ALKALOIDS FROM THREE SOUTH AFRICAN *CRINUM* SPECIES

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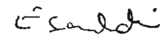
Preface

The experimental work in this thesis was carried out under the supervision of Professor J. van Staden and Professor S. E. Drewes in the Research Centre for Plant Growth and Development, School of Botany and Zoology, University of Natal, Pietermaritzburg.

The studies have not been submitted in any form to any other university and the result of my own original investigation, except where the work of others are acknowledged.



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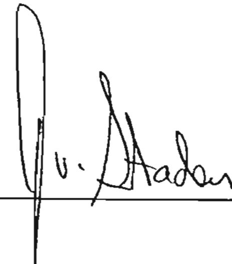

Supervisor
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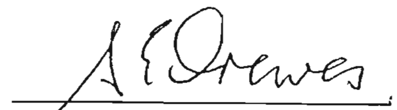

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Abstract

The alkaloid content of three *Crinum* species namely *C. bulbispermum*, *C. macowanii* and *C. moorei* was investigated. The ethanolic extracts of *C. bulbispermum* yielded seven compounds. The new alkaloids 8α-ethoxyprecriwelline, *N*-desmethyl-8α-ethoxypretazettine and *N*-desmethyl-8β-ethoxypretazettine were isolated for the first time from a natural source. In addition, the known alkaloids bulbispermine, crinamine, 6-hydroxycrinamine and 3-*O*-acetylhamayne were isolated in this study.

The ethanolic extracts of *C. moorei* were found to contain lycorine, 1-*O*-acetyllycorine, crinine, 3-*O*-acetyllycrinine, epibuphanisine, powelline, crinamidine, undulatine, epivittatine, 1-epideacetylbowdensine, cherylline and the new alkaloids mooreine and 3-[4'-(2'-aminoethyl)phenoxy]bulbispermine.

The alkaloids crinine, lycorine, bulbispermine, cherylline and hamayne were obtained from the ethanolic extracts of *C. macowanii*. In addition, the amine tyramine was identified during the isolation process.

Dilute HCl solution extraction followed by GC analysis was used to investigate organ-to-organ and seasonal variation of alkaloids in each *Crinum* species, as well as the interspecific variation in these alkaloids over two consecutive years. Twelve alkaloids were identified, including crinine, epibuphanisine, powelline, crinamine, crinamidine, 6-hydroxycrinamine, 1-epideacetylbowdensine, 3-*O*-acetylhamayne, undulatine, lycorine, 1-*O*-acetyllycorine and cherylline. Alkaloids were detected in all organs of *C. moorei* and *C. macowanii*. However, alkaloids were not detected in the leaves of *C. bulbispermum*.

Organ-to-organ and seasonal variations in the total yield and total ring types of these alkaloids were noticed. Organ-to-organ and seasonal statistical variations were also detected for some of the individual alkaloids detected in each of these species. The results also showed that *C. moorei* had the highest levels of all individual alkaloids except crinamine when compared to *C. bulbispermum* and *C. macowanii.* Quantitatively, the detected alkaloids chemotaxonomically separated *C. moorei* from *C. bulbispermum* and *C. macowanii.* The results also indicated that *C. macowanii* is more closely related to *C. bulbispermum.* Qualitatively, lycorine, 1-*O*-acetyllycorine, cherylline, crinamidine, 1-epideacetylbowdensine, crinine, crinamine and 3-*O*-acetylhamayne were detected in both *C.moorei* and *C. macowanii*, indicating the close relationship of these species.

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Abbreviations

$CDCl_3$	Deuterochloroform.
CD_3COOD	Deuteroacetic acid.
CD_3OD	Deuteromethanol.
CF_3COOD	Deuterated trifluoroacetic acid
DMSO	Dimethylsulphoxide.
DMSO-d_6	Deuterodimethylsulphoxide.
D_2O	Deuterowater.
D_2SO_4	Deuterosulphuric acid.
ED_{50}	Medium value of the individual effective dose.
FID	Flame ionization detector.
GC	Gas chromatography.
GC-MS	Gas chromatography-mass spectrometry.
Hep G2	Human hepatoma
HRMS	High resolution mass spectrometer.
i.d	internal diameter.
LD_{50}	Lethal dose for 50% of a group of living organisms.
LMTK	Murine alveolar non tumoral fibroblast.
Molt4	Human lymphoid neoplasm.
NMR	Nuclear magnetic resonance.
NPD	Nitrogen-phosphorus detector.
Pyridine-d_5	Deuteropyridine.
TLC	Thin layer chromatography.
VLC	Vacuum liquid chromatography.
TMS	Tetramethylsilane.

CHAPTER 1
Literature Review

1.1 Family Amaryllidaceae: Taxonomy and Distribution

Plants of the family Amaryllidaceae are perennial or biennial herbs with subterranean bulbs with thick, fleshy bulb scales or without a typical bulb but a rhizome as in *Scadoxus* and *Clivia*. The Amaryllidaceae are widely distributed. They are richly represented in the tropics and have pronounced centres in South Africa and to a lesser extend in Andean South America. Other groups have their centre in the Mediterranean. Groups of genera supposed to be phylogenetically related often have a particular geographic concentration (DAHLGREN *et al.* 1985)

The family is classified into nine tribes. The tribe Lycoridae is Asiatic in distribution while Stenomesseae and Euchardeae are South American in distribution. The tribe Pancratieae consists of Old World genera ranging from South Africa to Macronesia and the Mediterranean region and further eastwards into tropical Asia. The genus *Narcissus* of the tribe Narcisseae has a typically west Mediterranean distribution while the genus *Sterenbergia* ranges from the Mediterranean to Iran. The tribe Galantheae has a Mediterranean-western Asiatic distribution. Southern Africa is the centre of variation of the tribes Haemanthae and Amaryllidae. The latter being centred in the winter rainfall area of southern Africa, although the genus *Crinum* has a pantropical distribution (DAHLGREN *et al.* 1985).

The mostly African tribe Amaryllideae consists of 11 currently recognised genera and approximately 155 species. It is classified into two monophyletic sub-tribes. The sub-tribe Crininae has four genera *Boophane*, *Crinum*, *Ammocharis* and *Cybistetes* while the sub-tribe Amaryllidinae includes the genera *Amaryllis*, *Nerine*, *Brunsvigia*, *Crossyne*, *Hessea*, *Strumaria* and *Carpolyza* (SNIJMAN and LINDER 1996). Within the tribe Crininae, the genus *Cybistetes* is a Western Cape representative while *Ammocharis* is a widely distributed sub-Saharan genus (SNIJMAN and WILLIAMSON 1994). The g_nus *Boophane* is also known to occur in the temperate winter-rainfall region of southern Africa, although the species *B. disticha* occurs widely in Central Africa (VILADOMAT *et al.* 1997).

1.2 The Genus *Crinum*

The genus consists of herbaceous plants with large tunicated bulbs which are sometimes produced into a neck or rarely into a false stem made up of the

indurated sheathing bases of the old leaves. In some species new bulbs are readily produced laterally. Leaves are linear, lorate or ensiform, sheathing at the base, rosulate or distichous often dying back in the winter, the previous season's leaves growing back again in spring with a few new leaves in the centre. Inflorescence arising laterally, umbellate, 1-many-flowered, peduncle solid; spathe-valves 2, bracteoles at base of each flower narrowly linear. Flowers subsessile or pedicelled; perianth tube long; segments linear to broadly lanceolate, the inner slightly broader than the outer, spreading or connivent into a campanulate or trumpet shape. Stamens inserted in the throat of the perianth- tube; filaments arcuate, ascending or declinate; anthers versatile. Ovary inferior, obvious as a swelling between the apex of the pedicel and the base of the perianth tube, turbinate. Fruit subglobose, bulging with the large seeds eventually bursting irregularly to release the subglobose seeds, sometimes beaked with the persistent base of the perianth-tube. Seeds are subglobose (VERDOORN 1973).

The genus *Crinum* has a pantropical distribution which extends from Africa to Madagascar, the Mascarene and Pacific Islands and the tropics of America, Asia and Australia (SNIJMAN and LINDER 1996). According to DAHLGREN *et al.* (1985) there are 130 species in *Crinum*. Recent regional revisions of African taxa indicated that around half this number would be a better approximation (FANGAN and NORDAL 1993). A number of *Crinum* species are under cultivation, including the Indomalaysian *C. amabile*, the tropical Asiatic *C. latifolium* and *C. asiaticum*, the Himalayan *C. amoenum*, the American *C. americanum* and *C. erubescens*, the South African *C. bulbispermum* and *C. campanulatum*, the west African *C. laurentii* and *C. nutans* and the Australian *C. pedunculatum* (DAHLGREN *et al.* 1985).

1.2.1 *Crinum bulbispermum* (Brum.f.) Milne Redh.

Bulbs 7-13 cm in diameter near the base and narrowing gradually towards the apex. Leaves glaucous green, sheathing at the base to form a false stem up to 30 cm high, flaccid, arcuate, the outermost the broadest, up to 11 cm broad, all with a narrow cartilaginous teeth or hair, several to many new leaves complete with tip produced in the centre. Peduncle from 50-90 cm long, up to 2, 5cm and 1, 5cm thick. Spathe-valves up to about 8 cm long , 3, 5 cm at base, becoming chartaceous but not very thin and reflexed at least the upper half; bracteoles linear. Umbels 6-16 flowered. Pedicels of different length, 4-9 cm long. Perianth normally with a long cylindrical tube, up to 11 cm, rarely; segments conniving in a narrow funnel with the apices subspreading or slightly recurved, white with a dark red keel or entirely suffused with red and the keel very dark red, 6-10 cm long, the outer

about 2 cm wide, the inner about 3 cm wide. Stamens declinate white or partly suffused with deep pink; anthers greyish or light brown; style deep pink in the upper portion , stigma small. Fruit subglobose, green, partly suffused with red-purple, 3-7 cm, crowned with a ring but not beaked. Seeds large 1-2 cm in diameter, appearing smooth when turgid, reticulate later. The species was recorded in the Free state, KwaZulu- Natal and former Transvaal (VERDOORN 1973).

1.2.2 *Crinum macowannii* Bak.

Bulb varying in size, *large in some localities,* 6-25 cm diameter, fairly abruptly narrowed into a short to long neck, leaves variable, green or glaucous, spreading at ground level or arcuate from a short stem-like base, deeply canaliculate, from slightly to very strongly undulate, 80 cm long, or longer, 2-16 cm broad, margins with a narrow cartilaginous border and sparsely to fairly densely ciliate with short to about 1 mm cartilaginous hairs. Peduncle arcuate-erect or erect and arcuate at the base only, varying in length, 18-90 cm long, 1, 5-3 cm broad and 0, 6-1, 7cm thick. Spathe-valves 6-10 cm long, 2-4 cm broad just above the base, rather thick, becoming parchment-like but not very thin, eventually partly reflexed, bracteoles narrow linear. Umbels on the average 8-25-flowered. Pedicels varying in length, 1-4 cm long. Perianth with a long or short tube, the segments conniving in a wide trumpet shape with the apical portion recurved; tube 3-11 cm long, cernuous; segments about 7-11 cm long, white with a distinct rose coloured keel, rarely, in some areas, the whole segment rosy with a deep carmine keel; apical peak on outer segments about 5 mm long, on obtuse inner segments, short and broad. Stamens declinate, filaments usually white; anthers black. Style with apical portion red. Fruit subglobose, 3-6 cm diameter beaked with the basal remains of the perianth tube; seeds subglobose to depressed globose, about 2 cm in diameter in greatest width, very pale green or whitish, skin smooth becoming reticulate (VERDOORN 1973).

Crinum macowannii is distributed through the Zambesian and oriental domains of the Sudano-Zambesian region, that is from South Africa along the eastern flooded plains grassland, deciduous woodland within the savanna region and in montane grasslands. Generally it occurs in areas with large seasonal variation in water supply, mostly often on black cotton soils (FANGAN and NORDAL 1993). The species has been recorded in all provinces of South Africa but absent from the south -western Cape (VERDOORN 1973).

1.2.3 *Crinum moorei* Hook f.

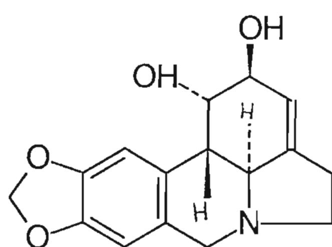
Bulb subglobose, varying in size, up to 19 cm diameter, narrowing abruptly into a long stem-like necks, 30-120 cm high, multiplying prolifically from the base. Leaves from the top of the false stem, ensiform, narrowing towards apex and base, about 65-150 cm long and 6-12 cm broad, parallel veins obvious and the midrib thickened on the lower surface, margins slightly undulate, smooth. Peduncle about 70 cm long or longer, somewhat flattened 1, 5-3 cm broad. Spathe-valves 5-12 cm long 1, 5-3 cm broad at base, narrowing towards apex; bracteoles narrowly linear. Umbel 5-10-flowered. Pedicels 1-8 cm long. Perianth with a long greenish cylindric tube, widening slightly at the apex, 8-10 cm long, erect at first, becoming cernuous as the flower matures; segments white, usually suffused with pink subspreading or forming a rather wide funnel, oblong-elliptic, about 8-10 cm long, 2, 4-4 cm broad, the inner slightly broader than the outer, peaked apex green, 3-6 mm long. Filaments declinate, tinged pink or red in the upper portion. Style red towards the apex. Fruit not beaked (VERDOORN 1973).

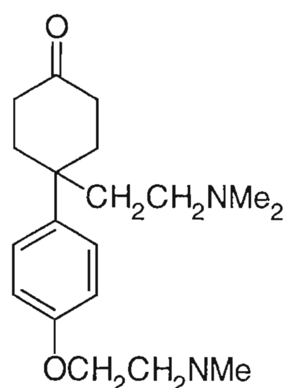
In South Africa, the species is found in the coastal and semi-coastal areas of the eastern Cape northwards as far as Ngome Forest in KwaZulu Natal.

1.3. Amaryllidaceae Alkaloids

1.3.1 Introduction

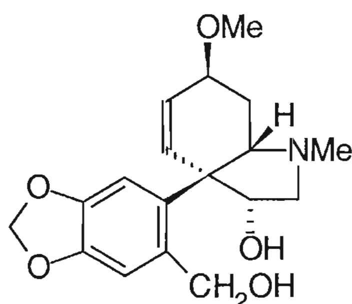
The poisonous and medicinal properties of certain species of the family Amaryllidaceae prompted many investigators in the nineteenth century to seek for active compounds from this family. The investigation of *Narcissus pseudonarcissus* in 1877 led to the isolation of lycorine **1** as the first alkaloid from this family (COOK and LOUDON 1952). Since then, more than 300 alkaloids have been reported of which more than 150 were isolated during the last two decades.

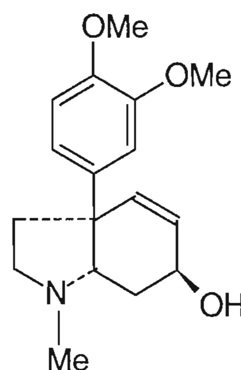




1 **2**

A characteristic feature of the Amaryllidaceae alkaloids is that they are produced exclusively by the members of this family (COOK and LOUDON 1952; GHOSAL *et al.* 1985; VILADOMAT *et al.* 1997). The only exceptions are amisine **2** from *Hymenocalis arenocola*, mesembrenol **3** from *Crinum oliganthum* and egonine **4** from *Hippeastrum equastre.* These three alkaloids belong to the mesembrine-type of alkaloids which were found originally in the family Aizoaceae (PHAM *et al.* 1999).





3 **4**

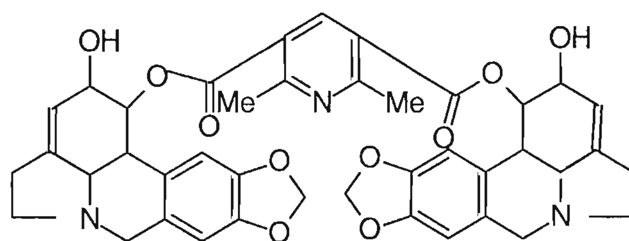
Most of the Amaryllidaceae alkaloids contain a ring system of fifteen carbon atoms which is divided into two parts. The first, containing seven carbon atoms, consists of the aromatic ring (ring A) and the benzylic carbon atom which is attached to either nitrogen or oxygen. The second contains an eight-carbon fragment and is composed of a six membered ring, either hydroaromatic or aliphatic, and a two carbon side chain which is attached to the nitrogen atom. These fragments have been referred to as C-6-C-1 and C-6-C-2 units respectively. The sources of these fragments were traced by radioactive precursors. Phenylalanine serves as a primary precursor of C-6-C-1 fragment while tyrosine is a precursor of ring C and the two- carbon side chain C-6-C-2 (WILDMAN 1968).

The two fragments invariably attach to each other and to the basic nitrogen atom to give the different ring types of the Amaryllidaceae alkaloids listed below:

1. Lycorine-type alkaloids derived from pyrrolo[3,2,1-*de*]phenanthridine/pyrrolo phenanthridone;
2. Lycorenine-type alkaloids derived from [2]benzopyrano[3,4*g*]indole) including both lycorenine and homolycorine;
3. Galanthamine-type alkaloids derived from a dibenzofuran ring;
4. Crinine-type alkaloids derived from 5, 10b-ethanophenanthridine;
5. Montanine type alkaloids derived from 5, 11b-methanomorphanthidine;
6. Cherylline-type alkaloids derived from tetrahydroisoquinoline;
7. Narciclasine-type alkaloids; and

8. Tazettine-type alkaloids derived from the [2]benzopyrano[3,4*c*]indole.

Dimer Amaryllidaceae alkaloids, which consist of two ring systems linked together have also been also isolated. Latindine **5** isolated from *Crinum latifolium,* and clivimine **6** and minitine **7** from *Clivia miniata,* are formed by two identical units linked together, possessing a central 2, 6-dimethyl-3,5-dicarboxylic unit. However, pallidflorine **8** is a heterodimer formed by two directly joined moieties, one of them being the alkaloid lycoramine **9**. The other moiety is the reverse form of the hemiacetal at C-11 of tazettine **10** (CODINA *et al.* 1990). Alkaloids dubiusine **11**, clivatine **12**, leucotamine **13** and O-methylleucotamine **14** have been reported to bear a 3-hydroxy butyryl radical which esterifies the 9-hydroxy group (BASTIDA *et al.* 1988).



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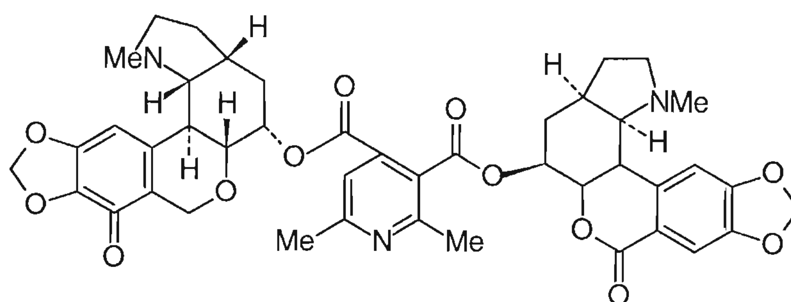

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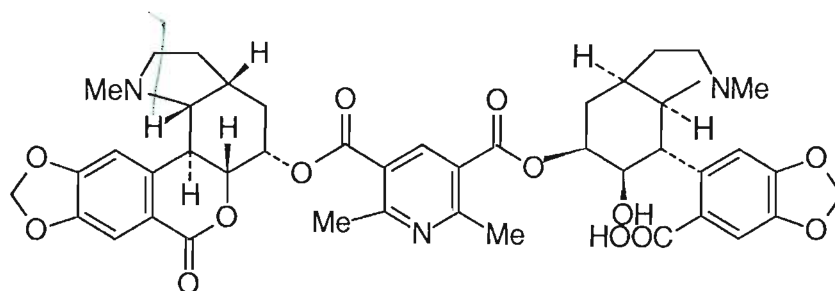

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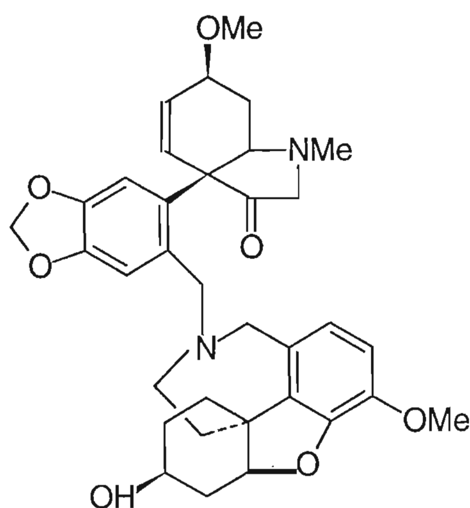

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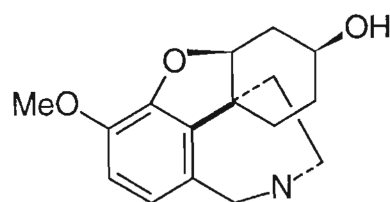

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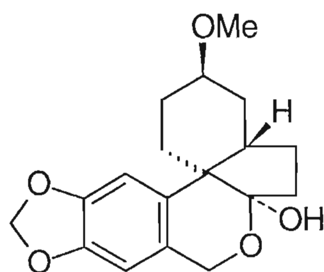

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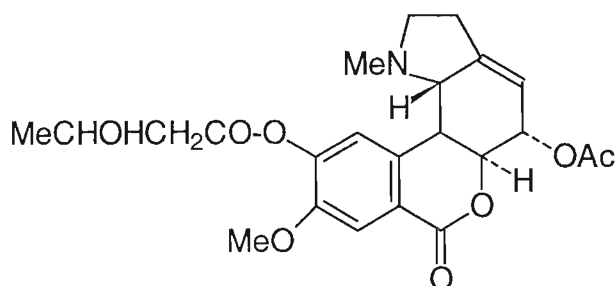

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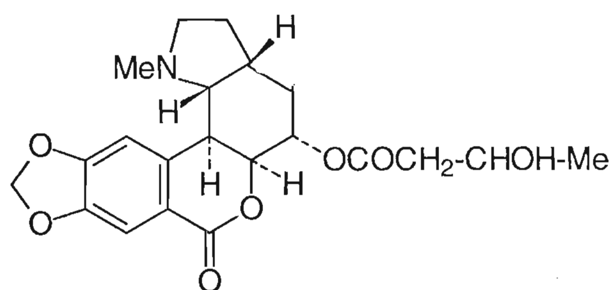

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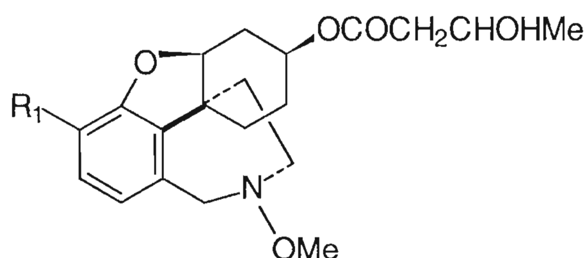
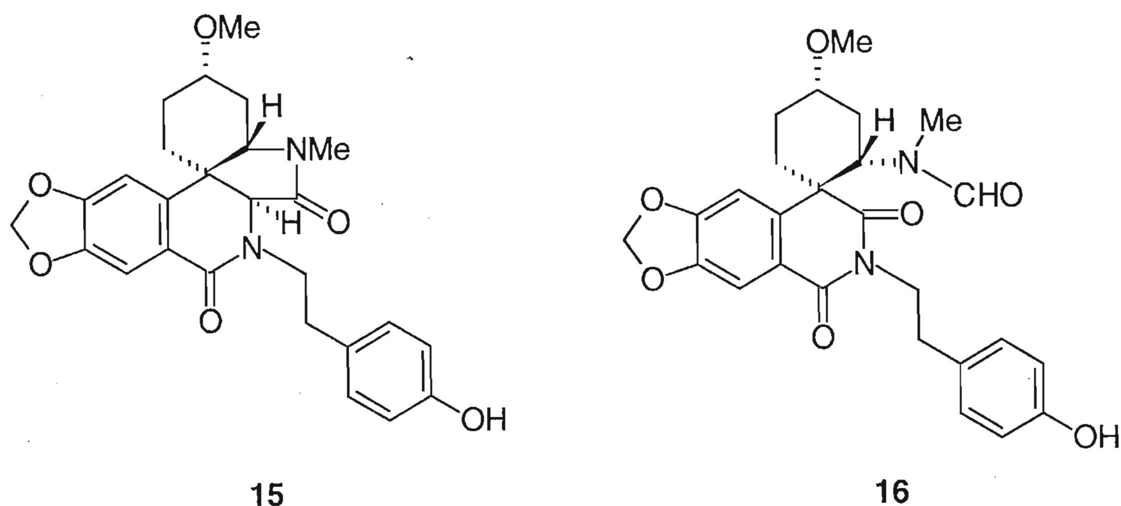


13 R_1 = H

14 R1 = OMe

Amaryllidaceae alkaloids are moderately weak bases. Each alkaloid contains only one nitrogen atom which is either secondary or tertiary (WILDMAN 1960). An exception is the dinitrogenous alkaloids (+)-plicamine **15** and (-)-secoplicamine **16** isolated from *Galanthus plicatus subspecies byzantinus* in which the oxygen atom in position 7 of a tazettine **10** molecule is replaced by a nitrogen atom substituted by a pendant 4-hydroxy phenethyl moiety (ÜNVER *et al.* 1999).



1.3.2 Identification and Structure Elucidation

1.3.2.1 NMR Spectra

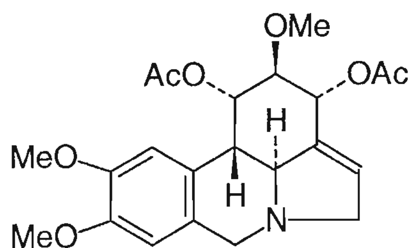
1.3.2.1.1 ^{1}H NMR Spectroscopy

Chemical methods have been used for the establishment of the structures of the major Amaryllidaceae alkaloids. Following the first report of the use of NMR spectra data by HAUGWITZ *el al.* (1965) to support structural and stereochemical data of Amaryllidaceae alkaloids, NMR proved to be a powerful tool in determining the structures and stereochemistry of new alkaloids obtained in small quantities.

Solvents to record NMR spectra of Amaryllidaceae alkaloids include $CDCl_3$, DMSO-d_6, CF_3COOD, pyridine-d_5, D_2O and their mixtures. The best resolution of NMR spectra of the alkaloid lycorine **1**, known for its insolubility in the common organic solvents, unstable in CF_3COOH and double signals in acidic solutions (1% D_2SO_4)-were obtained using CD_3OD-CD_3COOD (3 : 1) (EVIDENTE *et al.* 1983; GHOSAL *et al.* 1985). The NMR spectra of the parent ring types for each ring and the effect of different substituents at these rings will be discussed separately.

1.3.2.1.1.1 Aromatic hydrogen: Ring A

^{1}H NMR signals of the aromatic region appear at δ 6.5-8.3 relative to TMS. Almost in all ring types the lower field signal of the aromatic region is normally allocated to H-10 rather than H-7. This is due to the effect associated with the unsaturation of the 1, 2-double bond, since the 1,2-double bond would be expected to have a greater effect on the proximate 10- than 7-proton (HAUGWITZ *et al.* 1965). Exceptions to this are lycorenine-type alkaloids with a δ-lactone moiety where proton 8 appears normally downfield and proton -11 upfield due to the shielding effect of the peri-carbonyl group on H-8 (LATVALA *et al.* 1995).



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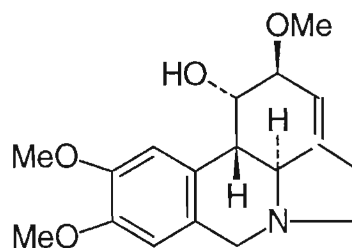
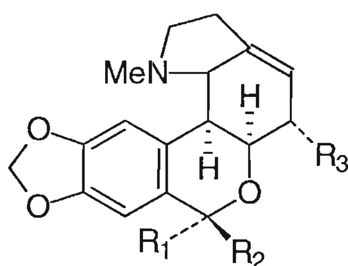
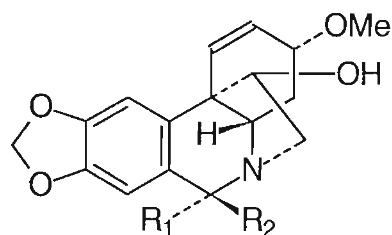


18

1.3.2.1.1. 2 Ring B

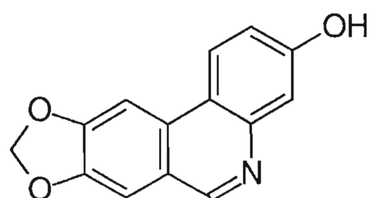
In the case of the unsubstituted benzylic protons, the most deshielded ones appear at δ 4.71 ppm as in diacetylnarcissidine **17** (KIHARA *et al.* 1995). The most shielded appear as upfield as δ 3.4 ppm as in galanthine **18** (BASTIDA *et al.* 1990). Substitution of either protons results in a downfield shift of the other proton depending on the kind and stereochemistry of the substituent. In the homolycorine series hydroxylation at the 7α-position resulted in a downfield shift of the H-7β as in oduline **19** while methylation at the same position resulted in an upfield shift by about δ 0.5 ppm in *O*-methyloduline **20** and 2α-hydroxy-6-*O*-methyloduline **21** (ALMANZA *et al.* 1996; KREH and MATUSCH 1995). The same was observed for 6-hydroxycrinamine. In 6β-hydroxycrinamine **22** the H-6α appeared as a singlet at δ5.01 ppm while H-6β in 6α-hydroxycrinamine **23** observed at a lowerfield (δ 5.59 ppm) (VILADOMAT *et al.* 1996). A further downfield shift of the C-6 proton occurred when the nitrogen was in conjugation with the aromatic ring as in 3-hydroxy-8,9-methylenedioxyphenanthridine **24** (δ 8.68 ppm) (RAZAFIMBELO *et al.* 1996) and δ 9.06 in 8,9-ethylenedioxophenanthridine **25** (SUAU *et al.* 1990). Furthermore, attachment of C-6 to a quaternary nitrogen shifted the benzylic proton to between δ 9.50-10.17, due to the salt effect, as in *N*-methyl-8,9-methylene-dioxy-phenanthridinum chloride **26** (SUAU *et al.* 1990), vasconine **27** (BASTIDA *et al.* 1992, roserine **28** (BASTIDA *et al.* 1992), and tortuosine **29** (BASTIDA *et al.* 1995).

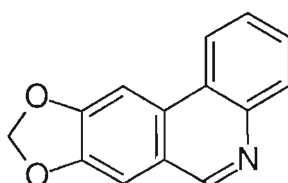


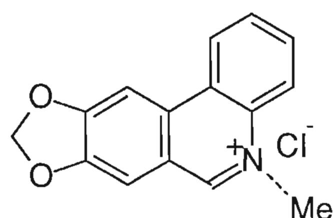


19 R_1 = OH, R_2 = R_3 = H
20 R_1 = OMe, R_2 = R_3 = H
21 R_1 = OMe, R_2 = H, R_3 = OH

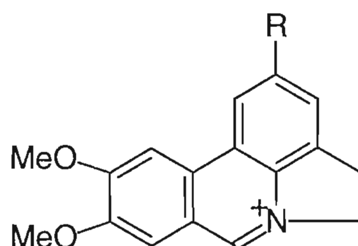
22 R_1 = OH, R_2 = H
23 R_1 = H, R_2 = OH

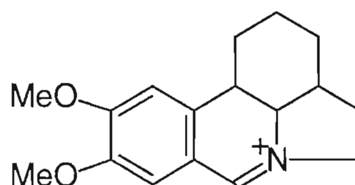






24 **25** **26**





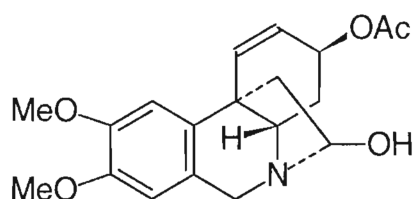
27 R = H

29 R = OH

28

1.3.2.1.1.3 Ring C

Protons attached to saturated C-1and C-2 appear at δ 1.88-2.16, and δ 1.82-1.98 respectively as in delagoenine **30** (NAIR *et al.* 1998) while those attached to C-3 appear at δ 1.48-1.61 as in amabiline **31** (LIKHITWITAYAWUID *et al.* 1993). In most of the crinine-type alkaloids unsaturation of C-1 and C-2 shifted the protons attached to these carbons far downfield to between δ 5.8-6.5 as in crinine **32**, hamayne **33**, and crinamine **34** as anticipated.



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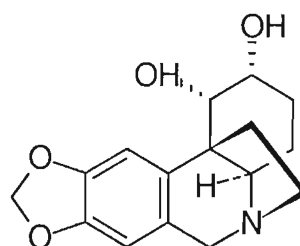

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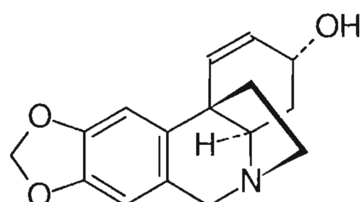

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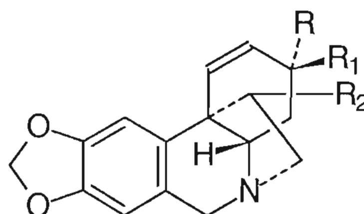
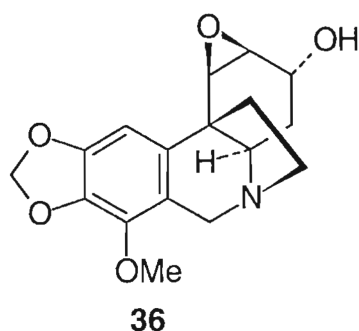


33 R = OH, R_1 = H, R_2 = OH

34 R = OMe, R_1 = H, R_2 = OH

35 R = R_2 = OMe, R_1 = OH

Hydroxylation or methylation of position 3 has a deshielding effect to around δ 4.0-4.5 as in 11-*O*-methylcrinamine **35** and crinamidine **36** (KOBAYASHI *et al.* 1984), while, acetylation at C-3 results in a downfield shift of H-3 as low as δ 5.99. However, hydroxylation of both C-1 and C-2, as observed in amabiline **31**, has a less pronounced effect on the chemical shift of H-1 (δ 3.88) and H-2 (δ 4.32) compared to that on H-3 (LIKHITWITAYAWUID *et al.* 1993). In augustine **83**, an epoxide ring bearing alkaloid, which normally occurs between C-1 and C-2, H-1 and H-2 appear around δ 3.98 and 4.08 (PHAM *et al.* 1998).



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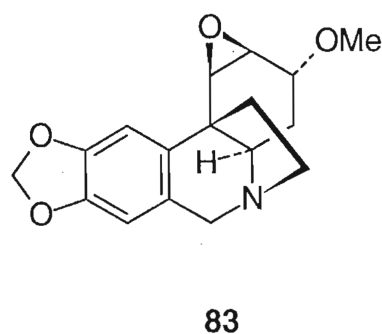
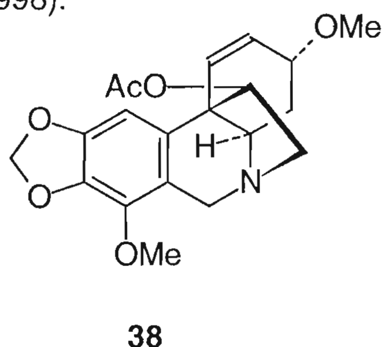
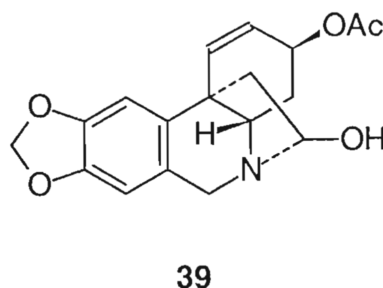


83

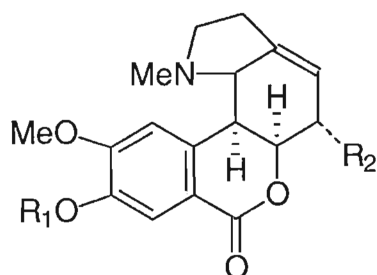
In crinine-type alkaloids, the ethano bridge protons appear at δ 1.94-2.01 and 2.19-2.23 for 11-protons. The protons attched to C-12 appear at 2.91-3.02 for H-12-*endo* and 3.36-3.56 for H-12-*exo*. Hydroxylation at C-11 results in a downfield shift to 4.02 in brunsbelline **46** and 4.39 in ambelline **51**. A further downfield shift of 11-*exo* (δ 5.11) due to acetylation was noticed in 11-*O*-acetylambelline **38** (VILADOMAT *et al.* 1995). Delagoensine **39** and delagoenine **30** are the only crinine-type alkaloids with a hydroxyl group in the C-12 position. In these two compounds H-12-endo was shifted to δ 5.04 and 5.20 respectively (NAIR *et al.* 1998).

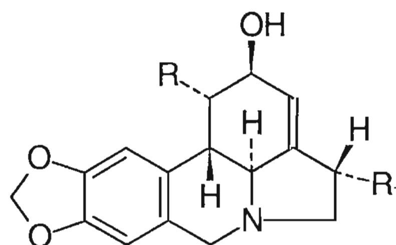




38 **39**

In homolycorine-types alkaloids the presence of two proton multiplets at δ 2.259, in 9-*O*-demethylhomolycorine **40** is an indication of lack of substituent at C-5. This normally shifts to a singlet around δ 3.93 in the case of substitution as in (+)-5-methoxy-9-*O*-demethylhomolycorine **41** (LATVALA et al. 1995).





40 $R_1 = R_2 = H$
41 $R_1 = H$, $R_2 = OMe$

42 R = OAc, $R_1 = OMe$
43 R = OAc, $R_1 = H$

The downfield shift of H-4 to δ 3.73 in lutessine **42** was used to differentiate it from 1-*O*-acetyllycorine **43** in which the signals appear normally as a two proton multiplet at δ 2.59 (EVIDENTE 1986).

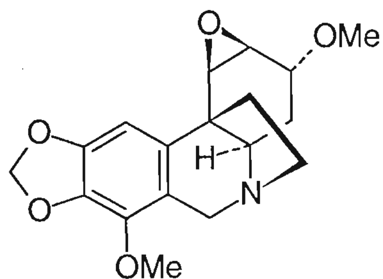
1.3.2.1.2 ^{13}C NMR Spectroscopy:

In line with ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy has been used in the establishment of the carbon skeleton of Amaryllidaceae alkaloids. Both proton noise decoupled (PND) and single frequency-off resonance decoupled (SFORD) were used in the establishment of multiplicities of the carbon signals. In addition, lanthanide shift reagents and empirical calculations have been used. The ^{13}C NMR spectra of Amaryllidaceae alkaloids were divided into two well defined regions: the lower field region (>δ 90 ppm) containing the carbonyl group signals, the unsaturated carbon signals (both aromatic and olefinic) and the methylenedioxy carbon signals. All the remaining saturated carbon resonances were located in the high field region (CRAIN *et al.* 1971; ZETTA *et al.* 1973).

1.3.2.1.2.1 Substituent effects

Substituents (OH, OMe, OAc) on the aromatic rings are of considerable importance in locating the positions of the functional groups (GHOSAL *et al.* 1985). Substitution of the methylenedioxy at both C-8 and C-9 results in a downfield shift of about 2 ppm as in delagensine **39** and delagoenine **30** (NAIR *et al.* 1998). Meanwhile, the presence of a methoxy group at C-7 produces a pronounced deshielding effect on C-7 by δ 35 ppm and a shielding effect on C-8 (δ 9 ppm), C-6a (δ 8 ppm) and C-10 (δ 2-5 ppm) in undulatine **44**, buphandrine **45**, brunsbelline **46** compared to their corresponding alkaloids, in the same series, lacking this substitution (VILADOMAT *et al.* 1995).

In the aliphatic region substitutions at C-3 with a methoxyl or an *O*-acetyl group exerts a downfield effect of at least δ 3 ppm more in 3-*O*-acetylhamayne **47**, and crinamine **34**, compared to that of a hydroxyl group at the same position in crinine



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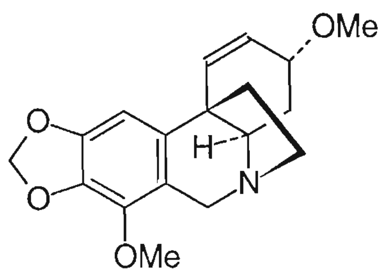

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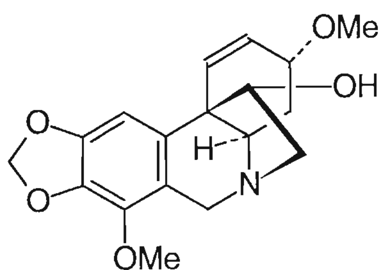

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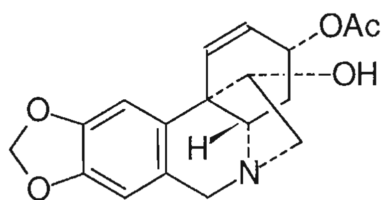

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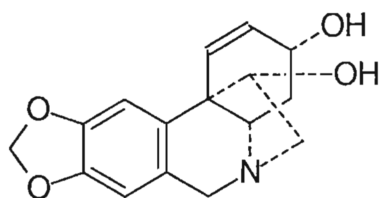

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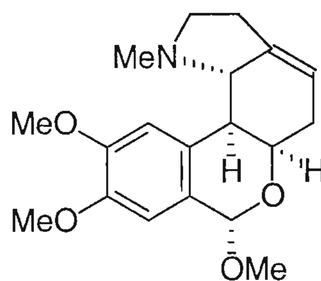

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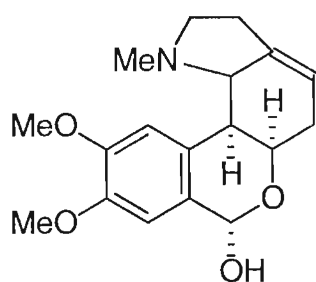

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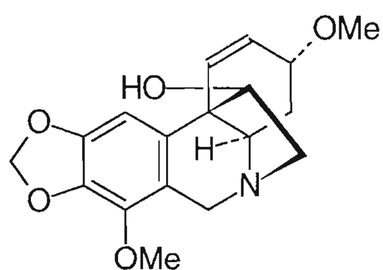


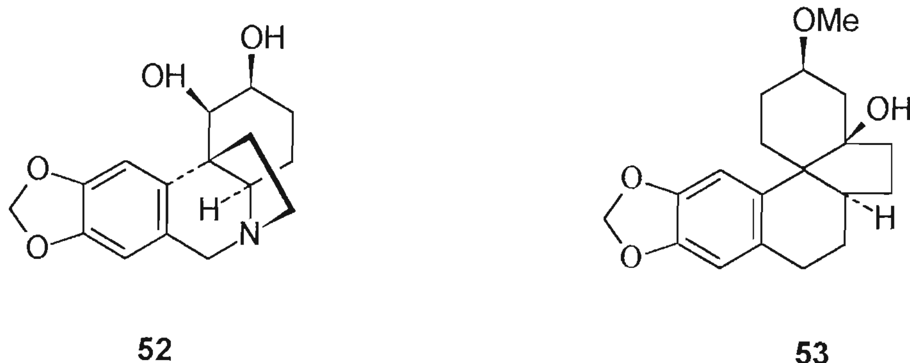
51

32, hamayne **33**, and bulbispermine **48**. The same effect was observed for lycorenine- type alkaloids, where in *O*-methyllycorenine **49** and 2α-hydroxy-6-*O*-methyloduline **21** C-6 appears downfield by δ 6-8 ppm which corresponds to the change from hydroxylation as in lycorenine **50** and oduline **19** to methoxylation. (ALMANZA *et al.* 1996; CODINA *et al.* 1993; KREH and MATSUCH 1995).

It appears that not only the kind of substituent has an effect on the differences in

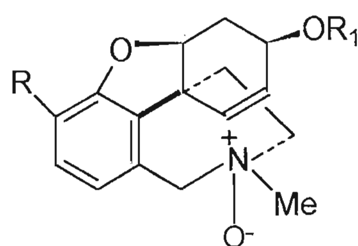
the ^{13}C signal chemical shifts. The stereochemistry of the same substituents may exert different chemical shifts of the same carbon at the same position. An example is the stereochemistry of a substituent at C-11 in crinine-type alkaloids. In 6-hydroxycrinamine (**22**, **23**), crinamine **34** and brunsbelline **46** the hydroxyl group at C-11 is in an *exo* position whereas in ambelline **51**, 11-OH is in an endo position. C-11 appears at δ 80 ppm in the first and around δ 88 ppm in the second (VILADOMAT *et al* 1995; VILADOMAT *et al.* 1996). The same effect was observed in 6α- and 6β-hydroxycrinamine (**22**, **23**) where in the first case C-6 appears δ 3 ppm upfield compared to that in 6β-hydroxycrinamine (VILADOMAT *et al.* 1996).

Changes in carbon chemical shift as a result of substituent was applied to differentiate between compounds differing in that substituent. As an example the presence of an hydroxyl group at C-4a in crinambine **37** shifted it to δ 96.8 ppm compared to that of 4a-dehydocrinambine **52** where it occurs at δ 69.7 ppm (PHAM *et al.* 1998). Change of hydroxylation in tazettine **10** from C-11 to C-4a to give littoraline **53** resulted in a downfield shift of C-4a by about δ 23 ppm and an upfield shift of C-11 by δ 27 ppm (CODINA *el al.* 1990; LIN *et al.* 1995).

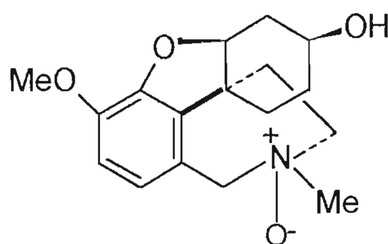


In the homolycorine series the deshielded chemical shift position of C-10b was used to determine hydroxylation at C-2 . It appears at about δ 4.5 ppm downfield in the hydroxylated compared to that of dehydroxylated ones (BASTIDA *et al.* 1992). Similarly, attachment of an oxygen atom to the N-atom as in galanthamine-*N*-oxide **54**, sanguinine-*N*-oxide **55** and lycoramine-*N*-oxide **56** shifted the carbons attached to N-atom (C-7, C-9, NMe) downfield by about δ10-15 ppm from the corresponding signals in galanthamine **81**, sanguinine **57**, and lycoramine **9** (KOBAYASHI *et al.* 1991). It was also noticed that 1,2-saturation in a crinine-type alkaloids with an oxiran ring resulted in a highfield shift of the C-4 signal (24.9 ppm) with respect to the corresponding signal in the 1,2-unsaturated alkaloids which is present at a lower field between δ 28-32.5 ppm (VILADOMAT *et al.* 1994). The most striking effect is the shift of C-6 from around δ 63 to 142-151 ppm as a result of its

attachment to a quaternary nitrogen with a double bond due to the minimum salt effect as in vasconine **27**, 8-*O*-demethylvasconine **58**, 3-hydroxy-8,9-methylenedioxyphenanthridine **24** (BASTIDA *et al.* 1995; RAZAFIMBELO *et al.* 1996).



54 R = OMe, R_1 = OH
55 R = OH, R_1 = OMe



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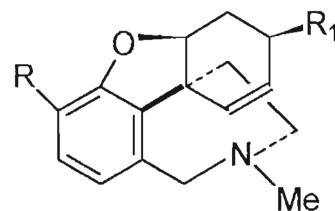
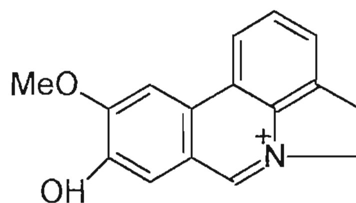


57 R = H = R_1 = H
81 R = OMe, R_1 = H



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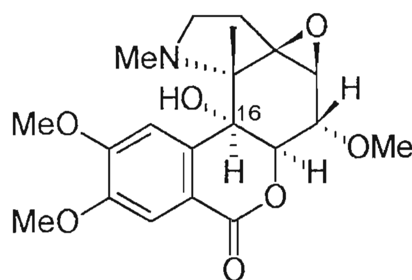

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1.3.2.1.3 Two dimensional NMR spectra

Both ^{1}H-^{1}H two dimensional spectra such as correlated spectroscopy (COSY), total correlated spectroscopy (TOCSY),rotating-frame Overhauser enhancement spectroscopy (ROSEY), nuclear Overhauser effect spectroscopy (NOESY) and ^{1}H-^{1}C NMR spectra including heteronuclear correlated spectroscopy (HETCOR), heteronuclear multiple-quantum spectroscopy (HMQC), and heteronuclear multiple bond coherence (HMBC) were used for the confirmation of the assignment and stereochemistry of different protons and carbons and their substituents.

1.3.2.1.3.1 NOESY Spectroscopy

Nuclear Overhauser effect spectroscopy indicates the spatial relationship of protons. It can be used in the deduction of the exact location of the aromatic substituents. The assignment of the methoxy group to C-9 in delagoenine **30** was deduced from its interrelation with H-10 and H-1eq while, the assignment Of the 8-OMe was supported by an NOE with H-7 (ÜNVER *et al.* 1999). The technique was also used to determine the stereochemistry of the hydroxyl group at C-1 in 4a-dehydroxycrinambine **52**. It shows the NOE correlation of the methine proton H-1

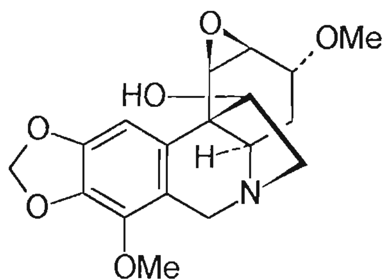
with H-2 and H-10 as well as with H-3α and with H-4a in the axial α-position. Accordingly, H-1 possesses the α-position and the OH group at C-1 must have a β-equatorial position (PHAM *et. al.*1998). NOESY allowed the assignment of the methoxy group at C-6 in 2α-hydroxy-6-*O*-methylodulline **21** (ALMANZA *et al.* 1996) as well as the aliphatic methoxy group to C-5 in (+)-16-hydroxygalwesine **59** (LATVALA *et al.* 1995).

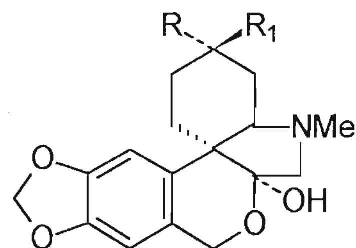
1.3.2.1.3.2 ROESY Spectroscopy

ROESY is used principally to afford information about the relative spatial distances of protons. It is used in the assignment of the aromatic singlet in delagoensine to H-10 due to the spatial proximity between H-10 and H-1eq. (NAIR *et al.* 1998). The allocation of the methoxy group to C-2 in tortuosine **29** was deduced from the spatial relation between H-1, H-10 and H-1 and the methoxy group protons (BASTIDA *et al.* 1995). In 1,2-β-epoxyambelline **60**, ROESY correlation observed in the ROESY spectrum of H-6α to H-4a and H-6β to H-12-*endo* allowed the assignment of both C-6 protons (MACHOCHO *et al.* 1999).

1.3.2.1.3.3 HMBC and HMQC

Both techniques show carbon-proton coupling as cross peaks between atoms which are separated by more than one bond. They are used to verify the allocation of protons and substituents specially in the aromatic region. HMBC allowed the assignment of the aromatic singlet at δ 6.73 ppm to H-10 in delagoenine **30** because of the three bond correlation with C-6a as well as with C-10b and C-8 (NAIR *et al.* 1998). The same was observed in buphandrine **45** which allow the assignment of the methoxy group to C-7. This assignment was further confirmed by the HMBC correlation between H-6 protons and C-7 (VILADOMAT *et al.* 1995). In tortuosine **29** the assignment of C-2, C-9 and C-8 was made because of their three bond correlation with their corresponding methoxy protons in the HMBC spectrum (BASTIDA *et al.* 1995).



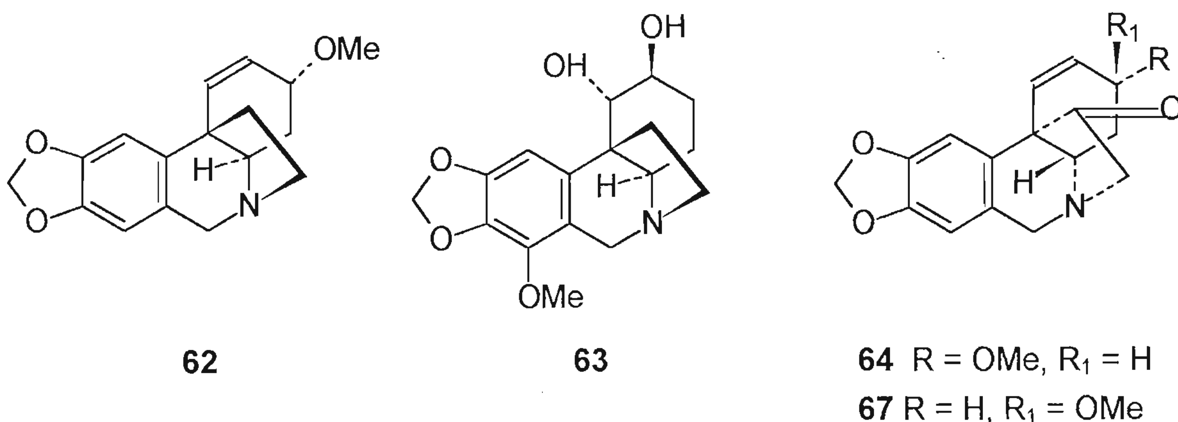


60

10 R = H, R1 = OMe
61 R = OMe, R1 = H

1.3.2.2 Mass spectrometry

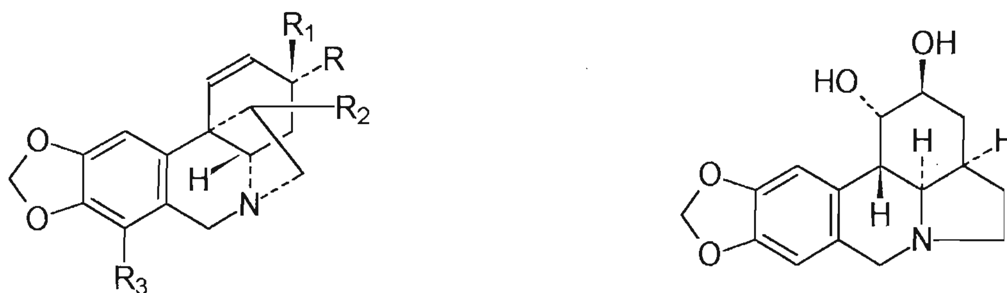
It was observed that minor changes in stereochemistry of Amaryllidaceae alkaloids are frequently sufficient to cause appreciable differences in the mass spectra of many of the stereoisomers (DUFFIELD *et al.* 1965). The differences between tazettine **10** and criwelline **61** as a result of the change in configuration of the methoxy group is sufficient to cause marked variations in relative abundance of the ions in the spectra of these alkaloids. The dominant ion in the spectra of tazettine occurs at mass 247 while the most abundant peak of criwelline occurs at mass 71 (DUFFIELD *et al.* 1965).



62 **63**

64 R = OMe, R_1 = H
67 R = H, R_1 = OMe

In crinine-type alkaloids with 5,10b-ethano bridge in a β-position such as ambelline **51**, buphanisine **62**, crinine **32** and deacetylbowdenisine **63** the base peak corresponds to the molecular ion. However, the stereochemistry at C-3 appears to govern the fragmentation pattern of alkaloids with the ethano bridge in an α-position. The spectra of 6-hydroxycrinamine (**22**, **23**), crinamine **34** and 11-oxocrinamine **64** (C-3 substitution at α-position) showed strikingly low abundance of the molecular ion and facile loss of methanol compared to their stereoisomers haemanthidine **65**, haemanthamine **66** and 11-oxohaemanthamine **67** with the substituents at C-3 in a β-position (DUFFIELD *et al.* 1965; LONGEVIALLE *et al.* 1973).

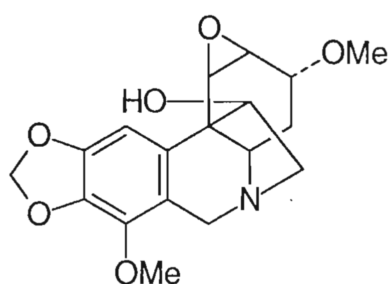


65 R = H, R_1 = OMe, R_2 = R_3 = OH
66 R = R_3 = H, R_1 = OMe, R_2 = OH

68

The spectra of lycorine-type alkaloids are not greatly affected by small structural changes and application to structure determination is exceptionally straightforward. All lycorine-type alkaloids containing 3, 3a-unsaturation gave very intense ions at masses 226 and 227 which is attributed to the loss of carbon atoms C1 and C2 and their substituents. In agreement with this, dihydrolycorine **68** and other derivatives lacking the 3, 3a-unsaturation do not lose the C1-C2 bridge (KINSTLE *et al.* 1966).

It was also noticed that in the crinine-type series containing C1-C2 unsaturation the substituent at C-11, mostly hydroxyl, governs almost entirely the fragmentation pattern of these alkaloids due to the ability of the hydroxyl group to bring about rearrangement (LONGEVIALLE *et al.* 1973). However, in alkaloids with a 1,2-epoxide group as in cavinine **69** the fragmentation pattern is governed by the presence of the epoxide ring rather than the hydroxyl group on the bridge (SAMUEL 1975).



69

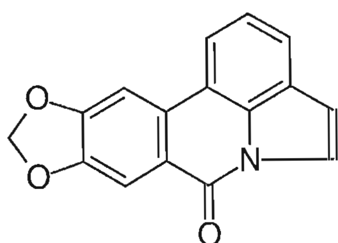
Field desorption (FD) mass spectrometry has given a single peak for lycorine and crinamine 34, corresponding to the molecular ion. It proved to be a useful tool for the study of nonvolatile compounds that undergo little fragmentation (ONYIRIUKA and JACKSON 1978).

Derivatisation using trimethylsilylation was used for GC-MS to study the composition of the crude extracts of *Crinum orantum*, *C. natans* (ONYIRIUKA and JACKSON 1978) and *Narcissus pseudonarcissus* (KREH *et al.* 1995). The study showed that trimethylsilylated alkaloids did not give more intense peaks than underivatized alkaloids and reliable identification was only possible for substances for which reference materials are available (KREH *et al.* 1995).

1.3.2.3 Chemical transformation

The structures of many Amaryllidaceae alkaloids were confirmed by conversion to previously known alkaloids. For instance, the structure of hippadine **72** was proved

by two chemical transformations. Firstly, reduction of hippadine by $LiAlH_4$ in ether-tetrahydrofuran afforded anhydrolycorine **73**. Secondly, dehydrogenation of an hydrolycorin-7-one **74**, on the other hand, by DDQ in anhydrous benzene under reflux afforded hippadine (GHOSAL *et al.*, 1981). However, opening of the methylenedioxy ring of hippadine into OH-OMe groups by heating with NaOMe in DMSO afforded pratorinine **75** (GHOSAL *et al.* 1981).



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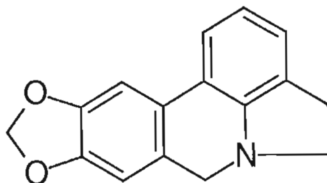

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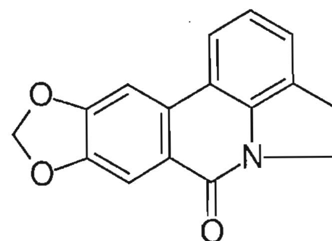
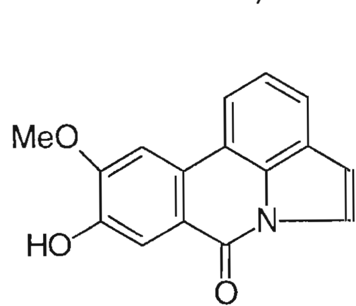


74

The epimeric alkaloids haemanthidine **65** and 6-hydroxycrinamine (**22**, **23**) are known to undergo rearrangement to give the corresponding C-3 epimers tazettine **10** and criwelline **61** , respectively, upon treatment with methyl iodide in acetone followed by dilute base (MURPHY and WILDMAN 1964). If methylation of 6-hydroxycrinamine is carried out with methyl iodide in refluxing methanol, followed by a base, O-methylcriwelline **76** is the major product. On oxidation with chromium trioxide in acetic acid, O-methyl criwelline give macronine **77** (MURPHY and WILDMAN 1964).



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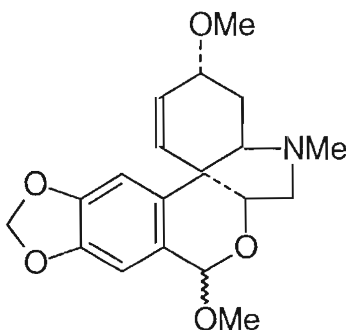

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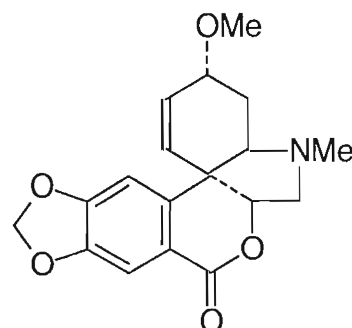

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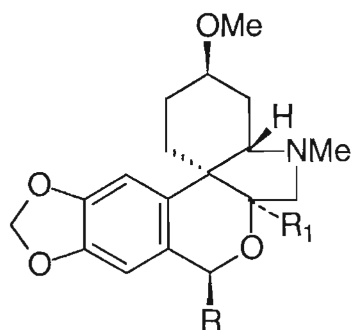
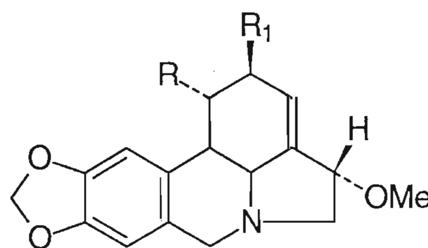
Tazettine **10** is known to arise as an artefact during isolation process due to the rearrangement of pretazettine **71** under basic conditions. Pretazettine is unstable as free base in solutions and gradually rearrange to tazettine upon standing. It can be converted readily into tazettine by chromatography on basic alumina or treatment with 0.1N sodium hydroxide at 25°C for 1 hour (WILDMAN and BAILY 1968).





10 R = H, R_1 = OH
71 R = OH, R_1 = H

78 R = R_1 = OAc
79 R = R_1 = OH

Chemical proof of the structure of 1,2-β-epoxyambelline **60** was provided by its conversion to ambelline **51**. On reduction with zinc-acetic acid, in the presence of sodium iodide, 1,2-β-epoxyambelline afforded ambelline. Heating with triethyl phosphate also afforded ambelline (GHOSAL *et al.*1984).

Acetylation with pyridine and acetic anhydride was used to confirm the identity of many acetylated alkaloids. For instance, treatment of lutessine **42** with pyridine and acetic anhydride gave diacetyllutessine **78** which on reduction with ethanolic KOH yielded the deacetyl derivative of lutessine **79** (EVIDENTE 1986).

1.3.3 Biological Activities

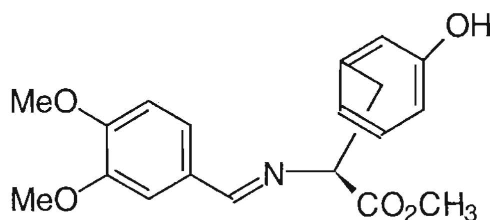
The biological activity associated with Amaryllidaceae alkaloids covers analgesic, central nervous system, antitumour, and antiviral effects (LEWIS 1990). Other effects have also been reported e.g. cardiovascular activity, immuno-stimulatory activity, antibacterial activity, antifertility activity, emetic and diaphoretic activity (CHATTOPADHYAH *et al.* 1983; GHOSAL *et al.* 1985; SPOEROKE and SMOLINSKE 1990; HARBORNE and BAXTER 1993).

1.3.3.1 Antiviral activity

A number of Amaryllidaceae alkaloids have shown antiviral activity. Littoraline **53** isolated from *Hymenocalis littoralis* showed inhibitory activity of HIV-1 reverse transcriptase (LIN *et al.* 1995). Lycorine **1** showed activity against Poliomyelitis at a concentration as low as 1 μg ml^{-1}, however, concentrations exceeding 25 μg ml^{-1} were found to be toxic. It also showed activity against Coxsackie, semliki forest, measles and herpes simplex viruses (IEVEN *et al.* 1982). On the other hand, pretazettine **71** inhibits avian myeloblastosis virus reverse transcriptase and showed activity against the Rauscher leukemia virus in mouse embryo cells (ANTOUN *et al.* 1993).

1.3.3.2 Central nervous system

Galanthamine **81** has shown cholinesterase inhibitory activity and is currently undergoing clinical trials for treatment of Alzheimer's disease (BASTOS *et al.* 1996). The alkaloid galanthamine showed anticholinesterase activity and an ability to amplify the nerve muscle transfer, which is greater than that of epigalanthamine **80** (GHOSAL *et al.* 1985). Galanthine **18** has been used in Russia in the treatment of myasthenia gravis, myopathy and diseases of the nervous system. Narwedine **82** increases the amplitude and frequency of respiratory movement (HARBORNE and BAXTER 1993)



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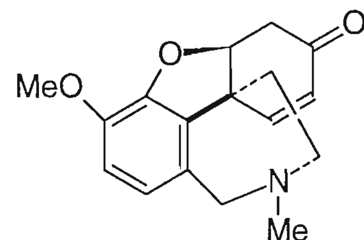

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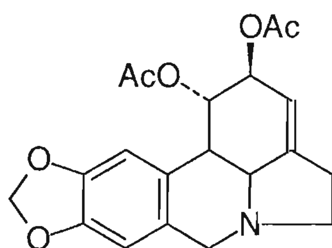
1.3.3.3 Antimalarial activity

Augustine **83** showed a significant antimalarial activity while lycorine and 1, 2-di-*O*-acetyllycorine **84**, and crinamine **34** showed a moderate activity against both chloroquine-sensitive and chloroquine -resistant strains of *Plasmodium falciparum.* The activity of augustine was attributed to the presence of the epoxide function which can result in the formation of adducts with nucleophiles in biological systems leading to non-selective toxicity. However, the activity shown by compounds lacking the oxiran ring, like crinamine, proved that other elements of the structure may play a role in activity and that epoxide functionality is not essential for activity (CAMPBELL *et al.* 1998; LIKHITWITAYAWUID *et al.* 1993). The compounds showed very low selectivity indices compared to chloroquine, quinine, and artemisinin.



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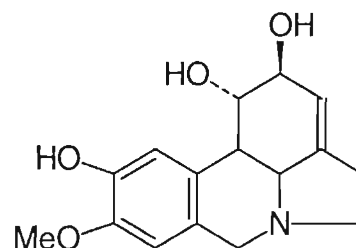

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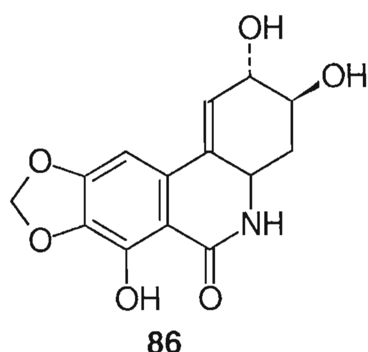
1.3.3.4 Anti-cancer activity

Lycorine **1** inhibited protein synthesis in eukaryotic cells by preventing peptide bond formation. Similar biological activities were reported for dihydrolycorine **68,** pseudolycorine **85** , narciclasine **86,** haemanthamine **66** and pretazettine **71** (GHOSAL *et al.* 1985; HARBORNE and BAXTER 1993). Pancratistatin **87** proved to be effective against the murine P-388 lymphocytic leukemia (38-106% life extension at 0.75-12.5 mg kg^{-1} dose levels) and markedly inhibited growth of the P-388 *in vitro* cell lines (ED50, 0.01 µg ml^{-1}) and *in vivo* murine M-5076 ovary sarcoma (53-84% life extension at 0.38-3.0 mg kg^{-1}) (PETTIT *et al.* 1986). Pancratistatin is now undergoing preclinical testing as an anti-cancer drug.



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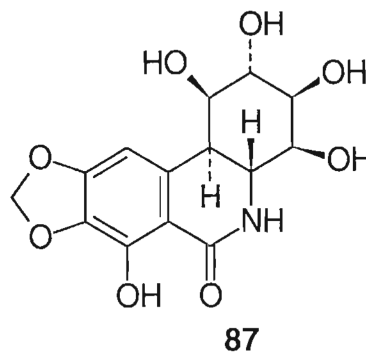

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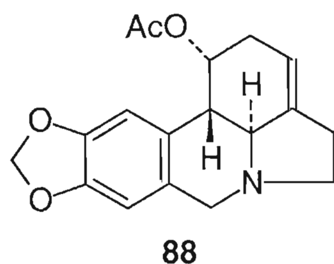
Lycorine, haemanthamine and augustine showed significant cytotoxic effects against human breast cancer, human fibro sarcoma, human lung cancer, human melanoma, human colon cancer, murine lymphoid neoplasm, human epidermoid carcinoma, hormone dependent human prostatic cancer, hormone dependent breast cancer, human glioblastoma cell lines. In addition to the above cell lines, lycorine was also active against human oral epidermoid carcinoma, and vinblastine-resistant human epidermoid carcinoma (LIKHITWITAYAWUID *et al.* 1993; LIN *et al.* 1995).

Haemanthidine **65** and hippeastrine **70** are significantly active against prostate and sarcoma cell lines. In addition, haemanthidine demonstrated activity against breast cancer cell lines, epidermoid carcinoma, nasopharyngeal carcinoma, lung and melanoma tumor cell lines. In the same study tazettine **10** showed activity against the colon cancer cell line while pretazettine **71** inhibited Hella cell growth as well as protein synthesis in eukaryotic cells (ANTOUN *et al.* 1993). Moreover, the alkaloids acetylcaranine **88,** ambelline **51,** and anhydrolycoronium **89** was found to have antineoplastc activity (PETTIT *et al.* 1984) while the alkaloids kalbretorine **90,** telastaside **91,** ambelline, 6-hydroxycrinamine **(22, 23)** and 1,2-*O*-di-acetyllycorine

84 showed some tumor inhibitory activity (GHOSAL *et al.* 1985 and 1990; NAIR *et al.* 1998).



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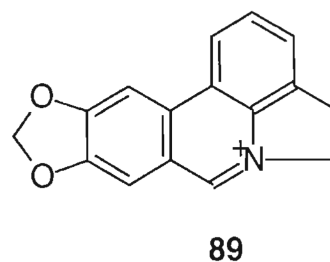

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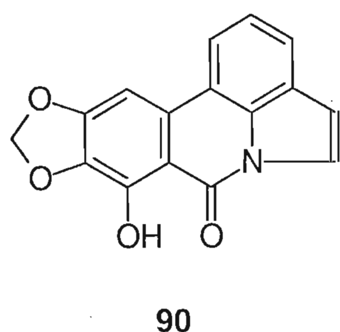

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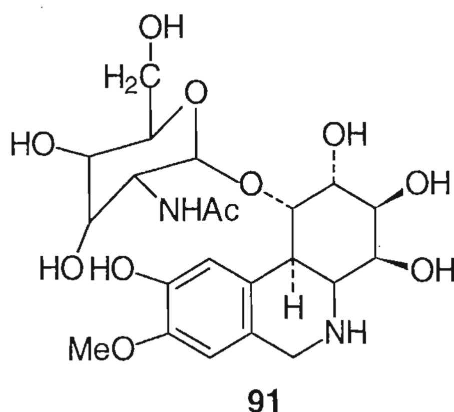
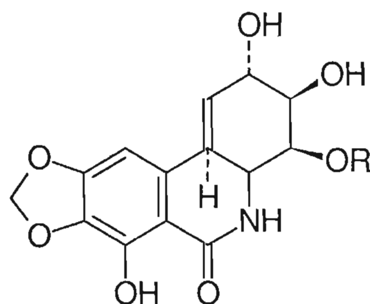


91

Narciclasine **86** and narciclasine-4-*O*-β-D-glucopyranoide **92** showed activity (LD50, 0.29 and 0.88 respectively) when tested against *Artemia salina*. In addition, both alkaloids showed anti-tumor activity indicated from its inhibition of crown gall tumor initiation when assayed on potato discs infected by *Agrobacterium fumefaciens* (ABU-DONIA *et al.* 1991).

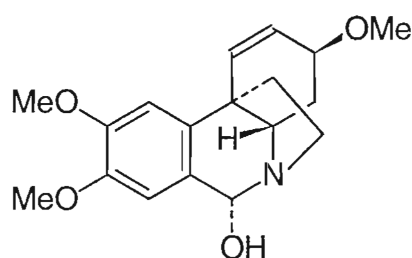


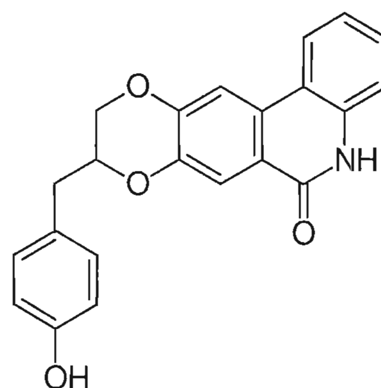
92 R = β-glucopyranoide

Significant differences in activity related to the alkaloid ring type was observed when 25 alkaloids belonging to 5 ring types including lycorine, homolycorine, lycorenine, crinine and tazettine-type alkaloids were evaluated for their cytotoxic activity against the murine non-tumorial cell line (LMTK) and two human tumorial cell lines (Molt 4 and Hep G2). The toxic effect of the tested alkaloids was more pronounced on the Molt 4 tumour cells and the LMTK cells than on the hepatoma

Hep G2. Almost all of the tested compounds showed cytotoxic activity against fibroblast LMTK cells. Pretazettine **71** was amongst the most active compounds on the Molt 4 lymphoid cells, but was inactive against Hep G2. In contrast, lycorenine **50** was the most cytotoxic compound against Hep G2 hepatoma but, inactive against Molt 4 cells (CAMPBELL *et al.* 1998).

In the same study, it was observed that stereochemistry was an important factor in the cytotoxic activity of alkaloids of the crinine-type. Crinine-type alkaloids with the ethano bridge in an α-position such as crinamine **34**, haemanthamine **66**, and papyramine **93** were specially active on Molt 4 cells and LMTK cells, whereas those having the ethano bridge in β-position like buphandrine **45** and ambelline **51** were inactive on all three cell lines tested (CAMPBELL *et al.* 1998).





93 **94**

1.3.3.5 Anti-bacterial activity

Both crinasiatine **94** and 8,9-methylenedioxy-6-phenanthridone **95** exhibited bacteriostatic activity (GHOSAL *et al.* 1985). Crinamine is the principal antibacterial constituents of the bulbs of *Crinum jagus* (VILADOMAT *et al.* 1996).

1.3.3.6 Anti-fertility activity

Hippadine **72** has proved to be a useful agent in fertility control. When applied to albino rats (3mg rat^{-1} day^{-1}), it resulted in the depletion of the DNA content of testes, loss of tissue weight of testes, activity of steroidogenic cell activity and an increase in the weight of the ventral prostate (GHOSAL *et al.* 1981).

1.3.3.7 Analgesic effect

The analgesic activity exhibited by the Amaryllidaceae alkaloids is attributed to their

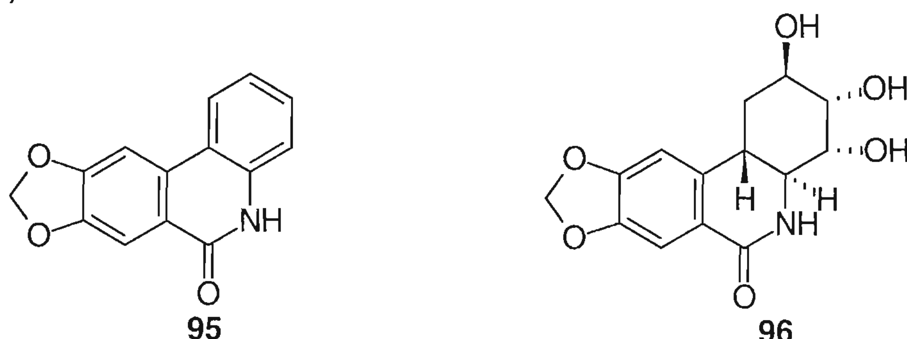
resemblance to morphine and codeine skeletons. The alkaloids belonging to the pyrrolophenanthridine, lycorenine, and pretazettine groups are less toxic than the dibenzofuran and ethano phenanthridine group. Narwedine **82** potentiated the pharmacological effects of caffeine, carbazole, arecoline and to a lesser extent of nicotine, in laboratory animals (GHOSAL *et al.* 1985). Galanthine **18** was reported to exhibit analgesic activity comparable to morphine (CORDELL 1981).

1.3.3.8 Emetic and diaphoretic activity

There are a few case reports of poisoning in the medical literature. However, the raw bulbs may cause nausea, persistent vomiting and diarrhoea when ingested. The low number of human poisonings reported is due to the small concentrations of the alkaloids in the plant (SPOEROKE and SMOLINSKE 1990).

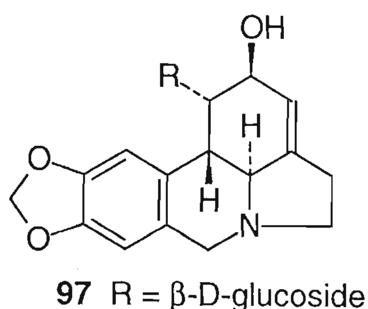
1.3.3.9 Insecticidal effect

Narciclasine **86,** hippeastrine **70,** galanthamine **81,** lycoramine **9** are potent anti-feedants against the larvae of the yellow butterfly (HARBORNE and BAXTER 1993).



1.3.3.10 Growth regulation

Lycorine **1** and lycoricidine **96** possess plant growth inhibitory properties (CORDELL 1981). A solution of lycoriside applied to bulbs of onion and other species stimulated the formation and linear growth of primary roots. Palmilycorine has only showed a slight growth promoting effect. Moreover, lycorine, lycorine 1-*O*-β-D glucoside **97**, lycoriside and palmilycorine inhibited leaf emergence (GHOSAL *et al.* 1985).



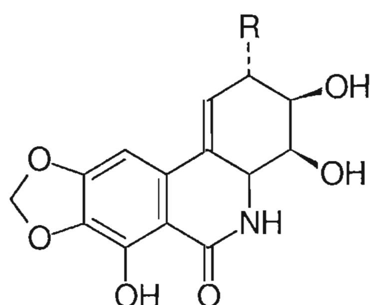
97 R = β-D-glucoside

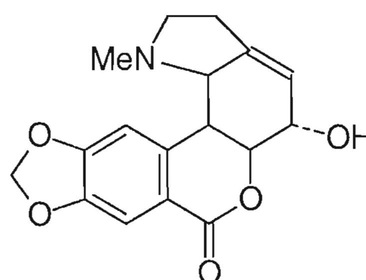
1.3.3.11 Cardiovascular activity

Narwedine **82** increases the amplitude and decreases the frequency of cardiac contractions. It shows hypotensive activity. Crinamine **34** is a powerfull hypotensive agent in dogs. Homolycorine **99** is also known to induce delayed hyper sensitivity in animals (GHOSAL *et al.* 1985).

1.3.3.12 Immuno-stimulatory activity

Lycorine -1-*O*-glucoside **97** produced varying degrees of mitogenic activation of splenic lymphocytes of mice. Lycorine -1-*O*-glucoside further showed a very low order of acute toxicity in albino rats. Lycorine-1-*O*-glucoside thus has potential as an immuno-stimulatory agent (GHOSAL *et al.* 1985). 1,2-β-epoxy ambelline **60** and kalbreclasine **98** produced a moderate activation of mouse spleen lymphocytes which was comparable to the activity of the known mitogen concanavalin A (GHOSAL *et al.* 1985).





98 R = O-β-glucose

99

1.4 Variations in alkaloids

It is from plants containing alkaloids that a large number of drugs are derived. The group, however, is a very varied one and it is only the chemical properties of a basic nitrogen that unify the many classes of alkaloids. A precise definition of the term alkaloid (alkali-like) is somewhat difficult because there is no clear-cut boundary between alkaloids and naturally occurring complex amines. A typical alkaloid is derived from plant source, basic, contain one or more nitrogen atoms, usually in a heterocyclic ring and have marked physiological actions in man or other animals (CORDELL 1981; ROBINSON 1981).

Alkaloids have been called nitrogenous waste products analogous to urea and uric acid in animals. On the other hand, the marked physiological effects of alkaloids when administered to animals led to speculation on their biological role in plants.

The debate that they protect the producing plant against physical and biotic environmental factors is gaining increasing support. It was postulated that plants accumulate protective secondary compounds in a tissue at risk of predation (CORDELL 1981; FRISCHKNECHT *et al.* 1986). In support of this, it was found that the purine alkaloids caffeine and theobromine have toxic effects on insects and fungi at concentrations found in the plant. The yield of these alkaloids is low when the leaf bud is protected by a resin layer and stipules. As the leaf emerges, alkaloid formation increases and reaches its maximum when the leaf is fully open (FRISCHKENECHT *et al.* 1986). The same was observed for conjugated alkaloids in the fruits of *Crinum asiaticum* during stress. Wounding of *C. asiaticum* fruits caused complete hydrolysis of the alkaloidal conjugates. Treatment of fruits with anaesthetic agents protected the alkaloidal conjugates from hydrolysis and oxidation. The qualitative and quantitative changes of the alkaloids in response to stress suggests their role in the protective mechanism of the producer plant (GHOSAL *et al.* 1990)

The formation of alkaloids varies notably from tissue to tissue within the same plant and also changes during the course of ontogeny. Investigation of the ontogenic variations of Amaryllidaceae alkaloids have shown marked fluctuations in respect of lycorine and its 1-*O*-glucoside. The concentration of lycorine 1-*O*-glucoside increased only during pre-to-post flowering time in a number of Amaryllidaceae species including *Crinum asiaticum*, *C. augustum*, *C. latifolium* and *C. rosea*. The same phenomenon was shown for pyrrolophenanthridine for *Crinum* species and for kalbretorine from *Haemanthus kalbreyeri* (GHOSAL *et al.* 1985).

A rapid gain and subsequent loss of the glucosyloxy alkaloids and their aglycones was observed in the flower stems of several Amaryllidaceae plants. A gradual increase in the concentration of the glyocosyloxy alkaloids and sugars was observed during the first three days of flowering whereafter there was a sharp fall in their concentration as the flowers started wilting (GHOSAL *et al.* 1985).

Ontogenic variation in alkaloids is not unique to the Amaryllidaceae. It has also been observed for other classes of alkaloids. In *Datura metal var. fastuosa* the percentage alkaloidal content of leaves reached a maximum during the period of vigorous leaf growth up to flowering bud formation. Afterwards a steady decline, during the post-flowering stages, which was at its lowst when fruit are ripening, was observed (GUPTA *et al.* 1973). Similar results were obtained for caffeine from the tea shrub *Camelia sinensis* (CLOUGHLEY 1982). In contrast the reproductive

stage of development was accompanied by the presence of the highest alkaloids in many species, including *Datura innoxia* (NANDI and CHATTERJEE 1975) and *Hyoscyamus muticus* (TYAGI *et al.* 1984). During the same developmental stage, different plant organs produce different levels of alkaloids. In *Datura innoxia* total alkaloids were highest in leaf tissues followed by the root and lowest in the stems. However, in *Catharanthus roseus,* the highest concentration was found in the root with minimal amounts in the stem (REDA 1978). Differences in the distribution of alkaloids vary between various parts of a specific plant organ. An ascending gradient in the thebaine content was found from the upper to the lower parts of the roots of *Papaver bracteatum* (LEVY *et al.* 1988). MORAES-CERDEIRA *et al.* (1996) studied the distribution of Amaryllidaceae alkaloids in bulb parts of *Narcissus cv. Ice Follies*. The study showed the highest concentrations of galanthamine, lycoramine and *N*-demethyl-lycoramine were present in the basal plates. Galanthamine was highest in the inner portion of the bulb. Lycorine had a distribution pattern similar to galanthamine.

Alkaloid composition was found to be influenced by genetic factors and developmental stage, rather than by environment. (GUPTA *et al.* 1973). The most important alkaloid-containing families are the Liliaceae, Amaryllidaceae, Compositae, Ranunculaceae, Loganiaceae Menispermaceae, Lauraceae, Papaveraceae, Leguminosae, Rutaceae, Solanaceae, Apocynaceae, and Rubiaceae (CORDELL 1981). Alkaloid chemistry has been used for the biochemical classification of plants belonging to these families for more than six decades. At first these data were applied at the tribal level and above (IZADDOOST 1975). Later, its application in chemotaxonomic classification extended to more advanced levels of the alkaloid-containing families. At the sub-tribal level, caffeine, chlorogenic acid and other 276 nm-absorbing compounds obtained from species within the tribe Coffeeae (Rubiaceae), were used to differentiate between different species. Within the tribe there is a continuous spectrum ranging from typical commercial coffees which produce seeds having large contents of caffeine and chlorogenic acid, through species with seeds containing little or no chlorogenic acid and caffeine but significant amounts of other 276 nm-absorbing components, to species with seeds lacking even these substances (CLIFFORD *et al.* 1989).

At the generic level, the presence of matrine alkaloids were used as markers to classify 25 species within the genus *Sophora* (Leguminosae) into four subgenera. Species containing only the cytisine series appear to represent the most primitive

members of the genus due to the universal occurrence of cytisine within the tribe Sophoreae. Those species containing both cytisine and matrine alkaloids form a second subgenus. The third group is distinguished only by the presence of the matrine alkaloids and a fourth group is characterized by the absence of all these alkaloids (IZADDOOST 1975).

The distribution of indole alkaloids in the leaves, stems and roots of ten African mainland *Rauvolfia* species allowed the classification of these species into two groups. Those with predominately normal(C-3Hα, C-20Hβ) configuration heteroyohimbines and related compounds. The second group comprises those with predominately allo(C-3Hα, C-20Hα) configuration heteroyohimbines and related compounds (COURT 1983).

The sole chemotaxonomic criterion that differentiates the genus *Ochrosia* from the closely related *Neisosperma* is the presence of the antitumor alkaloid ellipticine, or its derivatives in the former and their absence in the latter (AMARASEKERA and ARAMBEWELA 1986; SÉVENET 1991). Even the ratio of abundance in different plant organs have been helpful tools in chemotaxonomy. Example is the discovery of a large sanguinarine content in the roots of *Argemone subfusiformis* taxa, compared to the aerial parts. Such a variation does not occur in *A. polyanthemos* which is characterized by the presence of *N*-norchelerythrine (BANDONI *et al.* 1975).

Studies on variations in alkaloid content have been extended to infraspecific levels. The analysis of clonally propagated breeding lines of *Belladonna* for the alkaloid hyoscyamine revealed that the individual genotypes exhibited the greatest range of variability (DHAR and BHAT 1982). A significant difference in galanthamine content among four *Narcissus* cultivars was also found. The cultivars Ice Follice and Mount Hood had a high content of galanthamine compared to that of the cultivars Geranium and white cheerf (MORAES-CERDEIRA *et al.* 1997).

1.5 Aims of This Study

It becomes apparent from the above that ontogenic variation, variation among different plant organs and interspecific variation in alkaloids have been extensively studied. However, little work has been done to investigate variation in Amaryllidaceae alkaloids. So the aim of this study was to screen three *Crinum* species, namely *Crinum bulbispermum*, *Crinum macowanii* and *Crinum moorei* with

the following specific objectives:

1. To investigate the alkaloid content of each of the three species. This included isolation and characterization of any new alkaloids;
2. To study the variation in alkaloid content among different plant organs in each species;
3. To study the seasonal variation of these alkaloids; and
4. To study the interspecific variation in alkaloid content of the *Crinum* species in question.

CHAPTER 2
Materials and Methods

General Apparatus

An IKA Labortechnik (Janke & Kunkel) grinder was used for grinding plant material. Optical rotations were recorded on Perkin-Elmer 241 Polarimeter. Mass spectra were run on a Hewlett-Packard gas chromatographic mass spectrometer (HP 5988A). NMR spectra were recorded with a Varian Gemini 200 as well as a Varian 500 instrument with tetramethylsilane as internal standard. Melting points were recorded on Electrothermal IA 9300 Digital Melting Point Apparatus. Vacuum liquid chromatography columns were packed with silica gel (Merck 60, 230-400 mesh). Separations were monitored by TLC using strips of pre-coated plates (Merck 60, F_{254}). Ultraviolet light 254 and Dragendorff's reagent were used for spot detection. Compounds were scraped-off TLC plates and placed into a Pasteur pipette pre-packed with a celite filter cell at the bottom to avoid silica passage. Redistilled methanol was used for the recovery of purified compounds and as a solvent for GC analysis.

This chapter covers the methods used for the extraction and isolation of alkaloids from each *Crinum* species as well as the methods used for quantifying alkaloids to study organ-to-organ, seasonal, and interspecific variations.

2.1. Extraction and isolation of alkaloids

2.1.1 Plant material

Plants of *Crinum bulbispermum* (Brum.f.) Milne Redh., *Crinum moorei* HOOK f. and *Crinum macowanii* Bak. were obtained in December 1997 from Green Goblin Nursery, Durban, South Africa. Voucher specimens (Elgorashi 1 NU, Elgorashi 2 NU, Elgorashi 3 NU) were deposited in the University of Natal Herbarium, Pietermaritzburg.

2.1.2 Alkaloid Extraction and isolation

The plant material was sliced and dried in an oven at 55°C until constant dry weight was obtained and powdered using a grinder. The dried and powdered non-flowering whole plants (198.5 g for *C. bulbispermum*, 62.5 g for *C moorei* and 865 g for *C. macowanii*) were extracted ,according to the method of GHOSAL *et al.* (1983), using a Soxhlet apparatus for 40 h with:

1. Petroleum ether (60-80°); and
2. 95% ethanol.

The ethanolic extracts from each species were evaporated under reduced pressure and the residue was treated with 100 ml of 4% aqueous acetic acid. The aqueous acidic solution was filtered . The solution was basified with NH_4OH to pH 9.5 after removal of neutral material with diethyl ether (100 ml x 4) . The basified solution was extracted with diethyl ether, ethyl acetate, and n-butanol respectively (100 ml x 4 each) to give fractions A, B and C.

2.1.3 Purification of alkaloids

2.1.3.1 *Crinum bulbispermum*

Fraction A (532 mg) was subjected to VLC (8.5 x 3 cm) on 20 g of silica gel eluted with chloroform (100%) and then with chloroform enriched gradually with 5% methanol up to 50% methanol. Fractions eluted with 95% (Fraction 1) and 90% (Fraction 2) chloroform showed positive responses to Dragendorff's reagent. Further purification steps were carried out to purify alkaloids from these two fractions using preparative TLC.

Fraction 1 (204 mg) was developed on preparative TLC plates (2 mm) using chloroform: methanol (9:1) to give 6 fractions each of which was developed using chloroform: diethyl amine (20:1) to give compound **100** (10 mg), compound **101** (11 mg), compound **102** (14 mg), crinamine **34** (15 mg) and 3-*O*-acetyl hamayne **47**(28 mg).

Fraction 2 (99 mg) was developed on TLC plates (0.2 mm) using chloroform: methanol (4:1) followed by acetone : methanol (4:1) to give 6-hydroxycrinamine

(17 mg).

Fraction B (676 mg) was dissolved in 5 ml MeOH and kept at room temperature overnight. A powder (63 mg) was precipitated and the crude extract was subjected to VLC (20 g, 8.5 x 3 cm) using 100% chloroform and then chloroform enriched gradually with 2.5% methanol up to 50%. Fractions eluted with 92.5% (Fraction I), 87.5% (Fraction II) and 85% (Fraction III) chloroform responded positively to Dragendorff's reagent and were subjected to further purification.

Fraction I (187 mg) was developed on TLC plates (0.2 mm) using acetone : methanol (3:1) to give more crinamine. Fractions II (62 mg) and III (75 mg) were combined and subjected to TLC plates (0.2 mm) using chloroform : methanol (3:1) and then chloroform : methanol: diethyl amine (15:3:2) to give bulbispermine (70 mg).

2.1.3.2 Crinum moorei

Fractions A and B were combined and kept overnight in methanol at room temperature to precipitate lycorine as a powder (81 mg). The remaining crude extract was subjected to VLC on silica gel and eluted with chloroform and then with chloroform enriched gradually with methanol up to 50%. This yielded five fractions.

Fraction I (291 mg) was developed on a preparative TLC (2 mm) using chloroform : methanol (9:1) to give three bands . Band 1 was developed again using chloroform: diethyl amine (20:1) to give epibuphanisine (56 mg) and 1-*O*-acetyllycorine (32 mg). Band 2 was developed on TLC using chloroform: diethyl amine (40:1) to give undulatine (11 mg). Band 3 was developed on TLC using benzene : methanol (9:1) to give 3-*O*-acetylcrinine (8 mg).

Fraction II was developed on TLC plates (0.2 mm) with chloroform: diethyl amine (20:1) to give two bands. Band 1 was developed further on chloroform: diethyl amine (40:1) twice, to give epivittatine (53 mg) and cherylline (35 mg). Band 2

was developed on chloroform: methanol (10:1) to give crinamidine (7 mg).

Fraction III was developed on a preparative TLC plates (2.0 mm) using chloroform: diethyl amine (20:1) to give crinine (36 mg). Fraction IV was developed on a preparative TLC plates (2.0 mm) using chloroform: diethyl amine (20:1) to give powelline (20 mg). Fraction V was developed on a preparative TLC plates (2.0 mm) using chloroform: diethyl amine (20:1) to give 1-epideacetylbowdensine (16 mg).

Fraction C was subjected to VLC using silica gel and eluted with chloroform enriched gradually with methanol to give two fractions. Fraction I was developed on TLC plates (0.2 mm) using chloroform : methanol (2:1) to give mooreine **104** (7 mg). Fraction II was developed on a preparative TLC (2 mm) using chloroform: dichloromethane : ethanol: methanol (7:7:7:4) and ammonia vapour to give 3-[4'-(2'-aminoethyl)phenoxy]bulbispermine **103** (11 mg).

2.1.3.3 Crinum macowanii

Fractions A and B were combined and dissolved in 5 ml of methanol and kept at room temperature to precipitate lycorine as a powder (351 mg). The remaining crude extract was developed on a VLC packed with silica gel using chloroform and then chloroform enriched with 5% methanol and then with 2.5% methanol at a time. Fractions eluted with 90% (Fraction1), 85% (Fraction 2) and 82.5% (Fraction 3) chloroform responded positively to Dragendorff's reagent.

Fraction 1 was developed on a preparative TLC plates (2 mm) to give two bands. The first was developed on TLC using dichloromethane: methanol: ammonia (16:1:1) to give crinine (9 mg) and bulbispermine (7 mg). The second band was developed on TLC using chloroform: methanol (4:1) to give cherylline (12 mg).

Fractions eluted with 85% and 82.5% chloroform were combined and developed on a preparative TLC using chloroform: methanol (6:1) to give an impure fraction of 34 mg which further developed using chloroform: diethylamine (20:1) to give

hamayne (5 mg)

Fraction C was developed using VLC eluted with chloroform and then with chloroform enriched gradually with 5% methanol up to 50% methanol. Fractions eluted with 80% (Fraction 1) and 70% (Fraction 2) chloroform responded positively to Dragendorff's reagent.

VLC was used for further purification of Fraction 1 using chloroform and then chloroform mixed with 5% methanol up to 90% chloroform. The column was then eluted with chloroform enriched with 1% methanol up to 80% chloroform. The alkaloid fraction was purified on TLC developed with chloroform :methanol (15:2). A few drops of acetic acid were added to the solvent system to avoid band streaking, to yield tyramine 13 mg .

The second fraction was separated using VLC with chloroform mixed with methanol in a gradient of 5% to give 174 mg of alkaloid fraction. The fraction was run twice on TLC using chloroform: methanol (7:3) and then chloroform : methanol (2:1) to yield 4 mg of pure alkaloid. The alkaloid gave weak ^{1}H NMR signals and all attempts to obtain more information failed.

2.2 Quantification of alkaloids for the study of variations

2.2.1 Plant material

Plant material of the three *Crinum* species were obtained from Green Goblin Nursery in Durban, Good Will Farm, Howick and Pietermaritzburg. The collected plants were potted using a mixture of sand and compost (1:1) in the green house of the Botanical Garden of the University of Natal. Irrigation, weeding and insecticide practices were applied whenever necessary.

2.2.2 Sample collection

Sampling of plant material was carried out three times a year as follows:

1. Winter: The dormancy period (end of June);
2. Spring: The flowering period (late October for *C. bulbispermum*, early

November for *C. macowanii* and mid December for *C. moorei*); and

3. Summer: Early February.

The study was conducted during the years 1998/1999 and 1999/2000. In Winter plants were separated into bulbs and roots whereas in Spring they were separated into bulbs, leaves, roots and flowering stalks. In Summer plants were separated into bulbs, roots and leaves. The plants were washed thoroughly and dried at 55°C until constant dry weight was obtained. The dried plant material was ground to homogenous powders and kept at room temperature until further analysis.

2.2.3 Extraction and isolation of alkaloids

The method developed by BASTOS *et al.* (1996) for extraction and isolation of Amaryllidaceae alkaloids was followed with some modifications.

The dried powdered bulb material (500 mg) was mixed with 6 ml 0.05 N HCl solution. The mixture was placed on a shaker at 150 rpm and 40°C for 2.5 h. The mixture was then centrifuged at 3600 rpm for 5 min. Three ml of the supernatant was transferred to a centrifuge tube and basified with 1 ml 0.3 N NaOH. The mixture was mixed well with 4 ml $CHCl_3$ and centrifuged at 4500 rpm for 5 min. The $CHCl_3$ layer was removed and filtered through a Pasteur pipette containing Na_2SO_4 (1 cm from the bottom of the tube). The $CHCl_3$ extract was evaporated and the extract was dissolved in 400 μl methanol for *C. moorei* and 100 μl methanol for *C. macowanii* and *C. bulbispermum*.

The same procedure was used for extraction of alkaloids from leaves, roots and flowering stalks using 250 mg of powdered plant material. The final extract residue was dissolved in 200 μl methanol for *C. moorei* and 50 μl methanol for *C. bulbispermum* and *C. macowanii*.

2.2.4 GC-conditions

A Varian 3300 gas chromatograph equipped with FID and NPD detectors and a

DB-5 capillary column (30 m x 0.32 mm i.d. x 0.25 mm film thickness, J &W Scientific, CA) was used. The carrier gas was nitrogen at a head pressure of 40 KPa. Oven temperature was programmed at 220°C for 1.5 min. and increased at a rate of 3°C min^{-1}. up to 270°C. The program was left at 270°C for 5 min. Injector and detector temperatures were 270°C and 300°C respectively. Injected volume was 1 µl with split ratio of 1:15. The output was recorded using a Hewlett Packard HP integrator.

2.2.5 Linearity of response

The linearity of response of the reference compounds (Appendix 3) was plotted using a concentration range between 0.2-1.0 mg ml^{-1} of methanolic solution of, cherylline, undulatine, crinamidine, epibuphanisine, crinamine, crinine, powelline, 1-*O*-acetyllycorine and 1-epideacetylbowdensine. A concentration range between 0.2-1.2 mg ml^{-1} of methanolic solution was used for lycorine. A range between 0.2 -2.0 mg ml^{-1} for 3-*O*-acetylhamayne. A volume of 1 µl of each concentration was injected into the GC. The linear relation for each reference compound was obtained by plotting the area percent recorded for each concentration with the corresponding concentration. A methanol blank was used as a control concentration. The method was sensitive to the extent that a concentration as low as 10 µg ml^{-1} of crinine and epibuphanisine could be detected.

2.2.6 Recovery of alkaloids

Hyoscyamine was used as internal standard at a concentration of 100 µg ml^{-1} in 0.05 N HCl and extracted with the plant material the same way as in (2.2.3). Hyoscyamine thermally decomposed upon injection into the GC. Crinine was used as alternative for the evaluation of the extract recovery.

Crinine was dissolved in 0.05 N HCl at a rate of 100 µg ml^{-1} and extracted the same way as in (2.2.3). The recovered crinine was dissolved in 250 µl of methanol. One µl of the solution was injected into the GC. The area percent of the extracted crinine was extrapolated with the linear response curve of crinine for

the determination of the amount of recovered crinine.

2.2.7 Data processing

The GC results were recorded as an area percent of individual peaks to the total area of the resulting peaks of the chromatogram. As two injections were made for each sample, the mean area percent of each sample was converted to an area percent ml^{-1} of solvent. This was done by dividing the area percent of samples from *C. moorei* by 2.5, while those of *C. bulbispermum* and *C. macowanii* by 10. The resulting value was then converted to $\mu g\ ml^{-1}$ by substitution in the regression equation plotted using concentrations (2.2.5) for each alkaloid in question. Statistical analysis was carried out using Residual maximum likelihood (REML) with Genstat 5 Release (4.1) [Lawes Agricultural Trust (Rothamsted Experimental Station, 1998)]. Data were logarithmically transformed to ensure normality of distribution. Wald statistics was used to test the hypothesis of equality of all organs, seasons and species means. When significant, the least significant difference test was applied to rank the different means. The experiment was conducted using five plants from each species at each season.

CHAPTER 3

Identification and Structure Elucidation of *Crinum* Alkaloids

Introduction

The species under investigation are used by people of southern Africa to treat various ailments. *Crinum bulbispermum* is used by Zulu, Sotho, and Tswana people to treat rheumatism, aching joints, septic sores, varicose veins, and kidney and bladder infections. *C. macowanii* and *C. moorei* are used by Zulu healers against swelling of the body, for urinary tract problems and itchy rashes. In Zimbabwe, bulb infusions of *C. macowanii* are administered as emetics and to stimulate milk production in women and cows (HUTCHINGS et al. 1996; ROBERTS 1990).

Previous Chemical investigation of *C. bulbispermum* has revealed the presence of the alkaloids bowdensine, deacetylbowdensine, buphanamine, cherylline, crinamidine, crinamine, crinine, galanthamine, hamayne, hippadine, krepowine, lycorine, 1, 2-di-*O*-acetyllycorine, powelline, pratromine, pratorinine and vittatine (ALI *et al.* 1984; KOBAYASHI *et al.* 1984; VILADOMAT *et al.* 1997). The alkaloids acetylcaranine, cherylline, crinamidine, crinine, galanthamine, lycorine, 1-*O*-acetyllycorine, powelline, 9-*O*-methylpseudolycorine, tazettine and undulatine were isolated *from* *C. moorei* (VILADOMAT *et al.* 1997). Galanthamine has been detected using radioimmunoassy for the quantitative determination of galanthamine from *C. macowanii* (TANAHASHI *et al.* 1990).

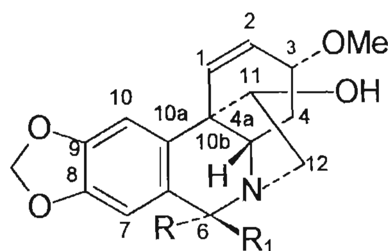
3.1 Alkaloids from *C. bulbispermum*

3.1.1 Results

Fractionation of crude ethanolic extracts of *Crinum bulbispermum* using VLC followed by preparative TLC yielded seven compounds. The known alkaloids bulbispermine **48** crinamine **34**, 6-hydroxycrinamine **(22, 23)** and 3-*O*-cetylhamayne **47** were isolated in the present study. In addition the new

alkaloids 8α-ethoxyprecriwelline**100**, *N*-desmethyl-8-αethoxypretazettine **101** and *N*-desmethyl-8β-ethoxypretazettine **102** were isolated for the first time from a natural source. The spectroscopic and physical data used for the identification of these compounds such as ^{1}H and ^{13}C NMR spectroscopy (Appendix 1), GC-MS , optical rotation and melting points are presented below for each alkaloid.

3.1.1.1 6-Hydroxycrinamine 22 , 23



22 R = OH, R_1 = H

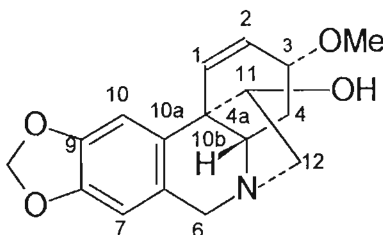
23 R = H, R_1 = OH

Amorphous compound (17 mg), $[\alpha]^{28}_{D}$ = +37.1°($CHCl_3$, c, 0.07), GC-MS 70 eV, m/z (% rel.int.): 313 [M^+, (<1], 285 (2), 284 (10), 269 (18), 268 (100), 255 (10), 209 (13). ^{1}H NMR ($CDCl_3$) α-6 -OH: 6.82 (1H, s, H-7), 6.76 (1H, s, H-10), 6.26 (1H, d, J = 10.5, H-1), 6.25 (1H, dd, J = 1.5, 10.5, H-2), 5.92 (2H, d, Distorted AB, OCH_2O), 5.04 (1H, s, H-6), 4.04 (1H, m, H-3), 3.90 (1H, m, H-11), 3.76 (1H, m, H-4a), 3.39 (3H, s, 3-OMe), 3.35 (1H, m, H-12*endo*), 3.30 (1H, m, H-12*exo*), 2.04-2.25 (2H, m, H-4). ^{13}C NMR ($CDCl_3$): 147.8 (s, C-9), 146.5 (s, C-8), 136.4 (d, C-1), 135.7 (s, C-10a), 127.2 (s, C-6a), 122.8 (d, C-2), 109.5 (d, C-7), 102.8 (d, C-10), 101.1 (t, OCH_2O), 88.0 (d, C-6), 78.1 (d, C-11), 75.9 (d, C-3), 59.5 (d, C-4a), 57.6 (t, C12), 56.0 (q, OMe), 50.4 (s, C-10b), 29.4 (t, C-4).

β -6-OH: ^{1}H NMR ($CDCl_3$): 6.97 (1H, s, H-7), 6.74 (1H, s, H-10), 6.26 (1H, d, J = 10.5, H-1), 6.25 (1H, dd, J = 1.5, 10.5, H-2), 5.90 (2H, d, distorted AB, OCH_2O), 5.62 (1H, s, H-6), 4.20 (1H, m, H-12*endo*), 3.95 (1H, m, H-3), 3.85 (1H, m, H-11), 3.41 (1H, m, H-4a), 3.39 (3H, s, 3-OMe), 3.01 (1H, m, H-12*exo*), 2.04-2.25 (2H, m, H-4). ^{13}C NMR ($CDCl_3$): 147.5 (s, C-9), 146.7 (s, C-8), 136.3 (d, C-1),

134.5 (s, C-10a), 128.6 (s, C-6a), 123.0 (d, C-2), 108.3 (d, C-7), 102.7 (d, C-10), 101.1 (t, OCH_2O), 85.5 (d, C-6), 78.9 (d, C-11), 75.6 (d, C-3), 64.8 (d, C-4a), 57.6 (t, C12), 55.9 (q, OMe), 50.8 (s, C-10b), 29.4 (t, C-4).

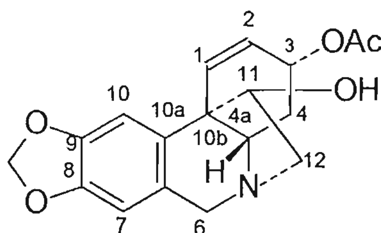
3.1.1.2 Crinamine 34



34

Colourless needles (15 mg), mp 192-194, $[\alpha]^{28}{}_{D}$ = +96.67° (CH_3OH, c, 0.15). GC-MS 70 eV, m/z (% rel.int.): 301 [M^+, (3.3)], 272 (100), 269 (12), 240 (12), 211 (20), 181 (29), 153 (10), 128 (11), 115 (12), 77 (8). ^1H NMR ($CDCl_3$): 6.79 (1H, s, H-10), 6.47 (1H, s, H-7), 6.24 (2H, s, H-1 & H-2), 5.89 (2H, s, OCH_2O), 4.29 (1H, d, J = 17, H-6), 3.98 (1H, m, H-3), 3.94 (1H, m, H-11), 3.69 (1H, d, J = 17, H-6), 3.45 (1H, m, H-12), 3.40 (3H, s, OMe), 3.33 (1H, m, H-4a), 3.24 (1H, m, H-12), 2.06 (2H, m, H-4). ^{13}C NMR ($CDCl_3$): 146.4 (s, C-9), 146.2 (s, C-8), 135.9 (d, C-1), 135.3 (C-10a), 126.6 (s, C-6a), 123.6 (d, C-2), 106.8 (d, C-7), 103.1 (d, C-10), 100.8 (t, OCH_2O), 80.0 (d, C-11), 76.0 (d, C-3), 66.1 (d, C-4a), 63.4 (t, C-6), 61.2 (t, C-12), 55.7 (q, 3-OMe), 50.2 (s, C-10b), 30.1 (t, C-4).

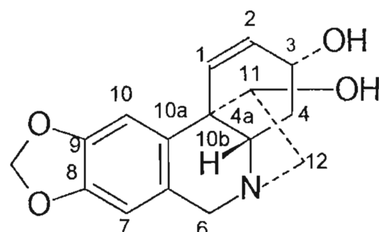
3.1.1.3 3-*O*-Acetylhamayne 47



47

Amorphous compound (28 mg), $[\alpha]^{28}{}_{D}$ = +104.8° (CH_3OH, c, 0.208). GC-MS 70 eV, m/z (% rel.int.): 329 [M^+, (18)], 281 (29), 240 (15), 225 (14), 224 (23), 223 (13), 207 (100), 181 (25), 153 (12), 115 (27), 73 (48). ^{1}H NMR ($CDCl_3$): 6.78 (1H, s, H-10), 6.45 (1H, s, H-7), 6.31 (1H, dd, J = 10, 2.2, H-1), 6.07 (1H, dd, J = 10, 1.0, H-2), 5.89 (2H, d, distorted AB, OCH_2O), 5.44 (1H, m, H-3), 4.28 (1H, d, J = 17, H-6), 3.94 (1H, m, H-11), 3.66 (1H, d, J = 17, H-6), 3.32 (2H, m, H-12), 3.23 (1H, dd, J = 6.4, 13, H-4a), 2.22 (2H, m, H-4), 2.07 (3H, s, OCOMe). ^{13}C NMR ($CDCl_3$): 170.0 (s, OCOMe), 146.5 (s, C-9), 146.3 (s, C-8), 134.0 (s, C-10a), 133.0 (d, C-1), 126.4 (s, C-6a), 125.0 (d, C-2), 106.9 (d, C-7), 103.2 (d, C-10), 100.9 (t, OCH_2O), 80.0 (d, C-11), 70.3 (d, C-3), 65.9 (d, C-4a), 63.5 (t, C-6), 61.1 (t, C-12), 50.0 (s, C-10b), 29.8 (t, C-4), 21.3 (q, OCOMe).

3.1.1.4 Bulbispermine 48

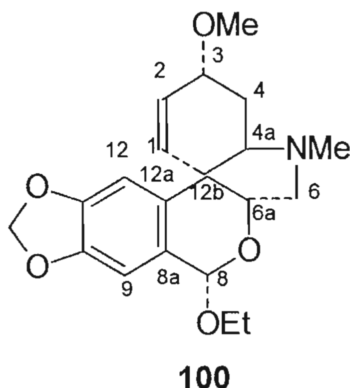


48

Amorphous compound (70 mg), $[\alpha]^{28}{}_{D}$ = +94.54° (CH_3OH, c, 0.604). GC-MS 70 eV, m/z (% rel.int.): 287 [M^+, (4)], 269 (4), 258 (100), 240 (10), 212 (10), 211(17), 181 (21), 173 (9), 153 (11),128 (19), 114 (20). ^{1}H NMR (CD_3OD): 6.83 (1H, s, H-10), 6.48 (1H, s, H-7), 6.23 (1H, dd, J = 2.2, 10.35, H-1), 6.02 (1H, d, J = 10.35, H-2), 5.86 (2H, s, OCH_2O), 4.30 (1H, ddd, J= 2, 4, 8, H-3), 4.22 (1H, d, J = 16.9, H-6), 3.93 (1H, dd, J = 2.9, 7, H-11), 3.68 (1H, d, J = 16.9, H-6), 3.41 (1H, dd, J = 7, 13.69, H-12), 3.32 (1H, m, H-12), 3.20(1H, m, H-4a), 2.04 (2H, m, H-4). ^{13}C NMR (CD_3OD): 148.3 (s, C-9), 147.9 (s, C-8), 137.7 (s, C-10a), 137.4 (d, C-1), 127.1 (s, C-6a), 125.2 (d, C-2), 108.1 (d, C-10), 104.5 (d, C-7), 102.5 (t, OCH_2O), 81.3 (d, C-11), 68.6 (d, C-3), 67.6 (d, C-4a), 64.0 (t, C-12), 61.7 (t, C-6), 51.6 (s, C-10b), 34.7 9t, C-4).

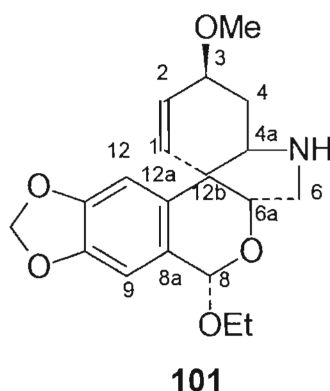
3.1.5 8α-Ethoxyprecriwelline 100

Amorphous compound (10 mg), $[\alpha]^{28}{}_{D}$ +116.6° ($CHCl_3$, c,0.06); ^1H and ^{13}C NMR data see Tables 3.1 and 3.2 (pages 45 and 46). GC-MS 70 eV, m/z(% rel.int): 359 [M^+,(41)], 329 [M-CH_2O, (78)], 314(50) 282 (16), 275 (47), (29), 181 (12), 149 (17), 113 (25), 89 (37), 82 (30), 70 (100). HRMS calculated for $C_{20}H_{25}NO_5$: 359.1719, found 359.1733.



100

3.1.1.6 *N*-Desmethyl-8α-ethoxypretazettine 101



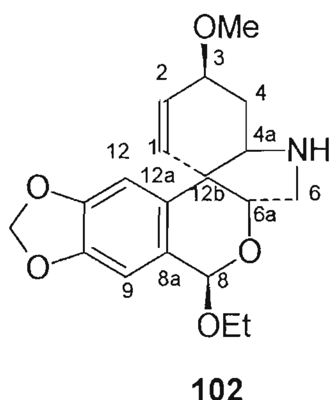
101

Amorphous compound (11 mg), $[\alpha]^{28}{}_{D}$ = +160.63° ($CHCl_3$, c, 0.09). ^1H and ^{13}C NMR data see Tables 3.1 and 3.2 (pages 45 and 46). GC-MS 70eV, m/z (% rel.int.): 345 [M^+, (41)], 316 (13), 315 (6), 314 (4), 300 (30), 289 (18), 268 (19), 244 (100), 215 (23), 181 (7), 141 (7), 115 (12), 89 (46), 61 (32). HRMS calculated for $C_{19}H_{23}NO_5$: 345.1576 found 345.1560.

3.1.1.7 *N*-Desmethyl-8β-ethoxypretazettine 102

Amorphous compound (14 mg), $[\alpha]^{28}{}_{D}$ = +34° ($CHCl_3$, c, 0.14), ^1H and ^{13}C NMR data see Tables 3.1 and 3.2 (Pages 45 and 46), GC-MS 70 eV, m/z (% rel.int.)

315 (<1%), 267 (28), 248 (60), 239 (24), 238 (100), 224(9), 223 (34), 210 (13), 180 (21), 152 (26), 95 (24), 75 (19).



102

3.1.2 Discussion

3.1.2.1 6-Hydroxycrinamine 22, 23

The compound occurs as a mixture of two epimers 6α- and 6β-hydroxcrinamine. Its NMR and GC-MS data (see 3.1.1.1) are in agreement with those reported for both epimers isolated from *Brunsvigia orientalis* (VILADOMAT *et al.*1996). Optical rotation of $[\alpha]_D$ +40° was reported by VILADOMAT *et al.* (1996) whereas $[\alpha]_D$ +37.1° was obtained in this study.

3.1.2.2 Crinamine 34

Crinamine was previously isolated from this species (ALI *et al.* 1984; El-MOGHAZI and ALI 1984; KOBAYASHI *et al.* 1984). ^{1}H and ^{13}C NMR spectra (see 3.1.1.2) were in full agreement with those reported in the literature (LIKHITWITAYAWUID *et al.* 1993; VILADOMAT *et al.* 1994). Crinamine showed optical rotation of $[\alpha]_D$ +96.6°. In the literature optical rotations $[\alpha]_D$ +96° (LIKHITWITAYAWUID *et al.* 1993) and $[\alpha]_D$ +180° (VILADOMAT *et al.* 1994) were reported.

Table 3.1. ^{1}H-NMR Data ($CDCl_3$) for 8α-ethoxyprecriwelline **100**, *N*-desmethyl-8α-ethoxypretazettine **101** and *N*- desmethyl-8β-ethoxypretazettine **102**.

H NO.	**100**	**101**	**102**
1	5.87 (1H, d, J = 10.3	5.73-5.90 (1H, m)	6.22 (1H, s)
2	5.99-6.06 (1H, m)	5.99 (IH, m)	6.22 (1H, s)
3	3.87 (1H, m)	3.80 (1H, m)	4.04 (1H, m)
4	2.32 (2H, m)	2.10-2.38 (2H, m)	2.03-2.09 (2H, m)
4a	2.87 (1H, bs)	3.46 (1H, bs)	3.52 (1H, bs)
6α	3.09 (1H, dd, J = 9.8, 11.2	2.83-2.98 (2H, m)	3.20 (2H, d, J = 4.92)
6β	2.66 (1H, J = 8.0, 9.8)	------------	----------
6a	4.35 (1H, dd, J = 8.0, 11.2)	4.46 (1H, 6.7, 11.2)	3.87 (1H, m)
8	5.68 (1H, s)	5.70 (1H, s)	4.50 (1H, s)
9	6.61 (1H, s)	6.56 (1H, s)	6.72 (1H, s)
12	6.76 (1H, s)	6.78 (1H, s)	6.73 (1H, s)
OCH_2O	5.80 (2H, s)	5.89-5.9 (2H, 2d, distorted AB	5.80-5.90 (2H, 2d, distorted AB
$OCHaCHbCH_3$			
Ha	3.94 (1H, dd, J = 9.7, 7.0)	3.92 (1H, dd, J = 9.7, 7.0)	4.03 (1H, dd, J = 9.7, 7.0)
Hb	3.70 (1H, dd, J = 9.7, 7.0)	3.70 (1H, dd, J = 9.7, 7.0)	3.66 (1H, dd, J = 9.7, 7.0)
CH_3	1.30 (3H, t, 7.0)	1.29 (3H, t, 7.0)	1.26 (1H, t, 7.0)
3-OMe	3.42 (3H, s)	3.40 (3H, s)	3.40 (3H, s)
NMe	2.50 (3H, s)	-----------	----------------

Table 3.2. ^{13}C-NMR Data ($CDCl_3$) 8α-ethoxyprecriwelline **100**, *N*-desmethyl-8α-ethoxypretazettine **101** and *N*- desmethyl-8β-ethoxypretazettine **102**.

C NO.	**100**	**101**	**102**
1	130.1d	131.5d	136.4d
2	125.9d	125.4d	123.2d
3	71.3d	71.3d	76.2d
4	29.3t	29.0t	29.0t
4a	62.5d	56.8d	60.0d
6	53.2t	44.9t	58.5T
6a	73.4d	76.2d	78.6d
8	99.1d	99.4d	94.0d
8a	127.2s	127.5s	127.1s
9	104.4d	104.6d	102.0d
10	146.4s	146.6s	146.3s
11	147.6s	147.6s	147.7s
12	108.1d	108.2d	109.0d
12a	134.8s	134.4s	137.6s
12b	45.3s	44.2s	50.0s
OCH_2O	101.1t	101.1t	101.0t
$O\underline{CH_2}CH_3$	63.8t	63.8t	64.0t
$OCH_2\underline{CH_3}$	15.3q	15.4q	15.0q
OMe	56.7q	55.4q	55.0q
NMe	42.5q	--------	-------

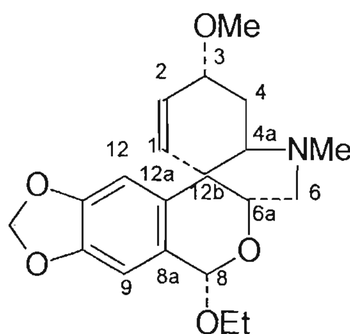
3.1.2.3 3-*O*-Acetylhamayne 47

The alkaloid was isolated first from *Crinum latifolium* (KOBAYASHI *et al.* 1984) and *Brunsvigia josphinae* (VILADOMAT *et al.* 1994). The ^{1}H and ^{13}C NMR and GC-MS data (see 3.1.1.3) are in agreement with those reported in the literature. In this study $[\alpha]_D$ +104.9° was obtained, while KOBAYASHI *et al.* (1984) and VILADOMAT *et al.* (1994) reported optical rotations of $[\alpha]_D$ of +123.5° and +123° respectively.

2.4.2.4 Bulbispermine 48

Crinum bulbispermum is the only source of this alkaloid (ALI *et al.* 1984). There are differences in the chemical shifts of both ^{1}H and ^{13}C NMR spectra (see 3.1.1.4) due to the use of CD_3OD instead of DMSO-d_6. In the literature an optical rotation of $[\alpha]_D$ +106.7° was reported while in this study $[\alpha]_D$ of +94.45° was obtained.

3.1.2.6 8-α-Ethoxyprecriwelline 100



100

The compound, $C_{20}H_{25}NO_5$, in its mass spectrum showed a molecular ion at m/z 359 and two fragment ions at m/z 329 and 70 (base peak). The latter two peaks are typical mass fragments characteristic of tazettine-type alkaloid with a 3-OMe in the α -position (DUFFIELD *et al.* 1965). The peak at m/z 314 (51%) represents the loss of the ethoxy group.

The ^{1}H-NMR (Table 3.1) spectrum in the downfield region showed 3 singlets at 6.76, 6.61 and 5.8 assigned to the aromatic protons H-12, H-9 and the two

methylenedioxy protons. The presence of a downfield multiplet at 5.99-6.06 and a doublet at 5.87 ppm indicated the presence of olefenic protons and were assigned to H-2 and H-1 respectively. In the aliphatic region, the triplet at 1.30 was assigned to a terminal methyl group while the singlets at 2.50 and 3.42 are typical signals for *N*-methyl and methoxy group protons respectively.

The presence of the three doublet of doublets at 4.35, 3.09 and 2.66 were assigned to H-6a, H-6α and H-6β. This is in agreement with signals for a tazettine-type alkaloid lacking substitution at 6a as observed in littoraline (ZE-LIN *et al.* 1995). The complete allocation of all protons was achieved through the use of COSY analysis. This showed positive correlations between H-2/H-3, H-6a/H-6α/6β, and also long range coupling between H-12/H-1 and H-2/H-4a.

In ^{13}C NMR spectrum (Table 3.2) the presence of one methoxy group carbon resonance at 56.7, one *N*-methyl group carbon resonance at 42.5 and one aliphatic carbon singlet at 45.3 together with four olefinic singlets and four doublets suggested the presence of tazettine-type of alkaloid (ZE-LIN *et al.* 1995). The DEPT spectrum allowed the multiplicities of the carbon signals to be obtained while HETCOR sequences allowed the assignment of different carbons to the corresponding protons.

The placement of the crucial ethoxy group at the C-8 position was based upon:

i) The triplet typical of a terminal methyl group at δ 1.3, J=7.0 Hz coupled to the doublet of quartets of Ha and Hb at δ 3.72 and δ 3.94, J=7.0 Hz. The two protons are diastereotopic and appeared as two complex signals resulting from the initial dd each being split into a quartet. The triplet was formed by the overlapping of the inner lines of the dd of the methyl group;

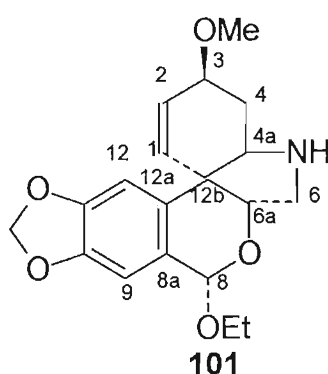
ii) The presence of a singlet proton signal at δ 5.68 typical of the chemical shift of a benzylic acetal hydrogen attached to C-8; and

iii) A ^{13}C NMR signal at 99.1 ppm, again characteristic of a benzylic carbon bearing an acetal moiety. By comparison, this carbon in 6-hydroxy crinine-type

alkaloids, in which it exists as a hemi-aminal, resonates at 85-88 ppm [BASTIDA *et al.* 1990; VILDOMAT *et al.* 1994).

The similarity of MS fragmentation pattern of 8α-ethoxyprecriwelline **100** with precriwelline allowed the stereochemistry at position 3 to be assigned the α-orientation. Moreover, comparison of the proton at position 8 of compound 8α-ethoxyprecriwelline **100** with those of papyramine **93** (BASTIDA *et al.* 1990), *O*-methyloduline **20** (KREH and MATUSCH1995), *O*-methyllycorenine **49** (CODINA *et al.* 1993), 2α-hydroxy-6-*O*-methyloduline **21** (ALMANZA *et al.* 1996) and 6α-hydroxycrinamine **22** (VILADOMAT *et al.* 1996) allowed the assignment of the ethoxy group in the α-orientation.

3.1.2.6 *N*-Desmethyl-8α-ethoxypretazettine 101



101

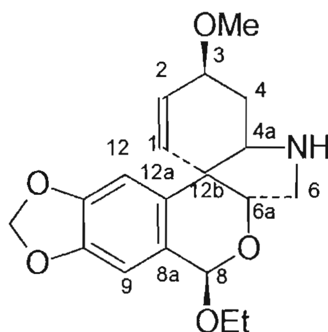
Apart from the absence of N-Me signals, the ^{1}H NMR (Table 3.1) and ^{13}C NMR (Table 3.2) spectra of this compound are very similar to that of compound 8α-ethoxyprecriwelline **100**. A downfield shift of H-4a by 0.59 ppm and upfield shift of C-4a by about 6 ppm were also observed. This strongly supports compound *N*-desmethyl-8α-ethoxypretazettine **101** being an *N*-desmethyl tazettine-type alkaloid.

The MS fragmentation pattern of *N*-desmethyl-8α-ethoxypretazettine **101** (see 3.1.1.2) showed a base peak at 244 and a molecular ion at m/z 345. This in agreement with the fragmentation pattern of tazettine-type alkaloid with a methoxy group in the β -position (DUFFIELD *et al.* 1965). The loss of the ethoxy

group, as in 8α-ethoxyprecriwelline **100**, from this compound is shown by a peak at m/z 300.

The similarity of the chemical shifts of both the proton and carbon at position 8 with those in 8α-ethoxyprecriwelline **100** allowed the assignment of the ethoxy group the α-position. Hence the only difference between precriwelline and pretazettine **71** is the stereochemistry at position 3. Compound *N*-desmethyl-8α-ethoxypretazettine **101** is the *N*-desmethyl isomer of 8α-ethoxyprecriwelline **100** and the name *N*-desmethyl-8α-ethoxypretazettine was proposed for **101**.

3.1.2.7 *N*-Desmethyl-8β-ethoxypretazettine 102



102

Compound **102** appears to be an isomer of *N*-desmethyl-8α-ethoxypretazettine **101** at C-8. In this instance H-8 resonates well upfield (δ 4.50) when compared to the same proton in 8α-ethoxyprecriwelline **100** and **101** (δ 5.70). Further differences in proton shifts for **101** are evident for all protons attached to sp^2 carbons (H-9, H-12, H-1, and H-2 (Table 3.1). ^{13}C NMR shifts for **100**,**101**,and **102** are very similar except C-12b in **102** which is shifted downfield (Table 3.2).

Again, comparison of proton shifts at position 8 of compound **102** with those of 6-epipapyramine (BASTIDA *et al.* 1990), 6β-hydroxycrinamine **23** (VILADOMAT *et al.* 1996) and 2α-hydroxy-6-*O*-methyloduline **21** (ALMANZA *et al.* 1996) allowed the assignment of the ethoxy group in the β-position for **102**.

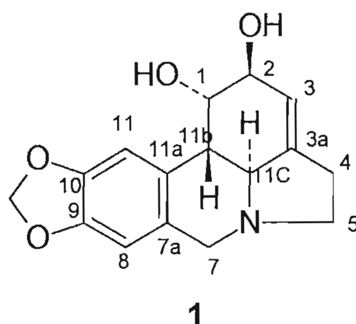
The presence of the ethoxy substituent at position 8 in **100**, **101**, **102** is not common in natural product chemistry. The possibility can not be excluded that the three compounds are artifacts formed during the extraction procedure

3.2 Alkaloids from *C. moorei*

3.2.1 Results

The ethanolic extracts of C. moorei were found to contain lycorine **1**, 1-*O*-acetyllycorine **43**, crinine **32**, 3-*O*-acetylcrinine **109**, powelline **73**, epibuphanisine **105**, crinamidine **36**, undulatine **44**, cherylline **106**, 1-epideacetylbowdenisine **108**, epivittatine **107** and the new alkaloids 3-[4'-(2'-aminoethyl)phenoxy]bulbispermine **103** and mooreine **104**. The spectroscopic and physical data used for the identification of these compounds such as ^{1}H and ^{13}C NMR (Appendix 1), GC-MS, optical rotation and melting points are presented below for each alkaloid.

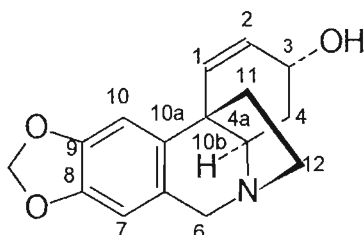
3.2.1.1 Lycorine 1



1

Amorphous powder (81 mg), $[\alpha]^{28}{}_{D}$ = -27.2° ($CHCl_3$, c, 0.011). GC-Ms 70 eV, m/z (% rel.int.): 287 [M^+, (30)], 286 (19), 268 (21), 227 (66), 226 (100), 211 (12). ^{1}H NMR (DMSO-D_6): 6.90 (1H, s, H-11), 6.84 (1H, s, H-8), 6.00-5.99 (2H, 2s, OCH_2O), 5.54 (1H, brs, H-3), 4.35 (1H, m, H-7), 4.05 (1H, m, H-7), 4.05 (1H, m, H-2), 3.40-3.30 (2H, m, H-5), 2.59 (1H, m, H-11b), 2.50 (2H, m, H-4). ^{13}C NMR (DMSO-D_6): 146.6 (s, C-9), 145.5 (s, C-10), 137.7 (s, C-3a), 129.7 (s, C-7a), 126.6 (s, C-11a), 120.8 (d, C-3), 107.3 (d, C-8), 105.1 (d, C-11), 100.8 (t, OCH_2O), 70.7 (d, C-2), 69.0 (d, C-1), 59.9 (d, C-11c), 53.6 (t, C-5), 53.1 (t, C-7), 37.8 (C-11b), 28.6 (t, C-4).

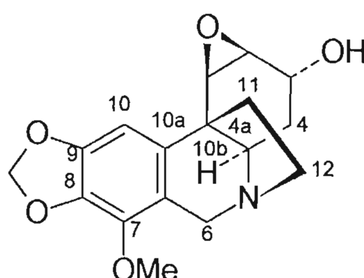
3.2.1.2 Crinine 32



32

Colourless needles (36 mg), mp 203-205, $[\alpha]^{28}{}_D$ = -12.9° ($CHCl_3$, c, 0.24). GC-MS 70 eV, m/z (% rel.int.): 271 [M^+, (100)], 270 (14), 254 (10), 242 (10), 228 (30), 215 (12), 200 (31), 199 (96), 186 (78), 173 (29), 172 (24), 129 (26), 128 (36). ^1H NMR ($CDCl_3$): 6.84 (1H, s, H-10), 6.47 (1H, s, H-7), 6.58 (1H, d, J = 10, H-1), 5.95 (1H, dd, J = 5.3, 10, H-2), 5.89 (2H, s, OCH_2O), 4.40 (1H, d, J = 17, H-6), 4.38 (1H, m, H-3), 3.77 (1H, d, J = 17, H-6), 3.42 (1H, m, H-4a), 3.30 (2H, m, H-12-*exo*), 2.89 (1H, m, H-12-*endo*),1.95 (1H, m, H-4), 1.94-2.06 (2H, m, H-11), 1.74 (1H, m, H-4). ^{13}C NMR ($CDCl_3$): 146.3 (s, C-9), 145.7 (s, C-8), 138.2 (s, C-10a), 132.1 (d, C-1), 127.5 (d, C-2), 126.2 (s, C-6a), 106.9 (d, C-7), 102.8 (d, C-10), 100.7 (t, OCH_2O), 64.00 (d, C-3), 62.7 (d, C-4a), 62.2 (t, C-6), 53.5 (t, C-12), 44.2 (s, C-10b), (t, C-11), 32.6 (t, C-4).

3.2.1.3 Crinamidine 36



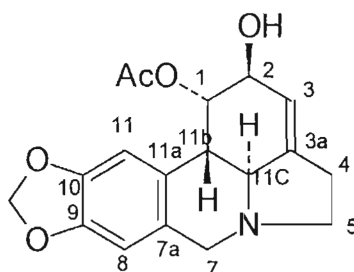
36

Amorphous compound (7 mg), $[\alpha]^{28}{}_D$ = -83.6° ($CHCl_3$, c, 0.049). GC-MS 70 eV, m/z (% rel. int.): 317 [M^+, 79)], 288 (100), 258 (23), 245 (26), 244(25), 217 (34), 205 (35), 204 (32), 203 (39), 189 (22), 173 (42), 115 (28), 56 (28). ^1H NMR

($CDCl_3$): 6.63 (1H, s, H-10), 5.88-5.87 (2H, 2d, distorted AB, OCH_2O), 4.50 (1H, m, H-3), 4.19 (1H, d, J = 17.3, H-6), 3.97 (3H,s, 7-OMe), 3.78 (1H, d, J = 3.4, H-1), 3.76 (1H, d, J = 17.49, H-6), 3.26 (1H, m, H-2), 3.17 (2H, m, H-4a & H-12-endo), 2.80 (1H, m, H-12-*exo*), 2.00 (1H, m, H-11-*endo*), 1.50-1.70 (2H, m, H-4). ^{13}C NMR ($CDCl_3$): 148.1 (s, C-9), 141.0 (s, C-7), 138.7 (s, C-10a), 133.3 (s, C-8), 117.0 (s, C-6a), 100.6 (t, OCH_2O), 96.3 (d, C-10), 65.4 (d, C-3), 60.8 (d, C-4a), 59.1 (q, 7-OMe), 58.5 (t, C-6), 56.4 (d, C-2), 53.8 (d, C-1), 52.5 (t, C-12), 41.6 (s, C-10b), 39.2 (t, C-11), 29.7 (t, C-4).

3.2.1.4 1-*O*-Acetyllycorine 43

Colourless needles (32 mg), mp 215-217, $[\alpha]^{28}{}_{D}$ = -59.68° (CH_3OH, c, 0.253). GC-MS 70 eV, m/z (% rel. int.): 329 [M^+, (9.7)], 268 (15.8), 227 (58), 226 (100),

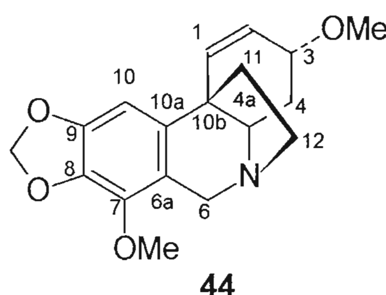


43

211 (5), 147 (10), 88 (10), 42 (48). ^1H NMR (CD_3OD): 6.90 (1H, s, H-11), 6.82 (1H, s, H-8), 6.095-6.085 (2H, s,OCH_2O), 5.87 (1H, d, J = 0.8, H-1 or H-2), 5.74 (1H, m, H-3), 4.31 (1H, d, J = 14.29, H-7), 4.33 (1H, dd, J + 0.8, 1.5, H-1 or, H-2), 3.75 (1H, d, J = 14.22, H-7), 3.50 (1H, m, H-5), 3.07 (2H, bs, H-11b & H-11c), 2.83 (2H, m, H-4), 2.67 (1H, m, H-5). ^{13}C NMR (CD^3OD): 172.4 (s, OCOMe), 148.4 (s, C-9), 148.2 (s, C-10), 143.9 (s, C-3a), 130.5 (s, C-7a), 128.6 (s, C-11a), 119.4 (d, C-3), 108.6 (d, C-8), 105.9 (d, C-11), 102.7 (t, OCH^2O), 73.6 (d, C-1 or C-2),70.5 (d, C-1 or C-2), 63.1 (d, C-11c), 57.8 (t, C-7), 54.9 (t, C-5), 40.3 (d, C-11b), 29.6 (t, C-4), 21.1 (q, OCOMe).

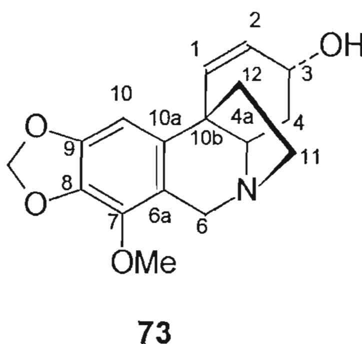
3.2.1.5 Undulatine 44

Amorphous compound (11 mg), $[\alpha]^{28}{}_D$ = -9.19° ($CHCl_3$, c, 0.087). GC-MS 70 eV, m/z (% rel.int.): 331 [M^+,(100)], 316 (7), 302 (8), 300 (9), 286 (13), 260 (10), 258 (31), 244(13), 232 (17), 219 (19), 217 (22), 205 (57). ^{1}H NMR ($CDCl_3$): 6.62 (1H, s, H-10), 5.87-5.85 (2H, 2d, distorted AB, OCH_2O), 4.20 (1H, d, J =17.5, H-6), 3.97 (3H, s, 7-OMe), 3.99-3.95 (1H, m, H-3), 3.77 (1H, d, J = 3.5, H-1), 3.71 (1H, d, J = 17.6, H-6), 3.43 (3H, s, 3-OMe), 3.43 (1H, m, H-12-*exo*), 3.33-3.30 (1H, m, H-2), 3.30 (1H, m, H-4a), 2.90-2.70 (1H, m, H-12-*endo*), 2.5-2.3 (1H, m, H-11-*exo*), 2.10-1.90 (2H, m, H-11-*endo* & H-4), 1.50-1.30 (1H, m, H-4). ^{13}C NMR ($CDCl_3$): 148.0 (s, C-9), 141.0 (s, C-7), 38.9 (s, C-10a), 133.3 (s, C-8), 117.8 (s, C-6a), 100.5 (t, OCH_2O), 96.3 d, C-10), 74.9 (d, C-3), 61.2 (d, C-4a), 59.1 (q, 7-OMe), 58.6 (t, 3-OMe), 55.1 (d, C-2), 53.9 (d, C-1), 52.5 (t, C-12), 41.4 (s, C-10b), 39.2 (t, C-11), 25.2 (t, C-4).



44

3.2.1.6 Powelline 73

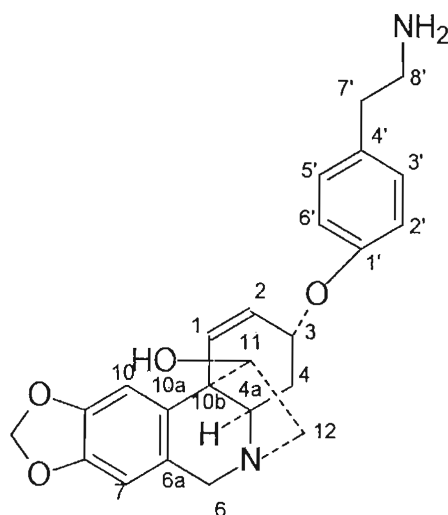


73

Colourless needles (20 mg), mp 200-202, $[\alpha]^{28}{}_D$ = -4° ($CHCl_3$, c, 0.138). GC-Ms 70 eV, m/z (% rel.int.): 301 [M^+, (100)], 258 (25), 246 (15), 229 (95), 217 (57). ^{1}H NMR ($CDCl_3$): 6.57 (1H, s, H-10), 6.54 (1H, d, J = 9.96, H-1), 6.00-5.90 (1H, dd, J = 5, 9.96, H-2), 5.86-5.85 (2H, 2d, distorted AB OCH_2O), 4.30 (1H, m, H-3),

4.24 (1H, d, J = 17.5, H-6), 3.96 (3H, s, OMe), 3.85 (1H, d, J = 17.5, H-6), 3.30-3.50 (2H, m, H-12), 2.80-3.00 (1H, m, H-4a), 1.70-2.00 (4H, H-4 & H-11). ^{13}C NMR ($CDCl_3$): 148.0 (s, C-9), 140.9 (s, C-7), 139.0 (s, C-10a), 133.4 (s, C-8), 132.0 (d, C-1), 127.4 (d, C-2), 116.9 (s, C-6a), 100.5 (t, OCH_2O), 96.7 (d, C-10), 63.8 (d, C-3), 62.4 (d, C-4a), 59.1 (q, 7-OMe), 58.4 (t, C-6), 53.6 (t, C-12), 44.1 (s, C-10b), 43.9 (t, C-11), 32.5 (C-4).

3.2.1.7 3-[4'-(2'-Aminoethyl)phenoxy] bulbispermine 103



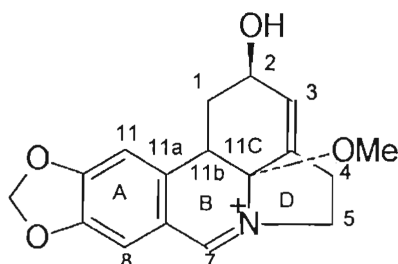
103

Amorphous compound (11 mg)$[\alpha]^{29}_{D}$+ 53.3° ($CHCl_3$, c, 0.045) ^{1}H and ^{13}CNMR see Tables 3.3 and 3.4. (Pages 57 and 58). GC-MS 70 eV, m/z (% rel.int.): 286 (<1%) 258 (100), 248 (26), 186 (18), 129 (9), 115 (14), 107 (11), 44 (35), 43 (56), 42 (17).

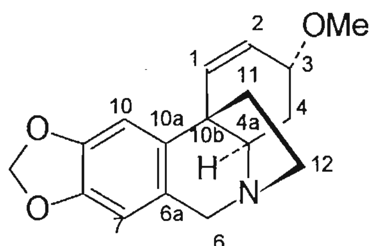
3.2.1.8 Mooreine 104

Amorphous compound (7 mg) ^{1}H and ^{13}CNMR see Table 3.5 (Page 59) electrospray (% rel.int.): 300 [M^{+}, 5), 292 (5), 284 (100), 266 (5).

104



3.2.1.9 Epibuphanisine 105



105

Colourless crystals (56 mg), mp (118-120), $[\alpha]^{28}_{D}$ + 118.5° ($CHCl_3$, c, 0.518). GC-MS 70 eV, m/z (% rel.int.): 285 [M^+, (81)], 270 (23), 254 (26), 230 (21), 215 (100), 201 (27), 185 (25), 172 (27), 167 (10), 157 (28), 149 (1.5), 129 (28). ^{1}H NMR ($CDCl_3$): 6.80 (1H, s, H-10), 6.49 (1H, s, H-7), 6.43 (1H, dd, J = 2.3, 9.9, H-1), 5.90-5.88 (2H, 2d, distorted AB OCH_2O), 5.85 (1H, dd, J = 1.3, 10.3, H-2), 4.40 (1H, d, J = 16.9, H-6), 4.00 (1H, m, H-3), 3.78 (1H, d, J = 16.9, H-6), 3.41 (3H, s, 3-OMe), 3.32 (1H, m, H-12-*end*), 2.80-3.00 (1H, m, H-12-*exo*), 3.16 (1H, m, H-4a), 2.23 (2H, m, H-11), 2.00 (1H, m, 4), 1.57 (1H, m, 4). ^{13}C NMR ($CDCl_3$): 146.5 (s, C-9), 146.1 (s, C-8), 138.6 (s, C-10a), 129.2 (d, C-2), 128.7 (d, C-1), 126.0 (s, C-6a), 106.9 (d, C-7), 102.7 (d, C-10), 100.7 (t, OCH_2O), 76.3 (d, C-3), 66.6 (d, C-4a), 62.2 (t, C-6), 55.9 (q, 3-OMe), 53.3 (t, C-12), 45.0 (t, C-11), 44.6 (s, C-10b), 31.0 (t, C-4).

3.2.1.10 Cherylline 106

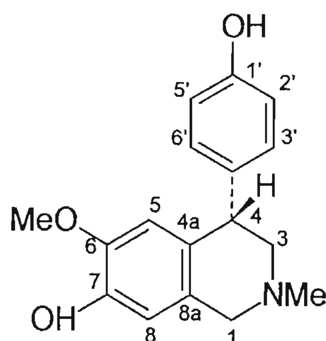


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Table 3.3. ^{1}H-NMR Data (CD_3OD) for 3-[4'-(2'-aminoethyl)phenoxy]bulbispermine **103** and bulbispermine.

H NO.	**103**	Bulbispermine
1	6.05 (1H, dd, J = 1.2, 10.3)	6.02 (1H, dd, J = 10.35
2	6.24 (1H, dd, J = 2.3, 10.3)	6.23 (1H, dd, J = 2.2, 10.35)
3	4.40 (1H, m)	4.30 (1H, ddd, J = 2, 4, 8
4	2.02 (2H, m)	2.03 (2H, m)
4a	3.32 (1H, m)	3.20 (1H, m)
6α	4.27 (1H, d, J = 16.9)	4.22 (1H, d, J = 16.9)
6β	3.72 (1H, d, J = 16.9)	3.68 (1H, d, J = 16.9)
7	6.53 (1H, s)	6.48 (1H, s)
10	6.86 (1H, s)	6.83 (1H, s)
11	3.95 (1H, dd, J = 3.6, 7)	3.93 (1H, dd, J = 2.9, 7)
12	3.32 (2H, m)	3.41 (1H, dd, J = 7, 13.69)
12'	---------	3.32 (1H, m)
2', 6'	7.05 (2H, d, J = 8.5)	---------
3', 5'	6.73 (2H, d, J = 8.6)	---------
7'	2.70 (2H, t, J = 7)	--------
8'	2.90 (2H, t, J =7)	--------

Table 3.4. ^{13}C-NMR Data (CD_3OD) for 3-[4'-(2'-minoethyl)phenoxy]bulbispermine **103** and bulbispermine.

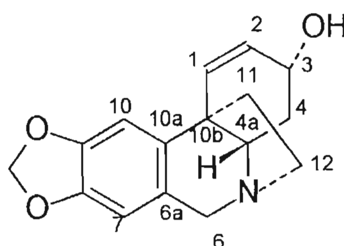
C NO.	103	Bulbispermine
1	137.4d	137.4d
2	125.2d	125.2d
3	68.6d	68.6d
4	34.7d	34.7t
4a	67.7d	67.6d
6	61.8t	61.7t
6a	127.1s	127.1s
7	104.5d	104.5d
8	147.9s	147.9s
9	148.4s	148.3s
10	108.1d	108.1d
10a	137.7s	137.7s
10b	51.7s	51.6s
11	81.3d	81.3d
12	63.9t	64.0t
1'	157.4s	----------
2', 6'	131.0d	----------
3', 5'	116.7d	----------
7'	37.9t	----------
8'	44.0t	----------

Table 3.5. ^{1}H-NMR and ^{13}C-NMR (CD_3OD) Data for mooreine **104**.

Atom NO.	^{13}C	^{1}H
1	54.8t	5.49 (1H, ddd, J = 0.69, 3.8, 5.3)
		5.48 (1H, ddd, J = 0.46, 1.14, 5.3)
2	72.0d	4.61 (1H, m)
3	127.6d	6.45 (1H, m)
3a	128.4s	-------
4	26.5t	3.32 (2H, m)
5	57.1t	5.00 (2H, m)
7	142.1d	9.36 (1H, s)
7a	134.2s	---------
8	106.1d	7.63 (1H, s)
9	158.5 s	-------
10	152.8s	-------
11	100.8d	7.77 (1H, s)
11a	139.3s	------
11b	68.9d	5.31 (1H, m)
11c	79.4s	--------
OMe	48.0q	3.3é (3H, s)

Amorphous compound (35 mg), $[\alpha]^{28}_{D}$ = -34° (CH_3OH, c, 0.24). GC-Ms 70 eV, m/z (% rel.int.): 285 [M^+, (36)], 242 (100), 241 (76), 225 (78), 211 (80), 210 (59), 43 (58). ^1H NMR (CD_3OD): 7.15 (2H, d, J = 8.69, H-3' & H-5'), 6.90 (2H, d, J = 8.69, H-2' & H-6'), 6.73 (1H, s, H-8), 6.50 (1H, s, H-5), 4.30(1H, d, J = 5.95, H-4), 3.80 (1H, d, J = 14.2, H-1), 3.61 (3H, s, OCH_3), 3.46 (1H, d, J = 14.6, H-1), 3.2 (1H, t, J = 5.95, H-3), 2.40-2.70 (1H, m, H-3). ^{13}C NMR (CD_3OD): 157.4 (s, C-6), 148.2 (s, C-7), 146.3 (s, C-4'), 136.4 (s, C-1'), 131.0 (d, C-2' & C-6'), 129.9 (s, C-8a), 128.4 (s, C-4a), 116.5 (d, C-3' & C-5'), 113.7 (d, C-8), 113.2 (d, C-5), 63.5 (t, C-1), 58.9 (t, C-3), 56.4 (q, OMe), 46.0 (q, NCH_3), 45.9 (d, C-4).

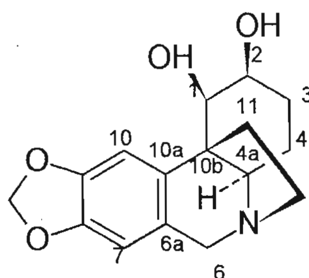
3.2.1.11 Epivittatine 107



107

Amorphous compound (53 mg), $[\alpha]^{28}_{D}$ = +98.4° (CH_3OH, c, 0.319). GC-Ms 70 eV, m/z (% rel.int.): 271 [M^+, (100)], 254 (10), 228 (22), 216 (9), 199 (65), 187 (65), 173 (21), 114 (12), 56 (10). ^1H NMR (CD_3OD): 7.03 (1H, s, H-10), 6.70 (1H, s, H-7), 6.62 (1H, dd, J = 2.4, 10.3, H-2), 6.04 (2H, s, OCH_2O), 5.93 (1H, d, 10.26, H-1), 4.51 (1H, d, J = 16.9, H-6), 4.50 (1H, m, H-3), 3.96 (1H, d, J = 16.9, H-6), 3.50 (1H, m, H-12), 3.35 (1H, m, H-4a), 3.10 (1H, m, H-12), 2.28 (2H, m, H-11), 2.28 (1H, m, H-4), 1.80 (1H, m, H-4). ^{13}C NMR (CD_3OD): 148.0 (s, C-9), 147.6 (s, C-8), 139.8 (s, C-10a), 133.0 (d, C-1), 129.5 (d, C-2), 126.5 (s, c-6a), 108.1 (d, C-7), 104.1 (d, C-10), 102.4 (t, OCH_2O), 68.3 (d, C-3), 67.9 (d, C-4a), 62.7 (t, C-6), 53.9 (C-12), 50.1 (s, C-10b), 46.0 (t, C-11), 35.3 (t, C-4).

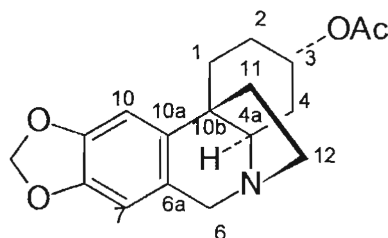
3.2.1.12 1- Epideacetyl bowdensine 108



108

Amorphous compound (16 mg), $[\alpha]^{28}_{D}$ = +36.5° ($CHCl_3$, c, 0.093). GC-Ms 70 eV, m/z (% rel.int.): 319 [M^+, (100)], 302 (10), 275 (30), 246 (20), 232 (76), 220 (33), 219 (27), 203 (21), 57 (17), 56 (34). 1H NMR ($CDCl_3$): 7.26 (1H, s, H-10), 5.86 (2H, s, OCH_2O), 4.33 (1H, d, J = 17, H-6), 4.15 (1H, m, H-2), 4.00 (1H, d, J = 4.4, H-1), 4.00 (3H, s, OMe), 3.87 (1H, d, J = 17, H-6), 3.60 (1H, m, H-12-*exo*), 3.10 (1H, m, H-4a), 2.90 (1H, m, H-12- *end*), 2.90 (1H, m, H-11-*exo*), 2.10 (1H, m, H-3 eq.), 2.02 (H-11-*endo*), 1.60 (2H, m, H-4). ^{13}C NMR ($CDCl_3$): 148.5 (s, C-9), 141.5 (s, C-10a), 139.9 (s, C-7), 133.4 (s, C-8), 114.9 (s, C-6a), 100.7 (t, OCH_2O), 99.7 (d, C-10), 72.4 (d, C-1), 69.0 (d. C-2), 67.5 (d, C-4a), 59.1 (q, 7-OMe), 56.9 (t, C-6), 51.0 (t, C-12), 49.3 (s, C-10b) 35.4 (t, C-11), 28.6 (t, C-3).

3.2.1.13 3-*O*-Acetylcrinine 109



109

Amorphous compound (8 mg), $[\alpha]^{28}{}_D$ = +24.4° (CH_3OH, c, 0.045). GC-Ms 70 eV, m/z (% rel.int.): 313 [M^+, (100)], 270 (25), 254 (61), 224 (28), 216 (49), 129 (14). ^1H NMR ($CDCl_3$): 6.90 &6.85 (1H, s, H-10), 6.68 (1H, d, J=10, H-1), 6.49 & 6.51(1H, s, H-7), 5.91 &5.90 (2H, s, OCH_2O), 5.89 (1H, m, H-2), 5.30 (1H, m, H-3), 4.35 &4.45 (1H, d, J=16.9, H-6), 3.70 &3.80 (1H, d, J=16.9, H-6), 3.40 &3.30 (2H, m, H-12), 2.80-3.00 (1H, m, H-4a), 2.10&2.006 (3H, s, OCOMe), 1.70-2.00 (4H, m, H-11, H-4). ^{13}C NMR ($CDCl_3$): 170.5 (s, OCOMe), 146.2 (s, C-9), 146.0 (s, C-8), 138.0 (s, C-10a), 134.3 (d, C-1), 126.0 (s, C-6a), 123.7 (d, C-2), 107.1 & 107.0 (d, C-7), 102.8 & 102.5 (d, C-10), 100.8 (t OCH_2O), 74.8 (d, C-3), 66.4 &63.2 (d, C-4a), 62.0t (t, C-6), 53.4& 52.2 (t, C-12), 44.2 (s, C-10b), 44.1 &39.1 (t, C-11), 29.7 (t, C-4), 21.2, (q, OCOMe)

3.2.1.14 Conversion of tyramine and 103 to their acetates

Tyramine (29 mg) and (5 mg) of **103** were heated with equal volumes of pyridine and acetic anhydride at 60øC for half an hour and kept overnight at room temp. to give tyramine-diacetate (39 mg) and bulbispermine-diacetate (6 mg) respectively .

Tyramine-diacetate

^1H NMR (CD_3OD): 7.26 (2H, d, J = 8.4, H-2 & H-6), 7.04 (2H, d, J = 8.4, H-3 &H-5), 3.41 (2H, t, J = 7.5, 2H-8), 2.81 (2H, t, J = 7.5, 2H-7), 202 & 1.93 (3H each, s, 2 OMe). ^{13}C NMR (CD_3OD): 173.2 &171.4 (s, 2 $O\underline{C}OCH_3$), 150.7 (s, C-1), 30.7 (d, C-2 &C-6), 138.9 (s, C-4), 122.6 (d, C-3 &C-5), 41.9 (t, C-8), 35.7 (t, C-7), 2.5 & 20.9 (q, 2Me).

Tyramine

^1H NMR (CD_3OD): 7.11 (2H, d, J = 8.42, H-2 and H-6), 6.79 (2H, d, J = 8.42, H-3 and H-5), 3.13 (2H, t, J = 7.14, 2H-8), 2.88 (2H, t, J = 7.09, 2H-7). ^{13}C NMR (CD_3OD): 157.2 (s, C-1), 130.7 (d, C-2 and C-6), 130.9 (s, C-4), 116.4 (d, C-3 and C-5), 44.3 (t, C-8), 39.0 (t, C-7).

3.2.2 Discussion

3.2.2.1 Lycorine 1

The NMR and GC-Ms data (see 3.2.1.1) were correlated with those reported by EVIDENTE *et al.* (1983).

3.2.2.2 Crinine 32

The compound showed a m.p of 203-205°, $[\alpha]_D$ -12.9°. In the literature, m.p. 08-210°, 204-205°; $[\alpha]_D$ -9°, $[\alpha]_D$ -17.5° (KOBAYASHI *et al.* 1984; VILADOMAT *et al.* 1995). The alkaloid was previously isolated from this species VILADOMAT *et al.* 1997). Its ^1H and ^{13}C NMR and GC-MS data (see 3.2.1.2) were in agreement with those reported for crinine (VILADOMAT *et al.* 1995).

3.2.2.3 Crinamidine 36

The alkaloid was previously isolated from *Crinum moorei* (VILADOMAT *et al.* 1997). Apart from the differences in optical rotation, the alkaloid showed identical spectroscopic data (see 3.2.1.3) as those reported for crinamidine.

3.2.2.4 1-*O*-Acetyllycorine 43

Changes in chemical shifts of ^1H and ^{13}C NMR (see 3.2.1.4) were observed due to the use of CD_3OD as solvent instead of $CDCl_3$ (EVIDENTE 1986). An optical rotation of $[\alpha]_D$= -59.68° was obtained in this study, while $[\alpha]_D$ -106° was reported (KOBAYASHI *et al.* 1984).

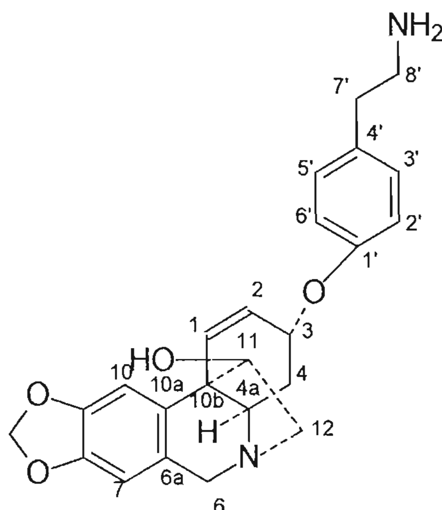
3.2.2.5 Undulatine 44

The alkaloid was previously reported from *Crinum moorei* (VILADOMAT *et al.* 1997). The alkaloid ^1H NMR and ^{13}C NMR signals (see 3.2.1.5) are in line with those reported in the literature (VILADOMAT *et al.* 1995). But differences in optical rotation were observed. The alkaloid showed an optical rotation of $[\alpha]_D$= -9.19° while VILADOMAT *et al.* (1995) and (1997) reported $[\alpha]_D$= -46°.

3.2.2.6 Powelline 73

The alkaloid showed ^{1}H NMR, ^{13}C NMR and MS data (see 3.2.1.6) identical with those reported for powelline (FRAHM *et al.* 1985, KOBAYASHI *et al.* 1984). The optical rotation of the alkaloid was $[\alpha]_D$= -4°, while $[\alpha]_D$= +3.9° and $[\alpha]_D$= + 0° were reported by ALI et al. 1984. and KOBAYASHI *et al.*(1984).

3.2.2. 7 3-[4'-(2'-Aminoethyl)phenoxy]bulbispermine 103



103

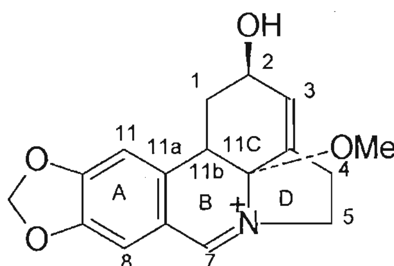
The ^{1}H NMR data of **103** (Table 3.3) showed the typical singlets associated with H-7, H-10, and the methylenedioxy group at 6.86, 6.53 and 5.87 respectively. From the HETCOR and COSY analysis the resonance of H-3 and H-11 could be assigned to δ 4.40 and 3.95 respectively. The first is clearly coupled to H-1 and H-2 and also to the methylene group at C-4 while the second is coupled to the methylene group at 3.32. These were the only likely positions to which the tyramine residue – whose presence was clearly indicated by the A_2X_2 system present at δ 6.73 and δ 7.05- could be attached. HMBC and NOESY were not helpful and no connectivities were observed between H-11 or H-3 and eitherC-6' and C-2' or C-7' and C-8'. Also, no NOE effects were observed between protons on the basic ring and the phenethyl moiety. Acetylation of the compound resulted in a downfield shift of both H-3 and H-11 to around 5.48 ppm and 4.97 ppm respectively. This indicates that acetylation occurred at both positions

which is further proved by the molecular ion of the acetate of a 371 mass unit with the loss of 60 and 43 mass units respectively. The possibility of the compound being a mixture of two compounds was rejected by developing bulbispermine and tyramine on a TLC plate using the same solvent system used for the purification of **103**. Tyramine has an R_f value of 0.67 while the value for bulbispermine is 0.59. From the above acetylation results, it is clear that the compound had undergone degradation at some stage during storage or acetylation. NOE information did not reveal much useful information but did show a good correlation between H-11 *exo* and H-12 *exo*. Construction of an accurate model using the NOE information showed, however, that the 3-position and 11-position were not sterically crowded and equally exposed to attack by a tyramine moeity. In these circumstances, the tyramine residue was attached at the more reactive allylic C-3. In addition, computer simulation programme ^{1}H NMR data, using Advanced Chemistry Development programme (ACD) from ALDRICH (1998), supported the attachment at C-3 as indicated in Table 3.6. It is suggested that the linkage to C-3 is via the phenolic group of the tyramine moiety. This is based on the observation that in both plicamine **15** and secoplicamine **16** (both bonded through N), C-8' resonates in the ^{13}C spectrum at 49.0 and 50.0 ppm, respectively (ÜNVER *et al.* 1999) whereas in **103** the relevant chemical shift is at 44.0 ppm. In addition, the shift of the ethylene group in authentic tyramine (36.7 and 43.4 for C-7' and C-8' respectively) corresponds closely to that observed for the corresponding group in compound **103**. Furthermore, acetylation of tyramine at both C-1 and NH_2 showed only a 0.15 ppm downfield shift of protons at C-2' and C-6' compared to 0.25 ppm downfield shift of protons at C-3' and C-5'. Protons attached to C-8' also shifted downfield by 0.28 ppm. Chemical shifts of C-2', C-6' remained unaffected while that of 3', 5' were shifted downfield by 6 ppm. The compound gave no molecular ion in its HRMS. However, it showed a fragment ion at 286.1058 ($C_{16}H_{16}NO_4$) which is the molecular ion of bulbispermine after loss of the tyramine moiety (see 3.2.1.7).

Table 3.6. Computer simulation programme ^1H NMR chemical shifts of protons at positions 3 & 11 for bulbispermine and bulbispermine with the tyramine attached either to C-3 or C-11 and **103**.

Proton NO.	bulbispermine	**103**	bulbispermine with tyramine moiet attached to C-3	C-11
3	4.37	4.40	4.68	4.21
11	3.91	3.95	3.91	5.12

3.2.2.8 Mooreine 104



104

Mooreine, under normal conditions of electron bombardment, showed no molecular ion. However, using an electrospray instrument it exhibited a molecular ion at m/z 300 (3.2.1.8). This corresponds to the proposed molecular formula of C_{17} $H_{18}NO_4$. The ^1H -NMR and ^{13}C-NMR data (Table 3.5) for ring A, B, D closely resemble those reported for the anhydrolycorinium ion **89** (PETTIT *et al.* 1984). The ^1H NMR spectrum showed 4 singlets at 9.36, 7.77, 7.63 and 6.38 assigned to H-7, H-11, H-8 and the methylenedioxy protons respectively. It also showed multiplets at 6.45, 5.31, 5.00, 4.61 and 3.32 assigned to H-3, H-11b, H-5, H-2 and H-4 respectively. The two doublet of doublet of doublet at 5.49 and 5.48 were assigned to the two protons at position 1. The multiplicities of the peaks relating to the aliphatic portion of the molecule were completely assigned by COSY analysis. The COSY spectrum showed a correlation between H-2 (4.61) and the one proton multiplet of H-3 at 6.41 and the doublet of doublet of

doublet of H-1 and H-1 at 5.49 and 5.48. In addition there was a correlation between the doublet of doublet of doublet of both protons at position 1 and that of H-11b at 5.31. The assignment of the signal at 5.31 to H-11b was supported by the long range coupling between the signal at 5.31 and that of H-11 (7.77). The deshielding of C-5 and C-4 protons is due to their α-and β-positions with respect to the nitrogen of the salt (BASTIDA *et al.* 1992).

The ^{13}C-NMR spectrum (Table 3.5) showed four methine sp^2carbons in the aromatic region at 142, 127, 105 and 101 ppm assigned to C-7, C-3, C-8, and C-11. The chemical shifts of the six quaternary carbons C-3a, C-7a, C-9, C-10, C-11a and C-11c are shown in Table 3.5. The placement of the methoxy group on C-11c was based on the chemical shift of this carbon atom and was in good agreement with predictions for ^{13}C chemical shifts derived from a commercially available modelling program. Lack of material and marked instability prevented a more detailed analysis of the compound and the proposed structure should be regarded as tentative.

3.2.2.9 Epibuphanisine 105

The alkaloid showed a m.p. of 118-120°, $[\alpha]_D$ +118.5°. Literature reports m.p.123-125°, $[\alpha]_D$ +129°. Its NMR and GC-MS data (see 3.2.1.9) are in line with epibuphanisine spectra isolated from *Boophane flava* (VILADOMAT *et al.* 1995).

3.2.2.10 Cherylline 106

Crinum moorei is known to contain this alkaloid (VILADOMAT *et al.* 1997). The only difference in spectroscopic and physical data (see 3.2.1.10) was observed in the optical rotation. The alkaloid has $[\alpha]_D$ -70.6° while $[\alpha]_D$ -34° was reported (KOBAYASHI *et al.* 1984).

3.2.2.11 Epivittatine 107

This is the first report of the alkaloid from *Crinum moorei.* The chemical shifts of ^{1}H and ^{13}C NMR (see 3.2.1.11) are slightly different due to the use of CD_3OD instead of DMSO-d_6. The alkaloid was isolated from *Boophane flava and Nerine*

bowdenii (VILADOMAT *et al.* 1995; VILADOMAT *et al.* 1997). Its showed $[\alpha]_D$ +98.4° while $[\alpha]_D$ +102° was reported for this compound (VILADOMAT *et al.* 995).

3.2.2.12 1-Epideacetylbowdensine 108

The only known source for this alkaloid is *Brunsvigia orientalis* (VILADOMAT *et al.* 1996). Its spectroscopic data, NMR and GC-MS (see 3.2.1.12), are in line with those reported in the literature. $[\alpha]_D$ +36.5° while $[\alpha]_D$ +22° was reported by VILADOMAT *et al.* (1996).

3.2.2.13 3-*O*-Acetylcrinine 109

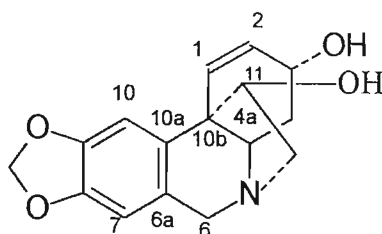
The compound is only known as a synthetic product and there are no reports for the isolation of the alkaloid from a natural source. The compound occurs as a mixture of two epimers and attempts at their separation failed. MS fragmentation pattern is in agreement with that reported in the literature (see 3.2.1.13), $[\alpha]_D$ +24.4°,while in the literature $[\alpha]_D$ +13.3° was obtained. The only report on proton and ^{13}C NMR spectra was incomplete (CAMPBELL *et al.* 1998).

3.3 Alkaloids from *C. macowanii*

3.3.1 Results

The alkaloids crinine **32**, lycorine **1**, bulbispermine , cherylline **106** and hamayne **33** were obtained from the ethanolic extracts of this species. In addition the amine tyramine was isolated during the isolation process. The spectroscopic and physical data of lycorine, crinine, bulbispermine and cherylline were identical with those obtained from *C. bulbispermum* and *C. moorei*.

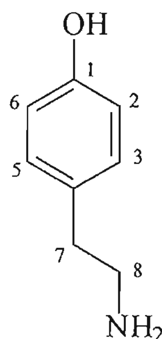
3.3.1.1 Hamayne 33



33

Amorphous compound (11 mg), $[\alpha]^{28}{}_{D}$ = +101.72° ($CHCl_3$, c, 0.058). GC-Ms 70 eV, m/z (% rel.int.): 287[M^+,(6)], 269 (35), 258 (100), 240 (19), 225 (9), 224 (13), 181 (40),115 (26), 77 (14), 56 (11), 55 (8). ^{1}H NMR ($CDCl_3$): 6.79 (1H, s, H-10), 6.47 (1H, s, H-7), 6.22 (1H, d, J = 10.4, H-1), 6.19 (1H, d, J = 10.5, H-2), 5.90-5.89 (2H, 2s, OCH_2O), 4.40 (1H, m, H-3), 4.30 (1H, d, J = 17, H-6), 3.98 (1H, dd, J = 3.2, 5.95, H-11), 3.69 (1H, d, J = 17, H-6), 3.35 (1H, dd, J = 6.4, 13.96, H-12), 3.32 (1H, dd, J = 3.2, 13.96, H-12), 3.22 (1H, dd, J = 4.5, 13.0, H-4a), 2.14-2.10 (2H, m, H-4). ^{13}C NMR ($CDCl_3$): 146.5 (s, C-9), 146.2 (s, C-8), 138.2 (d, C-2), 135.3 (s, C-10a), 126.4 (s, C-6a), 123.1 (d, C-1), 106.8 9d, C-7), 103.2 (d, C-10), 100.9 (t, OCH_2O), 79.9 (d, C-11), 67.6 (d, C-3), 66.2 (d, C-4a), 63.3 (t, C-6), 61.0 (t, C-12), 49.9 (s, C-10b), 34.0 (t, C-4).

3.3.1.2 Tyramine 110



110

Amorphous compound (13 mg). GC-Ms 70 eV, m/z (% rel.int.): 137 [M^+,(19)],108 (100), 107 (52), 91 (4), 77 (20), 51 (7). ^{1}H NMR (CD_3OD): 7.11 (2H,d, j=8.42, H-2 & H-6), 6.79 (2H, d, J=8.42, H-3 & H-5), 3.13 (2H, t, J=7.14, H-8), 2.88 (2H, t, J=7.14, H-7). ^{13}C NMR (CD_3OD): 157.5 (s, C-1), 130.7 (d, C-2 & C-6), 128.7 (s, C-4), 116.6 (d, C-3 & C-5), 42.3 (t, C-8), 34.1 (t, C-7).

3.3.2 Discussion

The ^{1}H NMR, ^{13}C NMR and GC-MS data of lycorine, crinine, cherylline and bulbispermine were identical with the spectra of the same alkaloids isolated from *C. moorei* and *C. bulbispermum* .

3.3.2.1 Hamayne

The alkaloid showed ^{1}H NMR and ^{13}C NMR signals (see 3.3.1.1) characteristic of a crinine-type alkaloid with substitution at position 3 and 11. The chemical shifts were the same as those reported for hamayne (VILADOMAT *et al.* 1994). The alkaloid showed an optical rotation, $[\alpha]_D$ +101.72°, while in the literature $[\alpha]_D$ +78.2° (KOBAYASHI *et al.* 1984) and $[\alpha]_D$ +79° (VILADOMAT *et al.* 1994) were reported.

During the course of alkaloid isolation the phenethylamine tyramine **110** was isolated. Its ^{1}H NMR and ^{13}C NMR and GC-Ms data are in agreement with an authentic sample.

Summary

The new alkaloids 8α -ethoxyprecriwelline, *N*-desmethyl-8α-ethoxypretazettine and *N*-desmethyl-8β-ethoxypretazettine, which probably are artifacts, were isolated from *C. bulbispermum*. While mooreine and 3-[4'-(2'-aminoethyl)phenoxy]bulbispermine were new alkaloids isolated from *C. moorei.* In addition, bulbispermine, 6-hydroxycrinamine and 3-*O*-acetylhamayne were isolated for the first time from *C. bulbispermum*. 1- Epideacetylbowdensine, epivittatine, epibuphanisine and 3-*O*-acetylcrinine were reported for the first time from *C. moorei*. None of the alkaloids isolated from *C. macowanii* were previously reported.

CHAPTER 4

Organ-to-organ and seasonal variation of alkaloids in *C. moorei*

4.1 Recovery of alkaloids

Hyoscyamine was used as standard to determine the recovery of extracted alkaloids. The alkaloid hyoscyamine decomposed to give three peaks when injected into a GC. As an alternative, crinine was used to determine the recovery of alkaloids and the efficiency of the methods employed. Crinine was extracted in the same way used for the extraction of crude alkaloids from the plant material. The percentage recovery was within the range 92.4-101.8% with an average of 97.7% (Table 4.1).

Table 4.1. Recovery of crinine after extraction with $CHCl_3$ from the basified HCl solution.

Sample No.	Mean area	Recovery (μg)	Recovery (%)
1	60562.5	1175.9	97.9
2	63093.5	1222.4	101.8
3	56938.0	1109.4	92.4
4	62382.0	1209.3	100.7

4.2 Variation of alkaloids from *C. moorei*

4.2.1 Total alkaloid yield

Eleven Amaryllidaceae alkaloids belonging to three ring types were identified (Appendix 2). Lycorine **1** and 1-*O*-acetyllycorine **43** represent the lycorine-type alkaloids; crinine **32**, crinamine **34**, epibuphanisine **105**, powelline **73**, 3-*O*-acetylhamayne **47**, undulatine **44**, crinamidine **36** and 1-epideacetylbowdensine **108** the crinine-type alkaloids and cherylline **106** the third ring type. The alkaloids

crinamine and 3-*O*-acetylhamayne gave broad peaks compared to the sharp peaks obtained for the other alkaloids isolated. The alkaloids were tentatively identified by comparison of their retention times and mass spectra with corresponding pure compounds as presented in Chapter 3.

The results of the variation in total alkaloids are presented as the percentage of total alkaloids of dry plant material, and also as the percentage of different ring types of the total alkaloid content (Table 4.2).

4.2.1.1 Year 1998/1999

There was no significant seasonal variation in the percentage of total alkaloids isolated from *C. moorei*. The highest alkaloid percentage was obtained in Winter (1.19%) and the lowest in Spring (1.1%). In addition, there were no variations in the different classes of Amaryllidaceae alkaloids, expressed as a percentage of the total alkaloid content. Crinine-type alkaloids were the dominant group detected in *C. moorei*. They represented more than 70% of the total alkaloid content throughout the year. Cherylline was the minor component. The percentage of this alkaloid ranged between 4.4-8.1%. Lycorine-type alkaloids ranged between 14.3-18.8% (Table 4.2).

Different organs had different percentages of total alkaloids and also of different ring types of alkaloids. Leaves had the highest total alkaloid content in Summer (2.2%) and Spring (2.1%) followed by bulbs in Winter (1.7%) (Figure 4.1). Roots contained the highest percentage of lycorine-type alkaloids. It represented 33.5-47.9% of the total alkaloid yield of the root while it was a minor component of the other organs. Leaves had the highest crinine-type alkaloids expressed as a percentage of the total alkaloid content throughout the year (Figure 4.2).

4.2.1.2 Year 1999/2000

In contrast to the results obtained in the first year of study, seasonal variation was noticeable in the total alkaloids (%) and in the different ring types expressed

as a percentage of the total alkaloid content. The total alkaloid level (%) was higher in Spring (1.6%) and lower in Winter (0.91%). Lycorine-type alkaloids (%) were higher in Winter (29.8%) and lower in Spring (7.9%). Crinine-type alkaloids showed the same pattern as those in the first year, with the highest levels in Spring (87.9%) and the lowest in Winter (65.7%). Cherylline content was higher during Summer (10.7%) (Table 4.1).

Again, roots followed by bulbs had the highest levels of lycorine-type alkaloids, particularly during the dormancy period. Leaves had the highest crinine-type alkaloids in Spring followed by bulbs, throughout the year (Figure 4.3).

4.2.2 Individual alkaloid variation

Crinamidine was the most abundant alkaloid in *C. moorei*. It was the major alkaloid in the leaves and flowering stalks with an average of more than gram per 100 g of plant material. There was no seasonal variation in the crinamidine content of the leaf. Crinine and crinamidine were the major alkaloids in the bulbs. In the first year of the study, bulbs had a significantly higher crinine level in Winter while, in the year 1999/2000, the bulbs had a significantly higher crinine level in Summer. Throughout the study, bulbs had a significantly lower crinine content in Spring. There was no significant seasonal variation detected for crinamidine levels in the bulbs. However, bulbs had the highest levels of this alkaloid in Winter for the year 1998/1999 and in Summer for the year 1999/2000. In all cases, bulbs had medium levels of crinamidine during Spring.

Lycorine and 1-*O*-acetyllycorine were the major alkaloids present in the roots. Throughout the study period, roots had a significantly higher lycorine content in Winter and lower levels in Spring. No significant seasonal variation was detected in the 1-*O*-acetyllycorine content of the roots. However, in all cases, roots had the lowest 1-*O*-acetyllycorine content in Spring.

Organ-to-organ variation in each alkaloid are shown in Table 4.4 and Table 4.5

Table 4.2. Total alkaloids (%) and different alkaloid ring types detected in different seasons for *C. moorei.*

Year	Season	Total[1] (%)	Alkaloid type[2]		
			Lycorine	Crinine	Cherylline
1998/1999	Winter	1.17	18.6	73.3	8.1
	Spring	1.10	14.3	80.5	5.2
	Summer	1.19	18.8	76.8	4.4
1999 / 2000	Winter	0.91	29.8	65.7	4.5
	Spring	1.60	7.9	87.9	4.1
	Summer	1.40	15.6	73.7	10.7

[1] Total alkaloid content expressed as percentage of the dried plant material analysed.

[2] Expressed as percentage of the total alkaloid component detected.

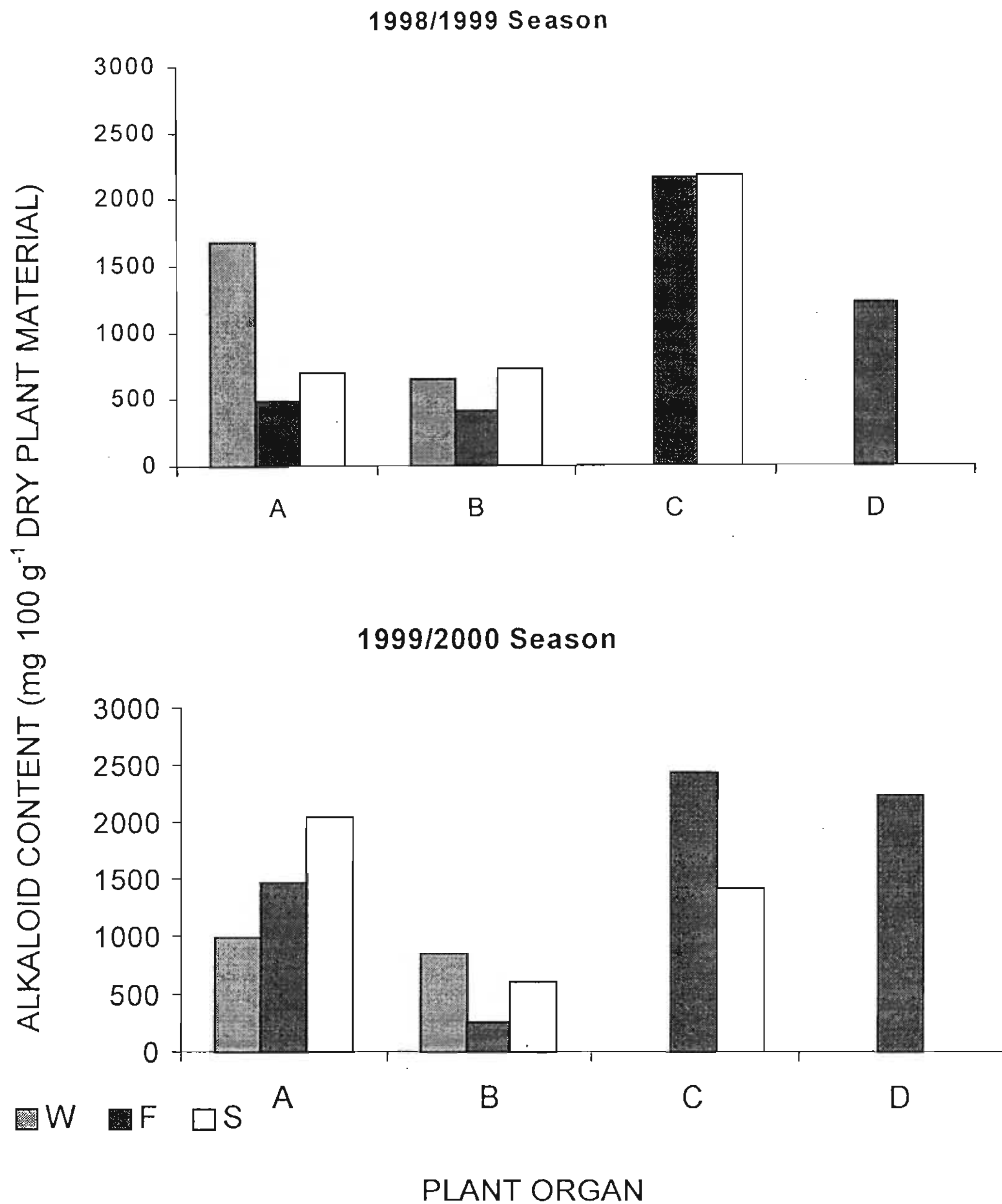


Figure 4.1. Alkaloids detected in different organs (A = bulb, B = root, C = leaf, D= flowering stalks) of *C. moorei* in different seasons (W = Winter, F = Spring, S = Summer) over the study period.

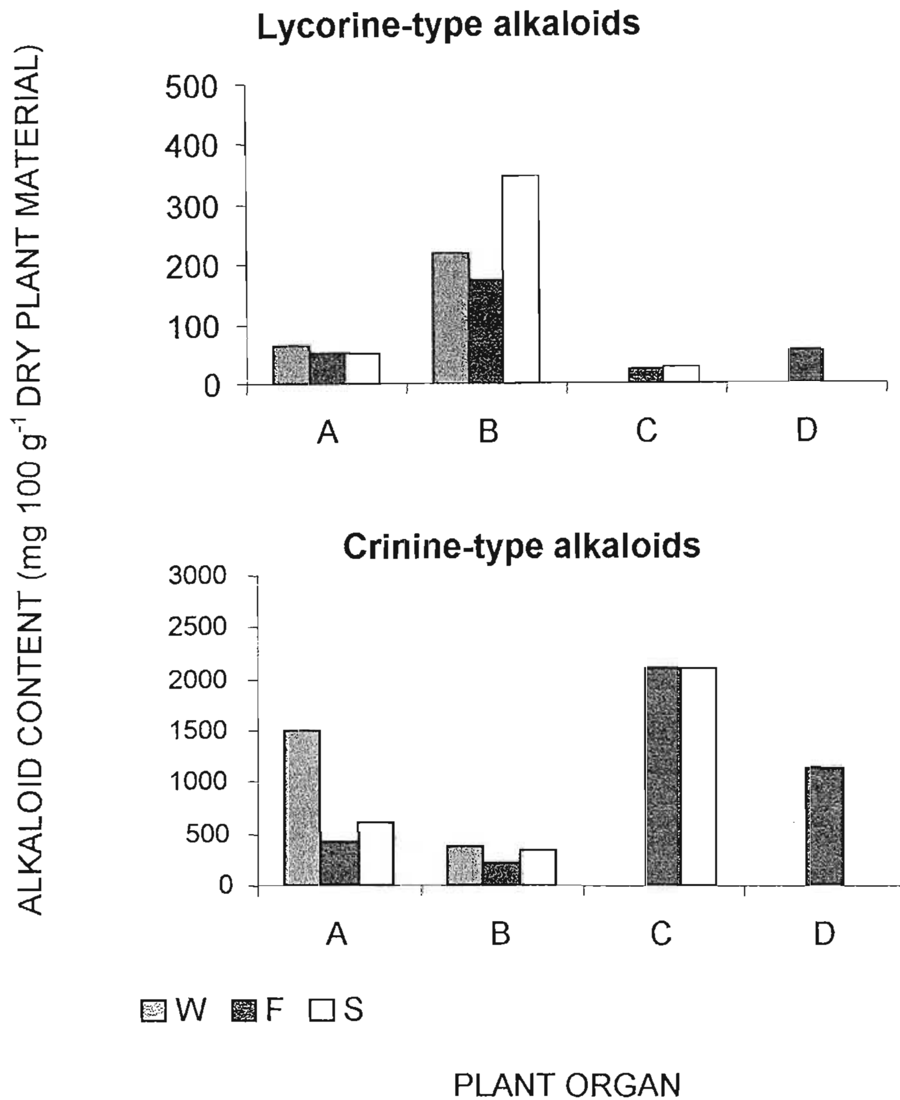


Figure 4.2. Different alkaloid ring types detected in different organs (A = bulb, B = root, C = leaf, D = flowering stalks) of *C. moorei* in different seasons (W = Winter, F = Spring, S = Summer) for the year 1998/1999.

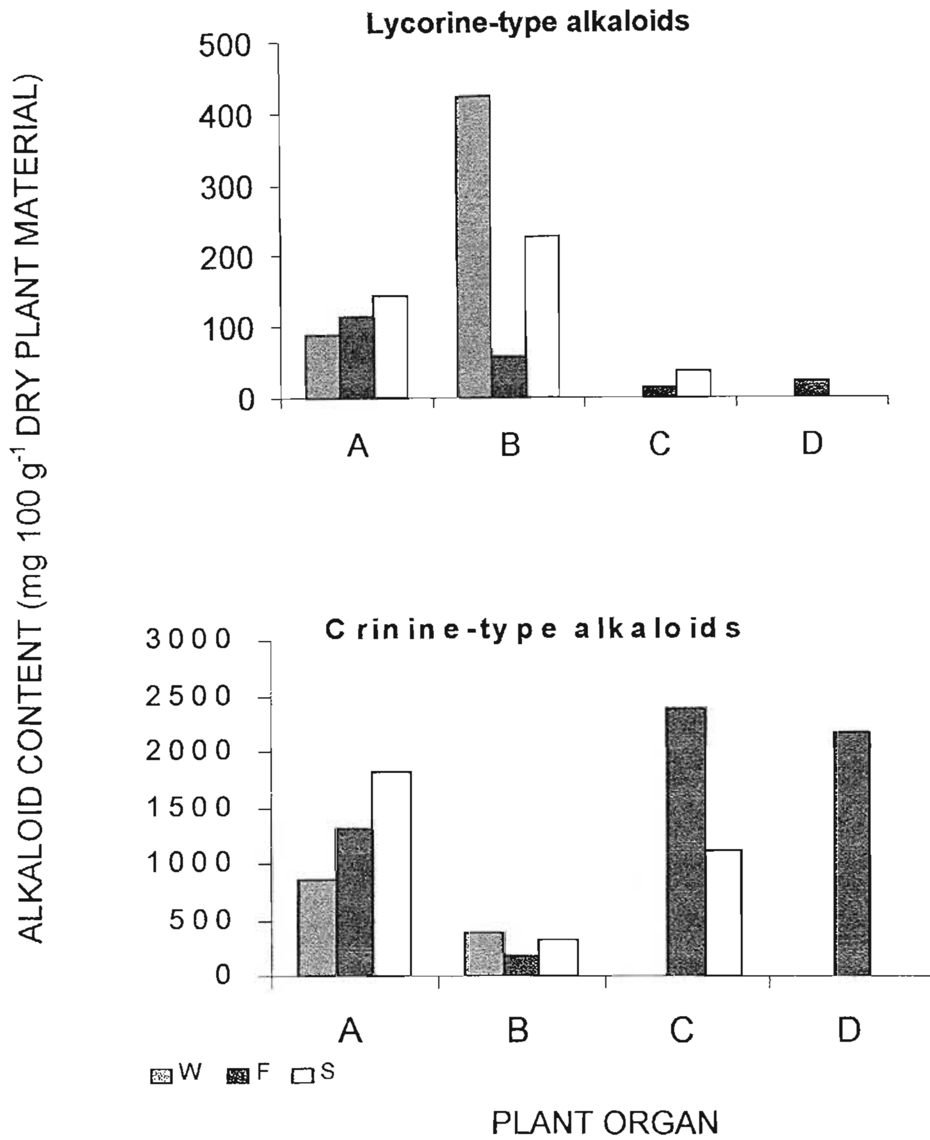


Figure 4.3. Different alkaloids ring types detected in different organs (A = bulb, B = root, C = leaf, D = flowering stalks) of *C. moorei* in different seasons (W = Winter, F = Spring, S = Summer) for the year 1999/2000.

for the year 1998/1999 and 1999/2000 respectively. Seasonal variations are presented in Table 4.6 and Table 4.7 for the same periods. Comparison of different means of logarithmically transformed data of individual alkaloids using the least significant difference test for organ-to-organ and seasonal variation are shown in Table 4.3.

4.2.2.1 Organ-to-organ variation

4.2.2.1.1 Lycorine 1

The roots had a significantly higher lycorine content than the flowering stalks and leaves. However, lycorine content of the leaves was significantly lower than that of the other organs. No significant differences in lycorine yield were observed between bulbs and roots on one hand and between bulbs and flowering stalks on the other hand.

4.2.2.1.2 Crinine 32

Bulbs had a significantly higher crinine content than the other organs. No significant differences were detected between leaves, roots and flowering stalks in the amount of crinine detected.

4.2.2.1.3 Crinamine 34

Crinamine was only detected in leaves and bulbs during Summer in the second year of the study period. Quantitatively, no significant differences were detected between the different plant organs in this alkaloid content.

4.2.2.1.4 Crinamidine 36

Wald statistics indicated the presence of significant differences between the differing organs in crinamidine content. Flowering stalks and leaves had a significantly high crinamidine content followed by the bulbs and then the roots.

4.2.2.1.5 1-*O*-Acetyllycorine 43

1-*O*-Acetyllycorine was not detected in the leaves, and flowering stalks

throughout the study period, and in bulbs collected in Summer of the year 1998/1999. 1-*O*-Acetyllycorine content was significantly higher in the roots than in the other organs.

4.2.2.1.6 Undulatine 44

Wald statistics revealed significant differences between the organs under investigation in undulatine content. Flowering stalks and bulbs yielded the highest undulatine levels. Bulbs and flowering stalks had a significantly higher undulatine yield than the roots. No statistical differences were detected in undulatine content between the roots and the leaves.

4.2.2.1.7 3-*O*-Acetylhamayne 47

During Winter of the first year of study, the bulbs had the highest 3-*O*-acetylhamayne content followed by the flowering stalks and leaves. In the second year of Study, leaves in Summer had the higest 3-*O*-acetylhamayne content followed by roots collected during Winter. No statistical differences were detected between different organs in their 3-*O*-acetylhamayne content.

4.2.2.1.8 Powelline 73

Wald statistics showed high variation in powelline content between different organs. Bulbs had a significantly higher powelline content, followed by the leaves, with the roots having the lowest yield. However, no significant differences in powelline content were detected between the flowering stalks and the leaves on one hand and between flowering stalks and roots on the other.

4.2.2.1.9 Epibuphanisine 105

There was no clear pattern of variation among different organs in the content of epibuphanisine. Bulbs in Winter had the highest epibuphanisine content during the study period followed by the bulbs during Summer in the first year and the bulbs during Spring in the second year. Epibuphanisine was not detected in the flowering stalks throughout the study period, in roots during Spring and or leaves in Summer during the second year of study. The change in quantity of

epibuphanisine in the different organs followed those of crinine and powelline in that an increase in the levels of crinine and powelline was accompanied by an increase of epibuphanisine content. Noticeable was that organs with low quantities of crinine were characterized by the absence of epibuphanisine.

Wald statistics revealed significant differences between the different organs in epibuphanisine content. Roots yielded a significantly lower epibuphanisine level than did the bulbs and the flowering stalks. No differences in epibuphanisine content were detected between bulbs and leaves on one hand and between roots and leaves on the other.

4.2.2.1.10 Cherylline 106

Cherylline is a minor alkaloid in all organs. Generally, the alkaloid yield ranged between 32-114 mg 100 g^{-1} during the first year compared to 25.9-264.5 mg 100 g^{-1} of plant material during the second year of study. No significant differences were observed for both season and organ. The bulbs yielded the highest yield in Winter and during flowering, while the leaf had the highest content in Summer. No clear pattern of variation was observed for either season or organ.

4.2.2.1.11 1-Epideacetylbowdensine 108

Flowering stalks and leaves had the highest content of 1-epideacetylbowdensine followed by the bulbs and the roots. No significant differences were detected between roots and the bulbs and between bulbs and the leaves in 1-epideacetylbowdensine content.

4.2.2.2 Seasonal variation

Seasonal variation in individual alkaloid content in different organs was detected only for crinine, lycorine and undulatine (Table 4.3).

4.2.2.2.1 Lycorine 1

No significant differences were detected in lycorine content of bulbs and roots in

Winter and in Spring. However, lycorine content during Spring was significantly lower than during Summer and Winter for roots and bulbs, and than in Summer for the leaves.

4.2.2.2.2 Crinine 32

Wald statistics showed highly significant seasonal differences in crinine content of the organs under investigation. Bulbs and roots had a significantly higher crinine levels in Winter and Summer followed by Spring. In the leaves, the crinine content was significantly higher during Summer compared to Spring.

4.2.2.2.3 Undulatine 44

Bulbs and roots had the highest undulatine content in Summer followed by the flowering season. No significant differences were observed in undulatine content of bulbs and roots in Summer and Spring, and between Spring and Winter. Significant differences were detected in undulatine yield of the bulbs in Winter and Summer. The same seasonal variation in undulatine yield was observed for the roots. Leaves were characterized by a lack of seasonal variation in undulatine content.

Conclusion

C. moorei is a rich source of Amaryllidaceae alkaloids on a dry weight basis. The total alkaloids represented more than one percent of the plant material analysed. No clear pattern of variation in total alkaloid yield and different ring types was detected. Crinine-type alkaloids were the major component of the alkaloid detected. It represented more than 65% of the total alkaloids throughout the study period. Leaves of *C. moorei* had the highest alkaloid levels throughout the study period. The major alkaloid in the leaves was crinamidine. It represented more than 80% of the total alkaloid yield of the leaves. An exception was the low yield (20%) of leaves in the Summer of the second year of the study. Crinamidine was also the major alkaloid of the flowering stalks. Roots had the highest lycorine-type alkaloid content. Bulbs could be reliable sources of the alkaloids crinine, epibuphanisine, powelline and cherylline. There was no clear pattern of

variation to allow for plant collection for alkaloid isolation at a specific time of the year. However, the abundance of alkaloids in flowering stalks and leaves made it economically feasible to collect the plant during Spring for alkaloid isolation.

Table 4.3. Variation of logarithmically transformed data for individual alkaloids in different seasons from different plant organs for *C. moorei*.

		Alkaloid detected										
		32	1	106	105	73	44	108	36	47	43	34
Organ	Bulb	6.908a	5.744ab	5.454a	176.1a	5.734a	5.559a	5.506ab	7.391a	88.2a	4.364a	4.087a
	Root	5.227b	6.075a	5.269a	8.64b	3.427c	4.676b	5.269b	6.171b	43.5a	5.975b	3.990a
	Leaf	5.353b	4.771c	5.441a	103.8ab	4.336b	5.142ab	6.027ac	8.261c	160.5a	3.966a	4.175a
	Flower*	5.249b	5.381b	5.565a	48.72a	4.044bc	5.866a	6.429c	8.699c	215.3a	4.124a	4.084a
	S.E	0.2943	0.2088	0.1806	81.24	0.3056	0.4152	0.186	0.3590	71.22	0.4185	0.1215
	LSD	0.6159	0.437	0.3779	170.04	0.6396	0.869	0.3892	0.7513	149.06	0.6174	0.2542
Season	Winter	6.286a	5.713a	5.556a	192.95a	4.856a	4.335a	5.999a	7.621a	160.1a	4.686a	4.051a
	Spring	5.115b	5.242b	5.171a	22.75a	3.922a	5.265ab	5.601a	7.709a	65.9a	4.376a	4.006a
	Summer	5.652c	5.523a	5.570a	37.23a	4.378a	6.334b	5.823a	7.561a	154.7a	4.759a	4.195a
	S.E	0.2560	0.1228	0.3137	87.05	0.3834	0.7090	0.1988	0.4039	71.11	0.2482	0.1162
	LSD	0.5196	0.2492	0.6368	176.71	0.7783	1.4392	0.4035	0.8199	144.35	0.5038	0.2358

Means of individual alkaloids levels detected in different organs and in different seasons. Values within the same column with the same letter are not significantly different from each other.

* Flower = flowering stalks.

Table 4.4. Organ-to-organ variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weighty) of *Crinum moorei* for the year 1998/1999.

Season	Organ	Alkaloid detected										Total	Yield (%)
		32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**43**		
Winter	Bulb	369.5a	60.8a	114.8a	58.7a	69.4a	92.5a	267.3a	598.6a	48.8a	3.5a	1683.9	1.7
	Root	111.8b	129.7a	60.5a	13.8b	7.5b	39.2b	47.0a	143.6b	10.5a	89.2b	652.8	0.7
Spring	Bulb	85.9a	44.6ab	38.8a	1.9a	22.3a	60.4a	34.2ab	199.3a	1.9a	5.3a	494.6	0.5
	Root	16.6b	108.2a	32.2a	0.0b	4.2b	36.8b	49.3a	86.2b	18.7a	65.0b	417.2	0.4
	Leaf	152.5b	23.1c	34.3a	34.5ab	17.4c	26.3ab	76.9cb	1746.7c	38.1a	0.0a	2149.2	2.2
	Flower*	23.1b	55.8b	42.4a	0.0a	8.5c	69.3a	82.9c	897.5c	40.1a	0.0a	1219.6	1.2
Summer	Bulb	210.9a	51.8ab	32.3a	51.6a	72.7a	44.1a	57.8ab	162.4a	14.7a	0.0a	698.3	0.7
	Root	77.8b	61.8a	44.3a	1.7b	23.7b	68.4b	50.4a	83.3b	26.0a	284.0b	721.4	0.7
	Leaf	27.6b	25.8b	50.9a	0.0ab	24.1c	74.5ba	155.1b	1768.1c	38.1a	0.0a	2164.2	2.2

Means of individual alkaloid levels detected in different organs in the same season. Values within the same column with the same letter are not significantly different from each other.

* Flower = flowering stalks.

Table 4.5. Organ-to-organ variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) of *Crinum moorei* for the year1999/2000.

Season	Organ	Alkaloid detected											Total	Yield (%)
		32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**43**	**34**		
Winter	Bulb	284.8a	79.0a	43.3a	88.2a	128.8a	35.9a	41.7a	250.8a	19.6a	11.0a	0.0a	982.6	1.0
	Root	139.4b	130.1a	38.4a	1.3b	32.9b	17.0b	48.7a	104.3b	34.1a	291.6b	0.0a	837.8	0.8
Spring	Bulb	114.5a	80.7ab	50.0a	10.2a	75.9a	121.3a	57.1ab	905.2b	18.3a	34.6a	0.0a	1467.8	1.5
	Root	19.9b	37.8a	25.9a	0.9b	3.4c	13.4b	25.9a	112.7c	1.1a	20.8b	0.0a	261.8	0.3
	Leaf	28.2b	14.2c	28.6a	0.9ab	44.0b	36.4ab	72.3bc	2195.7a	9.2a	0.0a	0.0a	2429.5	2.4
	Flower*	20.8b	23.8b	39.0a	0.0ab	6.3b	69.4a	127.6c	1909.8a	21.6a	0.0a	0.0a	2218.3	2.2
Summer	Bulb	305.5a	101.8ab	68.2a	2.7a	103.0a	117.8a	42.2ab	1221.1a	24.5a	41.0a	7.0a	2034.8	2.0
	Root	31.6b	117.7a	61.5a	3.6b	4.3c	75.5b	33.8a	157.4b	17.0a	108.4b	0.0a	610.8	0.6
	Leaf	37.9b	37.1b	264.5a	8.5ab	8.5b	617.6ab	77.4b	287.8c	50.4a	0.0a	13.5a	1409.1	1.4

Means of individual alkaloid levels detected in different organs in the same season. Values within the same column with the same letter are not significantly different from each other.

* Flower = flowering stalks.

Table 4.6. Seasonal variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) detected in different organs of *Crinum moorei* for the year 1998/1999.

Plant Organ	Season	Alkaloid detected									
		32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**43**
Bulb	Winter	369.5a	60.8a	114.8a	58.7a	69.4a	92.5a	267.3a	598.6a	48.8a	3.5a
	Spring	85.9c	44.6b	38.8a	1.9a	22.3a	60.4ab	34.2a	199.3a	1.9a	5.3a
	Summer	210.9b	51.8a	32.3a	51.6a	72.7a	44.1b	57.8a	162.4a	14.7a	0.0a
Root	Winter	111.8a	129.7a	60.5a	13.8a	7.5a	39.2a	47.0a	143.6a	10.5a	89.2a
	Spring	16.6c	108.2b	32.2a	0.0a	4.2a	36.8ab	49.3a	86.2a	18.7a	65.0a
	Summer	77.8b	61.8a	44.3a	1.7a	23.7a	68.4b	50.4a	83.3a	26a	284.0a
Leaf	Spring	152.5a	23.1a	34.3a	34.5a	17.4a	26.3a	76.9a	1746.7a	38.1a	0.0a
	Summer	27.6b	25.8b	50.9a	0.0a	24.1a	74.5a	155.1a	1768.1a	38.1a	0.0a

Means of individual alkaloid levels detected in the same organ in different seasons. Values within the same column with the same letter are not significantly different from each other.

Table 4.7. Seasonal variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) detected in different organs of *Crinum moorei* for the year 1999/2000.

Plant Organ	Season	Alkaloid detected										
		32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**43**	**34**
Bulb	Winter	284.8a	79.0a	43.3a	88.2a	128.8a	35.9a	41.7a	250.8a	19.6a	11.0a	0.0a
	Spring	114.5c	80.7b	50.0a	10.2a	75.9a	121.3ab	57.1a	905.2a	18.3a	34.6a	0.0a
	Summer	305.5b	101.8a	68.2a	2.7a	103.0a	117.8b	42.2a	1221.1a	24.5a	41.0a	7.0a
Root	Winter	139.4a	130.1a	38.4a	1.3a	32.9a	17.0a	48.7a	104.3a	34.1a	291.6a	0.0a
	Spring	19.9c	37.8b	25.9a	0.9a	3.4a	13.4ab	25.9a	112.7a	1.1a	20.8a	0.0a
	Summer	31.6b	117.7a	61.5a	3.6a	4.3a	75.5b	33.8a	157.4a	17.0a	108.4a	0.0a
Leaf	Spring	28.2a	14.2b	28.6a	0.9a	44.0a	36.4a	72.3a	2195.7a	9.2a	0.0a	0.0a
	Summer	37.9b	37.1a	264.5a	8.5a	8.5a	617.6a	77.4a	287.8a	50.4a	0.0a	13.5a

Means of individual alkaloid levels detected in the same organ in different seasons. Values within the same column with the same letter are not significantly different from each other.

CHAPTER 5

Organ-to-organ and seasonal variation of alkaloids in *C. macowanii*

Seven alkaloids belonging to three ring types of Amaryllidaceae alkaloids were identified during the first year of study. Crinine **32**, powelline **73**, crinamine **34** and 3-*O*-acetylhamayne **47** are crinine-type alkaloids. Lycorine **1** and 1-*O*-acetyllycorine **43** represent the lycorine-type. Cherylline **106** represents the third ring type of Amaryllidaceae alkaloids. In addition to the above, 1-epideacetylbowdensine **108** and crinamidine **36** were detected in the second year of study. 1-*O*- Acetyllycorine was excluded from the organ-to-organ and seasonal quantitative variation due to poor separation from an unidentified compound (Appendix 2).

5.1 Total quantified alkaloid content

In both years of study, the total quantified alkaloid content (%) was high in Winter and low during Summer. Expressed as a percentage of the plant material, the ranges were 0.05 to 0.12% and 0.08 to 0.17% in the first and second years of study respectively. In both years, Winter had the highest alkaloid content (%) followed by Summer (Table 5.1).

The crinine-type alkaloids represented 37.3-53.8% of the total alkaloids quantified during the first year of study (Table 5.1). In all seasons, bulbs had the highest crinine -type alkaloids (%), with the range 60.2-67.3% during Summer and Winter respectively . The roots contained 18.4- 40.2% of the crinine- type alkaloid during Spring and Winter respectively. Flowering stalks had a lower crinine- type alkaloid content (29.6%) (Table 5.3).

In the second year of study, the crinine-type alkaloids expressed as a percentage of the total alkaloids represented 53.6- 59.8% during Winter and Spring respectively. As in the first year, the bulbs contained the highest levels of crinine- type alkaloids, in all seasons, within the range 63.3-65% during Summer and Winter respectively. Percent wise, following the leaves (0%), the flowering stalks had a low level of the crinine- type alkaloids(24.8%). Bulbs had the highest level of crinine-type alkaloids. The roots had the highest crinine-type alkaloids yield (%) during Summer followed by Winter (Table 5.4).

5.2 Variation in individual alkaloid levels

5.2.1 Organ-to-organ variation

From data presented in Tables 5.5 and 5.6, it is clear that crinine and lycorine were the major alkaloids in the bulbs. Bulbs had significantly higher levels of lycorine in Winter compared to Summer. No significant seasonal variation was detected in lycorine content of the bulb. However, lycorine content was higher in Winter and lower in Spring throughout the study period. Lycorine was the major alkaloid in the roots. No significant seasonal variation and no clear pattern of variation were detected in lycorine content throughout the study period.

Organ-to-organ variation of the quantified alkaloids from *C. macowanii* are shown in Tables 5.3 and 5.4 for the years 1998/99 and 1999/2000 respectively. Comparison of different means of logarithmically transformed data of individual alkaloids using the least significant difference test for organ-to-organ and seasonal variation are shown in Table 5.2. Wald statistics (Appendix 4) showed significant differences between the different plant organs in their content of crinine, lycorine, powelline, crinamidine and crinamine.

5.2.1.1 Lycorine 1

Lycorine was the only alkaloid to be detected in trace amounts in the leaves of *C. macowanii* in the second year of study. Bulbs had a significantly higher lycorine content compared to the roots. The latter yielded significantly higher lycorine levels than the leaves and the flowering stalks.

5.2.1.2 Crinine 32

Crinine was detected in the bulbs and the roots throughout the study period and in the flowering stalks in the first year of study. Bulbs had a significantly higher crinine content than the other organs analysed. No significant differences were detected in crinine content of the flowering stalks and the roots. During Spring, flowering stalks ranked next to the bulbs in crinine levels.

5.2.1.3 Crinamine 34

Crinamine was a characteristic of the roots in all seasons during the study period. Exceptions were the bulbs collected during Spring which had a lower content.

5.2.1.4 Crinamidine 36

Crinamidine was detected in the bulbs collected in Summer and Spring as well as the roots collected in Summer in the second year of study. The bulbs had a significantly higher amount of crinamidine than the roots.

5.2.1.5 Powelline 73

Powelline was a minor alkaloid in *C. macowanii.* No clear pattern of its occurrence in the first year was observed. Powelline was detected in the bulbs and the roots during Winter, and in the roots during Summer in the first year . In the second year, powelline was detected in the roots in all seasons and in the bulbs during Spring. Roots had a statistically higher powelline content than the other organs in Winter and Summer, and in Spring in the second year of study.

The detected quantities of cherylline, 1-epideacetylbowdensine and 3-*O*-acetylhamayne were not very important in the overall alkaloid content of the different plant organs. 1-Epideacetylbowdensine was detected in the bulbs during Spring of the second year of study. Cherylline was present in the bulbs throughout the study period and in the flowering stalks during the second year of study. 3-*O*-Acetylhamayne was not detected in the leaves throughout the study

and in the flowering stalks and roots collected during Summer of the year 1998/99.

5.2.2 Seasonal variation in individual alkaloids

Seasonal variation were detected for crinine, 1-epideacetylbowdensine and crinamine. (Table 5.2). Seasonal variation of the detected alkaloids from *C. macowanii* are shown in Table 5.5 and Table 5.6 for the year 1998/99 and 1999/2000 respectively.

5.2.2.1 Crinine 32

The bulbs had a clear pattern of variation in crinine content in the different seasons throughout the study period. The bulbs contained a significantly higher crinine levels in Winter followed by Spring. This pattern of variation in crinine content was not similar in the roots . During the year 1998/1999, the roots had a higher crinine content during Winter followed by Summer. While in the second year of study, roots collected during Spring had the highest levels followed by Winter.

5.2.2.2 Crinamine 34

Crinamine content of the roots during Winter is significantly higher compared to that in Summer but not from that in Spring. No significant differences in crinamine content were detected between organs collected in Spring and Summer.

5.2.2.3 1-Epideacetylbowdensine 108

1-Epideacetylbowdensine was detected in the flowering bulbs during the second year of study. The amount of 1-epideacetylbowdensine detected was significantly high.

Conclusion

The total alkaloids (%) of *C. macowanii* was relatively low compared to that of *C. moorei*. Lycorine was the major alkaloid. It represented more than 40% of the

total alkaloid content throughout the study period. Collectively, crinine-type alkaloids represented 37.3-55.6% of the total alkaloids throughout the study period. As in *C. moorei*, no pattern of variation was observed in total alkaloid yield and different ring types was observed. The major alkaloids crinine and lycorine were abundant in winter. In addition, cherylline, powelline and 3-*O*-acetylhamayne were detected in appreciable levels in Winter. However, the alkaloids 1-epideacetylbowdensine and crinamidine were detected during Summer and Spring. From the above, the best collection time of plant material for alkaloid isolation depends very much upon the alkaloid to be isolated.

5.1. Total quantified alkaloid (%) and different alkaloid ring types for *C. macowanii.*

Year	Season	Total[1] (%)	Alkaloid type[2]		
			Lycorine	Crinine	Cherylline
1998/ 1999	Winter	0.12	42.3	53.8	3.9
	Spring	0.05	52.2	37.3	10.5
	Summer	0.06	51.3	48.7	0.0
1999/ 2000	Winter	0.17	44.5	53.6	1.9
	Spring	0.08	43.8	44.3	11.9
	Summer	0.12	41.2	55.6	3.2

[1] Total alkaloid content expressed as a percentage of the dried plant material analysed.

[2] Expressed as percentage of the total alkaloid component detected.

Table 5.2. Variation of logarithmically transformed data for individual alkaloids in different seasons from different plant organs of *C. macowanii.*

		Alkaloid detected							
		32	**1**	**106**	**73**	**36**	**108**	**47**	**34**
Plant Organ	Bulb	5.937a	5.537a	4.825a	2.540a	4.890a	4.415a	30.44a	4.024a
	Root	4.286b	5.272b	4.788a	2.645b	4.830b	4.406a	17.90ab	4.119b
	Leaf	4.244b	4.219c	4.797a	2.500a	4.806b	4.403a	1.00b	3.995b
	Flower*	4.279b	4.314c	4.874a	2.492a	4.811b	4.399a	21.24ab	4.017a
	S.E.	0.1354	0.08449	0.037	0.03654	0.0273	0.00576	13.99	0.02974
	LSD	0.2783	0.1737	0.0767	0.07512	0.05612	0.01184	28.763	0.06114
Season	Winter	4.840a	4.871a	4.842a	2.513a	4.798a	4.401a	6.952ab	4.017a
	Spring	4.695ab	4.801a	4.814a	2.568a	4.847b	4.413b	1.00b	4.027a
	Summer	4.524b	4.833a	4.807a	2.552a	4.857b	4.403a	17.775a	4.027b
	S.E.	0.1211	0.06344	0.0319	0.03087	0.02301	0.00465	6.733	0.0217
	LSD	0.243	0.1273	0.064	0.0619	0.04618	0.00934	13.515	0.0435

Means of individual alkaloid levels detected in different organs and in different seasons. Values within the same column with the same letter are not significantly different from each other.

* Flower = flowering stalks.

Table 5.3. Organ-to-organ variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) of *C.macowanii* for the year 1998/1999.

Season	Plant	Alkaloid detected						Crinine-type		Total	Total
		32	**1**	**106**	**73**	**47**	**34**	A	A(%)		
Winter	Bulb	80.1a	46.7a	14.6a	3.0a	43.6a	0.0a	126.7	67.3	188.0	0.19
	Root	11.4b	27.5b	0.0a	0.6b	0.3a	6.2b	18.5	40.2	46.0	0.05
Spring	Bulb	75.6a	41.1a	4.9a	0.0a	0.8a	5.8a	82.2	64.1	128.2	0.13
	Root	2.2b	39.6b	0.0a	0.0a	1.9a	4.8b	8.9	18.4	48.5	0.05
	Leaf	0.0b	0.0c	0.0a	0.0a	0.0b	0.0a	0.0	0.0	0.0	0.00
	Flower*	11.0b	15.9c	10.3a	0.0a	0.0a	0.0a	11	29.6	37.2	0.04
Summer	Bulb	70.1a	46.5a	0.0a	0.0a	0.3a	0.0a	70.4	60.2	116.9	0.12
	Root	10.7b	44.6b	0.0a	1.6b	0.0a	14.0b	26.3	37.1	70.9	0.07
	Leaf	0.0b	0.0c	0.0a	0.0a	0.0b	0.0a	0.0	0.0	0.0	0.00

Means of individual alkaloid levels detected in different organs in the same season. Values within the same column with the same letter are not significantly different from each other.

[1] A. Total crinine-type alkaloids. A(%). A expressed as a percentage of the total alkaloid levels.

[2] Expressed as percentage of the plant material analysed.

* Flower = flowering stalks.

Table 5.4. Organ-to-organ variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) of *C. macowanii* for the year 1999/2000.

Season	Plant	Alkaloid detected								Crinine-type[1]		total	Total[2]
		32	**1**	**106**	**73**	**108**	**36**	**47**	**34**	A	A (%)		
Winter	Bulb	168.3a	81.1a	9.8a	0.0a	0.0a	0.0a	0.2a	0.0a	168.5	65.0	259.4	0.26
	Root	13.3b	45.4b	0.0a	0.6b	0.0a	0.0b	7.5a	12.4b	33.2	42.2	78.6	0.08
Spring	Bulb	124.7a	47.3a	15.8a	0.7a	10.8a	16.3a	0.2a	0.0a	152.7	70.8	215.8	0.22
	Root	18.3b	47.6b	0.0a	3.2b	0.0a	0.0b	2.1a	4.7b	28.3	37.3	75.9	0.08
	Leaf	0.0b	0.0c	0.0a	0.0a	0.0a	0.0b	0.0b	0.0a	0.0	0.0	0.0	0.00
	Flower*	0.0b	8.2c	5.0a	0.0a	0.0a	0.0b	4.3a	0.0a	4.3	24.8	17.5	0.18
Summer	Bulb	45.6a	47.8a	10.0a	0.0a	0.0a	30.8a	23.4a	0.0a	99.8	63.3	157.6	0.16
	Root	12.2b	38.4b	0.0a	2.1b	0.0a	6.0b	1.5a	13.6b	35.4	48.0	73.8	0.07
	Leaf	0.0b	2.8c	0.0a	0.0a	0.0a	0.0b	0.0b	0.0a	0.0	0.0	0.0	0.00

Means of individual alkaloid levels detected in different organs in the same season. Values within the same column with the same letter are not significantly different from each other.

[1] A. Total crinine-type alkaloids. A (%). A expressed as percentage of the total alkaloid levels.

[2]Expressed as percentage of the plant material analysed.

* Flower = flowering stalks.

Table 5.5. Seasonal variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) detected in different organs of *Crinum macowanii* for the year 1998/1999.

Organ	Season	Alkaloid detected					
		32	**1**	**106**	**73**	**47**	**34**
Bulb	Winter	80.1a	46.7a	14.6a	3.0a	43.6ab	0.0a
	Spring	75.6ab	41.1a	4.9a	0.0a	0.8a	5.8a
	Summer	70.1b	46.5a	0.0a	0.0a	0.3b	0.0b
Root	Winter	11.4a	27.5a	0.0a	0.6a	0.3ab	6.2a
	Spring	2.2ab	39.6a	0.0a	0.0a	1.9a	4.8a
	Summer	10.7b	44.6a	0.0a	1.6a	0.0b	14.0b
Leaf	Spring	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	Summer	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

Means of individual alkaloid levels detected in the same organ in different seasons. Values within the same column with the same letter are not significantly different from each other.

Table 5.6. Seasonal variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) detected in different organs of *Crinum macowanii* for the year1999-2000.

Plant Organ	Season	Alkaloid detected							
		32	**1**	**106**	**73**	**108**	**36**	**47**	**34**
Bulb	Winter	168.3a	81.1a	9.8a	0.0a	0.0a	0.0a	0.2ab	0.0a
	Spring	124.7ab	47.3a	15.8a	0.7a	10.8b	16.3b	0.2a	0.0a
	Summer	45.6b	47.8a	10.0a	0.0a	0.0a	30.8b	23.4b	0.0a
Root	Winter	13.3a	45.4a	0.0a	0.6a	0.0a	0.0a	7.5ab	12.4a
	Spring	18.3ab	47.6a	0.0a	3.2a	0.0a	0.0b	2.1a	4.7a
	Summer	12.2b	38.4a	0.0a	2.1a	0.0a	6.0b	1.5b	13.6b
Leaf	Spring	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	Summer	0.0a	2.8a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

Means of individual alkaloid levels detected in the same organ in different seasons. Values within the same column with the same letter are not significantly different from each other.

CHAPTER 6

Organ-to-organ and seasonal Variation of alkaloids in *C. bulbispermum*

As indicated in Chapter 2, different organs of *C. bulbispermum* ,collected in three different seasons namely Summer, Winter and Spring, were extracted with dilute HCl solution and analysed for alkaloids using GC. The results of the GC chromatogram indicated the presence of crinine **32**, lycorine **1**, 3-*O*-acetylhamayne **47**, crinamine **34** and 6-hydroxycrinamine **22**, **23** (Appendix 2) . The alkaloid 6-hydroxycrinamine was decomposed and gave many peaks. These results are therefore excluded from the present calculations.

6.1 Total quantified alkaloid content

The concentration of quantified alkaloids of dry plant material ranged between 0.06% in Spring and 0.02% in Summer during the first year. In contrast, the total alkaloids (%) was the lowest during Spring (0.03%) and highest during Summer (0.04%) in the second year of the study. In all seasons, bulbs had the highest alkaloid content. The highest levels were detected during Spring and then Summer throughout the study period. The flowering stalks ranked second in their alkaloid content (Tables 6.1 and 6.2). The GC results used could not detect alkaloids in the leaves throughout the study period (Tables 6.1 and 6.2).

6.2 Individual alkaloid variation

6.2.1 Organ-to-organ variation

Lycorine was the major alkaloid in the bulbs, roots and flowering stalks of *C. bulbispermum*. Bulbs had a significantly higher lycorine content in Summer and Spring compared to Winter. Lycorine levels in the roots were significantly higher in Spring compared to those in Winter. Bulbs also had a considerably higher crinamine content. The levels of this alkaloid in the bulbs were higher in Spring compared to those in Summer and Winter (Table 6.4).

Organ-to-organ variation in the detected alkaloids from *C. bulbispermum* are shown in Tables 6.1 and 6.2 for the year 1998/99 and 1999/2000 respectively. Comparison of different means of logarithmically transformed data of individual alkaloids using the least significant difference test for organ-to-organ and seasonal variation are shown in Table 6.3. Wald statistics (Appendix 4) indicated significant differences between the different plant organs in the content of alkaloids under investigation.

6.2.1.1 Lycorine 1

Lycorine was detected in the bulbs, roots and the flowering stalks but not in the leaf extracts. Bulbs had a significantly higher lycorine content followed by the roots and the flowering stalks. No significant differences in lycorine levels were detected between the flowering stalks and the roots.

6.2.1.2 Crinine 32

Crinine was detected in the flowering stalks and the bulbs throughout the study period, and in the roots in Spring of the first year of the study. The flowering stalks had the highest crinine content followed by the bulbs.

6.2.1.3 Crinamine 34

The highest quantity of crinamine was detected in the bulbs during Spring followed by the roots of the flowering bulbs in 1998/1999, and by the flowering stalks in the second year of the study period. Crinamine, however, was not detected in the roots of the dormant bulbs and in the roots and leaves investigated during Summer in the first year of study. Bulbs had a significantly higher crinamine content compared to the roots .

6.2.1.4 3-*O*-Acetylhamayne 47

3-*O*-Acetylhamayne was a minor alkaloid in all plant parts. 3-*O*-Acetylhamayne was detected in the bulbs and the roots and as a trace in the flowering stalks. The highest quantity of this alkaloid was obtained from the bulbs collected during

Summer of the second year of study. However, throughout the study period, 3-*O*-acetylhamayne content of the bulbs was not significantly higher than that of the roots. The latter two organs yielded significantly more 3-*O*-acetylhamayne than in the flowering stalks.

6.2.2 Seasonal variation in individual alkaloids

Wald statistics indicated significant seasonal variation in the content of lycorine and 3-*O*-acetylhamayne detected in each plant organ (Table 6.4).

6.2.2.1 Lycorine 1

Wald statistics revealed highly significant differences in lycorine content of different organs in the three seasons. The bulbs contained more lycorine in Summer and Spring for the two successive years respectively, while bulbs collected in the dormancy period had the lowest amount. Lycorine content of the roots, was higher during Spring followed by Summer. Lycorine content of roots and bulbs collected in Spring and Summer were significantly higher than those collected in Winter. No significant differences were detected in lycorine yield of the roots and bulbs collected in Spring and Summer.

6.2.2.2 3-*O*-Acetylhamayne 47

3-*O*-Acetylhamayne was a minor component of the total alkaloid component of *C. bulbispermum*. Roots and bulbs had the highest 3-*O*-acetylhamayne content in Summer throughout the study period. No significant differences were detected in the 3-*O*-acetylhamayne content of bulbs and roots collected during Summer and Winter.

Conclusion

There was no clear pattern of variation in the total alkaloid levels of *C. bulbispermum*. The contradictory results between the first and second year of study in alkaloid percent made it difficult to pinpoint a specific season for alkaloid

isolation from this species. However, the profile was different for individual alkaloids. Results indicated that roots and flowering stalks had the highest lycorine content during the first year while bulbs and roots had the highest content during Spring of the second year of study. Crinine and crinamine were present in higher levels in the bulbs in Spring throughout the study period. From the above, it would appear to be more economical to collect the plant during Spring for alkaloid isolation.

Table 6.1. Organ-to-organ variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) of *C. bulbispermum* for the year 1998/1999.

Season	Plant Organ	Alkaloid detected					Total (%)[1]
		32	**1**	**47**	**34**	Total	
Winter	Bulb	12.2a	18.5a	0.4a	7.9a	39.0	0.03
	Root	0.0b	14.7b	5.3a	0.0b	20.0	
Spring	Bulb	12.7a	24.9a	3.9a	38.4a	79.9	0.06
	Root	6.5b	35.7b	5.7a	18.8bc	66.7	
	Leaf	0.0b	0.0c	0.0b	0.0c	0.0	
	Flower*	21.7c	38.2b	0.1b	16.5ab	76.5	
Summer	Bulb	11.7a	27.4a	0.5a	15.4a	55.0	0.02
	Root	0.0b	15.6b	1.6a	0.0b	17.2	
	leaf	0.0b	0.0c	0.0b	0.0b	0.0	

Means of individual alkaloid levels detected in different organs in the same season. Values within the same column with the same letter are not significantly different from each other.

[1] Total alkaloid yield expressed as a percentage of the plant material analysed.

* Flower = flowering stalks.

Table 6.2. Organ-to-organ variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) of *C. bulbispermum* for the year1999/2000.

Season	Plant Organ	Alkaloid detected				Total	Total (%)[1]
		32	**1**	**47**	**34**		
Winter	Bulb	10.4a	27.1a	2.6a	4.8a	44.9	0.04
	Root	0.0b	19.3b	7.5a	2.8b	29.6	
Spring	Bulb	14.5a	42.4a	2.0a	60.3a	119.2	0.03
	Root	0.0b	31.4b	1.0a	9.3bc	41.7	
	Leaf	0.0b	0.0c	0.0b	0.0c	0.0	
	Flower*	21.7c	25.4b	0.4b	16.5ab	64.0	
Summer	Bulb	13.4a	37.7a	19.2a	16.0a	86.3	0.04
	Root	0.0b	26.2b	5.5a	2.5b	34.2	
	leaf	0.0b	0.0c	0.0b	0.0b	0.0	

Means of individual alkaloid levels detected in different organs in the same season. Values within the same column with the same letter are not significantly different from each other.

[1] Total alkaloid expressed as a percentage of the plant material.

* Flower = flowering stalks.

Table 6.3. Variation of logarithmically transformed data for individual alkaloids in different seasons from different plant organs of *C. bulbispermum*.

		Alkaloid detected			
		32	**1**	**47**	**34**
Plant Organ	Bulb	4.117a	4.96a	24.33a	4.607a
	Root	3.676b	4.694b	26.44a	4.133bc
	Leaf	3.608b	3.268c	18.1b	3.847c
	Flower*	4.536c	4.706b	1.00b	4.456ab
	S. E.	0.07638	0.1226	8.987	0.1926
	LSD	0.157037	0.252066	18.477	0.3959
Season	Winter	3.917a	4.108a	1.423a	3.943a
	Spring	4.049a	4.6366b	2.193a	4.651b
	Summer	3.988a	4.477b	14.333b	4.187a
	S.E.	0.06256	0.08651	5.911	0.1513
	LSD	0.1322	0.173504	11.8551	0.303

Means of individual alkaloid levels detected in different organs and in different seasons. Values within the same column with the same letter are not significantly different from each other.

* Flower = flowering stalks.

Table 6.4. Seasonal variation in Amaryllidaceae alkaloid content (mg 100 g^{-1} dry weight) detected in different organs of *Crinum bulbispermum*.

Plant	Season	Year 1998/1999				Year 1999/2000			
		Alkaloid detected				Alkaloid detected			
		32	**1**	**47**	**34**	**32**	**1**	**47**	**34**
Bulb	Winter	12.2a	18.5a	0.4a	7.9a	10.4a	27.1a	2.6a	4.8a
	Spring	12.7a	24.9b	3.9a	38.4a	14.5a	42.4b	2.0a	60.3b
	Summer	11.7a	27.4b	0.5b	15.4a	13.4a	37.7b	19.2b	16.0a
Root	Winter	0.0a	14.7a	5.3a	0.0a	0.0a	19.3a	7.5a	2.8a
	Spring	6.5a	35.7b	5.7a	18.8a	0.0a	31.4b	1.0a	9.3b
	Summer	0.0a	15.6b	1.6b	0.0a	0.0a	26.2b	5.5b	2.5a
Leaf	Spring	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
	Summer	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

Means of individual alkaloid levels detected in the same organ in different seasons. Values within the same column with the same letter are not significantly different from each other.

CHAPTER 7
Inter-*Crinum* species variation in Amaryllidaceae alkaloids

7.1 Qualitative variation

As indicated in Chapters 4, 5 and 6, GC results revealed the presence of lycorine, cherylline, 1-*O*-acetyllycorine, crinine, powelline, crinamidine, 3-*O*-acetylhamayne crinamine and 1-epideacetylbowdensine, in *C. macowanii*. In addition to the abovementioned alkaloids, epibuphanisine and undulatine were detected in *C. moorei*. *Crinum bulbispermum* was found to contain crinine, crinamine, 6-hydroxycrinamine and lycorine.

Qualitatively, the presence of epibuphanisine and undulatine separated *C. moorei* from *C. macoawnii* and *C. bulbispermum*. While the presence of cherylline, crinamidine, 1-epideacetylbowdensine, powelline, 1-*O*-acetyllycorine separated *C. macowanii* and *C. moorei* from *C. bulbispermum*. 6-Hydroxycrinamine was detected only in *C. bulbispermum*.

7.2 Quantitative variation

Distribution of the detected alkaloids in *Crinum* species are presented in Table 7.3 and Table 7.4 for the year 1998/1999 and 1999/2000 respectively. Variation of logarithmically transformed data of the quantified individual alkaloids of *Crinum* species are shown in Table 7.1. The amounts of crinine, epibuphanisine, powelline, 1-epideacetylbowdensine, crinamidine, undulatine, 3-*O*-acetylhamayne, cherylline and lycorine were statistically higher in *C. moorei* compared to *C. bulbispermum* and *C. macowanii*. *C. bulbispermum* had a significantly higher crinamine content than *C. macowanii* and *C. moorei*. The levels of crinine and lycorine were statistically important in the separation of the three species in question from each other. The detected quantities of cherylline, powelline, crinamidine and 1-epideacetylbowdensine from *C. macowanii* were not quantitatively important in separating *C. macowanii* from *C. bulbispermum* as it

did not show any statistical differences.

7.3 Species-organ interaction

7.3.1 Bulbs

Wald statistics revealed significant variation in the amounts of the individual alkaloids detected in the bulbs of each of the three species under study. Variation due to species-organ interaction of individual alkaloids detected in *Crinum* species are shown in Table 7.2. Bulbs of *C. moorei* yielded significantly higher amounts of epibuphanisine, cherylline, powelline, 3-*O*-acetylhamayne, crinamidine, undulatine and 1-epideacetylbowdensine than the bulbs of *C. macowanii* and *C. bulbispermum*. No significant differences were detected in the yield of the abovementioned alkaloids for the bulbs of *C. macowanii* and *C. bulbispermum*. Bulbs of *C. moorei* also had the highest content of crinine and lycorine. The bulbs of *C. macowanii* which, in turn, had a significantly higher amounts of crinine and lycorine content than the bulbs of *C. bulbispermum*. Bulbs of *C. bulbispermum* contained significantly higher crinamine levels than the bulbs of *C. macowanii* and *C. moorei*, which were not statistically different from each other in their crinamine content.

7.3.2 Roots

The levels of lycorine and crinine in the roots were important in separating *C. moorei*, which had the highest levels, from *C. macowanii*, and *C. macowanii* from *C. bulbispermum*. No significant differences were detected between the *Crinum* species as far as the roots content of epibuphanisine, undulatine, 3-*O*-acetylhamayne and crinamine were concerned. The root content of cherylline, powelline, crinamidine and 1-epideacetylbowdensine was significantly higher in *C. moorei* than in *C. macowanii* and *C. bulbispermum*.

7.3.3 Leaves

Crinine, lycorine, cherylline, epibuphanisine, 3-*O*-acetylhamayne, powelline, undulatine, crinamidine and1-epideacetylbowdensine were detected in significantly higher amounts in the leaves of *C. moorei* compared to the leaves of

C. macowanii and *C. bulbispermum*. Leaves of *C. macowanii* contained statistically higher amounts of crinine and lycorine than the leaves of *C. bulbispermum*.

7.4 Flowering stalks

The amounts of crinine, lycorine cherylline, powelline, undulatine, crinamine crinamidine 1-epideacetylbowdensine and 3-*O*-acetylhamayne present in the flowering stalks of the three *Crinum* species were significant in separating the species under investigation. Flowering stalks of *C. moorei* had the highest content of the abovementioned alkaloids with no significant differences detected in the content of the flowering stalks of *C. macowanii* and *C. bulbispermum*. An exception was the statistically higher levels of lycorine in the flowering stalks of *C. macowanii* compared to that of *C. bulbispermum*. *C. bulbispermum* had the highest amount of crinamine in its flowering stalks.

Table 7.1. Inter-species variation of logarithmically transformed data for individual alkaloids detected in three *Crinum* species.

Species*	Alkaloid detected									
	32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**34**
moorei	5.527a	5.456a	5.412a	83.76a	4.160a	705.4a	5.772a	7.646a	141.64a	4.044a
macwa	4.659b	4.811b	4.876b	0.33b	2.441b	138.6b	4.403b	4.827b	12.87b	4.003a
bulbis	3.998c	4.490c	4.844b	0.29b	2.403b	137.1b	4.401b	4.808b	15.29b	4.363b
S.E	0.0999	0.07632	0.0684	21.92	0.1024	81.4	0.05109	0.1000	17.75	0.07947
LSD	0.1978	0.1511	0.1356	43.4016	0.2027	161.17	0.10115	0.198	35142	0.1573

Means of individual alkaloid levels detected in the different species. Values within the same column with the same letter are not significantly different from each other.

* moorei = *C. moorei*, macwa = *C. macowanii*, bulbis = *C. bulbispermum*.

Table 7.2. Inter-species variation of logarithmically transformed data for individually quantified alkaloids from different plant organs of Three *Crinum* species.

Plant Organ	Species*	Alkaloid detected									
		32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**34**
Bulb	moorei	6.888a	5.796a	5.487a	201.54a	5.611a	522.0a	5.529a	7.438a	112.4a	4.086a
	macwa	5.888b	5.540a	4.864b	1.01b	2.406b	125.4b	4.393b	4.878b	23.15b	4.044a
	bulbis	4.068c	4.963b	4.861b	1.01b	2.381b	125.4b	4.384b	4.812b	24.95b	4.627b
Root	moorei	5.205a	6.119a	5.301a	34.4a	3.295a	333.3a	5.293a	6.120a	68.51a	4.037a
	macwa	4.238b	5.276b	4.831b	1.19a	2.508b	131.3a	4.386b	4.823b	10.6a	4.138a
	bulbis	3.627c	4.697c	4.826b	1.01a	2.381b	125.4a	4.384b	4.812b	27.05a	4.153a
Leaf	moorei	5.128a	4.679a	5.413a	82.13a	4.042a	1278.3a	5.975a	8.218a	179.5a	4.082a
	macwa	4.205b	4.181b	4.851b	1.15b	2.415b	131.7b	4.405b	4.804b	0.50b	3.960a
	bulbis	3.680c	3.398c	4.851b	1.15b	2.415b	131.7b	4.405b	4.804b	0.50b	3.960a
Flower	moorei	4.889a	5.23a	5.449a	19.97a	3.693a	687.8a	6.289a	8.718a	210.12a	3.972a
	macwa	4.305b	4.246b	4.955b	0.97a	2.433b	166.0b	4.43b	4.805b	21.21b	3.871a
	bulbisp	4.619b	4.902c	4.875b	0.97a	2.433b	166.0b	4.43b	4.805b	12.65b	4.712b
S.E		0.1771	0.1372	0.1237	40.03	0.1793	143.3	0.09518	0.1886	31.87	0.1351
LSD		0.3531	0.2735	0.2466	79.819	0.3575	285.74	0.1897	0.376	63.548	0.2693

Means of individual alkaloid levels detected in the different species in the same organ. Values within the same column with the same letter are not significantly different from each other.

* moorei = *C. moorei*, macwa = *C. macowanii*, bulbis = *C. bulbispermum*.

Table 7.3. Distribution of the detected alkaloids in different seasons from different organs of *Crinum* species (mg100 g^{-1} dry weight) for the year1998/1999.

Season	Plant	Species*	Alkaloid detected										
			32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**43****	**34**
Winter	Bulb	*moorei*	369.5	60.8	114.8	58.7	69.4	92.5	267.3	598.6	48.8	3.5	0.0
		macwa	80.1	46.7	14.6	0.0	3.0	0.0	0.0	0.0	43.6	D	0.0
		bulbis	12.2	18.5	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	7.9
	Root	moorei	111.8	129.7	60.5	13.8	7.5	39.2	47.0	143.6	10.5	89.2	0.0
		macwa	11.4	27.5	0.0	0.0	0.6	0.0	0.0	0.0	0.3	D	6.2
		bulbis	0.0	14.7	0.0	0.0	0.0	0.0	0.0	0.0	5.3	0.0	0.0
Spring	Bulb	moorei	85.9	44.6	38.8	1.9	22.3	60.4	34.2	199.3	1.9	5.3	0.0
		macwa	75.6	41.1	4.9	0.0	0.0	0.0	0.0	0.0	0.8	D	5.8
		bulbis	12.7	24.9	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0	38.4
	Root	moorei	16.6	108.2	32.2	0.0	4.2	36.8	49.3	86.2	18.7	65.0	0.0
		macwa	2.2	39.6	0.0	0.0	0.0	0.0	0.0	0.0	1.9	D	4.8
		bulbis	6.5	35.7	0.0	0.0	0.0	0.0	0.0	0.0	5.7	0.0	18.8
	Leaf	moorei	152.5	23.1	34.3	34.5	17.4	26.3	76.9	1746.7	38.1	0.0	0.0
		macwa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		bulbis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 7.3. (Continued).

Spring	Flower	moorei	23.1	55.8	42.4	0.0	8.5	69.3	82.9	897.5	40.1	0.0	0.0
		macwa	11.0	15.9	10.3	0.0	0.0	0.0	0.0	0.0	0.0	D	0.0
		bulbis	21.7	38.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	16.5
Summer	Bulb	moorei	210.9	51.8	32.3	51.6	72.7	44.1	57.8	162.4	14.7	0.0	0.0
		macwa	70.1	46.5	0.0	0.0	0.0	0.0	0.0	0.0	0.3	D	0.0
		bulbis	11.7	27.4	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0	16.5
	Root	moorei	77.8	61.8	44.3	1.7	23.7	68.4	50.4	83.3	26	284.0	0.0
		macwa	10.7	44.6	0.0	0.0	1.6	0.0	0.0	0.0	0.0	D	14.0
		bulbis	0.0	15.6	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0
	Leaf	moorei	27.6	25.8	50.9	0.0	24.1	74.5	155.1	1768.1	38.1	0.0	0.0
		macwa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		bulbis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* moorei = *C. moorei*, macowa = *C. macowanii*, bulbis = *C. bulbispermum*.

**D = detected but not quantified.

Table 7.4. Distribution of the individual alkaloids detected in different organs of *Crinum* species in different (mg 100 g^{-1} dry weight) for the year 1999/2000.

Season	Organ	Species*	Alkaloid detected										
			32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**43****	**34**
Winter	Bulb	moorei	284.8	79.0	43.3	88.2	128.8	35.9	41.7	250.8	19.6	11.0	0.0
		macwa	168.3	81.1	9.8	0.0	0.0	0.0	0.0	0.0	0.2	D	0.0
		bulbis	10.4	27.1	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	4.8
	Root	moorei	139.4	130.1	38.4	1.3	32.9	17.0	48.7	104.3	34.1	291.6	0.0
		macwa	13.3	45.4	0.0	0.0	0.6	0.0	10.8	0.0	7.5	D	12.4
		bulbis	0.0	19.3	0.0	0.0	0.0	0.0	0.0	0.0	7.5	0.0	2.8
Spring	Bulb	moorei	114.5	80.7	50.0	10.2	75.9	121.3	57.1	905.2	18.3	34.6	0
		macwa	124.7	47.3	15.8	0.0	0.7	0.0	10.8	16.3	0.2	D	0.0
		bulbis	14.5	42.4	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	60.3
	Root	moorei	19.9	37.8	25.9	0.9	3.4	13.4	25.9	112.7	1.1	20.8	0
		macwa	18.3	47.6	0.0	0.0	3.2	0.0	0.0	0.0	2.1	D	4.7
		bulbis	0.0	31.4	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	9.3
	Leaf	moorei	28.2	14.2	28.6	0.9	44.0	36.4	72.3	2195.7	9.2	0.0	0.0
		macwa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		bulbis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 7.4 Continued.

	Flower	moorei	20.8	23.8	39.0	0.0	6.3	69.4	127.6	1909.8	21.6	0.0	0.0
		macwa	0.0	8.2	5.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0	0.0
		bulbis	21.7	25.4	0.4	0.0	0.0	0.0	0.0	0.0	0.4	0.0	16.5
Summer	Bulb	moorei	305.5	101.8	68.2	2.7	103.0	117.8	42.2	1221.1	24.5	41.0	7.0
		macwa	45.6	47.8	10.0	0.0	0.0	0.0	0.0	30.8	23.4	D	0.0
		bulbis	13.4	37.7	0.0	0.0	0.0	0.0	0.0	0.0	19.2	0.0	16.3
	Root	moorei	31.6	117.7	61.5	3.6	4.3	75.5	33.8	157.4	17.0	108.4	0
		macwa	12.2	38.4	0.0	0.0	2.1	0.0	0.0	6.0	1.5	D	13.6
		bulbis	0.0	26.2	0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.0	2.5
	Leaf	moorei	37.9	37.1	264.5	8.5	8.5	617.6	77.4	287.8	50.4	0.0	13.5
		macwa	0.0	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		bulbis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* moorei = *C. moorei*, macowa = *C. macowanii*, bulbis = *C. bulbispermum*.

**D = detected but not quantified.

CHAPTER 8

Variation in Amaryllidaceae alkaloids: Discussion

The method developed by BASTOS *et al.* (1996) was used for the quantitative analysis of alkaloids from different plant organs of *Crinum* species under investigation. The method uses dilute acid extraction and capillary gas chromatography. It is a rapid, simple, sensitive, reproducible technique and allows for the analysis of a large number of samples from limited plant material.

The high water content in fresh bulbs of Amaryllidaceae plants and the fluctuation of water content with season, age, tissue type and storage conditions makes the comparison of alkaloid content for fresh plant material difficult. The extraction of dried plant material using a dilute acid solution meant that conventional steps, such as the removal of acid and neutral fractions, could be avoided. Centrifugation was also used to remove large amounts of water soluble polysaccharides in the aqueous extracts which on occasions form emulsions in subsequent steps. In addition, the amount of non-alkaloids recovered were not significant enough to interfere with the results when the acid extract was partitioned with the organic solvent (BASTOS *et al.* 1996).

The use of 500 mg of bulbs and 250 mg of leaves, roots and flowering stalks was due to the fact that leaves, roots and flowering stalks have a large volume to weight ratio. Consequently, large volumes of the dilute acid solution would be absorbed by the plant material with the result that too little (less than the required 3 ml) would be available for subsequent steps. This could be overcome, either by increasing the dilute acid solution or reducing the amount of plant material used. Using half (250 mg) of the plant material made it possible to standardize the volume of acid extracts, 3 ml, used for further analysis. In addition, leaves, roots and flowering stalks were dissolved in half the volume used for the bulb for each species under investigation for GC analysis.

Capillary gas chromatography has been used for the analysis of complex mixtures of alkaloids including pyrrolizidine (WITTE *et al.* 1993), quinolizidine (WINK 1993), tropane (CHRISTEN *et al.* 1993; HARTMAN *et al.* 1982), tobacco (THOMPSON *et al.* 1982; LUANRATANA and GRIFFITH 1982), diterpenoid (MANNERS and RALPHS 1989), Lycopodium (GERARD and MACLEAN 1986), quinazoline (LAAKSO *et al.* 1990), indole (DAGNINO *et al.* 1991) and steroidal alkaloids (VAN GELDER *et al.* 1988). ONYIRIUKA and JACKSON (1978) used GC-MS for the analysis of Amaryllidaceae alkaloids derivatized as trimethylsilyl ethers. KREH *et al.* (1995) concluded that derivatized alkaloids did not give a better separation by GC and that the identification of alkaloids could not be easily achieved after derivatization.

Hyoscyamine was added as an internal standard at a concentration of 100 ug ml^{-1} to the plant material. However, decomposition of the alkaloid into three peaks made it unreliable as an internal standard . As an alternative, crinine, which is the most abundant alkaloid in the genus *Crinum,* was used to study the recovery of alkaloids. Crinine was added to the dilute acid solution at a rate of 100 ug ml^{-1} and extracted in the same way as the alkaloids from the plant material. A recovery of 97% was obtained. BASTOS *et al.* (1996) reported a recovery of 90% and 100% for galanthamine and deoxydihydrocodeine respectively.

For GC analysis, the crude alkaloid extracts were dissolved in methanol and injected into the GC. Each sample was injected twice as a precautionary against errors that may have resulted from manual injections. The coefficient of variation (percent standard deviation from the mean) between the two injections of each sample was less than 10%. This is to minimize variation due to manual injection. The duration of the analysis was 22 minutes. This is longer than the 15 minute analysis time reported by BASTOS *et al.* (1996). The time of the analysis is partly affected by the length of the column. BASTOS *et al.* (1996) used a 15 m long column while in this method a column 30 m long was used.

The sensitivity of the method was determined by the lowest concentration of

crinine and epibuphanisine that could be detected. Concentrations as low as 10 ug ml^{-1} of crinine and epibuphanisine could be detected. It is worth noting that the use of GC operation conditions with more sensitivity than that used resulted in poor resolution of the alkaloids under investigation. BASTOS *et al.* (1996) considered detection of 8 ug ml^{-1} of the Amaryllidaceae alkaloids galanthamine, *O*-methylmaritidine, lycoramine, N-demethyllycoramine, caranine, pluviine, haemanthamine, lycorine homolycorine hippeastrine and narcissidine as satisfactory. In addition, dissolving the crude extracts in 100 μl for *C. bulbispermum* and *C. macowanii* and 400 μl for *C. moorei* allowed the detection of levels as low as 1 μg ml^{-1} of crinine and epibuphanisine in the extracts of *C. bulbispermum* and *C. macowanii* and 4 μg ml^{-1} of these alkaloids from *C. moorei.*

Alkaloids were identified by comparing their retention times and mass spectra fragmentation patterns with those of authentic samples isolated from the three species under investigation as described in Chapter 3. This method of identification is well established and is a straight-forward method. It has been used for the identification of alkaloids from the genus *Narcissus* (BASTOS *et al.*1996; KREH *et al.* 1995).

Twelve alkaloids were identified including epibuphanisine, crinine, powelline, cherylline, lycorine, 1-*O*-acetyllycorine, undulatine, 3-*O*-acetylhamayane, crinamidine, crinamine, 1-epideacetylbowdenesine, and 6-hydroxycrinamine. Sharp peaks were obtained for crinine, epibuphanisine, powelline, undulatine, lycorine, 1-*O*-acetyllycorine, cherylline and 1-epideacetylbowdensine. Alkaloids having an ethano bridge in the α-position and 11- hydroxyl group such as crinamine and 3-*O*-acetylhamayne gave broader peaks, while 6-hydroxycrinamine decomposed to give a number of broader peaks. 6-Hydroxycrinamine occured as a mixture of two epimers: α- and β-6-hydroxycrinamine. The broader peaks given by the three alkaloids may be attributed to the expulsion of water from the molecule. DUFFIELD *et al.* (1965) reported the expulsion of water from 6-hydroxycrinamine and its isomer haemanthidine.

The use of the residual maximum likelihood method for statistical analysis instead of analysis of variance was based on the fact that the experimental design was unbalanced. The number of organs in the different seasons were unequal. Only roots and bulbs were available during Winter (the dormancy period). In Summer, leaves, bulbs and roots were collected. The three species flower once a year in South Africa, in Spring. The unbalanced design also makes the study of the interaction between organs and seasons difficult, and so each treatment was analysed separately.

Alkaloids were detected in all organs of *C. moorei* and *C. macowanii*. However, alkaloids were not detected in the leaves of *C. bulbispermum*. It is worth noting that only lycorine was detected in the leaves of *C. macowanii*. Most of the research conducted on the isolation of Amaryllidaceae alkaloids from other Amaryllidaceae species focused on the isolation of alkaloids from the bulbs or the whole plant, whereas potential screening of other organs received little attention. The few studies conducted on the alkaloid content of other organs include the isolation of lycorine from the roots of *Clivia miniata*, *Crinum asiaticum*, *Crinum pratense* and *Eucaris grandiflora*. Lycorine was also isolated from the root nodules of *Cyrtanthus pallidus* (COOK and LOUDON 1952). There are also reports on the occurrence of alkaloids in the flowers of *Lycoris incarnata* (LEWIS 1996), *L. incanata* (LEWIS 1995), *L. radiata* (LEWIS 1994), fruits of *C. asiaticum* (LEWIS 1992), leaves of *Narcissus* species 'Fortune' (LEWIS 1990) and seeds of *C. asiaticum* (COOK and LOUDON 1952).

In the first year of this study, the total alkaloid yield of *C. moorei* expressed as a percentage of the plant material analysed gradually decreased between Summer (high) and Winter (low). In the second year, the decrease in total alkaloid yield was between Spring (high) and Winter (low). There was no pattern in the variation in yield of different classes of Amaryllidaceae alkaloids namely, lycorine-type, crinine-type alkaloids with an ethano bridge in the α-position and cherylline. However, the yield, expressed as a percentage of plant material, of total crinine-type alkaloids, crinine-type alkaloids with an ethano bridge in the β-position with

saturation between positions 1 and 2, and those with an ethano bridge in the β-position without saturation between 1 and 2, showed a clear pattern of variation in an ascending gradient from Spring to Winter.

A lack of pattern was also observed in the yield of Amaryllidaceae alkaloids detected in *C. macowanii.* For instance, in the first year of study, the highest percent of lycorine-type alkaloids was obtained in Spring in the first year and in Winter in the second year. Furthermore, no pattern was observed in the content of individual alkaloids in different organs between seasons.

There is a lack of information on seasonal and organ-to-organ variation in alkaloid yield within the genus *Crinum.* The only report on the seasonal variation in Amaryllidaceae alkaloids was that of pancratistatin from *Hymenocallis littoralis* (PETTIT *et al.* 1995). The study clearly indicated that pancratistatin levels fluctuate markedly over the year and that these levels reflect the growth pattern of *H. littoralis.* The highest levels of pancratistatin were reached when the plant had undergone six months of growth. A drop in pancratistatin yield was reported when the plant produced flowers. Once the flowering stage ended pancratistatin yields increased rapidly. It was also found that levels of pancratistatin decreased drastically just after the end of dormancy when the plant is actively growing.

It is well known that a number of Amaryllidaceae species are self sterile and that few viable seeds are produced after self-fertilization. These include species in the African genera *Boöphane*, *Brunsvigia*, *Clivia*, *Cyrtanthus* and the American genera *Eucharis*, *Euchrosia*, *Hippeastrum*, *Rhodphiala* and *Ureceolina* (KOOPOWITZ, 1986). There is no information on the breeding systems of the Genus *Crinum.* The high variation among plants of the same species was particularly noticeable for those sampled in the same season. While some plants had high levels of a particular alkaloid, in other plants these alkaloids were not detected. For example, during Summer of the first year of study, cherylline was detected in two bulbs while epibuphanisine was detected in only one bulb. Even for alkaloids detected in all plants collected in a specific season, variation was

found to be high. For instance, during dormancy of the first year, crinine content of one bulb was 45% of the crinine content of the bulb having the highest level.

Leaves of *C. moorei* had the highest alkaloid content throughout the study period. Although ten alkaloids were detected in the leaves, the major alkaloid was crinamidine. Crinamidine represented more than 81.2% of the total alkaloid yield of leaves. An exception was the comparatively low yield (20%) of leaves in the Summer of the second year of the study. Generally, leaves of *C. moorei* were rich in crinine-type alkaloids with an ethano bridge in the β-position lacking a double bond between positions 1 and 2. These alkaloids represented 86-94.8% of the total alkaloid content. There was an exception in the second Summer where the yield of this class of alkaloids in the leaves was 69.7%. The alkaloid profile was quite different in *C. macowanii* and *C. bulbispermum* for the following reasons:

1. Alkaloids were not detected in the leaves of these plants during the study period;
2. Crinine- type alkaloids with an ethano bridge in the β-position lacking a double bond between 1 and 2 were not detected in all organs of *C. bulbispermum*;
3. Crinamidine was only detected in comparatively low levels in the bulbs and the roots; and
4. 1-Epideacetylbowdensine was detected in the flowering bulbs of *C. macowanii* only in the second year of study.

The alkaloids in these three *Crinum* species are reported to show different biological activities. Crinamine was found to possess anti-bacterial activity (VILADOMAT *et al.* 1996), while lycorine, crinamine, 6-hydroxycrinamine and undulatine showed anti-cancer activity (LIKHITWITAYAWUID *et al.* 1993; NAIR *et al.* 1998; PETTIT *et al.* 1984). Despite these biological activities, the ecological role of Amaryllidaceae alkaloids is not emphasized in many studies. GHOSAL *et al.* (1990) reported that the quantitative and qualitative changes in the Amaryllidaceae alkaloids in response to stress suggests a role in the protective mechanism of the producer plant. It was also postulated that plants accumulate

protective secondary compounds in a tissue at risk of predation (CORDEL 1981; FRISCHKNECHT *et al.* 1986). The fact that no lycorine or only traces of this compound were detected in the leaves of *C. bulbispermum* and *C. macowanii* respectively raises the question as to what role these alkaloids play in the plant. It is noteworthy that, in monocotyledonous plants, the organ at risk is the stem, mainly the apical meristem. The destruction of the apical meristem results in the death of the plant. As the modified leaves forming the bulb protect the apical meristem, it seems logical that the plant accumulates considerable amounts of the alkaloids in the bulb instead of in the leaves. The plant also undergoes dormancy during Winter for more than three months a year and the protection to the apical meristem is offered only by the bulb during this period as the leaves senesce.

The quantities of epibuphanisine in the different organs was similar to those of crinine and powelline. An increase in the levels of crinine and powelline was accompanied by an increase in epibuphanisine. Epibuphanisine is the methyl ether of crinine while powelline is the aromatic methoxy derivative of crinine. The biosynthetic relations of these alkaloids was shown by the aromatic demethoxylation of powelline and the acid hydrolysis of the allylic methyl ether buphanisine to give crinine (WILDMAN 1960). From the analysis of the differences in levels of powelline and epibuphanisine, it appears that the plant favors the conversion of crinine to powelline when the levels of crinine are low. A further increase in crinine is accompanied by the synthesis of epibuphanisine. This may also explain why epibuphanisine was not detected in both *C. macowanii* and *C. bulbispermum.* The levels of crinine detected in *C. macowanii* were relatively low compared to those detected in *C. moorei*, even though the conversion to powelline seems to be limited by low levels in *C. macowanii.* In *C. bulbispermum*, which contained the lowest amounts of crinine of the three species investigated even powelline was not detected and it seems the plant favored the accumulation of crinine rather than its conversion to its derivatives.

In the present study, the detected alkaloids chemically separated *C. moorei* from

C. bulbispermum and *C. macowanii*. Qualitatively, epibuphanisine and undulatine were detected in *C. moorei* but not in *C. macowanii* and *C. bulbispermum*. 6-hydroxycrinamine was only detected in *C. bulbispermum* while, powelline, cherylline, 1-epideacetylbowdensine, crinamidine and 1-*O*-acetyllycorine were detected in both *C. moorei* and *C. macowanii* but not in *C. bulbispermum.*

Quantitatively *C. moorei* had the highest levels of almost all the quantified alkaloids except crinamine. *C. macowanii* had higher levels of lycorine and crinine compared to *C. bulbispermum.*

The three *Crinum* species in question differ in many morphological characters. These differences include bulb size, leaf colour, leaf width, leaf ciliation and texture, number of flowers per umbel, the length of the perianth tube and whether the fruit is beaked by the persistent base of the perianth or not. In addition, *C. moorei* differs from all South African *Crinum* species including *C. macowanii* and *C. bulbispermum* in several respects. For instance, the neck of the bulb of *C. moorei* is made up of the thickened and hardened leaf-bases, which form a false stem. The bulb is only leafy at the apex. The leaves mature in one year, that is, they do not die back and grow out again the following year and the segments of the flowers do not form the usual narrow funnel but are more spreading when open and not keeled dorsally with a deeper coloured band (VERDOORN 1973).

The three *Crinum* species in question differ also in their habitat. *C bulbispermum* and *C. moorei* are found in damp places and along rivers and streams. *C. macowanii* is found in various types of habitat: mountain grass veld, stony slopes, grass veld in hard dry shade, gravel soil; sandy flats, along rivers and at the coast (VERDOORN 1973).

Differences in cytological characters and leaf anatomy have also been reported. In the three *Crinum* species the basic chromosome number is eleven. *C. bulbispermum* and *C. moorei* are diploids (2n = 22) while *C. macowanii* is a tetraploid (2n = 44) (VAN DER WALT *et al.* 1970). In a study of the anatomy of

many genera belonging to the family Amaryllidaceae, including the genus *Crinum*, VORSTER and SPREETH (1996) concluded that there is considerable variation within each genus.

Literature reports on interspecific variation in Amaryllidaceae alkaloids are rare and there is no information on the comparison of the chemical constituents between the three *Crinum* species. However, TANAHASHI *et al.* (1990) investigated the distribution of galanthamine in 45 species belonging to 11 Amaryllidaceae genera of South African origin, including *Ammocharis, Boöphane, Brunsvigia, Crinum, Cybistetes, Cyrtanthus, Gethyllis, Haemanthus, Hessia, Nerine* and *Strumaria*. Galanthamine was detected in most of the species but not in *Ammocharis coranica*, *Brunsvigia orientalis* and *C. macowanii*. Among the *Crinum* species studied, galanthamine yield were 0.043%, 0.009%, 0.006% and 0% for *C. powelii*, *C. bulbispermum*, *C.variabile* and *C. macowanii* respectively on a dry weight basis.

In another study, PETTIT *et al.* (1995) investigated seven species and one cultivated variety of the genus *Hymenocallis* as sources of pancratistatin. *Hymenocallis speciosa*, *H.* variegated, *H. pedalis*, *H. expansa*, *H. sonoranensis* were found to contain pancratistatin. Only *H.* x 'Tropical Giant' and *Ismene* x 'Advanced' failed to provide any member of the isocarbostyril series while *Ismene* x 'Sulphur Queen' yielded 7-deoxynarciclasine.

Studies on the variation in alkaloid content below the species level have been reported. A significant difference in galanthamine content between four *Narcissus* cultivars was found. The cultivars Ice follice and Mount Hood had a high level of galanthamine compared to that of the cultivars Geranium and White Cheerf (MORAES-CERDEIRA *et al.* 1997)

Conclusions

The Amaryllidaceae alkaloids represent a large and still expanding group of alkaloids which are found exclusively in plants belonging to this family. The genus *Crinum* is a true representative of the family as it exhibits all the chemical traits of the Amaryllidaceae (GHOSAL *et al.* 1985; VILADOMAT *et al.* 1997). In this study three *Crinum* species were investigated for alkaloid content namely *C. bulbispermum*, *C. macowanii* and *C. moorei.*

The ethanolic extracts of *C. bulbispermum* yielded seven alkaloids. The new alkaloids 8α-ethoxyprecriwelline, *N*-desmethyl-8α-ethoxypretazettine and *N*-desmethyl-8β-ethoxypretazettine were isolated for the first time from this species. In addition, the previously known alkaloids crinamine, 6-hydroxycrinamine, 3-*O*-acetylhamayne and bulbispermine were also identified. The isolation of crinamine, 6-hydroxycrinamine, and 8α-ethoxyprecriwelline is of biosynthetic significance. 6-Hydroxycrinamine on reductive transformations gave its 6-deoxy derivative crinamine and on facile conversions by methylation and treatment with dilute acid gave criwelline (DUFFIELD *et al.* 1965; KING *et al.* 1965).

The ethanolic extracts of *C. moorei* contained crinine, 3-*O*-acetyllycorine, powelline, epibuphanisine, crinamidine, undulatine, cherylline, 1-epideacetylbowdensine, epivittatine, lycorine, 1-*O*-acetyllycorine, and the new alkaloids mooreine and 3-[4'-(2'-aminoethylphenoxy]bulbispermine. The alkaloids crinine, powelline, buphanisine (an isomer of epibuphanisine), crinamidine, and undulatine are known to be interrelated through a combination of simple oxidation, reduction and dehydration reactions coupled with four specific techniques. These are: (1) aromatic demethoxylation by sodium and aryl alcohol; (2) replacement of OH by H via the action of lithium aluminium hydride on an intermediate chloro compound; (3) acid hydrolysis of allylic methyl ethers to alcohols; and (4) *O*-methylation of hydroxylic alkaloids with potassium and p-

toluene sulfonate (WILDMAN 1960). The isolation of the related alkaloids lycorine, 1-*O*-acetyllycorine and the quaternary alkaloid mooreine is also of biosynthetic importance. The presence of the quaternary alkaloids in plants proved to be vital intermediates with biosynthetic and translocation roles (COURT 1983).

C. macowanii contained lycorine, crinine, cherylline, hamayne and bulbispermine and the phenthylamine tyramine.

From the above it appears that, although they had been studied extensively, *Crinum* species continue to provide more information on Amaryllidaceae alkaloids.

The second part of the current study focused on organ-to-organ, seasonal and interspecific variation in *Crinum* alkaloids. Plant material was extracted with dilute HCl solution followed by GC analysis. Twelve alkaloids were detected from the *Crinum* species namely crinine, lycorine, epibuphanisine, powelline, cherylline, crinamidine, undulatine, 3-*O*-acetylhamayne, 1-epideacetylbowdensine, 1-*O*-acetyllycorine crinamine and 6-hydroxycrinamine. Variation among different organs was found in total alkaloid yield, crinine-type, lycorine-type and cherylline-type alkaloids respectively.

In this study, it was noticeable that biosynthetically related alkaloids were accumulated in a specific organ. This was more pronounced in *C. moorei* where lycorine-type alkaloids were present in higher amounts in the roots. Leaves had the highest amounts of crinine- type alkaloids lacking a double bond between 1 and 2. This showed the leaves with high levels of total alkaloids as well as the total crinine-type alkaloids.

The lack of a pattern and contradictory results on the stage of maximum individual alkaloid content led to the belief that either different stages of growth are

associated with high alkaloid yield or that no regular trend exists. The same results were reported from *Atropa belladonna* which is a cross pollinating plant and which exhibits a tremendous variation in all traits including alkaloid concentration. Further studies on *A. belladonna* indicated that the contradictory results were actually a developmental variation of heterogenous and heterozygous sources (DHAR and BHAT 1982).

In this study, it was found that *C. moorei* had the highest levels of all individual alkaloids except crinamine when compared to *C. bulbispermum* and *C. macowanii*. Quantitatively, the detected alkaloids chemotaxonomically separated *C. moorei* from *C. bulbispermum* and *C. macowanii*. The results also indicated that *C. macowanii* is apparently much closer related to *C. bulbispermum*. Qualitatively, lycorine, powelline, 1-*O*-acetyllycorine, cherylline, crinamidine, 1-epideacetylbowdensine, crinine, crinamine and 3-*O*-acetylhamayne were detected in both *C. moorei* and *C. macowanii*, indicating the closeness of these species to each other.

The results of this study indicated that *C. moorei* is a rich source of the alkaloids detected except for crinamine and 6-hydroxycrinamine which were detected in higher levels in *C. bulbispermum*. It is recommended that only leaves have to be sampled for any attempts to isolate crinine-type alkaloids having an ethano bridge in the β-position lacking a double bond between 1 and 2, namely crinamidine, undulatine and 1-epideacetylbowdensine. This has the advantage of avoiding the destruction of the plant. The roots of *C. moorei* are a rich source of lycorine-type alkaloids and it could be a reliable source of this kind of alkaloid if required for pharmaceutical use.

Although this work has offered an insight into the distribution of Amaryllidaceae alkaloids in *Crinum* species, further work is required using much larger samples and shorter time intervals for the evaluation of seasonal and organ-to-organ variation. This experiment was designed to study variation in alkaloid yield by

different species in three different seasons a year. Plants were sampled once during each season. Using shorter intervals between successive sampling (once every week or two weeks) will give a better understanding of the biosynthetic interconversion, translocation and accumulation of these alkaloids in the different organs and in different seasons.

The high variation between the individual plants sampled made it difficult to draw a clear conclusion on the organ and seasonal yield of the alkaloids. The use of homozygous samples raised from a single known source or closely related sources, would lead to more conclusive results.

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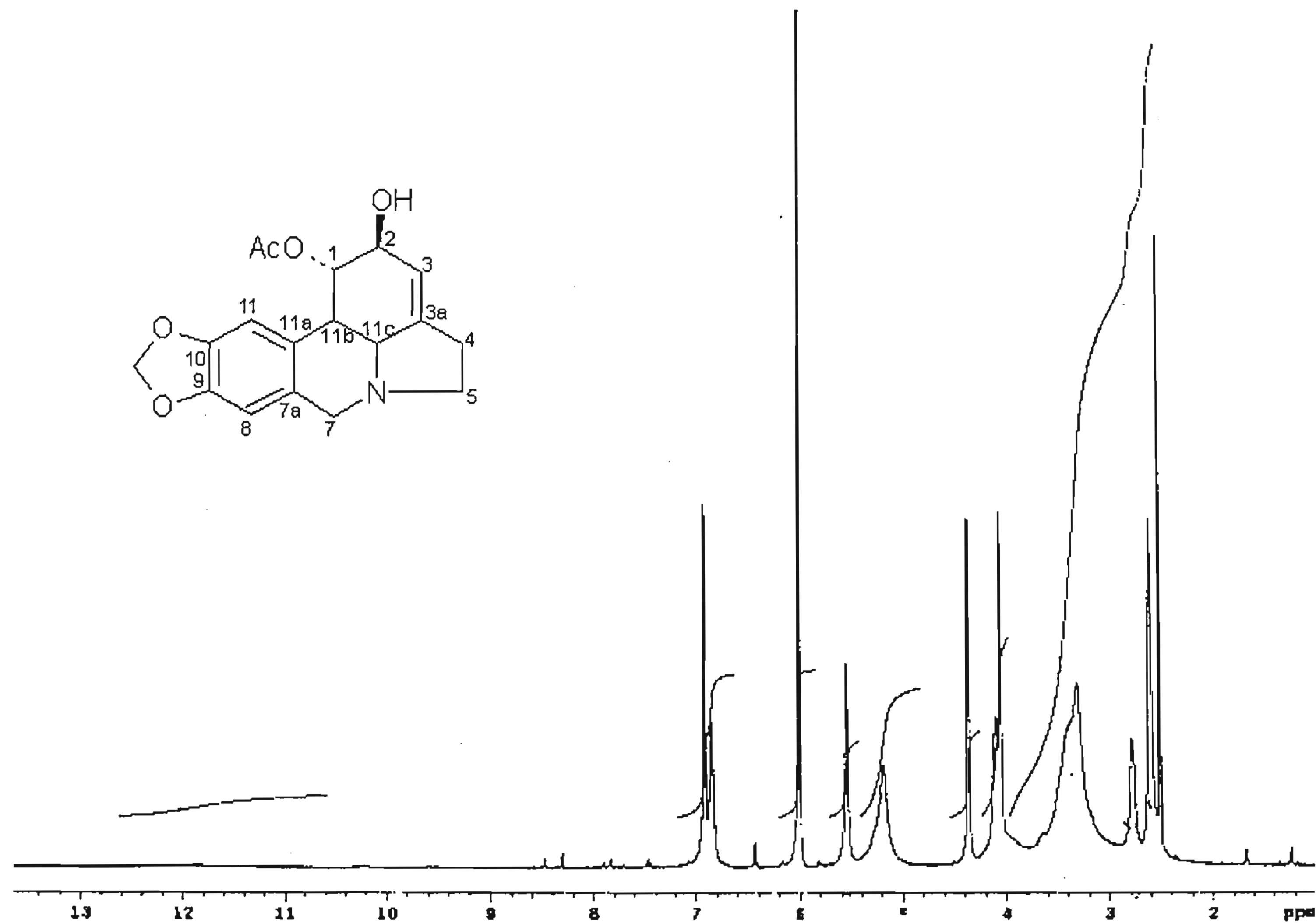
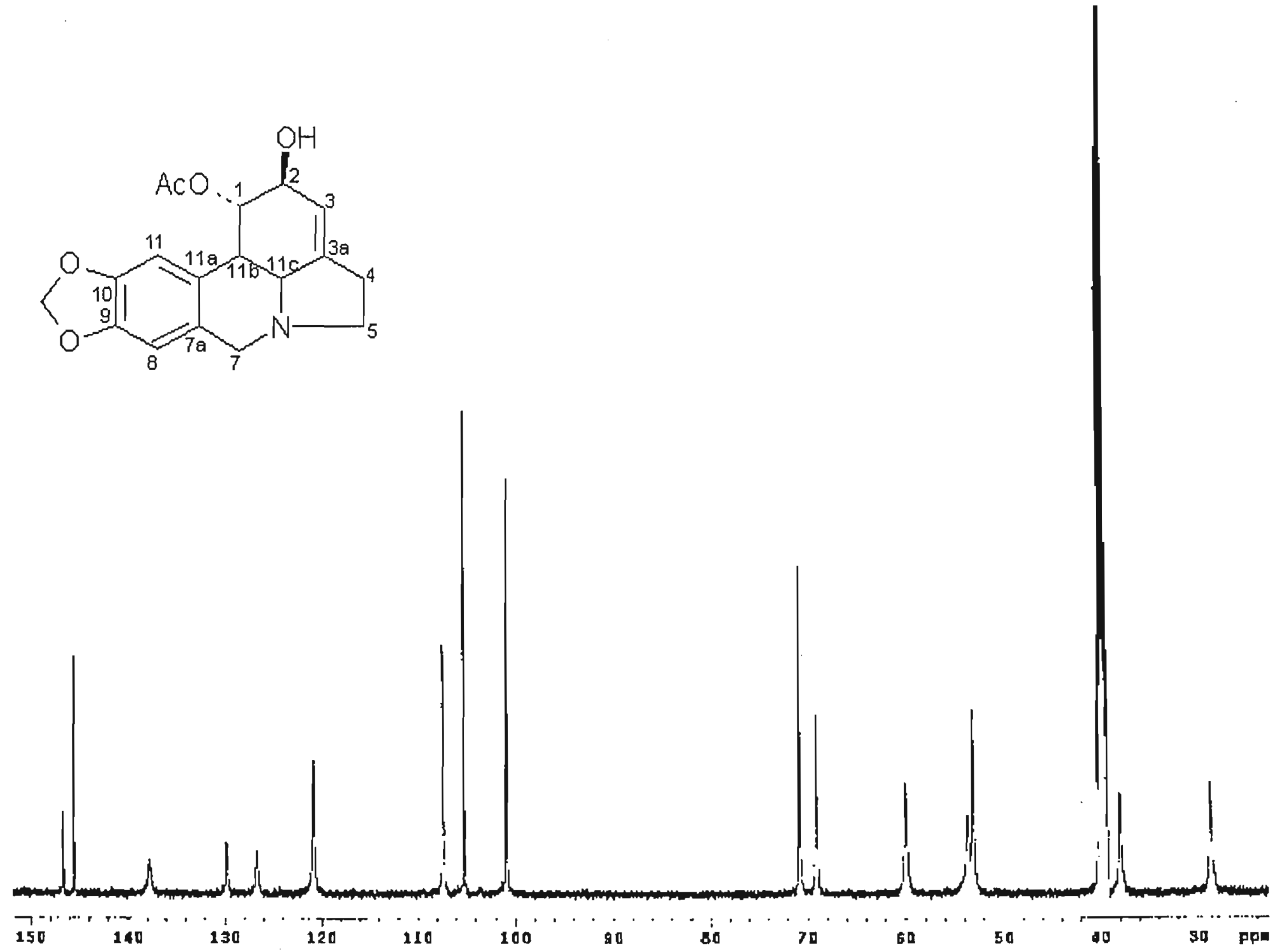
APPENDIX 1

^{1}H and ^{13}C NMR spectra of the isolated alkaloids arranged in numerical order of their appearance in the result sections of Chapter 3.



^{1}H NMR spectrum of lycorine **1** in DMSO-D$_6$



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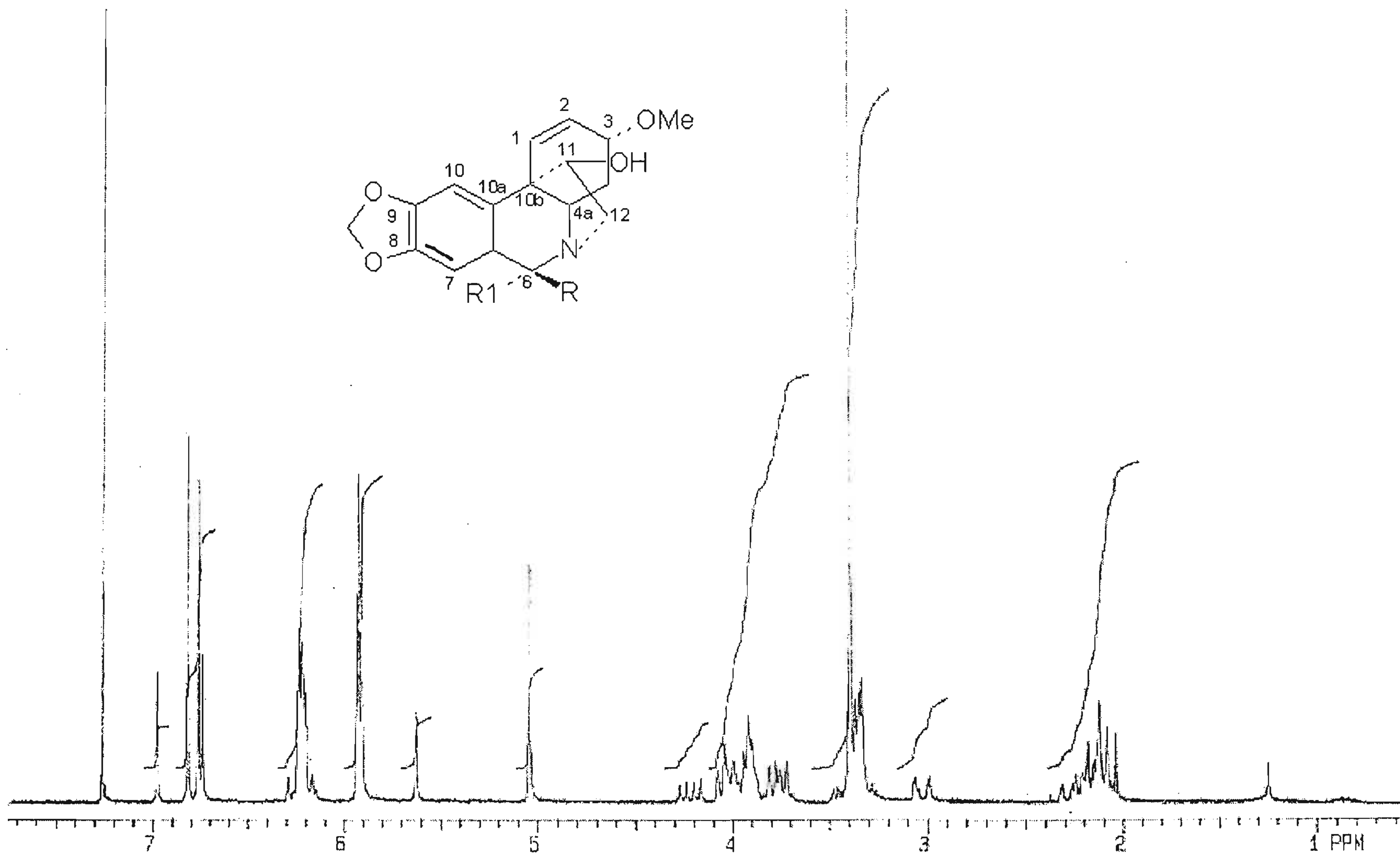
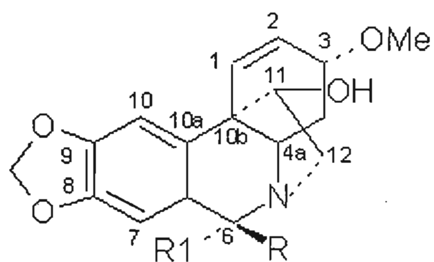
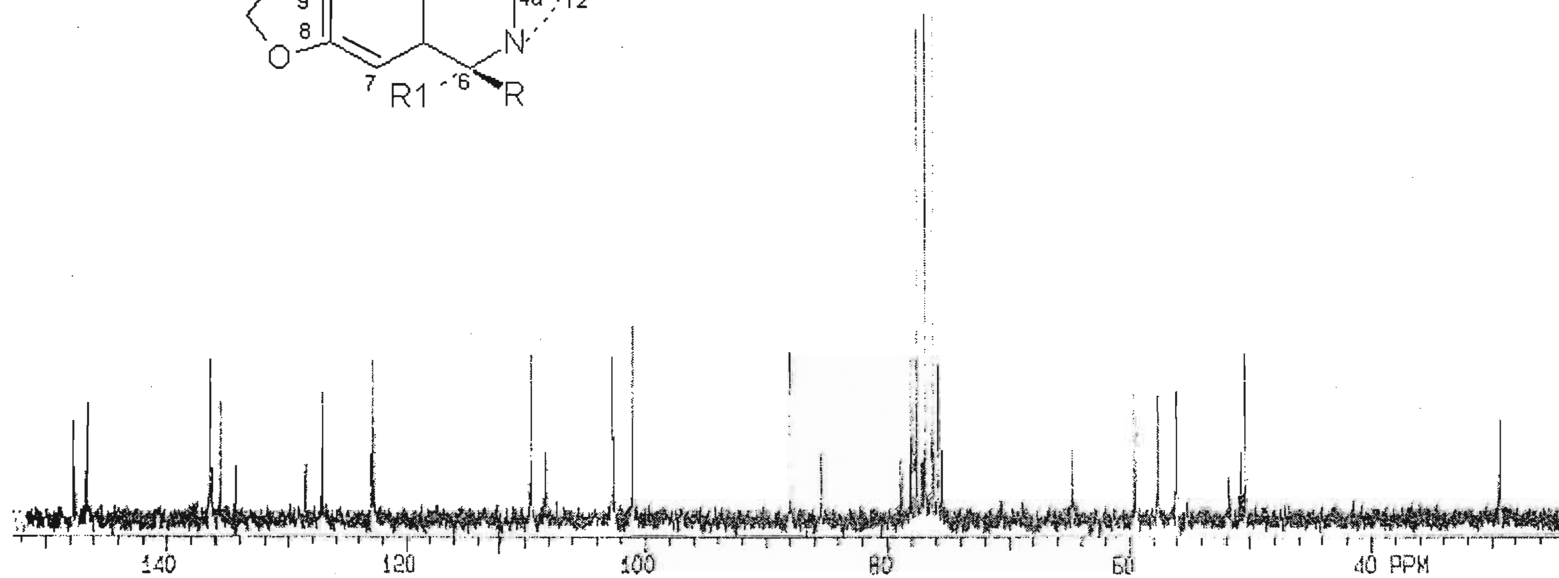
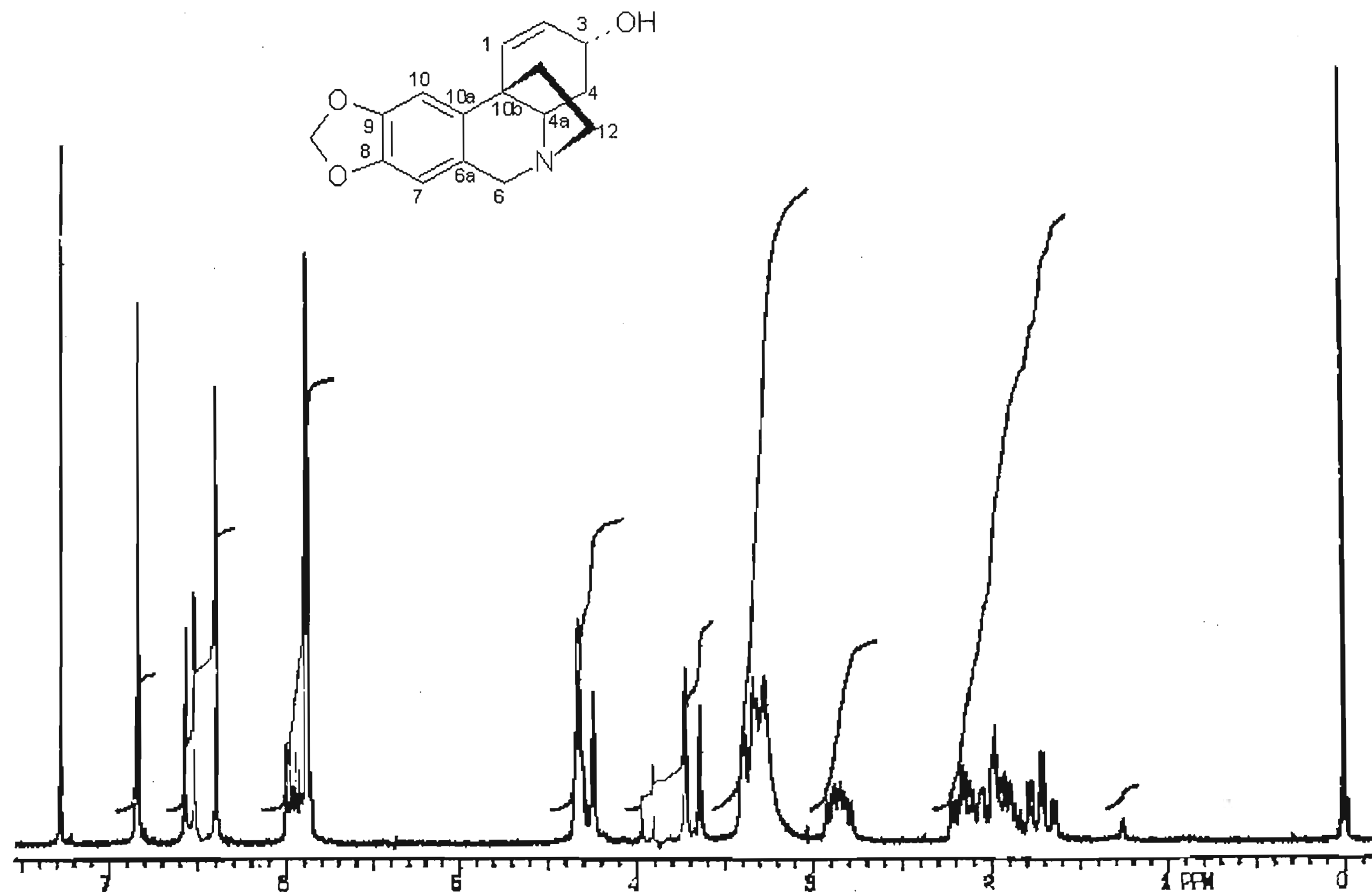


^{1}H NMR spectrum o 6-hydroxycrinamine **22**, **23** in $CDCl_3$

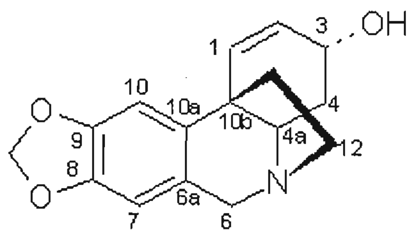


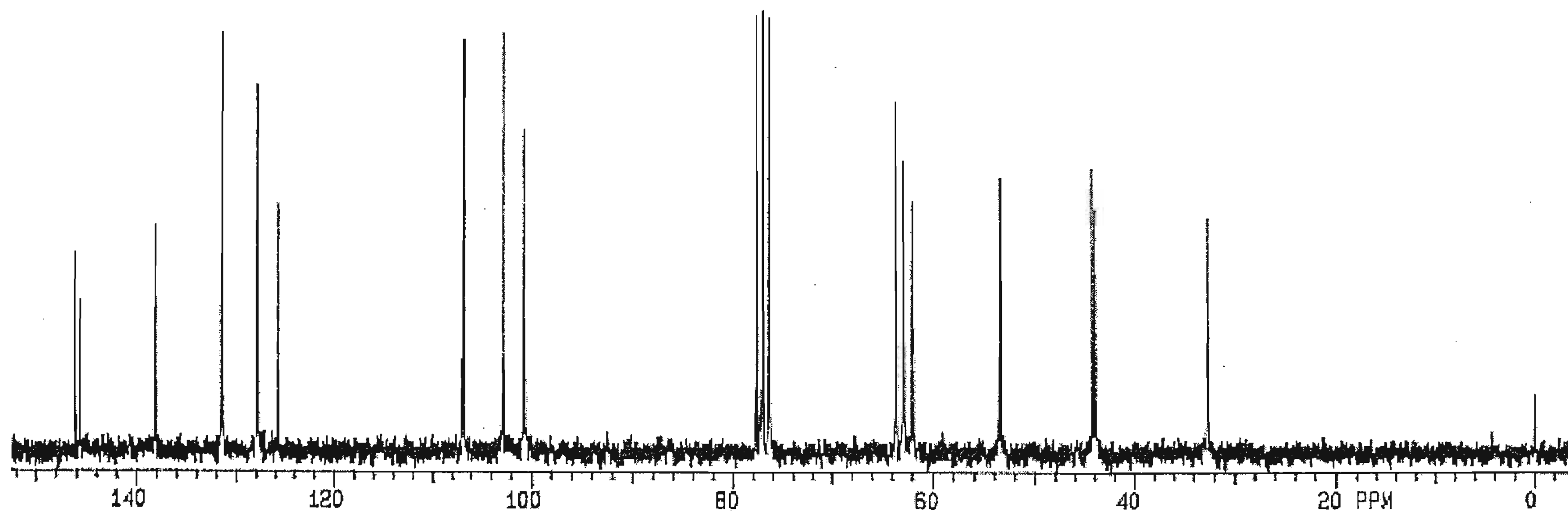


^{13}C NMR spectrum of 6-hydroxycrinamine **22, 23** in $CDCl_3$

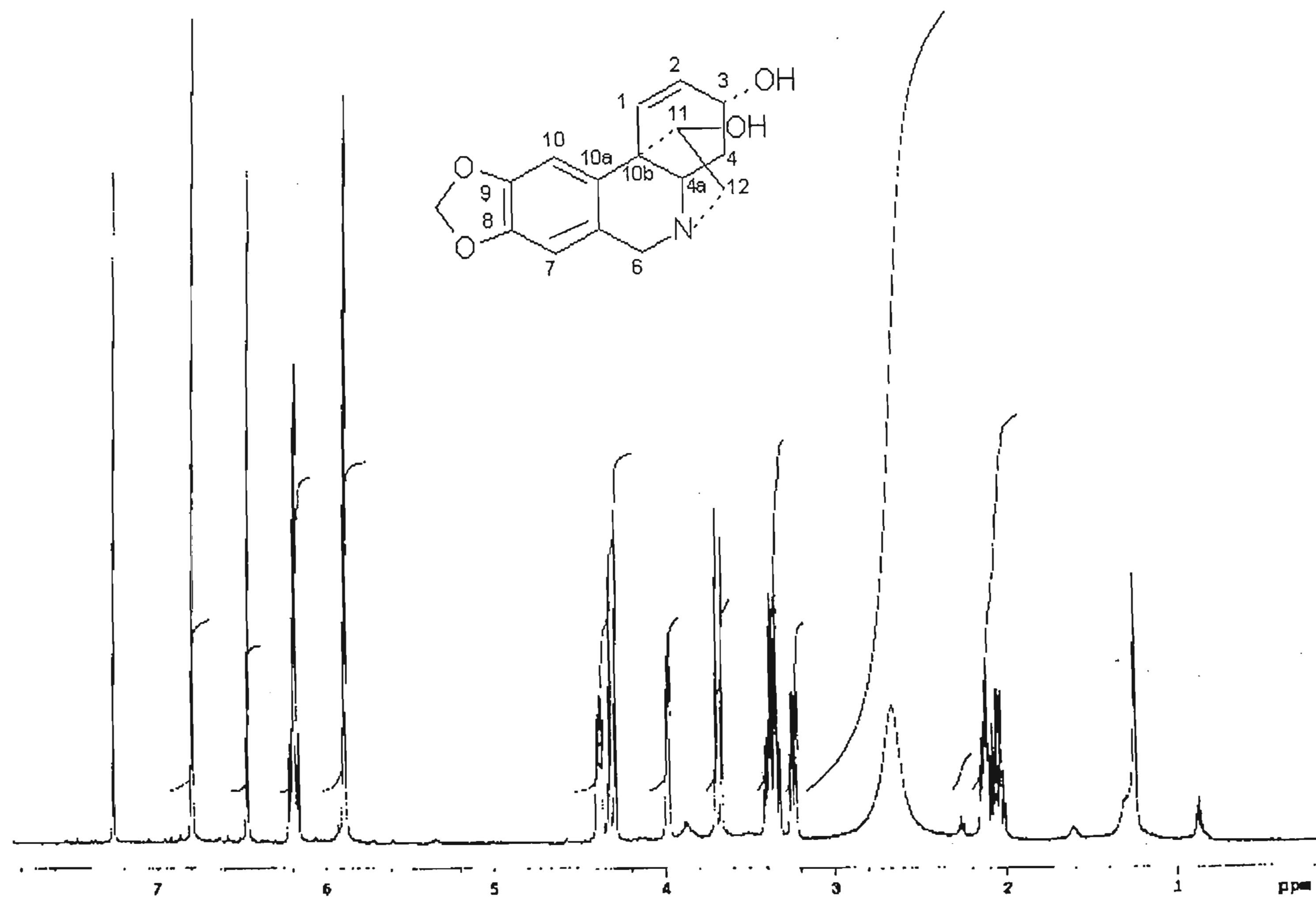


^{1}H NMR spectrum of crinine **32** in $CDCl_3$

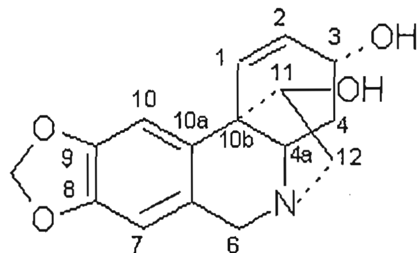




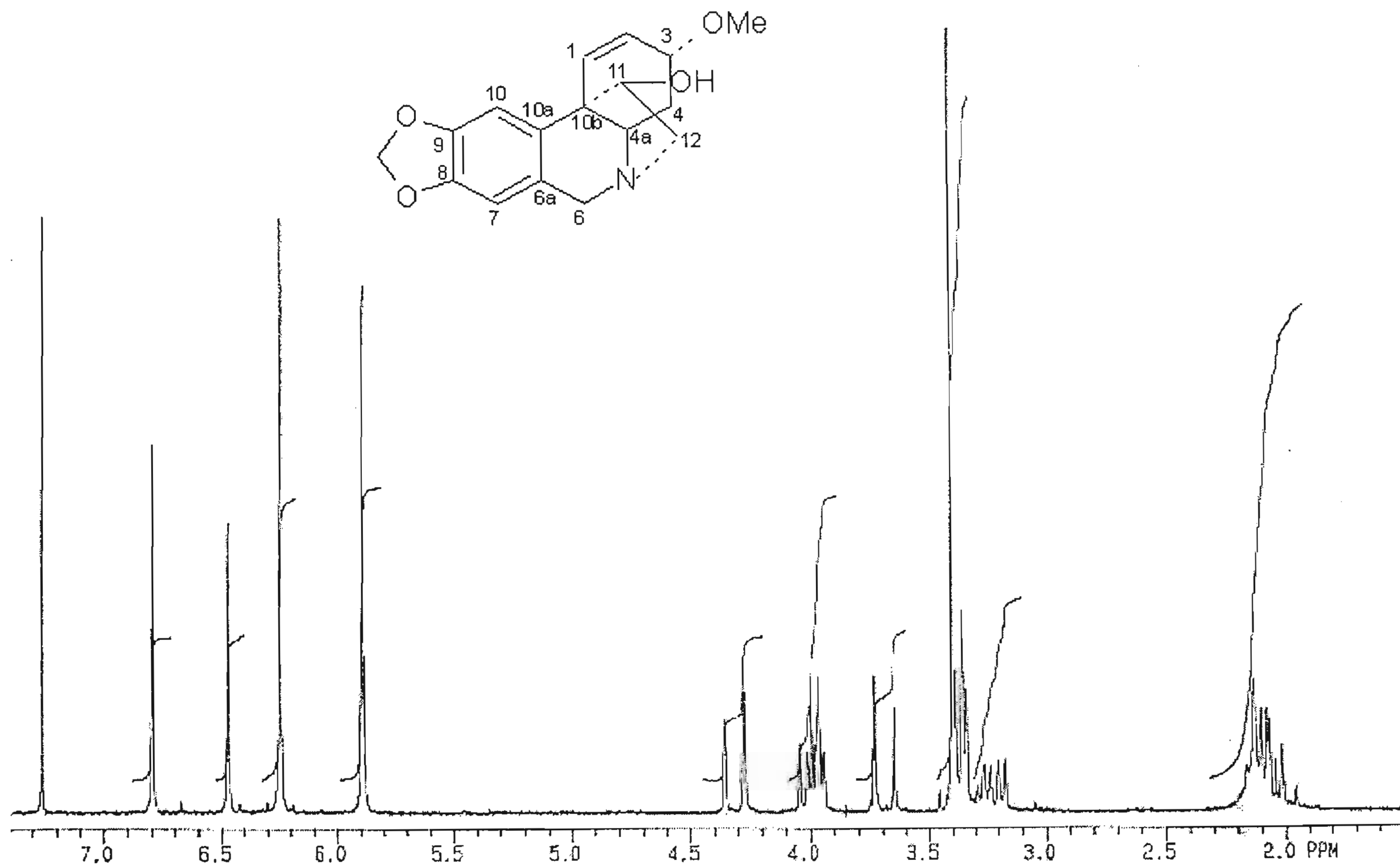
^{13}C NMR spectrum of crinine **32** in $CDCl_3$



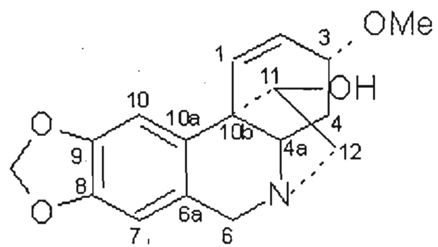
^{1}H NMR spectrum of hamayne **33** in $CDCl_3$

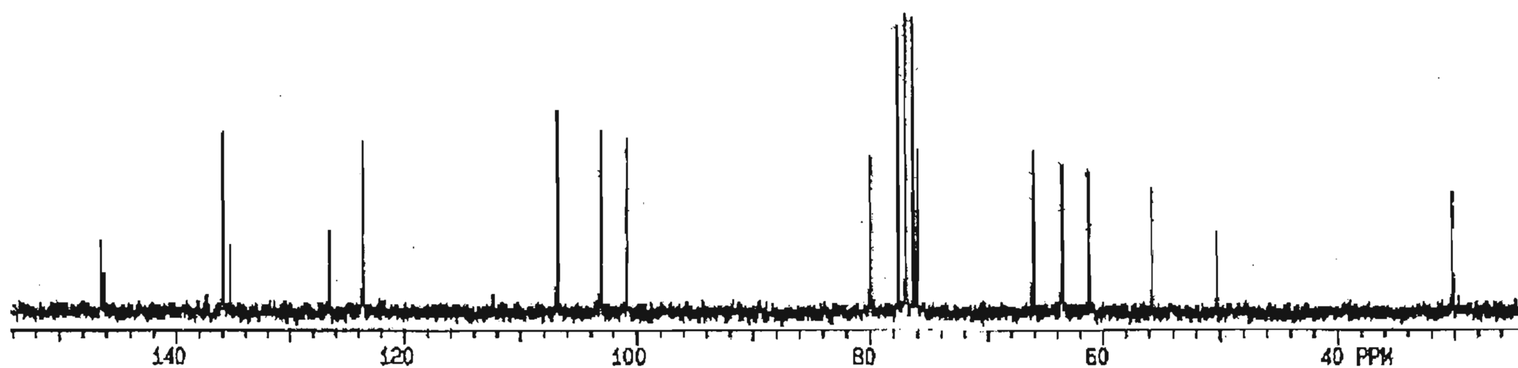


^{13}C NMR spectrum of hamayne **33** in $CDCl_3$

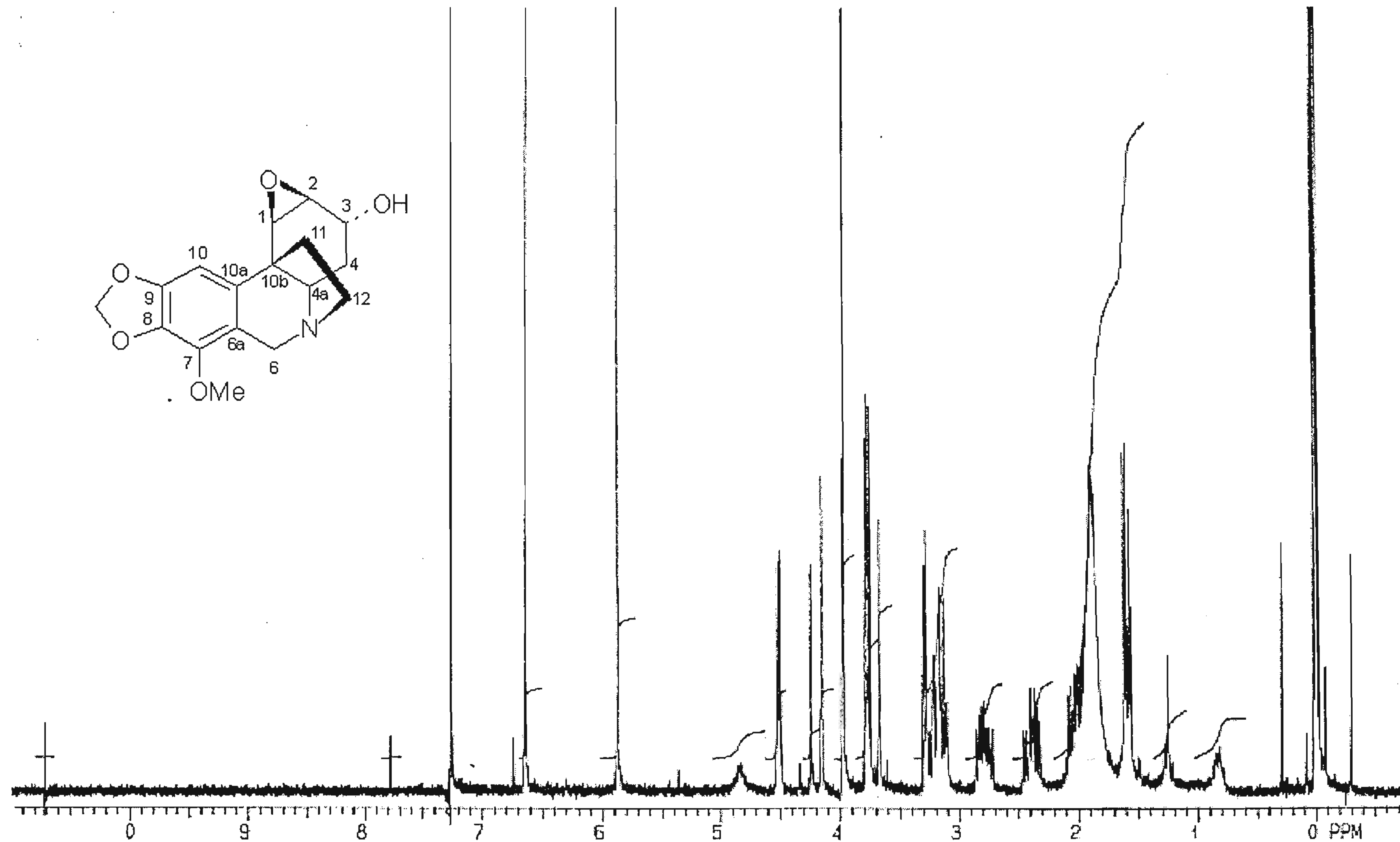


^{1}H NMR spectrum of crinamine **34** in $CDCl_3$

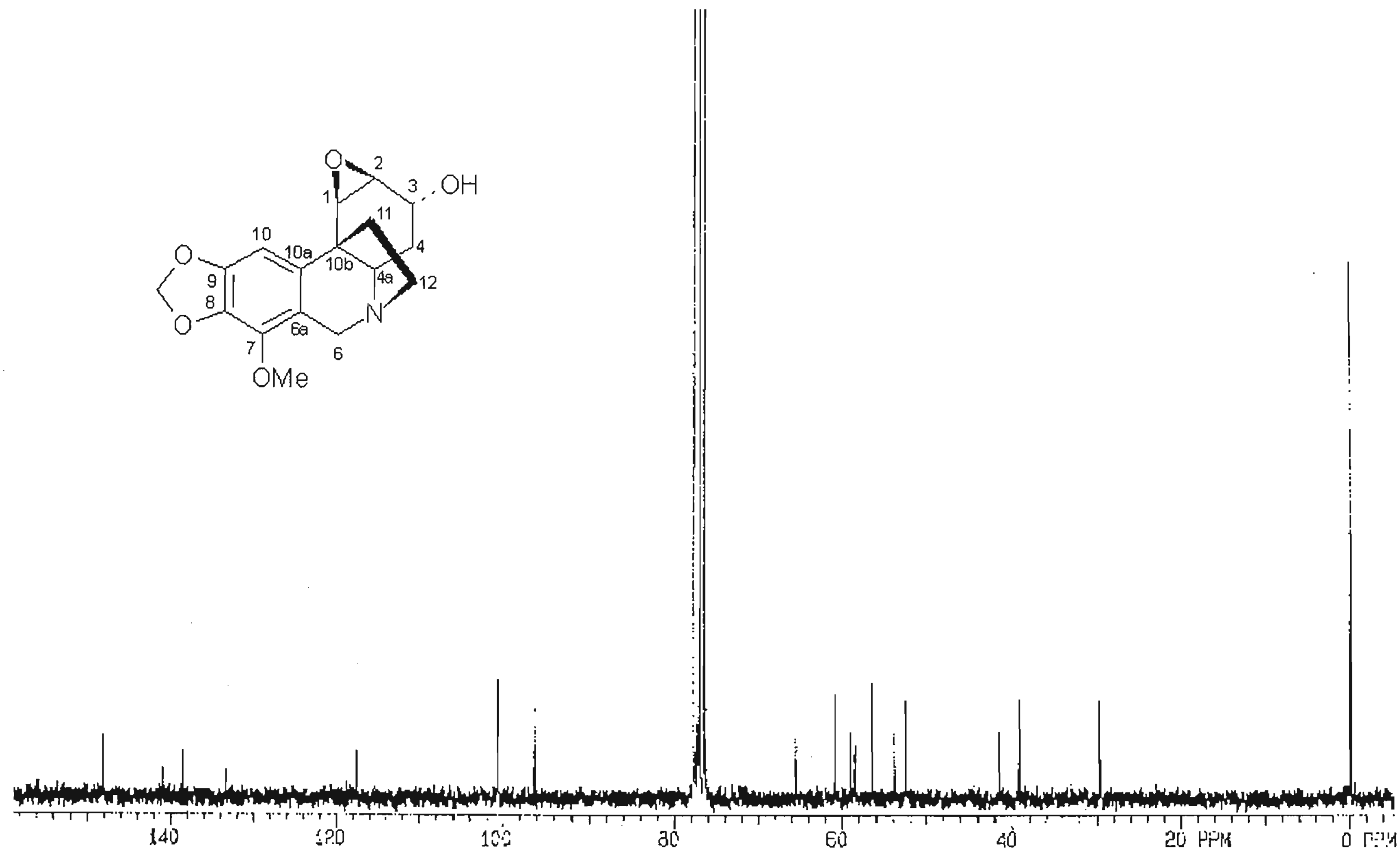




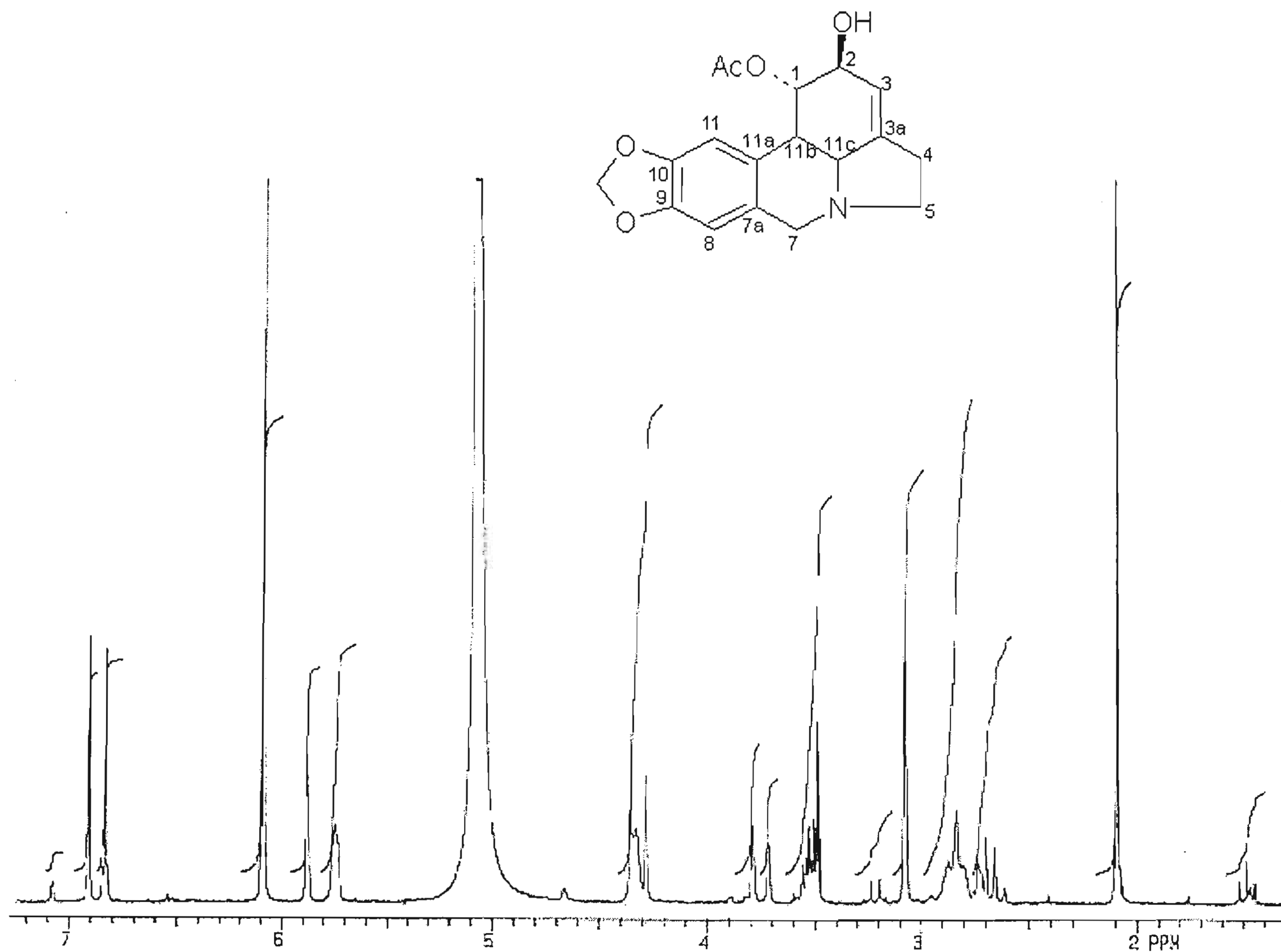
^{13} C NMR spectrum of crinamine **34** in $CDCl_3$



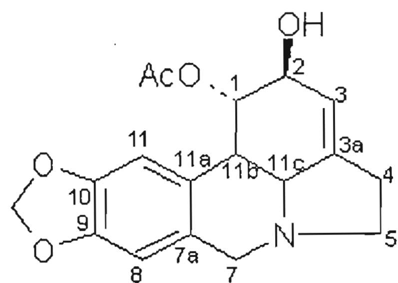
^{1}C NMR spectrum of crinamidine **36** in $CDCl_3$



^{13}C NMR spectrum of crinamidine **36** in $CDCl_3$



^1H NMR spectrum of 1-O-acetyllycorine **43** in CD_3 OD



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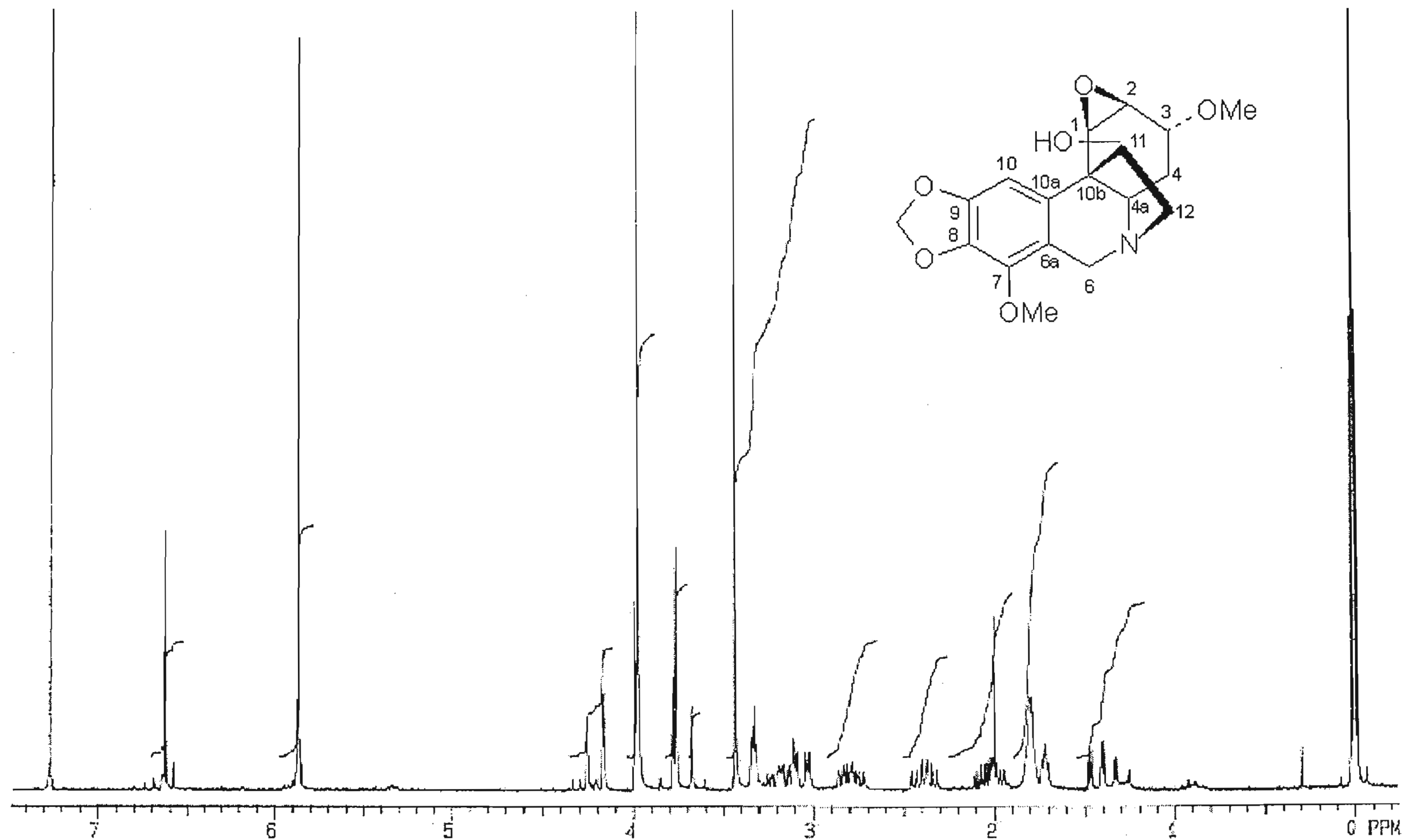
^{13}C NMR spectrum of 1-O-acetyllycorine **43** in CD_3OD



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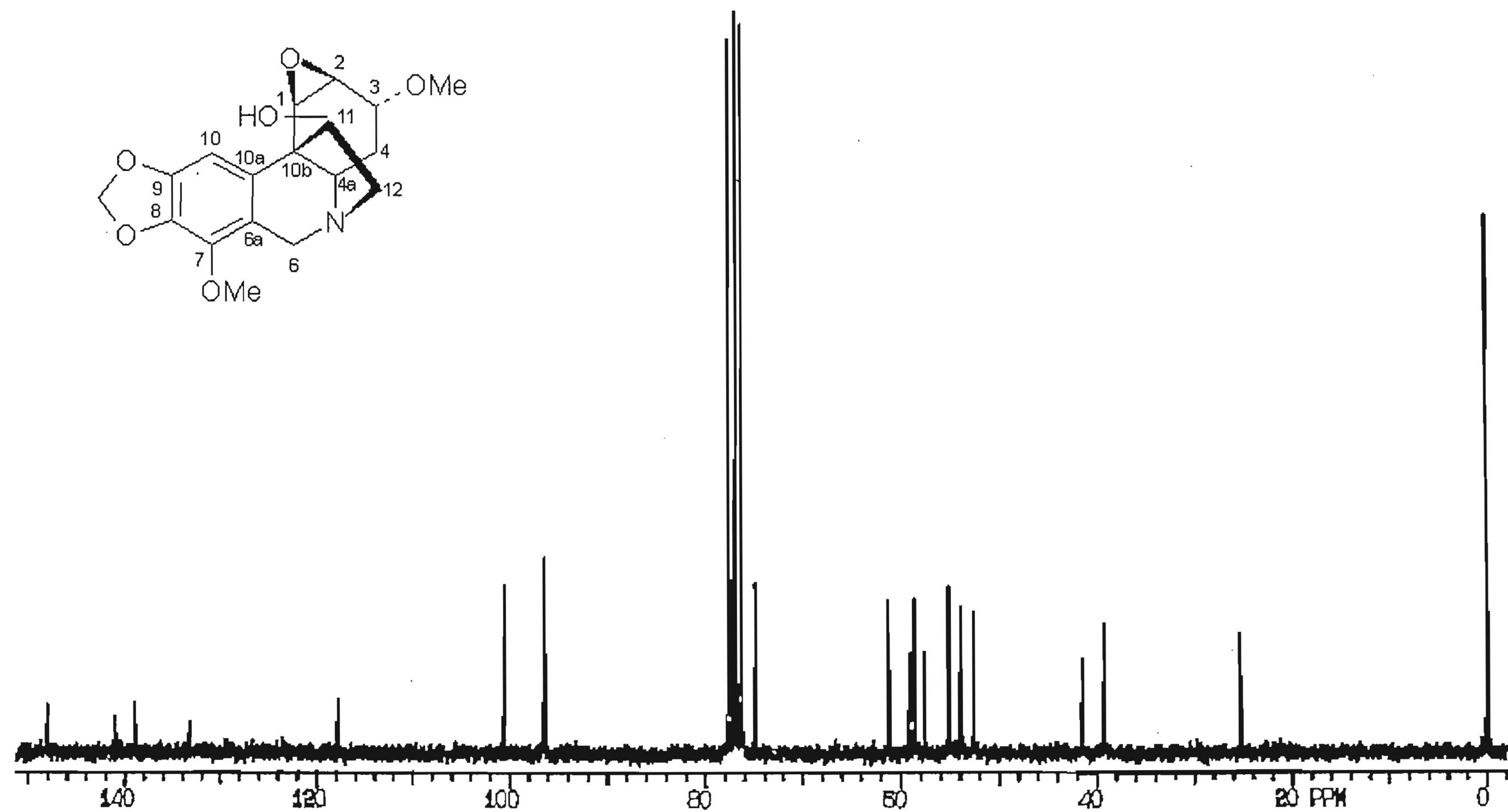
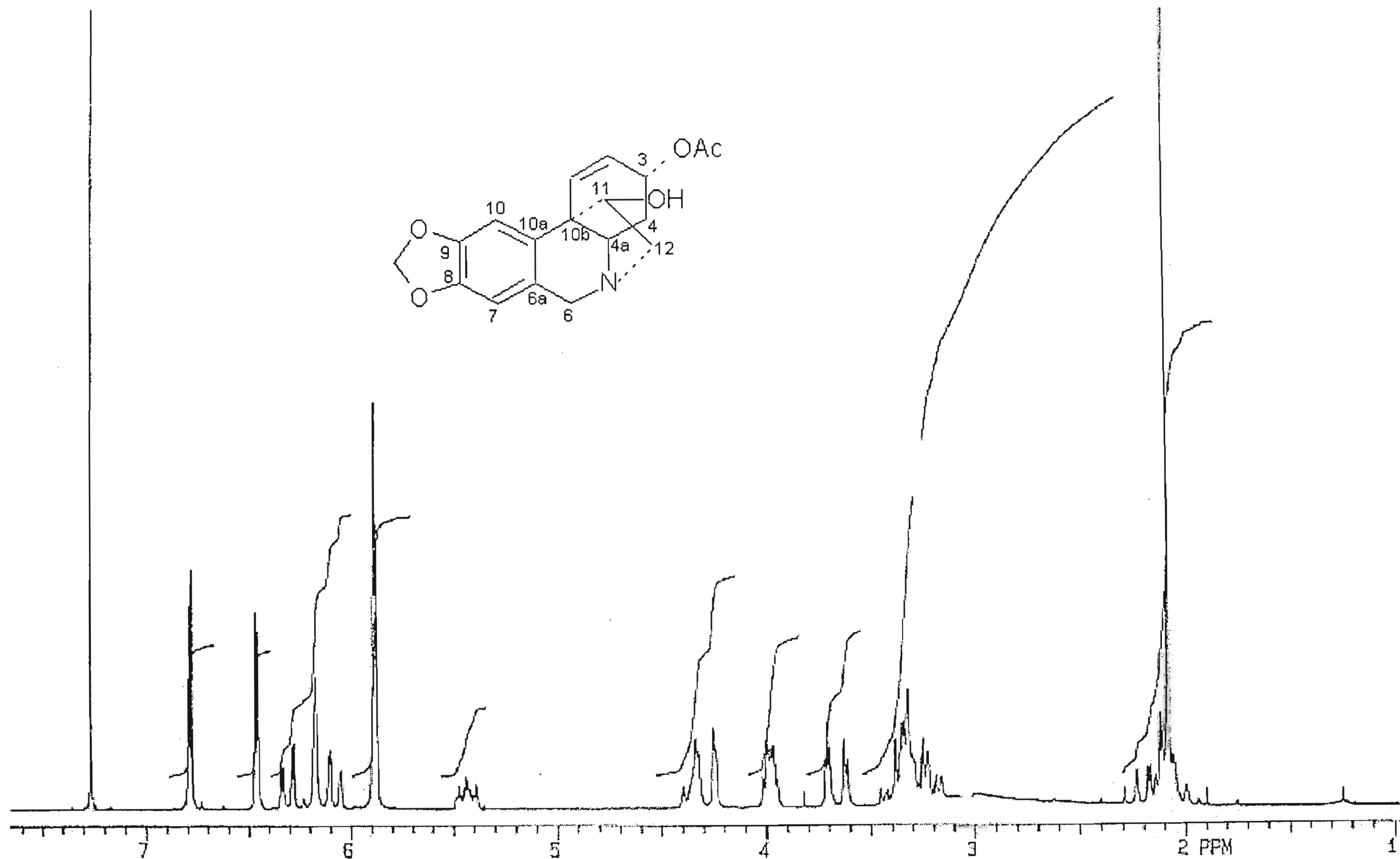


^{13}C NMR spectrum of undulatine **44** in $CDCl_3$



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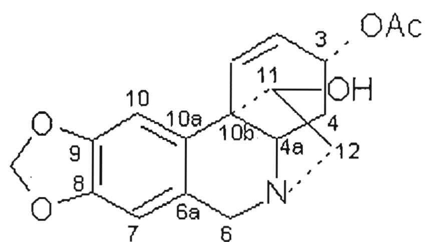

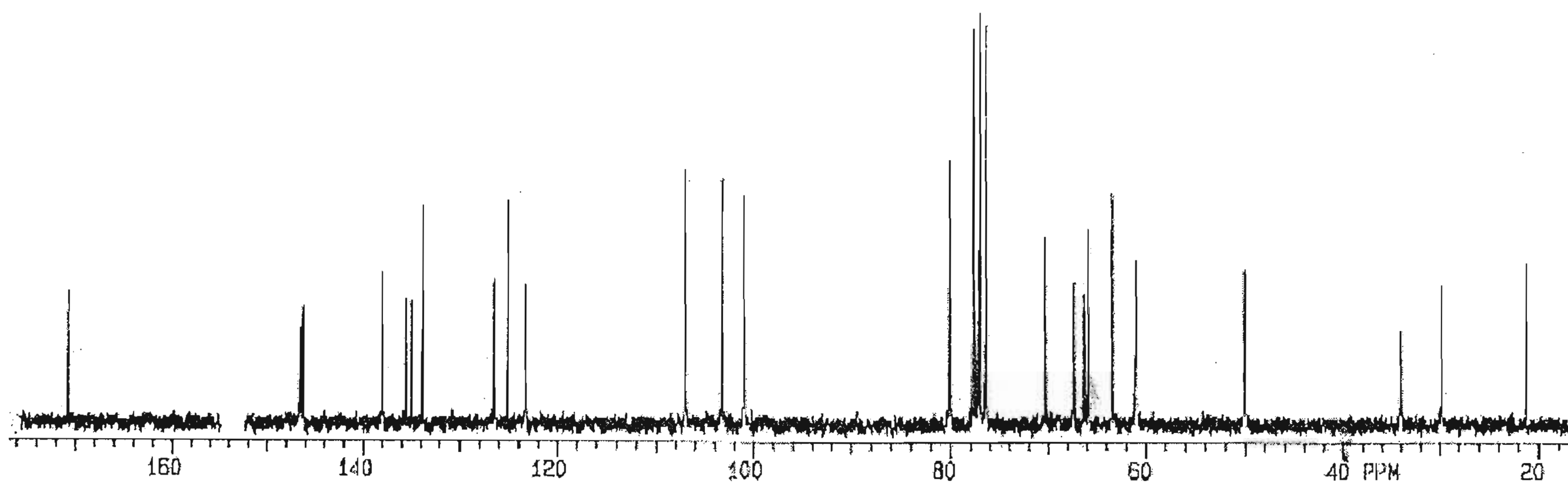

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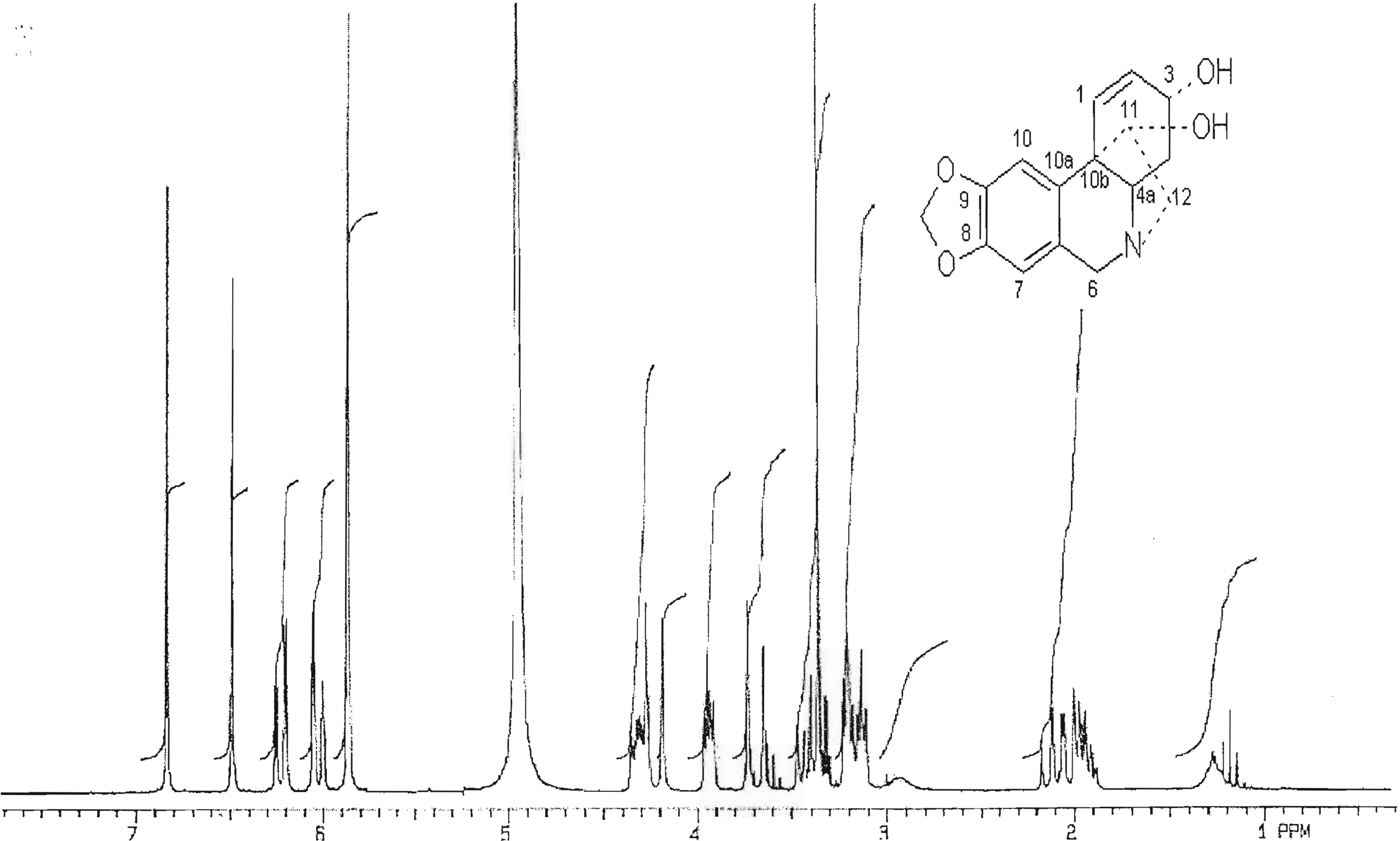
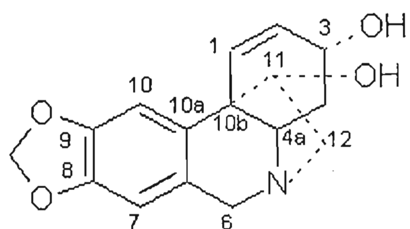
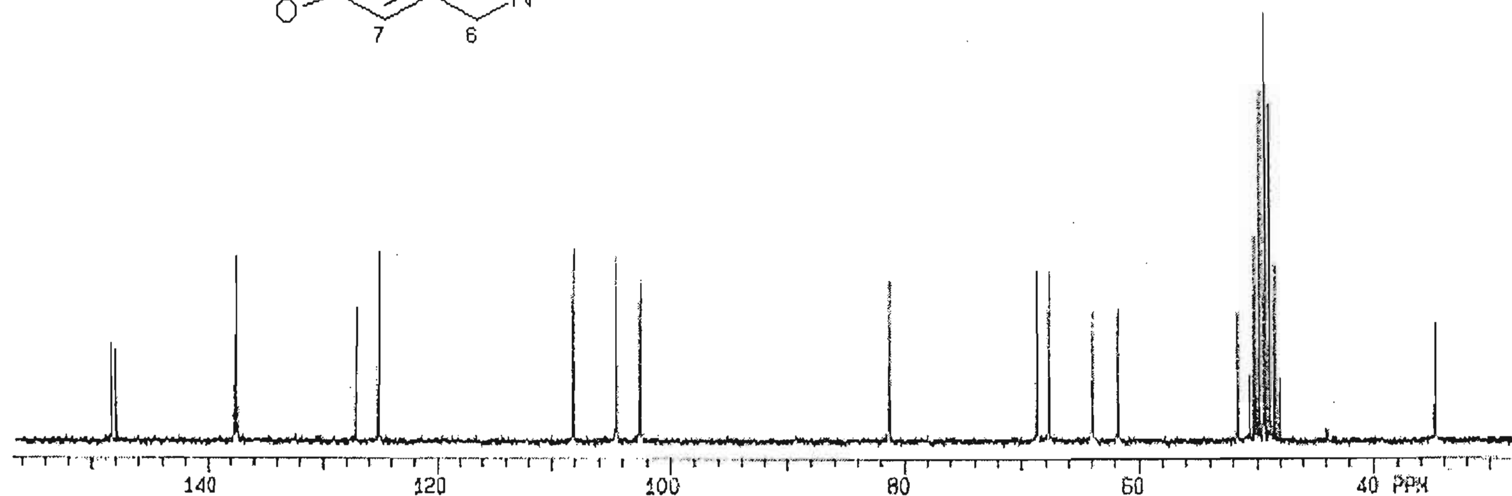
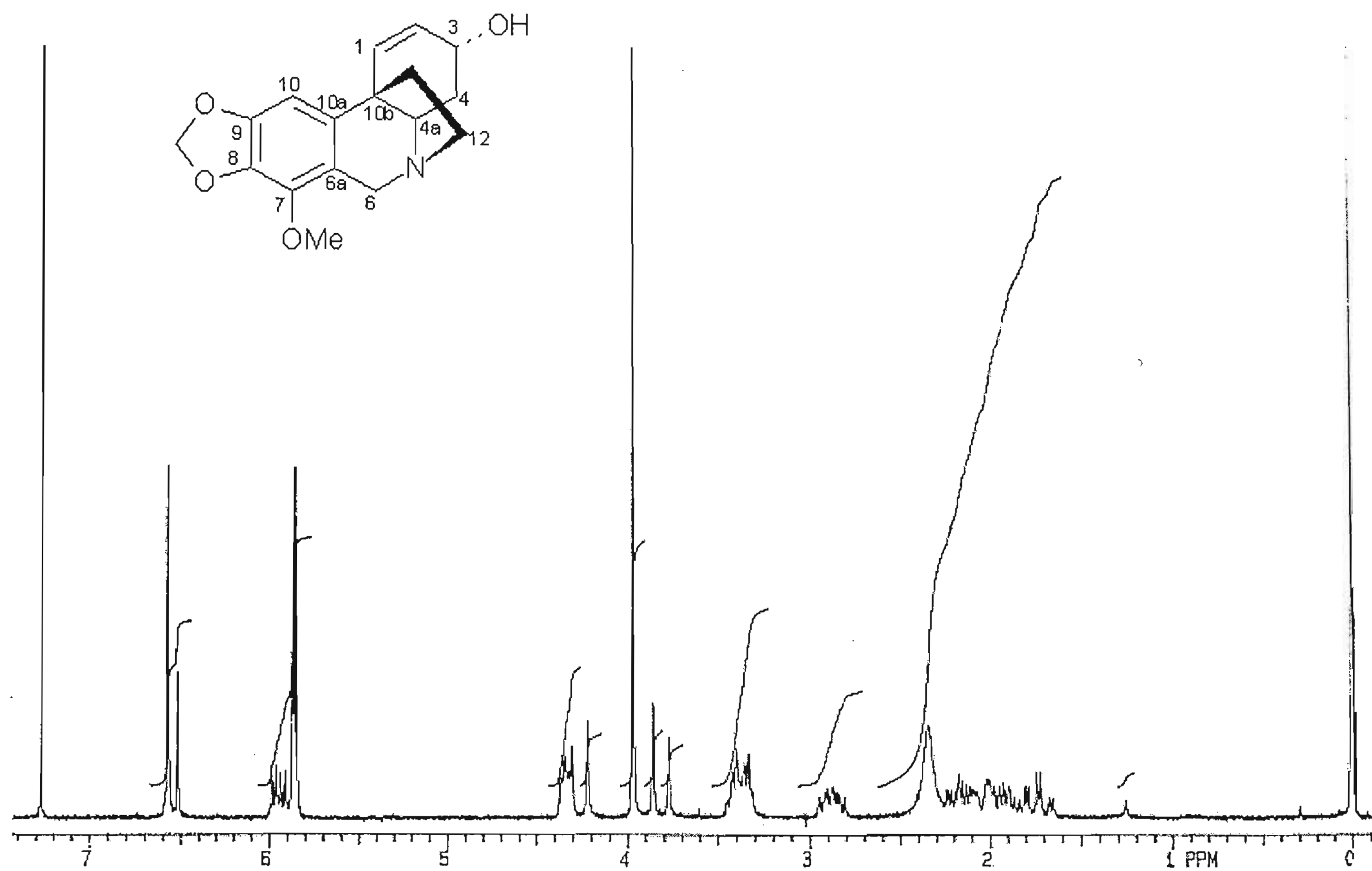


^{1}H NMR spectrum of bulbispermine **48** in CD_3OD

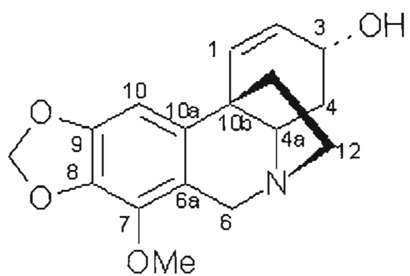


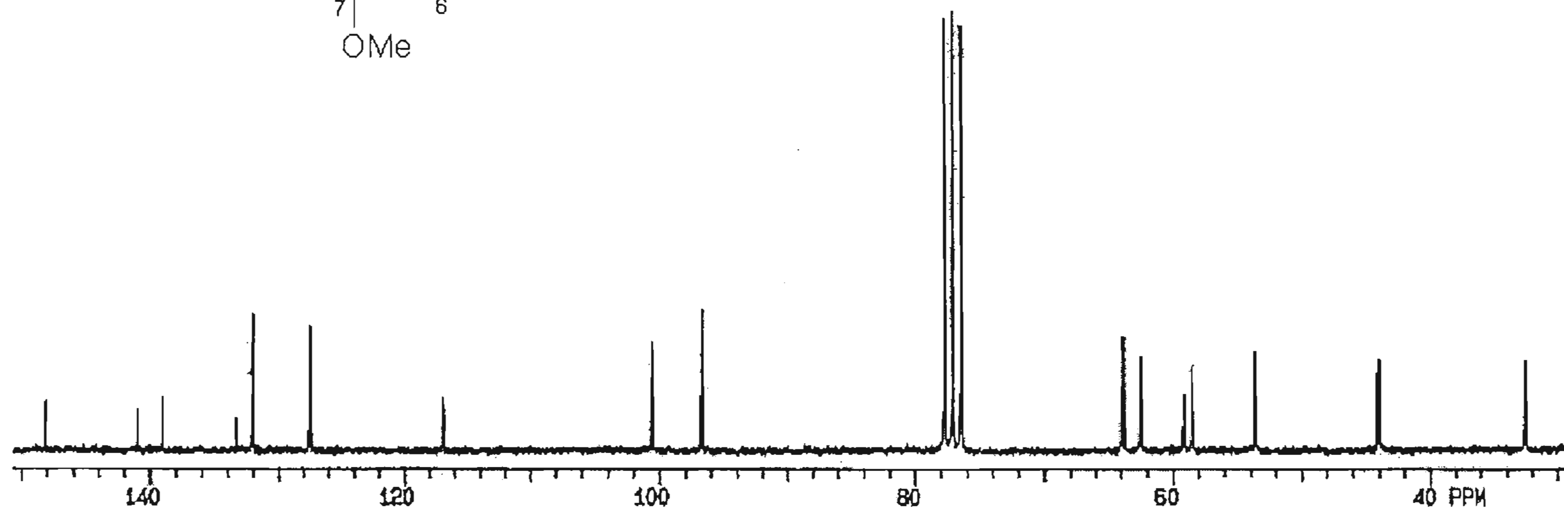


^{13}C NMR spectrum of bulbispermine **48** in CD_3OD

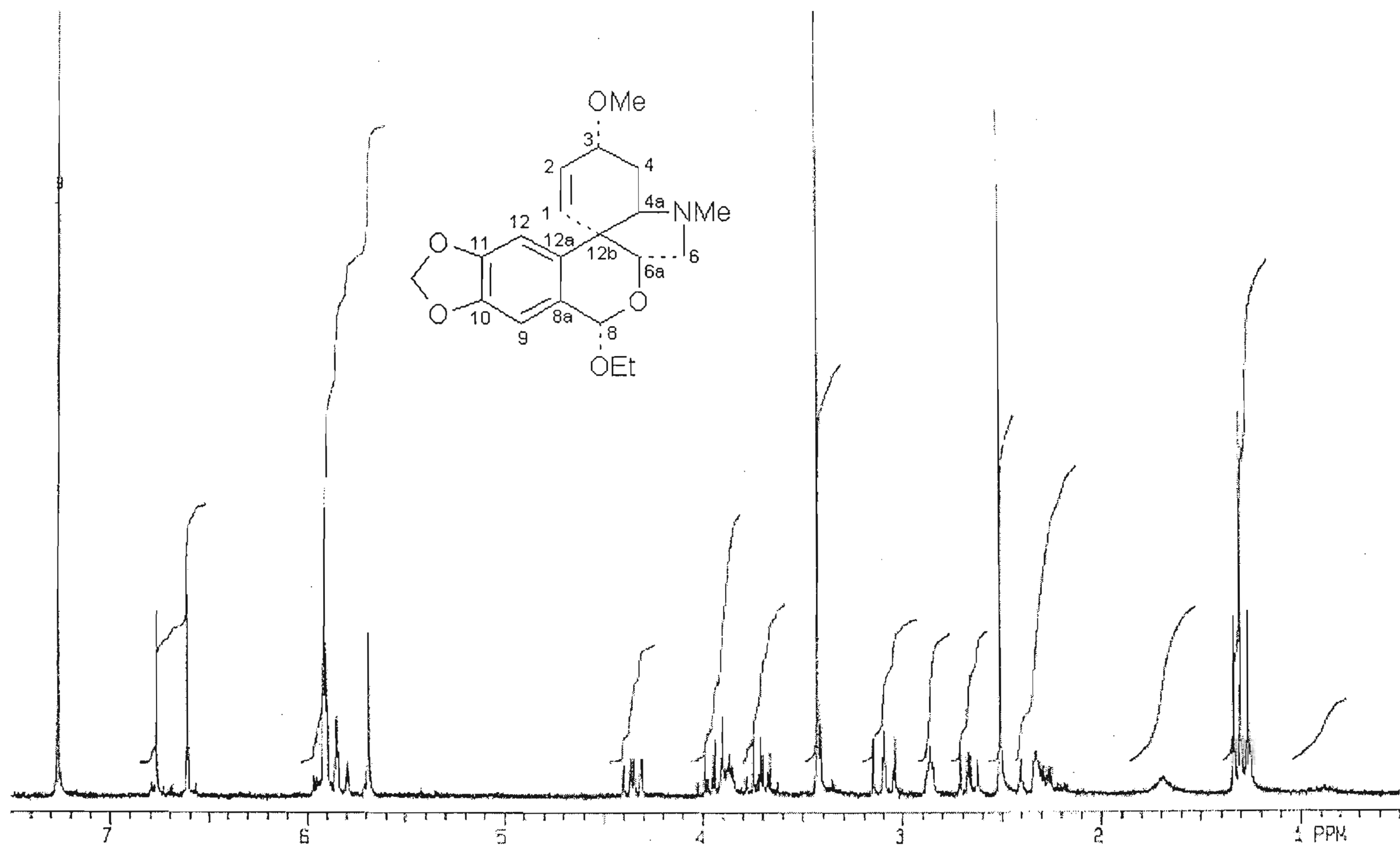


[1] H NMR spectrum of powelline **73** in $CDCl_3$

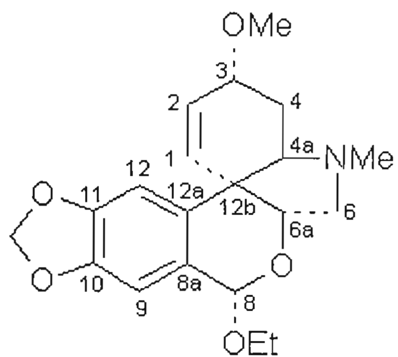




^{13}C NMR spectrum of powelline **73** in $CDCl_3$



[1] H NMR spectrum of 8α-ethoxyprecriwelline **100** in $CDCl_3$



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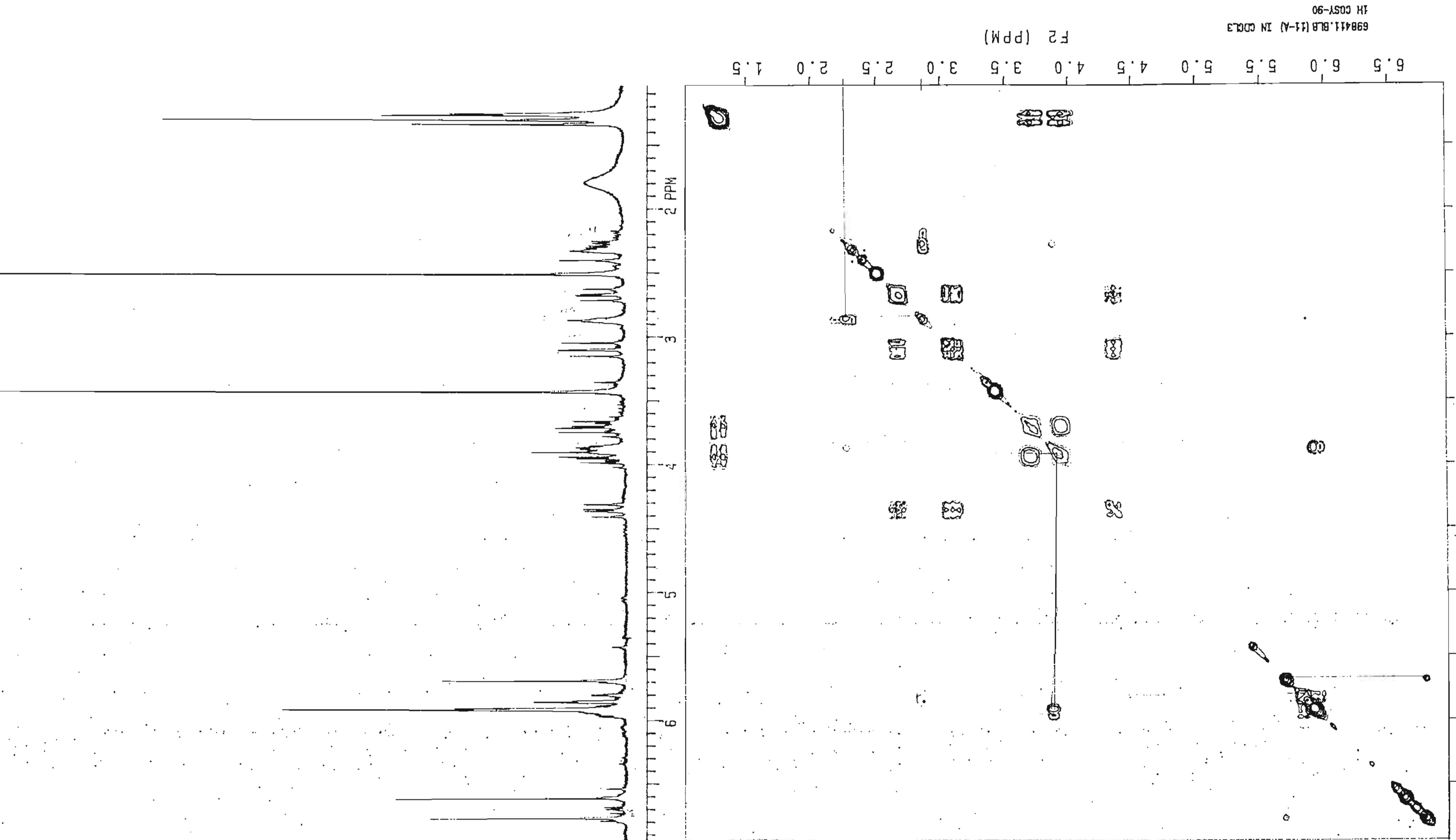
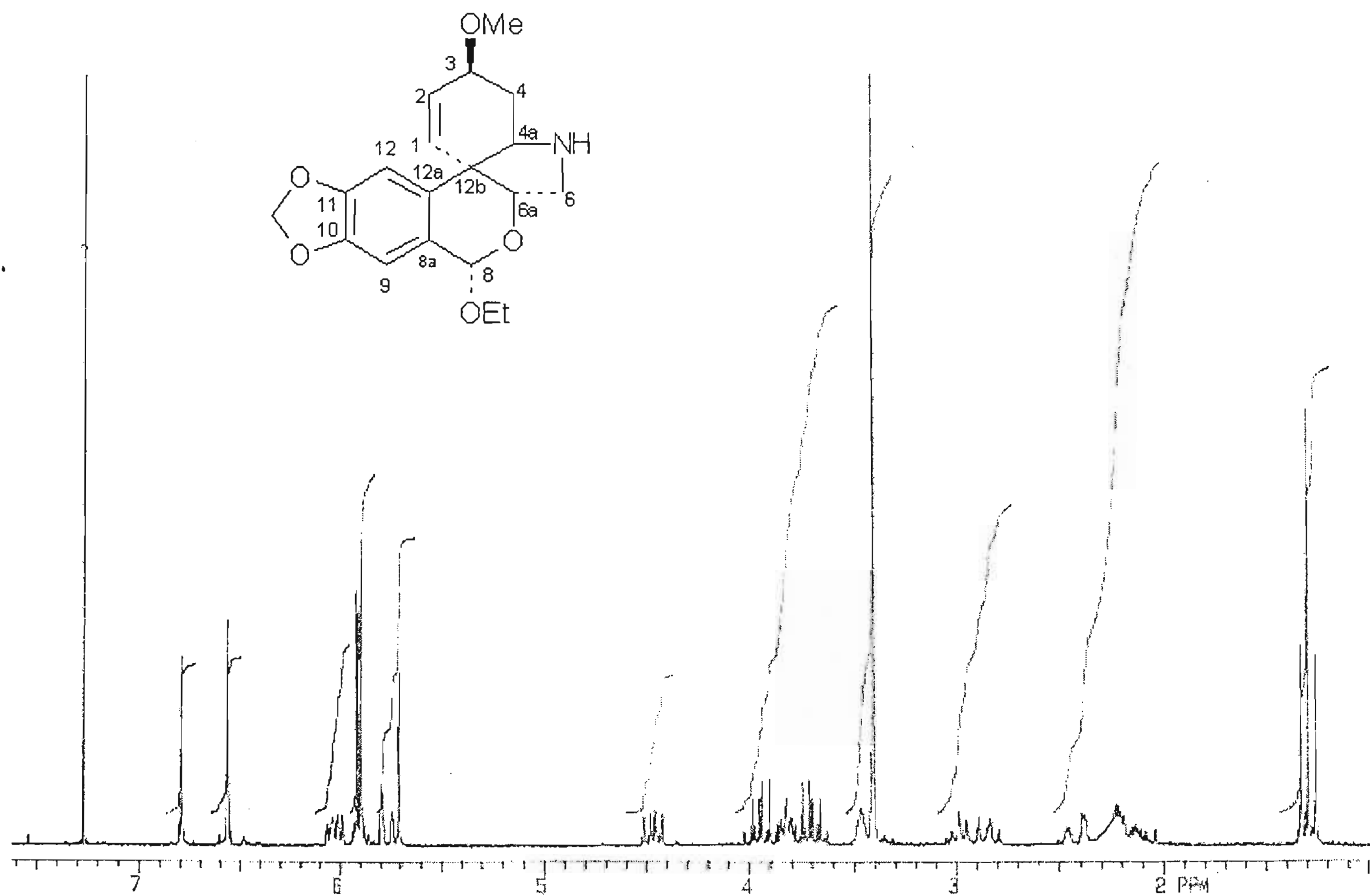
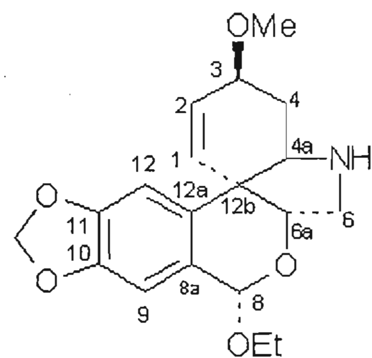
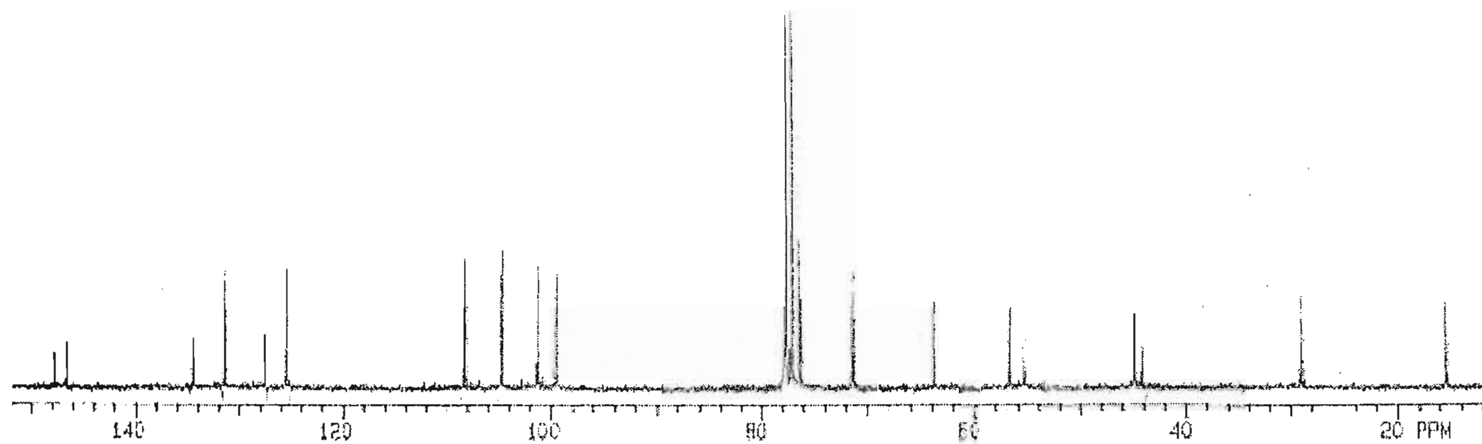


COSY spectrum of 8α-ethoxyprecriwelline **100** in $CDCl_3$



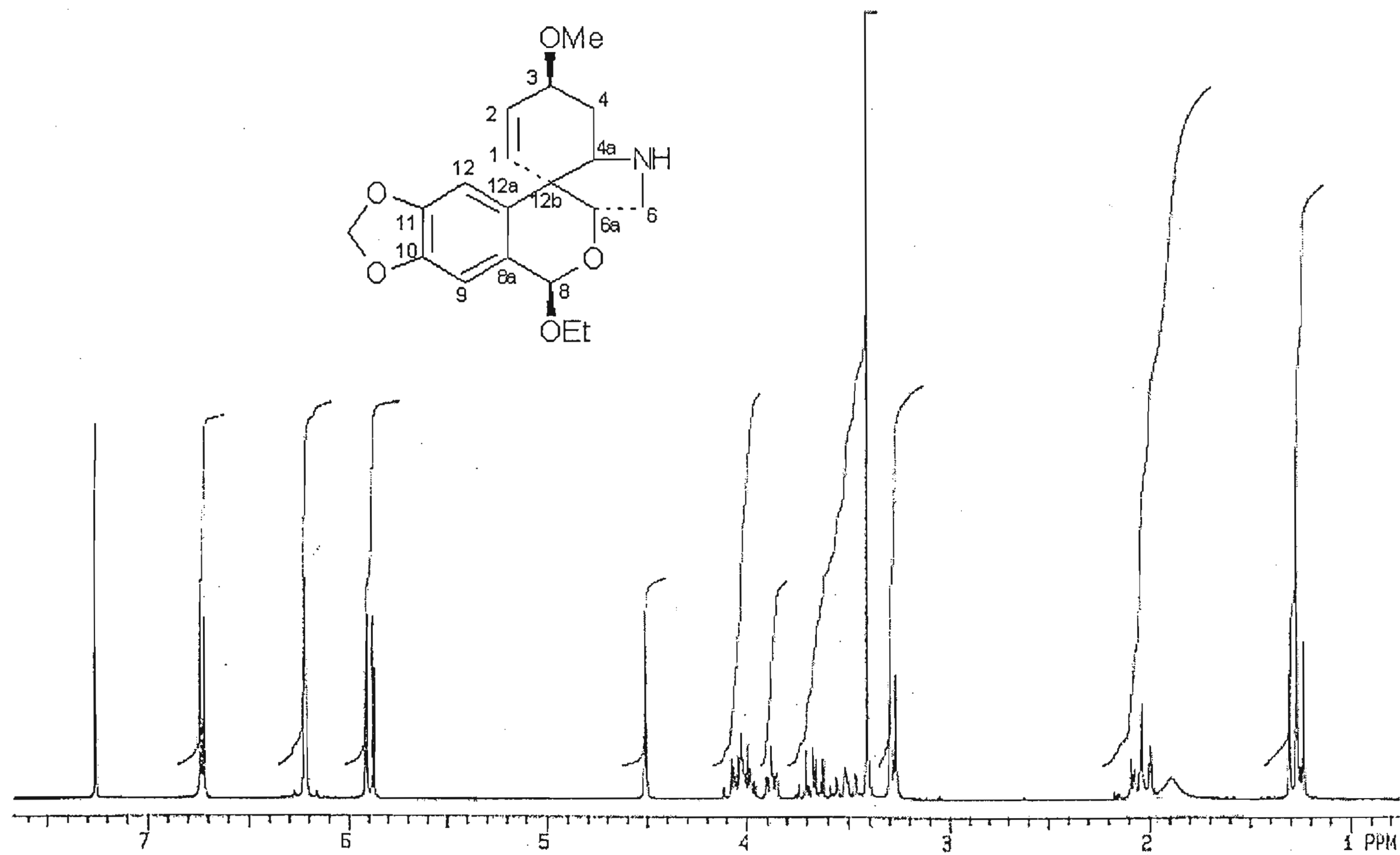
^{13}C NMR spectrum of N-desmethyl-8α-ethoxypretazettine **101** in $CDCl_3$



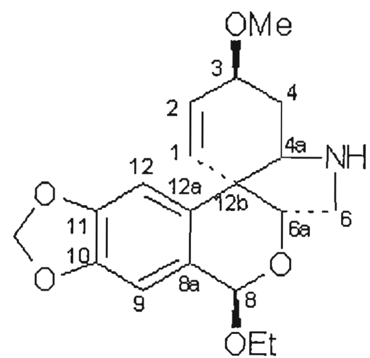


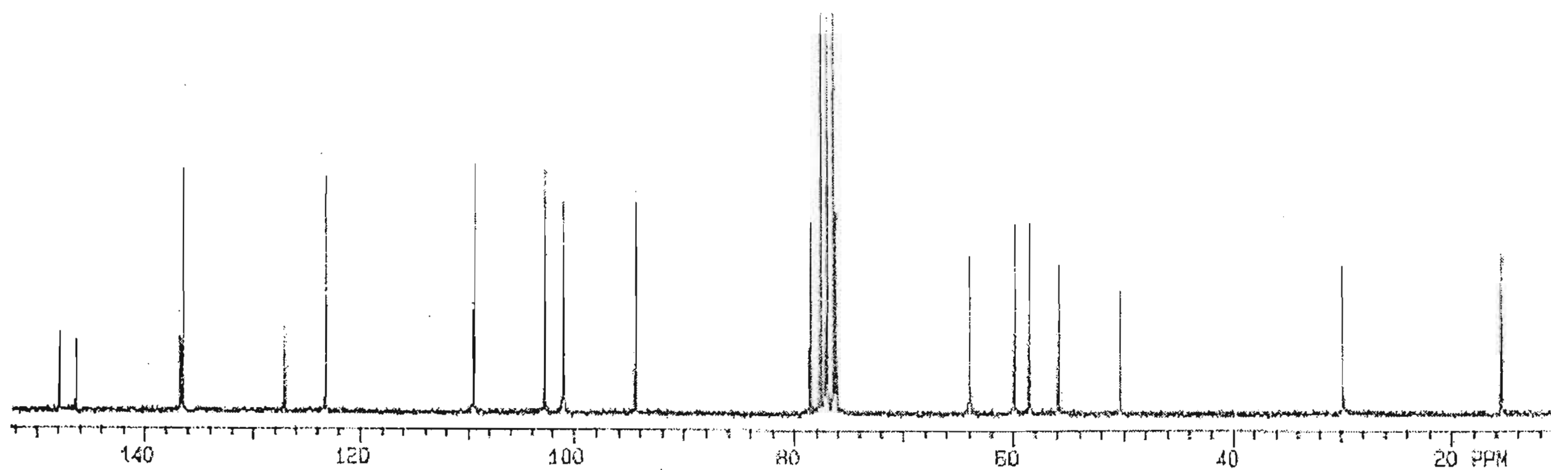
^{1}H NMR spectrum of N-desmethyl-8α-ethoxypretazettine **101** in $CDCl_3$

COSY spectrum of *N*-desmethyl- 8α-ethoxypretazettine **101** in $CDCl_3$



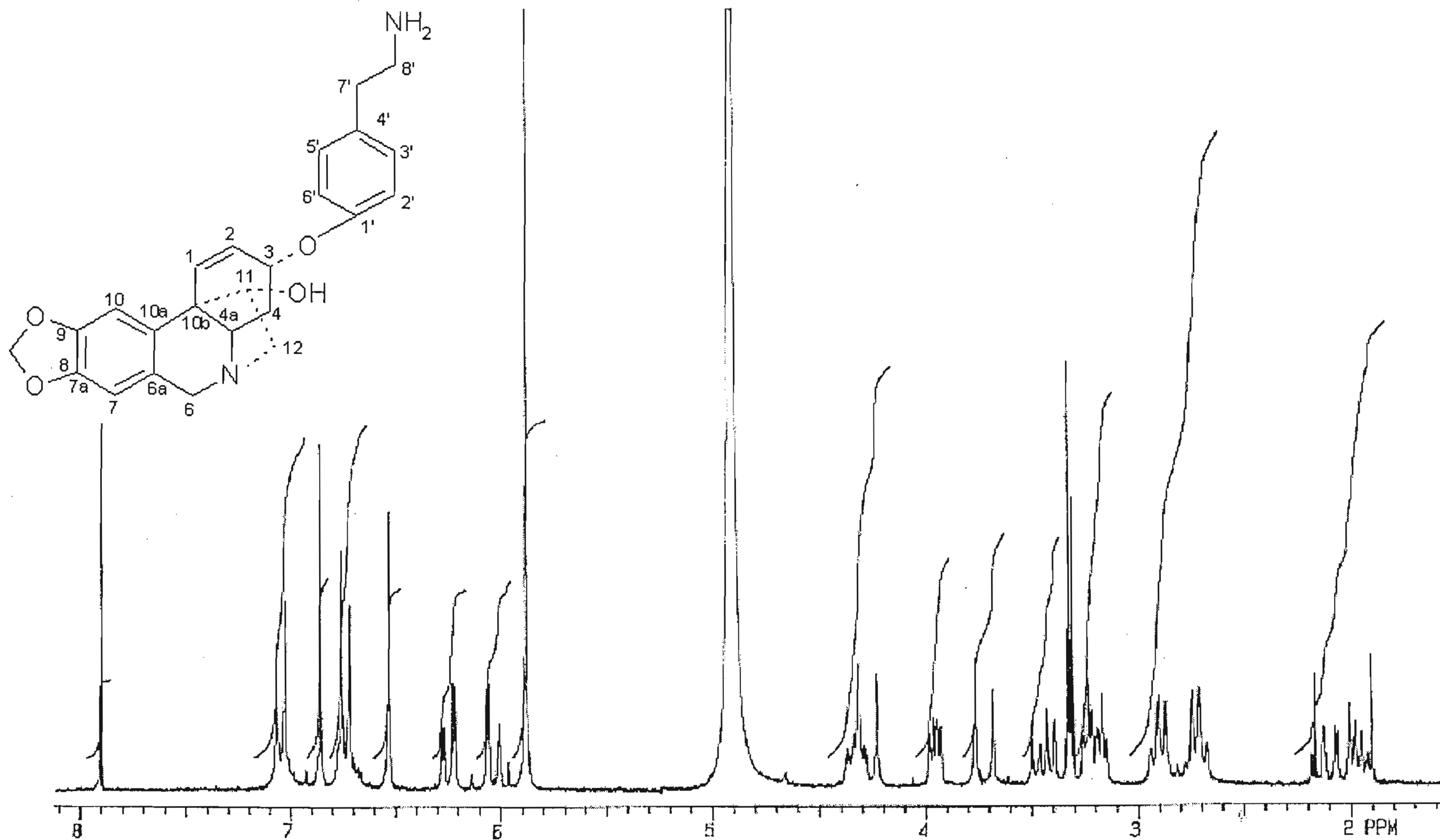
^{1}H NMR spectrum of N-desmethyl-8β-ethoxypretazettine **102** in $CDCl_3$



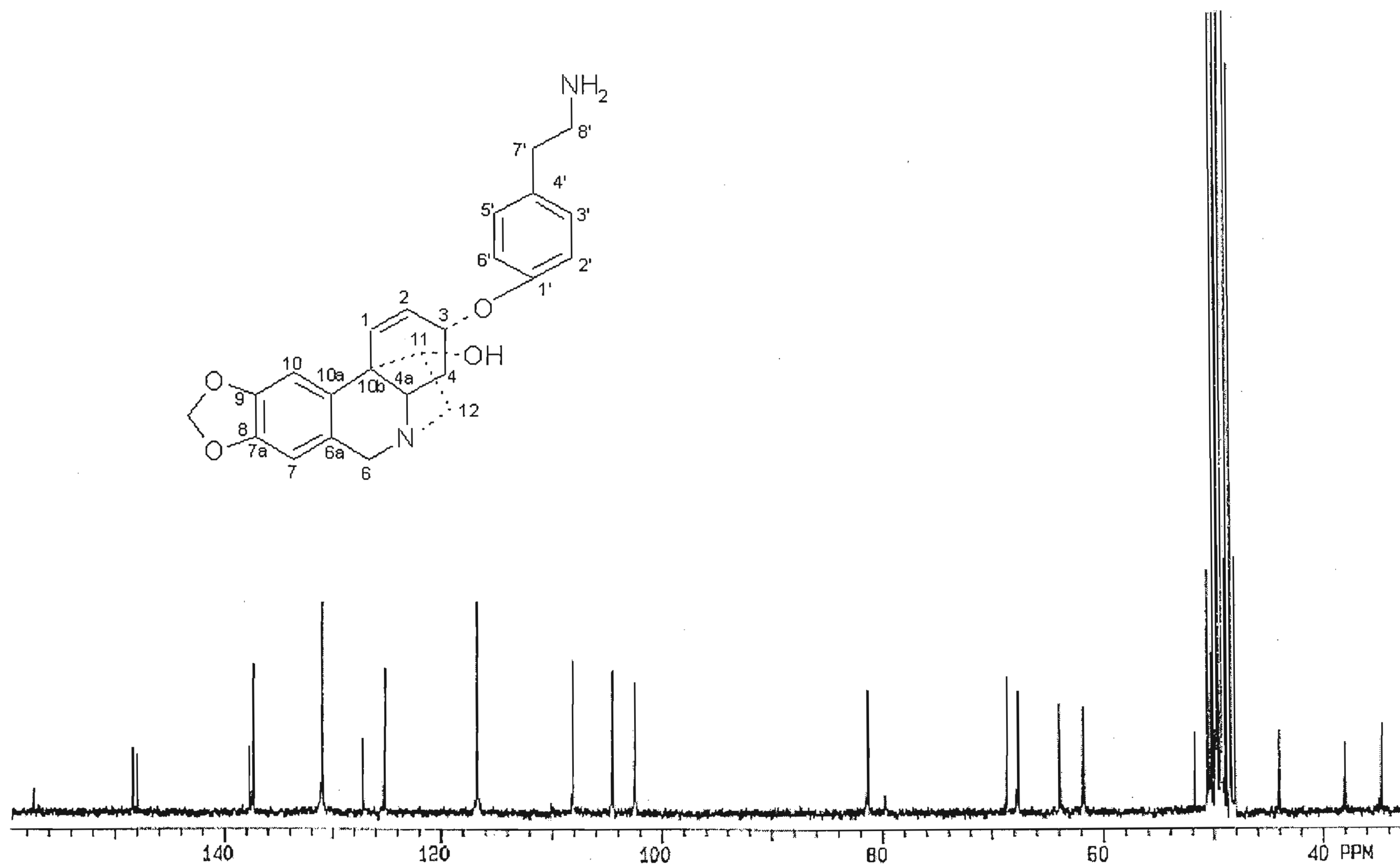


^{13}C NMR spectrum of N-desmethyl-8β-ethoxypretazettine **102** in $CDCl_3$

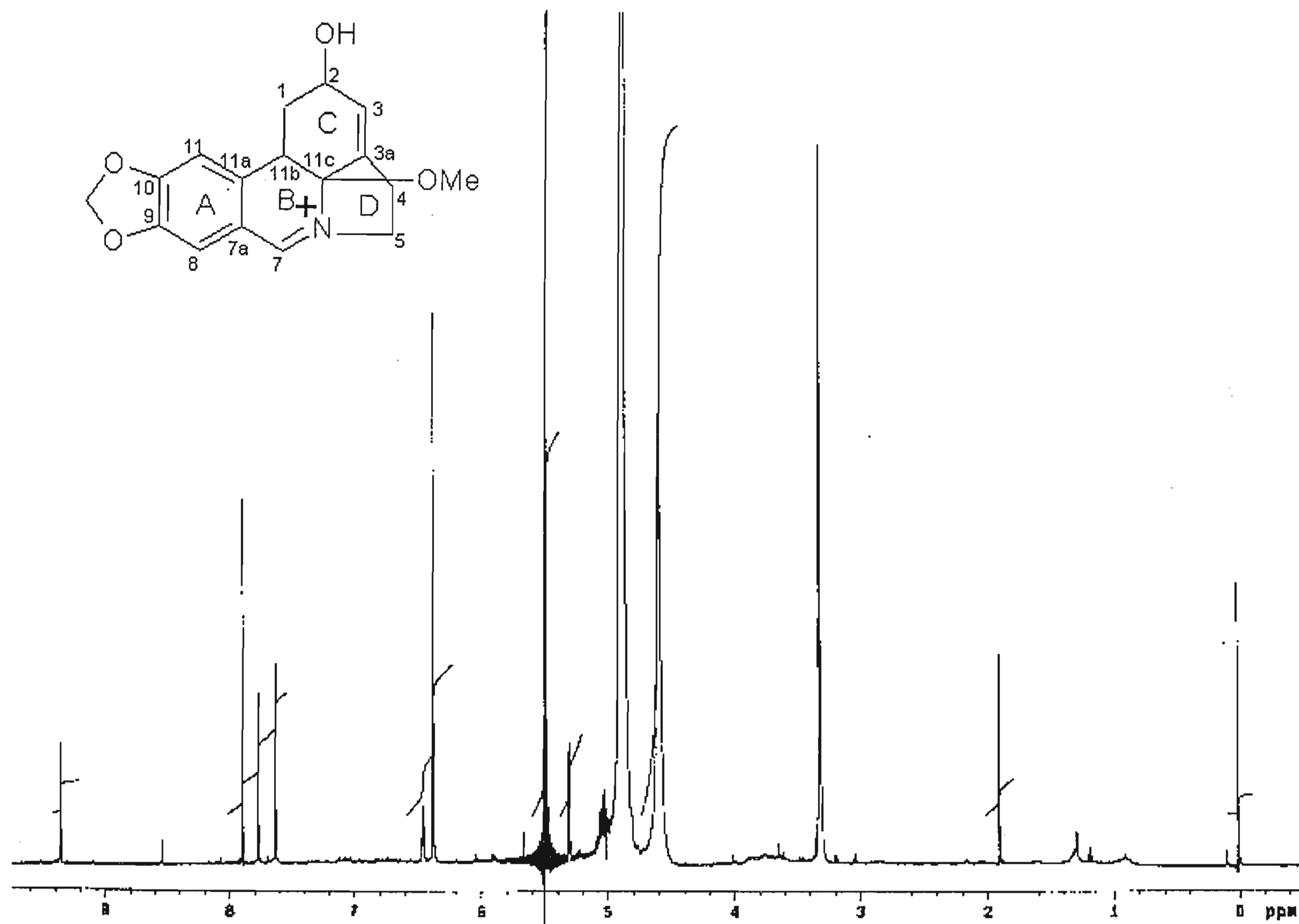
COSY spectrum of *N*-desmethyl- 8β-ethoxypretazettine **102** in $CDCl_3$



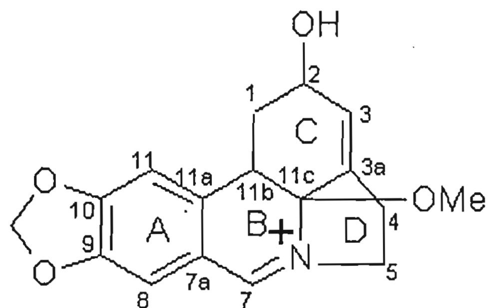
^{1}H NMR spectrum of 3-[4'-(8'-aminoethyl) phenoxy] bulbispermine **103** in CD_3 OD



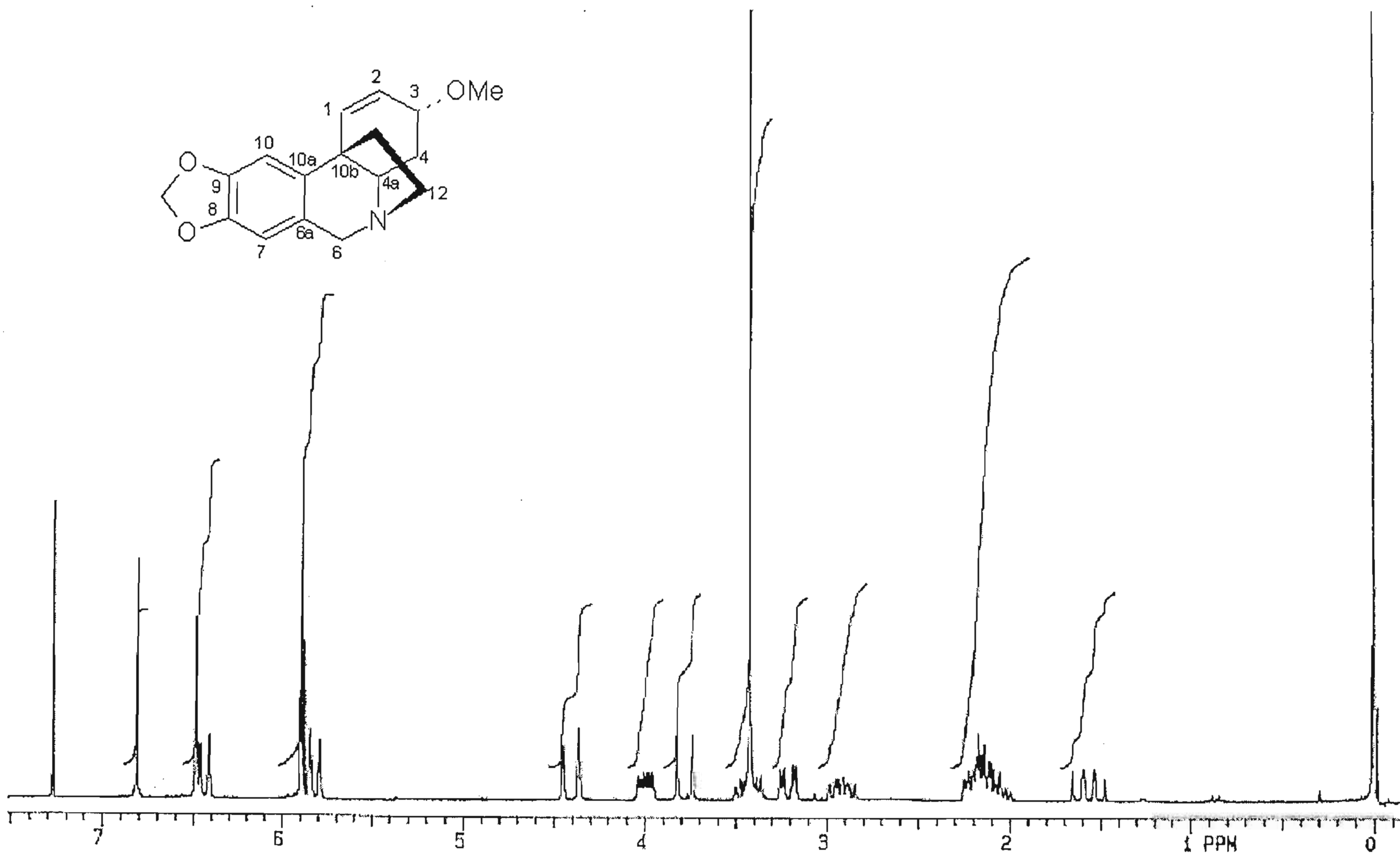
^{13}C NMR spectrum of 3-[4'-(8'-aminoethyl) phenoxy] bulbispermine **103** in CD_3OD



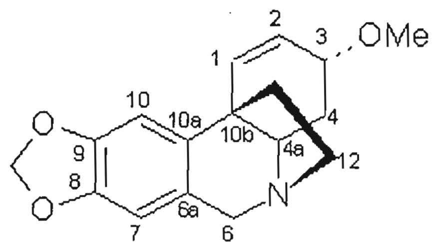
^{1}H NMR spectrum of mooreine **104** in CD_3OD

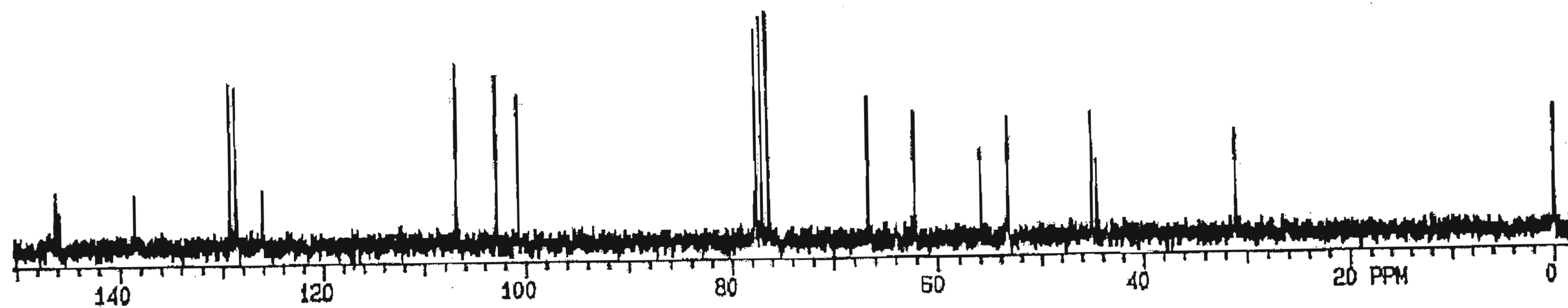


^{13}C NMR SPECTRUM OF MOOREINE **104** IN CD_3OD

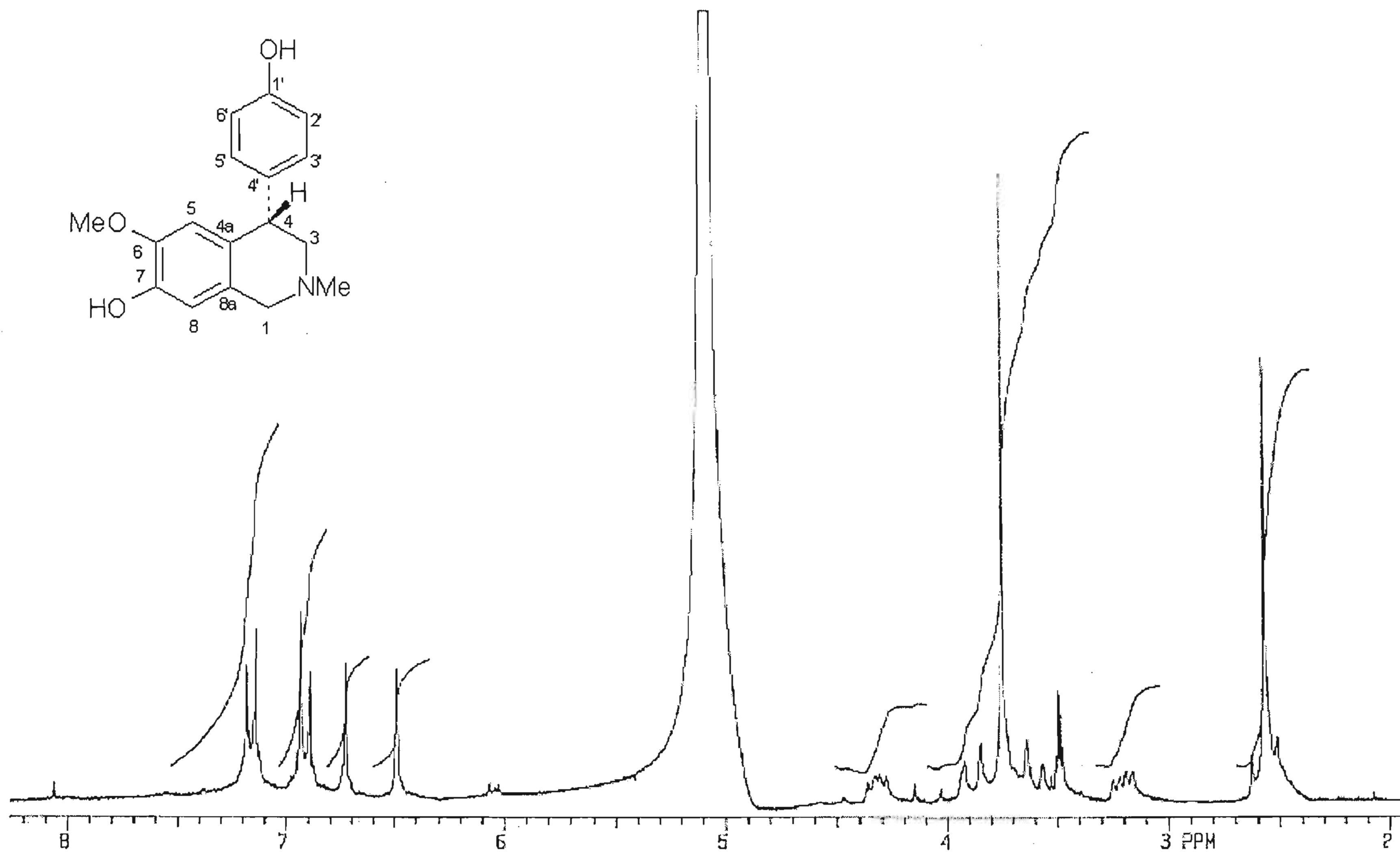


^{1}H NMR spectrum of epibuphanisine **105** in $CDCl_3$

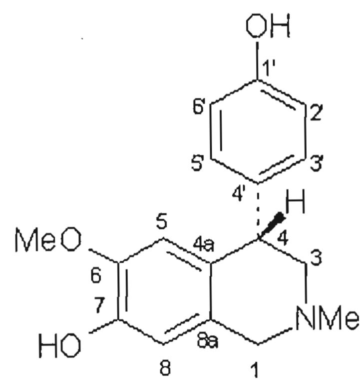




^{13}C NMR spectrum of epibuphanisine **105** in $CDCl_3$



^{1}H NMR spectrum of cherylline **106** in CD_3OD



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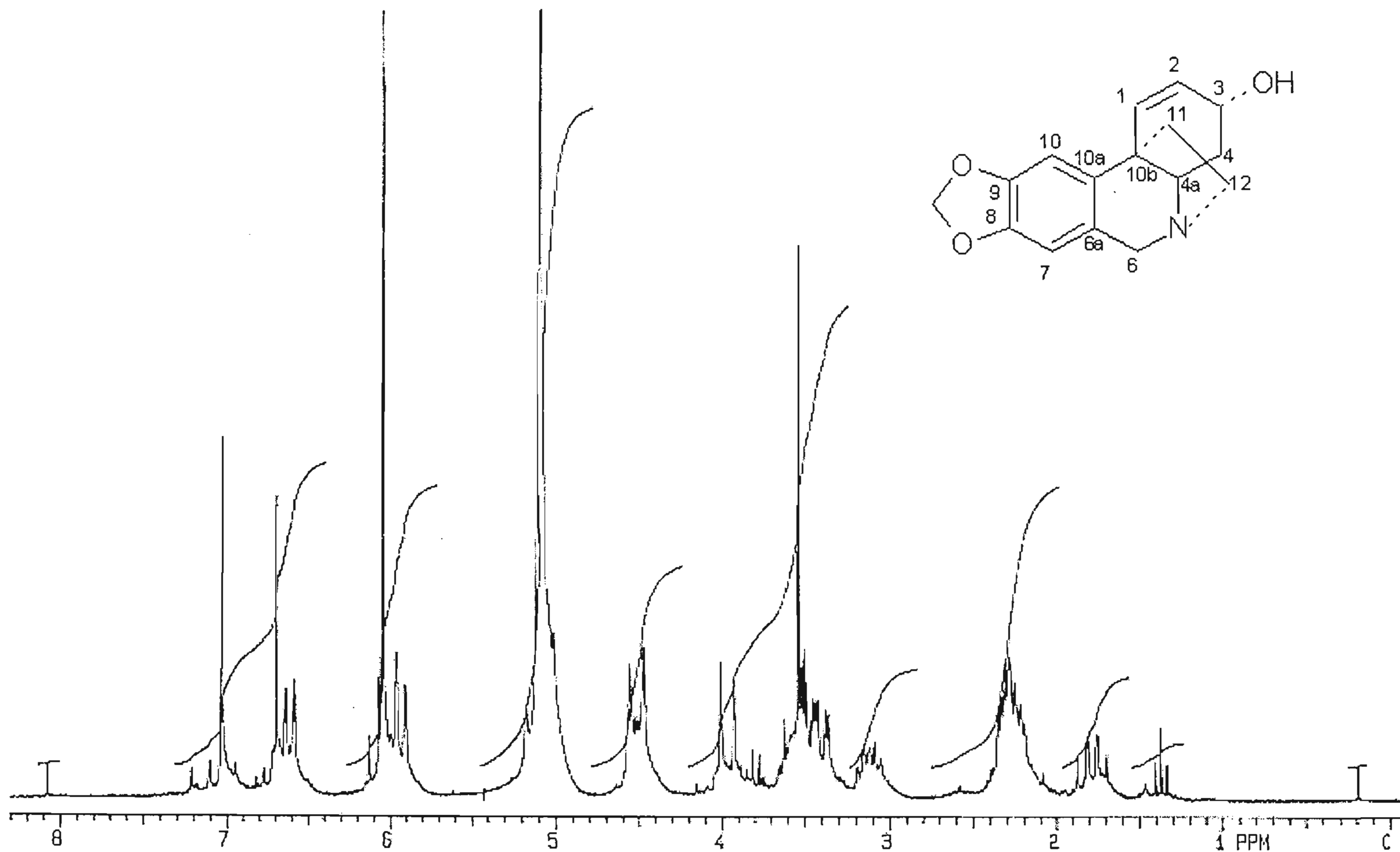
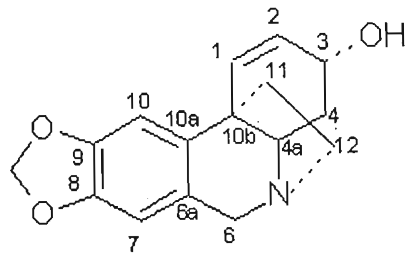
^{13}C NMR spectrum of cherylline **106** in CD_3OD



^1H NMR spectrum of epivittatine **107** in CD_3OD



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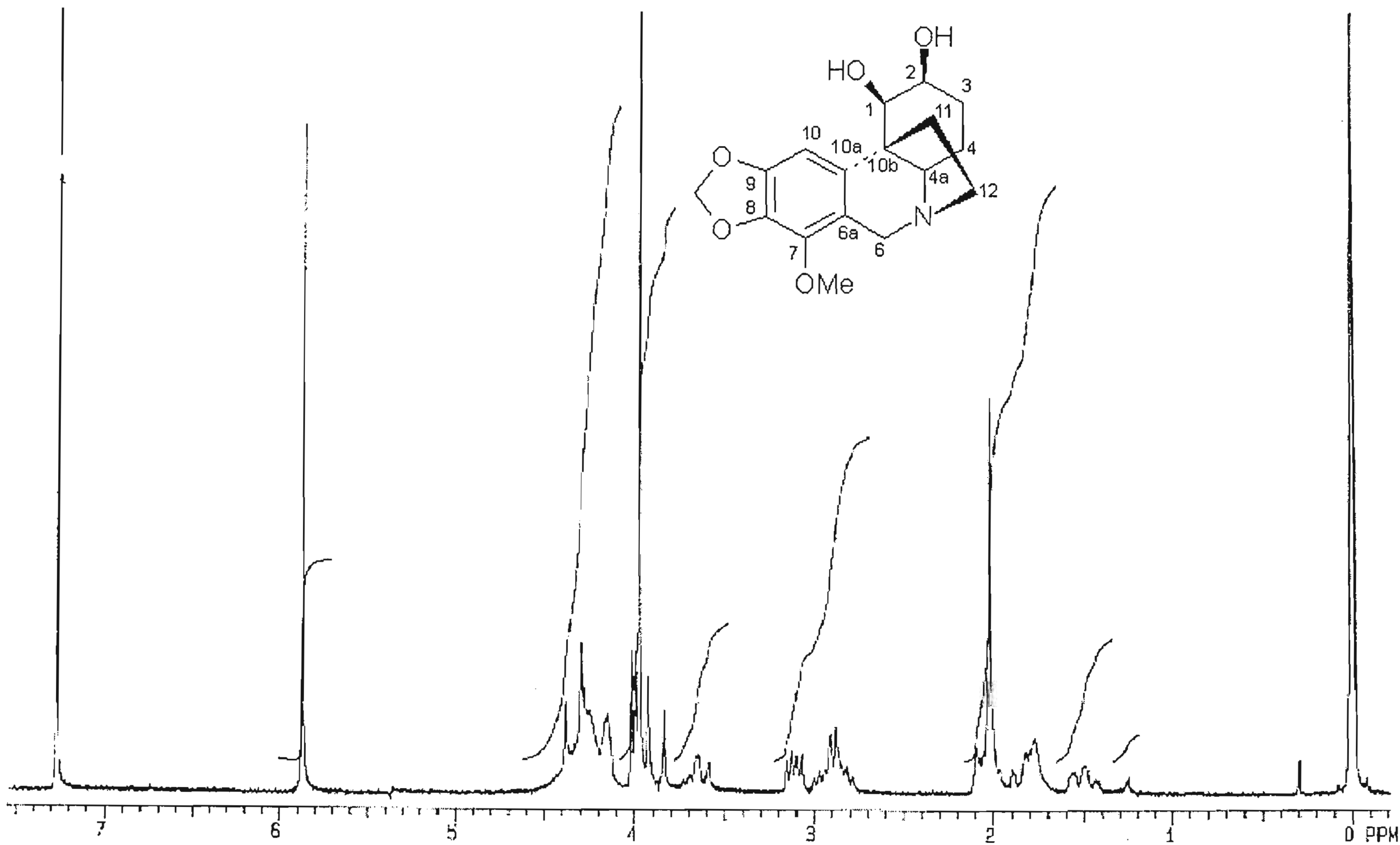
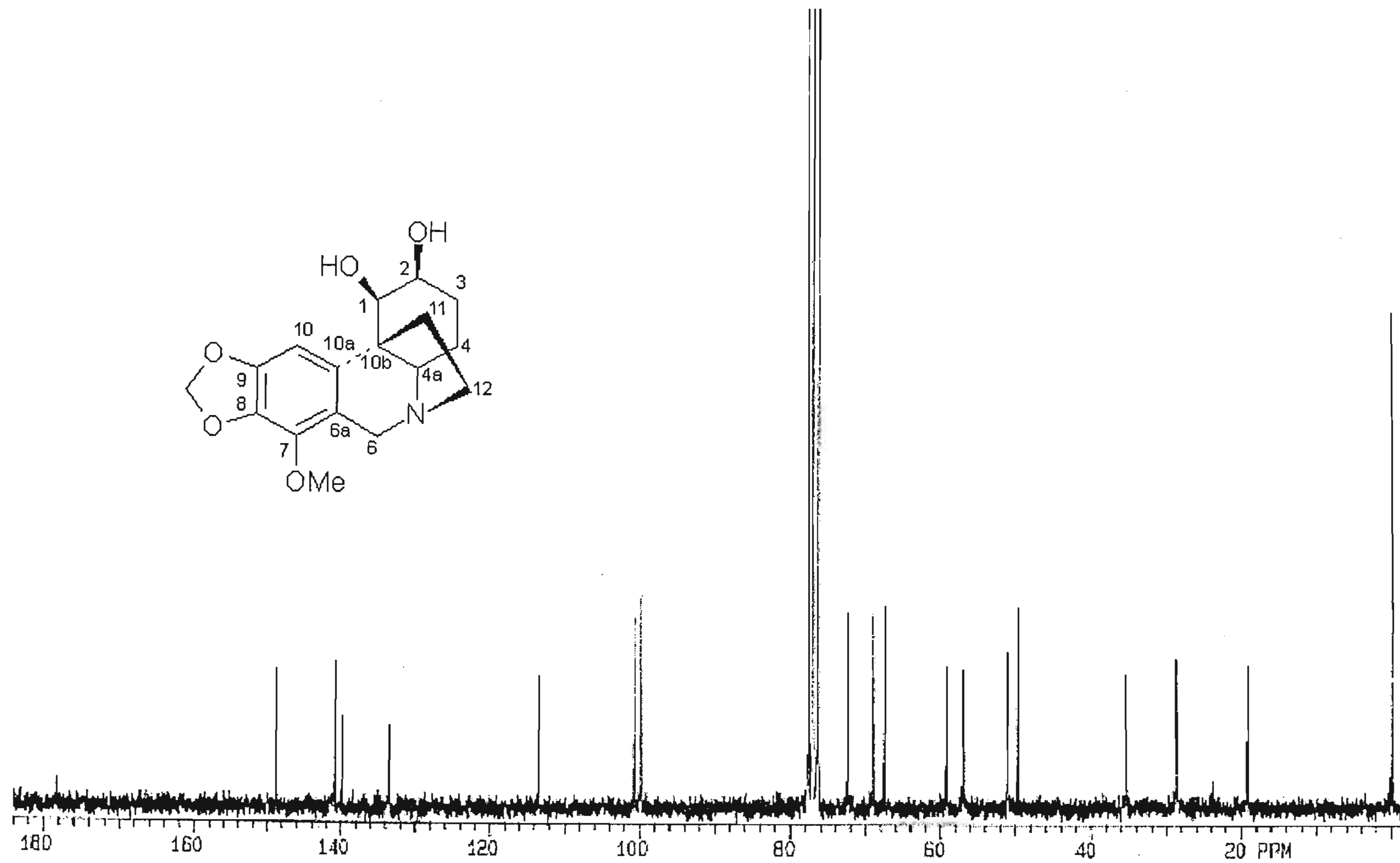
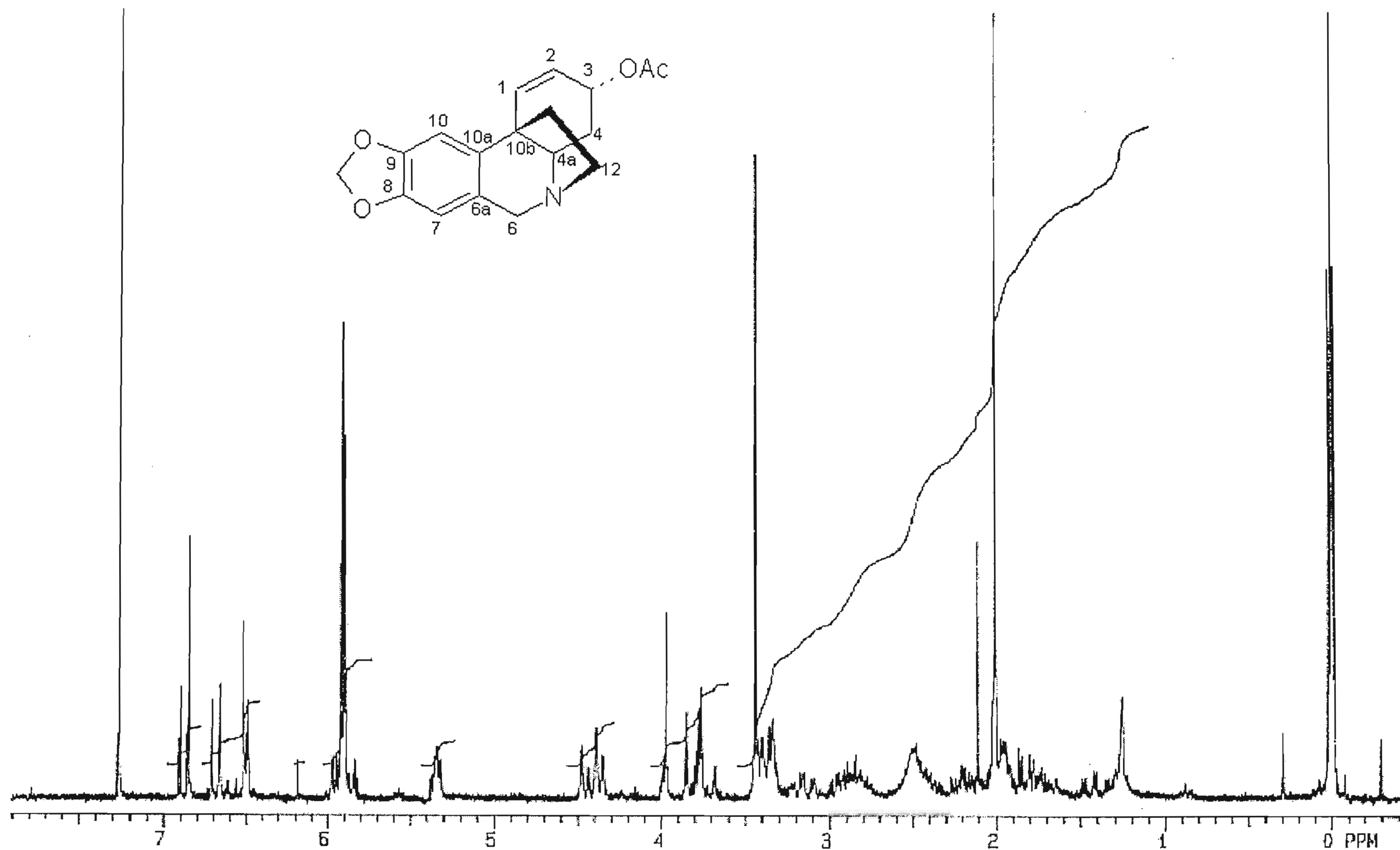
^{13}C NMR spectrum of epivittatine **107** in CD_3OD



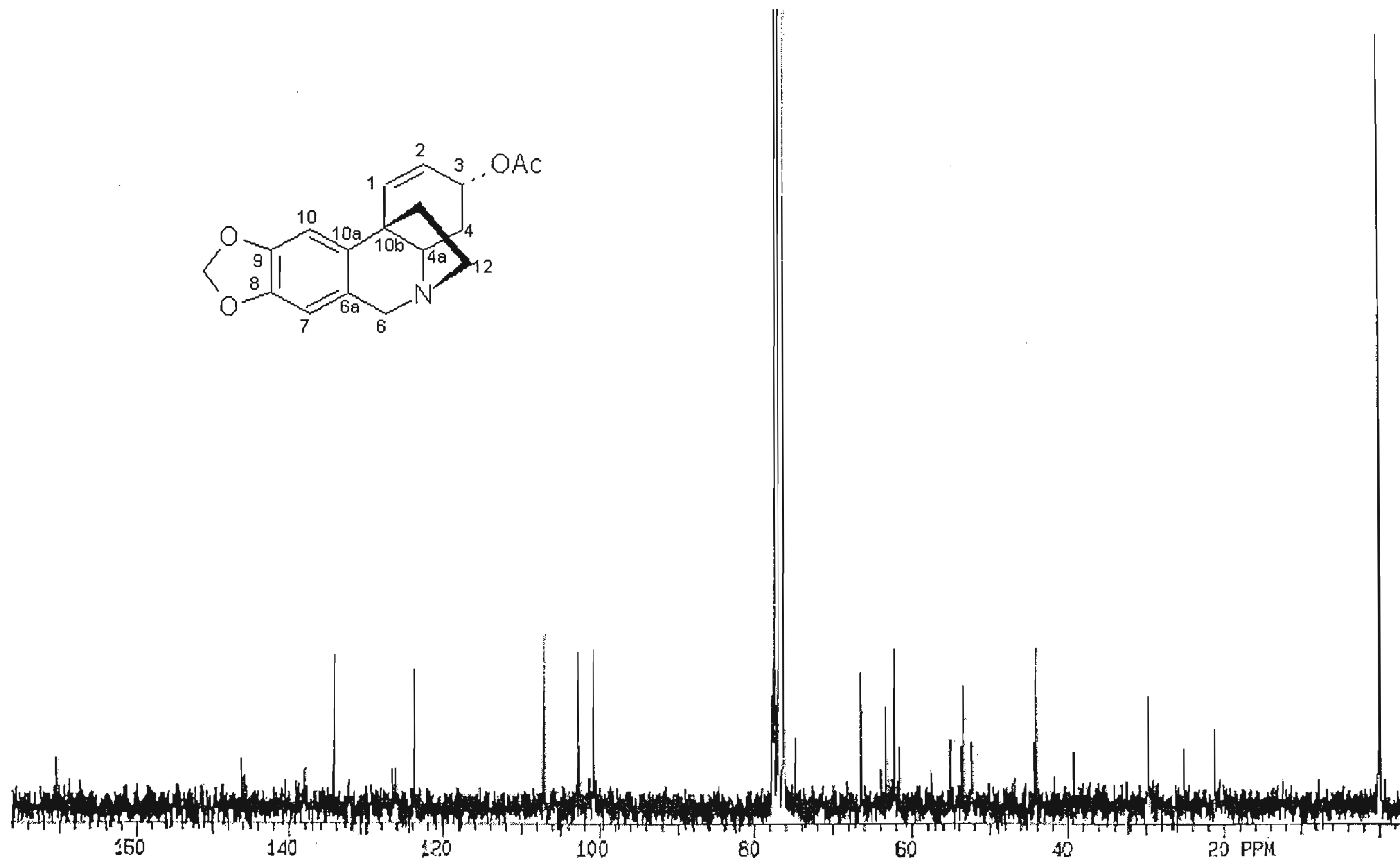
^{1}HNR spectrum of 1-epideacetylbowdensine **108** in $CDCl_3$



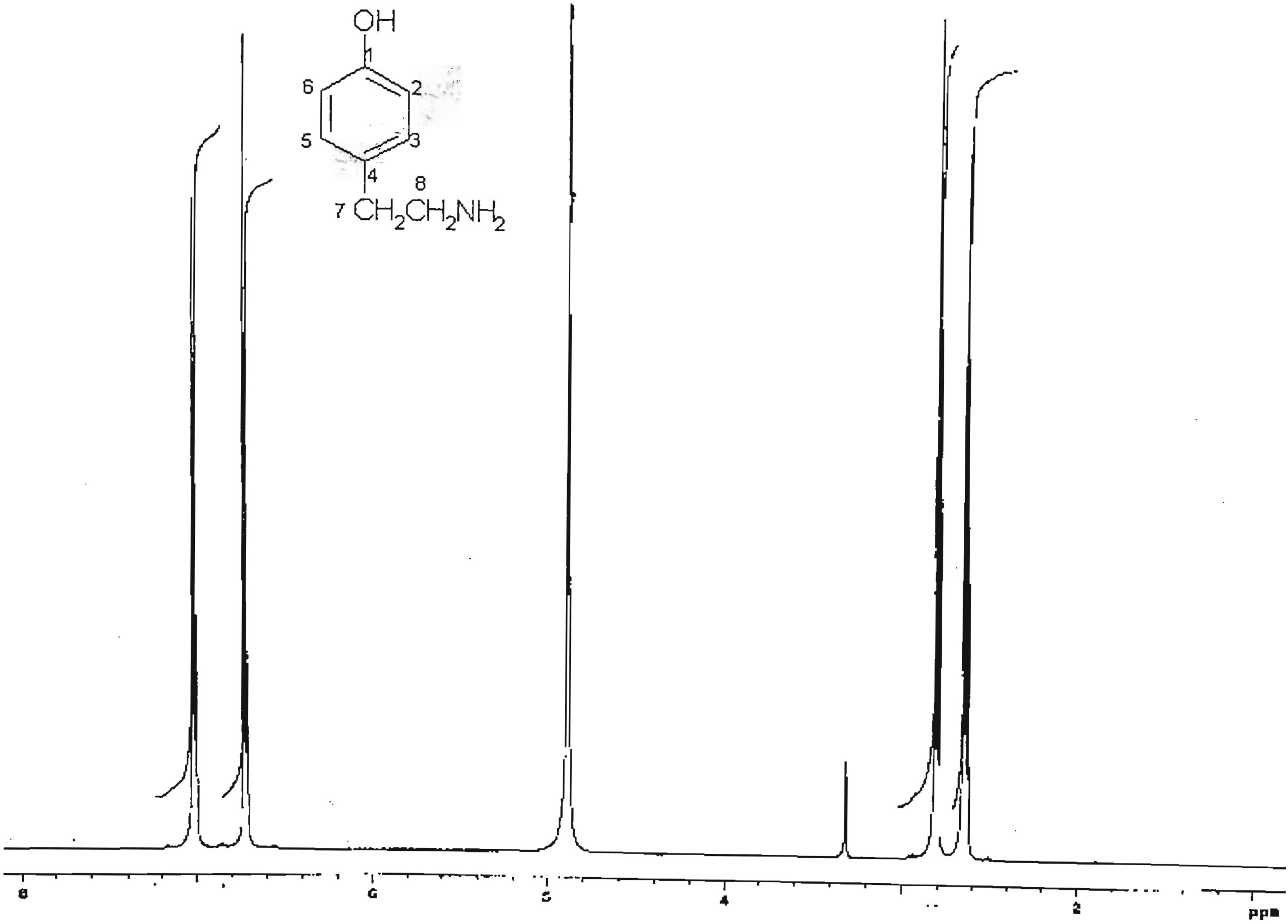
^{13}C NMR spectrum of 1-epideacetylbowdensine **108** in $CDCl_3$



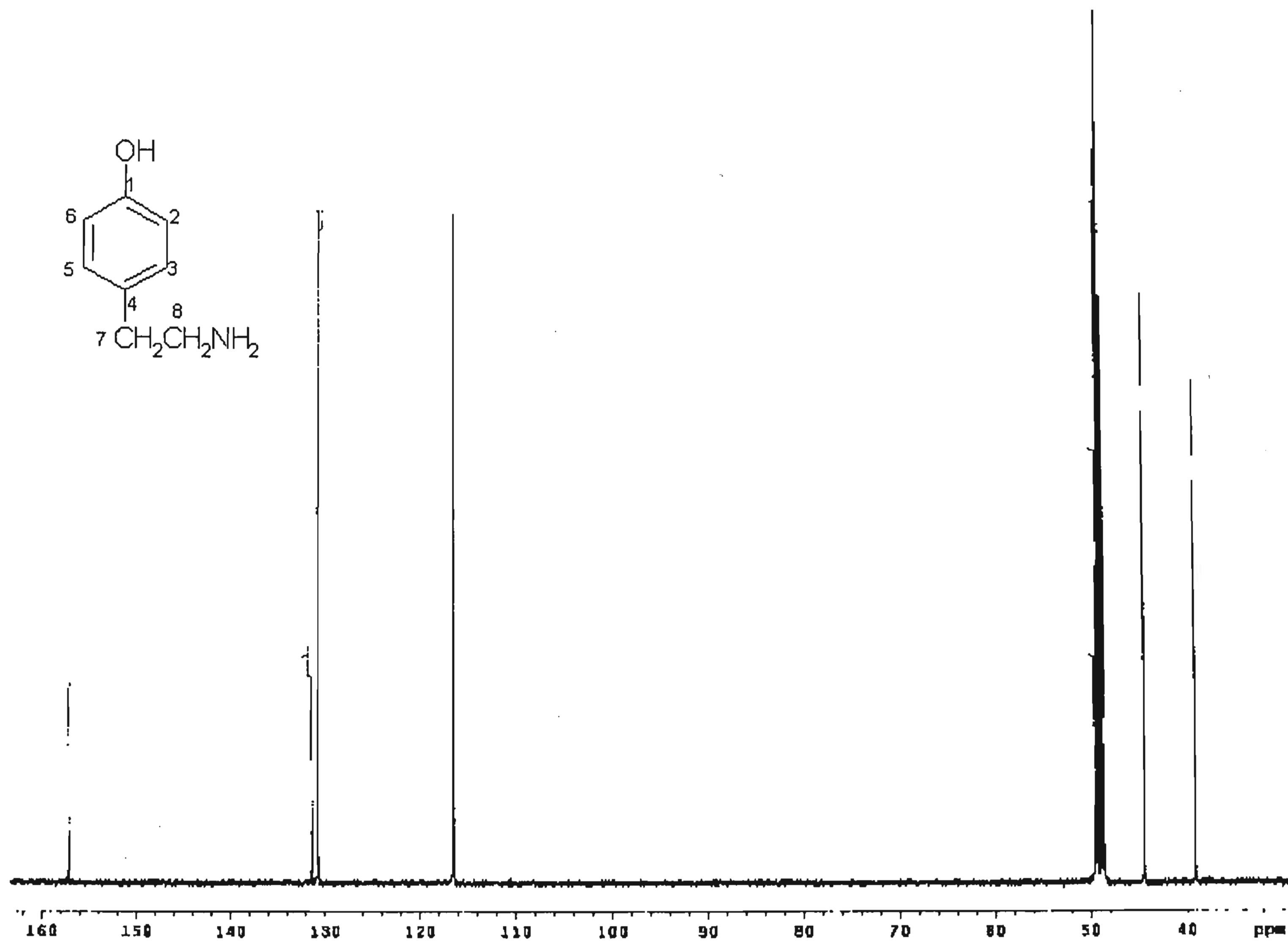
^{1}H NMR spectrum of 3-O-acetyllycorine **109** in $CDCl_3$



^{13}C NMR spectrum of 3-O-acetyllycorine **109** in $CDCl_3$



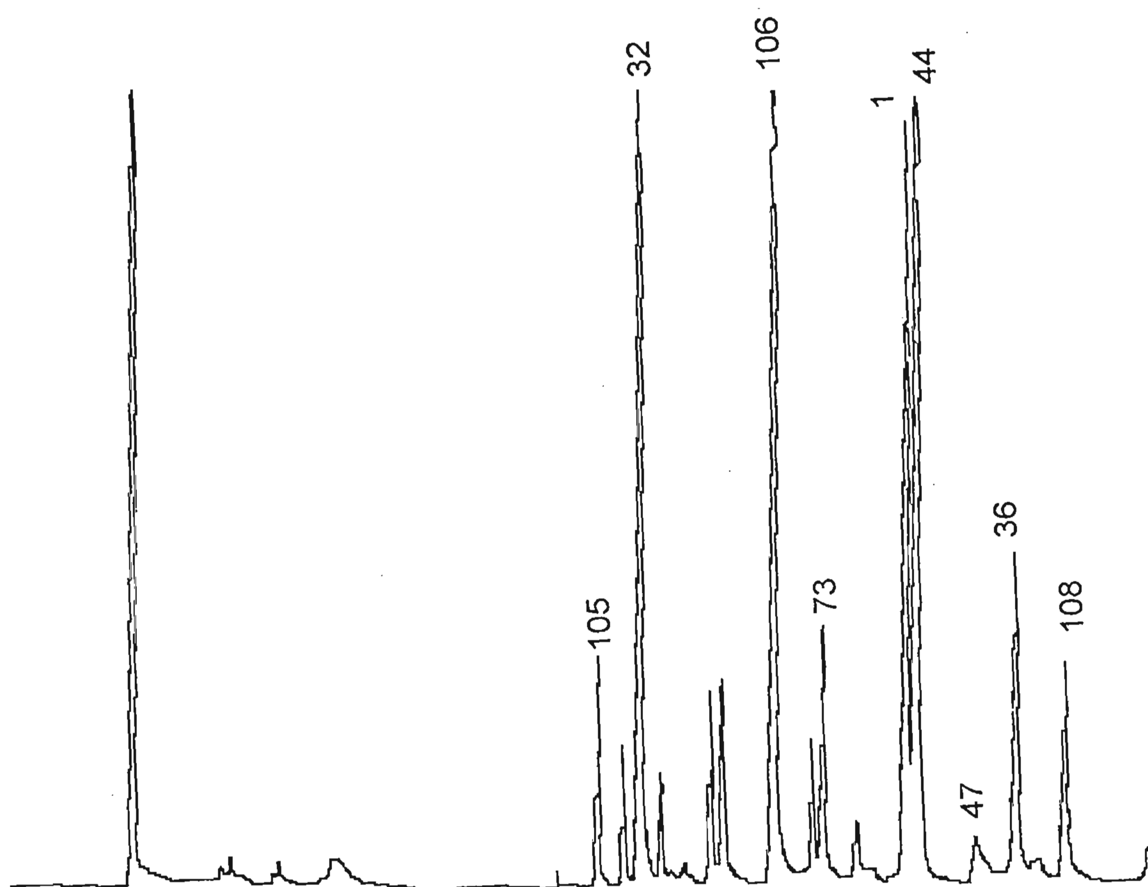
^{1}H NMR spectrum of tyramine **110** in CD_3OD



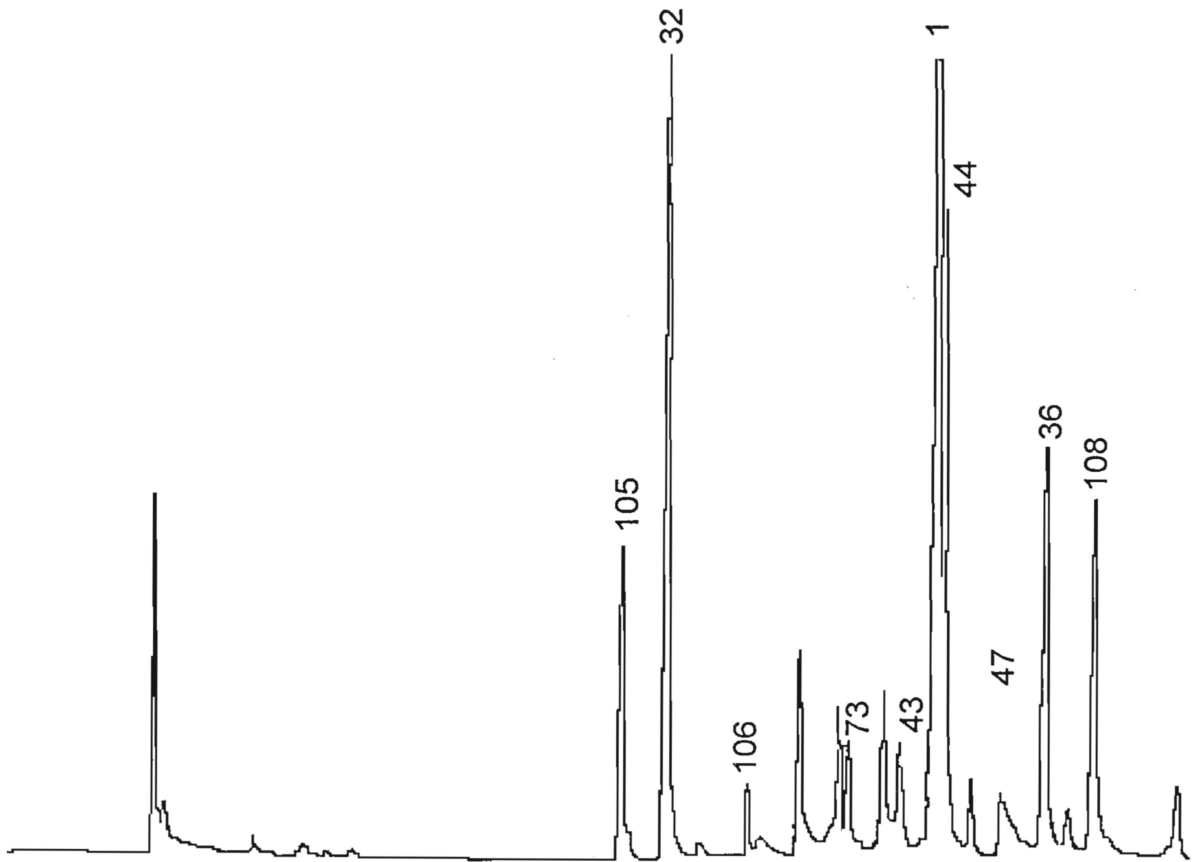
^{13}C NMR spectrum of tyramine **110** in CD_3OD

APPENDIX 2

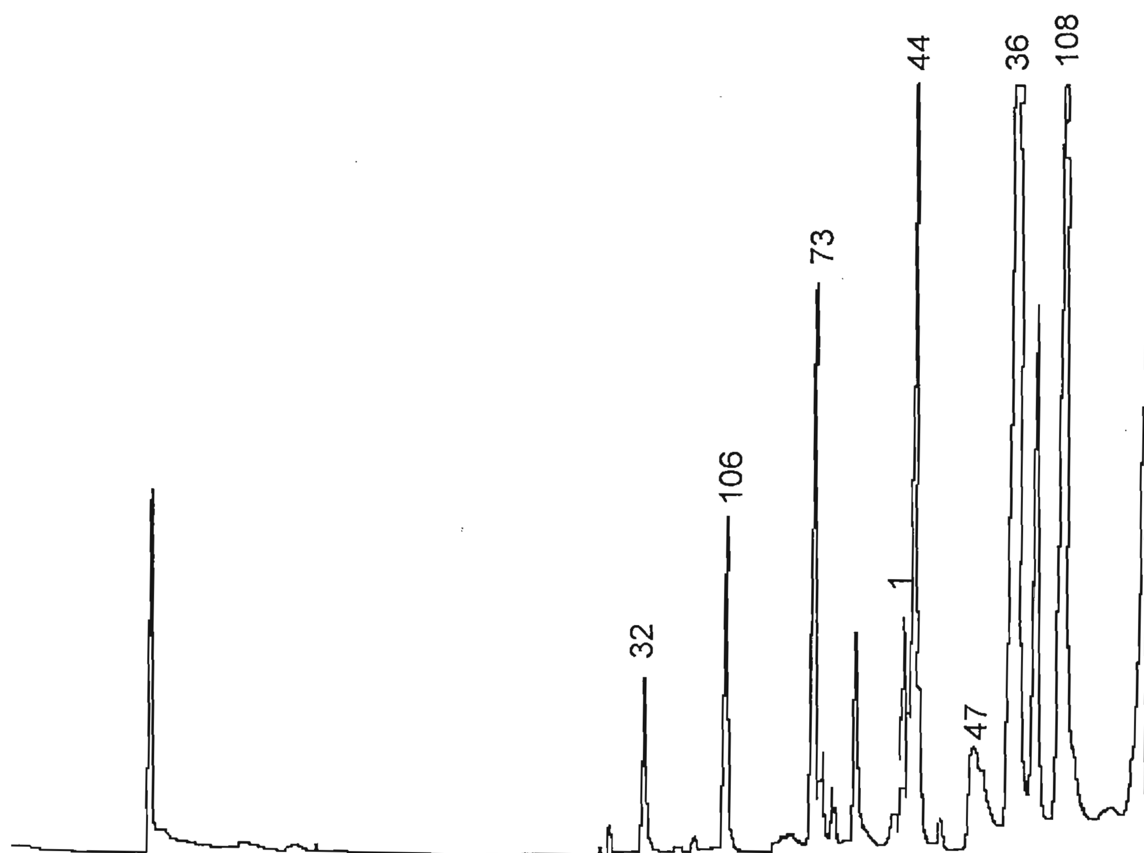
Selected GC-chromatograms of extacted alkaloids from different organs of the three *Crinum* species.



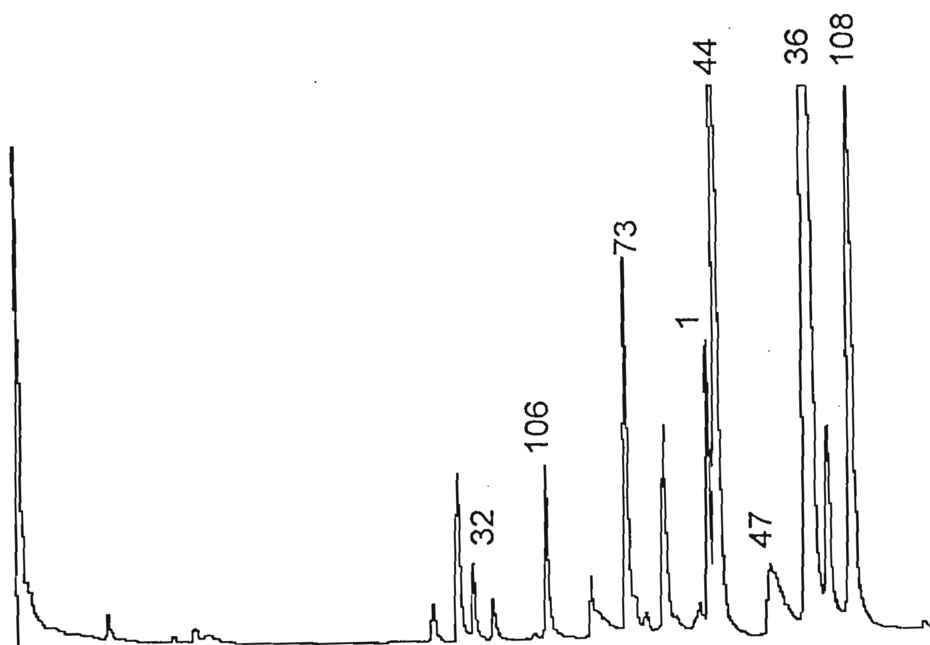
GC-chromatogram of extracted alkaloids from the bulb of *C. moorei*.



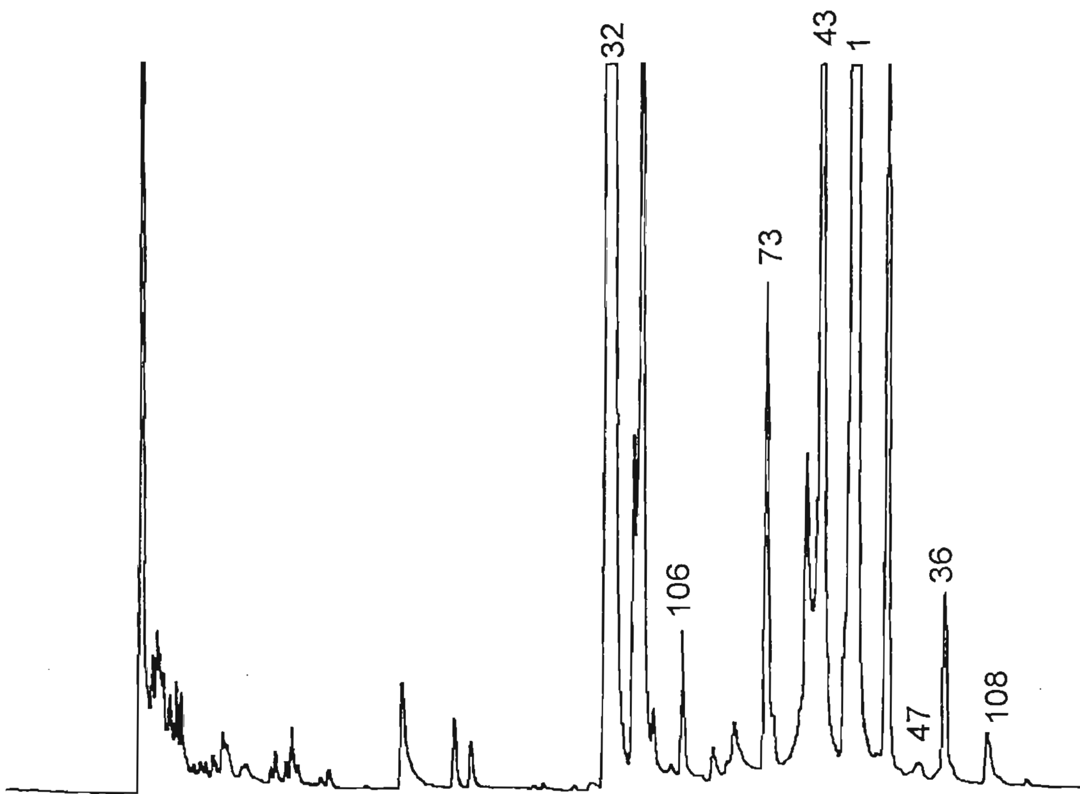
GC-chromatogram of extracted alkaloids from the root of *C. moorei*.



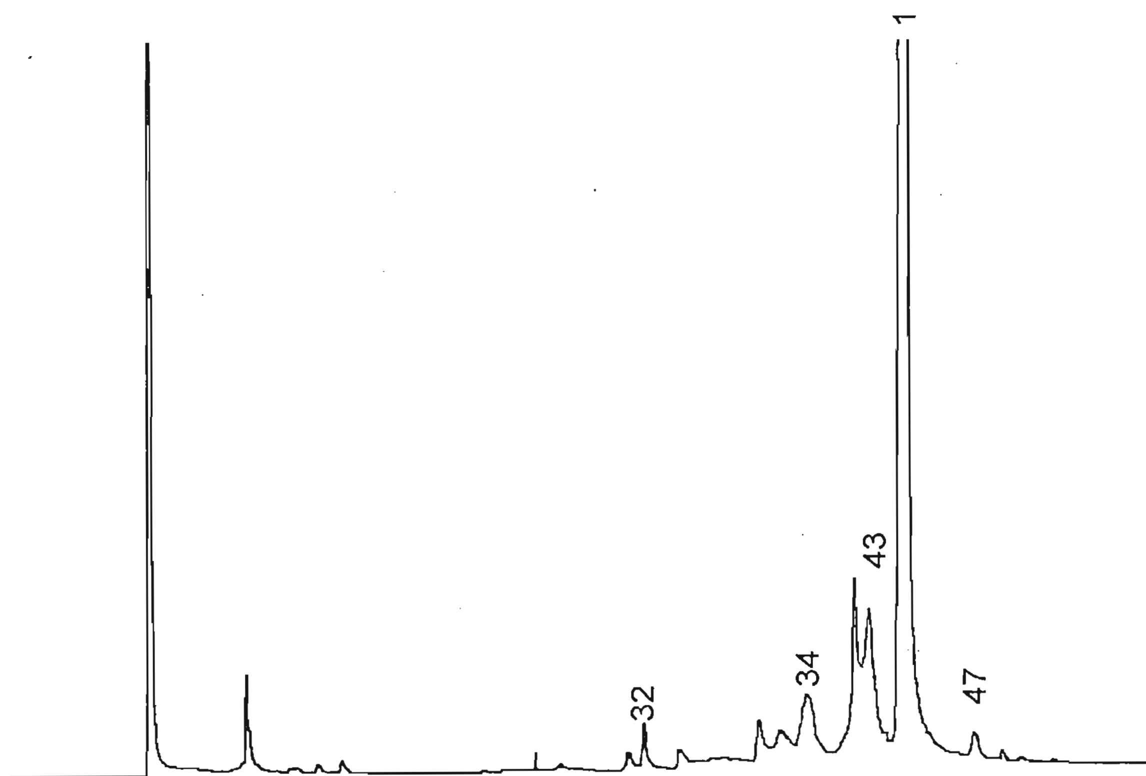
GC-chromatogram of extracted alkaloids from the leaf of *C. moorei.*



GC-chromatogram of extracted alkaloids from the flowering stalks of *C. moorei.*

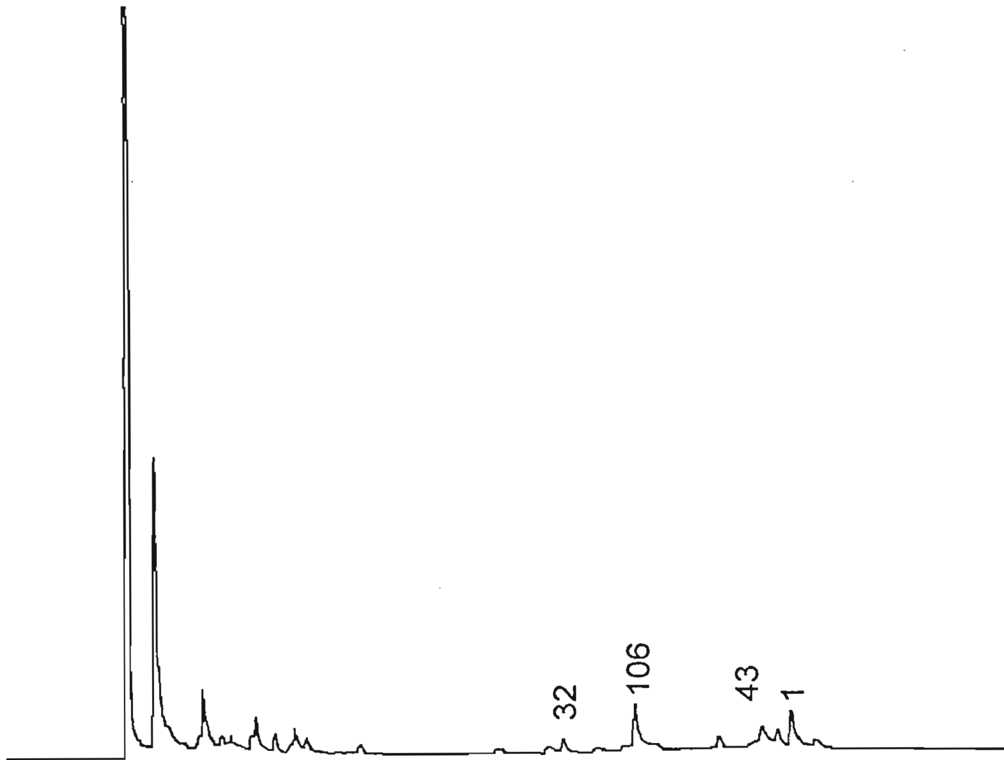


GC-chromatogram of alkaloid extracted from the bulb of *C. macowanii*.

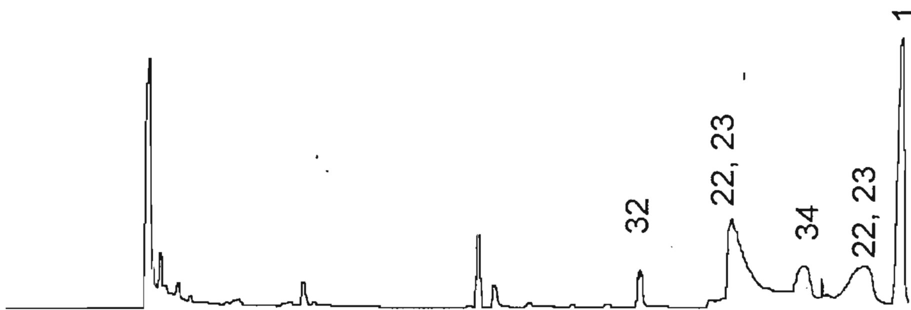


GC-chromatogram of extracted alkaloids from the root of *C. macowanii*.

GC-chromatogram of the extracts from the leaf of *C. macowanii*.

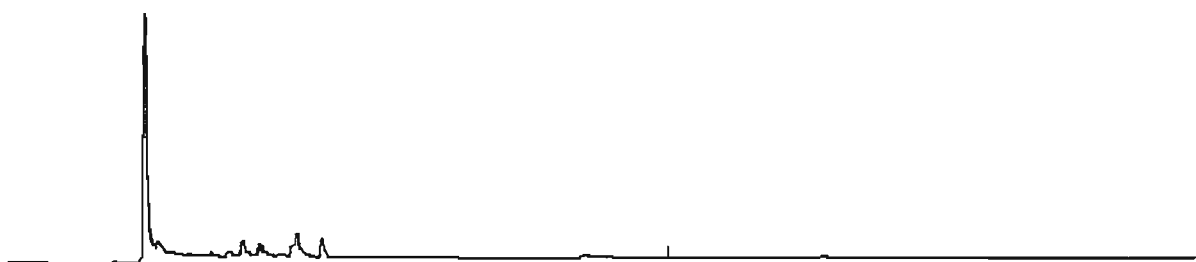


GC-chromatogram of extracted alkaloids from the flowering stalk of *C. macowanii.*

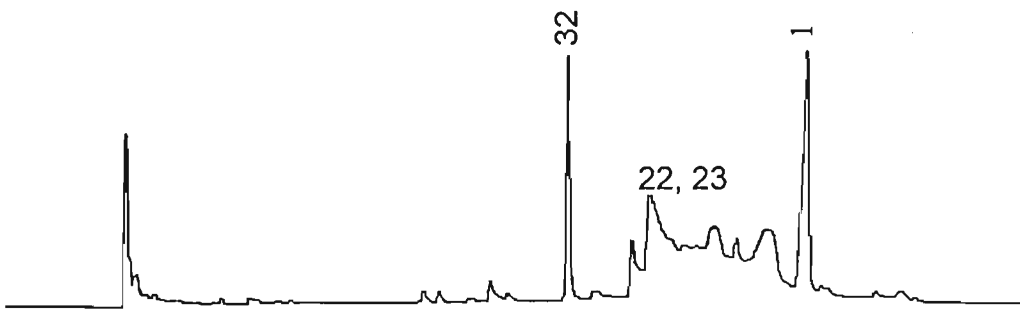


GC-chromatogram of extracted alkaloids from the bulb of *C. bulbispermum*

GC-chromatogram of extracted alkaloids from the root of *C. bulbispermum*.



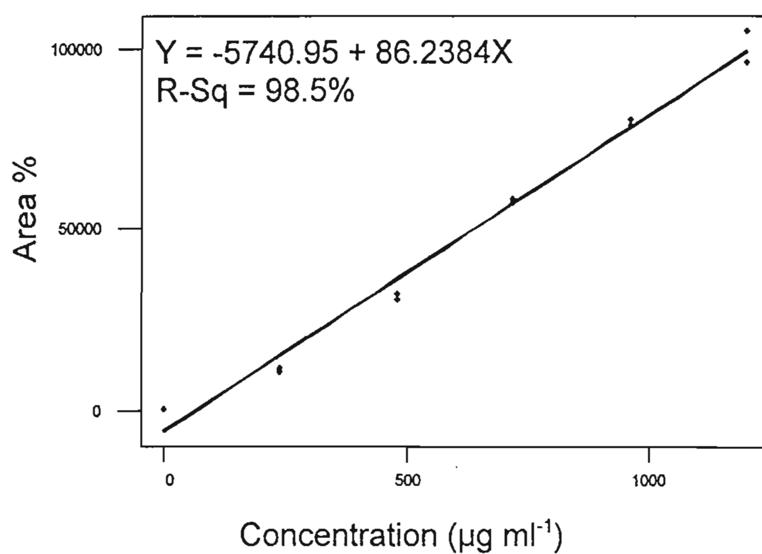
GC-chromatogram of extracted alkaloids from the leaf of *C. bulbispermum*.



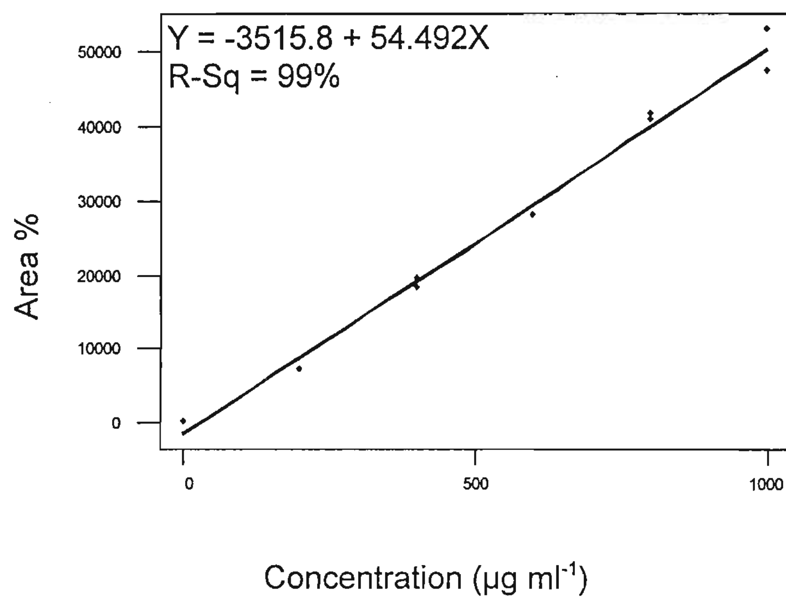
GC-chromatogram of extracted alkaloids from the flowering stalk of *C. bulbispermum*.

APPENDIX 3

Linearity of response of the alkaloid detected using GC arranged in numerical order.

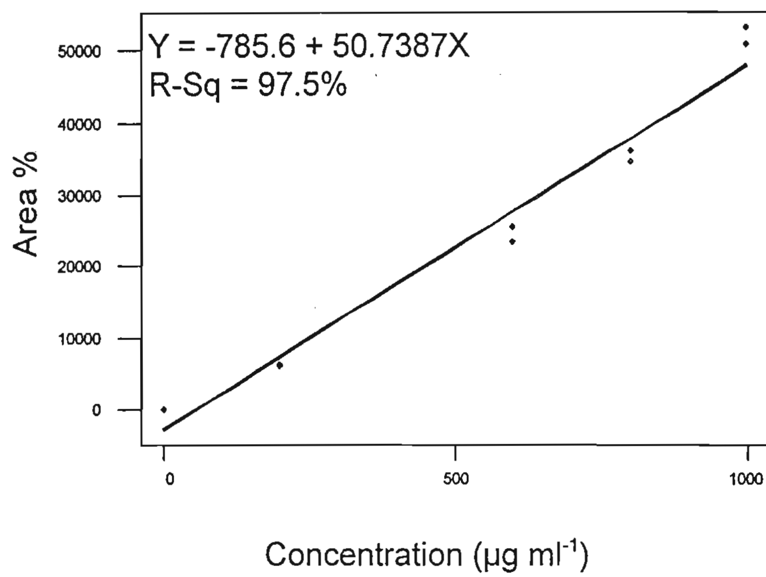


Linearity of response of lycorine **1**.



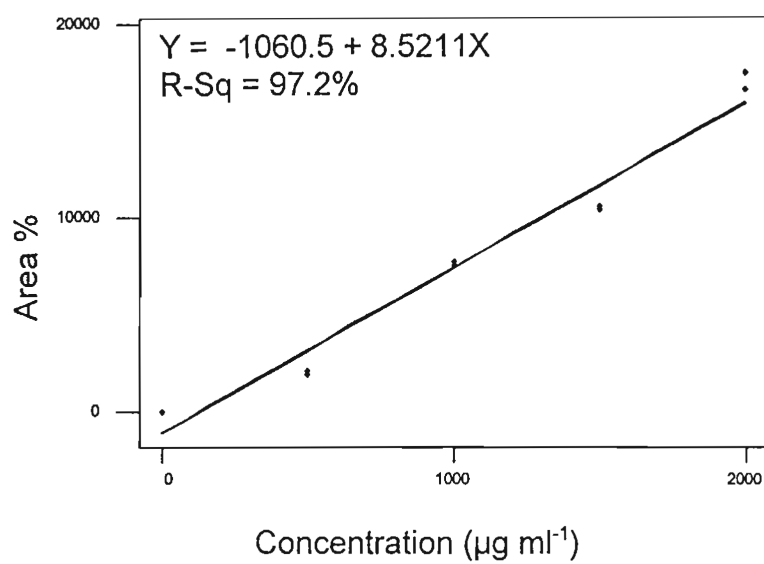
Concentration (μg ml^{-1})

Linearity of response of crinine **32**.

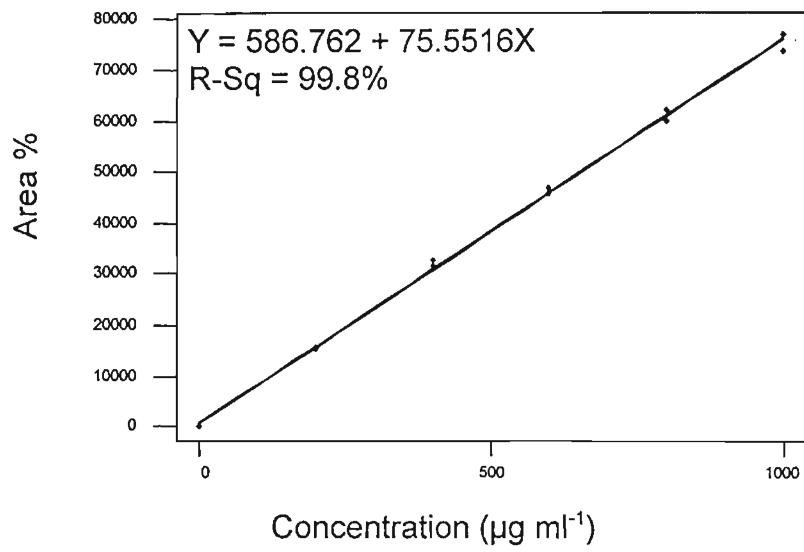


Concentration (μg ml^{-1})

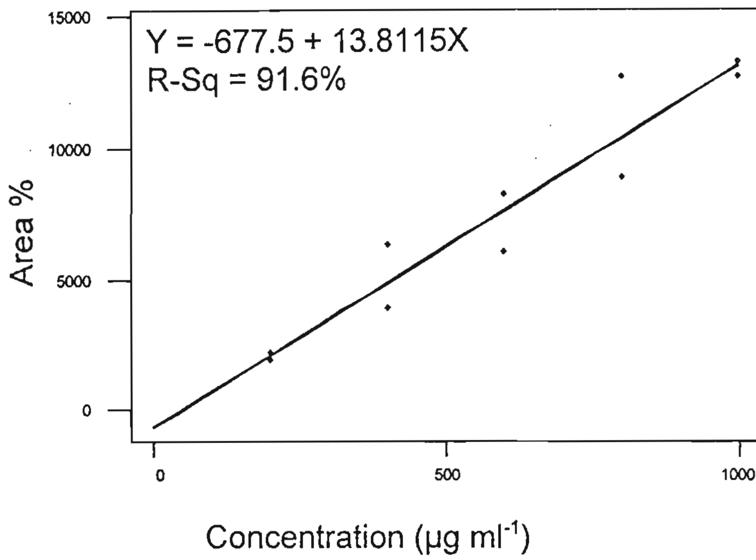
Linearity of response of crinamine **34**.



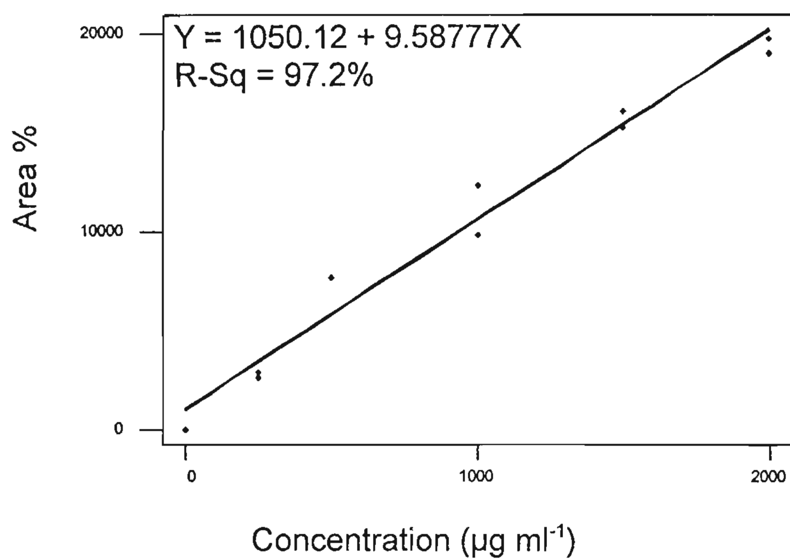
Linearity of response for crinamidine **36**



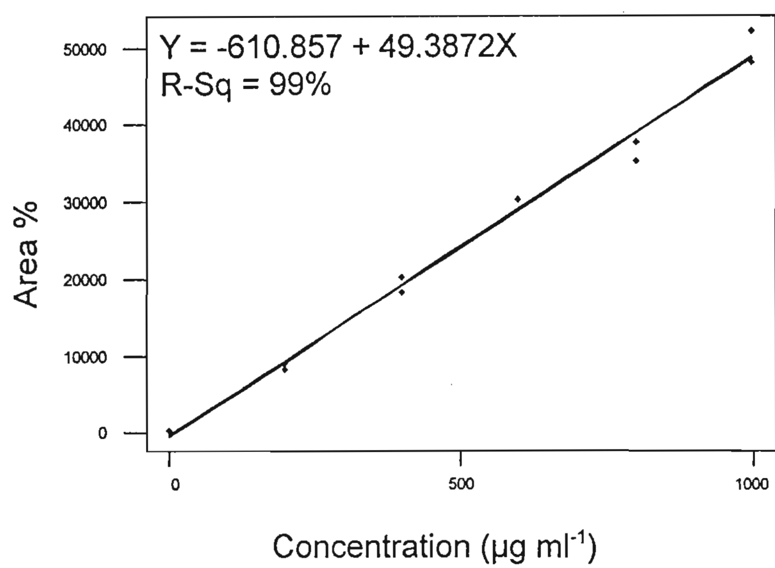
Linearity of response for undulatine **44**



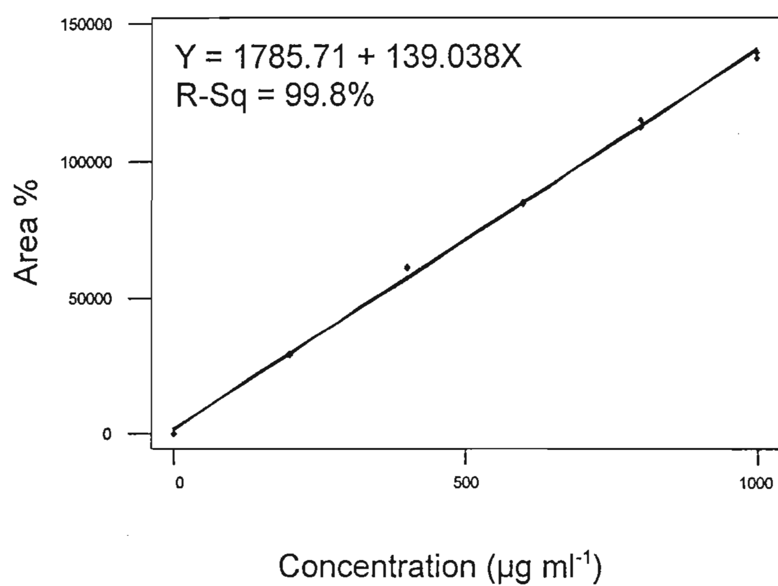
Linearity of response for 1-O-acetyllycorine **43**.



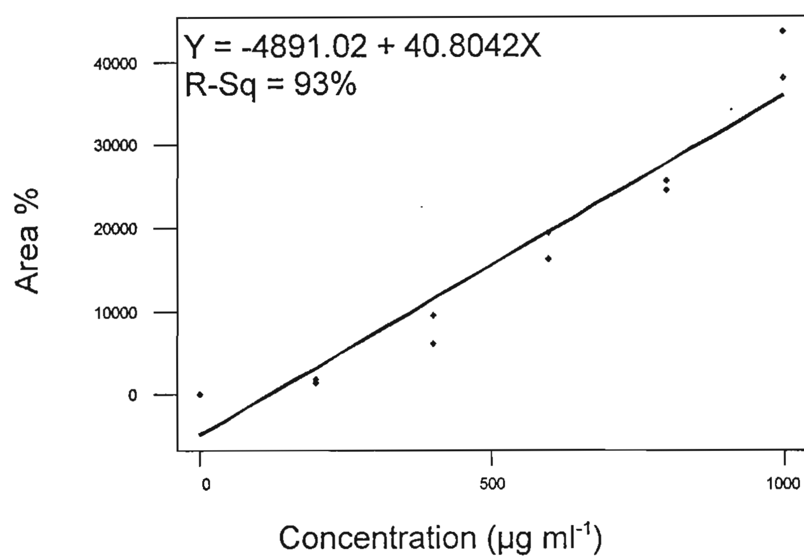
Linearity of response for 3-O-acetylhamayne **47**.



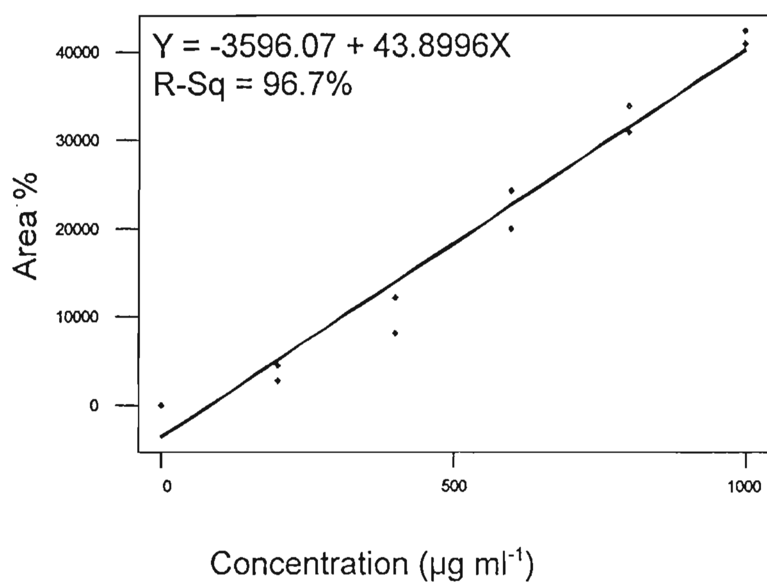
Linearity of response of powelline **73**.



Linearity of response for epibuphanisine **105**.



Linearity of response for cherylline **106**.



Linearity of response of 1-epideacetylbowdensine **108**.

APPENDIX 4

Wald statistics test of significance results for organ-to-organ, seasonal and interspecific variation of the three *Crinum* species.

Wald statistics test (X^2-test) for organ-to-organ and seasonal variation of alkaloids in *C. moorei*.

Source of variation	d.f	Alkaloid detected										
		32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**43**	**34**
Organ	3	103.9***	113.8***	2.8^{NS}	9.8**	126.7***	12.4***	34.6***	66.8***	7.6^{NS}	105.0***	4.4^{NS}
Season	2	19.1***	15.6***	2.4^{NS}	4.6^{NS}	5.4^{NS}	8.8**	3.9^{NS}	0.1^{NS}	2.2^{NS}	3.0^{NS}	3.5^{NS}

^{NS} = Not significant at 5% level of significance.

** = Significant at 2.5% level of significance.

***= Significant at 1% level of significance.

d.f = Degrees of freedom.

Wald statistics test (X^2-test) for organ-to-organ and seasonal variation of alkaloids in *C. macowanii.*

Source of variation	d.f	Alkaloid detected							
		32	**1**	**106**	**73**	**108**	**36**	**47**	**34**
Organ	3	367.8	508.6***	5.6^{NS}	25.4***	5.8^{NS}	12.7***	8.3*	31.5***
Season	2	6.7*	1.4^{NS}	1.1^{NS}	3.8^{NS}	7.4**	5.0^{NS}	6.3*	8.1**

^{NS} = Not significant at 5% level of significance.

* = Significant at 5% level of significance.

** = Significant at 2.5% level of significance.

*** = Significant at 1% level of significance.

d.f = degrees of freedom.

Wald statistics test (X^2-test) for organ-to-organ and seasonal variation of alkaloids in *C. bulbispermum*.

Source of variation	d.f	Alkaloid detected			
		32	**1**	**47**	**34**
Organ	3	187.1***	256.8***	32.3***	27.8***
Season	2	4.1^{NS}	32.4***	5.9^{NS}	22.8***

^{NS} = Not significant at 5% level of significance.

*** = Significant at 1% level of significance.

d.f = Degrees of freedom.

Wald statistics test (X^2-test) for interspecific variation in extracted alkaloids from the three *Crinum* species.

Source of variation	d.f	Alkaloid detected									
		32	**1**	**106**	**105**	**73**	**44**	**108**	**36**	**47**	**34**
Species	2	113.3***	277***	135.3***	23.3***	569.3***	76.8***	862.4***	926.2***	51.9***	32.4***
Organ. Species	6	90.8***	79.2***	3.2^{NS}	24.1***	220.7***	46.1***	69.0***	145.1***	17.5***	44.0***

^{NS} = Not significant at 5% level of significance.
*** = Significant at 1% level of significance.
d.f = Degrees of freedom.