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Bufadienolides from *Drimia robusta* and *Urginea epigea* (Hyacinthaceae)

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Abstract

Two bufadienolides, 6β-acetoxy-3β,8β,14β-trihydroxy-12-oxobufa-4,20,22-trienolide and 14β-hydroxybufa-3,5,20,22-tetraenolide were isolated from the dichloromethane extract of the bulbs of *Drimia robusta* and the methanol extract of the bulbs of *Urginea epigea*, respectively. The bulbs of *Drimia robusta* also yielded several known compounds, 6β-acetoxy-3β,8β,12β,14β-tetrahydroxybufa-4,20,22-trienolide (12β-hydroxyscillirosidin) from the dichloromethane extract and three common aromatic acids, 4-hydroxy-3-methoxybenzoic acid, 3,4-dihydroxybenzoic acid, and *trans*-3-(4′-hydroxyphenyl)-2-propenoic acid from the ethyl acetate extract.

Keywords: *Drimia robusta*; *Urginea epigea*; Hyacinthaceae; Bufadienolides; Bufatetraenolide; 6β-Acetoxy-3β,8β,14β-trihydroxy-12-oxobufa-4,20,22-trienolide; 6β-Acetoxy-3β,8β,12β,14β-tetrahydroxybufa-4,20,22-trienolide (12β-hydroxyscillirosidin); 14β-Hydroxybufa-3,5,20,22-tetraenolide; 4-Hydroxy-3-methoxybenzoic acid; 3,4-Dihydroxybenzoic acid; *trans*-3-(4′-Hydroxyphenyl)-2-propenoic acid

1. Introduction

Drimia robusta Bak. (syn. *D. alta* R.A. Dyer) from the subfamily Urgineoideae of the Hyacinthaceae is reportedly toxic (Van der Walt and Steyn, 1943) and possesses a caustic sap. As a cardiotonic (Sapeika, 1951), *D. robusta* has found widespread use in traditional South African medicine, particularly by the Zulu. Its various ethnomedicinal applications have earlier been summarised by Pohl et al. (2001). Plants are widely distributed in the moister eastern parts of southern Africa, and grow gregariously, exposed in shallow soil overlying rock, or close to forest margins in open grassland.

Phytochemical analyses of bulbs of *Urginea* Steinh. (syn. *Drimia* Jacq) (Urgineoideae; Hyacinthaceae) sourced from both Mediterranean and southern African regions have yielded chiefly bufadienolides. Within the Hyacinthaceae only representatives of the Urgineoideae reportedly produce this class of compounds; bufadienolides are accordingly recognised (Pohl et al., 2000) as useful sub-family chemotaxonomic markers. The clump-forming *Urginea epigea* Dyer inhabits semi-arid, rocky grasslands in a number of southern African countries, particularly along the eastern seaboard. As suggested by its specific epithet, bulbs are presented mostly above the soil surface, unlike its closest relative *Drimia altissima* (L.f.) Ker Gawl. (syn. *Urginea altissima* (L.f.) Bak.) which possesses largely subterranean perennating organs. As with *Drimia*, *Urginea* is known to pos-

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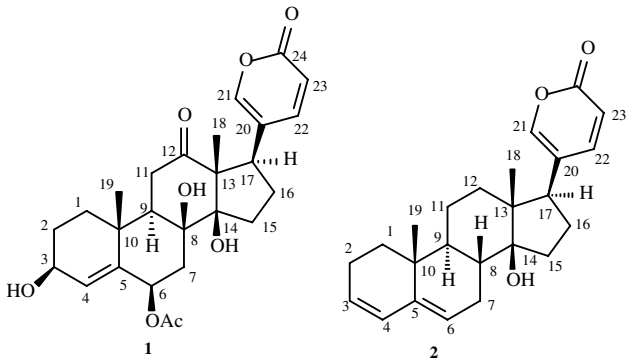
sess cardiotonic activity (Sapeika, 1951), a feature ascribed to the presence of bufadienolides. Although several regional *Urginea* species have been identified as poisonous to livestock (Kellerman et al., 1988), *U. epigea* has not thus far been implicated. However, as with many other southern African *Urginea* species, the bulb sap of *U. epigea* usually induces skin irritation upon handling, indicating the presence of bufadienolides. *U. epigea* is reportedly known as *lukhovu* to the Swati of Swaziland, who use it as a soap and for treating backache (Dlamini, 1981). To the north-west in Sekhukhuneland smoke from the smouldering bulbs of *U. epigea* is inhaled to relieve headaches (Dyer, 1947).

2. Results and discussion

The macerated bulbs of *D. robusta* were extracted using hexane, dichloromethane, ethyl acetate and methanol. Three common aromatic acids, 4-hydroxy-3-methoxybenzoic acid, 3,4-dihydroxybenzoic acid, and *trans*-3-(4′-hydroxyphenyl)-2-propenoic acid, were isolated from the ethyl acetate extract. The dichloromethane extract yielded two bufadienolides 6β-acetoxy-3β,8β,-12β,14β-tetrahydroxybufa-4,20,22-trienolide (12β-hydroxyscillirosidin), isolated previously from *Urginea maritima* Bak. (Kopp et al., 1996), *Drimia sanguinea* (Schinz) Jessop (syn. *Urginea sanguinea* Schinz) (Krenn et al., 1993) and *Drimia robusta* (Pohl et al., 2001) and 6β-acetoxy-3β,8β,14β-trihydroxy-12-oxobufa-4,20,22-trienolide, **1,** which has not been reported previously. No compounds of interest were isolated from the hexane and methanol extracts.

From the methanol extract of *U. epigea*, a bufatetraenolide, 14β-hydroxybufa-3,5,20,22-tetraenolide, **2**, was isolated. The isolation of two sesquiterpenoids, 1β,6α-dihydroxy-4(15)-eudesmene and 6α-hydroxy-4(15)-eudesmen-1-one from the dichloromethane extract of the bulbs were earlier reported (Koorbanally et al., 2004). Bufadienolides with a double bond at C-3 have previously been reported from *Scilla maritima* (Lichti et al., 1973) and *Bersama abyssinica* (Kupchan et al., 1971), however, this is the first reported natural occurrence of a bufadienolide with a 3,5-diene system although they have been synthesised previously by removing the sugar group from scillaglaucosid, isolated from *Scilla maritima* Baker L. (Lichti et al., 1973), by acid hydrolysis.

The high-resolution mass spectrum of compound **1**,6β-acetoxy-3β,8β,14β-trihydroxy-12-oxobufa-4,20,22-trienolide, revealed a parent ion peak at *m/z* 472.20981 which corresponded to the molecular formula $C_{26}H_{32}O_8$ (calculated 472.20972). ^1H, ^{13}C, COSY, HMBC and NOESY spectra were used to determine the structure of compound **1** and to assign all the ^1H and ^{13}C NMR resonances (Table 1).



The ^1H NMR spectrum of compound **1** was similar to that of the known bufadienolide,12β-hydroxyscillirosidin, with lactone ring coupled proton resonances at δ 7.47, δ 7.80 and δ 6.30, methyl resonances at δ 1.03 (3H-18) and δ 1.40 (3H-19), an olefinic resonance at δ 5.77 (H-4) and deshielded resonances at δ 4.10 (H-3), δ 5.47 (H-6) and δ 3.84 (H-17). The presence of an acetate group was indicated by the presence of a singlet methyl peak at δ 2.02 and a carbon resonance at δ 171.6, as in 12β-hydroxyscillirosidin. A correlation between the acetate methyl group proton resonance and the 3H-19 resonance in the NOESY spectrum resulted in the acetate group being placed at C-6β.

The deshielded proton resonance at δ 3.44 assigned to H-12 in 12β-hydroxyscillirosidin was now absent in the ^1H NMR spectrum and the C-12 carbon resonance experienced a shift from δ 76.7 to δ 215.2, indicating that a carbonyl group was now present at C-12. An HMBC correlation between the 3H-18 resonance and the carbonyl resonance at δ 215.2 supported this argument. The stereochemistry at C-3, C-8 and C-14 could not be determined using spectroscopic methods, but hydroxy groups present at these positions are usually in the β-orientation (Krenn and Kopp, 1998; Dewick, 2002). NOESY interactions between H-6α and H-4 and between H-4 and H-3 were observed. Computer and molecular models show that these interactions are possible with the hydroxy group at C-3 in the β-orientation.

The FAB mass spectrum of compound **2**, 14β-hydroxybufa-3,5,20,22-tetraenolide, showed a molecular ion peak at *m/z* 366.219581 which indicated a molecular formula of $C_{24}H_{30}O_3$. The ^1H NMR spectrum showed the presence of two methyl group proton resonances at δ_H 0.73 (3H-18) and δ_H 0.90 (3H-19) as well as a bufadienolide-type δ-lactone ring at C-17β by the coupled resonances at δ_H 7.82, δ_H 7.24 and δ_H 6.24.

The fully substituted carbon resonance at δ 85.9 was assigned to C-14 indicating that a hydroxyl group occurred at this position. This assignment was confirmed by a HMBC correlation between C-14 and the 3H-18 resonance at δ_H 0.73. The stereochemistry at C-14 could

Table 1
^{1}H, ^{13}C, COSY, HMBC and NOESY NMR data for compounds **1** (CD_3OD, 400 MHz) and **2** ($CDCl_3$, 400 MHz)

	Compound **1**						Compound **2**				
	^{1}H NMR	^{13}C	COSY	HMBC (H → C)	NOESY		^{1}H NMR	^{13}C	COSY	HMBC (H → C)	NOESY
1	1.78, *m*	35.9	2		3, 19	1α	2.30, *m*	33.6	2	5	2β, 8β, 11β
						1β	1.14, *m*				
2	1.66, *m*	28.8	1,3		19	**2**α	1.73, *m*	32.8	1		1b
						2β	2.05, *m*				
3	4.12, *m*	68.1	2		1, 4, 19	3	5.60, *m*	125.0	2β, 3		2β
4	5.78, *s*	134.9		2, 6, 10	3, 6	4	5.92, *br d* (*J* = 10.62)	128.5	3		3, 6
5		141. 5				5		140.4			
6	5.47, *dd* (*J* = 4.03, 2.75)	76.7	7		4, 7, 9	6	5.42, *t* (*J* = 3.66)	122.4	7		7
7α	2.45, *d* (*J* = 2.38)	38.7	6	6, 8	4, 6	7α	2.08, *m*	26.7	6 7α, 8β	5, 6	6 8β
7β	2.42, *d* (*J* = 2.20)					7β	2.39, *m*				
8		76.6				8	1.80, *m*	38.6	9α	9, 14	11β, 18, 19
9	1.76, *m*	48.7	11		6, 7	9	1.20, *m*	44.6	19		12α
10		37.9				10		35.4			
11α	2.30, *dd* (*J* = 14.10, 3.66)	35.4	9, 11β	9, 10, 12	9, 18, 19	11α	1.56, *m*	21.2	12, 9α 12		12β
11β	3.15, *d* (*J* = 14.10)		9, 11α			11β	1.34, *m*				
12		215.2				12α	1.50, *m*	40.1	11		17
						12β	1.40, *m*				18
13		64.6				13		48.3			
14		87.8				14		85.9			
15	1.80, *m*	38.5	16			15	2.12, *m*	22.9	16		16
16	1.92, *m*	29.5	15, 17		15, 17, 22	16α	2.20, *m*	28.1	17	13, 14, 17, 20	17, 15
						16β	1.75, *m*				
17	3.84, *t* (*J* = 8.97)	43.0	16	13, 16	16, 21	17	2.50, *m*	51.0	16	14, 20, 21, 22	12α, 16α
18	1.03, *s*	20.0		12, 13, 14, 17	11β, 22	18	0.73, *s*	16.3		8, 12, 13, 14, 17	21, 22, 8β, 12β
19	1.40, *s*	22.0		5, 9, 10	1, 2, 11β, $COCH_3$	19	0.90, *s*	18.7		1, 5, 9, 10	8β, 11β, 1β
20		123.1				20		122.6			
21	7.47, *d* (*J* = 2.57)	151.4	22	20, 22, 24	17	21	7.24, *d* (*J* = 2.56)	148.5	22	20, 24	18, 17
22	7.81, *dd* (*J* = 9.69, 2.57)	148.9	21, 23		16, 18, 23	22	7.82, *dd* (*J* = 2.56, 9.88)	146.8	21, 23	24	18, 16β, 23
23	6.30, *dd* (*J* = 9.69, 0.92)	115.9	22	20, 24	22	23	6.24, *d* (*J* = 9.88)	115.2	22	20, 24	
24		164.5				24		162.4			
$COCH_3$		171.6									
$CO\mathit{CH}_3$	2.03, *s*	21.5		$COCH_3$	19						

not be determined from the NMR spectra but, on biosynthetic grounds and because a literature search showed that bufadienolides in which hydroxy groups are present at this position have them in the β-orientation, it was assigned as such. The presence of a conjugated diene system was also evident from the alkene proton resonances at δ 5.60, δ 5.92 and δ 5.42 assigned to H-3, H-4 and H-6, respectively, as the resonances at δ 5.60 and δ 5.92 were coupled in the COSY spectrum and δ 5.92 and δ 5.42 showed correlations in the NOESY spectrum. The C-5 carbon resonance at δ 140.4 showed a correlation in the HMBC spectrum with the 3H-19 resonance at δ 0.90. These correlations supported the positioning of the diene system in the molecule.

Biosynthetically, triterpenoid derivatives would normally be oxygenated at C-3, usually having a hydroxy group, ketone or ester present. In compound **2**, however, the conjugated diene system could result from a dehydration reaction. The UV spectrum of this bufatetraenolide gave a distinct absorption maximum at 237 nm, which is in good agreement with that observed for a conjugated diene system (Bruice, 1995). The lactone ring also contains a conjugated diene system but this is affected by the carbonyl group and hence this second absorption maximum occurs at approximately 290 nm. The stereochemistry of H-8 was confirmed as β by the NOESY correlation seen between the H-8 and the 3H-18 and 3H-19 resonances. No NOESY correlation was seen between H-8 and H-9 confirming the α configuration for H-9 as expected. NMR data for compound **2** is given in Table 1.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Varian 400 MHz spectrometer. EIMS data were recorded on an Agilent MS 5973 instrument connected to a GC 6890, while HRMS data were recorded at the Cape Technikon on a Kratos HRMS 9/50 GC MS instrument and the FAB-MS was recorded using a Micromass 70-70E mass spectrometer with a *m*-nitrobenzyl alcohol matrix and xenon as the bombardment gas. Ultraviolet absorption spectra were obtained on a Varian DMS 300 UV visible spectrophotometer and Infrared spectra were recorded using a Nicolet Impact 400D Fourier-Transform Infra-Red (FT-IR) spectrometer.

3.2. Plant material and extraction

Bulbs of *Drimia robusta* Bak. (7.82 kg) were collected in February 2001 from God's Window, Mpumalanga, in South Africa and a voucher specimen (*N. Crouch 866*, NH.) retained. The material was dried and extracted with 2 L each of hexane, CH_2Cl_2, EtOAc and MeOH. The solvent was removed under reduced pressure to yield extracts of 2.36, 7.85, 2.54 and 3.59 g, respectively.

The bulbs of flowering plants of *U. epigea* R.A.Dyer (1.74 kg) were harvested near Vryheid in KwaZulu-Natal, South Africa during September 1999 and a voucher lodged for verification purposes (*Crouch 829*, NH). Finely chopped and dried material was extracted with methanol at room temperature for 24 h on a Labcon Mechanical shaker. Removal of the solvent under vacuum yielded 5.9 g of extract.

3.3. Chromatography

The hexane and MeOH extracts of *Drimia robusta* were fractionated using a hexane:CH_2Cl_2 and a CH_2Cl_2:MeOH step gradient, respectively, but did not contain any compounds of interest. The EtOAc extract (2.54 g) was separated using silica gel (Merck 9385) and eluted with an EtOAc:hexane step gradient. The acids, 4-hydroxy-3-methoxybenzoic acid, (4.21 mg), 3,4-dihydroxybenzoic acid, (8.70 mg) and *trans*-3-(4′-hydroxyphenyl)-2-propenoic acid, (4.03 mg) eluted with 2:3 EtOAc:hexane and were purified using repeated column chromatography. They were identified using NMR and mass spectrometry and by comparison of their physical and spectroscopic data against literature values (Dictionary of Natural Products on CD ROM, 2003; Pouchert and Behnke, 1993).

The CH_2Cl_2 extract of *Drimia robusta* (7.85 g) was separated in the same manner using a solvent system starting with 100% CH_2Cl_2 and then using an EtOAc:CH_2Cl_2 step gradient. 12β-Hydroxyscillirosidin (6β-acetoxy-3β,8β,12β,14β-tetrahydroxybufa-4,20,22-trienolide) (601.8 mg) and 6β-acetoxy-12-oxo-3β,8β,14β-trihydroxybufa-4,20,22-trienolide (4.63 mg), **1** were eluted with 1:4 CH_2Cl_2:EtOAc and purified with 100% EtOAc using repeated column chromatography. 12β-Hydroxyscillirosidin was identified using NMR and mass spectrometry and by comparison of its physical and spectroscopic data against literature values (Pohl et al., 2001).

The methanol extract of *U. epigea* (5.9 g) was dry-packed and separated over silica gel (Merck 9385) using CH_2Cl_2:MeOH (95:5) as the eluant to yield the bufadienolide, 14β-hydroxybufa-3,5,20,22-tetraenolide, **2** (10.6 mg). The structure of compound **2** was determined using 2D NMR and MS techniques.

6β-Acetoxy-3β,8β,14β-*trihydroxy*-12-*oxobufa*-4,20,22-*trienolide*, **1,** 4.63 mg, yellow amorphous compound. $\lambda_{max}^{CH_2Cl_2}$ nm (log ε): 296 (3.79). IR ν_{max}^{NaCl} cm^{-1}: 3460 (−OH), 2936, 1715(C=O), 1256, 1029. HRMS [M^+] at *m/z* 472.20981, $C_{26}H_{32}O_8$ requires 472.20972. EIMS 70 eV, *m/z* (rel. int.): 472 (11), 345 (18), 343 (22), 281(79), 271 (100), 215 (37), 199 (32), 194 (17), 174

(83), 169 (43), 156 (22), 145 (27), 99 (42). ^{1}H and ^{13}C NMR data are reported in Table 1.

14*β-Hydroxybufa*-3,520,22-*tetraenolide*, **2**, 10.6 mg, off-white, powdery solid. Mp: 215–217 °C. λ_{max}^{MeOH} nm (log ε): 237 (3.78) and 284 (3.46). IR ν_{max}^{NaCl} cm^{-1}: 3352, 2939, 1639, 1452, 1303, 1237, 1166, 1100. FAB-MS *m/z* 366.21958 ($C_{24}H_{30}O_3$ requires 366.21949). EIMS 70 eV, *m/z* (rel. int.): 366 (43), 323 (30), 145 (100), 91 (70), 55 (48). ^{1}H and ^{13}C NMR data are reported in Table 1.

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