ECDYSTEROIDS FROM *Silene claviformis*

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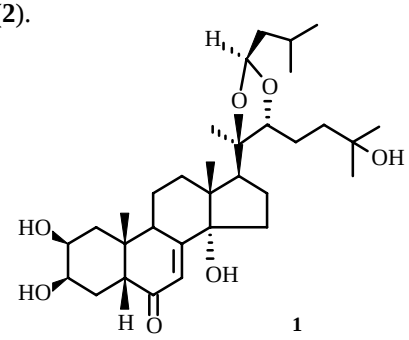
The ecdysteroid content of Silene claviformis *has been analyzed and the four major compounds have been identified as 20-hydroxyecdysone, 2-deoxy-20-hydroxyecdysone, 2-deoxyecdysone, and integristerone A. Two new minor compounds have also been identified.*

Key words: 2-Deoxyecdysone, 2-deoxy-20-hydroxyecdysone, 20-hydroxyecdysone, and integristerone A. NMR (1D ^{1}H and ^{13}C, 2D COSY, TOCSY, PFG-HMQC, and PFG-HMBC).

Silene claviformis is a perennial grassy plant (size 15-25 cm) growing on the slopes of the lower belt of Tashkent and Samarkand districts mountains. It is widespread in Middle Asia. The *Silene* genus has been much investigated and shown to contain many ecdysteroid-containing species [1-4], but no phytochemical data are available to date on this species. During the present study, besides already known ecdysteroids (i.e., 2-deoxyecdysone, 2-deoxy-20-hydroxyecdysone, 20-hydroxyecdysone, and integristerone A), the extract of *Silene claviformis* yielded two new ecdysteroids termed 20,22-isovaleriate-5β-cholest-7-ene-2β,3β,14α,20R,22R,25-hexahydroxy-6-one (**1**) and 20,22-episovaleriate-5β-cholest-7-ene-2β,3β,14α,20R,22R,25-hexahydroxy-6-one (**2**).



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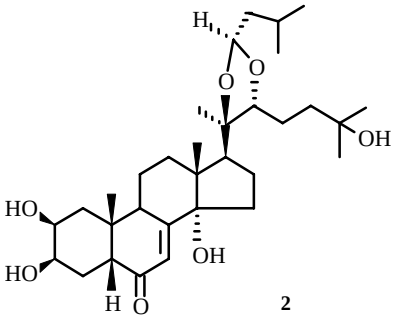

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The chemical ionization/desorption MS of compound **1** revealed the molecular mass 548, suggesting the empirical formula $C_{32}H_{52}O_7$. In its ^{1}H and ^{13}C NMR spectra (Table 1), the signals for the steroidal ring system were identical to those of 20-hydroxyecdysone, but signals for the 22-H (δ 3.82), C-20 (δ 86.4), and C-22 (δ 83.2) were more deshielded and thus suggested the modification of the side-chain at C-20 and C-22. The presence of two new doublet methyl signals in the region δ 0.7–1.3 ppm and of a new triplet signal hereafter termed α-H (δ 5.29; t : 5.1; 1H) was observed. The identity of the side-chain modification was deduced from this new triplet signal α-H and the two new doublet methyl signals which could be correlated thanks to ^{1}H 2D COSY and TOCSY experiments. The correlations observed are in agreement with the presence of an alkyl chain: (>CH–CH_2-CH(CH_3)$_2$). The ^{1}H and ^{13}C chemical shifts observed for the α-H (δ 5.29) and C-α (δ 104.30) are in accord with the formation of an acetal obtained from isovaleraldehyde (3-methylbutyraldehyde). The chemical shift deshielding for C-20 (δ 86.4) and C-22 (δ 83.2) as mentioned above support the attachment of the acetal unit at C-20 and C-22. This was confirmed from the ^{1}H-^{13}C long-range coupling observed in the PFG-HMBC spectrum between α-H and C-20 and C-22.

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TABLE 1. NMR Data of Compound **1** in D_2O (δ, ppm, J/Hz)

Position		Compound **1**	
		Carbon**	Proton
1	1-Ha	36.4	1.38
	1-He		1.88
2	2-Ha	*	3.99 (m, $w_{1/2}$ = 22)
3	3-He	68.2	4.08 (m, $w_{1/2}$ = 8)
4	4-Ha	32.3	1.75
	4-He		1.75
5	5-H	51.3	2.36 (t°)
6	-	*	
7	7-H	*	5.98 (d, 2)
8	-	*	
9	9-Ha	34.9	3.11 (m, $w_{1/2}$ = 23)
10	-	39.4	
11	11-Ha	*	1.73
	11-He		1.86
12	12-Ha	31.9	1.96
	12-He		
13	-	47.9	
14	-	*	
15	15-Ha	*	2.05
	15-Hb		1.68
16	16-Ha	*	1.95
	16-Hb		
17	17-H	50.7	2.32 (t, 8.1)
18	18-Me	17.8	0.85 (s)
19	19-Me	24.2	1.00 (s)
20	-	86.4	
21	21-Me	20.1	1.27 (s)
22	22-H	83.2	3.82 (m, $w_{1/2}$ = 16)
23	23-Ha	*	1.55
	23-Hb		1.73
24	24-Ha	41.4	1.55
	24-Hb		
25	-	72.7	
26	26-Me	28.7	1.237 (s)
27	27-Me	28.7	1.241 (s)
Other			
a	a-H	102.7	5.29 (t, 5.1)
b	b-H	24.1	1.54
c	c-H	44.0	1.75
d	d-Me	23.3	0.943 (d, 6.5)
d′	d′-Me	23.3	0.948 (d, 6.5)

**δ ^{13}C assigned from PFG-HMQC and PFG-HMBC spectrum [9].

t° Triplet-like, 4-H isochromous.

*Signal not detected, too low concentration.

Compound **2** had the same molecular mass as **1** and presented the same NMR features except a triplet (δ 5.07; t: 5.4; 1H) in place of the triplet signal α-H (δ 5.29; t: 5.1; 1H) for **1**. This triplet could be assigned to the signal observed for an epimer of **1** at the asymmetric carbon of the acetal function, and 2 was tentatively assigned to the epimer of **1**.

This unexpected result raises some doubts about the endogenous origin of compounds **1** and **2**, which could alternatively have arisen from a reaction of 20-hydroxyecdysone (the major ecdysteroid) with small amounts of isovaleraldehyde present in

the isoamyl alcohol used during sample processing. On the other hand, it should be mentioned that several natural ecdysteroid mono- and di-acetonides have been previously isolated from various plant sources [5].

The *Silene* genus comprises ca. 700 different species and its taxonomy appears very complex [6]. The question as to whether ecdysteroids may have a chemotaxonomic value for this genus can be addressed in two different ways : (1) a qualitative approach (i.e., their presence/absence), which was previously undertaken by Zibareva [4] and appeared very promising, and (2) a quantitative analysis of ecdysteroid patterns in positive species. A comparison of *Silene claviformis* with other previously investigated *Silene* species shows an ecdysteroid pattern very close to that observed in *S. otites* : the main characteristics are the predominance of 20-hydroxyecdysone and the presence of significant amounts of 2-deoxyecdysteroids (2-deoxyecdysone and 2-deoxy-20-hydroxyecdysone) and of integristerone **1** [3, 7, 8]. Other species such as *S. nutans* contain essentially 20-hydroxyecdysone and polypodine B (a 5β-OH derivative) and their 26-hydroxylated derivatives, but almost no 2-deoxyecdysteroids. Preliminary investigations performed on other *Silene* species (Zibareva, Volodin, Saatov, Lafont, and Dinan, in preparation) have shown that most of them belong to one of these two patterns, and it is expected that additional investigations will allow one to determine whether ecdysteroid patterns may indeed have a chemotaxonomic significance for the *Silene* genus.

EXPERIMENTAL

All ecdysteroids were identified by UV, EIMS, and NMR (1D ^{1}H and ^{13}C spectra and 2D COSY, TOCSY, PFG-HMQC, and PFG-HMBC). MS spectra were recorded on a Jeol MS 700 spectrometer equipped with a direct inlet probe. Spectra were recorded in the chemical ionization/desorption mode using ammonia as a reagent gas. NMR spectroscopy experiments were run at 500 MHz for ^{1}H, at 300 K, on a Bruker AMX 500 spectrometer equipped with a Silicon Graphics workstation. A presaturation of the solvent was used for all the 1D and homonuclear 2D ^{1}H experiments [9]. The samples were lyophilized twice in D_2O and then redissolved in D_2O. $TSPD_4$, 3-(trimethylsilyl) [2,2,3,3-d_4] propionic acid, sodium salt, was used as internal reference for the proton and carbon shifts.

An EtOH extract of stems and leaves (3.7 kg dry weight) was evaporated to dryness and the gummy residue (46.0 g) was sequentially extracted with chloroform (500 mL), ethyl acetate (250 mL), and finally isoamyl alcohol (250 mL). This last fraction was used for NP-HPLC analysis (semi-preparative Zorbax®-SIL column, 250 mm long, 9.4 mm i.d., solvent: dichloromethane-isopropanol-H_2O, 125:25:2, 4 mL/min) which yielded 4 major ecdysteroid-containing fractions (retention times: 11.2, 16.2, 36.6, and 46.8 min, respectively).

The three more polar ones were further purified by NP-HPLC (analytical Zorbax®-SIL column, 250 mm long, 4.6 mm i.d., solvent: cyclohexane-isopropanol-H_2O, 100:30:0.5, 1 mL/min) and yielded respectively pure 2-deoxy-20-hydroxyecdysone (ret. 16.2 min), 20-hydroxyecdysone (ret. 34.4 min, major ecdysteroid, ca. 0.15% of plant dry weight), and integristerone **1** (ret. 51.2 min), whereas the less polar one was further purified by RP-HPLC (analytical Spherisorb 5-ODS2 column, 250 mm long, 4.6 mm i.d., solvent: 45% acetonitrile-isopropanol (5:2) in H_2O, 1 mL/min) and yielded 2-deoxyecdysone (ret. 4.4 min) together with two unknown ecdysteroids (compounds **1** and **2**, ret. 21.3 and 23.8 min, respectively).

Compound 1. $C_{32}H_{52}0_7$. Mass spectrum: major ions at m/z 566 $(M+H+NH_3)^+$, 549 $(M+H)^+$, 531 $(M+H-H_2O)^+$, 464, 462, 447, 445, 429, and 427.

Compound 2. $C_{32}H_{52}0_7$. Mass spectrum: major ions at m/z 566 $(M+H+NH_3)^+$, 549 $(M+H)^+$, 531 $(M+H-H_2O)^+$, 464, 462, 447, 445, 429, and 427.

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