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Alkaloids from *Crinum macowanii*☆

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Abstract

Eleven alkaloids have been isolated from fresh bulbs of *Crinum macowanii* (Amaryllidaceae). Macowine is reported here for the first time. The structure and stereochemistry of this new alkaloid as well as of the known ones were determined by physical and spectroscopic methods.

Keywords: *Crinum macowanii*; Amaryllidaceae; Bulbs; Alkaloids; Macowine; Lycorine; Cherylline; Crinine; Krepowine; Powelline; Buphanidrine; Crinamidine; Undulatine; 1-Epideacetylbowdensine; 4a-Dehydroxycrinamabine

1. Introduction

About 20 species of the genus *Crinum* (Amaryllidaceae) are endemic to southern Africa (Snijman and Linder, 1996; Fangan and Nordal, 1993). *Crinum macowanii* Baker (also known as 'bush' or 'march lily') is widespread through the tropical and temperate regions of sub-Saharan Africa, mainly in the moist sites of the eastern and southern Africa. It is cultivated for its elegant flowers and used in interspecific breeding programmes amongst the Amaryllidaceae. Since vegetative reproduction under field conditions is slow, together with its exploitation in the traditional tribal practices, the continued existence of this species is being threatened. This has necessitated the application of tissue culture techniques (Slabbert et al., 1993, 1995) to meet the demands of the commercial sector. Extracts of *Crinum macowanii*, also referred to respectively as 'dururu' or 'umduze' in the Shona and Ndebele vernacular, have a number of applications in the traditional medicinal practices in Zimbabwe and South Africa, including the treatment of sexually-transmitted diseases and backache as well as to increase lactation in animal and human mothers (Duri et al., 1994; Gelfand et al., 1985). Pharmacologically, extracts of *Crinum macowanii* exhibited both antiviral (against a variety of exotic RNA viruses) and antifungal (in vitro against *Candida albicans*) activities (Duri et al., 1994; Gundidza, 1986). These encouraging observations led us to select this species as a viable candidate for phytochemical analysis, which forms part of our ongoing research of the amaryllidaceous plants endemic to southern Africa (Viladomat et al., 1997). The present investigation deals with the isolation and characterization of eleven alkaloids from fresh bulbs of *Crinum macowanii*, including the three principal constituents: lycorine, a common alkaloid in this genus with several biological properties (Gabrielsen et al., 1992; Bastida et al., 1998), crinine and powelline. A previous phytochemical investigation utilizing radioimmunoassay techniques indicated that no galanthamine is present in

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this species (Tanahashi et al., 1990). Interestingly, macowine (**1**), which is reported here for the first time, as well as the other known crinane-type alkaloids isolated during this investigation, possess a β-5,10b-ethano bridge. The structure of alkaloid **1** can be presumed to be the precursor of crinine in the biosynthetic pathway involving the conversion of the *O*-methoxyphenol to the methylenedioxy group, as was demonstrated in the conversion of norpluviine to caranine (Battersby et al., 1964).

2. Results and discussion

The absolute configuration of the 5,10b-ethano bridge in the crinane-type alkaloids was determined by measurement of their CD curves, which were all qualitatively similar to that of a β-5,10b-ethanophenanthridine alkaloid with a maximum around 250 nm (Ali et al., 1984; Wagner et al., 1996).

Macowine (**1**) had in its EIMS the molecular ion peak at *m/z* 273 correct for the formula $C_{16}H_{19}NO_3$, and the fragmentation pattern was consistent with compounds of the crinine series lacking a bridge substituent, and having a double bond in the 1, 2-position (Longevialle et al., 1973). The ^{1}H- and ^{13}C-NMR data (Table 1) were very similar to that of crinine (Viladomat et al., 1995), and only the differences ascribable to the substitution of the aromatic ring were significant. Thus, whereas crinine had the proton and carbon signals for the methylenedioxy group at δ 5.89–5.91 (2*d*) and δ 100.8 (*t*), respectively, these were replaced by the aromatic methoxyl group proton and carbon resonances (δ 3.86 (*s*) and δ 56.1 (*q*), respectively) in compound **1**. A three-bond HMBC correlation of the methoxyl group protons to C-9 justified this placement, which was supported by the ROESY contour between H-10 and the methoxyl protons (see Table 2). Three-bond HMBC correlations to either C-6a, C-8 and C-10b or C-6, C-9 and C-10a distinguished H-10 from H-7, which was supported by ROESY correlations of H-10 with H-1, and H-7 with 2H-6. The 3-hydroxyl group was assigned the pseudoaxial configuration due to the small coupling of H-3 with both H-4α (J = 1.5 Hz) and H-4β (J = 4.0 Hz), as well as the large *trans* diaxial coupling between H-4β and H-4a (J = 13.5 Hz). Further, ROESY contours linking H-4β with H-11*exo* and H-12*exo*; H-6α with H-4a, and H-6β with H-12*endo*, differentiated the methylene protons at C-4, C-6, C-11 and C-12. In addition, H-6α and H-12*exo* were shifted further downfield due to the nitrogen lone pair. The ^{13}C-NMR spectrum indicated a 16-carbon skeleton for compound **1**, the signals of which were assignable considering both HMQC (Bax and Subramanian, 1986) and HMBC (Bax and Summers, 1986) data. A slight shielding of both C-8 and C-9, relative to the corresponding signals in crinine (Viladomat et al., 1995), was attributable to the hydroxyl and methoxyl groups.

4a-Dehydroxycrinamabine ($C_{16}H_{19}NO_4$) (**2**) was the

Table 1
^{1}H-NMR, HMQC and HMBC data for macowine (**1**)[a]

Proton	$\delta_H$ppm (J in Hz)	Correlated C-atom	
		HMQC	HMBC
1	6.61 *d* (10.0)	132.1 *d*	C-3, C-4a, C-10a
2	5.96 *ddd* (10.0, 5.0, <1)	127.5 *d*	C-4, C-10b
3	4.33 *ddd* (5.0, 4.0, 1.5)	64.0 *d*	
4α	2.01 *dddd* (13.5, 4.0, 1.5, <1)	32.5 *t*	C-2
4β	1.72 *ddd* (13.5, 13.5, 4.0)	32.5 *t*	C-4a
4a	3.41 *dd* (13.5, 4.0)	62.9 *d*	C-12
6α	4.37 *d* (16.5)	61.6 *t*	C-6a, C-10a, C-12
6β	3.76 *d* (16.5)	61.6 *t*	C-4a, C-6a, C-7, C-10a, C-12
		125.4 *s* (C-6a)	
7	6.54 *s*	113.0 *d*	C-6, C-8, C-9, C-10a
		144.2 *s* (C-8)	
		145.1 *s* (C-9)	
10	6.79 *s*	104.9 *d*	C-1, C-6a, C-8, C-9, C-10a, C-10b
		136.3 *s* (C-10a)	
		44.0 *s* (C-10b)	
11*endo*	2.17 *ddd* (12.5, 9.0, 4.5)	44.0 *t*	
11*exo*	1.92 *ddd* (12.5, 10.5, 6.0)	44.0 *t*	C-10a
12*endo*	2.89 *ddd* (13.0, 9.0, 6.0)	53.4 *t*	C-6
12*exo*	3.37 *ddd* (13.0, 10.5, 4.5)	53.4 *t*	
9-OMe	3.86 *s*	56.1 *q*	C-9

[a] Chemical shifts in ppm rel. to TMS. Coupling constants (J) in Hz. C-multiplicities were determined by DEPT data.

Table 2
COSY and ROESY data for macowine (**1**)

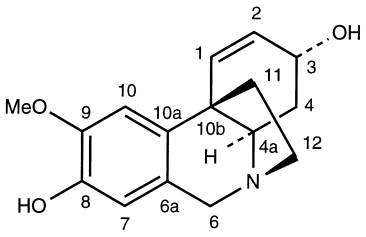
Proton	COSY	ROESY
H-1	H-2	H-2, H-10
H-2	H-1, H-3, H-4α	H-1, H-3
H-3	H-2, H-4α, H-4β	H-2, H-4α, H-4β
H-4α	H-2, H-3, H-4β, H-4a	H-3, H-4β, H-4a
H-4β	H-3, H-4α, H-4a	H-3, H-4α, H-11*exo*, H-12*exo*
H-4a	H-4α, H-4β	H-4α, H-6α
H-6α	H-6β	H-4a, H-6β, H-7
H-6β	H-6α	H-6α, H-7, H-12*endo*
H-7	–	H-6α, H-6β
H-10	–	H-1, 9-OMe
H-11*endo*	H-11*exo*, H-12*endo*, H-12*exo*	H-11*exo*, H-12*endo*, H-12*exo*
H-11*exo*	H-11*endo*, H-12*endo*, H-12*exo*	H-4β, H-11*endo*, H-12*endo*, H-12*exo*
H-12*endo*	H-11*endo*, H-11*exo*, H-12*exo*	H-6β, H-11*endo*, H-11*exo*, H-12*exo*
H-12*exo*	H-11*endo*, H-11*exo*, H-12*endo*	H-4β, H-11*endo*, H-11*exo*, H-12*endo*
9-OMe	–	H-10

subject of a recent article (Pham et al., 1998). Both the physical and spectral data of the compound we isolated were in close agreement with those that have been reported. However, we draw attention to certain peculiarities in the ^{1}H-NMR spectrum which we obtained in $CDCl_3$. In addition, the 2D COSY, ROESY and HMBC data (Tables 3 and 4) are afforded here. The chemical shifts reported (Pham et al., 1998), from the spectrum run in CD_3OD, for H-4a, (δ 3.37, *dd*), H-6α (δ 4.53, *d*), H-6β (δ 4.05, *d*), H-12*endo* (δ 3.10, *ddd*) and H-12*exo* (δ 3.62, *ddd*) are all deshielded ($\Delta\delta = +0.39, +0.16, +0.30, +0.30, +0.19$, respectively) relative to the corresponding signals we obtained for the compound. These differences cannot be explained in terms of a solvent effect since they persisted when we ran the spectrum in CD_3OD. Moreover, the data we obtained were very similar to that of 1-epideacetylbowdensine, isolated from this plant species and previously reported (Viladomat et al., 1996). Noticeably, these differences are more apparent for H-4a, 2H-6 and 2H-12 which are all vicinal to the nitrogen atom.

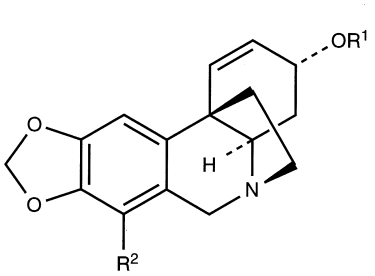
Table 3
^{1}H-NMR, HMQC and HMBC data for 4a-dehydroxycrinamabine (**2**)[a]

Proton	$\delta_H$ppm (J = Hz)	Correlated C-atom	
		HMQC	HMBC
1	4.05 *d* (4.5)	73.1 *d*	C-10a, C-10b, C-11
2	4.15 *ddd* (4.5, 3.5, 2.5)	69.9 *d*	
3ax	1.53 *dddd* (14.0, 12.5, 3.5, 2.5)	29.0 *t*	
3eq	2.02 *dddd* (14.4, 3.5, 3.5, 3.0)	29.0 *t*	
4ax	1.75 *dddd* (14.0, 12.5, 11.5, 3.5)	20.2 *t*	
4eq	1.58 *dddd* (14.0, 5.0, 3.5, 3.0)	20.2 *t*	
4a	2.98 *dd* (11.5, 5.0)	68.1 *d*	C-12
6α	4.37 *d* (16.5)	61.7 *t*	C-4a, C-6a, C-10, C-10a, C-12
6β	3.75 *d* (16.5)	61.7 *t*	C-6a, C-10a, C-12
		124.8 *s* (C-6a)	
7	6.42 *s*	106.0 *d*	C-6, C-9, C-10a
		145.9 *s* (C-8)	
		146.4 *s* (C-9)	
10	7.47 *s*	105.8 *d*	C-6a, C-8, C-10b
		140.8 *s* (C-10a)	
		49.5 *s* (C-10b)	
11*endo*	1.97 *ddd* (12.0, 8.5, 4.0)	36.4 *t*	
11*exo*	2.75 *ddd* (12.0, 9.0, 6.0)	36.4 *t*	C-10a, C-12
12*endo*	2.80 *ddd* (12.5, 8.5, 6.0)	51.5 *t*	
12*exo*	3.43 *ddd* (12.5, 9.0, 4.0)	51.5 *t*	
OCH_2O	5.88 *s*	100.8 *t*	C-8, C-9

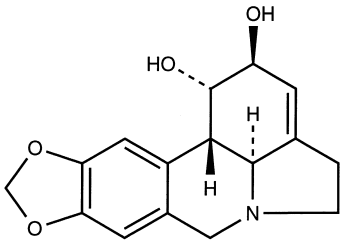
[a] Chemical shifts in ppm rel. to TMS. Coupling constants (*J*) in Hz. C-multiplicities were determined by DEPT data.



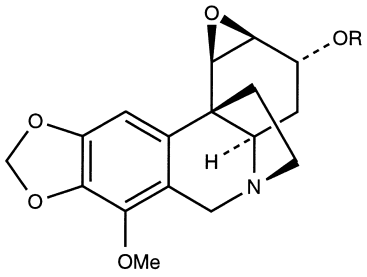
Macowine (**1**)



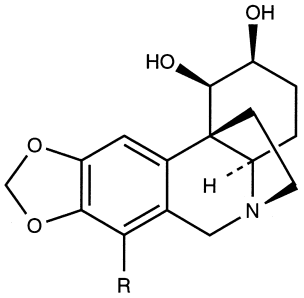
Crinine $R^1 = R^2 = H$
Krepowine $R^1 = Ac$, $R^2 = H$
Powelline $R^1 = H$, $R^2 = OMe$
Buphanidrine $R^1 = Me$, $R^2 = OMe$



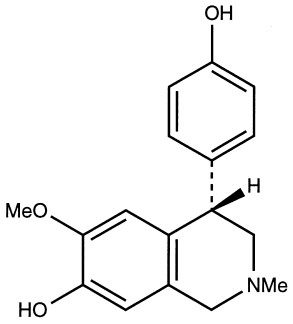
Lycorine



Crinamidine R = H
Undulatine R = Me



4a-Dehydroxycrinamabine (**2**) R = H
1-Epideacetylbowdensine R = OMe



Cherylline

Lycorine (Likhitwitayawuid et al., 1993; Spohn et al., 1994), cherylline (Kobayashi et al., 1984), crinine (Viladomat et al., 1995), krepowine (Ali et al., 1986; Campbell et al., 1998), powelline (Kobayashi et al., 1984; Frahm et al., 1985), buphanidrine (Viladomat et al., 1995), crinamidine (Viladomat et al., 1996), undulatine (Viladomat et al., 1995) and 1-epideacetylbowdensine (Viladomat et al., 1996) were identified by a comparison of their chromatographic and spectroscopic properties (TLC, IR, CD,

Table 4
COSY and ROESY data for macowine (**2**)

Proton	COSY	ROESY
H-1	H-2	H-2, H-3ax, H-4a, H-10
H-2	H-1, H-3ax, H-3eq, H-4eq	H-1, H-3ax, H-3eq
H-3ax	H-2, H-3eq, H-4ax, H-4eq	H-1, H-2, H-3eq, H-4eq, H-4a
H-3eq	H-2, H-3ax, H-4ax, H-4eq	H-2, H-3ax, H-4ax, H-4eq
H-4ax	H-3ax, H-3eq, H-4eq, H-4a	H-3eq, H-4eq, H-11exo, H-12*exo*
H-4eq	H-2, H-3ax, H-3eq, H-4ax, H-4a	H-3ax, H-3eq, H-4ax, H-4a
H-4a	H-4ax, H-4eq	H-1, H-3ax, H-4eq, H-6α
H-6α	H-6β	H-4a, H-6β, H-7
H-6β	H-6α	H-6α, H-7, H-12*endo*
H-7	–	H-6α, H-6β
H-10	–	H-1
H-11*endo*	H-11*exo*, H-12*endo*, H-12*exo*	H-11*exo*, H-12*endo*
H-11*exo*	H-11*endo*, H-12*endo*, H-12*exo*	H-4ax, H-11*endo*, H-12*exo*
H-12*endo*	H-11*endo*, H-11*exo*, H-12*exo*	H-6β, H-11*endo*, H-12*exo*
H-12*exo*	H-11*endo*, H-11*exo*, H-12*endo*	H-4ax, H-11*exo*, H-12*endo*
OCH_2O	–	–

MS, ^{1}H- and ^{13}C-NMR) with those of authentic samples obtained from other plant sources.

Lycorine showed a mild activity by in vitro assay screening against *Plasmodium falciparum* (strain NF-54, stage IEF) with an IC_{50} of 0.34 μg/ml. Compounds **1**, **2**, crinamidine and undulatine showed no activity. Chloroquine and qinghaosu were used as standards. No activity was reported against *Leishmania donovani* (strain MHOM-ET-67/L82, stage amastigotes) and *Trypanosma cruzi* (strain Tulahuen C4, stage trypomastigotes).

Compound **2** showed a very mild activity against *Trypanosoma brucei rhodesiense* (strain STIB-900, stage trypomastigotes) with an IC_{50} of 11.07 μg/ml. The other compounds, however, showed no activity. Melarsoprol was used as standard.

3. Experimental

3.1. General

Mps were uncorrected. IR spectra were measured on a Perkin Elmer 1600 FTIR series spectrometer in dry film. EIMS were run on a Hewlett Packard 5989A Mass Spectrometer at 70 eV. CD were measured on a Jasco J-700 Spectropolarimeter. ^{1}H-, ^{13}C-NMR, DEPT, COSY, HMQC, HMBC and ROESY spectra were recorded on a Varian VXR 500 in $CDCl_3$. Chemical shifts are reported in units of δ (ppm) relative to the TMS signal and coupling constants (J) in Hz. Silica gel SDS chromagel 60 A CC (230–400 mesh) and silica gel Merck (70–230 mesh) were used for flash CC and CC, respectively. Sephadex LH-20 (Pharmacia) was used for gel filtration, and silica gel SIL G/UV_{254} (Macherey-Nagel) for analyt. (0.25 mm) and SIL G-25 UV_{254} for prep. (0.25 mm) TLC. Spots on tlc were detected under UV light (254 nm) and by Dragendorff's reagent.

3.2. Plant material

Bulbs of *Crinum macowanii* Baker were obtained in December 1996 from the van den Berg Nurseries in Durbanville (Cape Province), South Africa. Samples were authenticated by the nursery proprietor, Mrs J. van den Berg.

3.3. Extraction and isolation of alkaloids

Fresh bulbs (2.9 kg) were crushed and macerated with EtOH for 48 h after which the extract obtained was evapd. under red. pres. The residue (142 g) was dissolved in H_2O (200 ml) and acidified to pH 4 with dil. HCl. After removing neutral material with Et_2O, the acidic soln. was extracted with $CHCl_3$ to provide extract A (0.85 g). Basifying the soln. to pH 8–9 with aq. NH_3 and extracting again with $CHCl_3$ afforded extract B (4.7 g), from which lycorine (908 mg) crystallized directly. Finally, a $CHCl_3$–MeOH (3:2) extraction of the basic soln. gave extract C (0.71 g), again from which a further 270 mg of lycorine was obtained by direct crystallization. Extracts A, B and C were combined (5 g) after TLC (MeOH–EtOAc (1:1)) indicated that they contained the same alkaloids, and subjected to flash CC on silica gel by gradient elution with *n*-hexane, *n*-hexane–EtOAc, EtOAc, EtOAc–MeOH and finally MeOH. Five fractions were generated in this manner; cherylline (31 mg) was obtained directly from fr. I by recrystallization with MeOH. Fr. II was rechromatographed by CC using an EtOAc–MeOH step gradient, followed by prep. TLC (Me_2CO) from which a further quantity of cherylline (14 mg) together with krepowine (5 mg), buphanidrine (5 mg), crinamidine (7 mg) and undulatine (7 mg) were obtained. Similarly, fr. III yielded crinamidine (15 mg) and powelline (180 mg) and fr. IV powelline (70 mg) and crinine (50 mg). Fr. V gave, in addition to crinine (10 mg) and powelline (6 mg), by further chromatography on Sephadex LH-20, macowine (9 mg), 1-epideacetylbowdensine (6 mg) and 4a-dehydroxycrinamabine (7 mg).

3.4. Macowine (*1*)

Found: C, 69.20; H, 7.36; N, 4.99. $C_{16}H_{19}NO_3$ requires: C, 70.31; H, 7.01; N, 5.12 %. Mp 115–117; $[\alpha]_D^{20}$ −34° ($CHCl_3$; *c* 0.235); CD $[\Theta]_{247}$ +491, $[\Theta]_{282}$ −2839. IR υ_{max} cm^{-1}: 3500–3000 (OH), 2928, 1582, 1507, 1471, 1315, 1277, 1220, 1134, 1114, 1096, 1040 (C–O–C), 999, 849, 814, 754. EIMS *m*/*z* (rel. int.): 273 [M^+] (60), 230 (23), 201 (68), 189 (47), 175 (21), 157 (18), 129 (20), 115 (26), 91 (19), 56 (100). ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (50 MHz, $CDCl_3$), (see Tables 1 and 2).

3.5. 4a-Dehydroxycrinamabine (*2*)

Found: C, 67.59; H, 6.83; N, 4.94. $C_{16}H_{19}NO_4$ requires: C, 66.41; H, 6.62; N, 4.84%. Mp, $[\alpha]_D$, EIMS, UV and CD data are in agreement with those reported recently (Pham et al., 1998). For ^{1}H-NMR (500 MHz) and ^{13}C-NMR (50 MHz) in $CDCl_3$ (see Tables 3 and 4).

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