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TWO BUFADIENOLIDES FROM *DRIMIA ALTISSIMA* (*URGINEA ALTISSIMA*)

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ABSTRACT. Two bufadienolides were isolated from the bulbs of *Drimia altissima* (*Urginea altissima*) and characterized on the basis of chemical degradation and spectroscopic evidence as arenobufagin-3-*O*-α-L-rhamnopyranoside and gamabufotalin-3-*O*-α-L-rhamnopyranoside, of which the former is a new compound. No alkaloids were detected in the material examined.

INTRODUCTION

Previously the genera *Drimia* Jacq. (s. str) and *Urginea* Steinh. (Family Liliaceae) comprised about 15 and 100 species, respectively. *Drimia* is wide-spread in South and East Africa and *Urginea* in Africa, Mediterranean area to India. It has now been proposed that the two be joined in one genus, *Drimia* [1] in the new family Hyacinthaceae. *Urginea altissima* (L.F.) Baker must now be referred to as *Drimia altissima* (L.F.) Ker-Gawl. This plant has been known to be poisonous to animals and often caused serious loss of stock in South and East Africa and Northern Zimbabwe [2]. Chemical investigations of some *Urginea* species have resulted in the isolation of flavonoids [3] and bufadienolides, which are known to have desirable cardiotonic properties [4,5].

Previous phytochemical studies on *U. altissima*, collected from Kenya, revealed the occurrence of six cardiotonic steroids [6,7]. In addition, the occurrence of two amaryllidaceae alkaloids has been reported [8]. However, Hegnauer [9] suggested that "the amaryllidaceous alkaloids lycorine and acetylcaranine ascribed to the bulbs of *U. altissima* [8], collected from Ethiopia, stemmed from misidentified amaryllidaceous bulbs". We have reinvestigated the bulbs of *U. altissima* in an attempt to prove the validity of the above suggestion. Thus, two bufadienolides were isolated and characterized as described below, however, no alkaloid was detected.

RESULTS AND DISCUSSION

The ground bulbs of *U. altissima* were extracted with ethanol and the residue obtained after removal of the solvent was taken up in 2% H_2SO_4. The soluble portion was extracted with $CHCl_3$. The aqueous acidic solution was then basified with aqueous NH_3 and extracted with $CHCl_3$. The two extracts were tested for the presence of alkaloids using the Dragendroff's reagent and gave negative results. Both $CHCl_3$ extracts were combined and subjected to

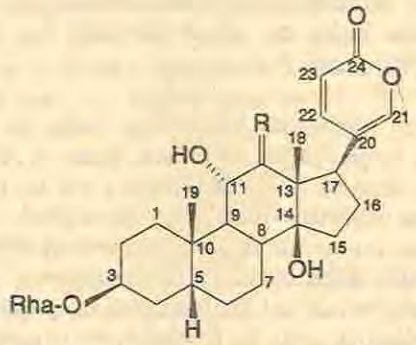
column chromatography over silica gel 60 to give two compounds as described in the Experimental section.

The ^{13}C NMR spectrum of compound **1** revealed the presence of 30 carbons and its molecular weight was determined to be 562 from its FABMS. The ^{1}H and ^{13}C NMR spectra together with ^{1}H-^{1}H COSY spectrum suggested compound **1** to be an *O*-glycoside, which was also substantiated by the MS data. The mass difference $[M+H]^+$-146 = $[G+H]^+$, which corresponds to the aglycone (*m/z* 417), required the presence of a 6-desoxyhexose as the sugar moiety [4,10]. The sugar was identified as L(+)-rhamnose on the basis of spectral data and further confirmed by co-PC after **1** was subjected to acid hydrolysis.

The downfield ABX pattern (δ 6.45 to 7.96) in the ^{1}H NMR spectrum together with the five carbon resonances at δ 115.9, 123.1, 149.2, 151.5 and 164.5 indicated the presence of a pyrone ring [11]. Furthermore, the signal observed at δ 214.9 confirmed the presence of a ketonic carbonyl group. With 30 carbons directly observed and 10 oxygens implied from the ^{13}C NMR spectrum of **1**, the molecular formula $C_{30}H_{42}O_{10}$ is required by its mass spectrum. Aside from the 11 carbon resonances of the rhamnose and pyrone units, the remaining 19 signals present a pattern which suggested that **1** contains a saturated steroid nucleus [12], a conclusion consistent with the molecular formula and with the presence of two upfield methyl signals in its ^{1}H NMR spectrum. This class of steroids, the bufadienolides, is characterized by *cis-anti-trans-syn-cis* steroid skeleton bearing 3ß- and 14ß-oxygen substituents and 17ß-pyrone substituent [10,11]. In addition to these, the carbon resonance at δ 214.9 and 73.7 in the ^{13}C NMR spectrum of **1** are assignable to the carbonyl group at C-12 and a hydroxylated C-11, respectively. The latter signal suggested 11α-hydroxylation, which is also compatible with the observed ß-effect of the neighbouring keto group and the remarkable upfield shifts of C-18 and C-19 [4]. Furthermore, the doublet at δ 4.4 (J = 11 Hz) assignable to 11ß-H proves the *trans* orientation of the protons at C-11 and C-9 and leads ultimately to the assignment of the aglycone moiety as 11α-hydroxy-12-oxo-bufalin or arenobufagin. The spectral data of the aglycone moiety of **1** were also found to correlate very well with those reported for arenobufagin [11,13]. The position of the glycoside linkage was confirmed by inspection of the glycosylation shifts. Thus, the C-3 resonance of arenobufagin appears at δ 64.4 [11,13] while that of **1** at 74.9. This pronounced deshielding requires placement of the rhamnose unit at this position. The anomeric proton of rhamnose appears at δ 4.8 as a doublet (J = 1.2 Hz) indicating the *eq-eq* relationship to the vicinal H-2', which clearly suggested the α-glycoside linkage [14]. This was further supported by the ^{13}C NMR data, in which the C-3' and C-5' signals appeared at significantly upper fields (δ 72.9 and 69.9, respectively) than the corresponding ß-anomer [15]. Moreover, the chemical shifts of C-2, 3 and 4 of **1** are also consistent with the expected α-rhamnosylation shifts [15].

On the basis of the above presented chemical and spectral evidence the structure of **1** was characterized as the new compound arenobufagin-3-*O*-α-L-rhamnopyranoside.

The molecular weight of compound **2** was determined to be 548 from its FABMS. The ^{1}H and ^{13}C NMR spectra showed very close patterns to that of compound **1** indicating that both compounds belong to the same class. Furthermore, following the same spectroscopic arguments forwarded for **1**, the bufadienolide-3-*O*-α-L-rhamnopyranosidenature of compound **2** was established. The only striking difference between the ^{13}C NMR spectra of **1** and **2** was the presence of a ketonic carbonyl resonance in **1**, which is not observed in that of **2**. The spectrum of **2**, however, revealed the presence of one additional methylene group at δ 51.5, consistent with the 14 amu difference in their molecular weights. The molecular formula of **2** was then established to be $C_{30}H_{44}O_9$. A more detailed examination of the spectral data of the aglycone moiety of **2** and comparison with that of **1** and reported bufalin derivatives, allowed



1 R = O
2 R = H_2

the characterization of the aglycone as gamabufotalin [5,16]. Based on the above discussed spectroscopic data and chemical degradation evidence compound **2** was identified as gamabufotalin-3-*O*-α-L-rhamnopyranoside, a known compound previously reported from *U. hesperia* [5].

Table 1. ^{13}C NMR data of compounds **1** and **2**

C	1	2	C	1	2
1	31.3	33.3	16	27.8	28.2
2	28.1	28.3	17	41.9	52.0
3	74.9	74.1	18	18.0	18.3
4	33.2	31.5	19	24.0	24.5
5	39.9	39.8	20	123.1	124.5
6	29.0	29.6	21	151.6	150.6
7	22.8	22.6	22	149.2	149.2
8	40.7	42.2	23	115.9	115.5
9	41.5	42.9	24	164.5	164.7
10	38.0	37.0	1'	99.9	99.9
11	73.7	69.2	2'	72.5	72.5
12	214.9	51.6	3'	72.9	73.0
13	63.7	50.0	4'	74.0	74.0
14	86.2	85.3	5'	69.9	69.9
15	33.7	34.1	6'	17.9	17.9

EXPERIMENTAL

General. Mps. are uncorrected. IR spectra were recorded as KBr discs. ^1H and ^{13}C NMR spectra were measured on Bruker WM 400 at 400 and 100 MHz, respectively.

Plant material. Bulbs of *D. altissima* were collected from Negele, Sidamo province , in Nov. 1986. A voucher specimen under the cipher ED-S030 was deposited in the National Herbarium, Addis Ababa University.

Extraction and isolation of compounds. The ground bulbs of *D. altissima* (1 kg) were extracted with EtOH at rt. by percolation for 3 days. Removal of the solvent yielded an oily residue (65 g) which was taken up in 2% H_2SO_4 (aq.) and stirred for 30 min. It was then filtered and the filtrate was extracted with $CHCl_3$ several times. The combined $CHCl_3$ was dried over Na_2SO_4 and concentrated to give a brown residue (560 mg). The aqueous layer was basified with dil. NH_3 solution and further partitioned with $CHCl_3$, which upon concentration gave 720 mg of extract and combined with the previous one. Both extracts were tested for the presence of alkaloids using the Dragendroff's reagent and gave negative results. The combined extract was subjected to column chromatography over silica gel 60 and elution was carried out using $CHCl_3$-MeOH mixtures of increasing polarities. A total of 20 fractions each 100 ml were collected and analyzed by TLC ($CHCl_3$-MeOH, 85:15). Fractions 4-7 (10% MeOH) contained mainly one spot on TLC which were combined and passed through Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) to give compound **1** (45 mg). Analogous treatment of fractions 13-17 (10% MeOH) yielded compound **2** (60 mg).

Acid hydrolysis of compounds 1 and 2. A solution of the sample and 2N HCl in MeOH was refluxed on a water bath for 1 h, which was then cooled and concentrated to dryness. The resulting residue was diluted with H_2O and extracted with $CHCl_3$. Paper chromatographic analysis of the aqueous layer revealed the presence of L(+)-rhamnose in both compounds **1** and **2**.

Arenobufagin-3-O-α-L-rhamnopyranoside (**1**). Colourless plates from MeOH, Mp 221-223°; IR υ_{max} cm^{-1}: 3500, 2970, 1740, 1720, 1640, 1550, 1460, 1390, 1250, 1060; ^1H NMR (400 MHz, MeOH-d_4) δ: 0.95 (3H, *s*, Me-18), 1.21 (3H, *s*, Me-19), 1.30 (3H, *d*, *J* = 6.3 Hz, Me-6'), 1.35-1.65 (10H, *m*), 1.70-2.25 (6H, *m*), 2.50 (1H, *bd*, H-5), 3.41 (1H, *dd*, *J* = 9, 9 Hz, H-4'), 3.70 (1H, *q*, *J* = 9, 6.3 Hz, H-5'), 3.74 (1H, *dd*, *J* = 9, 3.4 Hz, H-3'), 3.81 (1H, *dd*, *J* = 3.4, 1.2 Hz, H-2'), 3.97 (1H, *brs*, H-3α), 4.20 (1H, *t*, *J* = 7, 9 Hz, H-17α), 4.45 (1H, *d*, *J* = 10 Hz, H-11ß), 4.81 (1H, *d*, *J* = 1.2 Hz, H-1'), 6.36 (1H, *d*, *J* = 10 Hz, H-23), 7.57 (1H, *d*, *J* = 2 Hz, H-21), 7.96 (1H, *dd*, *J* = 10, 2 Hz, H-22); ^{13}C NMR (100 MHz, MeOH-d_4): see Table 1; FABMS *m/z* (rel. int.): 563 $[M+H]^+$ (100), 417 $[M+H-146]^+$ = $[G+H]^+$ (58), 399 $[G+H-H_2O]^+$ (82), 369 (26), 353 (33).

Gamabufotalin-3-O-α-L-rhamnopyranoside (**2**). Colourless plates from MeOH, Mp 122-125°; IR υ_{max} cm^{-1}: 3500, 2970, 1720, 1640, 1550, 1460, 1400, 1250; ^1H NMR (400 MHz, MeOH-d_4) δ: 0.78 (3H, *s*, Me-18), 1.11 (3H, *s*, Me-19), 1.28 (3H, *d*, *J* = 6.3 Hz, Me-6'), 1.40-2.20 (18H, *m*), 2.45 (1H, *d*, *J* = 10 Hz, H-5), 2.65 (1H, *t*, *J* = 7, 9 Hz, H-17α), 3.41 (1H, *dd*, *J* = 9, 9 Hz, H-4'), 3.6 (1H, *m*, H-11ß), 3.70 (1H, *q*, *J* = 9, 6.3 Hz, H-5'), 3.75 (1H, *dd*, *J* = 9, 3.4 Hz, H-3'), 3.80 (1H, *dd*, *J* = 3.4, 1.2 Hz, H-2'), 3.95 (1H, *brs*, H-3α), 4.80 (1H, *d*, *J* = 1.2 Hz, H-1'), 6.35 (1H, *d*, *J* = 10 Hz, H-23), 7.50 (1H, *d*, *J* = 2 Hz, H-21), 8.00 (1H, *dd*, *J* = 10, 2 Hz, H-22); ^{13}C NMR (100 MHz, MeOH-d_4): see Table 1; FABMS *m/z* (rel. int.): 549 $[M+H]^+$ (100), 403 $[M+H-146]^+$ =$[G+H]^+$ (20), 385 $[G+H-H_2O]^+$ (30), 367 (17), 349 (8).

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