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CRINUM ALKALOIDS: THEIR CHEMISTRY AND BIOLOGY*

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Key Word Index—*Crinum* alkaloids; Amaryllidaceae; distribution; isolation; spectral properties; structure; inter conversion; biological activity.

ract—The current status of research of free and glycosylated alkaloids occurring in members of the genus *Crinum* is wed. The distribution, isolation, spectral properties, structural patterns, inter conversions and biological activities ese alkaloids are presented.

INTRODUCTION

study of Amaryllidaceae alkaloids began with the tion of lycorine from *Narcissus pseudonarcissus* in [1]. Since that time, *ca* 150 species belonging to 36 ra of this family were examined for alkaloids [2, 3]. 100 structurally different alkaloids, comprising 12 uct ring types (Table 1), have so far been isolated from rent parts and at different periods of vegetation of ryllidaceae species (sub-family, Amaryllidioideae). identity of the homogeneous entities was established hemical transformation, spectroscopic analyses and, ost cases, by synthesis. An outstanding feature of the alkaloids of this family is that they are produced usively by members of this family. Conversely, true oids belonging to other plant families have not been untered in Amaryllidaceous species. The mesembrine oids (family Aizoaceae) are the only structurally ed group of compounds related to the Amaryllidae alkaloids. They also exhibit biosynthetic resembl- to Amaryllidaceae alkaloids in that tyrosine and ylalanine are the two primary building blocks in . However, some subtle aspects of biosynthesis of the mbrine alkaloids are still obscure [4]. In view of this, nly report of the isolation of mesembrenol from *um oliganthum* [5] warrants careful scrutiny.

ie genus *Crinum* is a true representative of the family t exhibits all the chief chemical traits of the ryllidaceae. Thus, the structures of the *Crinum* alids although divergent, are derived from the three amental nuclei, viz. N-(3,4-dioxybenzyl)-4-oxyethylamine (e.g. norbelladine), pyrrolo[de]phenridine (e.g. lycorine) and 5,10b-ethanophenanthridine vittatine/(−)-crinine). By modification of these nuclei (i) oxygenation, (ii) dehydrogenation, (iii) dehydn, (iv) cyclization, (v) *O,N*-methylation, (vi) addition phenylpropene, pyridine dicarboxylate moieties), and elimination (e.g. seco-reaction: lycorine → hippeastrine; apo-reaction: pyrrolo-phenanthridone → phenanthridone)] the various types of the *Crinum* alkaloids are produced. Table 1 summarizes the ring types and the representative members in each group.

art 14 in the series "Chemical Constituents of the yllidaceae". For part 13 see ref. [29].

DISTRIBUTION

A list of trivial names of the *Crinum* alkaloids, their structures, physical properties, distribution and literature references is given in Table 2. The list includes only those alkaloids that have been reported after the review by Fuganti [2]. Known alkaloids which have now been isolated from earlier unexplored sources are also included in the list.

NEW ALKALOIDS

In spite of the relentless catabolic reactions of Amaryllidaceae alkaloids *in vivo*, several reactive N-(3,4-dioxybenzyl) phenethylamine intermediates, e.g. belladine (**5**), latisoline (**28**), latisodine (**29**), *O*-methylnorbelladine (**33**) and ryllistine (**45**) were isolated from a number of *Crinum* species at the time of flowering [9, 12, 20, 22, 24]. Among these, the occurrence of ryllistine (**45**) deserves special mention. The possibility that 1,2-β-epoxy-5,10b-ethanophenanthridine alkaloids of the (−)-crinine/powelline type (**4**, **9**, **17**) are derived from a preformed oxygenated precursor(s) (and not by oxidation at the (−)-oxocrinine stage) was repeatedly considered before theoretically [9, 25, 26] but not tested experimentally until recently [20]. The first of such intermediates could conceivably be a dopamine-protocatechuic aldehyde derived 4-oxygenated norbelladine analogue. The isolation of ryllistine from *Amaryllis vittata* Ait and from a number of *Crinum* species now provides strong circumstantial evidence in favour of this hypothesis. The 1,2-β-epoxyalkaloid (**17**) also co-occurs with ryllistine in these plants. At the time of flowering, *C. asiaticum*, *C. augustum*, *C. latifolium* and *C. pratense* were found to contain a typical mixture (3:1) of ambelline (**1**) and 1,2-β-epoxyambelline (**17**) together with ryllistine (**45**) [8, 9, 20]. During the resting period, the first two alkaloids were

Table 1. Ring types and representative *Crinum* alkaloids

Ring type		Alkaloid (structure no.)	Reference
I.	N-(3,4-dioxybenzyl)-4-oxy-phenethylamine	*O*-Methylnorbelladine (**33**)	[12]
II.	N-(3,4-dioxybenzyl)-3,4-dioxyphenethylamine	Ryllistine (**45**)	[20]
III.	Pyrrolo[de]phenanthridine/ Pyrrolophenanthridone	Lycorine/Pratorinine (**30**)/(**40**)	[3, 27, 28] [6, 7]
IV.	Lycorenine	Hippeastrine (**3**)	[19]
V.	Galanthamine	Narwedine (**35**)	[19]
VI.	5,10b-Ethano-phenanthridine	Haemanthamine (**22**)	[3, 9, 27]
		Ambelline (**1**)	[8]
VII.	1,2-Epoxy-5,10b-ethanophenanthridine	1,2-β-Epoxyambelline (**17**)	[8]
VIII.	Pretazettine	Ornazidine (**37**)	[21]
IX.	Tetrahydroisoquinoline	Cherylline (**47**)	[2]
X.	Phenanthridone/ Lignoid-phenanthridone	Crinasiadine (**12**)/ Crinasiatine (**13**)	[18] [18]
XI.	Clivimine	Latindine (**27**)	[3, 9, 27]
XII.	Ismine	Ismine (**26**)	[9]

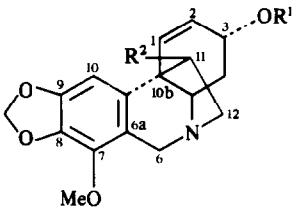
Table 2. Distribution of alkaloids* in the genus *Crinum*

Alkaloid (structure)	mp (°) $[\alpha]_D$	Plant source	Reference
Ambelline (**1**)	260–261 +32° ($CHCl_3$)	*C. asiaticum* L. *C. augustum* Roxb. *C. latifolium* L. *C. pratense* Herb.	[6–9]
Anhydrolycorine-7-one (**2**)	228–230	*C. pratense*	[6]
Augustamine (**3**)	173–175	*C. augustum*	[10]
Augustine (**4**)	174–176	*C. augustum*	[11]
Belladine (**5**)	—	*C. asiaticum* *C. latifolium*	[9, 12]
Buphanisine (**6**)	122–124 −26° (EtOH)	*C. augustum*	[13]
Buphanisine-6α-OH (**7**)	126–128	*C. augustum*	[14]
Buphanisine-6β-OH (**8**)	126–128	*C. augustum*	[14]
Crinalbine (**9**)	130–132 +24° ($CHCl_3$)	*C. bulbispermum* L.	[15]
Crinamine (**10**)	199–201 +157° ($CHCl_3$)	*C. asiaticum*	[9, 16, 17]
Crinamine-6-OH (**11**)	210 +46° ($CHCl_3$)	*C. asiaticum*	[9]
Crinasiadine (**12**)	276–278	*C. asiaticum*	[18]
Crinasiatine (**13**)	>270 (dec.)	*C. asiaticum*	[18]
Crinine (**14**)	209–210 −11° ($CHCl_3$)	*C. asiaticum*	[9]
Crinine-6α-OH (**15**)	268–270	*C. augustum*	[13]
Crinine-6β-OH (**16**)	268–270	*C. augustum*	[13]
1,2-β-Epoxyambelline (**17**)	245 +22.4° ($CHCl_3$)	*C. latifolium*	[8]
Galanthamine (**18**)	127–129 −121.4° (EtOH)	*C. amabile* Hort.	[19]
Galanthamine-*N*-demethyl (**19**)	156–158	*C. asiaticum* var. *japonicum* Baker	[16]

Table 2. (continued)

Alkaloid (structure)	mp (°) $[\alpha]_D$	Plant source	Reference
Galanthamine-*O*, *N*-diacetyl (**20**)	204–205	*C. asiaticum* var. *japonicum*	[17]
Galanthine (**21**)	166–167 −81.6° (EtOH)	*C. amabile*	[19]
Haemanthamine (**22**)	203 +19.7° (MeOH)	*C. asiaticum*	[9]
Haemanthidine (**23**)	189–190 −41° ($CHCl_3$)	*C. asiaticum*	[9]
Hamayane (**24**)	79–80	*C. asiaticum* var. *japonicum*	[17]
Hippeastrine (**25**)	214–215 +160° ($CHCl_3$)	*C. amabile*	[19]
Ismine (**26**)	99–100	*C. pratense*	[9]
Latindine (**27**)	>300 (dec.)	*C. latifolium*	[9, 20]
Latisoline (**28**)	— −48.5° (MeOH)	*C. latifolium*	[12]
Latisodine (**29**)	205–207	*C. latifolium*	[12]
Lycorine (**30**)	250–258 −83.8° (EtOH)	*C. amabile* *C. asiaticum* *C. augustum* *C. bulbispermum* *C. natans* L. *C. ornatum* Roxb. *C. pratense*	[6, 13, 15, 19, 21–24]
Lycorine-*O*, *O*-diacetyl (**31**)	212–213	*C. pratense*	[6]
Lycorine-1-*O*-glucoside (**32**)	— −92.4° (MeOH)	*C. asiaticum* *C. augustum* *C. latifolium* *C. pratense*	[9, 24]
O-Methylnorbelladine (**33**)	161–164	*C. asiaticum* *C. augustum*	[9, 22]
Narcissidine (**34**)	218–219 −32° (MeOH)	*C. pratense*	[6]
Narwedine (**35**)	188–189 +100° ($CHCl_3$)	*C. amabile*	[19]
Ornazamine (**36**)	—	*C. ornatum* Hort.	[21]
Ornazidine (**37**)	—	*C. ornatum*	[21]
Powelline (**38**)	200–201	*C. bulbispermum*	[15]
Pratorine (=hippadine) (**39**)	210–211	*C. asiaticum* *C. bulbispermum* *C. latifolium* *C. pratense*	[6, 15, 23]
Pratorinine (**40**)	265–267	*C. asiaticum* *C. latifolium* *C. pratense*	[6, 7]
Pratorimine (**41**)	263–265	*C. asiaticum* *C. augustum* *C. latifolium*	[6, 7, 9]
Pratosine (**42**)	232–233	*C. asiaticum* *C. augustum* *C. latifolium*	[6, 7, 9]
Pseudolycorine (**43**)	247–249 −53° (EtOH)	*C. asiaticum*	[9, 24]
Pseudolycorine-1-*O*-β-D-glucoside (**44**)	— −108° (EtOH)	*C. asiaticum* *C. latifolium*	[9, 24]
Ryllistine (**45**)	77–79	*C. augustum*	[20]
Tazettine (**46**)	202–203 +160 ($CHCl_3$)	*C. amabile*	[9, 19]

*Covers literature from 1976 until June 1984.



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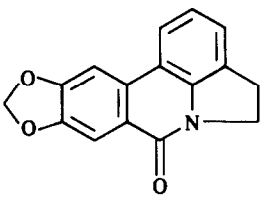
38 R^1 = R^2 = H
48 R^1 = Me, R^2 = Ac



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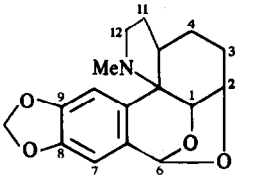

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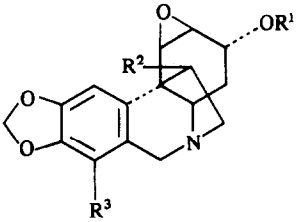
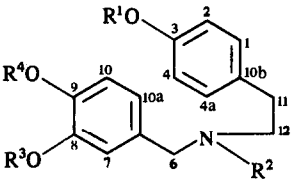
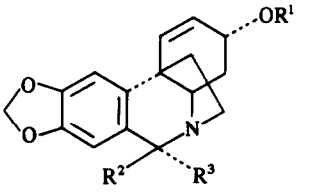


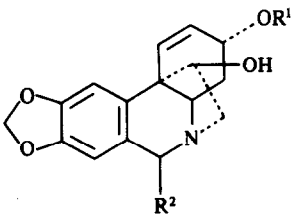
4 R^1 = Me, R^2 = R^3 = H
9 R^1 = R^2 = H, R^3 = OMe
17 R^1 = Me, R^2 = OH, R^3 = OMe
49 R^1 = Me, R^2 = OAc, R^3 = OMe



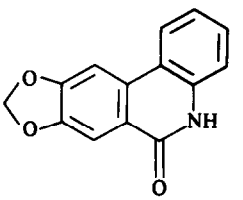
5 R^1 = R^2 = R^3 = R^4 = Me
28 R^1 = Glc, R^2 = H, R^3 = R^4 = Me
29 R^1 = R^2 = H, R^3 = R^4 = Me
33 R^1 = R^2 = R^3 = H, R^4 = Me



6 R^1 = Me, R^2 = R^3 = H
7 R^1 = Me, R^2 = H, R^3 = OH
8 R^1 = Me, R^2 = OH, R^3 = H
14 R^1 = R^2 = R^3 = H
15 R^1 = R^2 = H, R^3 = OH
16 R^1 = R^3 = H, R^2 = OH



10 R^1 = Me, R^2 = H
11 R^1 = Me, R^2 = OH



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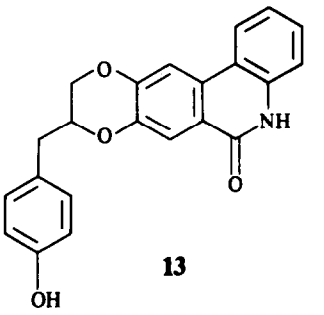

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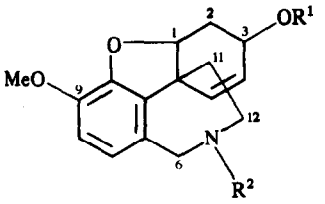
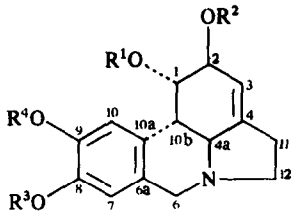
converted into their 11-*O*-acetyl derivatives (**48**/**49**). Although the subsequent catabolic sequence of the latter pair (**48**/**49**) was not definitely established analytical data (co-HPLC with synthetic markers, mass spectrometry) suggested that the C-6 position was oxygenated to give two more new alkaloids (**50**/**51**) [9]. Another new epoxy alkaloid reported from *Crinum* species was augustine (**4**) [11]. It co-occurs with the corresponding olefinic alkaloid, buphanisine (**6**) in *C. augustum* [13].

Four new pyrrolophenanthridone alkaloids, viz. pratorine (= hippadine, **39**), pratorinine (**40**), pratorimine (**41**) and pratosine (**42**), were isolated and characterized from *C. asiaticum*, *C. augustum*, *C. bulbispermum*, *C. latifolium* and *C. pratense* [6, 7, 9, 15, 23]. Additionally, the first trioxy-pyrrolophenanthridone alkaloid, kalbretorine (**52**), was isolated from *Haemanthus kalbreyeri* and its presence was also detected in *C. asiaticum* and *C. augustum* [29].

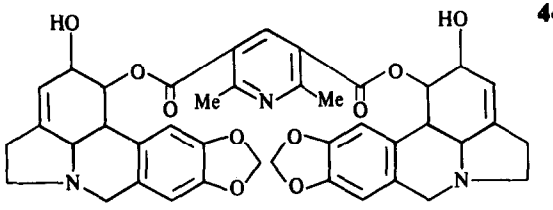
Lycorine-1-*O*-β-D-glucoside (**32**) was found as a major alkaloid in the roots of *C. asiaticum*, *C. augustum*, *C. latifolium* and *C. pratense* [9, 24]. This glucoalkaloid was encountered commonly in Amaryllidaceae plants at the time of flowering [9, 24]. It is, therefore, a little surprising that this compound eluded the notice of phytochemists until recently. Two factors may have contributed to this omission: (i) the gluco-alkaloid is produced in detectable amounts for a limited period of time during ontogeny of the producer plants; (ii) strongly polar extractives (e.g. *n*-



18 $R^1 = H, R^2 = Me$
19 $R^1 = R^2 = H$
20 $R^1 = R^2 = Ac$



21 $R^1 = H, R^2 = R^3 = R^4 = Me$
30 $R^1 = R^2 = H, R^3 + R^4 = -CH_2-$
31 $R^1 = R^2 = Ac, R^3 + R^4 = -CH_2-$
32 $R^1 = Glc, R^2 = H, R^3 + R^4 = -CH_2-$
43 $R^1 = R^2 = R^4 = H, R^3 = Me$
44 $R^1 = Glc, R^2 = R^4 = H, R^3 = Me$



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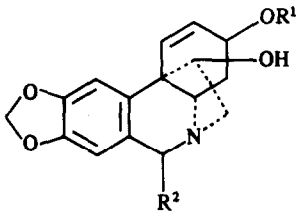
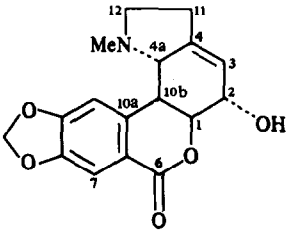


22 $R^1 = Me, R^2 = H$
23 $R^1 = Me, R^2 = OH$
24 $R^1 = R^2 = H$



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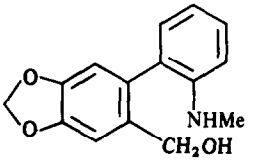

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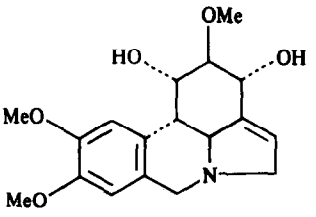

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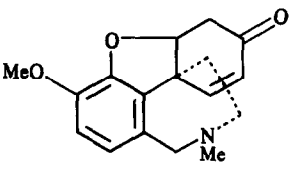


35

BuOH) which contained mainly gluco-alkaloid(s) were not examined. Another gluco-alkaloid, pseudolycorine-1-*O*-β-D-glucoside (**44**), previously reported from *Pancratium biflorum*, was detected in a number of *Crinum* species [9, 24].

Anhydrolycorine-7-one (**2**), which was known before as a synthetic compound, was isolated from *C. pratense* [8]. Likewise, 1,2-di-*O*-acetyllycorine (**31**), known before synthetically, was reported from a number of *Crinum* species [9, 24].

Structurally the most significant additions are the lignoid-phenanthridone alkaloid, crinasiatine (**13**), and the simple phenanthridone alkaloid, crinasiadine (**12**), from *C. asiaticum* [18], latindine (**27**) from *C. latifolium* [9, 20] and augustamine (**3**) from *C. augustum* [10]. Attachment of a phenylpropene (lignoid) moiety through a dioxane bridge was earlier observed only in naturally occurring oxygen heterocycles (e.g. flavone, xanthone and coumarino-lignans). Crinasiatine (**13**) is the first example of a lignoid alkaloid. The isolation of the simple phenanthridone alkaloid, crinasiadine (**12**), is also significant since it seems to involve 'apo-reaction' of a 4,5-ethanophenanthridone alkaloid. The absence of narciclasine in *Crinum* species lends credence to the contention that crinasiadine/crinasiatine are derived from a pyrrolophenanthridone alkaloid of the type **39–42** [9].

The structure of augustamine (3), if proved correct by further evidence (e.g. by chemical degradation, X-ray crystallography), will be of particular interest since this will be the first alkaloid, proposed to be formed from a 5,10b-ethano-phenanthridine intermediate [10], devoid of any oxygen substituent in the C-3 position of the hydroaromatic ring (C-ring). Such structures (devoid of C-3 oxygen function) previously assigned to haemultine and financine were later proved to be incorrect [31].

ISOLATION TECHNIQUES

Since Amaryllidaceae alkaloids are widely distributed and are found in a large variety of tissues, no one method of extraction can be said to be universally applicable and adopted as a standard technique. However, continuous solvent extraction (Soxhlet) of air-dried and milled plant material is still the usual procedure of extraction. Since the alkaloids vary so much in polarity and solubility, solvent-gradient extractions are often used. Extraction with petrol provides the least polar alkaloids, e.g. the pyrrolophenanthridones [6, 12, 29], while the strongly polar gluco-alkaloids require methanol or methanol–water for extraction [24]. Alkaloids of intermediate polarities are obtained from chloroform or acetone extracts or by CC of the above two extractives.

For isolation of reactive intermediates, e.g. the norbelladine analogues, and quaternary alkaloids, fresh tissues (leaves, bulbs, flower-stem) were used [8, 12, 24, 26–29]. The fresh tissues were immediately macerated and extracted with a solvent, e.g. acetone, methanol or ethanol. After one or two initial extractions with one of these solvents, the tissues were sufficiently dehydrated to permit the use of water-immiscible solvents for the final stages of extraction. In a typical extraction, fresh plant materials were cut into small pieces and homogenized (in a high-speed blender) with methanol for 1–2 min. Oxidation during the time of vigorous blending was avoided by directing a stream of N_2 into the vessel. The extract thus obtained was worked up in the usual way for the basic fraction (alkaloids). The marc was subsequently hot extracted with petrol and ethanol in succession to yield additional alkaloids [6, 12].

In yet another extraction procedure, flower-stem fluids of Amaryllidaceae plants were collected by a hypodermic syringe and dissolved in methanol. The extractives exhibited pronounced variations in the nature and quantity of alkaloids [8, 12, 20]. The flux in alkaloids was monitored by means of analytical HPLC. In cases of appreciable amounts of the methanol extractives, initial purification was carried out by passing the methanol concentrate through a column of Sephadex LH-20 (prepared with MeOH) to remove inorganic salts. The enriched alkaloidal fraction was then subjected to prep. TLC and/or semi-prep. HPLC [3, 20, 28]. CC over various types of adsorbents, e.g. silica gel, alumina and Florisil, was also carried out as a routine procedure for separation of alkaloids in larger quantities.

Separation by prep. TLC has been greatly improved over the years. Different varieties of silica gel TLC plates (prepared by standard procedures or purchased commercially) are employed as chromatostrips. Precoated plates (silica gel 60 with a fluorescent indicator F_{254}, e.g. Sil G/UV_{254}, Machery–Nagel) was conveniently used for separation of minor alkaloids on a number of occasions [3, 12, 20, 22, 24, 27, 28]. Prep. TLC on silica gel HF-254 using methanol–chloroform (1:9) was used for the separation of alkaloidal mixtures from *C. natans* and *C. ornatum* [21]. Inclusion of a basic modifier such as diethyl amine or Ag^+ (argentine silica gel G) provided better separation of alkaloids with similar polarity [28].

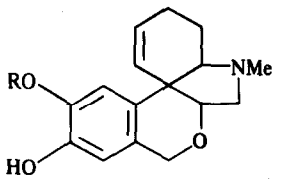
Another innovation in the isolation technique has been the Lobar liquid chromatography. This technique, with a prepacked column [LiChroprep. silica 60 (40–63 μm)/, was used for the separation of the alkaloids of *C. latifolium*. The alkaloid 'fraction B' from *C. latifolium*, consisting of a mixture of ambelline, 1,2-β-epoxy-ambelline and a strongly polar minor entity C, in the ratio of 70:25:5, was separated by Lobar chromatography using methanol–water (3:2) as eluant [8]. In another experiment, Lobar chromatography was successfully used for the separation of the gluco-alkaloid, latisoline (**28**) from its aglucone, latisodine (**29**) [12].

HPLC has been found to be of considerable value for detection as well as separation of Amaryllidaceae alkaloids as these compounds strongly absorb UV light. Reversed phase analytical HPLC was used to determine the flux of latisoline (**28**) and latisodine (**29**) when these were in dynamic equilibrium in *C. latifolium* [8]. Analytical HPLC was also applied for determining the levels of glyco-aglycone alkaloids in other genera of Amaryllidaceae [12, 24]. The composition (*ca* 3:1) of ambelline-1,2-β-epoxyambelline (**1/17**), at any time of the ontogeny of *C. asiaticum* and *C. latifolium*, was determined by analytical HPLC [8]. Likewise, the *in vivo* transformation of (**1/17**) into the 11-*O*-acetyl derivatives (**48/49**) and finally to the 6-α-hydroxy-11-*O*-acetyl derivatives (**51/52**) was monitored by analytical HPLC [3, 8, 9]. Reversed phase semi-prep. HPLC, using an RP-8 column and different proportions of methanol–water, was employed to isolate many rare and reactive alkaloids, e.g. *O*-methylnorbelladine and ryllistine from the flower-stem fluids of *C. augustum* and *A. vittata* [3, 9, 20, 24]; crinasiatine and crinasiadine from *C. asiaticum* [18]. Alkaloids of *C. natans* and *C. ornatum* were resolved by HPLC, using a Corasil II column with ethyl acetate–cyclohexane (5:95) as the eluant [21].

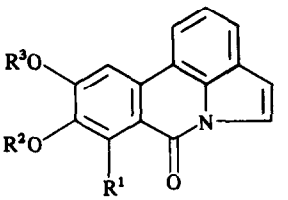
SPECTRAL PROPERTIES

UV spectra

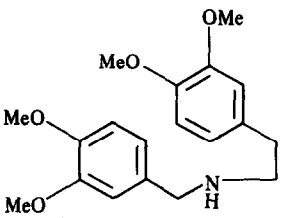
The UV spectra of Amaryllidaceae alkaloids, taken in a neutral solvent (e.g. methanol or ethanol), provide information regarding the type of the ring system (e.g. norbelladine/lycorine/lycorenine/5,10b-ethano-phenanthridine/pyrrolophenanthridone). The norbelladine alkaloids exhibit three high intensity UV maxima around λ225, 278 and 285 nm [12, 20, 22, 27]. Lycorine and crinine-type alkaloids, commonly having a methylenedioxy aryl chromophore and an isolated double bond, exhibit maximum or shoulder near λ240 nm and another maximum around λ280–290 nm (when no other unsaturation is present). Lycorenine-type alkaloids, e.g. hippeastrine, on the other hand, exhibit maxima in the region λ225, 265 and 310 (shoulder) nm. Alkaloids having additional unsaturation or aromatic ring-C exhibit high-intensity multiple maxima, the positions of which depend on the type of the ring system. Thus, cherylline, having an additional hydroxyphenyl moiety attached to the C-4 position of the tetrahydro-isoquinoline ring exhibits three



36 R = Ac
37 R = H



39 R^1 = H, $R^2 + R^3 = -CH_2-$
40 $R^1 = R^2$ = H, R^3 = Me
41 $R^1 = R^3$ = H, R^2 = Me
42 R^1 = H, $R^2 = R^3$ = Me
52 R^1 = OH, $R^2 + R^3 = -CH_2-$



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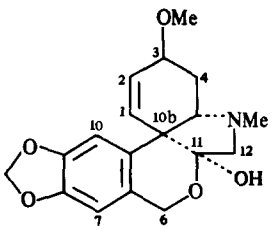

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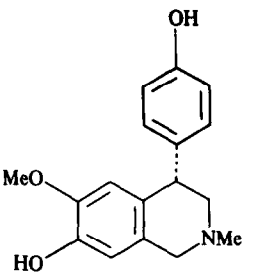

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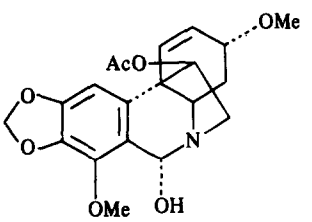

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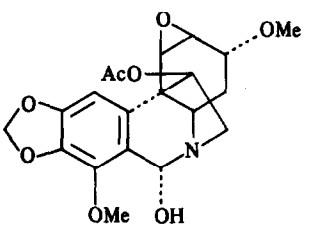


51

distinct maxima at λ227, 285 and 295 nm. The extinction value of the longer wavelength maximum of lycorine and crinine type alkaloids is generally greater except in cases where there is a C-7 methoxyl group and the C-11–C-12 bridge-head is upward (e.g. in case of ambelline, powelline and buphanidrine). The pyrrolophenanthridone alkaloids (**39–42**) exhibit several high-intensity maxima around λ228, 235, 248, 255, 300, 335, 360 nm because of the presence of the extended conjugation in the four-ring system [6, 7, 29]. The phenanthridone alkaloids (devoid of the etheno bridge) (**12** and **13**) also exhibit multiple maxima in the region λ245, 250, 265, 295, 305, 325, 335 nm but the intensities of the longer wavelength maxima were smaller than those of the pyrrolophenanthridone alkaloids [18]. The addition of the usual shift reagents [30] to an alcoholic (or methanolic) solution of the *Crinum* alkaloids induces considerable shift in the maxima of phenolic alkaloids, having a hydroxyl at the C-7, 8 or 9 positions. Thus, C-8/C-9 hydroxylated alkaloids of the pseudolycorine, pyrrolophenanthridone or simple phenanthridone type exhibit bathochromic shifts of both Band-I and Band-II [30], with sodium methoxide, by *ca* 7–20 nm. The extent of the bathochromic shift is dependent on the ring-type and the overall substitution pattern of the molecule. The presence of a hydroxyl at the C-9 position, *para* to carbonyl, in pyrrolophenanthridone alkaloids causes a considerable bathochromic shift of the longer wavelength maxima by the addition of even a weak

base, sodium acetate. The presence of a chelated hydroxyl group (C-7 hydroxyl) in kalbretorine (**52**) was detected by the shift of its longer wavelength maxima by the addition of anhydrous aluminium chloride; the shift remained unaltered on addition of HCl [29].

IR spectra

In recent years, IR spectra of Amaryllidaceae alkaloids have not been used for diagnostic purposes. However, the IR data are largely used to determine the nature of the oxygen functions (hydroxyl/methoxyl/methylenedioxy/epoxy/acetoxyl/glucosyl) in the different rings and are quoted in support of identities of newly isolated alkaloids with reference samples [6, 7, 13, 14, 17, 28, 32].

^{1}H *NMR spectra*

Although the structures of most of the major Amaryllidaceae alkaloids have been established by means of chemical methods, ^{1}H NMR spectra have proved to be of considerable value in aiding the structural assignments and in settling their stereochemistry. ^{1}H NMR has also helped in the structural elucidation of a number of *Crinum* alkaloids obtained in small quantities. Haugwitz *et al.* [33] first reported ^{1}H NMR spectral data to support structural and stereochemical assignments of alkaloids of the tazettine and vittatine/(−)-crinine types which are commonly found in this family. Since then, a number of compilations on the ^{1}H NMR spectra of Amaryllidaceae alkaloids have appeared [34–36].

^{1}H NMR spectra of Amaryllidaceae alkaloids are determined in various solvents, e.g. $CDCl_3$, DMSO-d_6, CF_3COOD, pyridine-d_5, methanol-d_4, D_2O and mixtures thereof, depending on the solubility of the compounds and the nature of information sought. However, the bulk of the published data refer to $CDCl_3$ and DMSO-d_6 as the solvents of choice. Occasionally, some uncommon solvents are also used. For instance, 8,9-methylenedioxy-6-phenanthridone, prepared synthetically, was taken in HMPT because of its poor solubility in common organic solvents [37]. For lycorine, the best resolution conditions were obtained in CD_3OD–CD_3COOD (3:1) since it was sparingly soluble in common organic solvents, unstable in CF_3COOH and double signals were observed in D_2O–D_2SO_4 (1%) solution due to interconversion of the two conformers of the protonated alkaloid [38]. Solvent-induced chemical shifts, having significant diagnostic value, have been reported for variously substituted Amaryllidaceae alkaloids [36, 39]. ^{1}H NMR signals of *Crinum* alkaloids appear at δ1.5–8.5 (δ scale relative to TMS). The informative δ regions, regarding A and C-ring substitutions, in the new *Crinum* alkaloids are given in Table 3.

Some important examples of the application of ^{1}H NMR spectra to structure elucidation of new *Crinum* alkaloids are cited below.

Ring-A protons. Pyrrolophenanthridone alkaloids

Table 3. Chemical shift regions for A- and C-ring protons of new *Crinum* alkaloids*

	Ring type (cf. Table 1)					
	I†	II†	III†	VI†	VII†	X‡
A ring (δ6.5–8.3) proton						
7	7.3–7.35 *s*†	6.9–6.92 *s*	7.6–7.75 *s*	6.7–6.9 *s*	6.5–6.6 *s*§	7.7–7.75 *s*§
10	6.8–6.9 *d*	6.7–6.75 *d*	7.9–8.3 *s*§	7.0–7.1 *s*	6.75–6.9 *s*	8.2–8.3 *s*
10a	6.80–6.82 *d*	6.6–6.7 *d*	—	—	—	—
C ring (δ1.4–8.3)						
1	6.9–6.95 *d*	6.7–6.75 —	7.9–8.3 *dd*	6.7–6.75 *d*	3.8–3.82 *d*	7.7–7.75 *dd*
2	6.5–6.6 *d*	—	7.4–7.5 *dd*	5.8–5.85 *m*	3.2–3.3 *m*	7.0–7.1 *dd*
3	—	—	7.7–7.85 *dd*	4.0–4.1 *m*	4.0–4.1 *m*	7.5–7.55 *dd*
4	6.5–6.6 *d*	6.7–6.75	—	1.4–1.7 *m*	1.4–1.7 *m*	—
4a	6.9–6.95 *d*	6.7–6.75	—	3.1–3.2 *m*	3.1–3.15 *dd*	—

*After the review of Fuganti [2]. For the sake of uniformity in assignments, a common numbering system, based on tenets of biogenesis of these compounds, is adopted (see structural formulae).

†Values (δ) in $CDCl_3$–DMSO-d_6 relative to TMS.

‡HMPT; $CDCl_3$.

§Line broadening. The exact chemical shift values of the individual protons in different compounds are dependent on the nature and number of oxygen substituents in the ring systems.

(39–42, 52). The assignments of the H-1 and H-10 resonances in these alkaloids (type III) were rationalized on the basis of the observed H-4 and H-5 resonances in the fully aromatic phenanthrene derivatives which are strongly deshielded [40]; the line-broadening effect of H-1 and H-10 resonances was due to their mutual long-range coupling. The H-10 signal in the pyrrolophenanthridine (e.g. lycorine) and 5,10b-ethanophenanthridine (vittatine) alkaloids always appears at a lower field than the corresponding H-7 signal. The H-10 proton being near in space to the C-ring experiences a diamagnetic shift from the unsaturation in that ring. This has been substantiated by measurements of nuclear magnetic double resonance (NMDR) and nuclear Overhauser effect (NOE) of the parent compounds and those of their dihydro derivatives. The location of the only hydroxyl function in pratorimine (41) was based on the maximum upfield shift (δ0.22) exhibited by the H-10 resonance in the presence of $NaOD–D_2O$. The location of the A-ring hydroxyl in cherylline was made on similar generalizations [41]. Furthermore, the observation that in *O*-acetylpratorimine it was the H-10 resonance which experienced maximum downfield shift (δ0.37) relative to the corresponding H-resonance in the parent compound and downfield shift by δ0.25 from that of the permethyl ether supported the above assignment. Irradiation of the only methoxyl signal in pratorimine and in *O*-acetyl pratorimine caused area enhancements (NOE) of their H-7 signal by 22% and 18%, respectively. Similar homonuclear NOE effects were observed in polyoxygenated xanthones which constituted a basis for determining the positions of their hydroxyl/methoxyl functions [42, 43]. The structures of two isomeric phenolic alkaloids, pratorinine (40) and pratorimine (41), occurring as minor alkaloids in several *Crinum* species, were confirmed by comprehensive spectroscopic analyses [3, 6, 7, 9].

Ring-C protons. 1,2-Olefinic and epoxy-5,10b-ethanophenanthridine alkaloids. Buphanisine (7) and augustine (4) showed close similarity of the ^{1}H NMR signals associated with the ring A protons and those of the oxygen substituents (OMe, OCH_2O). Also, both these compounds exhibited in their 60 MHz ^{1}H NMR spectra two doublets around δ4 ppm corresponding to the two benzylic protons (H_α, H_β 6). The major difference between the two spectra consisted of the signals associated with the H-1 and H-2 resonances. The spectrum of buphanisine exhibited signals assignable to an olefinic H-1, as a doublet at δ6.70 ($J_{1,2}$ = 10 Hz) and to the second olefinic proton H-2 at δ6.21 as a double doublet ($J_{1,2}$ = 10 Hz, $J_{2,3}$ = 5 Hz). The ^{1}H NMR spectrum of augustine did not show such olefinic proton signals indicating that its C-1, C-2 bond was saturated. That the two carbons are associated with an oxirane ring was indicated by one of the oxirane protons appearing as a doublet at δ3.83, assignable to H-1 [13]. The 90 MHz ^{1}H NMR spectrum of augustine exhibited distinct signals assignable to both the oxirane protons (H-1 and H-2). Furthermore, the 90 MHz spectrum provided good separation and some clarification of the signals in the complex region δ3.4 to 1.4 ppm which were recorded in the 60 MHz spectrum as a series of unresolved signals. The signals at δ3.83, assignable to H-1, appeared as a doublet by coupling with the signal appearing as a multiplet (*ddd*) at δ3.35, attributable to the second oxirane proton (H-2). Selective decoupling experiments and expanded spectral analyses finally decided the 1,2-β-configuration of the epoxy ring in augustine [11, 13]. The structure and 1β,2β-configuration of 1,2-epoxyambelline was established by a similar line of arguments [8].

^{13}C *NMR spectra*

^{13}C NMR spectroscopy has been liberally used in recent years for determining the carbon framework of Amaryllidaceae alkaloids. The assignments are made on the basis of chemical shifts and multiplicities of the signals in the proton-noise decoupled (PND) and single frequency off-resonance decoupled (SFORD) spectra. The use of shift reagents (lanthanide) is also made when polar groups, e.g. OH, COOH, NH_2, CO, present in the alkaloid allow binding to the lanthanide ion. The major contributions in this area are due to Evente *et al.* [38], Crain *et al.* [44] and Zetta *et al.* [45].

The ^{13}C NMR spectra of *Crinum* alkaloids, so far examined, can be divided into two broad regions. The low-field (> δ90) region containing the CO group signal, the unsaturated carbon signal (olefinic/aromatic) and the methylenedioxy signal. The saturated carbon resonances are in the high-field region. As a general guide, the range of chemical shifts for the different carbon-types encountered in the *Crinum* alkaloids are given in Table 4.

The ^{13}C chemical shifts observed for the different ring-types of *Crinum* alkaloids are given in Table 5. This should provide a basis for empirical calculations of the effects of substituent effects and for assigning structure to new alkaloids. Since all of these alkaloids are derived from a common $C_6–C_1–N–C_2–C_6$ intermediate, a uniform numbering system is adopted (see structural formulae) for uniformity in assignments.

Substituent effects. Substituent (OH, OMe, OAc) effects observed on the carbon resonances in the aromatic rings of the different ring-types (Table 5) are of considerable

Table 4. ^{13}C chemical shift ranges for different carbon-types in *Crinum* alkaloids

Carbon type	Chemical shift range [δ (ppm) from TMS at zero]
Aromatic	
(a) Oxygenated	160–155 (no *o/p* oxygen)
	149–139 (with *o/p* oxygen)
(b) Non-oxygenated	128–118 (no *o/p* oxygen)
	130–102 (with *o/p* oxygen)
Aliphatic	
(a) Oxygenated	90–65
Non-oxygenated	42–30
(b) C attached to N	60–44
Olefinic (>C=C<)	140–115
Carbonyl (>CO)	
(a) Amido	176–174
(b) Lactam	160–157
(c) Acetoxy	172–168
(d) Lactone	164–160
Methylenedioxy	102–100
Aromatic OMe	60–55
Aliphatic OMe	59–57
N–Me	42–39
Epoxy-C	54–51
Acetoxy-Me	23–20

Table 5. ^{13}C chemical shifts and multiplicities of the major ring-types of *Crinum* alkaloids*

Carbon	I	III	IV	V	VI	VIII	X
1	129*d*	70*d*	67*d*	88*d*	125*d*	131*d*	118*d*
2	114*d*	71*d*	32*t*	30*t*	128*d*	127*d*	124*d*
3	158*s*	122*d*	115*d*	62*d*	72*d*	72*d*	122*d*
4	114*d*	137*s*	140*s*	126*d*†	28*t*	27*t*	128*s*
4a	129*d*	62*d*	66*d*	126.8*d*†	63*d*	70*d*	130*s*
6	62*t*	54*t*	91*d*	60.5*t*	44*t*	65*t*	157*s*
6a	132*s*	130*s*	130*s*	132.6*s*†	126*s*	128*s*	131*s*
7	111*d*	108*d*	112*d*	132*s*†	106*d*	104*d*	107*d*
8	149*s*	149*s*†	148.5*s*†	144*s*	145.9*s*†	146*s*	148*s*
9	148*s*	148.5*s*†	148*s*†	145*s*	145.5*s*†	146*s*	152*s*
10	112*d*	106*d*	107*d*	110*d*	102*t*	109*d*	101*d*
10a	121*d*	125*s*	127*s*	121*d*	138*s*	125*s*	119*s*
10b	132*s*	38*d*	44*d*	48.2*s*	44*s*	50*s*	116*s*
11	33*t*	30*t*	28*t*	34*s*	44*t*	102*s*	—
12	58*t*	55*t*	57*t*	54*t*	53*t*	62*t*	—

*See Table 1 for ring-types and structural drawing for carbon-numbering.
†Values are interchangeable in the respective columns.

importance in locating the positions of the functional groups. The effects resulting from these substituents on the *Crinum* alkaloids are, as in simple aromatic compounds [46], largely additive and so more than one such function when present in the molecule can be taken into consideration for calculation purposes. Although a new substituent (or derivative) might have a modified influence, e.g. introduction of a methoxyl *ortho* to an existing methoxyl sometimes exhibit erratic effects where steric interaction is possible. The additivity principle of substituent effects was commonly used in locating the functional group(s) in *Crinum* alkaloids. Thus, introduction of an additional methoxyl function at the C-4 position of N-norbelladine afforded the alkaloid ryllistine (**45**) [20] which showed a downfield shift of the C-4 resonance (δ148) by 34 ppm which was consistent with the expected value (*ca* + 33 ppm). The *ortho* effect (−10 ppm), on introduction of the additional methoxyl, was smaller than the usual value, the shift being −17 ppm, while the *para* effect (−8 ppm) was again within the expected range (−7 to −8 ppm) [46]. Likewise, undulatine (≡ C-7 OMe augustine) exhibited the C-7 resonance at δ139.5 (*s*) [45] which was downfield by 32.5 ppm from the corresponding resonance (at δ107, *d*) in augustine [13] and was consistent with the expected value.

Acetylation of non-bonded phenolic hydroxyl groups produces marked changes in the aromatic carbon signals. Comparable effects of acetylation are observed in *Crinum* alkaloids. Thus, on acetylation of the C-9 hydroxyl in pratorimine, the corresponding signal was shifted upfield by 4.5 ppm while the *ortho* carbon (C-10) signal was moved downfield by 6.8 ppm [28]. The shifts were consistent with the expected values. Methylation of free phenolic hydroxyl groups in the A-ring of *Crinum* alkaloids produced variable effects [28].

^{13}C NMR spectroscopy was also used to analyse complex mixtures of alkaloids, e.g. of the (−)-crinine-powelline series without separating the components into homogeneous entities [45]. The method was successfully extended to the corresponding 1,2-olefin-epoxy pair of alkaloids which are not readily separable by conventional separation techniques [8]. The co-occurrence of the olefinic-epoxy pair of alkaloids in several *Crinum* species was detected from the ^{13}C NMR spectra of the appropriate fractions. Thus, the doublet signals in the range δ51–54 (C-1, C-2 of epoxy alkaloid) in association with the doublet signals at δ132–125 (assignable to olefinic C-1, C-2) suggested the co-occurrence of ambelline-epoxyambelline in a number of *Crinum* species. The assignment was further supported by the appearance of two triplet signals in the range δ34–28 (C-4 of olefinic) and 25–23 (C-4 of 1,2-epoxy) in the mixture of olefinic-epoxy alkaloids [8].

Mass spectroscopy

Amaryllidaceae alkaloids exhibit a wide range of structural variations and extensive studies on the mass spectra of these alkaloids having different ring types have been reported [7, 26, 47–52]. High resolution mass spectrometry and isotopic labeling have proved to be of considerable value in assigning structure and stereochemistry to Amaryllidaceae alkaloids. Of particular importance was the observation that even minor changes in stereochemistry were often sufficient to cause differences in the mass fragmentation patterns of these compounds. Thus, the difference in the configuration of the C-3 methoxyl in tazettine and criwelline caused marked variations in the fragmentation pattern and the relative abundance of the fragment ions [47]. The dominant ion in the mass spectrum of tazettine appeared at *m/z* 247 $[M-84]^+$. High resolution mass spectral measurements demonstrated the homogeneity and the composition of this ion as $[C_{13}H_{13}NO_4]^+$, which was shifted to *m/z* 250 in the spectrum of tazettine-N-d_3 indicating that the fragment ion originated, at least partially, from the $[M]^+$ in one-step decomposition. The prominent fragment-ion peak in the mass spectrum of criwelline, on the other hand, occurred at *m/z* 301 $[M-30]^+$ by elimination of formaldehyde from the $[M]^+$. Recognition of a metastable ion at *m/z* 274.0 ($301^2/331 = 273.7$), in the spectrum, was consistent with this assignment. Similar distinctive features of mass fragmentation were observed in the C-3 epimeric pairs of 5,10b-ethanophenanthridine alkaloids:

vittatine/(+)-epicrinine; haemanthamine/crinamine and maritidine/(+)-epimaritidine [26]. Thus, in the case of the first alkaloid in each pair, where the C-3 oxygen function is pseudo-axial, the $[M]^+$ appeared as a prominent peak while in the corresponding epimer (C-3 oxygen pseudo-equatorial) the $[M]^+$ was not detectable. In the case of these latter three alkaloids, the prominent peak appeared at *m/z* 269 by the loss of elements of water from (+)-epicrinine and (+)-epimaritidine, and methanol from crinamine. Chemical ionization (CI) mass spectra of these alkaloids exhibited $[M]^+$ as the base peak in each case.

The mass fragmentation of 1,2-epoxy-5,10b-ethanophenanthridine alkaloids is triggered by the epoxy function [52]. The most prominent peak in the mass spectrum of 1,2-β-epoxyambelline [8] was discernible at *m/z* 318 $[M-29]^+$ by the loss of CHO. A similar mode of fragmentation was observed in case of undulatine and augustine [13]. Since a large number of Amaryllidaceae alkaloids contain phenolic and/or aliphatic hydroxyl group(s), they are unsuitable for GC-MS analyses. To circumvent this drawback, mixtures of the alkaloids are converted into trimethylsilyl ether derivatives prior to their GC-MS analyses [21]. The M_r of the glucosyloxy alkaloid, hordenine-4-*O*-β-D-glucoside and those of lycorine-1-*O*-β-D-glucoside and pseudolycorine-1-*O*-β-D-glucoside, were determined following this procedure [24].

Several known Amaryllidaceae alkaloids have also been examined by field desorption mass spectrometry (FD-MS) and each gave only a single peak corresponding to the $[M]^+$ or $[MH]^+$ ion. Thus, lycorine exhibited only one peak at *m/z* 287 $[M]^+$ and crinamine showed the only peak at *m/z* 302 $[MH]^+$ in their FD-MS. Such $[M+1]^+$ ions are often observed in the FD-MS of the *Crinum* alkaloids. Since FD-MS generally produces only one ion ($[M]^+$ or $[M+1]^+$), qualitative analysis of a mixture of alkaloids from a number of *Crinum* species was carried out following this method [3, 21]. The other mass spectral techniques commonly used for similar purposes constituted CI and GC/EI-MS [21].

INTERCONVERSION OF *CRINUM* ALKALOIDS

The identities of a number of *Crinum* alkaloids were established by means of transformation into known alkaloids. Some recent examples are given below (Schemes 1–6).

Reduction of hippadine by lithium aluminium hydride in ether–THF afforded anhydrolycorine. Dehydrogenation, on the other hand, of a synthetic sample of anhydrolycorine-7-one by DDQ, in anhydrous benzene, under reflux, afforded hippadine in 75% yield [6] (Scheme 1).

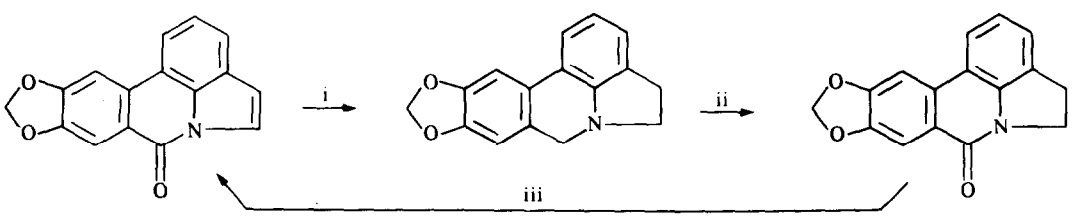
Hippadine (**39**) was transformed into pratorinine (**40**) by regioselective opening of the methylenedioxy ring by heating with sodium methoxide in DMSO at 150° [6]. Similar ring opening of the methylenedioxy group was known in acridone [53] and in piperonal and methylenedioxy-aromatic nitro compounds [54]. A concomitant opening of the lactam ring in **39** was expected and realized leading to indolopiperonylic acid. Treatment of pratorinine with ethereal diazomethane afforded pratosine (**42**), a minor alkaloid of several *Crinum* species [7] (Scheme 2).

The lignano-phenanthridone alkaloid, crinasiatine (**13**), on heating with pyridinium chloride, at 210°, produced 8,9-dihydroxy-6-phenanthridone (**53**) by opening of the dioxane ring. The latter (**53**), on treatment with chlorobromomethane and anhydrous potassium carbonate under reflux, afforded another alkaloid, crinasiadine (**12**) whose identity was established before by synthesis [18] (Scheme 3).

Selective demethylation of the C-3 methoxyl of buphanidrine (**54**) was carried out by using the $AlCl_3$–NaI–MeCN system when powelline (**55**) was produced [55] (Scheme 4). The reaction has precedent in aliphatic methyl ethers which are known to cleave by this system, at ambient temperature, without affecting aromatic methyl ether(s) or other active functions (e.g. ester or lactone) present in the molecule [56]. Methylation of **55** with MeI and NaH, in THF, regenerated **54** (Scheme 4).

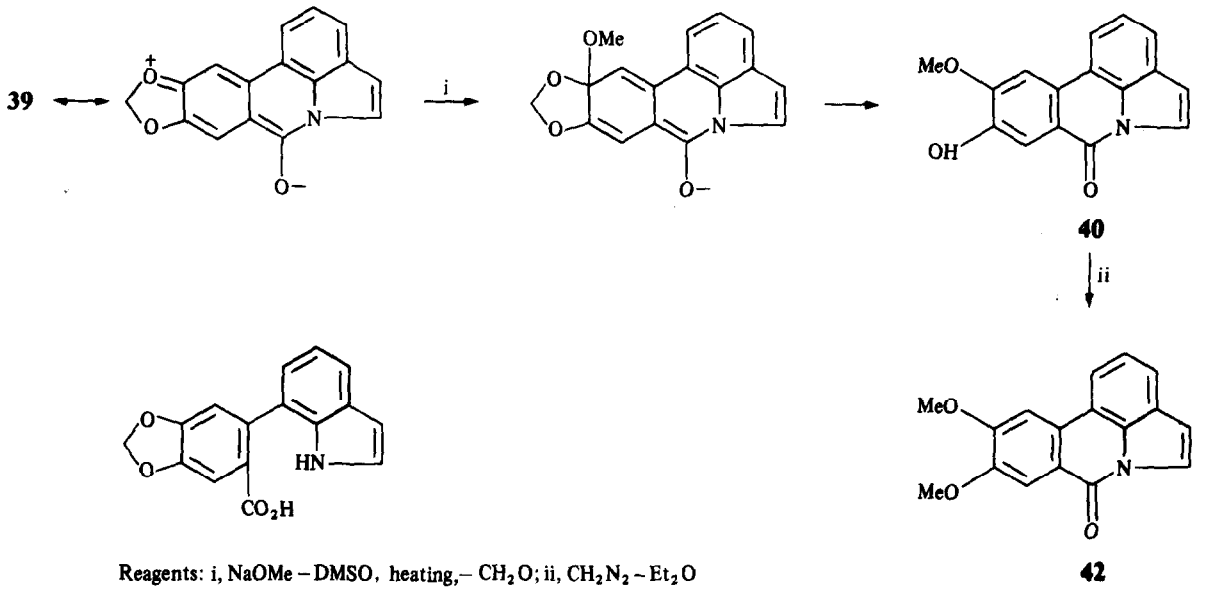
Although allylic rearrangement during acid treatment is extremely uncommon in the 5,10b-ethanophenanthridine alkaloids [57], partial success was achieved by means of this transformation in the synthesis of (±)-maritidine (**57**) (29%) by equilibrating (±)-epimaritidine (**58**) with aqueous HCl, under reflux for 1 hr [58]. Equilibration in the reverse direction, however, was more facile [26]. Heating of maritidine with aqueous HCl for a longer period of time (4 hr), afforded (+)-epimaritidine in 85% yield. It seems likely that thermodynamic stability of the *quasi*-equatorial C-3 hydroxyl in epimaritidine is responsible for the facile epimerization: maritidine ⇌ (+)-epimaritidine. Similar observation was made with two other alkaloids. Thus (+)-crinine (**60**) (C-3 hydroxyl *quasi*-equatorial) remained practically unaffected on heating with aqueous HCl, while vittatine (**59**) was partially converted to **60** under similar conditions (Scheme 5).

1,2-β-Epoxyambelline (**17**) resisted the normal Zn–AcOH reduction into ambelline (**1**) [59]. Treatment of **17** with Zn–AcOH–NaI, on the other hand, transformed it into **1** in 58% yield [8]. 11-*O*-Acetylambelline was oxidized with *m*-chloroperbenzoic acid, in dichloromethane, into 11-*O*-acetyl-1,2-β-epoxyambelline (Scheme 6).



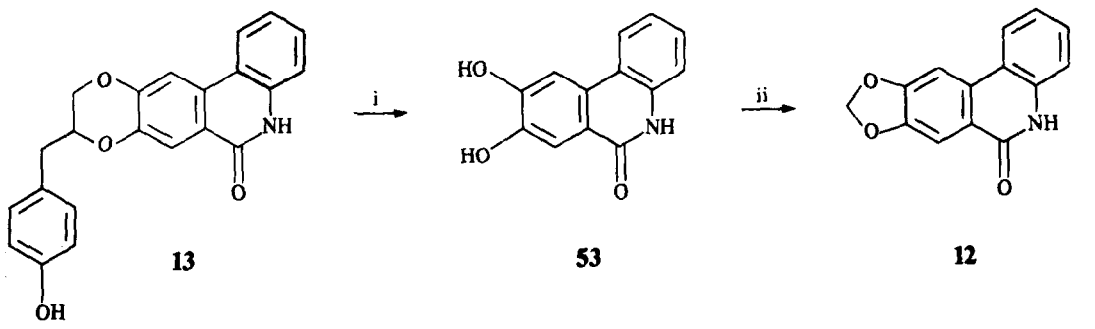
Reagents: i, $LiAlH_4$ – Et_2O–THF: ii, $K_3Fe(CN)_6$ – HO^-; iii, DDQ – C_6H_6, reflux

Scheme 1. Transformations of hippadine.



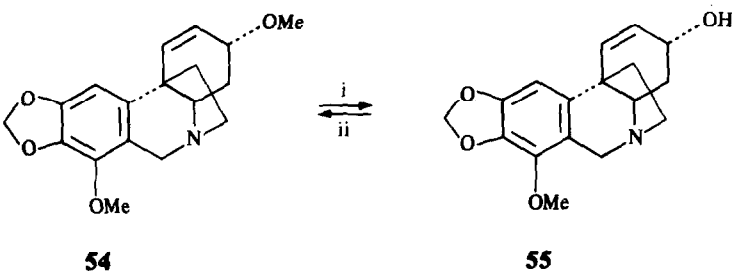
Reagents: i, NaOMe – DMSO, heating, – CH_2O; ii, CH_2N_2 – Et_2O

Scheme 2. Further transformations of hippadine.



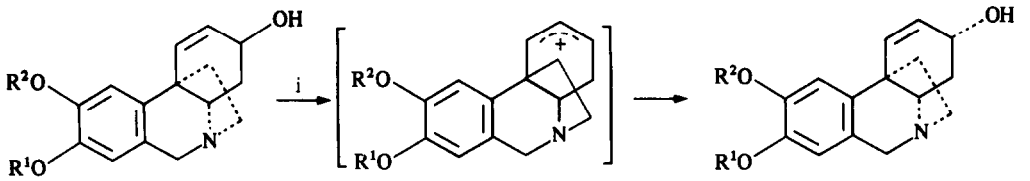
Reagents: i, $C_5H_5NH^+Cl^-$, 210°; ii, CH_2ClBr–K_2CO_3 – AcMe, reflux

Scheme 3. Transformations of crinasiatine.



Reagents : i, $AlCl_3$–NaI–MeCN, room temp. ; ii, NaH–THF–MeI–N_2, room temp.

Scheme 4. Transformations of buphamidrine.

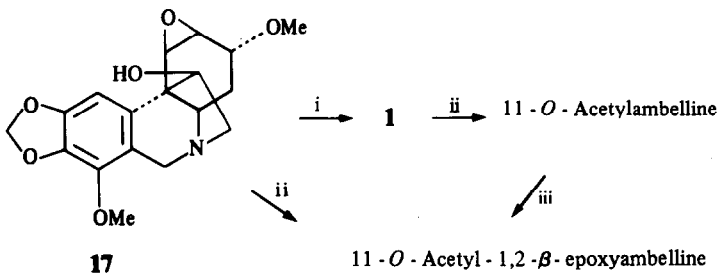


57 $R^1 = R^2 = Me$
59 $R^1 + R^2 = -CH_2-$

58 $R^1 = R^2 = Me$
60 $R^1 + R^2 = -CH_2-$

Reagents: aq. HCl (10%), reflux, 4 hr

Scheme 5. Transformations of maritidine, crinine and vittatine.



Reagents: i, Zn–AcOH–NaI; ii, $Ac_2O-C_5H_5N$; iii, *m* - chloroperbenzoic acid – CH_2Cl_2

Scheme 6. Transformations of 1,2-β-epoxyambelline.

ONTOGENESIS

Investigations of the ontogenic variations of Amaryllidaceae alkaloids have shown marked fluctuations in respect of lycorine and its 1-*O*-glucoside [24]. The amount of lycorine in *Pancratium biflorum* Roxb. was found to increase steadily from July up to January, followed by a very rapid increase up to April, and finally a sharp fall during May–June (flowering time of *P. biflorum*) every year. The concentration of lycorine-1-*O*-glucoside, on the other hand, increased only during the pre- to post-flowering time covering a span of *ca* 10 weeks. This was found to be a general phenomenon in members of the Amaryllidaceae. Thus lycorine-1-*O*-glucoside and several other glucosyloxy alkaloids were identified in *Amaryllis belladona*, *A. johnsonii*, *A. rosea*, *Crinum asiaticum*, *C. augustum*, *C. latifolium*, *C. pratense*, *Haemanthus kalbreyeri*, *Zephyranthes flava* and *Z. rosea* during their flowering time which were catabolized during the resting periods of these plants [3, 9, 24, 29, 60].

The concentrations of the pyrrolophenanthridone alkaloids (**39**–**42**) in *Crinum* species, and kalbretorine (**52**) in *H. kalbreyeri*, were maximum during the pre- to post-flowering stages covering a span of *ca* 40 days. In the resting bulbs, these alkaloids were present only in traces [6, 7, 9, 29].

Another noteworthy observation was the rapid gain and loss of the glucosyloxy alkaloids and their aglycones in the flower stems of several Amaryllidaceae plants. Sap-fluid samples of flower stems of *P. biflorum*, collected at daily intervals, for 9 days, showed (PPC and HPLC) the presence of both free and glycosylated alkaloids as well as free sugars. A gradual increase in the concentrations of the glycosyloxy alkaloids and sugars was observed up to day-3 of flowering which was followed by a sharp fall in their concentrations as the flowers started wilting (days 5–8). When this experiment was conducted at short intervals (every 2 hr), rapid changes in the contents and patterns of the alkaloids were noticed. Thus many minor alkaloids and glyco-alkaloids appeared and quickly disappeared. The gains and losses (flux) observed in respect of lycorine-1-*O*-glucoside, pseudolycorine-1-*O*-glucoside and their respective aglucones were in inverse proportions and thereby suggested the following equilibrium reaction: alkaloid aglycone + sugar ⇌ glycosyloxy alkaloid. The glycosyloxy alkaloids were subsequently found to accumulate, in association with phospholipids, in the roots and bulbs of *P. biflorum* [24]. Similar ontogenic variations in respect of free and glyco-alkaloids were observed in the *Crinum* species, *C. asiaticum*, *C. augustum*, *C. pratense* and *C. latifolium* [3, 8, 9, 18, 27]. Another noteworthy observation with the alkaloids of the fruits of *C. asiaticum* consisted of the presence of a lycorine–phospholipid conjugate in a very high concentration (*ca* 3% of fr. wt of fruits) [Ghosal, S., unpublished observation]. Lycorine, which is cytotoxic to both producer and many non-producer plants, becomes benign in the form of this conjugate. Latisoline (**28**), a rare glucosyloxy alkaloid found in *C. latifolium*, was produced only during the blooming of flowers. It was found to be present only in the fluid of the flower-stems. The corresponding aglucone, latisodine, also occurred during flowering time but was

distributed in all parts of the plant. The flower-stem fluid of *C. latifolium*, collected at short intervals (2 hr), exhibited a constant pattern of rise and fall in the concentrations of latisoline and latisodine during day-1 of flowering. The following equilibrium reaction was proposed [12]: latisoline ⇌ latisodine + D-glucose.

Although the biochemical significance of the above observations has not yet been entirely elucidated, the following connected observations might be of relevance for further analyses. (i) The glucosyloxy alkaloids form stable complexes with divalent metal ions (e.g. Cu^{2+}, Zn^{2+}, Fe^{2+}) and are able to translocate the metal ions from the rhizosphere to aerial parts. Alkaloids are known to elicit their activities by moulding the transport of metal ions across biological membranes [61]. The lycorine-1-*O*-glucoside–Cu^{2+} complex was found to produce significant root growth in *P. biflorum*, *Z. flava* and *Z. rosea* in hydroponic solutions. The lycorine–Cu^{2+} complex, on the other hand, was a strong inhibitor of root growth. (ii) The glucosyloxy alkaloids formed stable complexes with phytosterols, e.g. sitosterol, stigmasterol and campesterol. One of the biochemical possibilities of this sterol–alkaloidal complex was considered to be cell binding leading to modulation of the membrane phenomena (stabilization/cell viability). The corresponding agluccones, except the pyrrolophenanthridone alkaloids (**39–42, 52**), formed only weak complexes with the above mentioned phytosterols. The glucosyloxy alkaloid–sterol complexes showed pronounced root-growth in both producer and non-producer plants [24]. (iii) The glucosyloxy alkaloids were hydrolysed into the component alkaloid aglycones and sugars during their isolation due to the presence of large amounts of glycosidase enzymes in the green plants. Glycosidic enzyme(s) are relatively robust compounds that may be isolated by employing organic solvents (ethanol) as precipitating agents; heating at 60° and higher temperatures can only denature them [62]. Thus temporary deactivation or denaturation through freeze-drying or addition of organic solvents does not prevent a glycosidic enzyme from regaining its function once the deactivating environment is removed. This could be one of the reasons for the transformation of glycosyloxy Amaryllidaceae alkaloids into the aglycone and free sugars during their process of isolation [3, 24].

BIOLOGICAL EFFECTS

Lycorine was reported to inhibit protein and DNA syntheses in murine cells and *in vivo* growth of a murine transplantable ascites tumour [61, 63, 64]. In the *in vitro* studies, lycorine was found to markedly reduce the viability of tumour cells [28]. Lycorine-1-*O*-glucoside, in μg doses, produced varying degrees of mitogenic activation of splenic lymphocytes of mice. It further showed a very low order of acute toxicity (> 200 mg/kg ip) in albino rats. Lycorine-1-*O*-glucoside has thus a potential as an immunostimulatory agent [64]. Lycorine is a powerful inhibitor of growth and cell division in higher plants, algae and yeasts [65]. Lycorine-1-*O*-glucoside, on the other hand, is a potent promoter of root growth in higher plants [24]. This observation rationalizes the occurrence of lycorine-1-*O*-glucoside at the time of intense cellular activity of Amaryllidaceae plants. Lycorine inhibited ascorbic acid biosynthesis in plants [66], stopped the synthesis of poliovirus, precursors of poliovirus and specific poliopeptidase [67] and had pronounced antiviral activity. The alkaloid was reported to inhibit protein synthesis in eukaryotic cells by preventing peptide bond formation [68]. Similar biological activities were reported for dihydrolycorine, pseudolycorine, narciclasine, haemanthamine and pretazettine [69]. These alkaloids halted Hela cell growth and, at their minimum growth inhibitory concentrations, blocked protein synthesis in ascites cells and slowed down DNA synthesis. Pretazettine strongly inhibited the activity of RNA-dependent DNA polymerase (reverse transcriptase) which was associated with various oncogenic viruses. It acted by binding to the enzyme itself and not by interacting with the nucleic acid template [70].

Hippadine produced reversible inhibition of fertility in male rats. The alkaloid acted on the germ cells at an early stage of spermatocytogenesis [71]. Hippadine also markedly potentiated the viability of S-180 ascites tumour cells by membrane-stabilization [28]. The ability of its complexation with steroidal entities was considered responsible for the membrane-stabilizing action and for modulation of cellular metabolism. Hippadine did not produce any mortality in albino rats up to 200 mg/kg ip [28].

A concentration of 5 μg/ml of 1,2-β-epoxyambelline produced a moderate activation of mouse spleen lymphocytes. A mixture of 1:1 ambelline-1,2-β-epoxyambelline, in the same concentration, produced pronounced activation of the splenic lymphocytes which was comparable to the activity of the known mitogen, concanavalin A (con A) [8].

The analgesic activity exhibited by the Amaryllidaceae alkaloids was attributed to their resemblance to morphine and codeine skeletons. In this respect, the alkaloids belonging to pyrrolo[de]phenanthridine (e.g. galanthine), lycorenine and pretazettine groups have an advantage over the alkaloids of the dibenzofuran (e.g. galanthamine) and 5,10b-ethanophenanthridine group, the former being less toxic than the latter compounds [72]. Narwedine potentiated the pharmacological effects of caffeine, carbazole, arecoline, and to a lesser extent of nicotine, in laboratory animals. Narwedine and vittatine potentiated the analgesic effect of sub-optimal doses of morphine [28, 72].

Earlier studies had shown that a number of Amaryllidaceae alkaloids caused a transient fall in blood pressure in laboratory animals in high doses. Subsequent studies also did not reveal any significant hypotensive/anti-hypertensive effects by most of the Amaryllidaceae alkaloids. However, (+)-narwedine, galanthamine and epi-galanthamine were reported to produce significant hypotensive effects in mice [72, 73]. Interest is still being shown in the anticholinesterase activity of galanthamine. Its ability to amplify the nerve-muscle transfer, which is greater than that of epigalanthamine, is another noteworthy pharmacological activity [73].

The extracts (methanol) of several Amaryllidaceae plants, including those of *Crinum* species, also exhibit pronounced anti-bacterial and anti-fungal activities [28, 67].

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