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Alkaloids from *Crinum moorei*

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Abstract

Thirteen alkaloids were isolated from *Crinum moorei* two of which are new. These are 3-[4′-(8′-aminoethyl)phenoxy] bulbispermine and mooreine. The structures of the new alkaloids were determined by spectroscopic methods.

Keywords: *Crinum moorei*; Amaryllidaceae; Alkaloids; 3-[4′-(8′-Amino ethyl)phenoxy] bulbispermine; Mooreine

1. Introduction

Crinum moorei (Amaryllidaceae) is one of 21 *Crinum* species found in southern Africa (Verdoorn, 1973). *Crinum* species are used by Zulu healers against swelling of the body and for urinary tract problems (Hutchings et al., 1996). We report here on the isolation of the new compounds 3-[4′-(8′-aminoethyl)phenoxy] bulbispermine **1**, and **2** which we have designated as mooreine, systematic name [1,3]dioxolo [4,5-j]pyrrolo [3,2,1-de] phenanthridinum, 1,2,4,5,12b, 12c-hexahydro-2-hydroxy-12c-methoxy (Tables 1 and 2). Also isolated from this species were the known compounds crinine, undulatine (Viladomat et al., 1995a), 3-*O*-acetylcrinine (Campbell et al., 1998), powelline, cherylline (Kobayashi et al., 1984), crinamidine, epibuphanisine, epivittatine (Viladomat et al., 1995b), 1-epideacetyl-bowdensine (Viladomat et al., 1996), lycorine, and 1-*O*-acetyllycorine (Evidente et al., 1983).

2. Results and discussion

The ^{1}H NMR of **1** showed the typical singlets associated with H-7, H-10, and the methylenedioxy group at 6.86, 6.53 and 5.87, respectively. From the HETCOR and COSY analysis, the resonance of H-3 and H-11 could be pinpointed at δ 4.40 and 3.95, respectively. The first is clearly coupled to H-1 and H-2 and also to the methylene group at C-4 while the second is coupled to the methylene group at 3.32. These were the only likely positions to which the tyramine residue- whose presence was clearly indicated by the A_2X_2 system present at δ 6.73 and δ 7.05 — could be attached. HMBC and NOESY were not helpful and no connectivities were observed between H-11 or H-3 and either C-6′ and C-2′ or C-7′ and C-8′. Also no NOE effects were observed between protons on the basic ring and the phenethyl moiety. Acetylation of the compound resulted in a downfield shift of both H-3 and H-11 to around 5.48 and 4.97 ppm, respectively. This indicates that acetylation occurred at both positions which further proved by the molecular ion of the acetate of 371 mass unit with the loss of 60 and 43 mass units, respectively. The possibility of the compound being a mixture of two compounds was rejected by developing bulbispermine and tyramine on a tlc using the same solvent system used for the purification of 1. Tyramine has R_f value of 0.67 while bulbispermine R_f value of 0.59. From the above acetylation results, it is clear that the compound undergone degradation at some stage during storage or acetylation. NOE studies did not reveal much useful information but did show a good correlation between H-11 *exo* and H-12 *exo*. Construction of an accurate model using the NOE information showed, however, that the 3- and 11-positions were not sterically crowded

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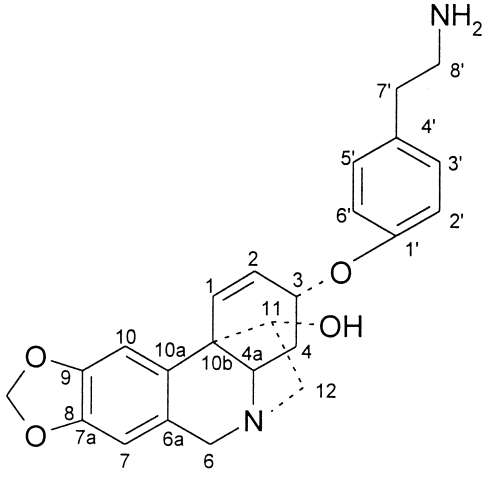
and equally exposed to attack by a tyramine moiety. In these circumstances, the tyramine residue was attached at the more reactive allylic C-3. In addition, computer simulation programme ^{1}H NMR data, using Advanced Chemistry Development programme (ACD) from Aldrich 1998, supported the attachment at C-3, as indicated in Table 3. It is suggested that the linkage to C-3 is via the phenolic group of the tyramine moiety. This is based on the observation that in both plicamine and secoplicamine (both bonded through N), C-8′ resonates in the ^{13}C spectrum at 49.0 and 50.0 ppm, respectively (Ünver et al., 1999) whereas in 1 the relevant chemical shift is at 44.0 ppm. In addition, the shift of the ethylene group in authentic tyramine (36.7 and 43.4 for C-7′ and C-8′, respectively) correspond closely to that observed for the corresponding group in compound 1. Furthermore, acetylation of tyramine at both C-1 and NH_2 showed only



Compound **1**

Table 1
^{1}H NMR data (CD_3OD) for compound **1**, and bulbispermine

	1	Bulbispermine
H-2′, H-6′	7.05 *d* (*J* = 8.5)	
H-7	6.86 *s*	6.83 *s*
H-3′, H-5′	6.73 *d* (*J* = 8.6)	
H-10	6.53 *s*	6.48 *s*
H-2	6.24 *dd* (*J* = 2.3, 10.3)	6.23 *dd* (*J* = 2.2, 10.35)
H-1	6.05 *dd* (*J* = 1.2, 10.3)	6.02 *d* (*J* = 10.35)
OCH_2O	5.87 *s*	5.86 *s*
H-3	4.40 *m*	4.3d *dd* (*J* = 2,4,8)
H-6α	4.27 *d* (*J* = 16.9)	4.22 *d* (*J* = 16.9)
H-11	3.95 *dd* (*J* = 3.6,7)	3.93 *dd* (*J* = 2.9, 7)
H-6α	3.72 *d* (*J* = 16.9)	3.68 *d* (*J* = 16.9)
H-12		3.41 *dd* (1H, *J* = 7, 13.69)
H-12′	3.32 *m* (2H)	3.32 *m* (1H)
H-4a	3.32 *m*	3.20 *m*
2H-8′	2.90 *t* (*J* = 7)	
2H-7′	2.70 *t* (*J* = 7)	
2H-4	2.02 *m*	2.03 *m*

Table 2
^{13}C NMR data (CD_3OD) for compound **1**, and bulbispermine

C no.	**1**	Bulbispermine
C-1′	157.4 *s*	
C-9	148.4 *s*	148.3 *s*
C-8	147.9 *s*	147.9 *s*
C-10a	137.7 *s*	137.7 *s*
C-1	137.4 *d*	137.4 *d*
C-2′, C-6′	131.0 *d*	
C-4−	130.9 *s*	
C-6a	127.1 *s*	127.1 *s*
C-2	125.2 *d*	125.2 *d*
C-3′, C-5′	116.7 *d*	
C-10	108.1 *d*	108.1 *d*
C-7	104.5 *d*	104.5 *d*
OCH_2O	102.4 *t*	102.5 *t*
C-11	81.3 *d*	81.3 *d*
C-3	68.6 *d*	68.6 *d*
C-4a	67.7 *d*	67.6 *d*
C-12	63.9 *t*	64.0 *t*
C-6	61.8 *t*	61.7 *t*
C-10b	51.7 *s*	51.6 *s*
C-8′	44.0 *t*	
C-7′	37.9 *t*	
C-4	34.7 *t*	34.7 *t*

Table 3
Computer simulation programme ^{1}H NMR chemical shifts of Protons at positions 3 and 11 for bulbispermine and bulbispermine with the tyramine attached either to C-3 or C-11

Proton no.	Bulbispermine	Bulbispermine with tyramine moiety attached to	
		C-3	C-11
3-H	4.37	4.68	4.21
11-H	3.91	3.91	5.12

Table 4
^{1}H NMR and ^{13}C NMR (CD_3OD) for compound **2**

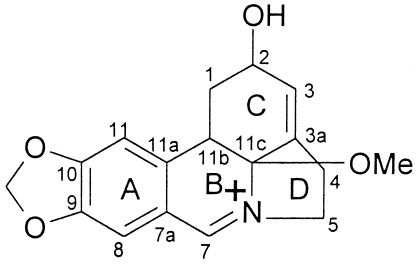
Atom no.	^{13}C	^{1}H
9	158.5 *s*	–
10	152.8 *s*	–
7	142.1 *d*	9.36 *s*
11a	139.3 *s*	–
7a	134.2 *s*	–
3a	128.4	–
3	127.6 *d*	6.45 *m*
8	106.1 *d*	7.63 *s*
OCH_2O	105.6 *t*	6.38
11	100.8 *d*	7.77 *s*
11c	79.4 *s*	–
2	72.0 *d*	4.61 *m*
11b	68.9 *d*	5.31 *m*
5	57.1 *t*	5.00 *m* (2H)
1	54.8 *t*	5.49 *ddd* (0.69, 3.8, 5.3)
		5.48 *ddd* (0.46, 1.14, 5.3)
4	26.5 *t*	3.32 *m* (2H)
OMe	48.0	3.32 *s* (3H)

0.15 ppm dowfield shift of protons at C-2′ and C-6′ compared to 0.25 ppm downfield shift of protons at C-3′ and C-5′. Protons attached to C-8′ also shifted downfield by 0.28 ppm. Chemical shifts of C-2′, C-6′ remained unaffected while that of 3′, 5′ were shifted downfield by 6 ppm.

The compound gave no molecular ion in its HRMS. However, it showed a fragment ion at 286.1058 ($C_{16}H_{16}NO_4$) which is the molecular ion of bulbispermine after loss of the tyramine moiety.

Compound **2** under normal conditions of electron bombardment showed no molecular ion. However, using an electrospray instrument it exhibited a molecular ion at m/z 300. This corresponds to the proposed molecular formula of C_{17} $H_{18}NO_4$ The ^{1}H NMR and ^{13}C NMR (Table 4) for ring A, B, D closely resemble those reported for the anhydrolycorinium ion (Petitt et al., 1984). The ^{1}H NMR showed four singlets at 9.36, 7.77, 7.63 and 6.38 assigned to H-7, H-11, H-8 and the methylenedioxy, respectively. It also showed multiplets at 6.45, 5.31, 5.00, 4.61 and 3.32 assigned to H-3, H-11b, H-5, H-2 and H-4, respectively. The two *ddd* at 5.49 and 5.48 were assigned to the two protons at position 1. The multiplicities of the peaks relating to the aliphatic portion of the molecule were completely assigned by COSY analysis. It showed a correlation between H-2 (δ 4.61) and the one proton multiplet of H-3 at δ 6.41 and the ddd of H-1β and H-1α at 5.49 and 5.48. In addition, there was a correlation between the *ddd* of both protons at position 1 and that of H-11b at δ 5.31. The assignment of the signal at 5.31 to H-11b was supported by the long range coupling between the signal at 5.31 and that of H-11 (7.77). The deshielding of C-5 and C-4 protons is due to their α- and β-positions with respect to the nitrogen of the salt (Bastida et al., 1992).

The ^{13}C NMR showed four methine sp^2 carbons in the aromatic region at 142, 127, 105 and 101 ppm assigned to C-7, C-3, C-8, and C-11. The chemical shifts of the six quaternary carbons C-3a, C-7a, C-9, C-10, C-11a and C-11c are shown in Table 4. The placement of the methoxy group on C-11c was based on the chemical shift of this carbon atom and was in good agreement with predictions for ^{13}C chemical shifts derived from a commercially available modelling program. Lack of material and marked instability prevented a more detailed analysis of the compound and the proposed structure should be regarded as tentative.



compound **2**

3. Experimental

NMR spectra were recorded in $CDCl_3$ and CD_3OD using TMS as internal standard at 500 and 200 MHz for ^{1}H and 50 and 125 MHz for ^{13}C. Chemical shifts are recorded in δ units and coupling constants (*J*) in Hz. Mass spectra were recorded on a Kratos MS 80 RF double-focussing magnetic sector instrument at 70 eV. Silica gel Merck (230–400 mesh) was used for VLC . Silica gel 60 F_{254} analytical and prep. TLC (2 mm) were used for additional separation. Spot detection by UV light (254) and Dragendorff's reagent.

3.1. Plant material

Crinum moorei (Hook f.) plants were obtained in December 1997 from Green Goblin Nursery, Durban, South Africa and their identity confirmed by Dr. T.J. Edwards, School of Botany and Zoology, University of Natal Pietermaritzburg. A voucher specimen (Elgorashi2 NU) was deposited in the University of Natal Herbarium, Pietermaritzburg.

3.2. Extraction and isolation

The dried and powdered non flowering whole plants (62.5 g) were extracted with Petr. ether (60–80°) and 95% EtOH for 40 h each using a Soxhlet apparatus. The ethanolic extract was evaporated under reduced pressure and the residue was treated with 4% aq. HOAc. The aqueous acidic solution was filtered. The solution was basified with NH_4OH to pH 9.5 after removal of neutral material with Et_2O. The basified solution was extracted with Et_2O, EtOAc, and *n*-BuOH to give fraction A, B, C, respectively (Ghosal et al., 1983).

Fractions A and B were combined and kept overnight in MeOH at room temp. to give lycorine as a powder (81 mg). The remaining crude extract subjected to VLC on silica gel eluting with $CHCl_3$ and then with $CHCl_3$ enriched gradually with MeOH up to 50% MeOH to give five fractions. Fraction I was developed on PLC (2 mm) using $CHCl_3$–MeOH (9:1) which were developed again using $CHCl_3$–Et_2NH (20:1), $CHCl_3$–Et_2NH (40:1), benzene–MeOH (9:1) to give epibuphanisine (56 mg), 1-*O*-acetyllycorine (32 mg), undulatine (11 mg) and 3-*O*-acetylcrinine (8 mg).

Fraction II was developed on TLC using $CHCl_3$–Et_2NH (20:1) to give two bands which were developed further on $CHCl_3$–Et_2NH (40:1) twice to give epivittatine (53 mg), cherylline (35 mg) and $CHCl_3$–MeOH (10:1) to give crinamidine (7 mg). Fractions III, IV, V were developed on PLC using $CHCl_3$–Et_2NH (20:1) to

give powelline (20 mg), crinine (36 mg), and 1-epideacetylbowdenisine (16 mg).

Fraction C was subjected to VLC using silica gel eluting with $CHCl_3$ enriched gradually with MeOH to give two fractions. Fraction I was developed on TLC using $CHCl_3$–MeOH (2:1) to give compound 2 (7 mg). Fraction II was developed on PLC (2 mm) using $CHCl_3$–CH_2Cl_2–EtOH–MeOH (7:7:7:4) and NH_3 vapour to give compound **1** (11 mg).

3.2.1. Conversion of tyramine to its acetate

Twenty-nine mg of tyramine were heated with equal volumes of pyridine and acetic anhydride at 60°C for half an hour and kept overnight at room temp. to give tyramine-diacetate (39 mg). ^{1}H NMR (CD_3OD): 7.26 *d* (8.4, H-2′ and H-6′), 7.04 *d* (8.4, H-3′ and H-5′), 3.41 *t* (7.5, 2H-8′), 2.81 *t* (7.5, 2H-7′), 2Me (*s*, 2.02 and 1.93). ^{13}C NMR (CD_3OD): 173.2 *s* and 171.4 *s* (2 O$\underline{C}$OCH_3), 150.7 *s* (C-1′), 130.7 *d* (C-2′ and C-6′), 138.9 *s* (C-4′), 122.6 *d* (C-3′ and C-5′), 41.9 *t* (C-8′), 35.7 *t* (C-7′), 22.5 and 20.9 *q* (2Me).

3.2.2. Tyramine

^{1}H NMR (CD_3OD): 7.11 *d* (8.42, H-2′ and H-6′), 6.79 *d* (8.42, H-3′ and H-5′), 3.13 *t* (7.14, 2H-8′), 2.88 *t* (7.09, 2H-7′). ^{13}C NMR (CD_3OD): 157.2 *s* (C-1′), 130.7 *d* (C-2′ and C-6′), 130.9 *s* (C-4′), 116.4 *d* (C-3′ and C-5′), 43.4t (C-8′), 36.7 *t* (C-7′)

3.2.3. Compound *1*

Amorphous compound (11 mg)$[\alpha]_D^{29} + 53.3°$ ($CHCl_3$, *c*, 0.045) ^{1}H and ^{13}CNMR (see Tables 1 and 2). GC–MS 70 eV, *m*/*z* (% rel. int.): 286 (< 1%) 258 (100), 248 (26), 186 (18), 129 (9), 115 (14), 107 (11), 44 (35), 43 (56), 42 (17).

3.2.4. Compound *2*

Amorphous compound (7 mg) ^{1}H and ^{13}C NMR (see Table 4), electrospray (% rel. int.): 300 [M^+, 5), 292 (5), 284 (100), 266 (5).

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