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Bufadienolides from *Drimia robusta* BAK. [+]

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From bulbs of *Drimia robusta* 7 bufadienolides were isolated, 3 of them new natural compounds. Structure elucidation was performed by comparison with authentic substances or by means of ^{1}H-, ^{13}C-NMR-spectroscopy and FAB-MS. The substances were identified as scilliroside (**1**), 12ß-hydroxyscillirosidin (**2**), 12ß-hydroxyscilliroside (**3**), hellebrigenin-3-0-ß-glucoside (**4**), 16ß-hydroxyhellebrigenin (**5**), 16ß-hydroxyhellebrigenin-3-O-ß-glucoside (**6**) and 5ß,16ß-dihydroxybufalin-3-O-ß-glucoside (**7**).

Keywords: Drimia robusta, *Hyacinthaceae, cardiac glycosides, bufadienolides*

Introduction

Drimia robusta BAKER (Hyacinthaceae) is a bulbous plant from the eastern and north-eastern parts of South Africa [1]. The plant is known as „inDONGANA-ZIBOMVANA" as constituent of „Intelezi", the „magical" mixtures in Zulu and Xhosa medicine. The leaves are used for cleaning of the bladder and diseases of the uterus [2]. Apart from the medicinal use *Drimia robusta* is reported to cause intoxications in stock [3]. Due to the close botanical relationship of the genus *Drimia* to the genus *Urginea*, bufadienolides were assumed to be responsible for the effects [1]. Thus we investigated *Drimia robusta* for the presence of cardiac glycosides. Only recently the first chemical investigation of this species was published, showing the presence of proscillaridin A in bulbs and leaves [4].

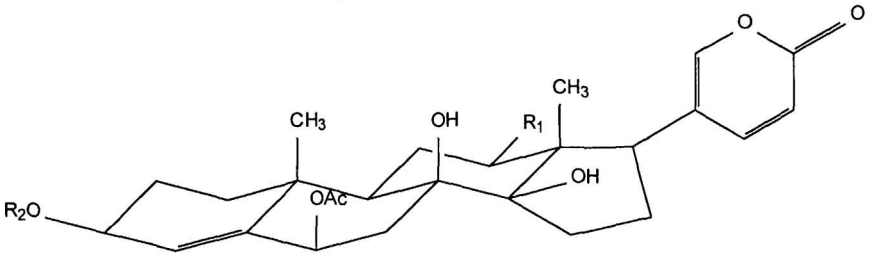
Results and Discussion

From a chloroform- and a chloroform-n-butanol extract from lyophilized bulbs of *Drimia robusta* seven bufadienolides were isolated by repeated CC. Along with four known substances (**1** - **4**) three new compounds (**5** - **7**) were obtained.

The identification of **1** - **4** was performed by direct comparison with authentic samples by TLC, **2** and **4** were additionally characterized by spectroscopic methods.

[+] XI[th] communication on bufadienolides; part of the diploma thesis V. Stapf, University of Vienna; X[th] communication see ref. [5]

Compound **1** was already known from samples of *Urginea maritima aggregate* from Turkey [6], Egypt [7] and Sardegna [8] as well as from *Urginea pancration* (STEINH.) DE PHILIPPE [9], *Urginea numidica* (JORD.& FOURR.) GREY [8] and from cultivated plants from California [10]. Substance **2** had been proved in *Urginea maritima agg.* from Egypt [7] as well as in *Urginea sanguinea* SCHINZ [11], **3** was isolated from *Urginea maritima agg.* from Egypt [7] and Sardegna [8] and from *Urginea sanguinea* SCHINZ [11] and *Urginea aphylla* (FORSKAL) SPETA [12]. Substance **4** had been found for the first time in *Urginea altissima* BAKER [13]. The structures of compounds **5** - **7** were determined on the basis of FAB mass, ^{1}H- and ^{13}C-NMR spectral studies.



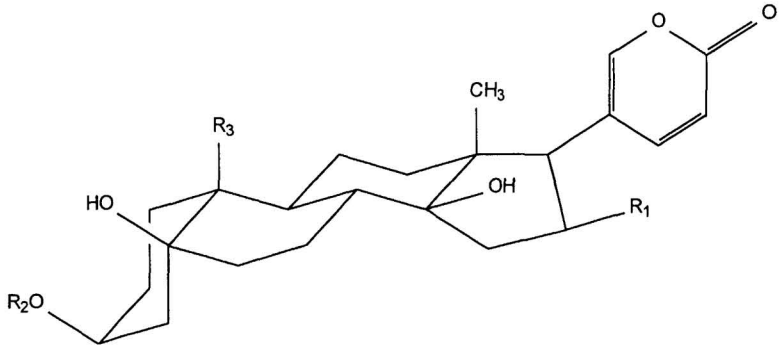
1 R_1 = H R_2 = -glucose
2 R_1 = OH R_2 = H
3 R_1 = OH R_2 = -glucose

The FAB-MS of **5** exhibited the $(MH)^+$ ion at m/z = 433. A signal at m/z = 405 = $(MH)^+$ - 28 indicated the presence of an aldehyde group, which was clearly identified by the characteristic ^{1}H-NMR resonance at δ = 10.08 ppm. The signal of the protons at C-18 was observed at 0.80 ppm. Due to the absence of signals between δ = 5 - 6 ppm in the ^{1}H-NMR spectrum the exsistence of a double bond in ring A could be excluded. The attachment of a hydroxyl moiety in position C-16 was proven by the doublet at δ = 2.70 ppm (17α-H). The coupling constant of 8 Hz indicated the cis-configuration of the protons at C-16 and C-17 [14]. The signal of a quarternary C at δ = 75.7 ppm in the ^{13}C-NMR spectrum pointed to a hydroxyl group at C-5. The comparison of the ^{13}C-NMR data of **5** and hellebrigenin [15] showed good correlations for the rings A, B and C, but variations in the substitution pattern of ring D. The chemical shift differences observed for C-15 ($\Delta\delta$ = +10.6 ppm), C-16 ($\Delta\delta$ = +45 ppm), C-17 ($\Delta\delta$ = +8.8 ppm) and C-20 ($\Delta\delta$ = -3 ppm) were in agreement with the connection of a hydroxyl group to position 16ß [16]. Based on these results **5** was identified as 16ß-hydroxyhellebrigenin.

After detection on the TLC-plates compound **6** showed the same colour as **5**, but higher polarity. By FAB-MS the molecular weight of 594 was determined. The fragmentation pattern of $(MH)^+$ -162 indicated **6** to be a glycoside with a hexose. The loss of 18 and

28 amu from the aglycone pointed to the presence of a hydroxyl and an aldehyde group. ^{1}H-NMR data of **6** were in good agreement with those of **5**. The doublet at δ = 4.67 ppm (J = 8 Hz) was assignable to the anomeric proton of the sugar moiety. By the upfield shift of C-2 ($\Delta\delta$ = -1.6 ppm) and C-4 ($\Delta\delta$ = -3.0 ppm) as well as the downfield shift of C-3 ($\Delta\delta$ = +5.9 ppm) in the ^{13}C-NMR spectrum the attachment of the sugar to O-3 was proven [16]. By its ^{13}C-NMR shifts the sugar unambiguously was determined as glucose. Thus, the structure of **6** was characterized as 16ß-hydroxyhellebrigenin-3-O-ß-glucoside.

From the FAB-MS of compound **7** a molecular weight of 580 was deduced. The loss of 162 amu indicated the presence of a hexose. The fragmentation pattern additionally proved the attachment of two hydroxyl groups to the genin. The ^{1}H-NMR spectrum showed the resonances of the methyl protons at C-18 and C-19 at δ = 0.84 ppm and δ = 1.04, respectively, and the doublet of the anomeric proton at δ = 4.67 ppm (J = 8 Hz). Like in **5** and **6,** the doublet at δ = 2.72 was assigned to the 17α-H, from the coupling constant of 8 Hz the ß-linkage of a hydroxyl group in position C-16 was deduced. This substitution pattern was confirmed by the ^{13}C-NMR shifts of C-14, C-15, C-16, C-17 and C-18. The signal of a quarternary C at δ = 81.9 ppm indicated the presence of a hydroxyl moiety at C-5. The calculated resonances of 5ß,16ß-dihydroxybufalin-3-O-ß-glucoside, based on the data of **6** and the shift differences of a methyl compared to an aldehyde group at C-10 [16], were in excellent agreement with the shifts determined, proving this structure for **7**.



4	R_1 = H	R_2 = -glucose	R_3 = -CHO
5	R_1 = OH	R_2 = H	R_3 = -CHO
6	R_1 = OH	R_2 = -glucose	R_3 = -CHO
7	R_1 = OH	R_2 = -glucose	R_3 = -CH_3

The main components of the bufadienolide complex of *Drimia robusta*, appr. 90 % of the total content, were **2** and **3**. In contrast to the results of Luyt et al. [4] proscillaridin A was not detected in *Drimia robusta* in this investigation. To sum up, it can be assumed, that bufadienolides are responsible for the effects of *Drimia robusta.*

Experimental

Plant material: Bulbs of *Drimia robusta* were obtained from a Zulu drug store in Durban. The material was identified by Doz. Franz Speta (OÖ Landesmuseum, Linz, Austria), a voucher specimen is on deposit at his private herbarium in Linz.

General: TLC: silica gel 60 F_{254} or RP-2 F_{254} precoated plates (Merck), system 1: $CHCl_3$-MeOH-H_2O (70:22:3.5), system 2: EtOAc-MeOH-H_2O 81:11:8, system system 3: $CHCl_3$-MeOH-H_2O (65:30:6), system 4: MeOH-H_2O 1:1 when using RP-2 plates. Detection: after 15 min. at 103-105°C spraying with 50% ethanolic H_2SO_4 [6]. CC: silica gel 60 (Merck, 0.063-0.200 mm). FAB-MS (positive ion mode [PIFAB-MS] and negative ion mode [NIFAB-MS]): Varian Mat 311 A; FAB - canon: Ion Tech Ltd., acceleration voltage = 2.2 kV, E(neutral): 6.0 keV, Xenon, T = 40°C, $p < 10^{-5}$ Torr, T_{inlet}: RT; matrix: glycerol. NMR: Bruker AM-250 spectrometer with Aspect-2000 computer; ^{1}H-NMR: SF = 250.13 MHz; ^{13}C-NMR: SF = 62.9 MHz, internal standard: TMS; solvent: $CDCl_3$-CD_3OD 1:1.

Extraction and isolation: The bulbs were cut and lyophilized (fr. wt. 5625 g, dry wt. 557 g). The powdered drug (total bufadienolide content = 0.26%, according to [17]) extracted as reported [18] yielding 2.4 g chloroform extract (bufadienolide content = 28.36%) and 19 g chloroform-n-butanol extract (bufadienolide content = 3.95%). The crude bufadienolide mixtures were fractionated by CC:

The less polar bufadienolide concentrate was submitted to CC on silica gel 60 Merck (8x75cm) with $CHCl_3$-MeOH-H_2O (90:3.5:0.2). The main bufadienolide each of fraction 9, 13, 15, 21, 23, and 24 was isolated by CC on silica gel with ethylacetate (watersaturated) - methanol mixtures of different polarity. The final purification of the substances was performed by CC on silica gel by using $CHCl_3$-MeOH-H_2O as mobile phase and led to the isolation of **1**, **2**, **3**, **4**, **5**, **7.** The chloroform-n-butanol extract was fractionated by CC on silica gel 60 Merck (8x 75cm) using $CHCl_3$-MeOH-H_2O (70:22:3.5) as mobile phase. The resulting fractions 2, 3, 7, 9 and 13 were purified by CC over silica gel, yielding **2**, **3**, **6** and **7.**

Scilliroside (**1**): amorphous, 13 mg, R_f values see reference [8].

12ß-hydroxyscillirosidin (**2**): amorphous, 236 mg, ^{1}H-NMR: 0.82 (s, 3H, H_3-18); 1.34 (s, 3H, H_3-19); 2.07 (s, 3H, $C\underline{H}_3$-CO at C-6); 5.82 (s, 1H, H-4); 6.32 (d, 1H, J = 10 Hz, H-23); 7.43 (d, 1H, J = 2 Hz, H-21); 7.94 (dd, 1H, $J_{21,22}$ = 2 Hz, $J_{22,23}$ = 10 Hz, H-22); ^{13}C-NMR: see table 1; FAB-MS: NIFAB-MS: m/z = 473 $(M - H)^-$; 431 = $(M - H)^-$ - 42; 413 = $(M - H)^-$ - 60; PIFAB-MS: m/z = 475 $(MH)^+$; 415 = $(MH)^+$ - 60.

12ß-hydroxyscilliroside (**3**): amorphous; 214 mg, R_f values see reference [8].

hellebrigenin-3-0-ß-glucoside (**4**): amorphous; 18 mg; ^{1}H-NMR: 0.70 (s, 3H, H_3-18); 4.42 (d, 1H, J = 8 Hz, H-1´); 6.30 (d, 1H, J = 10 Hz, H-23); 7.35 (d, 1H, J = 2 Hz, H-21); 7.89 (dd, 1H, $J_{21,22}$ = 2 Hz, $J_{22,23}$ = 10 Hz, H-22); 10.09 (s, 1H, C<u>H</u>O at C-10); ^{13}C-NMR: see table 1; FAB-MS: NIFAB-MS: m/z = 577 $(M - H)^-$; 549 = $(M - H)^-$ - 28; 531 = $(M - H)^-$ - 28 - 18; 415 = $(M - H)^-$ - 162; 397 = $(M - H)^-$ - 162 - 18; PIFAB-MS: m/z = 579 $(MH)^+$; 417 = $(MH)^+$ - 162; 399 = $(MH)^+$ - 162 - 18; 381 = $(MH)^+$ - 162 - 18 - 18;

16ß-hydroxyhellebrigenin (**5**): amorphous, 7 mg; ^{1}H-NMR: 0.80 (s, 3H, H_3-18); 2.70 (d, 1H, J = 8 Hz, H-17); 6.26 (d, 1H, J = 10 Hz, H-23); 7.35 (d, 1H, J = 2 Hz, H-21); 7.97 (dd, 1H, $J_{21,22}$ = 2 Hz, $J_{22,23}$ = 10 Hz, H-22); 10.08 (s, 1H, C<u>H</u>O at C-10); ^{13}C-NMR: see table 1; FAB-MS: NIFAB-MS: m/z = 431 $(M - H)^-$; 403 = $(M - H)^-$ - 28; PIFAB-MS: m/z = 433 $(MH)^+$; 415 = $(MH)^+$ - 18; 397 = $(MH)^+$ - 18 - 18;

16ß-hydroxyhellebrigenin-3-O-ß-glucoside (**6**): amorphous, 11 mg; ^{1}H-NMR: 0.80 (s, 3H, H_3-18); 2.72 (d, 1H, J = 8 Hz, H-17); 4.67 (d, 1H, J = 8 Hz, H-1´); 6.26 (d, 1H, J = 10 Hz, H-23); 7.39 (d, 1H, J = 2 Hz, H-21); 8.02 (dd, 1H, $J_{21,22}$ = 2 Hz, $J_{22,23}$ = 10 Hz, H-22); 10.09 (s, 1H, C<u>H</u>O at C-10); ^{13}C-NMR: see table 1; FAB-MS: NIFAB-MS: m/z = 593 $(M - H)^-$; PIFAB-MS: m/z = 595 $(MH)^+$; 433 = $(MH)^+$ - 162; 415 = $(MH)^+$ - 162 - 18; 387 = $(MH)^+$ - 162 - 18 - 28;

5ß,16ß-dihydroxybufalin-3-O-ß-glucoside (**7**): amorphous, 8 mg; ^{1}H-NMR: 0.84 (s, 3H, H_3-18); 1.04 (s, 3H, H_3-19); 2.74 (d, 1H, J = 8 Hz, H-17); 4.67 (d, 1H, J = 8 Hz, H-1´); 6.25 (d, 1H, J = 10 Hz, H-23); 7.40 (d, 1H, J = 2 Hz, H-21); 8.02 (dd, 1H, $J_{21,22}$ = 2 Hz, $J_{22,23}$ = 10 Hz, H-22); ^{13}C-NMR: s. table 1; FAB-MS: NIFAB-MS: m/z = 579 $(M - H)^-$; 417 = $(M - H)^-$ - 162; PIFAB-MS: m/z = 581 $(MH)^+$; 419 = $(MH)^+$ - 162; 401 = $(MH)^+$ - 162 - 18; 383 = $(MH)^+$ - 162 - 18 - 18;

Table 1: ^{13}C-chemical shifts in $CDCl_3$-CD_3OD (1+1) for **2** and **4 - 7**, in ppm, δ-values, TMS as internal standard

C-atom	2	4	5	6	7
1	38.2	18.6	18.0	18.4	25.0
2	28.5	25.5	27.3	25.8	25.0
3	67.8	74.2	67.2	73.1	74.8
4	134.5	35.6	38.1	35.1	30.8
5	141.0	74.2	75.7	75.5	81.9
6	76.7[a]	37.2	36.9	37.2	35.4
7	38.8	24.7	24.9	25.0	25.0
8	76.7	42.6	42.2	42.1	40.5
9	48.8	40.3	40.0	39.9	39.4
10	37.2	55.7	55.6	55.6	41.4
11	27.3	23.3	22.8	22.8	21.9
12	77.0[a]	41.1	41.7[a]	41.6	42.0
13	56.2	48.3	47.7	47.7	48.0
14	86.2	85.1	85.2	85.2	85.5
15	34.7	32.2	41.6[a]	41.6	42.0
16	29.7	29.4	73.2	73.1	73.4
17	47.7	51.4	58.7	58.7	58.8
18	12.7	16.2	16.8	16.8	16.9
19	21.6	209.5	209.8	209.0	16.9
20	124.4	124.0	119.5	119.5	119.7
21	150.4	149.4	151.7	151.6	151.7
22	148.8	148.6	151.7	151.6	151.7
23	115.2	115.6	113.0	113.0	113.1
24	164.4	164.9	162.3	164.0	164.3
$\underline{C}H_3COO$-	22.3				
$CH_3\underline{C}OO$-	170.8				
1'		101.3		101.3	107.1
2'		74.2		74.3	73.4
3'		77.2		77.3	77.4
4'		71.1		71.0	70.5
5'		77.7		77.5	77.4
6'		62.8		62.3	62.2

[a] Signal assignments in each column may be reversed

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