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Alkaloids from *Crinum bulbispermum*

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Abstract

In addition to crinamine, bulbispermine, 3-*O*-acetyl hamayne and 6-hydroxycrinamine, three new alkaloids 8α-ethoxy precriwelline, *N*-desmethyl 8α-ethoxy pretazettine and *N*-desmethyl-8β-ethoxy pretazettine were isolated from *Crinum bulbispermum*. The structures of the new alkaloids were established by specroscopic techniques. © 1999 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Crinum bulbispermum; Amaryllidaceae; Alkaloids; 8α-ethoxy precriwelline; *N*-desmethyl-8α-ethoxy pretazettine; *N*-desmethyl-8β-ethoxy pretazettine

1. Introduction

Crinum bulbispermum (Amaryllidaceae) is an indigenous species present in KwaZulu Natal, Gauteng, Northern Province and Western Cape Province of South Africa (Verdoorn, 1973). It is used by Zulu, Sotho and Tswana people to treat rheumatism, aching joints, septic sores, varicose veins and kidney and bladder infections (Roberts, 1990; Hutchings, Scott, Lewis & Cunningham, 1996). This report outlines the isolation of the new alkaloids 8α-ethoxy precriwelline **1**, *N*-desmethyl-8α-ethoxy pretazettine **2** and *N*-desmethyl-8β-ethoxy pretazettine **3** along with the previously isolated alkaloids, 3-*O*-acetyl hamayne (Kobayashi et al., 1984; Viladomat, Bastida, Codina, Campbell & Mathee, 1994), 6-hydroxycrinamine (two epimers) (Viladomat et al., 1996), crinamine (Kobayashi et al., 1984; Likhiwitayawuid et al., 1993) and bulbispermine (Ali, Ramadan & Frahm, 1984) from this species. Only crinamine and bulbispermine were previously isolated from this species (Ali et al., 1984; Kobayashi et al., 1984).

2. Results and discussion

Compound **1**, $C_{20}H_{25}NO_5$, showed in its mass spectrum a molecular ion at *m*/*z* 359 and two fragments ions at *m*/*z* 329 and 70 (base peak). The latter two peaks are typical mass fragments characteristic of tazettine type alkaloids with a 3-OMe in an α-position (Duffield, Alphin, Budzikiewicz, Djerassi & Wildman, 1965). The peak at *m*/*z* 314 (51%) represents loss of the ethoxy group.

The ^{1}H-NMR spectrum showed six singlets at 6.76, 6.61, 5.8, 5.68, 3.42, 2.85 assignable to the aromatic protons at H-12 and H-9, the methylenedioxy protons, H-8, 3-OMe and *N*-Me respectively. Examination of ^{13}C, COSY and HETCOR sequences allowed identification of the remaining protons and carbon atoms as shown in Tables 1 and 2. The placement of the crucial ethoxy group at the C-8 position was based upon

1. the triplet at δ 1.3, J = 7.0 Hz coupled to the doublet of quartets at δ 3.72 and 3.94, J = 7.0 Hz;
2. the presence of a singlet proton signal at δ 5.68 typical of the chemical shift of a benzylic acetal hydrogen; and
3. a ^{13}C signal at 99.1 ppm, again characteristic of a

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Table 1
^{1}H-NMR data ($CDCl_3$) for compounds **1**, **2**, **3** and pretazettine **4**

H No.	1	2	3	4
H-12	6.76 s (1H)	6.78 s (1H)	6.73 s (1H)	6.82 s (1H)
H-9	6.61 s (1H)	6.56 s (1H)	6.72 s (1H)	6.51 s (1H)
H-2	5.99–6.06 m (1H)	5.99 m (1H)	6.22 s (1H)	5.84 br (1H)
OCH_2O	5.80 s (2H)	5.89 and 5.9, 2 d (1H each, 1.4)	5.9 and 5.8, 2 d (1H each, 1.4)	5.92 s (2H)
H-1	5.87 d (1H, 10.3)	5.73–5.90 m (1H)	6.22 s (1H)	6.17 m (1H)
H-8	5.68 s (1H)	5.70 s (1H)	4.50 s (1H)	6.08 s (1H)
H-6a	4.35 dd (1H; 8.0, 11.2)	4.46 dd (1H; 6.7, 11.2)	3.87 m (1H)	
H-3	3.87 m (1H)	3.80 m (1H)	4.04 m (1H)	
$OCHaHbCH_3$				
Ha	3.94 dd (1H; 7.0, 9.7)	3.92 dd (1H; 7.0, 9.7)	4.03 dd (1H; 7.0, 9.7)	
Hb	3.70 dd (1H; 7.0, 9.7)	3.70 dd (1H; 7.0, 9.7)	3.66 dd (1H; J.0, 9.7)	
H-6α-	3.09 dd (1H; 9.8, 11.2)	2.83–2.98 m (2H)	3.20 d (2H, 4.92)	
H-6β-	2.66 dd (1H; 8.0, 9.8)	–	–	
3-OMe	3.42 s (3H)	3.40 s (3H)	3.4 s (3H)	3.4 s (3H)
H-4a	2.87 bs (1H)	3.46 bs (1H)	3.52 bs (2H)	
NMe	2.50 s (3H)	–	–	2.42 s (3H)
H-4	2.32 m (2H)	2.10–2.38 m (2H)	2.03–2.09 m (2H)	
OCH_2CH_3	1.30 t (3H, 7.0)	1.29 t (3H, 7.0)	1.26 t (3H, 7.0)	

benzylic carbon bearing an acetal moiety. By comparison, this carbon in 6-hydroxy crinine type alkaloids, in which it exists as a hemi-aminal, resonates at 85–88 ppm (Bastida et al., 1990; Likhiwitayawuid et al., 1993).

Compound **3**, $C_{19}H_{23}NO_5$, this is an epimer (at C-8) of **2**. In this instance H-8 resonates well upfield (δ 4.50) when compared to the same proton in **1** and **2** (δ 5.70). Further differences in proton shift for **2** are evident for all protons attached to sp^2 carbons i.e. H-9, H-12, H-1 and H-2 (Table 1). ^{13}C shifts for **1**, **2** and **3** are very similar except C-12b in **3** which is shifted downfield (Table 2).

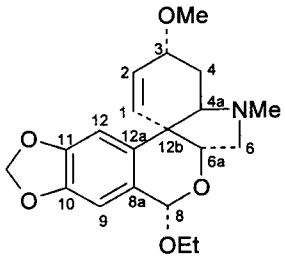
Table 2
^{13}C-NMR data ($CDCl^3$) for compounds **1**, **2** and **3**

C No.	1	2	3
C-11	147.6 s	147.6 s	147.7 s
C-10	146.4 s	146.6 s	146.3 s
C-12a	134.8 s	134.4 s	136.7 s
C-1	130.1 d	131.5 d	136.4 d
C-8a	127.2 s	127.5 s	127.1 s
C-2	125.9 d	125.4 d	123.2 d
C-12	108.1 d	108.2 d	109.0 d
C-9	104.4 d	104.6 d	102.0 d
OCH_2O	101.1 t	101.1 t	101.0 t
C-8	99.1 d	99.4 d	94.0 d
C-6a	73.4 d	76.2 d	78.6 d
C-3	71.3 d	71.3 d	76.2 d
OCH_2CH_3	63.8 t	63.8 t	64.0 t
C-4a	62.5 d	56.8 d	60.0 d
OMe	56.7 q	55.4 q	55.0 q
C-6	53.2 t	44.9 t	58.5 t c
C-12b	45.3 s	44.2 s	50.0 s
NMe	42.5 q	–	
C-4	29.3 t	29.0 t	29.0 t
OCH_2CH_3	15.3 q	15.4 q	15.0 q

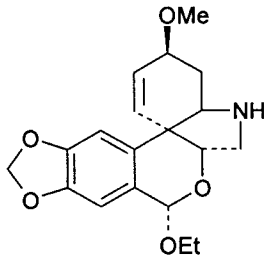
Attempts at obtaining a molecular peak from compound **3** failed. However, the compound showed a base peak at 238 the same as that of littroline (Lin et al., 1995). The fragmentation pattern is consistent with that of tazettine type alkaloids.

The proton and ^{13}C chemical shift values of **1** and **2** are very similar. However, the similarity of MS fragmentation pattern of **1** and **2** with precriwelline and pretazettine (Duffield et al., 1965; Ghosal, Kumar & Singh, 1984) respectively, provide a basis for different stereochemistry at position 3. For this reason the 3-methoxy group is tentatively assigned an α-configuration in **1** and a β-configuration in **2**. Compound **3** is a diastereomer of **2**. From differences in chemical shift in both proton and ^{13}C (Tables 1 and 2) at C-8 it appears to be epimeric at this centre compared to **1** and **2**.

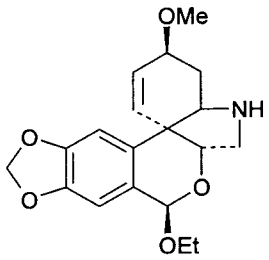
Comparison of protons at positions 8 of compounds **1**, **2**, **3** with those of papayramine, 6-epipayramine (Bastida et al., 1990), the two epimers of 6-hydroxy crinamine (Viladomat et al., 1996), *O*-methyl oduline (Kreh and Matusch, 1995), *O*-methyl lycorine (Codina et al., 1993) and 2α-hydroxy-6-*O*-methyloduline (Almanza et al., 1996) allowed the assignment of the ethoxy group in an α-position for **1** and **2** and in β-position for **3**. Hence the names 8α-ethoxy precriwelline; *N*-desmethyl-8α-ethoxy pretazettine; *N*-desmethyl-8β-ethoxy pretazettine are proposed for compound **1**, **2** and **3** respectively. The proton spectrum of pretazet-



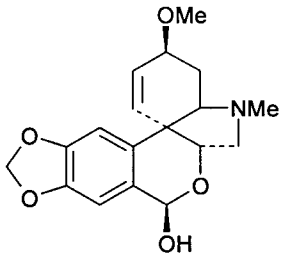
1



2



3



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tine **4** (Ghosal et al., 1984) is included for comparative purposes (Table 1).

3. Experimental

NMR spectra were recorded in $CDCl_3$ and CD_3OD using TMS as internal standard at 200 MHz for ^1H and 50 MHz for ^{13}C. Chemical shifts are reported 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 vacuum liquid chromatography. 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 bulbispermum ((Brum.f.) Milne-Redhead and Schweickerdt) plants were obtained in December 1997, from Green Goblin Nursery, Durban, South Africa and their identity confirmed by Dr. T.J. Edwards, Botany Department, University of Natal Pietermaritzburg. A voucher specimen {Elgorashi 1 (NU)} was deposited in the University of Natal Herbarium Pietermaritzburg.

3.2. Extraction and isolation

The dried and powdered nonflowering whole plants (198.5 g) were extracted with petrol-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 fractions A, B and C respectively (Ghosal, Saini & Frahm, 1983).

Fraction A was subject to vacuum liquid chromatography on silica gel eluting with $CHCl_3$ (100%) and then with $CHCl_3$ enriched gradually with 5% MeOH up to 50% MeOH. The first fraction was developed on PLC (2 mm) using $CHCl_3$:MeOH (9:1) to give 6 fractions each of which was developed using $CHCl_3$:Et_2NH (20:1) to give compound **1** (10 mg),

compound **2** (11 mg), compound **3** (14 mg), crinamine (15 mg) and 3-*O*-acetyl hamayne (28 mg).

The second fraction (90% $CHCl_3$) was developed using $CHCl_3$:MeOH (4:1) followed by Me_2CO:MeOH (4:1) to give 6-hydroxycrinamine (17 mg).

Fraction B was dissolved in 5 ml MeOH and kept at room temp. overnight to give a powder (63 mg) and the crude extract was subjected to vacuum liquid chromatography (20 g, 8.5 × 3 cm) using 100% $CHCl_3$ and then $CHCl_3$ enriched gradually with 2.5% MeOH up to 50%. The fraction eluted with 92.5% $CHCl_3$ was developed on TLC using Me_2CO:MeOH (3:1) to give more crinamine. Fractions eluted with 87.5% and 85% $CHCl_3$ were subjected to TLC using $CHCl_3$:MeOH (3:1) and then $CHCl_3$:Et_2NH:MeOH (15:3:2) to give bulbispermine (70 mg).

3.2.1. Compound **1**

Amorphous compound (10 mg), $[\alpha]_D^{28} + 116.6^\circ$ ($CHCl_3$, c, 0.06); ^1H and ^{13}C NMR see Tables 1 and 2. GC–MS 70 eV, *m*/*z* (% rel.int): 359 [M^+, (41)], 329 [M–CH_2O, (78)], 314 (50) 282 (16), 275 (47), (29), 181 (12), 149 (17), 113 (25), 89 (37), 82 (30), 70 (100). HRMS calcd. for $C_{20}H_{25}NO_5$: 359.1719, found 359.1733.

3.2.2. Compound **2**

Amorphous compound (11 mg);, $[\alpha]_D^{28} = +160.63^\circ$ ($CHCl_3$, c, 0.09). ^1H and ^{13}C NMR see Tables 1 and 2. GC–MS 70 eV, *m*/*z* (% rel.int.): 345 [M^+, (41)], 316 (13), 315 (6), 314 (4), 300 (30), 289 (18), 268 (19), 244 (100), 215 (23), 181 (7), 141 (7), 115 (12), 89 (46), 61 (32). HRMS calcd. for $C_{19}H_{23}NO_5$: 345.1576 found 345.1560.

3.2.3. Compound **3**

Amorphous compound (14 mg), $[\alpha]_D^{28} = +34^\circ$ ($CHCl_3$, c, 0.14), ^1H and ^{13}C NMR see Tables 1 and 2, GC–MS 70 eV, *m*/*z* (% rel.int.) 315 (< 1%), 267 (28), 248 (60), 239 (24), 238 (100), 224(9), 223 (34), 210 (13), 180 (21), 152 (26), 95 (24), 75 (19).

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