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The first two ecdysteroids containing a furan ring from *Serratula wolffii*

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Abstract

Two new ecdysteroids, named serfurosterone A and serfurosterone B, were isolated from a methanol extract of the roots of *Serratula wolffii*. Spectroscopic methods revealed that these compounds had previously unknown ecdysteroid structures with acetal functions in the side-chains.
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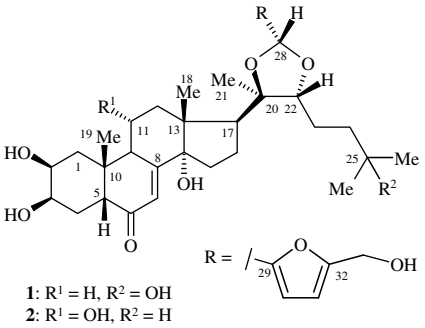
The cascade of morphological changes in insects is triggered by a group of steroid hormones known as ecdysteroids.[1] The phytoecdysteroids, compounds related to insect hormones, also occur in high concentration with diverse structures in several plant species.[2] *Serratula* species have proven to be rich sources of ecdysteroids.[3] *S. wolffii* Andrae (Asteraceae), which is native to the continental climate, is one of the most promising such species, which is cultivated in Hungary.[4]

Ecdysteroids are of great interest for their biological activities. Besides their beneficial pharmacological effects (e.g., anabolic action without androgenic side-effects, and also hypoglycemic and hypocholesterolaemic effects), phytoecdysteroids are inducers of the gene regulation system.[5]

We report here the isolation and structure elucidation of two ecdysteroids, serfurosterone A (**1**) and serfurosterone B (**2**), the first two ecdysteroids found to contain furan ring substituents.

The *S. wolffii* sample examined was collected from Herencsény, Hungary, in 2003. Its roots were extracted with methanol and the extract (208.9 g) was purified by fractional precipitation and column chromatography on polyamide. The fraction eluted with water (24.4 g) from the polyamide was subjected to low-pressure reversed-phase column chromatography on octadecyl silica. Further separation of the fractions containing the ecdysteroids (390 mg and 70 mg) was achieved by rotation planar chromatography on silica, using CH_2Cl_2–MeOH–C_6H_6 (50:10:6, v/v/v) and EtOAc–EtOH–H_2O (80:2:1, v/v/v) as mobile phases and by reversed-phase-HPLC. These separation steps furnished compounds **1** (0.5 mg, which represents 0.00024% of the extract) and **2** (0.5 mg, which represents 0.00024% of the extract).[6]

The structures of **1** and **2** (Fig. 1) were elucidated by using NMR, UV and MS measurements.[7] The UV spectra (DMSO) of **1** and **2** verified the presence of the 7-en-6-one



1: R^1 = H, R^2 = OH
2: R^1 = OH, R^2 = H

Fig. 1. Structures of compounds **1** and **2**.

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chromophore in both the structures.[8] The molecular formula, established by high-resolution measurements of the protonated molecular ion peaks in the HRESIMS, was $C_{33}H_{48}O_9$ in each case. The HRESIMS indicated pseudomolecular ions at *m/z* 589.3393 $[M+H]^+$ for **1** and at *m/z* 589.3389 $[M+H]^+$ for **2** (calcd: 589.3363). The peaks at *m/z* 519 $[M+K-R']^+$, 463 $[M+H-H_2O-R']^+$ and 445 $[M+H-2H_2O-R']^+$ in the ESIMS spectra of **1** and **2** supported the presence of a furan ring-containing substituent ($R' = C_6H_4O_2$) in the two molecules. The ^1H and ^{13}C NMR chemical shifts of **1** and **2** are presented in Table 1. Due to the low quantities of the samples of **1** and **2**, some quaternary signals remained under the noise level in the APT and ^{13}C NMR spectra. Their chemical shifts were determined from HMBC spectra. The characteristic HMBC correlations of the methyl signals over two and three bonds were utilized in their assignments. Their mutual HMBC correlations made the identification of the geminal Me-26 and Me-27 groups unambiguous. The singlet multiplicity of the signals of H_3-26 and H_3-27 and the high value of the ^{13}C chemical shift of C-25 (71.4 ppm) verified the existence of the 2-hydroxyisopropyl moiety in **1**. Differentiation between H_3-19 and H_3-18 was achieved by considering the coupling of the latter with C-17, which is also coupled to H_3-21. In both **1** and **2**, the high chemical shifts of C-20 and C-22 (83–86 ppm) proved the oxygen substitution. The H-22/H-28 NOESY correlation in **2** and the chemical shift of C-28 (96.5 ppm) verified the existence of an acetal-type five-membered ring. Moreover, the H-28/C-29, H-33/C-31 and H-33/C-32 HMBC cross-peaks and the H-28/H-30 NOESY correlations revealed a 5-hydroxymethyl-furfurylidene substituent on C-28. The characteristic ^{13}C chemical shifts and the low coupling value $^3J_{H\text{-}30,H\text{-}31} = 3.0$–$3.2$ Hz furnished further support for the structure.[9] The similar chemical shifts and signal multiplicity of H-28, H-30, H-31, H-33 and C-22 likewise indicated the presence of the 5-hydroxymethyl-furfurylidene unit in **1**.

Table 1
The ^1H (500 MHz) and ^{13}C (125 MHz) chemical shifts, multiplicities (m) and couplings constants (*J*) of compound **1** (MeOH-d_4) and compound **2** (DMSO-d_6) (δ in ppm, *J* in Hz)

No.		**1**			**2**		
		^{13}C	^1H	m; *J* (Hz)	^{13}C	^1H	m; *J* (Hz)
1	α	37.3	1.79	dt; 13.2, 4.2	38.2	2.46	dd; 12.5, 4.1
	β		1.42	dd; 13.2, 12.0		1.15	t; 11.9
2	α	68.5	3.84	dt; 12.0, 3.4	66.9	3.77	
3	α	68.3	3.95	s; br	66.5	3.76	
4	α	32.7	[a]1.75		32.0	1.62	
	β		[a]1.71			1.46	
5	β	52.1	2.39	dd; 12.6, 4.2	51.1	2.14	dd; 13.1, 3.8
6			—	—		—	—
7		122.0	5.82	d; 2.6	121.0	5.63	d; 2.6
8			—	—	162.6	—	—
9	α	35.0	3.15	ddd; 8.9, 2.6, 10.8	41.2	2.98	dd; 8.9, 2.6
10		39.1	—	—	40.5	—	—
11	α	31.7	1.62		67.2	—	—
	β		1.62			3.87	ddd; 10.9, 9.1, 5.7
12	α	32.2	2.11	td; 12.6, 4.2	42.0	2.08	dd; 12.1, 10.9
	β		1.85			1.93	dd; 12.1, 5.7
13		48.0	—	—	46.6	—	—
14		85.4	—	—	82.7	—	—
15	α	n.d.	n.d.		30.2	1.535	n.d.
	β		n.d.			1.85	n.d.
16	α	n.d.	n.d.		21.3	1.84	n.d.
	β		n.d.			1.84	n.d.
17	α	51.1	2.41	t; 9.1	49.2	2.30	t; 8.6
18	β	17.5	0.86	s	17.5	0.69	s
19	β	24.1	0.96	s	24.08	0.90	s
20		85.6	—	—	83.9	—	—
21		23.6	1.30	s	21.2	1.20	s
22		85.7	3.79	dd; 9.4, 2.3	83.4	3.69	dd; 9.4, 2.6
23	a	n.d.	n.d.		26.0	1.44	
	b		n.d.			1.51	
24	a	41.7	1.53	td; 13.2, 4.2	35.8	1.25	
	b		1.53	td; 13.2, 4.2		1.40	
25		71.4	—	—	27.5	1.58	dt; 13.3, 6.6
26		28.7	1.20	s	22.33	0.88	d; 7.0
27		29.2	1.21	s	22.44	0.89	d; 6.6
28		98.2	5.79	s	96.5	5.74	s
29			—	—	150.3	—	—
30			6.42	d; 3.0	109.9	6.45	d, 3.2
31			6.28	d; 3.0	107.5	6.25	d, 3.2
32			—	—	156.1	—	—
33	a,b	57.2	4.51	s	55.7	4.37	s

[a] Tentative, n.d. = no data observed.

In accordance with a 6-oxo-7-en-6-one moiety, H-7 correlates over $^3J_{C,H}$ couplings with C-5, C-9 and C-14. The hydrogen atoms of ring A form a common spin system analyzed by ^1H, ^1H-COSY and HMQC-TOCSY experiments. The signals of rings C and D, and of the side-chain on C-17, were assigned in an analogous way.

From the H_α-9/H_α-2 and H_3-19/H_β-5 NOESY correlations in **2**, the cis junction of rings A/B is clear. The H_β-12/H_3-18, H_β-12/H_3-21 and H_α-12/H_α-17 cross-peaks confirmed the trans junction of rings C/D. The H_3-18/H-11 NOESY cross-peak and the multiplicity of the H_α-9 signal verified the β-position of H-11.

In **2**, the H_β-12/H_3-21, H_3-18/H_3-21, H_3-18/H-30, H-22/H_2-16 and H-22/H-28 NOESY correlations revealed the steric arrangement of the side-chain and the absolute configurations of C-20 and C-22 as shown in Figure 2.

Compounds **1** and **2** are the first two ecdysteroids known to contain a furan ring: they are acetals of

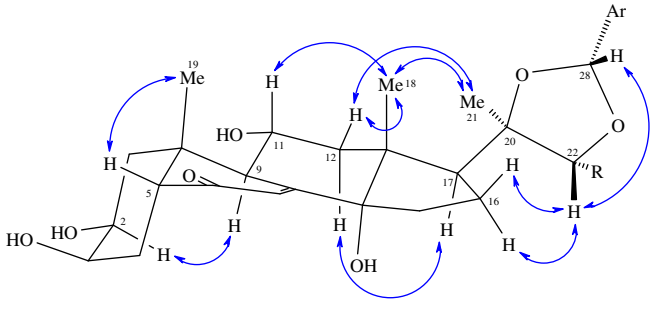

Fig. 2. Steric structure of compound **2**. Ar = 5′-hydroxymethyl-furfurylidene, R = $CH_2CH_2CHMe_2$.

5-hydroxymethyl-furfural and 20-hydroxyecdysone in the case of **1** and ajugasterone C in the case of **2**. Structurally related ecdysteroids with an acetal function in the side-chain, 20-hydroxyecdysone 20,22-ethylidene and ajugasterone 20,22-ethylidene, were isolated earlier from *Serratula coronata*.[10]

The plants often biosynthesize C-22 conjugated ecdysteroids to produce defensive constituents against insects. The two isolated compounds are a new type of C-22 conjugated ecdysteroid. These ecdysteroid derivatives are not detectable by the taste receptors of insects and might be hydrolyzed in the guts of insects to the active parent ecdysteroids.

Compound **2** contains an 11α-hydroxy group. Structure/activity experiments have confirmed that the 11α-hydroxy group in ecdysteroids is important for the manifestation of anabolic activity. The protein synthesis-enhancing the effect of turkesterone, an 11α-hydroxylated ecdysteroid, is comparable to that of Nerobol.[11]

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6. *Isolation of* **1** *and* **2**: The roots of *S. wolffii* (4.7 kg) were extracted with MeOH at room temperature, and the extract was subjected to fractional precipitation with acetone. The dry residue of the purified extract was chromatographed on a column of MN-polyamide SC 6 (Woelm, Eshwege, Germany). The fraction eluted with water (24.4 g) was separated by low-pressure reversed-phase column chromatography on octadecyl silica. The fraction eluted with 50% MeOH–H_2O (390 mg) was fractionated by repeated rotation planar chromatography (Harrison Model 8924 Chromatotron) on silica. In the second chromatographic step, the fraction eluted with EtOAc–EtOH–H_2O (80:2:1, v/v/v) was purified by reversed-phase HPLC (Zorbax SB C_{18} 250 × 4.6 mm i.d.; ACN–H_2O, 35:65, v/v, 0.8 ml/min) to obtain **1** (0.5 mg). The fraction (70 mg) eluted from the reversed-phase column with 60% MeOH–H_2O was separated by RPC (rotation planar chromatography). The fraction eluted with CH_2Cl_2–MeOH–C_6H_6 (50:10:6, v/v/v) was purified by reversed-phase HPLC (Zorax SB C_{18}, 5 μm, 250 × 4.6 mm i.d.; ACN–H_2O, 35:65, v/v, 1 ml/min) to yield **2** (0.5 mg).
7. *General procedure for NMR measurements*. NMR spectra were recorded in MeOH-d_4 (**1**) or in DMSO-d_6 (**2**) in Shigemi sample tubes at room temperature, using a Varian Inova-600 (**1**) or a Bruker Avance DRX-500 (**2**) spectrometer. The structures of the products were determined by comprehensive one- (1D) and two-dimensional (2D) NMR methods, using widely accepted strategies (Pretsch, E.; Tóth, G.; Munk, M. E.; Badertscher, M. In *Spectra Interpretation and Structure Generation*; Wiley-VCH: Weinheim, 2002; Duddeck, H.; Dietrich, W.; Tóth, G. Structure Elucidation by Modern NMR. In *A Workbook*; Springer-Steinkopff: Darmstadt, 1998).
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