Two New Ecdysteroids from *Serratula wolffii*

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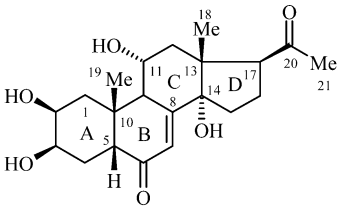
11α-Hydroxypoststerone (**1**) and herkesterone (**2**), two new natural ecdysteroids, were isolated from the herb *Serratula wolffii*. The former compound is the first 11-hydroxylated C_{21} ecdysteroid, while the latter is a new ecdysteroid with a 7,9(11)-dien-6-one chromophore. Their structures were determined using a combination of spectroscopic techniques.

Ecdysteroids are insect hormones responsible for the regulation of molting and control of embriogenesis and vitellogenesis. Phytoecdysteroids are structurally related to the main insect hormone ecdysone.[1] The diverse structural variations of phytoecdysteroids have been the basis of structure−activity experiments. These studies have proven that the ecdysteroids with a 7,9(11)-dien-6-one structural element show particularly high biological activities.[2] Ecdysteroid receptors have been engineered to be gene regulation systems and are induced by phytoecdysteroids to modulate gene expression.[3] Muristerone A[4] and ponasterone A[5] were found to be the two most active inducers, but efforts have been made to find additional inducers. The human gene therapy experiments have identified new ecdysteroids.

We report the isolation and structural elucidation of two new natural ecdysteroids from *Serratula wolffii* Andrae (Asteraceae), 11α-hydroxyposterone (**1**), and 5*β*,25-dihydroxydacryhainansterone (**2**). The last ecdysteroid has been given the trivial name herkesterone. Compound **1** is the first 11-hydroxylated ecdysteroid of the pregnane type that shows structural similarity to muristerone A. Herkesterone (**2**) is an ecdysteroid 7,9(11)-dien-6-one with potential insect hormone activity.

Compounds **1** and **2** were purified by solvent−solvent distribution, precipitation with acetone, and chromatographic purification from the methanolic extract of *S. wolffii*.[6] The IR spectrum of **1** showed typical absorption bands for OH and conjugated C=O, corresponding to common characteristics of ecdysteroids. The UV spectrum verified the presence of the 7-en-6-one chromophore of ecdysteroids.[7] The molecular formula, $C_{21}H_{30}O_6$, of **1** was established by the molecular ion peak, which was also consistent with the ^{1}H and ^{13}C NMR data (see Table 1). The ^{13}C NMR spectrum of **1** consist of 21 lines corresponding to three CH_3, five CH_2, seven CH, and six nonprotonated carbon atoms. Considering the chemical shifts of the CH signals, three are substituted with oxygen (HC−O) and one is =CH. Among the nonprotonated carbon atoms there are two C=O, one sp^2 =C, and three sp^3 C, where one is attached to oxygen. The HMBC correlations of the methyl hydrogens at 2.16 ppm with the C=O signal at 212.3 ppm and with the CH signal at 60.0 ppm revealed the presence



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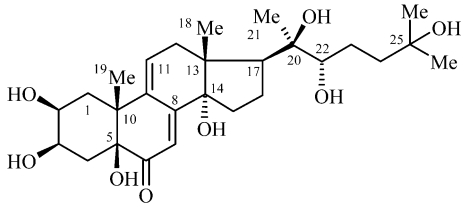

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of an acetyl group connected to the steroid skeleton. The methyl signal at 0.61 ppm gave HMBC cross-peaks with two quaternary carbon atoms (48.6, 84.8) one methylene (42.3), and one CH (60.0). The last correlation is consistent with the acetyl group being connected to C-17 and the methyl at position 18. The HMBC connectivities of the methyl (δ_H 1.05) revealed the assignment of the C-1, C-5, C-9, and C-10 atoms. The olefinic hydrogen showed HMBC correlations to C-5, C-9, and C-14, placing the conjugated carbonyl at position 6. The ^{1}H,^{1}H-COSY correlations starting from H_2-1 (δ 2.60, 1.38) revealed the connectivities of the hydrogen atoms located in the A ring, which comprises one spin system, whereas the H-9 (δ 3.18) correlation assigned the H-11 and H_2-12 signals (ring C). The large deshielding of the H-2, H-3, H-11 and C-2, C-3, C-11 resonances are consistent with the OH substitutions. The coupling pattern of these hydrogens indicated that 2-OH and 11-OH are located in equatorial positions and 3-OH is axial. The ^{1}H and ^{13}C assignment of the atoms in ring D was supported by an HMQC-TOCSY experiment.

The NOESY correlations (Figure 1) H_3-19/H_2-1, H_3-19/H-5, H-2/H-9, and H-4/H-9 verified the *cis*-type junction of the A/B ring system. The NOESY cross-peaks of H_α-12/H_α-17, H_3-18/H_β-15, and H_3-18/H_β-16 are in accordance with the *trans*-type connection of the C/D ring system and at the same time placed the acetyl group in the *β*-position.

The IR spectrum of **2** showed absorption bands for OH, C=C, and C=O. The UV spectrum supported the presence of the 7,9(11)-dien-6-one structure.[7] The molecular formula of **2** was established on the basis of HRESIMS. The ^{1}H and ^{13}C NMR chemical shifts of compound **2** are summarized

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectral Data of Compounds **1** and **2** (in MeOH-d_4 and DMSO-d_6 δ in ppm)

		1 (MeOH-d_4)			**2** (MeOH-d_4)		
position		^{13}C	^{1}H	mult., *J* (Hz)	^{13}C	^{1}H	mult., *J* (Hz)
1	α	39.2	2.60	dd; 13.0, 4.2	34.5	2.05	
	β		1.38	t; 12.3		2.05	
2	α	69.1	4.015	dt; 11.8, 3.8	68.6	3.83	ddd; 10.7, 5.4, 3.2
3	α	68.7	3.96	q; 2.9	70.3	3.88	q; 3.0
4	α	33.5	1.775	td; 13.6, 2.4	39.2	1.92	dd; 14.5, 2.8
	β		1.70			1.77	dd; ?, 3.4
5	*β*	53.0	2.345	dd; 13.1, 4.1	80.5		
6		206.6			203.1[a]		
7		123.3	5.807	d; 2.7	117.9	5.83	t; 1.2
8		164.4			156.5		
9	α	43.1	3.18	dd; 8.9, 2.7	137.9		
10		40.1			46.4		
11	*β*	69.4	4.08	ddd; 10.8, 8.9, 5.8	134.3	6.34	dt; 6.7, 2.0
12	α	42.3	2.406	t; 11.4	39.2	2.726	dd; 18.0, 1.8
	β		2.08	dd; 12.0, 5.8		2.436	dd; 18.2, 6.7
13		48.6			48.0		
14		84.8			84.5		
15	α	32.3	1.68		31.5	1.80	
	β		2.00			1.98	
16	α	22.4	1.90		21.8	1.79	
	β		2.245			1.99	
17	α	60.0	3.36	dd; 9.4, 8.1	50.7	2.49	t; 9.0
18	*β*	18.4	0.61	s	18.2	0.90	s
19	*β*	24.8	1.05	s	26.5	1.05	s
20		212.3			77.8		
21		31.6	2.16	s	20.9	1.208	s
22					78.6	3.35	dd; 10.3, 1.8
23	α				27.5	1.30	
	β					1.68	
24	α				42.5	1.44	ddd; 13.3, 11.6, 4.2
	β					1.81	
25					71.4		
26					29.1	1.19	s
27					29.9	1.206	s

[a] Very weak intensity in the ^{13}C spectrum.

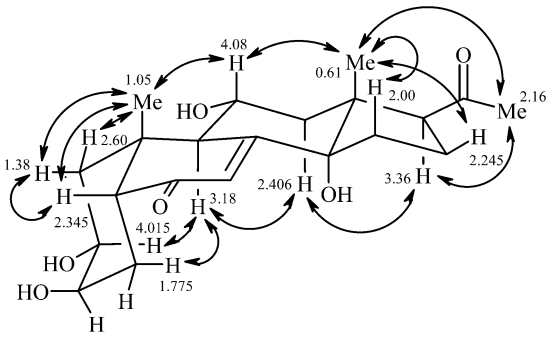

Figure 1. Stereostructure of compound **1**. The double arrows indicate the observed characteristic NOE correlations.

in Table 1. For the structure elucidation of compound **2** we utilized the same type of NMR measurements as described above. Here we discuss only the essential features of the structure elucidation. The presence of an α,*β*,*γ*,*δ* conjugated ketone was established on the basis of HMBC correlation of H-7 (5.83) with the quaternary =C signal at δ 137.9 and further supported by the H-11/H$_2$-12 correlations observed in the ^{1}H,^{1}H-COSY spectrum. The HMBC cross-peaks of H$_3$-19 (δ 1.05) and H-7 with the signal at δ 80.5 proved the presence of an OH substituent at C-5. The chain attached to C-17 is common in several ecdysteroids, and the observed chemical shifts for compound **2** are in accord with literature data.[7] H-2 is axial, as evident from its coupling constant of 10.7 Hz. The detected NOESY correlation between H-2 and H-11 is unique and provides straightforward evidence for the *cis*-type A/B ring junction (Figure 2). It is worth mentioning that the semiempirical calculation (HyperChem Release 7.0) showed 2.8 and 4.3 Å internuclear H-2 and H-11 distances for *cis*- and *trans*-type ring junctions, respectively. To gather further evidence for the structure and the *cis*-type A/B ring junction, a ROESY spectrum was measured in DMSO-d_6. The expected correlations of the 5-OH proton were not observed due to exchange; only the 22-OH gave COSY correlation.

Compound **1** is the first C$_{21}$ ecdysteroid with an important corticoid hydroxylation.[8] On the basis of the structure–activity studies the presence of the 7,9(11)-dien-6-one chromophore and 5*β*-hydroxyl and the absence of a 25-hydroxyl group usually increase the insect hormone activity

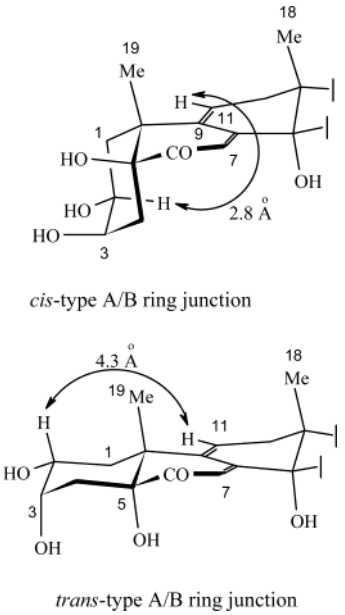

Figure 2. A/B ring junction structures of **2** and its 5α-OH isomer with the calculated (HyperChem Release 7.0) distances between H-11 and H_{ax}-2 (see double arrow).

of ecdysteroids.[2] Compound **2**, with a 7,9(11)-dien-6-one chromophore and 5*β*- and 25-hydroxylations, represents a new lead compound to study the common effects of these substitutions on activity.

Experimental Section

General Experimental Procedures. Melting points were measured with a Boetius apparatus (Dresden, Germany). Optical rotations were measured with a Perkin-Elmer 341 polarimeter. The UV spectra were recorded in MeOH using a Shimadzu UV 2101 PC spectrophotometer. FT-IR spectra (KBr) were recorded using a Perkin-Elmer Paragon 1000 PC FT-IR spectrophotometer. NMR spectra were recorded in MeOH-d_4 and in DMSO-d_6 in a Shigemi sample tube[9] at room temperature using a Bruker Avance DRX-500 spectrometer. Chemical shifts are given on the *δ*-scale and were referenced to the solvents (MeOH-d_4: $\delta_C = 49.1$ and $\delta_H = 3.31$; DMSO-d_6: $\delta_C = 39.5$ and $\delta_H = 2.51$). In the 1D measurements (^{1}H, ^{13}C, DEPT-135) 64K data points were used for the FID. The pulse programs of the 2D experiments [gs-COSY, gs-HMQC, HMQC-TOCSY (mixing time = 100 ms), gs-HMBC, NOESY (mixing time = 500 ms), ROESY (mixing time = 300 ms)] were taken from the Bruker software library, and the other parameters (pulse lengths and levels, delays, etc.) were in agreement with the parameters given in our previous work.[10] HRESIMS and FABMS were recorded on a Finnigan MAT 95SQ (Finnigan MAT, Bremen, Germany) hybrid tandem mass spectrometer. The stationary phase for the low-pressure reversed-phase column chromatography was Kovasil C18 (0.06−0.02 *μ*m, Chemie Uetikon, Uetikon, Switzerland), and a Zorbax-SIL column (5 *μ*m, DuPont, Paris, France) was used for HPLC.

Plant Material. The aerial parts of *Serratula wolffii* were collected in July 2001 from Herencsény, Hungary. A voucher specimen (collection number S94) was deposited at the Department of Pharmacognosy, University of Szeged, Hungary.

Extraction and Isolation. The dried herb (2 kg) was extracted with MeOH, purified with fractionated precipitation and solvent−solvent distribution,[6] and subjected to column chromatography on silica gel. Fractions eluted before 20-hydroxyecdysone [CH_2Cl_2−MeOH, 8:2 (4.3 g)] were separated by a combination of polyamide [H_2O, H_2O−MeOH, 75:25 (3.5 g)], alumina, and silica [CH_2Cl_2−MeOH, 9:1 (0.26 g)] and low-pressure reversed-phase column chromatography. Fractions eluted with MeOH−H_2O (40:60) from the reversed-phase column (0.13 g) gave **1** (2.2 mg). The 20-hydroxyecdysone-containing fractions eluted by CH_2Cl_2−MeOH (8:2) (13.6 g) from the first silica column were purified by column chromatography on alumina. Ecdysteroids eluted after 20-hydroxyecdysone with CH_2Cl_2−MeOH (9:1) (0.67 g) were subjected to reversed-phase column chromatography. Fractions eluted by MeOH−H_2O (45:55) (3 mg) were purified by normal-phase HPLC [CH_2Cl_2-i-PrOH−H_2O (125:40:3)] to give **2** (0.7 mg).

11α-Hydroxyposterone (1): colorless crystals, mp 174−176 °C; $[\alpha]^{28}_D$ +12° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 240 (4.116) nm; IR $(KBr)_{max}$ 3320, 1718, 1653 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); FABMS *m/z* 379 $[M + H]^+$ (100), 361 $[M + H - H_2O]^+$ (88), 343 $[M + H - 2H_2O]^+$ (40), 325 $[M + H - 3H_2O]^+$ (10), 299 (11), 282 (32), 277 (13), 249 (80), 231 (20); HRESIMS *m/z* 378.2045 (calcd for $C_{21}H_{30}O_6$, 378.2042).

Herkesterone (2): colorless crystals, mp 218 °C (dec); $[\alpha]^{28}_D$ +59° (*c* 0.1, DMSO); UV (MeOH) λ_{max} (log ϵ) 296 (4.02) nm; IR $(KBr)_{max}$ 3560−3200, 1650, 1602 cm^{-1}; ^{1}H and ^{13}C NMR (MeOH-d_4), see Table 1; ^{1}H NMR (DMSO-d_6, 500 MHz) *δ* 5.65 (1H, s, H-7), 6.18 (1H, s br; H-11*β*), 2.59 (1H, d; *J* = 17.8 Hz, H-12α), 2.29 (1H, dd; *J* = 17.8, 6.6 Hz, H-12*β*), 1.60* (1H, H-16α), 1.89* (1H, H-16*β*) (*assignment can be interchanged), 2.36 (1H, t; *J* = 9.3, H-17α), 0.77 (3H, s, CH_3-18*β*), 0.96 (3H, s b; CH_3-19*β*), 1.075 (3H, s, CH_3-21), 3.13 (1H, d; *J* = 10.2, H-22), 4.42 (1OH, OH-22) COSY to H-22, 1.13 (1H, H-23a), 1.49 (1H, H-23b),1.27 (1H, td; *J* = 12.0, 4.3, H-24a), 1.65 (1H, H-24b), 1.06 (3H, s, CH_3-26), 1.09 (3H, s, CH_3-27); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ* 78.4 (C-5), 116.5 (C-7), 136.3 (C-9), 131.7 (C-11), 37.5 (C-12), 46.3 (C-13), 82.0 (C-14), 20.4 (C-16), 48.7 (C-17), 17.3 (C-18), 25.8 (C-19), 75.5 (C-20), 20.7 (C-21), 76.3 (C-22), 26.1 (C-23), 41.4 (C-24), 68.7 (C-25), 29.0 (C-26), 30.0 (C-27); ESIMS *m/z* 495 $[M + H]^+$ (9), 477 $[M + H - H_2O]^+$ (10), 459 (23), 443 (26), 440 (11), 422 (8), 407 (49), 394 (17), 378 (9), 361 (26), 359 (10), 323 (18), 300 (100), 199 (11); HRESIMS *m/z* 494.2885 (calcd for $C_{27}H_{42}O_8$, 494.2880).

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