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Bufadienolides from the southern African *Drimia depressa* (Hyacinthaceae: Urgineoideae)

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Abstract

Two bufadienolides, 3β,16β-dihydroxy-5β-bufa-20,22-dienolide and 16β-hydroxy-5β-bufa-20,22-dienolide-3β-*O*-β-D-galactoside, have been isolated from bulbs of the poisonous South African geophyte *Drimia depressa* (Hyacinthaceae).

Keywords: Hyacinthaceae; *Drimia depressa*; Bufadienolide; Bufadienolide glycoside; 3β,16β-dihydroxy-5β-bufa-20,22-dienolide; 16β-hydroxy-5β-bufa-20,22-dienolide-3β-*O*-β-D-galactoside

1. Introduction

Drimia depressa (Bak.) Jessop (Hyacinthaceae) of the subfamily Urgineoideae is a widespread bulb of open upland grassveld in the summer–rainfall region of southern Africa. Information on this species has largely been documented under the synonyms *Urginea capitata* (Hook.) Bak. and *Urginea depressa* Bak. The bulbs are ovoid, hypogeal, and emergence of the tufted leaves is usually hysteranthous. These leaves have been reported as fatally toxic to cattle, inducing gastro-enteritis with severe haemorrhagic diarrhoea (Mitchell et al., 1934). This is in notable contrast with observations for the toxic *Drimia macrocentra* (Bak.) Jessop (syn. *Urginea macrocentra* Bak.) which causes a marked constipation in poisoned cattle (Mitchell, 1927). Recent findings for *D. macrocentra* reveal this taxon to similarly possess bufadienolides (Moodley et al., submitted for publication). Curson (1927) noted the toxicity of *D. depressa* to sheep (similarly causes gastro-enteritis) and Steyn (1934) of the plant to rabbits (pronounced dyspnoea).

To the Sotho, plants of *D. depressa* are known as moretele, meaning 'he who causes to glide or slip'. It is used in traditional medicine as a good luck and protective charm, and as a means of inflicting harm on adversaries. Phillips (1917) reported that such is the esteem with which this species was held by the Sotho that it was believed capable of affecting the wellbeing of their entire nation, in what is today the state of Lesotho.

The compounds hellibrigenin-β-D-glucoside and hellibrigenol-β-D-glucoside have been reported to occur in *D. depressa* (as *U. depressa*). However, these compounds were identified using paper chromatography against hellibrigenin-β-D-glucoside and hellibrigenol-β-D-glucoside standards which were synthesized from helligrigenin. No other physical data was provided and as the work was undertaken in 1959, no NMR data were provided (Rees et al., 1959). These compounds were not found in the present study.

2. Results and discussion

The methanol extract of bulbs of *D. depressa* yielded two novel bufadienolides: 5β-3β,16β-dihydroxybufa-

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20,22-dienolide (**1**) and its 3β-*O*-β-D-galactoside derivative (**2**). The isolation of these compounds is consistent with chemotaxonomic trends for the Urgineoideae (Pohl et al., 2000) and the well-documented toxic character of the source plant (Mitchell et al., 1934).

The high resolution mass spectrum of compound **1** showed a molecular ion peak at *m/z* 386.2462 which indicated a molecular formula of $C_{24}H_{34}O_4$. The IR spectrum showed the typical bufadienolide lactone carbonyl stretch at 1702 cm^{-1} and hydroxyl group stretch at 3395 cm^{-1}. The presence of the bufadienolide ring was indicated in the ^1H NMR spectrum by three double doublet resonances at δ 7.56 ($J = 1.0$, 2.5 Hz), δ 7.77 ($J = 2.6$, 9.7 Hz) and δ 6.25 ($J = 1.0$, 9.7 Hz) which were assigned to H-21, H-22 and H-23, respectively. These three resonances showed correlations in the HMBC spectrum with the C-24 lactone carbonyl carbon resonance at δ 164.8. The resonance at δ 118.8 was assigned to C-20 due to correlations in the HMBC spectrum with H-21 and H-23 and this resonance also showed a correlation with a resonance at δ 2.16 (*d*, $J = 7.5$ Hz) which was assigned to H-17. The H-17 resonance showed a correlation with a resonance at δ 4.39 (H-16) which showed further coupling with the two H-15 resonances at δ 2.32 and δ 1.29. The two H-15 resonances showed a further correlation in the COSY spectrum with the resonance at δ 1.05, which was assigned to H-14. The corresponding C-17 and C-16 resonances occurred at δ 58.2 and δ 73.9, respectively. The chemical shift of the C-16 resonance indicated the presence of a hydroxyl group at this position. The H-14 resonance showed further coupling with the H-8 resonance at δ 1.47, and was, in turn, seen to be coupled to the H-9 resonance at δ 1.57.

The methyl group proton resonance at δ 0.85 was assigned as 3H-18 (due to correlations in the HMBC spectrum with the C-17 and C-14 resonances) and the second methyl group proton resonance at δ 0.99 was thus assigned as 3H-19. The corresponding C-19 resonance at δ 24.4 showed a correlation in the HMBC spectrum with the H-5 resonance at δ 1.76. The remaining oxymethine resonance at δ 4.03 (*bs*) was assigned to H-3α and the second hydroxyl group was placed at C-3β on biosynthetic grounds. Using the COSY and HSQC spectra, all remaining methylene protons and corresponding carbon resonances could be assigned. The stereochemistry of H-16 and H-14 were assigned as α due to correlations seen between these protons and H-17 which is known to be α. Correlations in the NOESY spectrum between protons of the β-orientated C-19 methyl group and the H-5 resonance, and then the H-5 resonance and the H-8 resonance established H-5 and H-8 as β (see Fig. 1).

FAB MS of **2** indicated a molecular formula $C_{30}H_{44}O_9$. Comparison of the ^1H and ^{13}C NMR spectra for **2** with those of **1** showed that they differed from each other only in the presence of a hexose sugar at C-3β. The HMBC spectrum showed correlations between the C-3 resonance and H-1′ which occurred as a doublet at δ 4.31 (*d*, $J = 7.9$ Hz), and correlations were seen between H-3α and H-1′ in the NOESY spectrum, confirming the site of attachment of the sugar. To confirm the identity of the sugar, **2** was subject to enzymic hydrolysis using β-glucosidase and analysis of the sugar component of the resulting

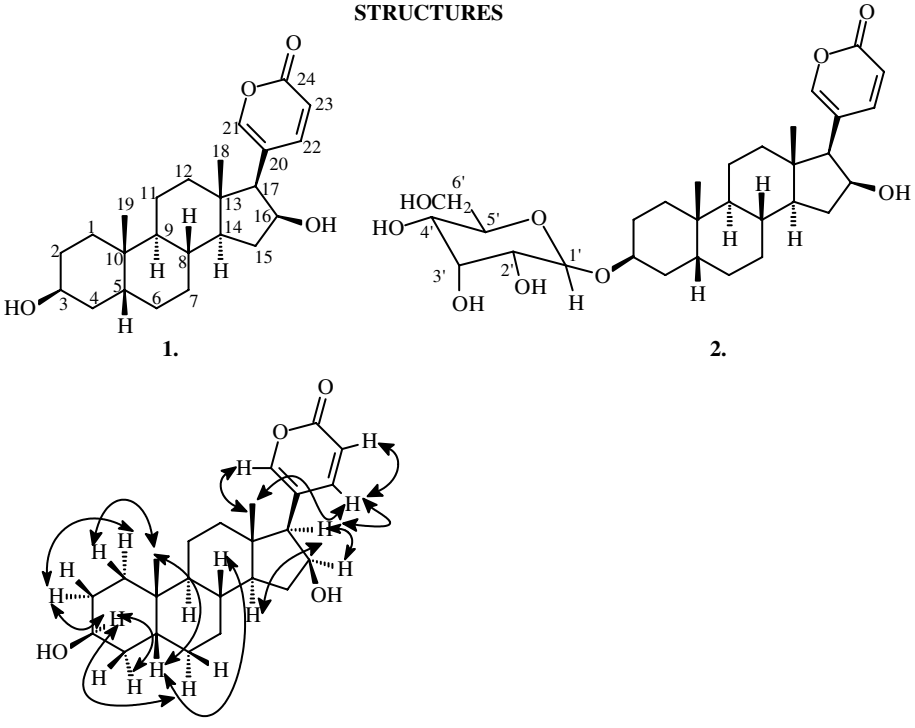

Fig. 1. NOESY correlations for **1**.

solution enabled its identification as β-D-galactose. The removal of β-D-galactose by β-glucosidase has been reported previously (http://www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/21.htm).

3. Experimental

Bulbs of *Drimia depressa* (Bak.) Jessop were collected from the Amatola Mountains near Stutterheim in the Eastern Cape Province of South Africa by R. McMaster during September 2001. A voucher has been lodged for verification purposes (*R. McMaster, s.n.*, NH). The finely chopped and dried material (873 g) was extracted successively with dichloromethane and methanol at room temperature for 48 h on a Labcon Mechanical shaker. The dichloromethane extract (6.47 g) was separated using gravity column chromatography over silica gel (Merck 9385) using a step gradient ($MeCl_2$/MeOH: 100/0 to $MeCl_2$/MeOH: 90/10) to yield 3β,16β-dihydroxy-5β-bufa-20,22-dienolide (**1**) (12.2 mg). The methanol extract (61.31 g) was separated in the same manner to yield 16β-hydroxy-5β-bufa-20,22-dienolide-3β-*O*-β-D-galactoside (**2**) (55.5 mg). Structures were determined using 2D NMR and MS techniques and the sugar by enzymatic hydrolysis of **2** followed by GC analysis of the sugar. The IR spectrum was recorded with a Nicolet Impact 400D spectrometer on sodium chloride plates and calibrated against an air background. The HRMS was obtained using a Kratos high resolution MS 9/50 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 400 MHz NMR spectrometer. The UV spectrum was recorded in methylene chloride on a Varian DMS 300 UV–visible spectrometer (see Table 1).

Table 1
NMR data for compounds **1** and **2** (CD_3OD, 400 MHz)

1			**2**		
Carbon number	^1H NMR data (400 MHz)	^{13}C NMR data (100 MHz)	Carbon number	^1H NMR data (400 MHz)	^{13}C NMR data (100 MHz)
1α	1.27 (1H, *m*)	31.0 (CH_2)	1	1.48 (2H, *m*)	31.5 (CH_2)
1β	1.44 (1H, *m*)				
2α	1.45 (1H, *m*)	28.5 (CH_2)	2	1.65 (2H, *m*)	27.7 (CH_2)
2β	1.54 (1H, *m*)				
3	4.03 (1H, *bs*)	67.7 (CH)	3	4.06 (1H, *m*)	75.5 (CH)
4α	2.00 (1H, *m*)	34.3 (CH_2)	4α	1.86 (1H, *m*)	31.0 (CH_2)
4β	1.30 (1H, *m*)		4β	1.54 (1H, *m*)	
5	1.76 (1H, *m*)	37.9 (CH)	5	1.82 (1H, *m*)	37.9 (CH)
6α	1.96 (1H, *m*)	27.6 (CH_2)	6α	1.65 (1H, *m*)	27.4 (CH_2)
6β	1.20 (1H, *m*)		6β	1.48 (1H, *m*)	
7	1.16 (1H, *m*)	27.8 (CH_2)	7α	1.17 (1H, *m*)	27.5 (CH_2)
			7β	1.92 (1H, *m*)	
8	1.47 (1H, *m*)	41.4 (CH)	8	1.46 (1H, *m*)	41.6 (CH)
9	1.57 (1H, *m*)	36.8 (CH)	9	1.56 (1H, *m*)	36.8 (CH)
10	–	36.4 (C)	10	–	36.1 (C)
11α	1.43 (1H, *m*)	21.5 (CH_2)	11α	1.46 (1H, *m*)	21.5 (CH_2)
11β	1.26 (1H, *m*)		11β	1.26 (1H, *m*)	
12α 12.β	1.11 (1H, *m*) 1.50 (1H, *m*)	39.3 (CH_2)	12α 12β	1.12 (1H, *m*) 1.50 (1H, *m*)	39.3 (CH_2)
13	–	45.0 (C)	13	–	45.0 (C)
14	1.05 (1H, *m*)	54.3 (CH)	14	1.06 (1H, *m*)	54.3 (CH)
15α 15β	2.32 (1H, *m*) 1.29 (1H, *m*)	37.8 (CH_2)	15α 15β	2.33 (1H, *m*) 1.30 (1H, *m*)	37.8 (CH_2)
16	4.39 (1H, *m*)	73.9 (CH)	16	4.37 (1H, *m*)	73.9 (CH)
17	2.16 (1H, *d*, $J = 7.5$ Hz)	58.2 (CH)	17	2.16 (1H, *d*, $J = 7.5$ Hz)	58.2 (CH)
18	0.85 (3H, *s*)	15.2 (CH_3)	18	0.85 (3H, *s*)	15.2 (CH_3)
19	0.99 (3H, *s*)	24.4 (CH_3)	19	0.98 (3H, *s*)	24.2 (CH_3)
20	–	118.8 (C)	20	–	118.8 (C)
21	7.59 (1H, *dd*, $J = 1.0$, 2.5 Hz)	152.3 (CH)	21	7.59 (1H, *dd*, $J = 2.5$, 1.0 Hz)	152.2 (CH)
22	7.77 (1H, *dd*, $J = 2.6$, 9.7 Hz)	151.2 (CH)	22	7.77 (1H, *dd*, $J = 2.5$, 9.7 Hz)	151.2 (CH)
23	6.25 (1H, *dd*, $J = 1.0$, 9.7 Hz)	114.4 (CH)	23	6.25 (1H, *dd*, $J = 1.0$, 9.7 Hz)	114.4 (CH)
24	–	164.8 (C)	24	–	164 (C)
			1′	4.31 (1H, *d*, $J = 7.9$ Hz)	102.6 (CH)
			2′	3.18 (1H, *m*)	75.1 (CH)
			3′	3.32 (1H, *m*)	78.2 (CH)
			4′	3.26 (1H, *m*)	71.6 (CH)
			5′	3.22 (1H, *m*)	77.8 (CH)
			6′a	3.64 (1H, *m*)	62.7 (CH_2)
			6′b	3.82 (1H, *m*)	

3.1. 3β,16β-Dihydroxy-5β-bufa-20,22-dienolide (**1**) (12.2 mg)

White amorphous, HRMS *m/z* 386.2462 ($C_{24}H_{34}O_4$ requires 386.245710). EIMS: *m/z* 386, 368, 355, 341, 328, 300, 273, 256, 231, 161, 140, 122, 109 (100%), 93, 81 (100%), 69, 55, 41. Optical rotation: $[\alpha]_D = +16.7°$ ($c = 0.006$ g/100 cm^3; CH_3OH).

3.2. 16β-Hydroxy-5β-bufa-20,22-dienolide-3β-O-β-D-galactoside (**2**) (55.5 mg)

Cream powdered substance, FAB MS: [M$^+$] at *m/z* 548, ($C_{30}H_{44}O_9$ requires 548). IR: ν_{max} (NaCl) cm^{-1}: 3395, 2928, 1702, 1454, 1028. Optical rotation: $[\alpha]_D = +66.4°$ ($c = 0.226$ g/100 cm^3; $CHCl_3$).

3.3. Enzymatic hydrolysis of **2**

A mixture of **2** (16 mg) and β-glucosidase (5 mg) (from *Cadocellum saccharolyticum* Sigma G6906) was shaken in a waterbath at 37 °C for 7 days. The resultant mixture was run through a high throughput sugar gas chromatography analysis system and the sugar was identified as galactose by comparing it to a range of standards. The instrument used was a Perkin–Elmer HPLC with Dionex Anion exchange column run with pure water. A post column adjustment to pH 14 (350 mM NaOH) was performed and pulsed amperometric detection was used.

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