Ecdysteroids from *Serratula wolffii* Roots

Erika Liktor-Busa,[†] András Simon,[‡] Gábor Tóth,[‡] Gábor Fekete,[§] Zoltán Kele,[⊥] and Mária Báthori*,[†]

Department of Pharmacognosy, University of Szeged, Szeged, Eötvös utca 6, H-6720, Hungary, Department of Inorganic and Analytical Chemistry, Budapest University of Technology and Economics, Budapest, Szt. Gellért tér 4, H-1111, Hungary, Plant Protection Institute of Hungarian Academy of Sciences, Budapest, Herman Ottó út 15, H-1525, Hungary, and Department of Medical Chemistry, University of Szeged, Szeged, Dóm tér 8, H-6720, Hungary

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Two new natural ecdysteroids, 20,22-didehydrotaxisterone (**1**) and 1-hydroxy-20,22-didehydrotaxisterone (**2**), were isolated from the roots of *Serratula wolffii*. Their structures were elucidated by 1D and 2D NMR spectroscopy and mass spectrometry. The biological activities of these compounds were determined via oral aphid (*Acyrthosiphon pisum* (Harris)) tests. Compound **1** was inactive and compound **2** exhibited very low toxicity in the oral aphid test. The activities of these two ecdysteroids were in agreement with those of other 22-deoxyecdysteroids.

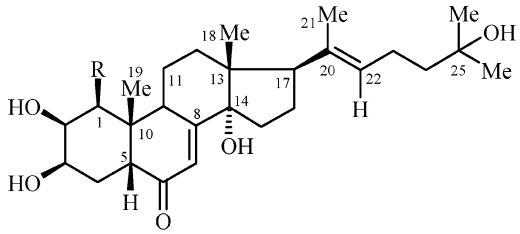
Zooecdysteroids are steroid hormones that control the molting of arthropods.[1] Phytoecdysteroids, compounds structurally related to zooecdysteroids, are widely distributed secondary constituents of plants.[2] Many plant species biosynthesize phytoecdysteroids for protection against insects. Ecdysteroids are lead compounds for the development of selective invertebrate pest control agents.[3] The occurrence of ecdysteroids in relatively large amounts in plant species has made investigation of their pharmacological effects possible. Ecdysteroids exert significant anabolic action without androgenic side effects.[4] Recent research into ecdysteroids has been intensified as a consequence of their application in gene expression systems.[5]

Serratula species are rich sources of ecdysteroids.[6,7] Eighteen ecdysteroids have been reported to occur in S*erratula wolffii* Andrae (Asteraceae).[8] The aerial parts of this species produce not only several known biologically active ecdysteroids containing 11-hydroxy and 7,9(11)-dienone moieties, but also a series of minor new ecdysteroids. These results stimulated our interest in the possible presence of ecdysteroids in the roots of *S. wolffii*. The isolation of known ecdysteroids from this part of this plant has already been published.[9]

We now report the isolation and structure determination of two new 20,22-didehydro derivatives of taxisterone:[10] 20,22-didehydrotaxisterone (**1**) and 1-hydroxyl-20,22-didehydrotaxisterone (**2**). These compounds were tested for toxicity on the L_1-L_2 larvae of *Acyrthosiphon pisum* (Harris) via oral uptake.

A methanol extract of the roots of *S. wolffii* was purified by a multistep isolation procedure[9] including precipitation, column chromatography on polyamide and on octadecyl-silica, and rotation planar chromatography. The final chromatographic step using preparative HPLC afforded compounds **1** and **2**.

Compound **1** was assigned the molecular formula $C_{27}H_{42}O_5$ (using HRESIMS). Its UV spectrum revealed absorption at 242 nm ($\log \epsilon = 4.387$) characteristic of an α,β-unsaturated ketone. The electrospray mass spectrum demonstrated a quasimolecular ion at *m*/*z* 485 $[M + K]^+$. The characteristic fragment ions were formed from the intact parent compound by the loss of water: *m*/*z* 429 $[M + H - H_2O]^+$, *m*/*z* 411 $[M + H - 2H_2O]^+$, and *m*/*z* 393 $[M + H - 3H_2O]^+$.



1 R = H
2 R = OH

On the basis of the molecular ion peak observed by HRESIMS, compound **2** was assigned the molecular formula $C_{27}H_{42}O_6$. ESIMS indicated pseudomolecular ions at *m*/*z* 501 $[M + K]^+$ and 463 $[M + H]^+$. The UV spectrum of compound **2** was consistent with the presence of a 7-en-6-one ecdysteroid chromophore (241 nm ($\log \epsilon = 3.95$)).

The structures of **1** and **2** were determined from the ^1H and ^{13}C NMR data. The ^1H and ^{13}C data for compounds **1** and **2** are summarized in the Experimental Section. The singlet methyl signals in the ^1H NMR spectrum aided in their assignments using the characteristic HMBC correlations of these signals over two and three bonds. Identification of the geminal Me-26 and Me-27 groups was unambiguous owing to their mutual HMBC correlation, whereas Me-21 correlated with two olefinic carbon atoms exhibiting strong deshielding (δ 127.7, 134.8). Differentiation between the H_3-19 and H_3-18 atoms of the methyl groups was achieved considering the coupling of the latter with C-17, which also coupled to H_3-21. In accordance with a 6-oxo-$\Delta^{7,8}$-moiety, the H-7 olefinic hydrogens of **1** and **2** (δ 5.81, 5.83) correlated with C-5, C-9, and C-14. The high value of the ^{13}C chemical shift for C-1 in compound **2** (δ 76.6) justifies assignment of an OH group attached to this atom. The hydrogen atoms of ring A form a common spin system, which was analyzed by $^1H,^1H$-COSY and HMQC-TOCSY experiments. The ^1H signal assignments of rings C and D, as well as the side chain attached to C-17, were obtained in an analogous way. Since the amount of compound **2** was limited, signals of C-6 and C-8 remained under noise level. The existence of a conjugated C═O unit was supported by comparison with the ^{13}C chemical shifts of compounds from previous work.[11] The H_α-9/H_α-2 and H-19/H_β-5 correlations in the NOESY spectrum of **1** proved a *cis*-type junction of rings A/B, and the H_β-12/H-18, H_β-12/ H-21, and H_α-12/H_α-17 cross-peaks verify the *trans*-type junction of rings C/D.

In compound **2**, β-orientation of the OH group attached to C-1 was justified by two reasons. First, H_α-2 is axial and its multiplicity

* Corresponding author. Tel: 0036-62-545558. Fax: 0036-62-545704. E-mail: bathori@pharm.u-szeged.hu.
[†] Department of Pharmacognosy, University of Szeged.
[‡] Budapest University of Technology and Economics.
[§] Plant Protection Institute of HAS.
[⊥] Department of Medical Chemistry, University of Szeged.

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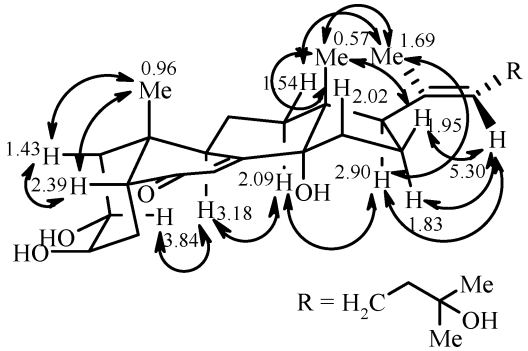

Figure 1. Steric view of compound **1**. Arrows show characteristic proximities obtained by NOESY experiment.

and coupling constant (t; 3.1 Hz) preclude the axial (β) orientation of H-1 because of the absence of an axial/axial coupling constant (9−10 Hz). Second, many ^{1}H and ^{13}C NMR signals of the atoms in ring "A" and methyl-19 are broad, as opposed to the corresponding signals of compound **1**, indicating hindered conformational motion of ring "A". The *trans* arrangement of C-21 and H-22 was proved by the NOESY correlations H-22/H$_\alpha$-16, H-22/H$_\beta$-16, H-22/H$_\alpha$-17, H$_3$-21/H$_\beta$-12, and H$_3$-21/H$_3$-18 (Figure 1).

Compounds **1** and **2** are the first ecdysteroids known to possess an extra double bond in the side chain at position 20(22).[12] These ecdysteroids are also of interest in view of their unusual hydroxylation pattern. The natural ecdysteroids generally contain an OH group in the side chain at position 20 and/or 22. Fourteen ecdysteroids have been isolated previously that do not contain the 20,22-diol structure. Five of these compounds were isolated from plants, mainly from species of the family Cactaceae.[13,14]

Compound **1** (LC_{50} > 100 ppm on day 4) proved inactive, and compound **2** (LC_{50} = 48.5 ppm) exhibited low oral activity (mortality) against aphid larvae (*Acyrthosiphon pisum* (Harris)) in comparison with the active, main phytoecdysteroid, 20-hydroxyecdysone (LC_{50} = 1.07 ppm). These results verify that the presence of the 20,22-diol is an essential structural requirement for ecdysteroids to attain high mortality in this test. An earlier investigation of the structure−activity relationship indicated that oxygen functions at C-20 and C-22 are important molecular features for activity.[1,15,16]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. The 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 a Bruker Avance DRX-500 spectrometer. The structures of the products were determined by means of comprehensive 1D and 2D NMR methods, using widely accepted strategies.[17,18] Chemical shifts are given on the δ-scale and were referenced to the solvent (MeOH-d_4: δ_C = 49.15 and δ_H = 3.31). 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 = 80 ms), gs-HMBC, 1D NOESY (mixing time = 350 ms), 2D NOESY (mixing time = 400 ms)] were taken from the Bruker software library; the other parameters (pulse length and levels, delays, etc.) were the same as given in our previous work.[17,18] The 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 high 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). 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). A Zorbax-SIL column (5 μm, DuPont, Paris, France) was used for normal-phase HPLC.

Plant Material. Roots of *Serratula wolffii* were collected in August 2003 from Herencsény, Hungary. A voucher specimen (collection number S94) has been deposited at the Department of Pharmacognosy, University of Szeged, Hungary.

Extraction and Isolation. Fresh roots (4.7 kg) were extracted with MeOH and purified by fractionated precipitation with acetone.[9] The dry residue (137.5 g) of the purified extract was applied to a polyamide column (MN-polyamide SC 6, Woelm, Eshwege, Germany). The fraction eluted with water (24.4 g) was subjected to low-pressure reversed-phase column chromatography on octadecyl-silica (0.06−0.02 μm, Chemie Uetikon, Uetikon, Switzerland). Fractions eluted with 60% MeOH−H_2O (70 mg) were further purified by rotation planar chromatography. From the fractions eluted with CH_2Cl_2−MeOH−C_6H_6 (50:5:3) (1.5 mg), compound **1** (0.5 mg) was obtained and was further purified by normal-phase HPLC [C_6H_{12}−i-PrOH−H_2O (100:40:3)]. Fractions eluted with MeOH were repeatedly separated by RPC. Fractions eluted with EtOAc−EtOH−H_2O (80:5:2) (3 mg) were purified by normal-phase HPLC [C_6H_{12}−i-PrOH−H_2O (100:40:3)] to give compound **2** (2.5 mg).

20,22-Didehydrotaxisterone (1): colorless crystals; mp 231−233 °C; $[\alpha]^{28}_D$ +71 (*c* 0.025, MeOH); UV (MeOH) λ_{max} (log ϵ) 242 (4.387) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.81 (1H, d, *J* = 2.6 Hz, H-7), 5.30 (1H, t, *J* = 7.1 Hz, H-22), 3.96 (1H, m, H-3α), 3.84 (1H, ddd, *J* = 12.0, 4.2, 3.3 Hz, H-2α), 3.18 (1H, ddd, *J* = 11.2, 7.2, 2.6 Hz, H-9α), 2.90 (1H, t, *J* = 9.2 Hz, H-17α), 2.39 (1H, dd, *J* = 12.7, 4.5 Hz, H-5β), 2.13 (2H, m, H-23), 2.09 (1H, td, *J* = 13.0, 8.0 Hz, H-12α), 2.02 (1H, m, H-15β), 1.95 (1H, tdd, *J* = 12.0, 9.2, 2.3 Hz, H-16β), 1.84 (1H, m, H-11α), 1.83 (1H, m, H-16α), 1.79 (1H, m, H-1α), 1.76 (1H, m, H-4α), 1.70 (1H, dt, *J* = 14.2, 4.5 Hz, H-4β), 1.69 (3H, s, H-21), 1.66 (1H, m, H-15α), 1.63 (1H, m, H-11β), 1.54 (1H, ddd, *J* = 13.0, 5.3, 1.7 Hz, H-12β), 1.50 (2H, m, H-24), 1.43 (1H, dd, *J* = 13.4, 12.2 Hz, H-1β), 1.20 (6H, s, H-26, H-27), 0.96 (3H, s, H-19), 0.57 (3H, s, H-18); ^{13}C NMR (CD_3OD, 125 MHz) δ 206.7 (C, C-6), 167.9 (C, C-8), 134.9 (C, C-20), 127.7 (CH, C-22), 121.9 (CH, C-7), 85.0 (C, C-14), 71.5 (CH, C-25), 68.85 (CH, C-2), 68.67 (CH, C-3), 54.3 (CH, C-17), 52.0 (CH, C-5), 48.9 (CH, C-13), 45.0 (CH_2, C-24), 39.5 (C, C-10), 37.6 (CH_2, C-1), 35.4 (CH, C-9), 33.0 (CH_2, C-4), 32.3 (CH_2, C-15), 31.1 (CH_2, C-12), 29.3 (CH_3, C-26, C-27), 24.6 (CH_3, C-19), 24.4 (CH_2, C-23), 24.1 (CH_2, C-16), 21.8 (CH_2, C-11), 18.4 (CH_3, C-21), 17.7 (CH_3, C-18); ESIMS *m*/*z* 485 $[M + K]^+$ (69), 447 $[M + H]^+$ (93), 429 $[M + H - H_2O]^+$ (100), 411 $[M + H - 2H_2O]^+$ (6), 393 $[M + H - 3H_2O]^+$ (7), 347 (6), 320 (4); HRESIMS *m*/*z* 447.3025 $[M + H]^+$ (calcd for $C_{27}H_{43}O_5$, 447.3021).

1-Hydroxy-20,22-didehydrotaxisterone (2): colorless crystals; mp 218−220 °C; $[\alpha]^{28}_D$ +10 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 241 (3.95) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.84 (1H, d, *J* = 2.5 Hz, H-7), 5.30 (1H, t, *J* = 7.0 Hz, H-22), 4.04 (1H, br, H-3α), 3.88 (1H, t, *J* = 3.1 Hz, H-2α), 3.82 (1H, br, H-1α), 3.10 (1H, t, *J* = 8.8 Hz, br, H-9α), 2.89 (1H, t, *J* = 9.2 Hz, H-17α), 2.61 (1H, dd, *J* = 12.8, 4.5 Hz, H-5β), 2.13 (2H, m, H-23), 2.06 (1H, m, H-12α), 2.05 (1H, m, H-15β), 1.95 (1H, tdd, *J* = 12.0, 9.2, 2.2 Hz, H-16β), 1.83 (1H, m, H-4β), 1.82 (1H, m, H-16α), 1.78 (1H, m, H-4α), 1.72−1.69 (2H, m, H-11α, H-11β), 1.68 (3H, s, H-21), 1.66 (1H, m, H-15α), 1.52 (1H, m, H-12β), 1.50 (2H, m, H-24), 1.20 (6H, s, H-26, H-27), 1.07 (3H, s, br, H-19), 0.59 (3H, s, H-18); ^{13}C NMR (CD_3OD, 125 MHz) δ 134.8 (C, C-20), 127.7 (CH, C-22), 122.0 (CH, C-7), 84.8 (C, C-14), 76.6 (CH, C-1), 71.5 (CH, C-25), 71.1 (CH, C-3), 68.6 (CH, C-2), 54.3 (CH, C-17), 48.9 (CH, C-13), 47.0 (CH, C-5), 44.9 (CH_2, C-24), 44.0 (C, C-10), 36.0 (CH, C-9), 33.7 (CH_2, C-4), 32.3 (CH_2, C-15), 31.1 (CH_2, C-12), 29.3 (CH_3, C-26, C-27), 24.3 (CH_2, C-23), 24.0 (CH_2, C-16), 22.2 (CH_2, C-11), 20.2 (CH_3, C-19), 18.4 (CH_3, C-21), 17.7 (CH_3, C-18); ESIMS *m*/*z* 501 $[M + K]^+$ (26), 463 $[M + H]^+$ (4), 445 $[M + H - H_2O]^+$ (100), 427 $[M + H - 2H_2O]^+$ (8), 408 $[M + H - 3H_2O]^+$ (2), 391 $[M + H - 4H_2O]^+$ (14), 374 (4), 363 (5), 336 (4); HRESIMS *m*/*z* 463.2976 $[M + H]^+$ (calcd for $C_{27}H_{43}O_6$, 463.2970).

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References and Notes

(1) Dinan, L. In *Studies in Natural Products Chemistry, Bioactive Natural Products (Part J)*; Rahman, A., Ed.; Elsevier: Amsterdam, 2003; Vol. 29, pp 3−71.
(2) Lafont, R. *Russ. J. Plant Physl.* **1998**, *45*, 276−295.
(3) Dhadialla, T. S.; Carlson, G. R.; Le, D. P. *Annu. Rev. Entomol.* **1998**, *43*, 545−569.
(4) Sláma, K.; Lafont, R. *Eur. J. Entomol.* **1995**, *92*, 355−377.
(5) Lafont, R.; Dinan, L. *J. Insect Sci.* **2003**, *3:7*, 1−30.
(6) Odinokov, V. N.; Kumpun, S.; Galyautdinov, I. V.; Evrard-Todeschi, N.; Veskina, N. A.; Khalilov, L. M.; Girault, J-P.; Dinan, L.; Maria, A.; Lafont, R. *Collect. Czech. Chem. Commun.* **2005**, *70*, 2038−2052.
(7) Rudel, D.; Báthori, M.; Gharbi, J.; Girault, J. P.; Rácz, I.; Melis, K.; Szendrei, K.; Lafont, R. *Planta Med.* **1992**, *58*, 358−364.
(8) Hunyadi, A.; Gergely, A.; Simon, A.; Tóth, G.; Veress, G.; Báthori, M. *J. Chromatogr. Sci.* **2007**, *45*, 76−86.
(9) Kalász, H.; Liktor-Busa, E.; Janicsák, G.; Báthori, M. *J. Liq. Chromatogr. Relat. Technol.* **2006**, *29*, 2095−2109.
(10) Nakano, K.; Nohara, T.; Tominatsu, T.; Nishikawa, M. *Phytochemistry* **1982**, *21*, 2749−2751.
(11) Simon, A.; Pongrácz, Z.; Tóth, G.; Mák, M.; Máthé, I.; Báthori, M. *Steroids* **2004**, *69*, 389−394.
(12) www.ecdybase.org.
(13) Djerassi, C.; Knight, J. C.; Brockman, H. *Chem. Ber.* **1964**, *97*, 3118−3130.
(14) Knight, J. C.; Pettit, G. R. *Phytochemistry* **1969**, *8*, 477−482.
(15) Dinan, L.; Hormann, R.; Fujimoto, T. *J. Comput.-Aided Mol. Des.* **1999**, *13*, 185−207.
(16) Ravi, M.; Hopfinger, A. J.; Horman, R. E.; Dinan, L. *J. Med. Chem.* **2001**, *41*, 1587−1604.
(17) 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.
(18) Duddeck, H.; Dietrich, W.; Tóth, G. *Structure Elucidation by Modern NMR*; A Workbook; Springer-Steinkopff: Darmstadt, 1998.

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