C-29 Ecdysteroids from *Ajuga reptans* var. *reptans*

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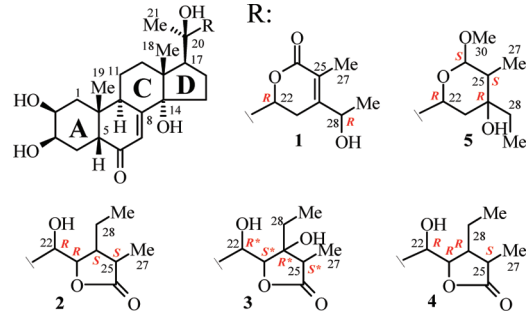
Investigation of the ecdysteroid constituents of the herb *Ajuga reptans* var. *reptans* resulted in the isolation of three new ecdysteroids, named reptanslactone A (**2**), reptanslactone B (**3**), and sendreisterone (**5**), and the known 24-dehydroprecyasterone (**1**) and breviflorasterone (**4**). The structures of compounds **1**−**5** were determined by spectroscopic methods including one- and two-dimensional NMR measurements.

The ecdysteroids are a growing class of biologically active steroids of great scientific interest because of their prospective use in both conventional and modern gene therapy. A number of studies have proved their effectiveness in enhancing protein synthesis in mammalian tissues with few or no negative hormonal consequences.[1−3] The result has been intensive advertising of ecdysteroids as growth promoters on the Internet. Ecdysteroids also exert other beneficial pharmacological effects on mammals, such as decreasing the cholesterol level and glycemia in diabetic animals, preventing myocardial ischemia and arrhythmia, wound-healing activity, etc. These compounds also display important physiological (hormonal) effects on insects, such as the regulation of molting and metamorphosis, reproduction, and differentiation.[4] Extensive study on the structure−insect hormone activity relationships of a series of ecdysteroids revealed some functional analogues of ecdysteroids, such as bisacylhydrazines, which are used as insecticides.[4]

Ajuga species are noted as rich sources of structurally diverse ecdysteroids.[5] Several *Ajuga* species have been used in traditional medicine, and their ecdysteroids are often responsible for the effectiveness of these plants.[6,7] The anabolic activities of turkesterone and cyasterone (characteristic constituents of *Ajuga* species) are comparable with those of the well-known anabolic agent Nerobol.[8] Cyasterone also possesses antitumor activity.[9] 20-Hydroxyecdysone, the predominant ecdysteroid, and turkesterone are interferon-inducing agents.[10] In an ongoing effort to discover new natural compounds and to establish the ecdysteroid profile of *Ajuga reptans* var. *reptans* (Lamiaceae), an investigation of the ecdysteroids of this plant was undertaken. In the present work we describe the isolation and structure elucidation of three new and two known ecdysteroids with the rare C_{29}-ecdysteroid skeleton.

The isolation of compounds **1**−**5** from the plant extract was based on an optimized sequence of chromatographic techniques: column chromatography on alumina and octadecyl silica, rotation planar chromatography (RPC), and preparative HPLC. The structures of compounds **1**−**5** were identified via NMR, UV, and MS measurements. Their UV spectra proved the presence of α,β-unsaturated ketone groups in these molecules. A pseudomolecular ion at *m/z* 519.2939 $[M + H]^+$ in the HRESIMS of compound **1** indicated the molecular formula $C_{29}H_{42}O_8$, in accordance with the ^1H and ^{13}C NMR data. Characteristic fragment ions were formed from the parent compound by loss of water: *m/z* 501 $[M + H - H_2O]^+$, 482 $[M - 2H_2O]^+$, and 464 $[M - 3H_2O]^+$. The molecular formula of **2** was determined as $C_{29}H_{44}O_8$ by HRESIMS of the protonated molecular ion peak at *m/z* 520.3029 (calcd 520.3024). HRESIMS indicated the pseudomolecular ion for **3** at *m/z* 559.2861 $[M + Na]^+$, consistent with the molecular formula $C_{29}H_{44}O_9$. On the basis of the molecular ion peak at *m/z* 520.3018 observed by HRESIMS, compound **4** was assigned the molecular formula $C_{29}H_{44}O_8$. Characteristic peaks at *m/z* 503 $[M + H - H_2O]^+$, 487 $[M - H_2O - CH_3]^+$, and 484 $[M - 2H_2O]^+$ in the ESIMS of **4** supported its structure. Compound **5** was assigned the molecular formula $C_{30}H_{48}O_8$ through use of HRESIMS. The ESIMS of **5** demonstrated a quasimolecular ion at *m/z* 559 $[M + Na]^+$.



The ^1H and ^{13}C chemical shifts and assignments of compounds **1**−**5** are summarized in Tables 1−3. Fortunately, the alicyclic skeleton of compounds **1**−**5** is the same, and this fact facilitated the structure elucidation and determination of the absolute configuration of the stereogenic centers of the ring system. Characteristic HMBC correlations of methyl ^1H signals over two and three bonds and the olefinic hydrogen (H-7) were utilized in the assignments. The H_3-18/C-14, H-7/C-14, H-7/C-5, H-7/C-9, H_3-19/C-1, H_3-19/C-5, and H_3-19/C-9 HMBC correlations made possible the distinctions between the Me-18 and Me-19 groups. The H_3-18/C-17 and H_3-21/C-17 cross-peaks identified Me-21. The hydrogen atoms of ring A as well as rings B, C, and D form common spin systems that were analyzed by $^1H,^1H$-COSY and HMQC-TOCSY experiments.

The HMBC cross-peaks of H_3-27 and H_3-29 in compounds **1**−**4** and H_3-27, H_3-29, and H_3-30 in compound **5** indicated the connectivity of the side chain from C-24 to C-29/C-30. The H-22/H_2-23 COSY correlation and H-23/C-24 ^2J HMBC cross-peak in compounds **1**, **3**, and **5** as well as the H-22/H-23 and H-23/H-24 COSY correlations in compounds **2** and **4** linked the skeleton and side chain of the compounds. The H-23/C-26 HMBC correlation in compounds **2**−**4** proved the existence of the five-membered

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Table 1. ^{13}C NMR (125 MHz) Chemical Shifts (δ) of Compounds **1**−**5** in Methanol-d_4

no.	1	2	3	3[a]	4	5
1	37.5	37.5	37.5	38.4	37.5	37.5
2	68.8	68.8	68.8	68.5	68.8	68.9
3	68.7	68.7	68.7	68.5	68.6	68.7
4	33.0	33.05	33.0	33.0	33.0	33.0
5	51.9	52.0	52.0	51.9	51.9	52.0
6	206.6	206.7	206.8	204.0	206.6	206.6
7	122.4	122.0	122.0	121.6	122.2	122.3
8	167.9	168.6	168.6	167.2	168.1	168.1
9	35.2	35.3	35.3	34.9	35.2	35.2
10	39.4	39.4	39.4	39.0	39.4	39.4
11	21.63	21.65	21.7	21.5	21.6	21.65
12	32.6	32.5	32.5	32.3	32.6	32.6
13	48.8	48.9	48.9	48.6	49.2	48.6
14	85.3	85.3	85.2	84.4	85.3	85.4
15	31.9	31.8	31.8	32.0	31.8	31.9
16	21.7	21.9	22.0	22.1	21.6	22.1
17	50.6	50.7	50.7	50.4	51.2	50.7
18	18.3	18.6	18.7	18.8	18.3	18.3
19	24.5	24.5	24.5	24.9	24.5	24.5
20	76.6	77.5	77.4	76.8	77.1	77.4
21	21.58	21.68	21.5	22.2	22.0	21.84
22	84.4	72.8	72.9	72.5	78.1	77.5
23	25.2	81.4	87.2	87.5	84.4	36.8
24	158.3	48.9	81.2	80.6	42.3	74.2
25	121.0	41.4	44.3	43.9	38.8	49.2
26	169.3	182.3	180.2	178.6	182.9	106.4
27	12.0	15.45	9.4	9.9	10.6	8.8
28	67.0	21.4	28.2	28.3	22.7	25.3
29	21.3	12.0	8.4	9.1	12.0	7.5
30						57.2

[a] In pyridine-d_5.

lactone rings. In the HMBC spectrum of compounds **1** and **5** a H-22/C-26 cross-peak was not seen, but the chemical shifts of H-22 and C-22 in compound **1** were in good agreement with those of ajugalactone, 2-dehydroajugalactone, and 3-dehydroajugalactone, containing six-membered lactone moieties.[5] The chemical shifts of H-22 and C-22 (^1H: δ 3.29−3.39, ^{13}C: δ 78.4−78.7) in the corresponding "open chain" 22-hydroxy compounds are characteristically high-field shifted.[11,12] In compound **5** the H-22/H-26 NOESY correlation verified the presence of a six-membered ring in the side chain.

The *cis* junction of rings A/B was indicated from the H_α-9/H_α-2 and H_3-19/H_β-5 NOESY correlations in compounds **1**−**5**. The H_3-18/H_β-12, H_β-12/H_3-21, H_α-12/H_α-17, H_3-18/H_β-15, and H_3-18/H_β-16 NOESY cross-peaks of these compounds confirmed the *trans* junction of rings C/D. It should be mentioned that, in the case of 14-epi steroids, steric interactions resulted in a characteristic 9 ppm shift of C-12 and C-15 (e.g., 20-hydoxyecdysone and 14-epi-20-hydroxyecdysone).[5]

The H_β-12/H_3-21, H_3-18/H_3-21, H-22/H_2-16, H_{eq}-23/H_2-16, and H_3-29/H_α-16 NOESY correlations in compounds **1** and **5** revealed the absolute configuration of C-20 and C-22 as shown in the chemical structures (Figure S1, Supporting Information). In the NOESY spectrum of compound **1** the H_3-27/H-28 NOESY correlation was very strong and the H_3-27/H_3-29 NOESY cross-peak was weak, proving that H-28 is oriented toward H_3-27 in the preferred conformation. A NOESY cross-peak was observed between H_3-29 and H_{eq}-23, but there was no H_3-29/H_{ax}-23 NOESY proximity. These facts described the positions of 28-OH and Me-29 in relation to the plane of the lactone ring.

In compound **5** the $^3J_{H\text{-}22,Ha\text{-}23} = 12.0$ Hz proved the antiperiplanar arrangement of these hydrogens. The H-22/H-26, H-23/H-25, and H-26/H_3-27 NOESY correlation indicated the antiperiplanar arrangement of H-25 and H-26 and also determined the positions of the Me-27 and 26-OMe groups. The H_3-29/H_{eq}-23 NOESY cross-peak indicated that the conformation around the C-24−C-28 bond was similar to that of compound **1**.

Compounds **2** and **4** are diastereomers. In compound **2** the $^3J_{H\text{-}22,H\text{-}23} = 9.9$ Hz and the H_3-18/H_3-21, H_3-21/H-23, and H-22/H_2-16 NOESY correlations proved the antiperiplanar arrangement of H-22 and H-23 and the absolute configuration of C-20. The H-22/H_2-28, H-23/H_3-27, and H-25/H_3-29 NOESY cross-peaks verified the *trans* arrangement of Me-27 and 24-Et. Four isomers, by changing the configuration of C-22 and C-23, were taken into account using PM3 semiempirical calculations (Hyperchem 7). In the NOESY spectrum of compound **2** an H-23/H-17 cross-peak was detected, which eliminated two of the isomers because of a distance of 4.3 Å between these hydrogens.

The distinction between the remaining two isomers was achieved considering the fact that a 24-Et group under ring "D" should result in strong spatial congestion. In addition, no NOESY steric proximities were detected between the hydrogens of "D" and the lactone ring. Moreover, the antiperiplanar arrangement of H-22 and H-23 was ruled out on the basis of our PM3 calculation. The structure of compound **2** was assigned to the isomer with the 20*R*,22*R*,23*R*, 24*S*,25*S* configuration (Figure S2, Supporting Information).

In the isomeric compound **4** H_3-18/H_3-21, H_3-21/H-23, H-22/H_2-16, and the H-23/H-17 NOESY correlations determined the absolute configuration of C-20 and C-22. Strong H-23/H_3-29 and H-24/H-25 NOESY cross-peaks and the absence of a H_3-27/H_2-24 correlation verified the *cis* arrangement of the H-23, Me-27, and 24-Et groups. The $^3J_{H\text{-}22,H\text{-}23} = 4.3$ Hz indicated their *gauche* arrangement. The very strong H-23/H-17 NOESY correlation indicated predominance of the conformer where the C-23−H-23 bond points toward ring D. The strong H_3-21/H-24 NOESY response made possible the determination of the absolute configuration in the lactone ring. Castro et al.[6] reported breviflorasterone, an ecdysteroid with the same structure as **4**. On their assumption of a common biosynthesis, the relative configuration was considered the same as the cyasterone (24*R*,25*S*). Unfortunately, they gave the ^1H and ^{13}C chemical shifts in pyridine-d_5, and our data were detected in methanol-d_4; that is, the identity of brevisterone and compound **4** does not follow from the NMR measurements. The $[\alpha]_D$ values of compound **4** and breviflorasterone are very different, indicating that the configurations in the lactone rings may be different (Figure S3, Supporting Information).

In compound **3** the H_3-29/H-25 NOESY cross-peaks verified the *trans* arrangement of the 24-Et and Me-27 groups. The measured $^3J_{H\text{-}22,H\text{-}23} = 9.7$ Hz and the H-22/H-25 and H-23/H_3-27 NOESY correlation proved the antiperiplanar arrangement of H-22 and H-23. The measured H_3-21/H-23 and H-22/H_2-16 correlations verified the configuration of C-20. We could not detect a H-23/H-17 NOESY response and were not able to determine the configuration of C-22. Considering the similarity of the spatial structure and the $^3J_{H\text{-}22,H\text{-}23}$ values of compounds **2** and **3**, the configuration of C-22 should be "*R**" (the change of configurations of C-23 and C-24 follows from the H→OH substitution); that is, the possible spatial arrangement is the same as depicted for compound **2** (24-OH instead of H-24).

Compounds **1**−**5** contain the stigmastane-type C_{29}-steroid skeleton. Only 15% of the known ecdysteroids isolated from plants have a C_{29} skeleton containing a (possibly substituted) ethyl group at C-24 in their side chain.[5] Four of the isolated ecdysteroids possess a lactone ring, where the 26 carboxyl group forms a lactone ring with a 22- or 23-hydroxy group in the side chain. These rare lactone-ring-containing ecdysteroids are mainly biosynthesized in plants. Interestingly, they are characteristic constituents of the taxonomically very distant *Ajuga* and *Cyathula* species. Apart from these two genera, only *Eriophyton wallchii, Leuzea chartamoides, Rhaponticum uniflorum*, and two *Silene* species each synthesize one lactone-ring-containing ecdysteroid.[5] The free 23-OH group is also rare in ecdysteroids: only three such compounds are known in plants, in *Ajuga iva*, *Raphonticum carthamoides*, and *Serratula tinctoria*.[5] The 23-OH group is mainly bound in the lactone ring form in the ecdysteroid derivatives in plants.

Table 2. ^{1}H NMR Chemical Shifts of the Skeleton of Compounds **1**−**5** in Methanol-d_4 and Multiplicities and Coupling Constants (J) of Compound **1**

		1		**2**	**3**	**3**[a]	**4**	**5**
no.		δ (ppm)	m; J (Hz)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)
1	α	1.80		1.79	1.79	2.18	1.81	1.80
	β	1.43	dd; 13.5, 12.4	1.42	1.42	1.97	1.43	1.43
2	α	3.85	ddd; 12.1, 4.3, 3.3	3.85	3.86	4.31	3.84	3.84
3	α	3.96	q; 2.8	3.95	3.95	4.31	3.95	3.95
4	a	1.71		1.70	1.70	1.94	1.71	1.75
	b	1.77		1.75	1.75	2.14	1.78	1.70
5	β	2.39	dd; 13.0, 4.5	2.38	2.37	3.08	2.38	2.39
7		5.82	d; 2.6	5.80	5.79	6.31	5.81	5.81
9	α	3.17	ddd; 11.5, 7.1, 2.6	3.16	3.17	3.68	3.16	3.16
11	α	1.82		1.80	1.80	1.85	1.82	1.81
	β	1.71		1.69	1.68	1.73	1.70	1.70
12	α	2.20	td; 13.1, 5.0	2.19	2.20	2.73	2.16	2.17
	β	1.84		1.87	1.88	2.00	1.87	1.85
15	α	1.65		1.60	1.58	1.95	1.63	1.63
	β	2.01		1.97	1.96	2.20	1.98	2.00
16	α	1.79		1.78	1.71	2.17	1.81	1.81
	β	2.03		2.00	2.00	2.49	2.00	2.01
17	α	2.50		2.75	2.73	3.54	2.57	2.50
18	β	0.90	s	0.89	0.89	1.25	0.88	0.87
19	β	0.97	s	0.96	0.96	1.11	0.97	0.97
14-OH						6.38		

[a] In pyridine-d_5.

Table 3. ^{1}H NMR Chemical Shifts of the Side Chain, Multiplicities, and Coupling Constants (J) of Compounds **1**−**5** in Methanol-d_4

		1		**2**		**3**		**3**[a]		**4**		**5**	
no.		δ (ppm)	m; J (Hz)	δ (ppm)	m; J (Hz)	δ (ppm)	m; J (Hz)	δ (ppm)	m; J (Hz)	δ (ppm)	m; J (Hz)	δ (ppm)	m; J (Hz)
21		1.34	s	1.315	s	1.33	s	1.72	s	1.30	s	1.27	s
22		4.14	dd; 13.5, 3.2	3.59	d; 9.8	3.54	d; 9.7	4.12	d; 9.5	3.62	d; 4.3	3.26	dd; 12.0, 1.2
23	a	2.33[b]	ddq; 18.1, 13.5, 2.5	4.40	dd; 10.0, 2.5	4.14	d; 9.7	4.81	d; 9.5	4.34	dd; 4.3, 2.0	1.2[b]	t; 12.4
	b	2.56[c]	dd; 18.1, 3.1									2.02[c]	
24				2.11						2.62	tdd; 8.7, 5.1, 2.1		
25				2.485	qd; 7.5, 3.0	2.605	q; 7.3	2.90	q; 7.2	3.10	dq; 8.2, 7.6	1.53	
26												4.13	d; 8.9
27		1.86	d; 2.2	1.29	d; 7.6	1.18	d; 7.3	1.48		1.13	d; 7.6	0.92	d; 7.0
28	a	4.84	q; 6.6	1.27		1.73		2.01		1.35	14.2, 8.9, 7.3	1.52	
	b			1.90		1.93		2.34	dq; 14.4, 7.3	1.59		1.57	
29				0.98	t; 7.3	1.04	t; 7.3	1.35	q; 7.3	1.00	t; 7.3	0.933	t; 7.4
30		1.30	d; 6.6									3.50	s
22-OH								6.88	s				
24-OH								6.49	s				

[a] In pyridine-d_5. [b] Axial. [c] Equatorial.

Compound **1** is a 24-dehydroprecyasterone[5] (24,25-didehydroprecyasterone) containing a six-membered δ-lactone ring, the 22-OH group forming a lactone ring with the 26-carboxyl group. This compound was isolated earlier from *Ajuga iva.*[13] Compound **2**, named reptanslactone A, and compound **3**, named reptanslactone B, contain five-membered γ-lactone rings involving a lactone bridge between the 23-OH and 26-carboxyl groups. Compound **3** is the 24-OH derivative of compound **2**, but the 23*R**, 24*S** configuration was assigned to compound **2** and the 23*S**, 24*R** configuration to compound **3**.

Compound **4** (breviflorasterone), previously isolated from *Ajuga macrosperma* var. *breviflora,*[6] is a diastereomer of compound **2**; they differ from each other in configuration at C-24. Compound **5**, named sendreisterone, has a structure closely related to ajugacetalsterone A,[14] but the 26-OH group is methylated. This compound displays a unique cyclic acetal structure in the side chain.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were recorded in MeOH with a Shimadzu UV 2101 PC spectrophotometer. NMR spectra were recorded in MeOH-d_4 in a Shigemi sample tube at room temperature with Bruker Avance DRX-500 and Varian Mercury plus 400 and VNMRS-800 spectrometers. The structures of **1**−**5** were determined by means of comprehensive one- and two-dimensional NMR methods, using widely accepted strategies.[15,16] Chemical shifts are given on the δ scale and were referenced to the solvents (methanol-d_4: δ_C = 49.15 and δ_H = 3.31, pyridine-d_5: δ_C = 150.35 and δ_H = 8.74). In the 1D measurements (^{1}H, ^{13}C, APT, and DEPT-135), 64K data points were used for the FID. The pulse programs of all experiments [gs-COSY, phase-sensitive DQF-COSY, gs-HMQC, HMQC-TOCSY (mixing time = 80 ms), edited gs-HSQC, gs-HMBC, NOESY (mixing times = 400, 500, and 750 ms), and 1D gs-NOESY (mixing time = 300 ms)] were taken from the Bruker and Varian software libraries. Mass spectrometric measurements were performed on a Finnigan TSQ 7000 tandem mass spectrometer (Finnigan MAT, San Jose, CA) equipped with a laboratory-built nanoelectrospray ion source. A voltage of about 1000 V was used in the ion source. The instrument was scanned in the normal MS mode over the mass range 10−1500, with a scan time of 2 s. HRESIMS recordings were made on a Finnigan MAT 95SQ tandem mass spectrometer (Finnigan MAT, Bremen, Germany). HPLC analyses were performed with a Jasco model PU-2080 pump and Jasco model UV-2070/2075 detector. A Zorbax-SIL column (5 μm, 4.6 mm × 250 mm DuPont, Paris, France) was used for normal-phase HPLC, and a Zorbax SB C18 column (5 μm, 4.6 mm × 250 mm DuPont, Paris, France) was used for reversed-phase HPLC. Rotation planar chromatography (RPC) was carried out on a Harrison model 8924 Chromatotron instrument (Harrison Research, Palo Alto, CA). The stationary phase for RPC was silica gel 60 GF_{254} (E. Merck, Darmstadt, Germany). Column chromatography (CC) support: Chemie Ueticon-C-Gel octadecyl-silica (0.06−0.02 μm, Chemie Ueticon, Ueticon, Switzerland) and aluminum oxide (Brockman II neutrale, Reanal, Budapest, Hungary).

Plant Material. The herb *Ajuga reptans* var. *reptans* was collected in August 2003 from the environs of Budapest, Hungary, and identified

by one of the authors (L.P.). A voucher specimen (collection number A0308) has been deposited at the Department of Ecotoxicology, Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary.

Extraction and Isolation. The dry plant material (1.24 kg) was extracted with MeOH, and the extract was purified by precipitation using acetone and solvent−solvent distribution between aqueous MeOH and C_6H_{12}. The dry residue of the aqueous MeOH phase (99.9 g) was applied to a polyamide column (MN-polyamide SC 6, Woelm, Eschwege, Germany). The fraction eluted with water (62.3 g) was subjected to low-pressure reversed-phase CC on octadecyl-silica. The fraction eluted with MeOH−H_2O (40:60, v/v) (0.12 g) was further purified by RPC (CH_2Cl_2−MeOH−C_6H_6, 25:5:3) and repeated normal-phase HPLC on silica (c-C_6H_{12}−i-PrOH−H_2O, 100:50:4) and CH_2Cl_2−i-PrOH−H_2O, 125:40:3) (UV detection at 245 nm) to give **1** (0.7 mg). Another fraction (0.17 g) eluted from the reversed-phase column with MeOH−H_2O (45:55) was purified by a combination of RPC and normal-phase HPLC. From the fraction (2.1 mg) eluted with CH_2Cl_2−MeOH−C_6H_6 (50:5:3) by RPC, compound **2** (1.3 mg) was obtained by normal-phase HPLC.

Fractions eluted from the polyamide column with H_2O−EtOH (9:1 and 8:2) (7.8 g) were further purified by normal-phase CC on alumina. The fraction (0.61 g) obtained by elution from the alumina with CH_2Cl_2−EtOH (9:1) was further separated by low-pressure reversed-phase CC on octadecyl-silica. The fraction eluted with MeOH−H_2O (35:65) (25.2 mg) was separated by RPC (EtOAc−EtOH−H_2O, 400:10:5, and MeOH). The residue from elution with MeOH (2.3 mg) was purified by repeated normal-phase HPLC (c-C_6H_{12}−i-PrOH−H_2O (100:60:4.5) and CH_2Cl_2−i-PrOH−H_2O (125:30:2), resulting in compound **3** (1.5 mg). The reversed-phase CC gave a fraction (0.39 g) (MeOH−H_2O (40:60 and 45:55 v/v)), which was purified by RPC (CH_2Cl_2−MeOH−C_6H_6 (50:5:3)) to obtain compound **4** (2.7 mg). Another fraction (0.33 g) eluted from the reversed-phase column gave compound **5** (1.6 mg).

24-Dehydroprecyasterone (1): colorless crystals; mp 275−277 °C; $[\alpha]^{25}_D$ +7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 243 (4.013) nm; ^{1}H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) (see Table 1). The MS data for compound **1** agreed with that in the literature.[13]

Reptanslactone A (2): colorless crystals; $[\alpha]^{25}_D$ +17 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 239.5 (3.931) nm; ^{1}H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) (see Table 1); ESIMS *m*/*z* 543 $[M + Na]^+$ (41), 521 $[M + H]^+$ (11), 505 $[M - CH_3]^+$ (11.5), 503 $[M + H - H_2O]^+$ (100), 484 $[M - 2H_2O]^+$ (9), 452 $[M + H - 3H_2O - CH_3]^+$ (15), 437 (24), 413 (23), 391 (37.5), 365 (17); HRESIMS *m*/*z* 520.3029 $[M]^+$ (calcd for $C_{29}H_{44}O_8$, 520.3024).

Reptanslactone B (3): colorless crystals; $[\alpha]^{25}_D$ +3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 242 (3.5952) nm; ^{1}H NMR (MeOH-d_4, pyridine-d_5, 500 MHz) and ^{13}C NMR (MeOH-d_4, pyridine-d_5, 125 MHz) (Table 1); HRESIMS *m*/*z* 536.2961 $[M]^+$ (calcd for $C_{29}H_{44}O_9$, 536.2973), 559.2861 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_9Na$: 559.287).

Breviflorasterone (4): colorless crystals; mp 268−270 °C; $[\alpha]^{25}_D$ +15 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 242 (3.9459) nm; ^{1}H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) (Table 1); MS data were in accordance with those in the literature.[14]

Sendreisterone (5): colorless crystals; mp 253−255 °C; $[\alpha]^{28}_D$ +22 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.62) nm; ^{1}H NMR (MeOH-d_4, 500 MHz) and ^{13}C NMR (MeOH-d_4, 125 MHz) (Table 1); ESIMS *m*/*z* 559 $[M + Na]^+$ (100), 560 $[M + Na + H]^+$ (27), 537 $[M + H]^+$ (25.5), 521 $[M - CH_3]^+$ (14), 519 $[M + H - H_2O]^+$ (26), 505 (19.5), 482 $[M - 3H_2O]^+$ (18), 445 (20.7), 437 (16.5), 413 (27.9), 391 (7.5), 365 (37.9), 356 (14.8); HRESIMS *m*/*z* 537.3424 $[M + H]^+$ (calcd for $C_{30}H_{49}O_8$, 537.3414).

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Supporting Information Available: Figure S1 shows the major conformer of the side chain of compound **1**. Figure S2 shows the possible spatial arrangement of the side chain in compound **2**. Figure S3 shows the possible spatial arrangement of the side chain in compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Dinan, L. *Phytochemistry* **2001**, *57*, 325–339.
(2) Bathori, M.; Pongracz, Z. *Curr. Med. Chem.* **2005**, *12*, 153–172.
(3) Lafont, R.; Dinan, L. *J. Insect Sci.* **2003**, *3*, 1–30.
(4) Dinan, L. In *Studies in Natural Products Chemistry, Bioactive Natural Products (Part J)*; Rahman, A. Ed.; Elsevier: Amsterdam, 2003; Vol. 29, pp 3−71.
(5) Lafont, R.; Harmatha, J.; Marion-Poll, F.; Dinan, L.; Wilson, I. D. *The Ecdysone Handbook*; 2002; http://www.ecdybase.org/ (continuously updated) (accessed January 30, 2009).
(6) Castro, A.; Coll, J.; Tandrón, Y. A.; Pant, A. K.; Mathela, C. S. *J. Nat. Prod.* **2008**, *71*, 1294–1296.
(7) Filippova, V. N.; Zorinyants, S. E.; Volodina, S. O.; Smolenskaya, I. N. *Russ. J. Plant Physiol.* **2003**, *50*, 501–508.
(8) Syrov, V. N.; Saatov, Z.; Sagdullaev, Sh. Sh.; Mamatkhanov, A. U. *Pharm. Chem. J.* **2001**, *35*, 667–671.
(9) Takasaki, M. ; Tokuda, H.; Nishino, H.; Konoshima, T. *J. Nat. Prod.* **1999**, *62*, 972–975.
(10) Syrov, V. N.; Saiitkulov, A. M.; Khushbaktova, Z. A.; Saatov, Z. *O'zbekiston Respublikasi Fanlar Akademiyasining Ma'ruzalari* **2003**, *2*, 53–57.
(11) Hunyadi, A.; Tóth, G.; Simon, A.; Mák, M.; Kele, Z.; Máthé, I.; Báthori, M. *J. Nat. Prod.* **2004**, *67*, 1070–1072.
(12) Simon, A.; Tóth, G.; Liktor-Busa, E.; Kele, Z.; Takács, M.; Gergely, A.; Báthori, M. *Steroids* **2007**, *72*, 751–755.
(13) Wessner, H.; Champion, B.; Girault, J.-P.; Kaouadji, N.; Saidi, B.; Lafont, R. *Phytochemistry* **1992**, *31*, 3785–3788.
(14) Coll, J.; Tandrón, Y. A.; Zeng, X. *Steroids* **2007**, *72*, 270–277.
(15) Pretsch, E.; Tóth, G.; Munk, M. E.; Badertscher, M. *Computer-Aided Structures Elucidation. Spectra Interpretation and Structure Generation*; Wiley-VCH Verlag GmbH & Co. KgaA: Weinheim, 2002.
(16) Duddeck, H.; Dietrich, W.; Tóth, G. *Structure Elucidation by Modern NMR*, A Workbook; Springer-Steinkopff: Darmstadt, 1998.

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