extracts were concentrated (cyclone, then rotary evaporator) to a dark green gum which was partitioned between 0.25 M aq H_2SO_4 (70 mL) and $CHCl_3$ (100 mL). The aq. phase was washed with more $CHCl_3$ (3 x 100 mL) and then stirred with zinc dust (ca. 10 g) at room temperature overnight. After filtering off the zinc dust and washing the filter with a little 0.25 M aq. H_2SO_4, the filtrate and washings were chilled by adding some crushed ice, brought to pH *ca.* 10 (indicator paper) with conc. aq. NH_4OH, and extracted with $CHCl_3$ (6 x 50 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and then evaporated to dryness under reduced pressure to yield the crude "reduced bases" as a light yellow glass (14.1 g, 1.1%). A TLC analysis ($CHCl_3$-MeOH-NH_4OH (50:10:1)) of this revealed five components, R_f 0.37, 0.33, 0.27, 0.22 and 0.07; while GLC analysis showed nine peaks, R_T 8.7 (52%), 9.2 (3%), 10.0 (1%), 10.6 (34%), 11.8 (5%), 12.4 (3%), 13.8 (1%) , 14.5 (0.5%) and 17.5 min. (0.5%).

3.3.1.2 Without Reductive processing

Powdered plant material (1.08 Kg) was defatted with hexanes and extracted with 95% EtOH (see section 3.3.1.1 above). The processing of the ethanolic extract differed from that in section 3.3.1.1 only in that the treatment with aq. H_2SO_4 and Zn dust was omitted. The 0.25 M aq. H_2SO_4 extracts were basified to pH *ca.* 10 (indicator paper) with NH_4OH, and extracted with $CHCl_3$ (6 x 50 mL). Evaporation of the combined dried

($MgSO_4$) $CHCl_3$ extracts gave the "unreduced bases" as a pale brown glass (2.3 g, 0.2%). Analysis of this material by TLC and GLC again revealed 9 components chromatographically indistinguishable from those in section 3.3.1.1, but with some quantitative variations in their relative amounts, e.g. R_T 8.7 (45%), 9.2 (3%), 10.0 (1%), 10.6 (40%), 11.8 (3%), 12.4 (6%), 13.8 (1%), 14.5 (1%) and 17.5 (0.5%) min.

The residue left after extracting with $CHCl_3$ was extracted with n-butanol (7 x 100 mL). The n-butanol extracts were dried ($MgSO_4$) and concentrated (rotary evaporator) to give a brown residue (10.9 g).

3.3.2 Separation Of The Individual Alkaloids

3.3.2.1 Separation of the Reduced Bases.

Initial separations of the components of the reduced alkaloid mixture were carried out using RCTLC and VLC.

3.3.2.1.1 Separation of the alkaloids mixture by RCTLC

The crude alkaloid mixture (0.85 g) was subjected to RCTLC using $CHCl_3$-MeOH-NH_4OH (100:20:1) as eluant. The fractions were monitored by TLC and GLC and pooled accordingly (see table 51). The components of fractions 1-10 were non alkaloidal and were not examined any further.

The other fractions were subjected to the following separations:

Fraction 11 showed mainly one spot on TLC analysis, R_f 0.33. This material was subjected to PTLC, with double elution, to yield the main component as a white powder (8 mg) which formed colourless crystals from Me_2CO and was identified as rosmarinine (**68**) on the basis of the following properties : R_f 0.33, R_T 10.6 min, mp 208-209^0 C from

Table 51: Radial centrifugal thin layer chromatography of crude alkaloid mixture (0.85 g). The eluant was $CHCl_3$-MeOH-NH_4OH (100:20:1)

Fraction No.	Volume collected	Weight eluted
	(mL)	(mg)
1-10	50	11.0
11	5	12.0
12	5	30.0
13	,,	42.6
14	,,	80.0
15	,,	100.0
16	,,	120.0
17	,,	60.0
18	,,	51.0
19	,,	44.0
20	,,	38.0
21	,,	30.0
22	,,	45.4
23	,,	25.0
24	,,	24.0
25	,,	10.0
26	,,	30.0
27	,,	30.0
28	,,	80.0
29	,,	21.3
30	,,	10.0
31-41	100	109.2
		1003.5

Me_2CO [lit [73] 203-204^0 C, [75] 209^0 C]; $[\alpha]_D$ -121^0 (c = 4, $CHCl_3$), [lit [73] $[\alpha]^{20}_D$ -120^0 (c = 1, $CHCl_3$)]: ^1H and ^{13}C NMR (see Tables 3 and 4]; IR υ_{max} (KBr) 3408 (br, s, OH), 1743 (s, C=O), 1719 (s, C=O), 1655 (w); EIMS m/z (%) [M^+] 353 (1), 156 (18), 154 (28), 138 (38), 98 (16), 83 (12), 82 (43), 81 (18), 44 (100) and 43 (68); CIMS (NH_3) [M+H] 354 (100), 227 (19), 199 (3), 156 (23), 154 (22), 138 (55),1 22 (11), 112 (7), 98 (5), 82 (18).

Fraction 12 showed two main spots and one minor one on TLC analysis with R_f values 0.37, 0.33 and 0.27. This material was purified by PTLC to yield three components.

The component with R_f 0.37, 12-O-acetylrosmarinine (**86**) (8 mg), was obtained as a pale yellow oil, R_T 14.5 min., $[\alpha]_D$ -36.1° (c = 1.8, EtOH); IR υ_{max} ($CHCl_3$) 3543 (br, m), 2981 (m), 2966 (m), 1750 (s, C=0), 1729 (s, C=O), 1708 (s, C=O), 1448 (m), 1370 (m), 1188 (s), 1164 (s), 1110 (s); ^1H and ^{13}C NMR (see Table 14), EIMS m/z (%) [M^+] 395.1939 (2) (calc. for $C_{20}H_{29}NO_7$ 395.1945), 336 (1), 270 (1), 226 (18), 154 (40), 138 (28), 117 (10), 110 (10), 98 (12),86 (15), 84 (25), 82 (22), 55 (18), 44 (100), 43 (78) and 41 (20).

The second component, R_f 0.33 (13 mg) was the main one in the mixture. The compound was identified as rosmarinine (**68**) on the basis of spectral data (^1H and ^{13}C NMR).

The third component, (6.0 mg), R_f 0.27 , R_T 8.7 mins. was obtained as a pale yellow oil and identified as hadiensine (**70**) on the basis of spectral data (^1H and ^{13}C NMR see Table 5, see p 137 for the other properties).

Fraction 13 was not purified as TLC analysis revealed it to be similar in composition to fractions 12 and 14.

Fraction 14 was purified by PTLC giving rise to four zones. The material (18.3

mg) recovered from the zone with R_f 0.37, appeared to be pure (GLC analysis). Spectroscopic analysis (^1H and ^{13}C NMR) revealed this component to be 12-O-acetylrosmarimine (**86**) (see pg 133). Materials extracted from the last three zones were found to be impure (GLC analysis) and were not analysed further.

Fraction 15 yielded a white powder upon concentration. This was purified by PTLC to yield two substances of which the less polar (67 mg) R_f 0.33 was identified as rosmarinine (**68**) on the basis of its mp 208-9^0 C (Me_2CO) and spectroscopic properties (^1H and ^{13}C NMR).

The more polar component (11 mg), R_f 0.27, was similarly identified as hadiensine (**70**).

Fraction 16 was purified by PTLC on silica gel plates ($CHCl_3$-MeOH-NH_4OH (50:5:1), double development) giving rise to four components.

The least polar component, R_f 0.37, was found to be impure (GLC analysis). Further purification by PTLC ($CHCl_3$-MeOH-NH_4OH (50:5:1)) resulted in the isolation of the major component as a pale yellow oil (*ca.* 1.0 mg). It was identified as 12-O-acetylrosmarinine (**86**) on the basis of its spectroscopic properties (^1H and ^{13}C NMR).

The second component, R_f 0.33 was obtained as a white powder (56 mg) which crystallized from Me_2CO to give white crystals, mp 208-9° C. This component was found to be rosmarinine (**68**) (^1H and ^{13}C NMR).

The third component, R_f 0.27 was a pale yellow oil (19.0 mg). Spectral analysis (^1H and ^{13}C NMR) revealed this compound to be hadiensine (**70**).

The fourth component was observed as a very faint narrow band under UV light (short range). From it was isolated an oil (*ca.* 0.8 mg) which appeared to be

2-hydroxyhadiensine (**82**). The spectral properties were as follows: ^{1}H and ^{13}C NMR (see Table 11); CIMS m/z (%) $[M+1)^{+}]$ 370 (100), 336 (27), 199 (31), 153 (85), 118 (19), 93 (20), 82 (44), 81 (11) and 43 (10).

Fraction 17 was subjected to PTLC giving rise to two components. The less polar component (19 mg), R_f 0.33 was found to be identical in all respects with rosmarinine (**68**).

The second component (3 mg), R_f 0.27, had spectroscopic characteristics (^{1}H and ^{13}C NMR) identical with hadiensine (**70**).

Fraction 18 was purified by PTLC ($CHCl_3$-MeOH-NH_4OH (100:10:3 drops)) triple development, giving rise to two components. That with R_f 0.33 (3.2 mg) was identified by its spectroscopic characteristics (^{1}H and ^{13}C NMR) as rosmarinine (**68**) (see pgs. 22 and 23), while the more polar components (10.5 mg), R_f 0.27, had spectral properties (^{1}H and ^{13}C NMR) identical to those of hadiensine (**70**).

Fractions 19 and 20 were pooled after GLC analysis revealed their similar compositions. Purification by PTLC ($CHCl_3$-MeOH-NH_4OH (100:10, 3 drops)), triple development) gave rise to two components.

The less polar component (11.2 mg), R_f 0.33, was found (^{1}H and ^{13}C NMR) to be rosmarinine (**68**),while the more polar component (53.2 mg), R_f 0.27, was similarly identified as hadiensine (**70**) (see p 25 and 137).

Fractions 21 and 22 were purified separately by PTLC. Each fraction gave rise to two zones. The zones with identical R_f values were pooled. The less polar component (6.8 mg), R_f 0.33, was identified as before as rosmarinine (**68**), while the more polar

component (3.8 mg) was similarly found to be hadiensine (**70**).

Fractions 23 and 24 were pooled and purified by PTLC ($CHCl_3$-MeOH-NH_4OH (100:10:1)), double development) giving rise to two components. The less polar component (3.3 mg) was found (^1H and ^{13}C NMR) to be rosmarinine (**68**), while the more polar one, obtained as a pale yellow oil (46.1 mg) was similarly shown to be hadiensine (**70**).

Fractions 25 and 26 were pooled and purified by PTLC $CHCl_3$-MeOH-NH_4OH (100:10:1), double development) giving rise to two zones. The less polar component (6.3 mg) was (^1H and ^{13}C NMR) rosmarinine (**68**), while the more polar component which was obtained as a pale yellow oil (30.7 mg) was shown by spectroscopic analysis (^1H and ^{13}C NMR as in Table 5, p. 25) to be hadiensine (**70**).

Fraction 27 was purified by PTLC giving rise to two zones. The less polar component was isolated as a white powder (2.8 mg) and identified as rosmarinine (**68**) on the basis of spectroscopic analysis (^1H and ^{13}C NMR). The more polar component was a pale yellow oil (18.5 mg) similarly identified as hadiensine (**70**).

Fraction 28 was purified by PTLC, double development to yield the main component as a pale yellow oil (32.6 mg) which was identified (^1H and ^{13}C NMR see page 25) as hadiensine (**70**)

Fraction 29 was purified as fraction 28 above to give more hadiensine (**70**) (^1H and ^{13}C NMR) as a pale yellow oil (9.3 mg).

Fraction 30 was purified by PTLC (double development), to yield the major component as a pale yellow oil (10.0 mg). Spectroscopic analysis similarly revealed this component to be hadiensine(**70**).

Preparation of hadiensine perchlorate salt.

A crystalline perchlorate salt of hadiensine was prepared as follows: Pooled hadiensine (101.8 mg) was dissolved in the minimum of EtOH. A 5% solution of perchloric acid in ethanol was then added dropwise until the mixture was just acidic (as judged by spot tests on Congo red indicator paper i.e until this turned blue). The salt was then precipitated by the addition of Et_2O. After centrifugation, the ether solution was decanted and the solid left to air dry (3 hrs.). Recrystallisation was effected by dissolving the solid in MeOH-EtOH. Upon cooling the solution yielded white crystals (93.6 mg) : mp 279-80^0 C, $[\alpha]_D$ ($HClO_4$ salt) -83+1^0 (c = 1.6, MeOH); ^1H and ^{13}C NMR (for the free base) (see table 5); IR ν_{max} ($HClO_4$) 3483 (br, s, OH), 3081 (m), 2987 (m), 2852 (m), 1732 (s, C=O), 1640 (w, C=C), 1455 (m), 1378 (m), 1343 (w), 1326 (w), 1320 (w), 1301 (w), 1250 (m), 1227 (m), 1146 (s), 1110 (s), 1053 (m), 1020 (m), 1002 (s), 996 (w), 930 (m), 907 (w), 853 (w), 876 (w), 833 (w), 811 (w), 796 (w), 782 (w), 763 (w) and 626 (m); EIMS (for the free base) m/z (%) [M^+] 353.1829 (13) ($C_{18}H_{27}NO_6$ req. 353.1839), 338 (10), 336 (12), 282 (18), 180 (15), 156 (8), 154 (12), 138 (22), 137 (82), 98 (18), 82 (100), 68 (14), 55 (26), 53 (15), 44 (32), 43 (66) and 41 (30).

Preparation of methiodide salt.

A crystalline methiodide salt of hadiensine was prepared by refluxing hadiensine (70 mg) and methyl iodide (0.2 mL) under a cold-finger condenser for 5 mins. After the mixture had cooled, the excess methyl iodide was decanted and the residue recrystallised from ethanol/water to give colourless needles (65 mg) : mp 233-4° C; IR υ_{max}

(methiodide) 3567-3410 (br, m, OH), 2986 (w), 2963 (w), 1750 (s, C=O), 1707 (s, C=O), 1655 (w), 1458 (w), 1449 (w), 1439 (w), 1422 (w), 1389 (w), 1366 (w), 1345 (w), 1240 (m), 1200 (w),1186 (w), 1163 (m), 1123 (s), 1105 (m), 1061 (w), 1011 (w) .

Fractions 31-41 were pooled and evaporated to give a mixture (109.2 mg) (GLC analysis). This was purified by PTLC to yield three components.

The least polar component was isolated as a white powder (16 mg) whose spectroscopic properties (^{1}H and ^{13}C NMR) revealed it to be rosmarinine (**68**).

The more polar component was isolated as a pale yellow oil (19 mg). Spectroscopic analysis (^{1}H and ^{13}C NMR) revealed this to be hadiensine (**70**).

The most polar component, petitianine (**87**), was isolated as a white powder (10 mg), R_f 0.07, R_T 17.5 min, mp 207-209^0 C from MeOH; $[\alpha]_D$ - 60^0 (c = 0.5, MeOH); IR ν_{max} ((KBr) 3491 (br, m, OH), 3259 (br, m), 2970 (m), 2939 (m), 2874 (s), 1745 (s, C=O), 1717 (s, C=O), 1654 (w, C=C), 1445 (m), 1373 (w), 1346 (w), 1325 (w), 1305 (w), 1263 (m), 1229 (s), 1206 (s), 1165 (s), 1148 (s), 1126 (s), 1089 (s), 1055 (s), 1000 (m), 966 (w), 907 (w), 750 (w) and 572 (w); ^{1}H and ^{13}C NMR (see Table 16); EIMS m/z (%) [M^+] 369.1802 (5) (calcd. for $C_{18}H_{27}NO_7$ 369.1788), 354 (1), 352 (1), 333 (5), 243 (8), 98 (37), 82 (85), 68 (25), 67 (20), 55 (33), 53 (28), 43 (50) and 41 (48).

3.3.2.1.2 Separation of the alkaloid mixture by Vacuum Liquid Chromatography (VLC) [144]

The alkaloid mixture (3.5 g), was subjected to VLC as described in section 3.1. The column was eluted using $CHCl_3$-MeOH-NH_4OH, 50:10:1 → 50:20:1 → 50:30:1 and finally MeOH. Each portion of solvent was sucked through the column and removed from the receiver before the vacuum was reapplied and the next eluent poured onto the column. A total of 27 fractions were collected (see Table 52). Fractions were monitored by GLC

and pooled accordingly (see Tables 52 and 53).

Fraction 3 was a brown oil. GLC analysis revealed two peaks (see Table 53). This was purified further by VLC as described above. This time a smaller sintered glass Buchner filter funnel (15 mL) was used. The solvent system used was $CHCl_3$-MeOH-NH_4OH (50:10:1). Fractions 8-10 were pooled, after monitoring by GLC, to give a pale yellow oil (20 mg). This was purified further by PTLC ($CHCl_3$-MeOH-NH_4OH (50:10:1)) giving rise to two components. The less polar component was 12-O-acetylrosmarinine (**86**), obtained as a pale yellow oil (3.1 mg), with spectroscopic properties as recorded on pg 45 and 133. The more polar component, a white powder (11.6 mg), was rosmarinine (**68**) (^1H and ^{13}C NMR).

Fractions 4, 5 and 6 were pooled to give a mixture (1.03 g) (GLC analysis). Trituration of this material with acetone gave a white solid. The supernatant was decanted and the residual solid recrystallized from acetone to give rosmarinine (**68**) as white crystals (695.8 mg) with mp and spectroscopic properties as recorded previously (see page 22, 23, 131 and 133).

The mother liquor was concentrated *in vacuo* to yield a brown oil (300 mg). Part of the brown oil (129 mg) was purified by PTLC to yield three substances.

The least polar component, R_f 0.37, was isolated as a pale yellow oil (0.9 mg) and was identified as 12-O-acetylrosmarinine (**86**) on the basis of spectroscopic data (^1H and ^{13}C NMR) (see p 45).

The second component, R_f 0.33, was obtained as an amorphous white powder (45.7 mg). Spectroscopic analysis (^1H and ^{13}C NMR) revealed this compound to be identical to rosmarinine (**68**).

The third component, R_f 0.27, was obtained as a pale yellow oil (30 mg).

Table 52: Vacuum column chromatography for the fractionation of crude alkaloid mixture (3.5 g)

Fraction No.	Eluant	Volume Collected (mL)	Weight eluted (mg)
1	$CHCl_3$-MeOH-NH_4OH (50:10:1)	50	-
2	,,	,,	-
3	,,	,,	33.4
4-6	,,	3 X 50	1029.8
7	,,	50	286.9
8	,,	,,	326.5
9	,,	,,	260.8
10	,,	,,	214.7
11	,,	,,	224.8
12	,,	,,	116.4
13 - 15	,,	3 X 50	695.0
16	,,	50	304.1
17	,,	,,	121.9
18	50:20:1	,,	103.1
19	,,	,,	4.3
20	,,	,,	0.5
21	,,	,,	
22	,,	,,	
23	50:30:1	100	
24	,,	,,	1.0
25	,,	,,	
26	,,	,,	
27	,,	,,	
		Total	3723.2

Table 53: PA profile for fractionation of crude alkaloid mixture (3.5 g) of *S. hadiensis* by vacuum column chromatography

Fraction	GLC retention time (min)								
	8.7	9.2	10.0	10.6	11.8	12.4	13.8	14.5	17.5
3	-	-	-	43.1	-	-	-	16.0	-
4	1.0	-	-	75.7	9.1	7.5	1.3	3.7	-
5	2.7	0.5	-	77.1	11.1	5.9	1.1	0.8	-
6	17.6	2.6	-	64.3	10.1	4.0	0.9	0.1	-
7	39.7	4.5	-	46.4	6.7	1.8	0.5	-	-
8	59.8	5.4	-	28.2	4.4	1.1	0.3	-	-
9	70.3	5.8	-	19.5	3.1	0.7	0.2	-	-
10	75.5	5.2	0.5	15.6	2.1	0.6	0.2	-	-
11	79.1	5.1	0.9	12.3	1.6	0.5	0.1	-	-
12	83.0	4.7	1.5	8.2	0.8	0.3	-	-	-
13	95.5	-	0.7	3.3	0.2	-	-	-	-
14	95.5	4.2	3.6	2.3	0.2	-	-	-	-
15	90.3	3.0	3.5	1.1	0.1	-	-	-	-
16	91.0	2.8	3.3	0.7	-	-	-	-	-
17	81.9	1.5	1.7	1.0	0.3	-	4.4	-	2.9
18	29.0	-	-	12.5	-	-	20.0	-	29.0
19	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
21-27	-	-	-	-	-	-	-	-	-

Spectroscopic analysis (^1H and ^{13}C NMR) revealed this compound to be hadiensine (**70**).

Part of the remaining portion of the material from the mother liquor (140 mg) was also purified by PTLC giving rise to three components.

The least polar component, R_f 0.37, was obtained as a pale yellow oil (3.2 mg). Spectroscopic analysis (^1H and ^{13}C NMR) revealed this compound to be identical to 12-O-acetylrosmarinine (**86**) (see pg 45).

The second component, R_f 0.33, was isolated as an oil (136.3 mg). GLC analysis revealed this to be a mixture of three components. Further purification by PTLC (Et_2O-MeOH-NH_4OH (160:19:5) gave rise to three zones. The least polar component, R_T 10.6 min on GLC analysis, was obtained as a white amorphous solid (97.8 mg). Spectroscopic analysis revealed this component to be rosmarinine (**68**).

The more polar component, R_T 11.8 min. on GLC analysis, afforded neorosmarinine (**78**) as a pale yellow oil (26.3 mg), $HClO_4$ salt mp 205-207° C from EtOH (for the preparation of this salt see the procedure used for preparing the hadiensine salt p 137); $[\alpha]_D$ ($HClO_4$ salt) -12° (c = 0.6, EtOH); IR υ_{max} (KBr) ($HClO_4$ salt) 3425 (br, s, OH), 3072 (w), 2973 (m), 2931 (m), 1729 (s, C=O), 1637 (w, C=C), 1280 (s), 1225 (s), 1147 (s), 1111 (s), 1089 (s) and 627 (s); ^1H and ^{13}C NMR (for the free base) (see Table 7); EIMS (for the free base) m/z (%) [M^+] 353.1829 (2) (calcd. for $C_{18}H_{27}NO_6$ 353.1838), 282 (1), 242 (2), 227 (6), 156 (30), 154 (40), 138 (60), 122 (20), 112 (15), 111 (17), 98 (25), 82 (75), 68 (15), 55 (27), 54 (15), 53 (20), 43 (100) and 41(39).

The next polar component, R_f 0.27, yielded 12-O-acetylhadiensine (**81**) as a pale yellow gum (8.3 mg): R_T 12.4 min on GLC analysis, for ^1H and ^{13}C NMR data (see Table 9), EIMS m/z (%) [M^+] 395.1938 (1) (calcd. for $C_{20}H_{29}NO_7$ 395.1945), 336 (1), 308 (1), 290 (1), 270 (1), 252 (2), 226 (3), 180 (20), 138 (23), 137 (72), 136 (20), 125 (17), 117 (70), 106 (31), 104 (26), 90 (56), 89 (43), 82 (50), 67 (31), 60 (62), 55 (45), 45 (75), 44 (60), 43 (100) and 41 (57).

The most polar component, R_f 0.27, was isolated as a pale yellow oil (150 mg), spectroscopic analysis (^1H and ^{13}C NMR) revealed this component to be identical to hadiensine (**70**).

The last portion (30 mg) of the material recovered from the liquors after trituration of the pooled fractions 4, 5 and 6 was also purified by PTLC (Et_2O- MeOH-NH_4OH (160:19:5) giving rise to five substances. Each of these was analysed by GLC. The component with R_f 0.33, R_T 11.8 min, was isolated as a pale yellow oil (6 mg). Spectroscopic analysis (^1H and ^{13}C NMR) revealed this alkaloid to be neorosmarinine (**78**).

The component with R_f 0.33, R_T 10.6 min, was isolated as a white amorphous solid (9.3 mg) identical in all respects with rosmarinine (**68**).

The component with R_f 0.27, R_T 12.4 min, was isolated as a pale yellow oil (3.1 mg) identical in its spectroscopic properties with 12-O-acetylhadiensine (**81**).

The component with R_f 0.27, R_T 8.7 min, was obtained as a pale yellow oil (8.9). Its spectral properties (^1H and ^{13}C NMR) showed it to be hadiensine (**70**).

The component with R_f 0.27, R_T 9.2 min, was also isolated as a pale yellow oil (*ca.* 2 mg) whose spectroscopic properties (^1H and ^{13}C NMR) showed it to be identical to 2-hydroxyhadiensine (**82**) (see pg 33).

Fraction 7 was triturated with acetone and filtered to give a white solid (75.2 mg), which was recrystallized from acetone to give rosmarinine (**68**) (^1H and ^{13}C NMR).
Part of the material (180 mg) recovered from the mother liquor was purified by PTLC giving rise to four zones, from which only two substances were obtained in pure form: one component, obtained as a pale yellow oil (7 mg) (R_f 0.27, R_T 12.4 min.) was 12-O-acetylhadiensine (**81**) (spectroscopic properties as on p. 35 and 142), and the other (R_f 0.27, R_T 9.2 min.), also obtained as a yellow oil (1.3 mg) was similarly shown to be

2-hydroxyhadiensine (**82**).

Fraction 8 was purified by PTLC ($CHCl_3$-MeOH-NH_4OH (50:10:1)) using 1 mm thick layers of silica gel. Two substances were isolated but each was found to be impure (GLC and ^1H NMR analyses).

The less polar component was purified further by PTLC (Et_2O-MeOH-NH_4OH (160:19:5)), three developments). Three zones were separated.

The least polar component, R_f 0.33, R_T 11:8 mins. neorosmarimine (**78**) was isolated as an oil (1.5 mg).

A more polar component, R_f 0.33, R_T 10.6 min. rosmarinine (**68**), was isolated as a white solid (6.6 mg).

The most polar component, 12-O-acetylhadiensine (**81**) R_f 0.27, R_T 12.4 min, was isolated as a pale yellow oil (1.1 mg).

The more polar component showed mainly one component. This was also purified by PTLC (Et_2O-MeOH-NH_4OH (160:19:5)) to give as the major component hadiensine (**70**) as a pale yellow oil (35.0 mg), R_f 0.27, R_T 8.7 min.

Fraction 9 was triturated with acetone and filtered to afford a white amorphous solid which was recrystallized from acetone to give rosmarinine (**68**) as white crystals (17.2 mg).

Evaporation of the mother liquor from this trituration gave a solid (239 mg) which was purified by PTLC giving rise to two zones. Both were impure (GLC analysis) and were purified further as follows:

The less polar component was purified by PTLC (Et_2O-MeOH-NH_4OH (160:19:5)) to afford two zones: neorosmarinine (**78**), the component with R_f 0.33, R_T 11.8 mins, was isolated as an oil (*ca.* 2 mg), and rosmarinine (*68*) R_f 0.33, R_T 10.6 min.,

was isolated as a white amorphous solid (7.8 mg).

The more polar component was purified by PTLC (Et_2O-MeOH-NH_4OH (160:19:5)) to afford hadiensine (***70***) as the major component, obtained as a pale yellow oil (16 mg).

Fractions 10, 11 and 12 were not studied further as they showed more or less the same components as those seen in fractions 7-9 (see Table 53).

Fractions 13-15 (each fraction was greater than 90% one component (see Table 53)). These were pooled to afford a pale yellow oil (695 mg). The sample was dissolved in the minimum amount of ethanol and a solution of $HClO_4$ in EtOH (*ca.* 5%) added until the mixture was just acidic to Congo red indicator paper. Et_2O was then added to precipitate the salt. After centrifugation, the supernatant was decanted and the salt resuspended in fresh Et_2O and again separated by centrifugation. This process was repeated thrice. The resulting solid was air dried and then recrystallized from ethanol/methanol to afford white crystals (388 mg). This was dissolved in methanol, basified with aq. $NaHCO_3$, to pH *ca.* 9 (indicator paper) and extracted with $CHCl_3$ (4 x 50 mL). The extracts were pooled, dried ($MgSO_4$) and concentrated (rotary evaporation) to yield hadiensine (**70**) as a pale yellow oil (380.8 mg), R_f 0.27, R_T 8.7 mins.

The ethanol/methanol mother liquor was basified ($NaHCO_3$ pH *ca.* 9 [indicator paper]) and extracted with $CHCl_3$ (4 x 50 mL). The extracts were pooled, dried ($MgSO_4$) and concentrated to give more hadiensine as a pale yellow oil (49.2 mg), R_f 0.27, R_T 8.7 min.

Fraction 16 Part of this material (120 mg) was purified by PTLC giving rise to two zones. The minor component was obtained as a white amorphous solid (28 mg). GLC

analysis revealed this to be a mixture of two components with one (R_T 10.6 mins) predominating. Further purification as above resulted in the isolation of this substance which proved to be (^1H and ^{13}C NMR) rosmarinine (**68**).

The major component, R_f 0.27 and R_T 8.7 min was isolated as a pale yellow oil (60.4 mg). On the basis of spectral data (^1H and ^{13}C NMR), the compound was identified as hadiensine (**70**)

Fraction 17 was purified by PTLC giving rise to two zones:

The component with R_f 0.33, was concentrated to give a pale yellow oil (29 mg), identified as before as hadiensine (**70**).

The component with R_f 0.07, R_T 17.5 mins was concentrated to give petitianine (**87**) (^1H and ^{13}C NMR) as a white powder (10.5 mg).

Fraction 18 was purified by PTLC giving rise to two zones:

The less polar component, R_f 0.27, on concentration gave hadiensine (**70**) as a pale yellow oil (28.7 mg).

The more polar component, R_f 0.07, on concentration gave petitianine (**87**) as a white powder (17.0 mg).

Fractions 19 and 20 were pooled and the material obtained (4.8 mg) was then purified by PTLC to yield petitianine (**87**) as a white powder (2.2 mg).

Fraction 21-27 were not examined further because of insufficient material collected (see Table 52).

3.3.2.1.3 Fractionation of the remaining "reduced" alkaloid mixture from *S. hadiensis*.

The remaining alkaloid mixture (7.0 g) from the reductive processing of *S. hadiensis* was triturated with acetone (*ca.* 50 mL) and filtered to give a white amorphous solid (2.15 g). The filtrate was concentrated and an additional quantity of white amorphous solid (220 mg) was collected. Both solids were identified (^1H and ^{13}C NMR) as rosmarinine (**68**)

The mother liquors remaining after the separation of rosmarinine (**68**) were diluted with ethanol and treated with an ethanolic solution of $HClO_4$ (*ca.* 5%) until acidic (to Congo red indicator). Then Et_2O was added, and the resulting precipitate filtered off. The precipitate was recrystallized (MeOH) to afford hadiensine perchlorate as white crystals (2.7 g): The filtrate and mother liquors remaining from the precipitation of the perchlorate salt (ether and methanol filtrates) were pooled and concentrated, then diluted with MeOH, basified (aq. $NaHCO_3$) and extracted with $CHCl_3$ (6 x 50 mL). The pooled $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated to give a brown oil (684 mg).

Part of this brown oil (130 mg) was purified by PTLC (Et_2O-MeOH-NH_4OH (160:19:5), 5 developments) giving rise to three zones:

The least polar component, R_f 0.33, R_T 11.8 min, neorosmarinine (**78**), was isolated as a pale yellow oil (6.7 mg).

The more polar component, R_f 0.27, R_T 12.4 min, 12-O-acetylhadiensine (**81**), was isolated as a pale yellow oil (10 mg).

The most polar component (83 mg) was found to be a complex mixture (GLC analysis). Further purification was performed by PTLC (CHC_3-MeOH-NH_4OH (95:4:1), on aluminium oxide F_{254}, type E). The least polar component, R_F 0.27, R_T 13.8 mins, was isolated as a pale yellow oil (*ca.* 1 mg). This material was 12-O-acetylneohadiensine (**83**). For ^1H and ^{13}C NMR data see Table 12; EIMS m/z (%) [M^+] 395.1948 (1) (calcd. for $C_{20}H_{29}NO_7$ 395.1945), 336 (3), 308 (2), 234 (31), 180 (37), 154 (23), 153 (22), 138 (45),

137 (89), 136 (20), 117 (28), 83 (23), 82 (73), 81 (20), 67 (15) and 43 (100).

3.3.2.2 Separation of the "unreduced" bases of *S. hadiensis*

3.3.2.2.1 Chloroform Extract

Part of the sample (120 mg) was purified by PTLC giving rise to five zones:

The least polar component R_f 0.37, was isolated as a pale yellow oil (10.6 mg) and identified (^1H and ^{13}C NMR) as 12-O-acetylrosmarinine (**86**).

The second component R_f 0.33. R_T 10.6 min, was isolated as a white amorphous solid (45.0 mg) and identified (^1H and ^{13}C NMR) as rosmarinine (**68**).

The third component was isolated as a mixture (GLC analysis) and was purified further by PTLC (aluminium oxide, $CHCl_3$-MeOH-NH_4OH (95:4:1), triple development) giving rise to three zones. The least polar component, R_f 0.27, R_T 8.7 min, was isolated as a pale yellow oil (22.8 mg) and was identified as hadiensine (**70**) (^1H and ^{13}C NMR).

A more polar one, R_f 0.27, R_T 12.4 min, was isolated as an oil (*ca.* 1 mg) and identified as 12-0-acetylhadiensine (**86**) (^1H and ^{13}C NMR).

The most polar polar component was a yellow oil (16.7 mg), was identified as 12-O-acetylhadiensine N-oxide (**89**): ^1H and ^{13}C NMR (see tables 17 and 18); FAB-MS m/z (%) [M^+] 412 (100), 398 (14), 397 (28), 394 (23), 352 (11), 336 (13), 153 (14), 138 (45), 120 (19), and 98 (25).

The fourth component was a mixture (^1H NMR). This was purified further by PTLC (aluminium oxide 60 F_{254}, type E, $CHCl_3$-MeOH-NH_4OH (95:4:1) to yield a colourless oil (15.0 mg) (R_f 0.17) which was identified (by ^1H and ^{13}C NMR) as hadiensine N-oxide (**90**). A crystalline perchlorate derivative was prepared in the usual way (see pg 137) and obtained as white crystals, mp 160-1° C (Et_2O); $[\alpha]_D$ ($HClO_4$ salt) -78.8° (c = 2.22, EtOH); for ^1H and ^{13}C NMR (data for free base) (see Tables 19 and 20); IR ν_{max} ($HClO_4$ salt) 3466 (br, m, OH), 2996 (m), 2974 (m), 2946 (m), 1745 (s, C=O),

1716 (s, C=O), 1655 (w, C=C),1473 (m), 1465 (m), 1457 (m), 1382 (m), 1303 (m), 1291 (m), 1256 (s), 1222 (s), 1149 (s), 1115 (s), 1017 (w), 978 (w), 937 (w) and 626 (m) cm^{-1}; FAB-MS (for the free base) m/z (%) 370 (100, M+1), 354 (75, (M+1)-16), 156 (16), 138 (39).

The fifth component was also a mixture (^1H NMR). Further purification by PTLC (aluminium oxide, 60 F_{254}, type E, $CHCl_3$-MeOH-NH_4OH (95:4:1) yielded two zones:

The less polar component was isolated as an oil (*ca.* 0.5 mg) and identified (^1H NMR) as 2-hydroxyhadiensine (**82**).

The more polar component was isolated as a mixture (^1H NMR) and was not examined any further.

3.3.2.2.2 <u>The n-Butanol Extract.</u>

Part of the extract (120 mg) was purified by PTLC (aluminium oxide plates, type E, F_{254} EtOAc-MeOH (1:1), double development, to yield two zones (UV light). The less polar component, R_f 0.17, was isolated as a colourless gum (54.6 mg). This compound was found to be identical in all respects with the alkaloid earlier identified as hadiensine N-oxide.

The more polar component R_f 0.09, rosmarinine N-oxide (**91**) was isolated as a colourless gum (60.9 mg) which on addition of H_2O followed by evaporation resulted in white crystals: mp 152^0 C (dec.) [lit [101] 169° C (dec)]; ^1H and ^{13}C NMR (see Tables 21 and 22); IR υ_{max} (KBr) 3422 (br, m), 2949 (m), 1720 (s, C=O), 1686 (w) 1655 (w, C=C), 1474 (w), 1458 (w), 1449 (w), 1377 (w), 1366 (w), 1258 (m), 1221 (m), 1152 (s) and 1113 (m) cm^{-1}; EIMS m/z (%) 353 (6), 242 (6), 227 (18), 156 (50), 154 (70), 153 (34), 139 (24), 138 (81), 137 (23), 136 (21), 135 (39), 122 (42), 98 (35), 85 (55), 83 (98), 82 (77), 81 (54), 68 (29), 55 (48), and 43 (100); CIMS m/z (%) $[M+1]^+$ 370 (8), 355 (22), 354 (100), 334 (7), 227 (15), 199 (74), 153 (36), 138 (43) and 82 (19).

3.4 Chemical transformation studies with the *S. hadiensis* alkaloids

Saponification of hadiensine (**70**) - A solution of hadiensine (**70**) (1 g) and $Ba(OH)_2.8H_2O$ (4.4 g) in H_2O (50 mL) was boiled under reflux for 5 h. After the solution had cooled to room temperature, CO_2 was bubbled in until no further precipitation occurred. The mixture was filtered, the filtrate acidified with aq. H_2SO_4 and extracted continously with Et_2O. Evaporation of the ether extract afforded a solid (0.6 g): which was recrystallized from Et_2O to give senecic acid (**25**), mp 147-149° C from Et_2O [lit [74] 145-146° C (EtOAc-petrol), [75] 151° C]; $[\alpha]_D$ +16.7 (c = 0.48, EtOH) [lit [74] $[\alpha]_D$ + 12.7° (c = 0.0834, EtOH [75] $[\alpha]_D$ +11.8; [76] $[\alpha]_D$ + 19.6° (= 0.0138, EtOH)]; IR ν_{max} (KBr) 3452 (s, OH), 2992 (s), 1734 (s), 1695 (s), 1674 (s), 1647 (m), 1637 (w), 1457 (m), 1444 (m), 1434 (m), 1431 (m), 1410 (m), 1413 (w), 1379 (w), 1305 (m), 1280 (m), 1260 (m), 1236 (m), 1219 (m), 1172 (m), 1137 (m), 1122 (m), 1096 (m), 1067 (w), 1020 (w), 965 (w), 953 (w), 941 (w), and 924 (w) cm^{-1}; ^1H NMR (DMSO), δ 0.74 (3H, d, J = 6.1 Hz, H-10), 1.20 (3H, s, H-9), 1.73 (IH, d, J = 7.5 Hz, H-4), 1.85 (1H, m, H-3), 1.85 (3H, d J = 7 Hz, H-8) 2.34 (2H, d, J = 9.4 Hz, H-4), 5.85 (1H, d, J = 7 Hz, H-7); ^{13}C NMR (DMSO): δ 12.3 (C-10), 15.2 (C-8), 23.3 (C-9), 36.0 (C-3), 39.2 (C-4), 75.5 (C-2); 132.6 (C-5), 134.3 (C-7), 169.0 (C-6), 177.3 (C-1); EIMS m/z (%) 198 $[M^+-18]^+$ (36), 180 (14), 162 (16), 154 (31), 153 (72), 152 (36), 137 (28), 136 (15), 135 (18), 134 (30), 111 (23) 110 (42), 109 (55), 107 (41), 83 (54), 82 (53), 81 (78), 55 (60), and 43 (100).

The aqueous solution remaining after the Et_2O extraction was passed through a column (0.8 x 13 cm) of Dowex-SBR anion exchange resin (20-50 mesh, OH form), and the column washed with water. Evaporation of the eluates under reduced pressure gave a solid which was extracted with Me_2CO-MeOH. Evaporation of these extracts gave a crystalline product which was recrystallised from Me_2CO to give hadienecine (**74**) (419

mg) as colourless crystals: mp 140-1° C; IR ν_{max} (KBr) 3343 (br, s), 2945-2885 (br s), 1350 (w), 1341 (w), 1319 (w), 1281 (m), 1260 (s), 1192 (s), 1125 (s), 1076 (w), 1061 (w), 1045 (s), 1020 (s), 974 (w), 882 (w), 835 (s), 746 (s), 669 (m), 602 (m), 534 (m), 488 (m) and 475 (m) cm^{-1}; ^1H and ^{13}C NMR (see Table 6); EIMS m/z (%) [M^+] 173.1050 (23) (calc. for $C_8H_{15}NO_3$ 173.1052), 155 (38), 129 (27), 112 (19) 99 (91), 98 (95), 82 (100), 70 (26), 68 (23), 57 (20), 56 (25), 55 (25), 42 (40) and 41 (43).

Conversion of hadiensine (**70**) to senecionine (**4**) - To a solution of hadiensine (15 mg) in Py (2 mL) was added MsCl (40 μL) and the mixture was then boiled under reflux for 30 min. After removing excess reagents under reduced pressure, the residue was partitioned between aq. Na_2CO_3 (*ca.* 5 mL) and $CHCl_3$ (10 x 5 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to yield a solid residue, judged by TLC and GLC analysis to be a mixture of hadiensine (**70**) and senecionine (**4**). Separation by PTLC ($CHCl_3$-MeOH-NH_4OH (50:10:1) resulted in the isolation of recovered hadiensine (5.6 mg) and senecionine (3.9 mg, 44%).

The latter was obtained as colourless crystals, mp 229-230° C from EtOH [lit [150] 233° C (Me_2CO), [37] 245° C], IR ν_{max} (KBr): 3457 (w), 3082-2816 (br, s), 1739 (s), 1715 (s), 1659 (w), 1444 (m), 1365 (m), 1244 (s), 1237 (s), 1219 (s), 1205 (s), 1190 (s), 1163 (s), 1141 (s), 1108 (s), 1095 (s), 1073 (s), 1006 (m), 969 (m), 958 (m), 946 (m) and 824 (m); ^1H and ^{13}C NMR (see Tables 29 and 30), EIMS m/z (%) [M^+] 335 (6), 291 (1), 290 (1), 248 (4), 247 (2), 246 (5), 220 (16), 153 (13), 138 (28), 137 (20), 136 (58), 121 (25), 120 (53), 119 (49), 117 (70), 95 (28), 94 (34), 93 (52), 90 (34), 89 (27), 81 (32), 80 (23), 44 (44),43 (100) and 40 (77).

Preparation of 1-O-acetylhadiensine (**81**)

A solution of hadiensine (30 mg) in Ac_2O (0.5 mL) and Py (0.5 mL) was stirred for

14 h. at room temperature. Excess Ac_2O and Py were removed *in vacuo*, and the residue was then basified (aq. $NaHCO_3$, pH *ca.* 9 (indicator paper)) and extracted repeatedly with small volumes of $CHCl_3$ (total volume *ca.* 50 mL). Evaporation of the dried ($MgSO_4$) extracts follwed by PTLC ($CHCl_3$-MeOH-NH_4OH (50:10:1)) gave 1-O-acetylhadiensine (**79**) (22.5 mg, 67%) as colourless crystals: mp 142-144° C; $[\alpha]_D$ - 67°(c = 0.5, EtOH); IR ν_{max} (KBr): 3423 (br, w), 2968 (m), 1740 (s, C=O), 1729 (s, C=O), 1463 (m), 1457 (m), 1376 (m), 1367 (m), 1251 (s), 1241 (s), 1217 (s), 1163 (m), 1148 (s), 1115 (s), 1097 (m), 1058 (m), 1021 (m), cm^{-1}; ^1H and ^{13}C NMR (see Table 8); EIMS m/z (%) [M^+] 395.1948 (1) (calcd. for $C_{20}H_{29}NO_7$ 395.1945), 378 (1), 220 (10), 180 (18), 138 (35), 136 (30), 121 (100), 99 (47), 82 (70), 55 (27), 43 (72) and 41 (30). The nOe measurements used in the attempt to determine the stereochemistry at position 1 in hadiensine were carried out on this sample.

Saponification of Petitianine (**87**)

Petitianine (10 mg) was added to a solution of $Ba(OH)_2.8H_2O$ (115 mg) in H_2O (10 mL) and the mixture was boiled under reflux for 5 h. The solution was allowed to cool, treated with CO_2 and then filtered. The filtrate was acidified (aq. 0.25 M H_2SO_4) and extracted continously with Et_2O overnight. The ether extract was concentrated to afford a solid (3.9 mg, 38%). The ^1H and ^{13}C NMR spectra for this sample and that of isatinecic acid obtained from retrorsine (Sigma Chem. Co) (see next experiment) were identical: ^1H NMR (CD_3OD) δ 0.86 (3H, d, J = 6.3 Hz, H-10), 1.94 (3H, d, J = 7.1 Hz, H-8), 2.02 (1H, m, H-3), 2.02 (1H, dd, J = 10.2 Hz, H-4), 2.42 (1H, dd, J = 10.2, Hz, H-4), 3.74 (1H, dd, J= 10.6, 11.1 Hz, H-9), 5.98 (1H, q, J = 7.3 Hz, H-7) [lit [93] in $CDCl_3$-CD_3OD]; ^{13}C NMR (CD_3OD): δ 13.0 (C-10), 15.9 (C-8), 38 (C-3), 38 (C-4), 67.7 (C-9), 81.9 (C-2), 133.1 (C-5), 171.5 (C-6) and 177.8 (C-1).

The residual acidic solution was passed through a column (i.d. 0.8 cm, length 13

cm) of Dowex-3X anion exchange resin (20-50 mesh, OH form) and the column washed with water. Evaporation of the solvent under reduced pressure gave a pale yellow oil (3.2 mg). The ^{1}H and ^{13}C NMR spectra for this sample and that of rosmarinecine (**14**) obtained from rosmarinine (see saponification of rosmarinine for spectral properties) were identical.

Saponification of retrorsine (**76**)

A solution of retrorsine (45.8 mg) (Sigma Chemical Co) and $Ba(OH)_2.8H_2O$ (305.0 mg) was refluxed for 5 h. The solution was treated as before with CO_2 and then extracted with ether to yield a white solid (36 mg) which was recrystallised from Et_2O-EtOAc mixture to afford isatinecic acid (**26**) with spectroscopic properties as reported earlier (see saponification of petitianine above).

The residual acidic solution was treated as in the previous two cases (see saponification of hadiensine (**70**) and petitianine (**87**)) to yield retronecine (**15**) (7 mg, 35%) as white crystals from Me_2CO: mp 119-120° C [lit [37] 117-118° C]; IR υ_{max} (KBr) 3460 (br, s), 2976-2851 (s), 1128 (s), 1101 (s), 1040 (s), 1003 (s), 841 (s) and 746 (s) cm^{-1}; EIMS m/z (%) 155 (48), 111 (70), 94 (49), 93 (37), 82 (33), 81 (49), 80 (100), 68 (52), 67 (31), 53 (49) and 41 (66); ^{1}H NMR ($CDCl_3$) δ 5.70 (1H, br s, H-2), 3.83 (1H, d, J = 15.5 Hz, H-3), 3.41 (1H, dd, J = 5.0, 15.5 Hz, H-3), 3.22 (1H, m, H-5), 2.73 (1H, m, H-5), 1.93 (2H, m, H-6), 4.30 (1H, br s, H-7), 4.16 (1H, d, J = 11.7 Hz), 4.32 (1H, d, J = 11.7 Hz, H-9) and 4.09 (1H, d, J = 11.7 Hz, H-9) [lit [151]]; ^{13}C NMR δ 137.0 (C-1), 127.0 (C-2), 62.0 (C-3), 54.1 (C-5), 35.4 (C-6), 71.1 (C-7), 79.5 (C-8) and 58.9 (C-9) [lit [93, 106]];

Saponification of rosmarinine (**68**).

Rosmarinine (44.0 mg) was added to a solution of $Ba(OH)_2.8H_2O$ (296.0 mg) in H_2O (10 mL) and the mixture boiled under reflux for 5 h. The solution was allowed to cool, treated with CO_2 gas and then filtered. The filtrate was acidified (aq. 0.25 M H_2SO_4

and extracted continously with Et_2O for 10 h. The ether extract was concentrated to afford a solid (19.4 mg). Recrystallization afforded white feathery crystals. The mp, $[\alpha]_D$, IR, ^1H and ^{13}C NMR for this sample were the same as those of senecic acid (**25**) obtained from hadiensine (**70**).

The residual acidic solution was treated as in the previous cases to yield rosmarinecine (**14**) with spectroscpic properties as given in the preceding experiment. This material was recrystallized from ethanol/acetone to yield white prisms (15.7 mg, 73%): mp 168-170° C [lit [75, 76] 171-172° C]; $[\alpha]_D$ -121 (c = 4.9, EtOH) [lit [76] $[\alpha]_D$ -116.5^0 (c = 0.0106, EtOH), lit [75] -118^0; IR ν_{max} (KBr) 3171-3503 (s, OH), 2859-2972 (s), 1458 (m), 1439 (m), 1410(w), 1398(w), 1375 (w), 1360 (w), 1343 (w), 1325 (w), 1240 (w), 1167 (w), 1140 (w), 1099 (s), 1063 (m), 1051 (w), 1034 (w), 1003 (s), 789 (m), 756 (m), 681 (w) and 561 (w) cm^{-1}; ^1H NMR (D_2O) δ 2.40 (1H, m, H-1), 4.46 (1H, m, H-2), 3.41 (1H, dd, J = 6.7, 12.3 Hz, H-3), 3.32 (1H, dd, J = 7.5, 12.3 Hz, H-3), 3.18 (2H, m, H-5), 2.00 (2H, m, H-6), 4.46 (1H, m, H-7), 4.06 (dd, J = 6.8,*ca.* 4.0 Hz, H-8), 3.88 (1H, dd, J = 7.1, 11.3 Hz, H-9) and 3.77 (1H, dd, J = 4.8, 11.3 Hz, H-9); ^{13}C NMR (D_2O) δ 49.0 (C-1), 70.4 (C-2), 61.5 (C-3), 54.7 (C-5), 34.7 (C-6), 70.8 (C-7), 73.3 (C-8) and 58.3 (C-9); EIMS m/z (%) [M^+] 173 (37), 155 (26), 129 (63), 112 (20), 99 (46), 98 (100), 86 (21), 83 (25), 82(61), 80 (35), 68 (53), 55 (45), 42 (62) and 41 (65).

Photolysis of rosmarinine (**68**).

Rosmarinine (15.9 mg) in EtOH (1.5 mL) contained in a quartz tube was photolysed for 2.75 h. Analysis of the reaction product by GLC revealed 3 components (two of which corresponded to rosmarinine (**68**) and neorosmarinine (**78**)), while two were discernible by TLC. Separation by PTLC (Et_2O-MeOH-NH_4OH (160:19:5)) gave rosmarimine (2 mg) and a mixture (10 mg) whose ^1H was consistent with it being a *ca.* 3:2 mixture of neorosmarinine (**78**) (see Table 7, p. 31) and probably compound (**77**) which

was not characterised.

Conversion of 12-O-acetylhadiensine (**81**) to 1,12-di-O- acetylhadiensine (**80**)

A solution of 12-O-acetylhadiensine (**81**) (*ca.* 5 mg) in Py (0.1 mL) and Ac_2O (0.1 mL) was stirred overnight at room temperature. The reaction mixture was concentrated (rotary evaporator) and then basified (aq. $NaHCO_3$, to pH *ca.* 9 (indicator paper) and then extracted with $CHCl_3$ (5 x 2 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated under reduced pressure to afford the diacetate (**80**) (*ca.* 5.0 mg) (^1H and ^{13}C NMR see Table 10 p. 36).

Conversion of hadiensine to 1,12-di-O-acetylhadiensine (**80**)

A mixture of hadiensine (30 mg), anhydrous sodium acetate (15 mg) and acetic anhydride (1 mL) was boiled under reflux overnight and the excess acetic anhydride then removed under reduced pressure (rotary evaporator). The residue was partitioned between aq.Na_2CO_3 (4 mL), pH *ca.* 10 (indicator paper) and $CHCl_3$ (5 x 5 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was subjected to PTLC ($CHCl_3$-MeOH-NH_4OH (50:10:1) to afford a product (4.9 mg, 13%) whose spectral data (^1H and ^{13}C NMR) see Table 10) showed it to be identical with that obtained by acetylation of 12-O-acetylhadiensine (**81**) (see the previous experiment).

Photolysis of 12-O-acetylhadiensine (**81**)

A solution of 12-O-acetylhadiensine (10 mg) in EtOH (1.3 mL) contained in a quartz tube was photolysed for 2 1/2 h. The solution was then concentrated under reduced pressure and the residue subjected to PTLC on alumina (Merck type E, F_{254}, $CHCl_3$-MeOH-NH_4OH (95:4:1) to give a substance (2 mg) with ^1H and ^{13}C NMR spectroscopic properties (see Table 12, p. 41) identical with those of 12-O-acetyl-

neohadiensine (**83**) isolated from *S. hadiensis*.

Conversion of 12-O-acetylrosmarinine (**86**) to 2,12-di-O-acetylrosmarinine (**85**)

A solution of 12-O-acetylrosmarinine (2 mg) in pyridine (0.1 mL) and acetic anhydride (0.1 mL) was stirred for 8.5 h. at room temperature. After work-up by addition of aq. Na_2CO_3, extraction with $CHCl_3$ (6 x 2 mL) and removal of solvent from the combined dried ($MgSO_4$) $CHCl_3$ extracts, 2,12-di-O-acetylrosmarinine (**85**) was obtained as a pale yellow oil with ^1H and ^{13}C NMR data as in Table 15, p. 46; EIMS m/z (%) [M^+] 437.2069 (11) (calcd. for $C_{22}H_{31}NO_8$ 437.2050), 378 (5), 377 (4), 342 (3), 311 (15), 268 (38), 180 (30), 153 (20),138 (18), 137 (25), 136 (59), 122 (20), 121 (55), 119 (60), 118 (20), 117 (4 8), 106 (30), 95 (25), 94 (35), 93 (38), 90 (25), 83 (34), 82 (50), 81 (36), 80 (37), 79 (15), 77 (10), 71 (25), 69 (35), 68 (21), 67 (23), 60 (31), 57 (43), 55 (50), 53 (22), 51 (15), 46 (32), 45 (85), 44 (45), 43 (100), 42 (38).

Conversion of rosmarinine (**68**) to 2,12-di-O-acetylrosmarinine (**85**)

A stirred solution of rosmarinine (22 mg) in Ac_2O (0.5 mL) and Py (0.5 mL) containing DMAP (5 mg) was kept at 90±10° C for 48 h. The dark reaction mixture was cooled to room temperature then poured into excess aq. Na_2CO_3 and extracted with $CHCl_3$ (5 x 10 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to yield a dark oil from which 2,12-di-O-acetylrosmarinine (**85**) (1.5 mg, 6%) was isolated by PTLC (Et_2O-MeOH-NH_4OH (160:9:5)) as a pale yellow oil with ^1H and ^{13}C NMR spectra identical to those of the product obtained above (see Table 15, p. 46).

Conversion of rosmarinine (**68**) to 2-O-acetylrosmarinine (**84**)

A solution of rosmarinine (20 mg) in Ac_2O (0.2 mL) and Py (0.2 mL) was stirred and kept at room temperature for 24 h. Excess reagents were removed under high vacuum

and the residue was partitioned between aq. Na_2CO_3 (3 mL) and $CHCl_3$ (5 x 5 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to yield colourless crystals of 2-O-acetylrosmarinine (17 mg, 76%): mp 153-154° C, $[\alpha]_D$ -113° (c = 1.8, MeOH); IR ν_{max} (KBr) 3452 (br, w, OH), 2935 (m), 2896 (w), 1741 (s, C=O), 1713 (s, C=0), 1465 (w), 1449 (w), 1370 (m), 1250 (s), 1217 (s), 1160 (s), 1149 (s), 1115 (s), 1102 (m), 1050 (m), 1042 (m), 1019 (w), 971 (w) and 752 (w) cm^{-1}; ^1H and ^{13}C NMR (see Table 13, p 44); EIMS m/z (%) [M^+] 395.1948 (18) (calcd. for $C_{20}H_{29}NO_7$ 395.1945), 380 (3), 378 (3) , 336 (12), 335 (10), 269 (22), 220 (20), 198 (30), 180 (32), 153 (25), 138 (32), 137 (28), 136 (40), 119 (100), 108 (30), 108 (30), 80 (38), 67 (30) and 43 (50).

Preparation of hadiensine N-Oxide (**90**) from hadiensine (**70**).

A solution of hadiensine (26.3 mg) and MCPBA (17.9 mg) in $CHCl_3$ (10 mL) was stirred for 30 min. at room temperature. The solvent was removed under reduced pressure (rotary evaporator) and the resulting residue dissolved in H_2O (10 mL), then extracted with Et_2O (6 x 10 mL). The aq. solution was concentrated *in vacuo* to yield the N-oxide as a colourless oil (17.9 mg, 65%) with ^1H and ^{13}C NMR spectra identical to those of the product obtained from the *S. hadiensis*.

Preparation of rosmarinine N-oxide (**91**) from rosmarinine (**68**)

A solution of rosmarinine (72.1 mg) and MCPBA (35.2 mg) in $CHCl_3$ (10 mL) was stirred continously while kept at room temperature for 30 min. The solvent was removed (rotary evaporator) and the residue dissolved in H_2O (10 mL) and then extracted with Et_2O (6 x 20 mL). The residual aqueous solution was then evaporated *in vacuo* to yield the N-oxide (70.9 mg, 94%) as white crystals. The ^1H and ^{13}C NMR spectra were identical to those of the natural product.

Preparation of 12-O-acetylhadiensine (**81**) from 12-acetyl-O-acetylhadiensine N-oxide (**89**).

The N-oxide (5.0 mg) was dissolved in 0.25 M aq. H_2SO_4 (1 mL) and then stirred with zinc dust (*ca.* 10 mg) at room temperature overnight. The zinc dust was filtered off and the filter was washed with a few drops of aq. H_2SO_4. The resulting aq. solution was then basified with conc. aq. NH_4OH to pH *ca.* 10 (indicator paper), and then extracted with $CHCl_3$ (3 x 10 mL). The combined extracts were dried ($MgSO_4$) and then evaporated to dryness to yield an oil (*ca.* 3 mg). The ^1H and ^{13}C NMR of this material were identical to those of 12-O-acetylhadiensine (**81**).

3.5 Isolation of the alkaloids of S. syringifolius O. Hoff.

3.5.1 With reductive processing

The fresh plant material was extracted by repeated maceration in 95% EtOH in a Waring blender. The combined EtOH extracts were concentrated (cylone and rotary evaporator) to a dark green gum. The dark green gum was freeze-dried to give a dark black solid material (120.5 g). This was dissolved in $CHCl_3$ (1 L) and extracted with 0.25 M H_2SO_4 (10 x 65 mL). The aq. phase was stirred with excess zinc powder (*ca.* 10 g) overnight, then filtered through Celite, and the resulting solution basified (NH_4OH, to pH *ca.* 11, indicator paper) and extracted with $CHCl_3$ (10 x 200 mL). The $CHCl_3$ extracts were dried ($MgSO_4$), filtered and concentrated (rotary evaporator) to yield a white amorphous solid (11.6 g). Further purification of the sample was achieved by dissolving it in 0.05 M H_2SO_4 (30 mL) and washing this solution with $CHCl_3$ (10 x 100 mL). The aq. phase was then basified (NH_4OH, indicator paper) and extracted with $CHCl_3$ (10 x 150 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$), filtered and concentrated to yield the crude "reduced" bases as white amorphous solid (10.2 g). GLC analysis revealed four components (see Table 54).

Table 54: PA profile for *S. syringifolius* as determined by GLC analysis	
GLC retention time (min)	Percent of total PA
11.9	32.7
12.4	32.0
13.2	7.6
16.5	26.3

3.5.1.1 Separation of the individual alkaloids.

In a preliminary investigation of this mixture a portion of the alkaloid (40 mg) was subjected to PTLC (Et_2O-MeOH-NH_4OH (380:20:20)) to give 3 components.

The least polar component, R_f 0.37, was isolated as a pale yellow oil (15 mg). This alkaloid was identical in all respects to the alkaloid earlier isolated from *S. hadiensis* and shown to be 12-O-acetylrosmarinine (**86**).

The more polar component, R_f 0.33, was isolated as a white amorphous solid (8 mg) and identified as rosmarinine (**68**) on the basis of spectral data (^1H and ^{13}C NMR).

The most polar component R_f 0.30, was isolated as a white amorphous powder (11.0 mg). Recrystallization from EtOAc furnished angularine (**92**) as white crystals; mp 197-9^0 C from EtOAc [lit [76] 200-201^0 C], $[\alpha]_D$ -96^0 (c = 1.28, EtOH) [lit [76] $[\alpha]_D^{25^\circ}$ -98^0 (c = 0.0223, EtOH)]: ^1H and ^{13}C NMR (see Table 23); IR ν_{max} (KBr) 3423 (m, OH), 3095-2870 (m), 1741 (s, C=O), 1723 (s, C=O), 1654 (w, C=C), 1452 (m), 1365 (m), 1348 (m), 1318 (m), 1304 (m), 1385 (m), 1244 (m), 1226 (m), 1210 (m), 1183 (s), 1156 (s), 1135 (s), 1108 (s), 1096 (m), 1052 (m), 1004 (m), 987 (m), 958 (m), 925 (m) and 908 (m), EIMS m/z (%) [M^+] 351 (3), 227 (2), 154 (24), 151 (30), 138 (44), 122 (17), 109 (13), 98 (21), 82 (39), 43 (100) and 40 (53); CIMS m/z (%) $[M+1]^+$ 352 (100), 211 (16), 174 (16),

154 (23), 151 (23), 139 (26), 138 (99), 129 (9), 122 (40), 112 (9), 98 (17), and 82 (24).

Another portion of the alkaloid mixture (200 mg) was triturated with Me_2CO to afford a white amorphous solid (70 mg) which was recrystallized from Me_2CO to yield colourless crystals of rosmarinine (**68**) and the mother liquor remaining from the separation of rosmarinine was then purified by PTLC (Et_2O-MeOH-NH_4OH (380:20:20)) to give three components.

The least polar component, R_f 0.37, was isolated as a pale amber coloured oil (84.3 mg). ^1H and ^{13}C NMR revealed this sample to be 12-O-acetylrosmarinine (**86**).

The more polar component, R_f 0.33, was isolated as a white amorphous solid (16.3 mg) and identified from its spectral characteristics (^1H and ^{13}C NMR) as rosmarinine (**68**).

The most polar component, R_f 0.30, was isolated as a two - component mixture (^1H NMR and GLC analysis [with R_T 12.4 and 13.2 mins]). Attempts to separate these two substances using different solvent and support systems failed. However, the ^1H NMR suggested the mixture to contain angularine (**92**) and probably its geometrical isomer which was not further characterised.

The remaining alkaloid mixture (*ca.* 10 g) was triturated with acetone to afford a white amorphous solid (*ca.* 4 g). GLC analysis revealed a mixture of two components with R_f 0.33 and 0.30 [R_T 11.9 and 12.4 min respectively].

The mother liquor was concentrated further to yield more solid (3.25 g). Part of this material (400 mg) was purified by PTLC (Et_2O-MeOH-NH_4OH (380:20:20)) to yield four components.

The least polar component, 12-O-acetylrosmarinine (**86**), R_f 0.37, was isolated as an oil (180.3 mg)

The second least-polar component, R_f 0.33, was neorosmarinine (**78**), isolated as a

pale yellow oil (5.3 mg) identified on the basis of its ^{1}H and ^{13}C NMR spectra.

The third component, R_f 0.33, was rosmarinine (**68**) (^{1}H and ^{13}C NMR) and was isolated as white amorphous solid (130.6 mg).

The most polar component, R_f 0.30, was also isolated as a white amorphous solid (20.2 mg). GLC analysis revealed two components, R_T 12.4 and 13.2 min. Recrystallization from EtOAc gave a product (53.6 mg) mp 197-199^0 C, whose ^{1}H and ^{13}C NMR spectra were the same as those for angularine (**92**).

3.5.2 Without reductive processing

The dried ground aerial parts of *S. syringifolius* (548.5 g) were subjected to repeated extraction in a Waring blender with 95% EtOH (10 L) until the resulting extract was pale yellow. The combined ethanolic extracts were then concentrated to dryness (cyclone, then rotary evaporators). The residue was dissolved in $CHCl_3$ (100 mL) and extracted with 0.05 M aq H_2SO_4 (4 x 50 mL). The combined aq. extracts were then basified (NH_4OH, to pH *ca.* 10 (indicator paper)) and extracted with $CHCl_3$ (8 x 100 mL). The $CHCl_3$ extracts were combined, dried ($MgSO_4$) and concentrated to yield a light yellow oil (0.78 g, 0.14%). Further extraction of the aqueous basic solution with n-butanol (10 x 100 mL), followed by evaporation of the combined, dried ($MgSO_4$) n-BuOH extract gave a bright brown, almost red residue (9.7 g).

3.5.2.1 Separation of the "unreduced" bases of *S.syringifolius* (chloroform extract)

Part of the chloroform extract (140 mg) was purified by PTLC (EtOAc-MeOH (1:1) to give three subatances.

The least polar of these (R_f 0.37) was isolated as an oil (16 mg). ^{1}H NMR suggested it to be a mixture and further purification by PTLC (Et_2O-MeOH-NH_4OH (190:20:2) led to the isolation of 12-O-acetylrosmarinine (**86**) as the major component :

obtained as a pale yellow oil (*ca.* 2 mg) and identified by its ^{1}H NMR.

The more polar substance R_f 0.33 was also isolated as a mixture (90.6 mg) (^{1}H NMR). Further purification by PTLC (aluminium oxide, type E, Et_2O-MeOH-NH_4OH (190:10:10) resulted in the isolation of two components.

The less polar component, rosmarinine (**68**) was isolated as an amorphous powder (56.3 mg) with spectroscopic properties as on p. 22 and 23.

The more polar component, angularine (**92**), R_f 0.30 was isolated as a white amorphous powder (49.6 mg) (^{1}H and ^{13}C NMR as p 65).

The third substance was obtained as an oil which was a mixture, as revealed by ^{1}HNM analysis. Further purification was performed by PTLC (aluminium oxide, 60 F_{254} type E, EtOAc-MeOH (4:1), four developments). Two zones were observed (UV light). The least polar component was isolated as an oil (9.6 mg). ^{1}H NMR analysis revealed this to be a mixture of two components. Further separation by PTLC (aluminium oxide, 60 F_{254}, $CHCl_3$-MeOH-NH_4OH (95:4:1)) resulted in the isolation of these components. The less polar component, angularine N-oxide (**93**), R_f 0.09 was isolated as an oil (*ca.* 3.3 mg). Addition of H_2O followed by evaporation under reduced pressure gave rise to a white solid: mp 138° C (dec); $[\alpha]_D$ -85.2° (c = 0.61, $CHCl_3$),^{1}H and ^{13}C NMR (see Tables 24 and 25); IR ν_{max} (KBr) 3414 (m, br, OH), 2982 (m), 1728 (br, s, C=O), 1647 (w, C=C), 1448 (w), 1364 (w), 1252 (m), 1232 (m), 1148 (s, N-O), 1109 (m), 994 (w) and 755 (w) cm^{-1}; EIMS m/z (%) [M$^+$] 367 (1), 351 (53), 334 (11), 331 (5), 154 (61), 138 (69), 135 (65), 122 (61) , 112 (39), 85 (84), 83 (100), 82 (68), 47 (71)and 43 (84); CIMS (NH_3) m/z (%) 368 (2), 352 (100), 334 (3), 197 (23), 154 (16), 151 (18), 138 (37), 122 (15), 82 (15) and 43 (5)

The more polar component, 12-O-acetylrosmarinine N-oxide (**94**), was isolated as an oil (*ca.* 4.5 mg). Addition of H_2O followed by evaporation resulted in a colourless solid: mp 135-7^0 C (dec.); ^{1}H and ^{13}C NMR (see Tables 26 and 27); IR υ_{max} (KBr) 3389

(br, m, OH), 2980 (m), 1740 (s, C=O), 1458 (m), 1449 (m), 1371 (m), 1246 (s, N-O), 1221 (s), 1155 (s), 1115 (s), 1072 (w) and 1028 (w) cm^{-1}; FAB-MS m/z (%) [M+1] 412 (100), 397 (14), 396 (58), 354 (7), 353 (4) 352 (9), 351 (9), 337 (4), 336 (5), 306 (4), 155 (6), 154 (31), 153 (12), 152 (10), 138 (38), 137 (14), 136 (41), 135 (7), 134 (6), 125 (7), 124 (11), 122 (8), 121 (6), 120 (15), 119 (15), 118 (12), 108 (14), 107 (9), 106 (12), 105 (5), 103 (10), 98 (23), 96 (10) and 94 (12).

The more polar component, rosmarinine N-oxide (**91**), was isolated as a pale yellow oil (6.5 mg) (^1H, and ^{13}C NMR as pg 60 and 61).

3.6 Chemical transformation studies with *S. syringifolius* alkaloids

Preparation of angularine N-oxide (**93**) from angularine (**92**).

A solution of angularine (60 mg) and MCPBA (29.5 mg) in $CHCl_3$ (10 mL) was stirred at room temperature for 45 mins. The solvent was then removed (rotary evaporator), the residue dissolved in H_2O (10 mL) and then extracted with Et_2O (6 x 20 mL). The aq. solution was evaporated *in vacuo* to yield the N-oxide (59.7 mg, 95%) with spectroscopic properties identical with those of the sample isolated from *S. syringifolius*.

Preparation of 12-O-acetylrosmarinine N-oxide (**94**) from 12-O-acetylrosmarinine (**86**)

A solution of 12-O-acetylrosmarinine (54.7 mg) and MCPBA (23.9 mg) in $CHCl_3$ (10 mL) was stirred at room temperature for 30 mins. The solvent was then removed (rotary evaporator) and the residue dissolved in H_2O (10 mL) and extracted with Et_2O (6 x 10 mL). The aq. solution was then evaporated in vacuo to yield the N-oxide (7.5 mg 13%) whose spectroscopic data (^1H and ^{13}C NMR) were identical to those for the N-oxide from the *S. syringifolius*.

3.7 Isolation of the alkaliods of *S. canus* Hook.

The fresh plant material was extracted repeatedly with 95% EtOH (3 x 4 L) in a Waring blender until the extracts were nearly colourless. The filtered extracts were concentrated (cyclone evaporator, then rotary evaporator) to give the crude extract (208.5 g).This was partitioned between 0.25 M H_2SO_4 (100 mL) and $CHCl_3$ (100 mL). The $CHCl_3$ layer was removed, and the aq. phase further extracted with $CHCl_3$ (3 x 100 mL). The aq. phase was then stirred with zinc dust (*ca.* 10 g) overnight. The excess zinc dust was filtered off, and the filtrate basified (NH_4OH, to pH *ca.* 10 (indicator paper)). The basic solution was extracted with $CHCl_3$ (6 x 100 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to afford the crude alkaloids (0.9 g). This was purified by dissolving it in 0.25 M H_2SO_4 (10 mL), and washing this solution with $CHCl_3$ (10 x10 mL). The aq. acid solution was then basified (NH_4OH, to pH *ca.* 11 (indicator paper)) and extracted with $CHCl_3$ (10 x 10 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to afford the crude alkaloid (490.3 mg). GLC analysis revealed three components (see Table 55)

Table 55: PA profile for *S. canus* as determined by GLC analysis

GLC retention time (mins.)	Percent of total PA
8.2	76.0
8.7	3.6
9.3	17.4

The two major components were isolated as follows: Part of the sample (100 mg) was purified by PTLC giving rise to two zones.

The component with R_f 0.45 was isolated as a white crystalline solid (40 mg) which was identified as senecionine (**4**) on the basis of physcio-chemical data (mp, IR,

^1H, ^{13}C NMR and MS) (see pgs. 77, 78 and 151).

The component with R_f 0.24 was isolated as white amorphous solid and recrystallized from Me_2CO to afford retrorsine (**76**) (17.3 mg) as colourless crystals: mp 208-9^0 C from Me_2CO [lit [71] 213-5^0, [37] 216^0, [150] 210^0]; for ^1H and ^{13}C NMR (see Tables 31 and 32); IR υ_{max} (KBr) 3572 (m, OH), 2972-2820 (m), 1742 (s, C=O), 1713 (s, C=O) ,1655 (w, C=C), 1458 (w), 1439 (w), 1252 (m), 1231 (m), 1217 (m), 1190 (m), 1163(s), 1103 (w), 1078 (w), 1005 (m), 943 (w), 910 (w), 824 (w) and 602 (w) cm^{-1}, this spectrum was superimposible upon that of authentic retrorsine (Sigma Chem. Co.); EIMS m/z (%) [M^+] 351 (20), 320 (11), 306 (5), 248 (12), 246 (21), 221 (15), 220 (27), 218 (11), 139 (18), 138 (66), 137 (89), 122 (32), 121 (59), 120 (95), 1 19 (87), 118 (27), 117 (22), 116 (6), 109 (33), 108 (17), 106 (18), 104 (96) 95 (74), 94 (88), 93 (100), 92 (9), 91 (17), 90 (12), 89 (14), 83 (7), 82 (8) ,81 (23), 80 (58), 79 (15), 77 (14), 68 (23), 67 (28), 66 (14), 63 (13), 55 (23), 54 (33), 53 (53), 43 (25) and 41 (47).

3.8 Isolation of the alkaloids of *S. foetidus* Howell (*S. hydrophiloides* Rydb.)

The fresh flowering tops of this plant were extracted with 95% EtOH (40 L) in a Waring blender until the extracts were nearly colourless. The extracts were concentrated (cyclone evaporator, then rotary evaporator). The resulting residue was partitioned between 0.25 M H_2SO_4 (1600 mL) and $CHCl_3$ (4 L). The aq. phase was stirred with excess zinc dust overnight (12 hrs.); then filtered through a pad of Celite and the resulting solution basified (NH_4OH, pH *ca.* 10 (indicator paper)) and extracted with $CHCl_3$ (8 x 500 mL). The $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated (rotary evaporator) to afford a dark residue (20.6 g). This was partitioned between 0.25 M H_2SO_4 (5 x 20 mL) until the acid extracts were negative when tested for alkaloids (Mayer's reagent). The pooled aq. extracts were basified (NH_4OH, to pH *ca.* 11, indicator paper) and extracted with $CHCl_3$ (10 x 20 mL). The $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated to

afford the "reduced" bases (13.9 g). GLC analysis revealed at least seven peaks (see Table 56).

Table 56: PA profile for *S. foetidus* as determined by GLC analysis

GLC retention time (min)	Percent of total PA
3.1	6.0
3.4	15.0
3.6	15.0
8.2	4.0
9.6	27.0
9.9	15.0
10.3	3.0

3.8.1 Separation of the reduced bases of *S. foetidus*

Preliminary separation of 20 mg of the reduced bases was undertaken by PTLC (Et_2O-MeOH-NH_4OH (190:19:5)) giving rise to two zones:

The less polar component was isolated as an oil shown by GLC analysis to be a mixture. Further purification by PTLC (Et_2O-MeOH-NH_4OH (190:19:5) gave rise to two zones, with the less polar one being predominant. This was 7-O-senecionyl-9-O-sarracinylretronecine (**99**) isolated as an oil (3.8 mg), R_f 0.47; $[\alpha]_D$ + 10.5 (c = 2.1, MeOH) [lit [109] $[\alpha]_D$ $+11^0$ (c = 1, MeOH)]; ^1H and ^{13}C NMR (see Tables 33 and 34); EIMS m/z (%) [M^+] 335 (6), 237 (30), 221 (5), 220 (34), 219 (10), 154 (10), 138 (8), 137 (20), 136 (100), 121 (20), 120 (56), 119 (64), 118 (35), 117 (77), 100 (44), 94 (77), 93 (93), 83 (97), 82 (29), 80 (41), 55 (73), 53 (65), 41 (68), and 39 (88).

A minor component, 7-O-angeloylretronecine (**103**), was also isolated as an oil (*ca.* 0.9 mg): R_f 0.39; [α] +40° (c = 0.4, EtOH) [lit [37] $[\alpha]_D$ +49° (EtOH); for ^1H and ^{13}C NMR data see Tables 39 and 40; EIMS m/z (%) [M^+] 237 (5), 219 (5), 154 (4), 138 (6), 137 (22), 136 (23), 124 (29), 111 (41), 106 (45), 94 (27), 93 (13), 80 (100), 55 (56).

Another minor component, sarracine (**67**) was also isolated as an oil (*ca.* 0.3 mg): for ^1H and ^{13}C NMR (see Tables 41 and 42); EIMS m/z (%) [M^+] 337 (1), 237 (17), 222 (15), 219 (9), 207 (3), 156 (6), 140 (26), 139 (29), 138 (100), 123 (25), 122 (47), 121 (29), 120 (31), 119 (22), 106 (18), 100 (13), 97 (35), 96 (5), 83 (30), 82 (96), 80 (18), 55 (65), 53 (37) and 39 (34) .

3.8.2 Separation of the alkaloid mixture by vacuum liquid chromatography

Large scale separation of the alkaloid mixture was undertaken by subjecting the mixture to vacuum liquid chromatography (see section 3.1 for the procedure). Table 57 summarises the results obtained. All fractions were analysed by TLC and visualised with UV light and iodine. On the basis of these results, fractions 8 and 9; 12 and 13 and 18 and 19 were combined. Fractions 2-5; 10 and 11 and 14-17 were similar mixtures but were not pooled. Further chromatographic separations were undertaken as follows:

Fraction 1: this was purified by PTLC ($CHCl_3$-MeOH-NH_4OH (100:20:1), double development) to yield 7-O-senecioyl-9-O-sarracinylretronecine (**99**), as the main component as an oil (7 mg) with ^1H and ^{13}C NMR as on p 86 and 87.

Fraction 2: part of this material (295 mg) was purified by PTLC (Et_2O-MeOH-NH_4OH (190:19:5)) to yield three components:

The least polar component, 7-O-senecioyl-9-O-sarracinylretronecine (**99**), R_f 0.47, R_T 9.6 mins, was isolated as an oil (71.3 mg).

Table 57: Vacuum column chromatography for the fractionation of crude alkaloid mixture (5.3 g) from *S. foetidus*.

Fraction No.	Eluant	Volume Collected (mL)	Weight eluted (mg)
1	$CHCl_3$-MeOH-NH_4OH (70:10:1)	125	15.4
2	,,	,,	550.1
3	,,	,,	13.9
4	,,	,,	282.6
5	,,	,,	215.3
6	,,	,,	1116.5
7	,,	,,	384.0
8	,,	,,	8.8
9	,,	,,	0.2
10	,,	,,	604.9
11	,,	,,	460.7
12	,,	,,	61.0
13	,,	,,	158.1
14	,,	,,	953.3
15	,,	,,	178.8
16	,,	,,	55.3
17	,,	,,	20.6
18	,,	,,	9.6
19	,,		1.0
20	MeOH	250mL	29.4
		Total	5119.5

A more polar component, foetidine (**106**), R_f 0.46, was also isolated as an oil (2.8 mg): ^1H and ^{13}C NMR (see Tables 45 and 46); EIMS m/z (%) [M^+] 335.1734 (4) (calcd. for $C_{18}H_{25}NO_5$ 335.1726), 237 (24), 220 (20), 219 (8), 137 (210), 136 (91), 120 (36), 119 (37), 118 (23), 117 (64), 100 (34), 94 (40), 93 (73), 83 (100), 82 (26), 81 (34), 80 (36), 69 (73), 56 (71), 43 (48), 41 (80) and 39 (55).

The most polar component was isolated as a mixture (^1H and ^{13}C NMR). Further purification by PTLC ($CHCl_3$-MeOH-NH_4OH (100:20:1)) yielded the major component, 7-O-senecioylretronecine (**105**), as an oil (2.2 mg) with ^1H and ^{13}C NMR data as given on p 104 and 105; EIMS m/z (%) [M^+] 237 (10), 219 (8), 156 (26), 154 (19), 138 (53), 137 (63), 136 (50), 124 (57), 106 (74), 95 (80), 94 (73), 93 (83), 83 (70), 82 (84), 81 (46), 80 (100), 55 (96) and 53 (75).

Fraction 3 was also purified by PTLC ($CHCl_3$-MeOH-NH_4OH (100:20:1)) to yield as the major component, 7-O-senecioyl-9-O-sarracinylretronecine (**99**) (^1H and ^{13}C NMR see p 86 and 87), as an oil (8.3 mg), R_f 0.47.

Fraction 4 was purified by PTLC ($CHCl_3$-MeOH-NH_4OH (100:20:1) , double development) to yield three zones:

The least polar component, R_f 0.47, R_T 9.6 min, isolated as an oil (15.8 mg) was identified as before (^1H and ^{13}C NMR) 7-O-senecioyl-9-O-sarracinylretronecine (**99**).

A more polar component on concentration yielded an oil (153.6 mg). ^1H NMR analysis revealed this component to be a mixture. No further purification was undertaken.

The most polar component, sarracine (**67**), was obtained as an oil (9.3 mg), R_f 0.35, R_T 3.11 min.

Fraction 5 was purified by PTLC ($CHCl_3$-MeOH-NH_4OH (100:20:1), three

developments) which resulted in two zones:

The least polar component, R_f 0.47, R_T 9.6 mins, 7-O-senecioyl-9-O-sarracinylretronecine (**99**) was obtained as an oil (7.3 mg).

The more polar component was isolated as an oil (79.5 mg) and found to be a mixture (GLC analysis). Further purification by PTLC ($CHCl_3$-MeOH-NH_4OH (100:20:1)) resulted in the isolation of two components which were identified as sarracine (**67**) (56.7 mg) (see p. 165) and 7-O-angeloylretronecine (**103**) (18.5 mg) (see p 166).

Fractions 6-13 were not examined as TLC and GLC revealed components already isolated and present in fraction 14 (see below).

Fraction 14 was observed to consist of two oils of different density. The more dense layer was removed using a pipette and then purified by PTLC ($CHCl_3$-MeOH-NH_4OH (100:20:1), 3 developments) to yield two zones:

The less polar component, an oil (67.0 mg), R_f 0.16, R_T 3.4 mins, was 7-O-angeloylplatynecine (**100**) : ^1H and ^{13}C NMR (see Tables 35 and 36); EIMS m/z (%) [M^+] 239 (1), 221 (2),156 (32), 140 (7), 139 (40), 138 (12), 137 (6), 114 (6), 113 (10), 108 (10), 106 (8), 100 (8), 96 (9), 95 (17), 83 (16), 82 (100) and 55 (47).

The more polar component, R_f 0.08, R_T 3.6 mins, was also isolated as an oil (16.3 mg). Spectral analysis (^1H and ^{13}C NMR) revealed the sample to be 9-O-angeloylplatynecine (**101**): ^1H and ^{13}C NMR (see Tables 37 and 38): EIMS m/z (%) [M^+] 239 (30), 221 (44), 195(25), 156 (51), 140 (49), 139 (65), 138 (51), 122 (47), 96 (72), 95 (88), 83 (68), 82 (100) and 55 (87).

The less dense layer was purified by PTLC ($CHCl_3$-MeOH-NH_4OH (100:20:1), triple development) to yield two zones:

The less polar component, 7-O-angeloylplatynecine (**100**), R_f 0.16, R_T 3.4 min,

was isolated as an oil (39.3 mg) with spectroscopic properties as on p. 90 and 91

The more polar component, R_f 0.08, was also isolated as an oil (53.7 mg). This alkaloid was identical in all respects to the alkaloid identified above as 9-O-angeloylplatynecine (**101**).

3.9 Isolation of the alkaloids of *D. cottonii* J.Hutch. and G.Tayl

The dried ground leaves of *D. cottonii* (90 g) were subjected to repeated extraction in a Waring blender (quart size) using 95% EtOH until the resulting solution was pale yellow. The ethanolic extracts were concentrated to dryness to yield the crude extracts (30.9 g).

The above material (30.9 g) was dissolved in 0.5 M H_2SO_4 (60 mL) and extracted with $CHCl_3$ (6 x 15 mL). The $CHCl_3$ extract was then washed with 1.0 N H_2SO_4 (3 x 30 mL) and then added to the original acid solution to give the aq. phase (160 mL).

3.9.1 Separation of the reduced bases of *D. cottonii*

Part of the aq. phase (100 mL) was stirred with zinc dust (5 g) at room temperature overnight, then filtered through Celite and the filtrate basified (NH_4OH, pH. *ca.* 11 (indicator paper)) and extracted with $CHCl_3$ (6 x 50 mL). The $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated (rotary evaporator) to give the crude alkaloid mixture (240 mg, 1.24%). GLC analysis showed one major peak and two minor ones (see Table 58)

Part of the chloroform extract (140 mg) was purified by PTLC ($CHCl_3$-MeOH-NH_4OH (95:4:1), double elution) to yield three zones:

The major component, R_T 11.1 min, deoxycinchonidine (**111**) was isolated as an oil (25.7 mg): $[\alpha]_D$ -18.7^0 (c = 1.6, EtOH, [lit [137] $[\alpha]_D$ -27.3^0 (c = 2.089, EtOH), [150] $[\alpha]_D^{13}$ -29.9^0 (c = 2.006, 99% alcohol)]; IR ν_{max} (neat) 2940 (s), 2932 (s), 2924 (s), 2888

Table 58: Alkaloid profile for *D.cottonii* as determined by GLC analysis	
GLC retention time (min)	Percent of total
11.1	82.5
14.2	3.2
15.3	6.7

(s), 1680 (m), 1591 (m), 1568 (m), 1559 (m), 1508 (m), 1456 (w), 1449 (w), 1422 (w), 1321 (w), 1045 (w), 991 (w) and 909 (m) cm^{-1}; ^{1}H and ^{13}C NMR (see Tables 47 and 48), EIMS m/z (%) [M^{+}] 278 (20), 277 (9), 263 (4), 237 (18), 169 (22), 168 (39), 167 (30), 156 (22), 155 (15), 154 (50), 143 (58), 142 (18), 137 (50), 136 (100), 115 (25), 82 (23), 81 (39), 79 (23), 77 (23), 55 (47) and 41 (53).

The second major component, R_T 15.3 mins. was also isolated as an oil (*ca.* 1.2 mg) but was not characterised.

The third component (*ca.* 1.2 mg) was isolated as an oil too, which GLC analysis revealed to be a mixture and it was not examined further.

3.9.2 Separation of the unreduced bases of *D. cottonii.*

The unreduced extract was basified (NH_4OH, to pH *ca.* 11 (indicator paper)) and extracted with $CHCl_3$ (7 x 30 mL). The extracts were dried ($MgSO_4$) and concentrated (rotary evaparator) to give a solid residue (176.5 mg). The sample was recrystallized from EtOH to give cinchonidine (**110**) as white crystals (100 mg): mp 209-210^{0} C [lit [141] 205^{0} C, [141] 210.5^{0} C)]; $[\alpha]_D$ -102.5° (c = 2.8, EtOH) [lit [141] $[\alpha]_D$ -110 (EtOH)]; ^{1}H and ^{13}C NMR (see tables 49 and 50); IR ν_{max} (KBr) 3200 (m), 2926 (s), 2866 (m), 2764 (m), 2735 (m), 2718 (m), 1581 (m), 1510 (m), 1454 (m), 1098 (s), 1037 (m), 999 (m), 901 (m), 883 (m), 826 (m), 804 (m) and 756 (s) cm^{-1}; EIMS m/z (%) [M^{+}] 294 (6), 159 (22),

156 (15), 136 (100), 128 (21), 95 (16), 81 (41), 79 (19), 77 (14), 55 (28) and 42 (54).

3.10 Conversion of cinchonidine to deoxycinchonidine using zinc powder

Cinchonidine (**110**) (1.00 g) was dissolved in 0.5 M H_2SO_4 (15 mL) and zinc powder (2 g) added at room temperature. The solution immediately turned yellow and then brown. The solution was stirred for 1hr. and then monitored by TLC which suggested the reaction to be complete. The reaction was continued for another 0.5 h. and the reaction mixture worked up as follows: After being filtered through Celite, the acid solution was basified (NH_4OH, pH *ca.* 11 (indicator paper)) and then extracted with $CHCl_3$ (7 x 30 mL), the extracts dried ($MgSO_4$) and concentrated to give the product (930 mg). GLC analysis revealed a single peak.

Part of the sample (120 mg) was purified by PTLC ($CHCl_3$-MeOH-NH_4OH (95:4:1)) giving rise to a single zone:

The component was isolated as an oil (106.0 mg). Spectroscopic analysis revealed the compound to be identical to deoxycinchonidine (**111**).

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