Structures of 9-*O*-Demethylhomolycorine and 5α-Hydroxyhomolycorine. Alkaloids of *Crinum defixum*, *C. scabrum*, and *C. latifolium*. Assignment of Aromatic Substitution Patterns from ^{1}H-Coupled ^{13}C Spectra[1]

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Received September 24, 1984

The isolation and characterization of the major crystalline alkaloids of three *Crinum* species, *C. defixum*, *C. scabrum*, and *C. latifolium*, are reported. A new alkaloid, 5α-hydroxyhomolycorine (1) has been isolated from *C. defixum*. The latter plant also contains an alkaloid identified as 9-*O*-demethylhomolycorine which differs in physical properties from that previously reported for this compound. Evidence is provided for the structure of 9-*O*-demethylhomolycorine by ^{1}H and ^{13}C NMR studies. In the latter, the exploitation of long-range ^{1}H coupling in the ^{13}C spectra of lactones in this series is found to be diagnostically useful in assigning aromatic substitution patterns. A survey of the CD spectra of lactone alkaloids of the benzopyrano[3,4-*g*]indole system indicates that this technique can provide useful structural information.

The *Crinum* genus of the amaryllidaceae has a wide geographical distribution in the temperate and subtropical regions. The species *C. defixum* Ker-Gawl, *C. latifolium L.*, and *C. scabrum* Herb. are indigenous to the Indian subcontinent. Several preliminary reports[2] have established the presence of lycorine in each of these species and a more definitive study[3] of *C. defixum* has shown the presence of caranine, crinamine, crinine, galanthamine, galanthine, haemanthamine, and hippeastrine from *C. defixum* grown in Holland.

Results and Discussion

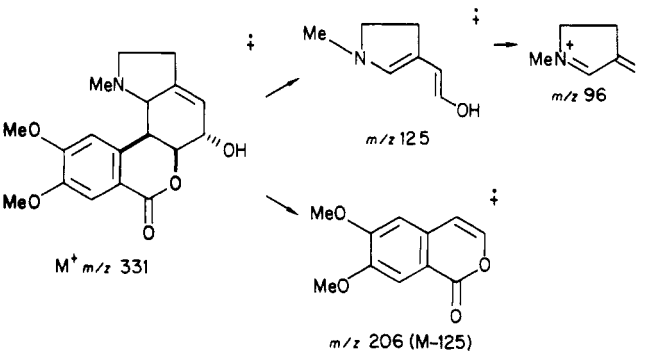
This paper reports on the major crystalline alkaloids found in the above mentioned *Crinum* species. Dried samples of each of these species were received from collections made from their natural habitat through an arrangement made by Professor Govindachari at the Ciba Research Institute, Bombay, India. The plants were processed by extraction with alcohol shortly after arrival. Recovery of the alkaloid fraction from the alcohol extract followed standard procedures which are described briefly in the Experimental Section. Following the removal of lycorine by filtration of a chloroform–methanol concentrate of the crude alkaloid fraction, the solvent was evaporated and the residual oil chromatographed over alumina in benzene–ethyl acetate and ethyl acetate–methanol solvent combinations to yield the crystalline alkaloids listed in Table I. *C. latifolium* and *C. scabrum* afforded alkaloids of known structure. It should be noted that *C. scabrum* seems to be a particularly rich source of crinamine, the alkaloid being present to the extent of 0.073%.

The alkaloids isolated from *C. defixum* include two crystalline bases in addition to lycorine and homolycorine. The isolation of these alkaloids is in marked contrast to those previously reported in this plant by Dopke et al.[3] (vide supra): we have not isolated or detected the presence of any of the alkaloids which they reported to occur in this species. The species they examined was reportedly obtained from Holland and may have been a cultivated variety whereas the species which we studied was obtained from its natural habitat.

Table I. Alkaloids Isolated from *C. defixum*, *C. latifolium*, and *C. scabrum*

C. defixum	lycorine
	homolycorine
	5α-hydroxyhomolycorine, mp 168–170 °C
	9-*O*-demethylhomolycorine, mp 138–140 °C
C. latifolium	lycorine
	hippeastrine
C. scabrum	crinamine
	6-hydroxycrinamine
	lycorine

Scheme I. EI Mass Spectral Fragmentation of Homolycorine



5α-Hydroxyhomolycorine (1). 5α-Hydroxyhomolycorine (1), mp 168–170 °C, was obtained from chromatographic column fractions following the elution of homolycorine. The mass spectrum of the new base displays a molecular ion at m/z 331 and abundant fragment ions at m/z 125 and m/z 96 together with a less intense fragment at m/z 206 (see Scheme I). This fragmentation pattern is characteristic[4] of lactone alkaloids of the benzopyrano[3,4-*g*]indole series which exhibit a retro-Diels–Alder cleavage of ring C to give abundant ions representing the pyrrolidine ring fragment (m/z 125) and a less intense

(1) Part 27 in the series Alkaloids of the Amaryllidaceae. For previous paper see: Jeffs, P. W.; Hansen, J. F.; Dopke, W.; Bienert, M. *Tetrahedron* **1971**, *27*, 5065.

(2) Ranyaswami, S.; Rao, E. V. *Curr. Sci.* **1954**, *23*, 265. Ranyaswami, S.; Suryanarayana, M. *Indian J. Pharm.* **1951–1955**, *17*, 229. Kinel, F. A.; Trocoso, V.; Rosenkranz, G. *J. Org. Chem.* **1957**, *22*, 574. Reichert, B. *Arch. Pharm.* **1938**, *276*, 328.

(3) Boit, H. G.; Dopke, W.; Stender, W. *Chem. Ber.* **1957**, *90*, 2203.

(4) Schnoes, H. K.; Smith, P. H.; Burlingame, A. L.; Jeffs, P. W.; Dopke, W. *Tetrahedron* **1968**, *24*, 2825.

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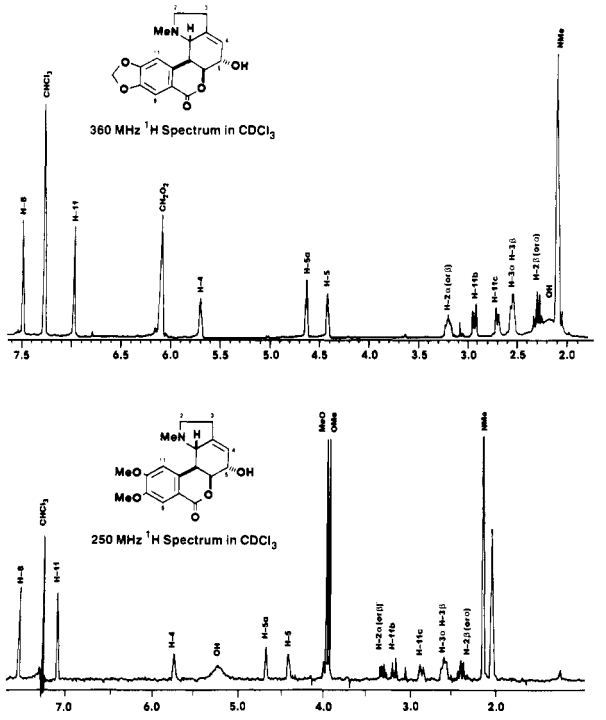
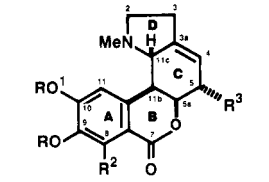


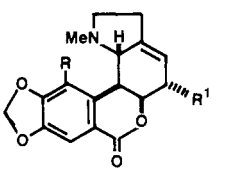
Figure 1. Comparison the ^1H NMR spectra of (a) hippeastrine and (b) 5α-hydroxyhomolycorine.

fragment from the other part of the molecule which encompasses the aromatic lactone moiety (*m/e* 206). Both the IR (3600 cm^{-1}, OH and 1708 cm^{-1}, CO) and the UV (λ_{max} 225, 269, and 303 nm) spectra of **1** provide supporting evidence for the presence of an aryl conjugated lactone and alcoholic hydroxyl functions. The presence of an alcoholic hydroxyl group was confirmed by the formation of an O-acetyl derivative. At present two stereochemically different modifications of the benzpyrano[3,4*g*]indole skeleton are exhibited by the alkaloids of this series. The majority belong to a single enantiomeric series containing a cis B:C ring junction. A much smaller group is represented by the alkaloid clivonine (**2**) and related bases which possess a trans B:C ring fusion.[5] Previous studies[6] have established the value of ^1H NMR spectroscopy in providing the stereochemical details of the lactone alkaloids of the series and the nature of the B:C ring fusion in particular is readily ascertained by this technique. The ^1H NMR spectrum of 5α-hydroxyhomolycorine resembles closely the spectrum of its congener, homolycorine (**4**). The major difference in the spectra of these two compounds is the presence of a one proton multiplet at δ 4.40 in the spectrum of **1** which is attributable to the H-5 signal. The stereochemistry of **1** was readily ascertained on the basis of chemical shifts and peak profiles of H-4, H-5, H-5a, H-11b, and H-11c with analogous signals in the spectrum of hippeastrine (**3**), all of which showed a remarkable similarity (see Figure 1). Additional evidence in support of the proposed stereochemistry was provided by the coupling constants of H-4, H-5, H-5a, H-11b, and H-11c (see Experimental Section) which were obtained from ^1H spin decoupling experiments.

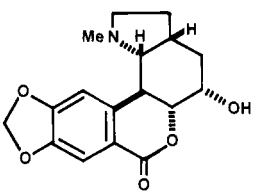
The absolute configuration of 5α-hydroxyhomolycorine was established by its CD spectrum. Aside from one study[7] on the lycorine alkaloids relatively little use[8] has been made of CD spectral results in the amaryllidaceae alkaloid field for structural purposes. We have found, as discussed later, that this technique can be used to differentiate the various stereochemical modifications extant in the lactone alkaloids of the benzopyrano[3,4-*g*]indole ring system. In this particular instance it was sufficient to compare the CD spectra of homolycorine and **1**. The close relationship of the CD spectra of **1** and **4** (see Table IV) indicate that 5α-hydroxyhomolycorine has the absolute stereochemistry indicated in structure **1**.



1. $R = R^1 = Me$, $R^2 = H$, $R^3 = OH$
4. $R = R^1 = Me$, $R^2 = R^3 = H$
7. $R = R^2 = R^3 = H$, $R^1 = Me$
9. $R = Me$, $R^1 = R^2 = R^3 = H$
11. $R = R^1 = Me$, $R^2 = OMe$, $R^3 = H$



3. $R = H$, $R^1 = OH$
10. $R = OMe$, $R^1 = H$
13. $R = OMe$, $R^1 = OH$
14. $R = R^1 = H$
16. $R = OMe$, $R^1 = OAc$



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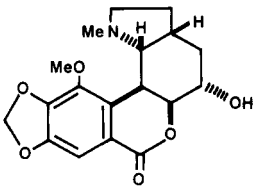

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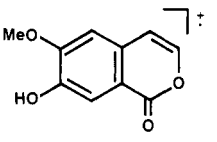
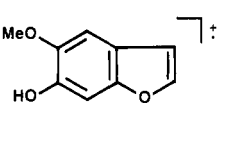
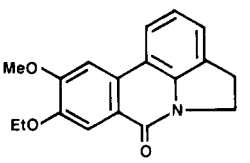


5 m/z 192



6 m/z 164



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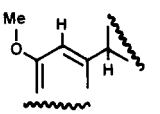

12

9-*O*-Demethylhomolycorine. The fractions eluted immediately following those containing 5α-hydroxyhomolycorine contained a crystalline base, mp 138–140 °C, which was readily identified as a member of the lactone series from its spectral properties. The mass spectrum of the compound exhibited a molecular ion at *m/z* 301 corresponding to $C_{17}H_{19}NO_4$ and exhibited the typical fragmentation pathway characteristic[4] of the benzopyrano-[3,4-*g*]indole series involving the retro-Diels–Alder fragmentation of ring C giving rise to the aromatic fragment ion at *m/z* 192 and its daughter ion at *m/z* 164 whose structures are represented by **5** and **6**, respectively. With the ubiquitous occurrence of a 9,10-aromatic oxygenation pattern in the amaryllidaceae lactone alkaloids, the mass spectral data suggested that the alkaloid was either 9-*O*-demethyl- or 10-*O*-demethylhomolycorine. The ^1H NMR

(5) Jeffs, P. W.; Hansen, J. F.; Dopke, W.; Bienert, M. *Tetrahedron*, 1971, *27*, 5065.
(6) Hawksworth, W. A.; Jeffs, P. W.; Tidd, B. K.; Toube, T. P. *J. Chem. Soc.* **1965**, 1991.
(7) Kuriyama, K.; Iwata, T.; Moriyama, M.; Kotera, K.; Hamuda, Y.; Mitsui, R.; Takeda, K. *J. Chem. Soc. B* **1967**, 46.
(8) An elegant study of the pH dependent equilibrium between pretazettine and methohaemanthidine was readily followed by CD and clearly indicated the utility of the technique in characterizing the two different ring systems involved.[9]
(9) Wildman, W. C.; Bailey, D. T. *J. Org. Chem.* **1968**, *33*, 3749.

spectrum of the alkaloid provided strong support for this contention in that not only was the occurrence of one-proton singlets at δ 7.59 and 7.08 consistent with the 9,10-oxygenation pattern, but the remaining features of the spectrum overlapped directly with the corresponding signals of the homolycorine spectrum. The only significant variation was the expected intensity difference of the *O*-methyl signal at δ 3.97. The CD spectra of the phenolic alkaloid and homolycorine were virtually superimposable indicating that the new base and homolycorine belong to the same enantiomeric series. Confirmation of the structure of *C. defixum* alkaloid as 9- or 10-*O*-demethylhomolycorine was obtained by its conversion to homolycorine on methylation with diazomethane.

A compound, mp 213–214 °C, first isolated from *Lycoris radiata*[10] was reported to be 9-O-demethylhomolycorine (**7**). The relative position of the 9,10-oxygen functions in this compound appeared to have been firmly established by its degradation to the phenanthridone **8**. Since the *O*-demethylhomolycorine isolated in this current study when recrystallized from several different solvents consistently gave crystals, mp 138–140 °C, we concluded that it is not identical with the compound previously described as 9-*O*-demethylhomolycorine and presumed that it must, therefore, be the isomeric 10-*O*-demethylhomolycorine (**9**). Confirmation of this structure was sought by further NMR studies.

Differentiation of the H-8 and H-11 signals is readily made in the homolycorine series by virtue of the deshielding of H-8 by the lactone carbonyl group.[6] For example, the effect of the *peri*-carbonyl group of the lactone in the cis B:C series on the H-8 proton signal forms the basis of assigning the 9,10,11-trioxyaryl oxygenation pattern in oxokrigenamine (**10**) and the 8,9,10-oxygen substitution of the aromatic ring in albomaculine (**11**).[11] Therefore, irrespective of whether the lactone possesses structure **7** or **9**, the H-8 and H-11 signals in the compound are confidently assigned at δ 7.59 and 7.08, respectively.

Two ^{1}H NMR experiments indicated that the compound which we have isolated is 9-O-demethylhomolycorine (**7**). Firstly, irradiation at the methoxy resonance results in a 10% NOE of the *upfield* aromatic proton at δ 7.08 in accord with the proximity of H-11 and a C-10 methoxy as reflected in structure **7**. Secondly, when the alkaloid was heated in CD_3OD–D_2O (1:1), the ^{1}H NMR spectrum of the resulting deuterated compound shows the absence of the *downfield* aromatic singlet at δ 7.59 again in consonance with the result expected for structure **7**.

Although the ^{1}H NOE experiment in conjunction with spectral changes associated with deuterium exchange appear to provide strong evidence for structure **7** for the alkaloid, if the proton chemical shift assignments for H-8 and H-11 were reversed, the results would then lead to structure **9** for the alkaloid isolated in this study. An independent experiment, which does not depend on interpeting the relative chemical shifts of the H-8 and H-11 proton signals is therefore desirable. Although this is not so important in the lactones of this family containing a cis B:C ring junction where the H-8 and H-11 proton signals are clearly distinct and assignable, these proton resonances are not so readily assigned in the trans B:C lactones, cf. clivonine, or in the nonlactone members of the benzopyrano[3,4-*a*]indole series. An alternative approach is to establish atom connectivities through utilizing both dipolar

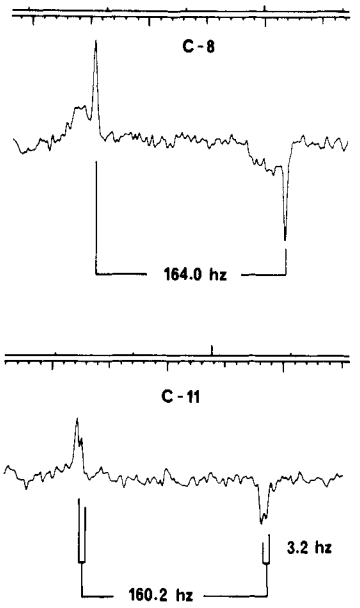

Figure 2. C-8 and C-11 proton-coupled ^{13}C signals of 9-*O*-demethylhomolycorine obtained by the Ernst–Doddrell INEPT sequence.

and scalar couplings in the region of interest. With the relationship between the methoxyl group and the aromatic proton at δ 7.08 established by the ^{1}H NOE experiment, it remains to ascertain the chemical shift of the carbon to which this proton is linked and then to define whether the signal from this carbon originates from C-8 or C-11. Complete assignments of ^{13}C chemical shifts of the carbons of the aromatic ring were made for the ^{13}C spectra of the 9-*O*-demethylhomolycorine and representative members of this family containing the 9,10-, 8,9,10- and 9,10,11-aromatic oxygenation patterns (see Table II) from chemical shift theory and model compounds.[13]

In several cases, possible ambiguities in assignments were resolved through examination of the ^{1}H-coupled spectra. A comparison of the ^{1}H-decoupled ^{13}C spectra of homolycorine and hippeastrine with the corresponding ^{1}H-coupled ^{13}C spectra for the two alkaloids shows that the proton bearing sp^2 carbons at C-4, C-8, and C-11 can be clearly assigned. In the coupled spectrum of 9-*O*-demethylhomolycorine C-4 appears as a doublet at 116.1 ppm with further fine splittings, C-8 occurs as a doublet at 115.9 ppm, and a doublet of doublets at 110.5 ppm is observed for C-11 (see Table III). The $^3J_{CH}$ coupling of the C-11 carbon resulting from the H-11b proton could also be discerned in the spectra of albomaculine (**11**), where the C-11 resonance appears as a doublet of doublets ($^1J_{CH}$ = 161.1, $^3J_{CH}$ = 5.6), whereas in oxokrigenamine (**10**) it appears as a simple doublet ($^3J_{CH}$ = 4.0).

In the above experiments the acquisition of the coupled and decoupled ^{13}C spectra was obtained by using either the Ernst-Doddrell INEPT procedure[15] where the sensitivity enhancement provided by this technique was invaluable for obtaining the coupled ^{13}C spectra on samples at the 1–2-mg level or by employing the appropriate gated pulse sequence. The two signals of interest resulting from C-8 and C-11 obtained by the INEPT procedure in the spectrum of *O*-demethylhomolycorine are shown in Figure 2.

(10) Uyeo, S.; Yanaihara, N. *J. Chem. Soc.* **1959**, 172.
(11) The 8,9,10-oxygenation pattern deduced by ^{1}H NMR was confirmed by subsequent chemical degradation experiments.[12]
(12) Jeffs, P. W.; Toube, T. P. *J. Org. Chem.* **1966**, *31*, 189.
(13) Published ^{13}C shift assignments for the hemiacetal base, lycorenine provided a useful model.[14]
(14) Shamma, M.; Hindenlang In "Carbon-13 NMR Shift Assignments of Amines and Alkaloids"; Plenum: New York, 1979; p 190.
(15) Doddrell, D. M.; Pegg, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 6388.

Table II. Compilation of ^{13}C Chemical Shifts of Lactone Alkaloids of the [2]Benzopyrano[3,4-*g*]indole Series[a]

compd	carbon																	
	2	3	3a	4	5	5a	7	7a	8	9	10	11	11a	11b	11c	N-CH_3	C9-OCH_3	C10-OCH_3	C11-OCH_3	CH_2O_2
homolycorine (**4**)	56.6	28.1	140.9[b]	115.2	31.3	77.7	165.9	116.9	111.9	148.9	153.1	110.8	137.8[b]	43.8	66.6	44.2	56.2[c]	56.4[c]		
hippeastrine (**3**)	55.9	27.6	138.9	119.4	66.7	82.1	164.6	118.5	109.9	148.1	152.0	108.8	143.3	38.6	67.3	43.2				102.2
masonine (**13**)	56.3	27.9	139.8[b]		31.1	77.3	165.1	118.6	109.6	147.7	151.6	108.4	140.8[b]	43.6	66.6	44.4				101.9
oxokrigenamine (**10**)	56.3	27.9	139.5	114.7	31.2	78.0	165.4	119.2	104.2	148.8	140.1	140.8	131.7	36.8	67.7	42.2			59.3	101.8
9-*O*-demethyl-homolycorine (**7**)	56.2	27.8	139.6[b]	116.1	31.2	77.2	165.4	117.6	115.9	145.7	150.8	110.5	136.2[b]	43.1	66.7	43.3		56.4		
albomaculine (**11**)	56.3	27.8	140.0	115.7	30.8	76.0	162.1	111.3	157.1	142.5	156.1	107.3	140.4	45.0	65.9	43.4	61.9[b]	56.3	61.1[c,d]	

[a] Chemical shifts reported as ppm downfield for Me_4Si (taken as 76.9 ppm from deuterochloroform (center)). [b,c] Tentative assignment only. These pairs may be interchanged. [d] Assignment for C8.

Table III. ^{13}C-^1H Coupling Constants for the C-8 and C-11 Carbon Signals in Representative Lactone Alkaloids

compd	$^1J_{C8-H8}$, Hz	$^1J_{C11-H11}$, Hz	$^3J_{C11-H11b}$, Hz
homolycorine (**4**)	161.5	160.0	3.1
hippeastrine (**3**)	169.2	166.1	4.6
oxokrigenamine (**10**)	170.7		4.0
albomaculine (**11**)		161.1	5.6
9-*O*-demethylhomolycorine (**7**)	164.0	160.2	3.7

The foregoing results clearly substantiate that the carbon chemical shift assignments in the *O*-demethylhomolycorine spectrum are as indicated in Table II. Therefore, all that remained was to identify the chemical shift of the proton attached to C-11. This was accomplished by selective irradiation of the ^1H signal at δ 7.08, which resulted in the collapse of the $^1J_{CH}$ = 160 Hz coupling in the carbon signal at 110.5 ppm and left the doublet at 115.9 ppm unperturbed. In a complementary experiment, selective irradiation of the downfield ^1H signal at δ 7.59 resulted in the collapse of the doublet at 115.9 ppm to a singlet in the ^{13}C spectrum.

A summary of these results, which are entirely independent of carbon or proton chemical shift assignments, establishes the connectivity pattern indicated in the partial structure **12**. This is clearly only consistent with structure **7** and confirms that the alkaloid is correctly represented as 9-*O*-demethylyhomolycorine.

There is a large discrepancy in the melting point of the latter compound reported in this current study with that previously reported for 9-*O*-demethylhomolycorine, mp 213–214 °C. The issue with regard to the previous report on 9-*O*-demethylhomolycorine has been further confused by a subsequent publication on the isolation of an *O*-demethylhomolycorine from *Galanthus caucasius*.[16] In the latter case, no attempt was made to define the relative positions of the phenolic hydroxy and aromatic methoxy group; however, the physical constants of the *Galanthus* alkaloid reportedly corresponded closely with the *O*-demethylhomolycorine isolated from *L. radiata* and have been taken to indicate the identity of the two samples. Unfortunately spectral or sample comparisons with the *O*-demethylhomolycorine reported in the current work with those isolated previously have not been possible.[17]

CD Spectra. A compilation of the CD spectra of lactone alkaloids of the homolycorane–masonane series is presented in Table IV. The alkaloids of this series containing 3a,4-unsaturation forms a stereochemically homogeneous group containing aryl oxygen substituents at the 9,10-positions and with a methoxy substituent, when present, occupying the 8- or 11-positoins, cf. oxokrigenamine (**10**)[5] and albomaculine (**11**). A further structural variation is found through the occurrence of alkaloids with a 5α-hydroxy group, e.g., hippeastrine[18] (**3**) and neronine (**13**)[5], while several additional further stereochemical variations are found in the 3a,4-dihydro series including alkaloids possessing a trans B:C ring fusion, cf. clivonine (**2**).[1]

The CD spectra show a consistent pattern in that all eight lactone bases containing 3a,4-unsaturation, which appear in Table IV, exhibit Cotton effects with negative maxima at 232–238 nm and 272–276 nm and positive maxima at 250–258 nm of approximately the same am-

(16) Tsakadze, D. M.; Kiparenko, T. N.; Tsitsishvili, N. S.; Abdusamatov, A.; Yunusov, S. Yu. *Soobshch. Akad. Nauk Gruz. SSR* **1969**, *56*, 305.

(17) Prof. Uyeo informed us that his sample of *O*-demethylhomolycorine and its spectra were destroyed in 1962 in a fire in the laboratory.

(18) Kotera, K.; Hamada, Y.; Nakane, R. *Tetrahedron* **1968**, *24*, 759.

Table IV. Circular Dichroism Spectral Data on Lactone Bases of the [2]Benzopyrano[3,4-*g*]indole Series

alkaloid	CD maxima, nm [θ]			
homolycorine (4)	232	250	272.5	307
	−27 100	+17 320	−16 740	−2 890
5α-hydroxyhomolycorine (1)	232	251	273	302
	−30 980	+14 420	−18 160	−4 270
9-*O*-demethylhomolycorine (7)	232.5	250	272	314
	−31 000	+10 750	−11 170	−1 370
albomaculine (11)	233	253	276	291sh
	−17 070	+16 830	−9 480	−5 450
masonine (14)	232	258	273	304
	−41 640	+8 990	−15 990	+3 310
hippeastrine (3)	233	254	275.5	307
	−41 440	+6 880	−14 630	+1 220
oxokrigenamine (10)	234	258	280	314
	−37 910	+4 920	−4 528	−1 790
O-acetylneronine (16)	235	261	283	314
	−30 980	+1 550	−5 880	−2 010
clivonine (2)	231	248	271	300
	+8 980	−5 130	+9 050	−280
5α-hydroxymasan-7-one (15)	232	268		304
	+1 814	−1 650		−1 220

plitudes. Such variations that occur appear in the region 295–314 nm and may be correlated with the type of aromatic substitution; homolycorine (**4**) and the two related homolycoranes **1** and **7** show a negative maxima in the range 302–307 nm; whereas, the symmetry related (methylenedioxy)aryl lactones, masonine (**14**) and hippeastrine (**3**), surprisingly exhibit a positive band at 304 nm. The CD spectra of albomaculine (**11**), which has a 8,9,10-trimethoxyaryl chromophore, and the 11-methoxy-9,10-(methylenedioxy)aryl lactones **10** and **16** are virtually identical with the CD of homolycorine.

The CD maxima of the four bands within each spectrum are of the same order of magnitude and are, therefore, all reasonably associated with the transitions of the aryl-conjugated lactone chromophore. The absence of electron-transfer bands involving the nitrogen was ascertained from the observation that the CD spectra of the lactone alkaloids and their hydrochlorides were identical.

The diagnostic utility of the CD spectral properties for deriving structural information is clearly revealed by comparing the last two entries in Table IV with the other entries. The 5a-epimeric 3a,4-dihydro bases **2** and **15** are not only quite different from each other in their CD properties, but are also clearly distinguishable from the spectra of the 3a,4-unsaturated lactone alkaloids.

When these empirical correlations are used, the CD spectra of lactone bases of this series are capable of providing important stereochemical information. It is obvious that a more extensive compilation of CD spectra of the lactones in this series is desirable before sector rules can be defined.

Experimental Section

Fourier transform ^{13}C NMR spectra were obtained on a JEOL FX-90Q instrument at 22.6 MHz. The instrument was equipped with a cross polarization modification kit to enable the use of INEPT pulse sequences. A typical experiment utilized a 6300-Hz (280 ppm) spectral width, 9-μs pulse width, and 6-s pulse delay. Coupled spectra were obtained with a gated decoupling irradiation mode, which turned the decoupler off during data acquisition retaining the benefits of Overhauser enhancements. Decoupled spectra of **2**, **3**, **4**, and **10** were obtained on this instrument, as were coupled spectra of **3** and **4**. INEPT spectra of homolycorine were also run on this instrument with a spectral width of 6300 Hz, 90° pulse width of 17-μs, and a $^1/_4J$ value of 1.8 ms. The samples were dissolved in deuterochloroform (0.1 M solutions) with the deuterium used for an internal heteronuclear lock and tetramethylsilane as an internal standard.

Coupled spectra of **4** and **10** were obtained on a Bruker WM-250 instrument at 62.9 MHz. A 15151-Hz (240 ppm) spectral width, 6-μs pulse width, and 10-s delay were used. Coupled spectra of **7** (INEPT) and **11** (gated coupled) were run aon a JEOL GX 270 at 67.8 MHz. A spectral width of 14005 Hz (200 ppm), a 9-μs pulse width, and a 3.0-s pulse delay were used.

Extraction of the Alkaloids from *Crinum defixum*. Dried powdered bulbs (2.3 kg) were extracted by cold percolation with ethanol. The combined extracts were concentrated under reduced pressure and acidified with 5% tartaric acid (pH 2). The aqueous acidic solution was extracted successively with $CHCl_3$ (10 × 500 mg) and $CHCl_3$–MeOH (3:1) (3 × 100 mL). The $CHCl_3$ extracts were combined, washed with saturated NaCl solution, and dried over anhydrous Na_2SO_4. The solution was concentrated to a small volume and at this stage lycorine precipitated (3.5 g). The precipitate was filtered off and its identity was confirmed by the formation of a picrate,[19] mp 195–197 °C, and *O,O*-diacetate,[20] mp 220–222 °C. The filtrate of the crude alkaloid fraction was freed from the solvent by distillation leaving a residue of crude alkaloid weighing 5.1 g (0.374%).

Separation of the Alkaloids by Column Chromatography. The crude alkaloid fraction (4.2 g) after filtration of lycorine was dissolved in $CHCl_3$ and evaporated over alumina placed on the topof a wet packed column of neutral alumina (Activity 2, 120 g) in benzene and eluted with the two linear gradients A and B followed by the solvent mixture C.

A: benzene (2 L)–EtOac (2 L). B: EtOAc (2 L)—EtOAc–MeOH (9:1), v/v, 2 L). C: EtOAc–MeOH (8:2, v/v, 1 L). A total of 60 fractions (150 mL) were collected and combined as indicated below on the basis of the results of their analysis by TLC on silica gel in $CHCl_3$–EtOAc–MeOH (2:2:1). Fraction 1–7, nonalkaloidal material (0.4 g); 8–14, homolycorine (0.3 g); 15–33, oil, mostly nonalkaoidal (1.15 g); 34–35, 5α-hydroxyhomolycorine (0.3 g); 36, mixture of 5α-hydroxyhomolycorine and 9-*O*-demethylhomolycorine (0.2 g); 37, 9-*O*-demethylhomolycorine (0.2 g); 38–39, 9-*O*-demethylhomolycorine and unidentified alkaloid mixture (0.3); 40–60, unidentified alkaloids (1.1 g).

Homolycorine. Fractions 8–14 afforded homolycorine (35 mg), mp 173–175 °C, from benzene. Its identification was based upon spectral comparisons (IR, CD, NMR, MS and UV), TLC, and a mixed melting point with an authentic sample.

5α-Hydroxyhomolycorine (1). Fractions 34–35 crystallized from Me_2CO–MeOH to give 1 (30 mg): mp 168–170 °C; IR ($CHCl_3$) 3600, 3400 (OH), 1708 cm^{-1} (lactone CO); UV max (EtOH) 228 (22 100), 265 (8030), 302 nm (4070); CD (EtOH) $[\theta]_{233}$ −30 980; $[\theta]_{242}$ 0; $[\theta]_{251}$ +14 420; $[\theta]_{273}$ −14 420, $[\theta]_{302}$ −4270 $[\theta]_{326}$ 0; ^1H NMR ($CDCl_3$–CD_3OD) δ 2.09 (s, 3 H, NMe), 3.02 (m, 1 H, H-11c), 3.32 (m, 1 H-11b), 3.92 (s, 3 H, OMe), 3.96 (s, 3 H, OMe), 4.40 (bs, 1 H H-5), 4.63 (bs, 1 H H-5a), 5.72 (bs, OH, H-4), 7.07 (s, 1 H, H-11), 7.50 (s, 1 H, H-8); the following coupling constants were obtained from ^1H spin decoupling studies $J(4,11c)$ = 1.5 Hz, $J(4,5)$ = 1.2, $J(5,5a)$ = 2.0, $J(5a,11b)$ = 2.0, $J(11b,11c)$ = 10.0; MS, m/z 331 (M^+), 313 (M − H_2O), 206 (M − 125), 125 ($C_7H_{11}NO$), 109 ($C_7H_{11}N$), 96 (C_6H_{10}); calcd for $C_{18}H_{21}NO_5$ 331.1419, found M^+, m/z 331.1423. Acetylation of 5α-hydroxyhomolycorine (5 mg) in pyridine (0.5 mL)–acetic anhydride (0.2 mL) at 25 °C for 24 h gave on workup an oily *O*-acetate (IR 1735 (OAc), 1710 cm^{-1} (lactone CO)), which could not be induced to crystallize.

9-*O*-Demethylhomolycorine (7). Fraction 37 gave crystals from acetone–methanol (20:1): mp 138–140 °C (70 mg); IR ($CHCl_3$) 3540 (OH), 1715 cm^{-1} (conjugated δ-lactone); UV (EtOH) 238 (12 300), 267 (5130), 308 nm (3090); CD (MeOH) $[\theta]_{215}$ 0, $[\theta]_{232}$ −31 000, $[\theta]_{241}$ 0, $[\theta]_{250}$ +10 750 $[\theta]_{259}$ 0, $[\theta]_{272}$ −11 170, $[\theta]_{296}$ 0, $[\theta]_{314}$ −1370; ^1H NMR 206 (s, 3 H, NMe), 3.26 (m, 1 H, H-11c), 3.97 (s, 3 H, OMe), 4.77 (bs, 1 H, H-5a), 5.53 (bs, 1 H, H-4), 7.08 (s, 1 H, H-11), 7.59 (s, 1 H, H-8); MS, m/z 301 (M^+), 285 (M − 1 − Me), 192 (M − 109, ion **5**), 164 (ion **6**), 109 ($C_7H_{11}N$), 94 (C_6H_8N), 82 (C_5H_8N); calcd for $C_{17}H_{19}NO_4$ 301.1314, found M^+, m/z 301.1311. The singlet at δ 7.59 was absent in the NMR spectrum of a sample

(19) Cook, J. W.; Loudon, J. D.; McClosky, P. *J. Chem. Soc.* **1954**, 4176.

(20) Hunger, A.; Reichstein, T. *Helv. Chim. Acta* **1953**, *36*, 824.

of 9-O-demethylhomolycorine (5 mg) which had been heated at 60 °C for 17 h in D_2O–CD_3OD (1:1) (0.5 mL).

Methylation of 9-O-Demethylhomolycorine. To a solution O-demethylhomolycorine (2 mg) in CH_3OH–Et_2O (1:1) (1 mL) was added 3 separate aliquots of 2 mL of ethereal CH_2N_2 solution over a period of 3 days. Removal of the exces CH_2N_2 and solvent left a solid residue (2 mg) which on crystallization from benzene gave homolycorine, mp 170–172 °C. The TLC and mass spectrum of this sample was identical in every respect with TLC and mass spectrum of authentic homolycorine.

Extraction of the Alkaloids from *C. scabrum*. The dry powdered plant (1.5 kg) was processed as described above for the extraction of *C. defixum*. After removal of the lycorine (0.52 g, 0.035%) by filtration, a crude alkaloid fraction (6.4 g) remained.

Chromatography of Crude Alkaloid Fraction from *C. scabrum*. The crude alkaloid extract (6.4 g) was preabsorbed on Al_2O_3 (neutral, grade II) in benzene. Fractions (150 mL) were collected by using the following linear solvent gradients. A: benzene–5% EtAc(1.5 L)–EtAc (1.5 L). B: EtOAc (1.5 L)–MeOH (8:2, v/v, 1.5 L). C: EtOAc–MeOH (1:1, 1 L). A total of 43 fractions were collected and combined as follows based upon analysis by TLC on silica gel with the solvent system ($CHCl_3$–EtOAc–MeOH (2:2:6). Fractions 1–12, unidentified alkaloid (s) (1.3 g); 13–24, crinamine (2.0 g); 25–26, mixture of lycorine and 6-hydroxycrinamine (1.1 g); 27–30, 6-hydroxycrinamine (0.3 g); 31–43 mixture of four unidentified alkaloids (1.4 g).

The alkaloids crinamine and 6-hydroxycrinamine were identified by IR, NMR, and MS spectral comparisons with the spectra of authentic samples and by the preparation of the following crystalline derivatives O-acetylcrinamine, mp 160–161 °C, 6-hydroxycrinamine methiodide, mp 170–172 °C, crinamine picrate, mp 271–271 °C, and 6-hydroxycrinamine methopicrate, mp 146 °C.

Extraction of the Alkaloids *C. latifolium*. Utilizing the same procedures as described for *C. defixium* and *C. scabrum*, the leaves (12.3 kg) and bulbs (700 g) of *C. latifolium* were extracted separately. The leaves afforded lycorine (1.8 g) and a crude alkaloid fraction (16.8 g) while the bulbs similarly gave lycorine (0.28 g) and a crude alkaloid residue (2.6 g). A TLC examination of the two crude alkaloid fractions indicated they contained the same alkaloids.

Chromatographic Separation of the Alkaloids of *C. latifolium*. A portion of the crude alkaloid fraction (10 g) was preabsorbed on Al_2O_3 (50 g) and placed on the top of a column containing Al_2O_3 (1 kg neutral, Activity II). The solvents used for linear gradient elution were the same as used for the chromatography of the *C. scabrum* alkaloids. Individual fractions (150 mL) were collected and combined as indicated below on the basis of their analysis by TLC on silica gel in $CHCl_3$–EtOAc–MeOH (2:2:6). Fraction 1–19, nonalkaloid material (0.9 g); 20–49, noncrystalline mixture of two bases (0.6 g); 50–59, crude hippeastrine and three other uncharacterized alkaloids (1.15 g). This fraction on standing gave crystalline material which on crystallization gave hippeastrine (50 mg), mp 215–217 °C, identified by suitable spectral comparisons with an authentic sample. Fraction 60–69, mixture of alkaloids (0.5 g), which onstanding deposited lycorine (12 mg); 70–75, unidentified alkaloids (0.3 g); 76–104, noncrystalline mixture of alkaloids (1.6 g).

Acknowledgment. We are indebted to the Egyptian Government for a fellowship to A.A. for a leave of absence from the Department of Pharmocognosy, Alexandria University, and to the National Institutes of Health for a predoctoral traineeship to D.C. We are most grateful to Dr. C. A. Evans, JEOL, for the ^{13}C-INEPT and ^1H NOE experiments on 9-O-demethylhomolycorine.

Registry No. 1, 13255-05-5; 3, 477-17-8; 4, 477-20-3; 7, 6879-81-8; lycorine, 476-28-8; crinamine, 639-41-8; 6-hydroxycrinamine, 545-66-4.

Chemistry of Acronycine X. Oligomers of Noracronycine

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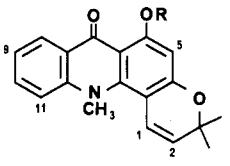
Received July 25, 1984

Treatment of noracronycine (3) with methanolic hydrochloric acid as well as yielding the dimers AB-1 (6) and AB-2 (7) and the trimer AB-3 (8) also afforded AB-5. Plasma desorption mass spectrometry indicated this product to be a mixture of tetrameric and pentameric species which were separated and characterized. The pentamer AB-5A was deduced through spectroscopic interpretation to be the all-linear isomer 10 and the tetramer, AB-5B, was shown to have a partially rearranged linear–angular–angular–angular structure (11). The dihydro derivative of AB-5B was synthesized through the union of dihydro AB-1 (12) and AB-2 (7).

Acronycine (1), an alkaloid isolated from the bark of



	R
1	CH_3
3	H

Acronychia baueri Schott (Rutaceae),[1-4] possesses the broadest spectrum of in vivo antineoplastic activity of any alkaloid thus far tested.[5,6] In spite of this, very little is known of its chemistry or mode of action.[3,4]

Acronycine (1) is an acridone alkaloid with an additional hemiterpene unit attached at C-4 at the parent nucleus and cyclized to form a pyran ring. Initially there was a question as to whether acronycine had a linear or angular structure, and the presently accepted structure was de-

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(1) Hughes, G. K.; Lahey, F. N.; Price, J. R.; Webb, L. J. *Nature (London)* **1948**, *162*, 223.
(2) Lahey, F. N.; Thomas, W. C. *Aust. J. Sci. Res.* **1949**, *2A*, 423.
(3) Brown, R. D.; Drummond, L. J.; Lahey, F. N.; Thomas, W. C. *Aust. J. Sci. Res.* **1949**, *2A*, 622.
(4) Drummond, L. J.; Lahey, F. N. *Aust. J. Sci. Res.* **1949**, *2A*, 630.
(5) Svoboda, G. H.; Poore, G. A.; Simpson, P. J.; Boder, G. B. *J. Pharm. Sci.* **1966**, *55*, 758.
(6) Svoboda, G. H. *Lloydia* **1966**, *29*, 206.

0022-3263/85/1950-1737$01.50/0