9α,20-Dihydroxyecdysone, a New Natural Ecdysteroid from *Silene italica* ssp. *nemoralis*

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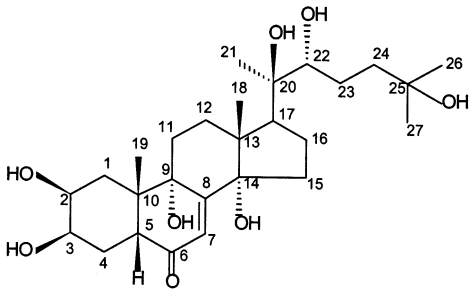
Received November 1, 2002

A new natural compound, 9α,20-dihydroxyecdysone (**1**), and two known related compounds, 20-hydroxyecdysone and ecdysone, were isolated from the herb *Silene italica* ssp. *nemoralis*. Compound **1** is the first C-9 hydroxylated ecdysteroid with a *cis*-fused A/B ring junction to have been isolated from a plant source, and its structure was determined using a combination of spectroscopic techniques.

There is a growing interest in ecdysteroids due to their important human pharmacological effects and insect-molting hormone activity. Anabolic, adaptogenic, and antidepressant ecdysteroid-containing preparations have greatly increased in sales in the past few years.[1] Previous work has shown that certain *Silene* species (Caryophyllaceae) are rich sources of biologically important ecdysteroids of diverse structure.[2] In an ongoing effort to discover new natural products, an investigation of the ecdysteroids of the herb *Silene italica* (L.) Pers. ssp. *nemoralis* (Waldst. & Kit.) Nyman was undertaken.[3] In the present paper, we report the isolation and structure elucidation of a new natural ecdysteroid, 9α,20-dihydroxyecdysone (**1**), and the identification of two known analogues, 20-hydroxyecdysone and ecdysone, from *S. italica* ssp. *nemoralis*. Compound **1** is the first C-9 hydroxylated natural ecdysteroid with a *cis*-fused A/B ring to have been isolated.



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Compound **1**, 20-hydroxyecdysone,[4] and ecdysone[4] were purified from a methanol-soluble extract of *S. italica* ssp. *nemoralis* by a series of clean-up and chromatographic steps. The IR spectrum of **1** showed characteristic absorption bands for OH and conjugated C=O. The UV spectrum supported the presence of the 7-en-6-one chromophore group of ecdysteroids.[1] The ESIMSMS of compound **1** showed a lithiated molecular ion at *m/z* 503 $[M + Li]^+$ along with ions formed by the loss of H_2O units from the intact parent compound. The molecular formula $C_{27}H_{44}O_8$ was deduced from the ^{13}C NMR spectra and exact mass of the fragment ion at *m/z* 460.5998 $[M - 2\ H_2O]^+$ as determined in the HREIMS. The ^1H and ^{13}C NMR chemical shifts of compound **1** are summarized in Table 1. Unambiguous ^1H and ^{13}C NMR assignments were made on the basis of COSY, HMBC, HMQC-TOCSY, and NOESY experiments. The Me-19 protons gave HMBC correlations to two quaternary carbons at 43.0 ppm (C-10) and 76.6 ppm in accord with OH substitution at C-9. Differentiation between the two oxygenated CH signals (δ_{C-2} 69.7 and δ_{C-3} 69.8) was achieved from the COSY cross-peaks observed for the H-2/H-1 protons and the H-3/H-4 protons, respectively. The *cis* A/B and *trans* B/C and C/D ring junctions and the configurations of C-20 (*R*) and C-22 (*R*) were determined by comparison with NMR data of related compounds[4] and by NOESY NMR observations (Figure 1). To avoid the strong steric interactions between Me-18/Me-21 and Me-18/OH-20 groups in the preferred conformation around the C-17−C-20 bond, the H-17 and C-22 bonds were assigned with a *syn*-planar arrangement. It was concluded that compound **1** is the new natural compound 9α,20-dihydroxyecdysone. Three C-9 hydroxylated ecdysteroids have been isolated before the present work, but these ecdysteroids have *trans*-fused A/B rings. Suksamrarn et al. described the partial synthesis of compound **1** from 20-hydroxyecdysone.[5] Our melting point and IR, UV, and MS spectral data match the previous values, but ^{13}C NMR data were not reported.

Experimental Section

General Experimental Procedures. Melting points were measured with a Boetius apparatus (Dresden, Germany). The optical rotation was 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 taken in MeOH-d_4 using a Bruker Avance DRX-500 spectrometer. HREIMS were recorded on a Finnigan MAT 95SQ (Finnigan MAT, Bremen, Germany) hybrid tandem mass spectrometer and ESIMSMS using a Finnigan TSQ 7000 tandem mass spectrometer (Finnigan Ltd., San Jose, CA). Column chromatographic support: Chemie Urticon-C-Gel (C_{18}), C-560. HPLC support: Zorbax-SIL column (DuPont, Paris, France).

Plant Material. The aerial parts of *Silene italica* ssp. *nemoralis* were collected in May 1997 from the Botanical Garden, Vácrátót, Hungary. A voucher specimen (collection number: 227) was deposited at the Department of Pharmacognosy, University of Szeged, Hungary.

Extraction and Isolation. The dried herb (4.5 kg) was extracted with MeOH, purified with fractionated precipitation

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10.1021/np0205194 CCC: $25.00
Published on Web 03/07/2003

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

position		^{13}C	^{1}H [m, *J* (Hz)]	position		^{13}C	^{1}H [m, *J* (Hz)]	position		^{13}C	^{1}H [m, *J* (Hz)]
1	α	37.6	2.06 (d)	11	α	30.9	1.95	19	*β*	29.6	1.01 (s)
	β		1.38 (t)		*β*		2.04	20		78.0	
2	α	69.7	4.45 (dt; 10.4, 3.9)	12	α	29.6	2.19 (td; 13.1, 4.6)	21		21.2	1.22 (s)
3	α	69.8	3.96 (q; 2.6)		*β*		1.83 (d)	22		78.6	3.32 (dd)
4	α	36.3	2.44 (td; 13.3, 2.3)	13		49.1		23	a	27.5	1.31 (q)
	β		1.74 (d)	14		87.2			b		1.66 (t)
5	*β*	51.4	2.56 (dd; 13.3, 3.9)	15	α	31.6	1.67	24	a	42.5	1.44 (td; 11.6, 4.2)
6		206.7			*β*		1.98		b		1.81 (t)
7		123.8	5.85 (s)	16	a	21.4	1.71	25		71.4	
8		162.6			b		2.01	26		29.1	1.19 (s)
9		76.6		17	α	50.4	2.45	27		29.9	1.205 (s)
10		43.0		18	*β*	18.5	0.89 (s)				

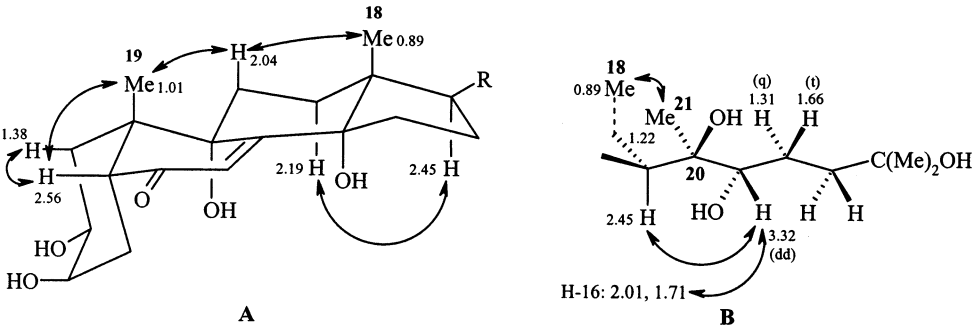

Figure 1. Selected NOESY correlation for the skeleton (**A**) and the side chain (**B**) of compound **1** (R: side chain of compound **1**).

and solvent−solvent distribution, and subjected to column chromatography on alumina.[3] Fractions eluted by CH_2Cl_2−MeOH (8:2) were used to purify 20-hydroxyecdysone by crystallization. The mother liquid (1.9 g) was subjected to reversed-phase column chromatography. Fractions eluted with 45% MeOH−H_2O (0.13 g) were purified by preparative TLC [Si gel, EtOAc−EtOH−H_2O (8:1:0.5)]. The crude compound **1** and ecdysone were purified by normal-phase HPLC [CH_2Cl_2−i-PrOH−H_2O (125:40:3)] to give **1** (4.5 mg) and ecdysone (20 mg).

9α,20-Dihydroxyecdysone (1): colorless crystals, mp 269−271 °C; $[\alpha]^{24}_D$ −10° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 228 (3.87) nm; IR (KBr) ν_{max} 3460, 3380, 1660 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1 and ref 6); ESIMSMS *m*/*z* 503 [M + Li]$^+$, 485 [M + Li − H_2O]$^+$; EIMS *m*/*z* 460 (2), 442 (4), 424 (2), 410 (4), 392 (4), 379 (1), 374 (6), 361 (59), 343 (43), 325 (19), 316 (1), 301 (94), 283 (96), 250 (54), 232 (28), 211 (40), 161 (22), 143 (46), 125 (40), 99 (62), 81 (100), 69 (63); HRESIMS *m*/*z* 460.5998 (calcd for $C_{27}H_{40}O_6$, 460.5962).

Acknowledgment. This project was supported by Hungarian National Research Fund (OTKA D32830, T032618 and T035054). A.S. is grateful for Bolyai, Békésy and Rohr Fellowships.

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NP0205194