9-Hydroxyscilliphaeoside, a New Bufadienolide from *Urginea maritima*[1]

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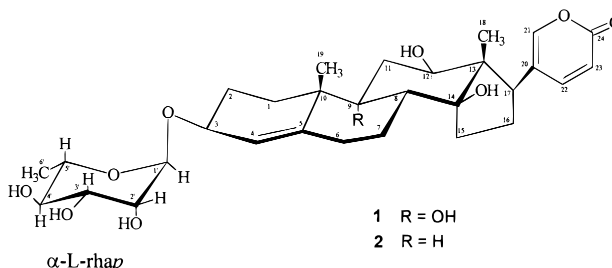
From bulbs of *Urginea maritima* (L.) *s.l.* was isolated 9-hydroxyscilliphaeoside (**1**) and its structure established by use of 2D NMR techniques (HH-COSY, TOCSY, ROESY, TOCSY−ROESY, HMQC, HMBC).

Urginea maritima (L.) Baker, *s.l.* (Hyacinthacea), commonly known as squill, can be found all over the Mediterranean coast. The plant has been used in medicine since early times because of its powerful digitalis-like cardiac effect. Bulbs of *U. maritima* from Israel and Egypt showed a very complex bufadienolide pattern, which differs widely from that of *Urginea aphylla* from Greece and Turkey and from that of *Urginea numidica* from Tunisia.[1] The investigation of samples from Egypt led to the isolation of more than 40 bufadienolides.[2] In this paper we report the isolation and structural investigation of a bufadienolide with a 9-hydroxyl function, which was obtained for the first time from nature.

The FAB-MS of **1** revealed a molecular weight of 562. The loss of a fragment of 146 mass units pointed to the presence of a 6-deoxyhexose. The fragment at *m/z* 417 in the positive ion FABMS represented a dihydroxylated bufadienolide genin. In the ^{1}H-NMR the doublet at δ 1.23 ppm ($J = 6$ Hz) corresponded to the protons of C-6 of the deoxyhexose; the one at δ 4.84 ($J = 1.5$ Hz), the anomeric proton. Beside the common signals of an α,β unsaturated γ-lactone in position 17β, the vinylic proton of C-4 appeared at δ 5.54 ppm. From the shift of the protons at C-18 (δ 0.62 ppm), as well as from the characteristic ^{13}C NMR resonances of C-13 and C-18, the attachment of a 12β-hydroxyl group was determined.[3] A further signal of a quaternary carbon atom at δ 76.4 ppm suggested the linkage of the second hydroxyl unit to C-8 or C-9. From the upfield shift of C-7 and C-12 as well as the downfield shift of C-10, compared to scilliphaeoside **2**, the hydroxylation in position C-9 was suggested. In the corresponding C-8 hydroxylated compound, which had been isolated from the same source and differed from its chromatographic behavior and color after detection,[2] C-7 and C-12 were shifted downfield although C-10 remained uninfluenced. The ^{1}H-NMR and ^{13}C-NMR shifts of the sugar moiety were in good agreement with those of α-L-rhamnopyranose.[2]

The use of 2D NMR techniques allowed the assignment of all the ^{1}H- and ^{13}C-NMR resonances and a clear determination of the structure of 9-hydroxyscilliphaeoside **1**. By means of an H,H-COSY[4] spectrum the partly overlapping resonances were assigned to six spin systems (1′-H/2′-H/3′-H/4′-H/5′-H/6′-H_3, 1-H_2/2-H_2/3-H/4-H, 6-H_2/7-H_2/8-H, 11-H_2/12-H, 15-H_2/16-H_2/17-H, 21-H/22-H/23-H) and two methyl groups. The spin system 6-H_2/7-H_2/8-H proved the hydroxylation in position C-9. These results were supported by a 2D TOCSY experiment.[5,6] The mixing time was long so that every proton gave correlation signals with all the other protons of the corresponding spin system. On the basis of the known 1H chemical shifts and proton−proton couplings, the unambiguous assignment of the protonated carbon atoms of the aglycon and the sugar moiety was carried out using a HMQC spectrum,[7] which reveals the one-bond C−H connectivities. The HMBC spectrum[8] enabled us to determine the sugar linkage site and the assignment of all of the non-protonated carbon atoms. Thus, the three-bond connectivity of C-9 at δ 76.4 ppm with the methyl C-19 protons at δ 1.17 ppm in the HMBC spectrum proved the hydroxylation in position 9. The ^{1}H and ^{13}C data of **1** are presented in Table 1, while further selected two- and three-bond connectivities of carbon atoms of the aglycon are given in Table 2. A 2D ROESY[9] and a 2D TOCSY-ROESY[10] experiment confirmed the closeness of the anomeric hydrogen atom of the rhamnopyranose to 3-H of the aglycon. The equatorial orientation of H-1 of the rhamnose unit was confirmed by the absence of ROESY cross peaks between 1-H and 3-H or 5-H (a cross peak is observed between 3-H and 5-H). The α-glycosidic linkage of the L-rhamnopyranosyl residue was also deduced from the chemical shifts of C-3′ (δ 71.8 ppm) and C-5′ (δ 69.0 ppm). These shifts allow one to distinguish between α- or β-glycosidic linkage.[11] Additionally, the sugar moiety was identified by GLC-MS analysis after methylation, acidic hydrolysis, and trimethylsilylation.[12] The structure of the isolate 9-hydroxyscilliphaeoside was determined to be that of **1**.



[1] Dedicated to Prof. Dr. Max Wichtl on the occasion of his 70th birthday.

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Table 1. ^{1}H- and ^{13}C-NMR Assignments for 9-Hydroxyscilliphaeoside (**1**)

Pos.	Carbon	δ (^{1}H)[a] **1**	δ (^{13}C) **1**	δ (^{13}C) **2**[16]
1	CH_2	1.95 (obsc.)/1.34 (td, 14, 4)	28.1	36.0
2	CH_2	1.95 (obsc.)/1.50 (br td)	27.8	27.5
3	CH-O-α-L-rha*p*	4.09 (br quint.)	74.0	74.3
4	CH=	5.54 (s)	126.5	121.3
5	C=		143.5	147.6
6	CH_2	2.26 (obsc.)/2.09 (dd, 15, 3)	31.8	33.0[d]
7	CH_2	1.90 (obsc.)/1.45 (dd, 14, 5)	23.3	29.3[e]
8	CH	1.94 (obsc.)	43.1	42.2
9	C-OH		76.4	47.5
10	C		44.8	37.8
11	CH_2	1.65/1.60 (obsc.)	34.5[b]	30.2
12	CH_2	3.64 (obsc.)	71.8[c]	75.8
13	C		54.8	55.1
14	C-OH		85.1	85.4
15	CH_2	2.31/1.58 (obsc.)	34.4[b]	32.8[d]
16	CH_2	2.04 (br t)/1.70 (br d)	29.6	29.2[e]
17	CH	3.06 (t)	46.5	46.7
18	CH_3	0.62 (s)	9.9	10.2
19	CH_3	1.17 (s)	21.9	19.2
20	C		124.0	124.1
21	CH=	7.38 (d, 2.5)	150.0	148.8
22	CH=	7.82 (dd, 2.5, 9.7)	148.7	148.8
23	CH=	6.23 (d, 9.7)	115.0	115.0
24	C=O		164.2	164.3
α-L-rhamnopyranose				
1′		4.84 (d, 1.5)	99.9	100.0
2′		3.79 (dd 1.5, 3.4)	71.7	71.9
3′		3.64 (obsc.)	71.8[c]	71.9
4′		3.34 (t, 9.7)	73.5	73.5
5′		3.66 (obsc.)	69.0	69.1
6′		1.23 (d, 6.0)	17.6	17.7

[a] Signal multiplicity and coupling constants (Hz) are in parentheses; obsc. indicates overlapping resonances. [b–e] Assignments with the same superscript are interchangeable.

Table 2. Selected HMBC Connectivities[a]

hydrogen	correlated carbon atom
4-H	C-2, C-6, C-10
12-H	C-17, C-18
17-H	C-12, C-13, C-20, C-21, C-22
18-H	C-12, C-13, C-14, C-17
19-H	C-1, C-5, C-9, C-10
1′-H	C-3

[a] One HMBC experiment optimized for $J = 7$ Hz was carried out.

Experimental Section

General Experimental Procedures. FABMS (positive-ion-mode = PIFABMS and negative-ion-mode = NIFABMS): 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 Spectroscopy. NMR spectra were recorded with a Bruker AMX-500 spectrometer (^{1}H frequency: 500.13 MHz, ^{13}C frequency: 125.76 MHz). An Aspect X32 computer employing UXNMR software was used for data processing: 5-mm inverse probehead; solvent; [D_4]MeOH/[D_1]$CHCl_3$ (1:1); temperature, 310 K. The MeOH signal was used as an internal standard (^{1}H, δ 3.3; ^{13}C, δ 49.0). The parameters were as follows: 90° pulses: ^{1}H, 10.2 μs; ^{13}C, 10.3 μs; WALTZ[13] ^{1}H-decoupling pulse, 112 μs; GARP[14] ^{13}C-decoupling pulse, 60 μs. **COSY**: 45° mixing pulse. **TOCSY**: phase-sensitive adjustment, TPPI,[15] mixing time 100 ms (100 MLEV-17 cycles plus 2 trim pulses of 2.5 ms each), 10 dB attenuation. **ROESY**: phase-sensitive adjustment, TPPI, spin-lock cw pulse (250 ms), 28 dB attenuation. **HMQC**: phase-sensitive using TPPI, BIRD sequence, GARP-decoupled. **HMBC**: phase-sensitive using TPPI, delay to achieve long-range couplings 71 ms ($J_{C,H} = 7$ Hz). We used NMR programs of Bruker except for 2D-TOCSY-ROESY. Pulse sequence for 2D TOCSY-ROESY, 90° $-t_1$ (incremented) − [MLEV 17] − t_2 (15 ms) − [spin lock] FID.

Plant Material. The bulbs of *Urginea maritima* (*L.*) *s.l.* were collected in El Arish, Egypt, in 1986. A voucher specimen is on deposit at the Institute of Pharmacognosy, University of Vienna, Austria.

Isolation. Extraction and fractionation were performed as previously described[3]: lyophilized bulbs (1.6 kg, bufadienolide content ca. 1.2%) yielded 6 g $CHCl_3$ (bufadienolide content 66%) and 19 g $CHCl_3$-EtOH (bufadienolide content 45%) extract. The crude glycoside mixtures were submitted to column chromatography on Si gel with $CHCl_3$-MeOH-H_2O mixtures of increasing polarity from 90:3.5:0.2 to 65:30:6, fraction size, 200 mL. 9-Hydroxyscilliphaeoside (**1**) was isolated from fractions 84−90 by column chromatography on Si gel with EtOAc-H_2O-saturated MeOH (95:5). The final purification of the substance was performed by DCCC using $CHCl_3$−MeOH−H_2O (5:10:6) in the ascending mode and led to the isolation of 12 mg pure, amorphous 9-hydroxyscilliphaeoside. The compound showed on Si gel TLC hundredfold R_f value (hRf) 40 using $CHCl_3$−MeOH−H_2O (70:22:3.5) and hRf 35 using EtOAc−MeOH−H_2O (81:11:8). GLC-MS was performed according to De Bettignies-Dutz.[12]

9-Hydroxyscilliphaeoside (1): amorphous, $C_{30}H_{42}O_{10}$ (562.6); PIFABMS, m/z 563 $(MH)^+$; 417 $(MH)^+$ −146; 399 $(MH)^+$ −146 − 18; 381 $(MH)^+$ −146 − 18 − 18; NIFABMS, m/z 561 $(M - H)^-$; 415 $(M - H)^-$ −146; ^{1}H- and ^{13}C-NMR, see Table 1.

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