26-Hydroxylated Ecdysteroids from *Silene viridiflora*

Noémi Tóth,[†] András Simon,[‡] Gábor Tóth,[‡] Zoltán Kele,[§] Attila Hunyadi,[†] 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. Gellérrt tér 4, H-1111, Hungary, and Department of Medical Chemistry, University of Szeged, Szeged, Dóm tér 8, H-6720, Hungary

Received March 5, 2008

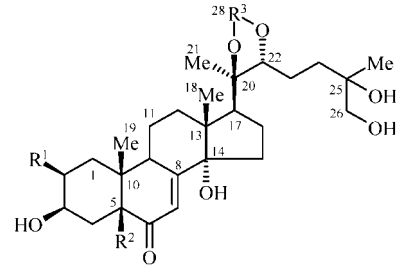
Four new 26-hydroxylated phytoecdysteroids, 2-deoxy-5,20,26-trihydroxyecdysone (**1**), 5,20,26-trihydroxyecdysone 20,22-acetonide (**2**), 2-deoxy-5,20,26-trihydroxyecdysone 20,22-acetonide (**3**), and 20,26-dihydroxyecdysone 20,22-acetonide (**4**), were isolated from the herb *Silene viridiflora*, and their structures were elucidated by means of one- and two-dimensional NMR and mass spectrometry.

The phytoecdysteroids comprise a large group of biologically active plant steroids than have structures similar to those of the insect-molting hormones.[1] They are distributed in plants as secondary metabolites that afford protection against phytophagus insects.[1] Even though they do not bind to the vertebrate steroid receptors,[3] ecdysteroids seem to exert various biological activities on vertebrates, such as anabolic, adaptogenic, immunostimulatory, and hepato- and renoprotective effects.[4] Their various pharmacological effects on mammals have led to the appearance of ecdysteroid-containing health improvement preparations on the market.[4]

Of the more than 300 phytoecdysteroids detected to date,[5] approximately 40 were isolated first from various *Silene* species.[5] The genus *Silene* biosynthesizes a complex spectrum of ecdysteroids. *Silene viridiflora* L. is characterized by a high accumulation and a broad structural diversity of ecdysteroids, with many having 2-deoxy- and 26-hydroxylated structures. Nine ecdysteroids have been previously isolated from this herb.[6,7] We report herein the isolation and structure identification of four new ecdysteroids from the herb *S. viridiflora*, namely, 2-deoxy-5,20,26-trihydroxyecdysone (**1**), 5,20,26-trihydroxyecdysone 20,22-acetonide (**2**), 2-deoxy-5,20,26-trihydroxyecdysone 20,22-acetonide (**3**), and 20,26-dihydroxyecdysone 20,22-acetonide (**4**).

A methanolic extract of the herb *S. viridiflora* was purified by a multistep isolation procedure,[8,9] including precipitation and column chromatography on octadecyl silica gel, polyamide, and cyano (CN) phases. The final chromatographic step was normal- and CN-phase HPLC, which afforded compounds **1**−**4**.

The molecular formula of **1** was established by HRESIMS (*m*/*z* 497.3108 $[M + H]^+$) as $C_{27}H_{44}O_8$. Its UV spectrum revealed an absorption at 243 nm (log ε 4.02). ESIMS demonstrated a quasimolecular ion at *m*/*z* 535 $[M + K]^+$. On the basis of the molecular ion peak observed by HRESIMS, **2** was assigned the molecular formula $C_{30}H_{48}O_9$. The ESIMS exhibited pseudomolecular ions at *m*/*z* 553 $[M + H]^+$ and 575 $[M + Na]^+$. The UV spectrum of **2** was consistent with the presence of a 7-en-6-one ecdysteroid chromophore [242 nm (log ε 3.76)]. For **3**, the molecular formula $C_{30}H_{48}O_8$, was demostrated. The UV spectrum gave a maximal absorption at 238 nm (log ε 4.08), which is in accordance with the presence of a 7-en-6-one chromophore of an ecdysteroid. The ESIMS displayed prominent peaks at *m*/*z* 559 $[M + Na]^+$ and 537 $[M + H]^+$. The molecular formula of **4**, $C_{30}H_{48}O_8$, was likewise established by HRESIMS. The maximal absorption in the UV spectrum was observed at 242 nm (log ε 4.01). ESIMS of **4** revealed quasimolecular peaks at *m*/*z* 575 $[M + K]^+$ and 560 $[M + H + Na]^+$.



	R^1	R^2	R^3
1	H	OH	2 H
2	OH	OH	Me_2C
3	H	OH	Me_2C
4	OH	H	Me_2C

The characteristic HMBC correlations of the methyl signals over two and three bonds were utilized in the assignments of **1**−**4**. The mutual HMBC correlations made the identification of the geminal Me-26 and Me-27 (in compound **3**) and that of the geminal hydroxymethyl-26 and Me-27 (in compound **2**) groups straightforward. 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.

Both in **2** and **3**, the diagnostic chemical shifts of C-20 and C-22 (86.0 and 83.6 ppm, respectively) proved oxygen substitution at these positions. The chemical shift of C-28 (108.2 ppm) and the H-22/H_3-29 NOESY correlation verified the presence of an acetal-type five-membered ring. This NOESY correlation also allowed the spatial differentiation between Me-29 and Me-30. In accordance with a 6-oxo-$\Delta^{7,8}$ moiety, H-7 correlated over $^3J_{C,H}$ couplings with the C-5, C-9, and C-14 atoms. The hydrogen atoms of ring A formed a common spin system analyzed by ^{1}H,^{1}H-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 NOESY correlation in compound **2** the *cis* junction of rings A/B was unambiguous. In compound **3** H_α-2 appeared overlapped, but the ^{1}H and ^{13}C NMR chemical shifts of rings A and B were in good agreement with values measured for 2-deoxy-5,20-dihydroxyecdysone (known also under the name 2-deoxypolypodin B).[5,10]

The similar ^{1}H and ^{13}C NMR chemical shifts of rings C and D and the side chain in compounds **2** and **3** indicated a similar structure and stereochemistry of these moieties. The H_β-12/H_3-18, H_β-12/H_3-21, and H_α-12/H_α-17 NOESY NMR spectroscopic cross-peaks of compound **2** confirmed the *trans* junction of rings C/D.

* 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.
[§] Department of Medical Chemistry, University of Szeged.

10.1021/np800139q CCC: $40.75
Published on Web 07/29/2008

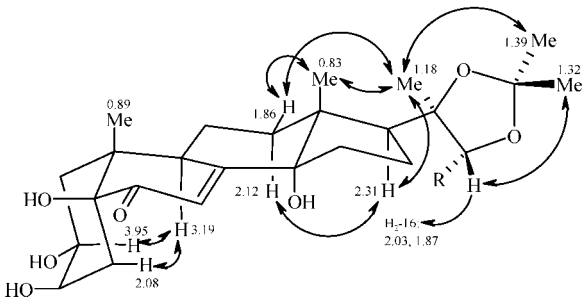


Figure 1. Spatial structure of compound **2**. The double arrows indicate the observed characteristic NOE correlations.

The H_β-12/H_3-21, H_β-16/H_3-29, H_3-21/H_3-30, H-22/H_3-29, and H-22/H_2-16 NOESY correlations verified the configurations of C-20 and C-22, respectively, and the conformation of the side chain as depicted in Figure 1.

The available low quantities of samples **1** and **4** did not allow the direct detection of ^{13}C NMR signals. Several ^{13}C NMR chemical shifts were determined from the DEPT-135, HMQC, and HMBC spectra. On the basis of the well-separated and characteristic signals of the methyl groups, we managed to assign many of the signals utilizing the HMBC correlations. Comparing the ^{13}C NMR chemical shifts with those measured for compounds **2** and **3**, and also with literature data, the structures of compounds **1** and **4** were determined as shown.

The ^{1}H and ^{13}C NMR chemical shifts of compounds **1** and **3** were similar with the exception of the values of the atoms at position 20−22. The lack of the Me_2C signals indicated that compound **1** is not an acetonide, and the characteristic 5−8 ppm low-frequency shift of signals C-22 and C-20 is in accord with the vicinal dihydroxy substitution as observed for its analogue integristerone A.[11]

The MS and NMR investigations for compound **4** showed that it is the 5-deoxy derivative of compound **2**. Comparison of the ^{1}H and ^{13}C NMR chemical shifts of compounds **2** and **4** showed close similarities. Characteristic differences were observed in the case of **4**: the appearance of the H-5 signal at 2.42 (dd; J = 12.6, 4.4 Hz) and the paramagnetic shift (3.2 ppm) of C-1, which can be well explained by the absence of the *γ-gauche* effect of the HO-5 group. It is worth mentioning that for 20,22-didehydrotaxisterone similar H-5 (2.39 ppm, dd; J = 12.7, 4.5 Hz) and C-1 (37.6 ppm) signals were detected.[12]

The presence of 26-hydroxylated ecdysteroids in nature is not unusual. They have previously been described in both insects and plant species from the *Ajuga*, *Cyathula*, *Sida*, and *Podocarpus* genera, and in certain ferns (Pteridophyta division).[5] Plants produce 26-hydroxylated ecdysteroids of greater structural variety than those in insects. The 26-hydroxylated ecdysteroids frequently occur in *Silene* species in 2-deoxy, 22-deoxy, and further hydroxylated forms of ecdysone or as acetate derivatives of the basic molecules.[5] The series of 26-hydroxylated ecdysteroids has been expanded hereby by four new members isolated from this plant. The number of known ecdysteroid acetonides is continuously increasing. They have been reported from *Leuzea carthamoides* and from *Vitex*, *Serratula*, *Rhaponticum*, and *Cyathula* species and additionally from *Silene brachuica*,[5] but this is their first identification in *S. viridiflora*.

Study of the biosynthetic relationships among the isolated *S. viridiflora* ecdysteroids may allow the conclusion that the final hydroxylation steps in plants probably occur at C-2 and C-5, in accordance with an earlier hypothesis.[1] However, the common occurrence of our isolated compounds proves also that the hydroxylation at C-26 may proceed at an earlier stage of the biosynthesis than the hydroxylation at C-2 or C-5. Further studies of a wider range of species are required to affirm the suggested order of these biosynthetic hydroxylation steps.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. The UV spectra were recorded in MeOH using a Shimadzu UV 2101 PC spectrophotometer. NMR spectra were recorded in MeOH-d_4 in a Shigemi sample tube at room temperature using a Burker Avance DRX-500 spectrometer. The structures of the products were determined by means of comprehensive 1D and 2D NMR experiments, using widely accepted strategies.[13,14] Chemical shifts are given on the δ-scale and are referenced to the solvent (MeOH-d_4: δ_C 49.15 and δ_H 3.31). (In the 1D measurements (^{1}H, ^{13}C, APT, 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, 2D NOESY (mixing time = 400 ms)] were taken from the Bruker software library. 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 amu, with a scan time of 2 s. HRESIMS recordings were made on a Finnigan MAT 95SQ tandem mass spectrometer (Finnigan MAT, Bremen, Germany). The stationary phases for column chromatography were octadecyl silica gel for vacuum reversed-phase chromatography (0.06−0.02 μm, Chemie Uetikon, Uetikon, Switzerland), MN-polyamide (SC 6, Woelm, Eshwege, Germany) for polyamide column chromatography, and CN-phase (0.063−0.200 mm, Chemie Uetikon C-gel, Uetikon, Switzerland) for cyano-phase column chromatography. HPLC was performed on an Agilent 1100 Series isocratic pump (Agilent Technologies Inc. Palo Alto, CA) coupled with a JASCO UV-2075 Plus detector (JASCO Corporation, Tokyo, Japan). Zorbax SB-CN (5 μm, Agilent Technologies Inc.) at 1 mL/min and Zorbax RX-Sil columns (5 μm, Agilent Technologies Inc.) at 1 mL/min flow were used for HPLC.

Plant Material. The aerial parts of *Silene viridiflora* were collected in June 2002 from Vácrátót, Hungary, and identified by Vilmos Miklóssy Váry. A voucher specimen (SV-020612) has been deposited at the Department of Pharmacognosy, University of Szeged, Hungary.

Extraction and Isolation. The dried herb (1.2 kg) was extracted with methanol (12 L), and the crude extract was purified by fractionated precipitation with acetone. The dried residue (60.3 g) of the purified extract was subjected to reversed-phase column chromatography on octadecyl silica gel. The fraction eluted with MeOH−H_2O (1:1) (2.94 g) underwent repeated reversed-phase column chromatography on octadecyl silica gel. The fractions eluted with MeOH−H_2O 1:1 (1.23 g) were subjected to polyamide column chromatography, and the fraction eluted with water (1.16 g) was purified further by repeated CN-phase column chromatography. From the fractions eluted with n-C_6H_{14}−Ac (8:2) (98 mg) compounds **1** (1.2 mg) **2** (1.6 mg), **3** (1.0 mg), and **4** (2.0 mg) were obtained. The compound **1**-containing fractions were further purified by CN-phase HPLC [C_6H_{12}−i-PrOH (100:14)]. The fractions containing compounds **2**−**4** (55 mg) were subjected to vacuum reversed-phase column chromatography, compounds **2** and **3** [from the fractions eluted with MeOH−H_2O (3:2) (12 mg)] were purified by normal-phase HPLC [CH_2Cl_2−i-PrOH−H_2O (125:40:3)], and compound **4** [from fractions eluted with MeOH−H_2O (65:35) (15 mg)] was purified by normal-phase HPLC [CH_2Cl_2−EtOH−H_2O (125:11:2)].

2-Deoxy-5,20,26-trihydroxyecdysone (1): colorless crystals; $[\alpha]^{25}_D$ +41 (*c* 0.05 MeOH); UV (MeOH) λ_{max} (log ε) 243 nm (4.02) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.85 (1H, d, J = 2.2 Hz, H-7), 4.08 (1H, s, br, H-3α), 3.37 (2H, s, H-26a, H-26b), 3.34 (1H, m, H-22), 3.27 (1H, m, H-9α), 2.39 (1H, t, J = 8.8 Hz, H-17α), 1.19 (3H, s, H-21), 1.14 (3H, s, H-27), 0.90 (3H, s, H-18*β*), 0.89 (3H, s, H-19*β*); ^{13}C NMR (CD_3OD, 125 MHz) δ 120.7 (CH, C-7), 85.2 (C, C-14), 81.0 (C, C-5), 78.7 (CH, C-22), 77.9 (C, C-20), 73.6 (C, C-25), 70.8 (CH_2, C-26), 67.2 (CH, C-3), 50.5 (CH, C-17), 48.8 (C, C-13), 43.3 (C, C-10), 37.9 (CH, C-9), 37.2 (CH_2, C-24), 32.6 (CH, C-12), 25.3 (CH_2, C-1), 23.6 (CH_3, C-27), 21.1 (CH_3, C-21), 18.2 (CH_3, C-18), 17.2 (CH_3, C-19); ESIMS m/z 535 $[M + K]^+$ (100); HRESIMS m/z 497.3108 $[M + H]^+$ (calcd for $C_{27}H_{45}O_8$, 497.3102).

5,20,26-Trihydroxyecdysone 20,22-acetonide (2): colorless crystals; $[\alpha]^{25}_D$ +89 (*c* 0.05 MeOH); UV (MeOH) λ_{max} (log ε) 242 (3.76) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.86 (1H, d, J = 2.8 Hz, H-7), 3.99 (1H, q, J = 3.0 Hz, H-3α), 3.95 (1H, ddd, J = 10.0, 7.4, 3.6 Hz, H-2α),

3.695 (1H, t, J = 6.0 Hz, H-22), 3.375 (1H, d, J = 11.0 Hz, H-26b), 3.355 (1H, d, J = 11.0 Hz, H-26a), 3.19 (1H, ddd, J = 11.3, 7.0, 2.7 Hz, H-9α), 2.31 (1H, dd, J = 9.4, 8.1 Hz, H-17α), 2.12 (1H, td, J = 13.1, 5.0 Hz, H-12α), 2.075 (1H, dd, J = 14.7, 3.0 Hz, H-4α), 2.03 (1H, m, H-16β), 1.96 (1H, dd, J = 12.4, 6.5 Hz, H-15β), 1.87 (1H, m, H-16α), 1.86 (1H, m, H-12β), 1.81 (1H, m, H-11b), 1.77 (1H, dd, J = 14.9, 3.0 Hz, H-4β), 1.74 (1H, m, H-11α), 1.73 (2H, m, H-1α, H-1β), 1.71 (1H, m, H-24b), 1.61 (1H, m, H-15α), 1.55 (1H, m, H-23b), 1.53 (1H, m, H-23a), 1.52 (1H, m, H-24a), 1.39 (3H, s, H-30), 1.32 (3H, s, H-29), 1.18 (3H, s, H-21), 1.15 (3H, s, H-27), 0.915 (3H, s, H-19β), 0.83 (3H, s, H-18β); ^{13}C NMR (CD_3OD, 125 MHz) δ 202.5 (C, C-6), 167.4 (C, C-8), 120.7 (CH, C-7), 108.2 (C, C-28), 86.0 (C, C-20), 85.3 (C, C-14), 83.6 (CH, C-22), 80.4 (C, C-5), 73.6 (C, C-25), 70.7 (CH_2, C-26), 70.4 (CH, C-3), 68.6 (CH, C-2), 50.5 (CH, C-17), 48.7 (C, C-13), 45.5 (C, C-10), 39.2 (CH, C-9), 37.2 (CH_2, C-24), 36.3 (CH_2, C-4), 34.3 (CH_2, C-1), 32.6 (CH_2, C-12), 31.8 (CH_2, C-15), 29.5 (CH_3, C-30), 27.3 (CH_3, C-29), 24.0 (CH_2, C-23), 23.9 (CH_3, C-27), 22.7 (CH_3, C-21), 22.65 (CH_2, C-11), 22.5 (CH_2, C-16), 17.8 (CH_3, C-18), 17.1 (CH_3, C-19); ESIMS m/z 575 $[M + Na]^+$ (46), 553 $[M + H]^+$ (100), 537 $[M - CH_3]^+$ (5), 535 $[M + H - H_2O]^+$ (2), 520 $[M + H - H_2O - CH_3]^+$ (2), 495 $[M + H -$ acetone$]^+$ (59), 481 (1), 477 (3), 437 (3), 359 (3), 328 (14); HRESIMS m/z 553.3366 $[M + H]^+$ (calcd for $C_{30}H_{49}O_9$, 553.3363).

2-Deoxy-5,20,26-trihydroxyecdysone 20,22-acetonide (3): colorless crystals; $[\alpha]^{25}_D$ +25 (c 0.05 MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.08) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.86 (1H, s, br, H-7), 4.08 (1H, s, br, H-3β), 3.70 (1H, m, H-22), 3.37 (1H, d, J = 11.0 Hz, H-26b), 3.36 (1H, d, J = 11.0 Hz, H-26a), 3.28 (1H, m, H-9α), 2.32 (1H, t, J = 8.7 Hz, H-17α), 2.12 (1H, td, J = 12.4, 5.7 Hz, H-12α), 2.04 (1H, m, H-16β), 2.035 (1H, m, H-4b), 1.97 (1H, m, H-15β), 1.96 (1H, m, H-2b), 1.88 (1H, m, H-16α), 1.86 (1H, m, H-12β), 1.84 (1H, m, H-1b), 1.77 (1H, m, H-2a), 1.73 (1H, m, H-11a), 1.72 (1H, m, H-24b) 1.61 (2H, m, H-4a, H-15α), 1.55 (2H, m, H-23a, H-23b), 1.53 (1H, m, H-24a), 1.50 (1H, m, H-1a), 1.39 (3H, s, H-30), 1.32 (3H, s, H-29), 1.18 (3H, s, H-21), 1.15 (3H, s, H-27), 0.89 (3H, s, H-19β), 0.83 (3H, s, H-18β); ^{13}C NMR (CD_3OD, 125 MHz) δ 167.9 (C, C-8), 120.7 (CH, C-7), 108.2 (C, C-28), 86.0 (C, C-20), 85.4 (C, C-14), 83.6 (CH, C-22), 81.2 (C, C-5), 73.6 (C, C-25), 70.7 (CH_2, C-26), 67.2 (CH, C-3), 50.6 (CH, C-17), 48.7 (C, C-13), 43.25 (C, C-10), 38.1 (CH, C-9), 37.2 (CH_2, C-24), 36.9 (CH_2, C-4), 32.6 (CH_2, C-12), 31.8 (CH_2, C-15), 29.5 (CH_3, C-30), 29.3 (CH_2, C-2), 27.3 (CH_3, C-29), 25.6 (CH_2, C-1), 24.05 (CH_2, C-23), 23.8 (CH_3, C-27), 22.7 (CH_3, C-21), 22.5 (CH_2, C-11), 22.5 (CH_2, C-16), 17.8 (CH_3, C-18), 17.3 (CH_3, C-19); ESIMS m/z 559 $[M + Na]^+$ (100), 537 $[M + H]^+$ (36), 518 $[M - H_2O]^+$ (3), 541 $[M + Na - H_2O]^+$ (12), 501 $[M + H - 2H_2O]^+$ (2), 445 (10), 385 $[M + H - H_2O - C_6O_3H_{14}]^+$ (3), 315 (12), 304 (24); HRESIMS m/z 537.3420 $[M + H]^+$ (calcd for $C_{30}H_{49}O_8$, 537.3414).

20,26-Dihydroxyecdysone 20,22-acetonide (4): colorless crystals; $[\alpha]^{25}_D$ +145 (c 0.005 MeOH); UV (MeOH) λ_{max} (log ε) 242 (4.01) nm; ^{1}H NMR (CD_3OD, 500 MHz) δ 5.86 (1H, d, J = 2.6 Hz, H-7), 3.70 (1H, m, H-22), 3.38 (1H, d, J = 10.9 Hz, H-26b), 3.36 (1H, d, J = 11.0 Hz, H-26a), 2.42 (1H, dd, J = 12.6, 4.0 Hz, H-5β), 2.33 (1H, dd, J = 9.2, 8.6 Hz, H-17α), 1.39 (3H, s, H-30), 1.32 (3H, s, H-29), 1.18 (3H, s, H-21), 1.15 (3H, s, H-27), 0.96 (3H, s, H-19β), 0.83 (3H, s, H-18β); ^{13}C NMR (CD_3OD, 125 MHz) δ 121.8 (CH, C-7), 85.9 (C, C-20), 85.5 (C, C-14), 83.6 (CH, C-22), 73.2 (C, C-25), 70.7 (CH_2, C-26), 50.5 (CH, C-17), 49.3 (C, C-13), 37.1 (CH_2, C-24), 32.55 (CH_2, C-12), 29.4 (CH_3, C-30), 27.3 (CH_3, C-29), 24.4 (CH_3, C-19), 23.7 (CH_3, C-27), 22.7 (CH_3, C-21), 17.8 (CH_3, C-18); ESIMS m/z 575 $[M + K]^+$ (14), 560 $[M + H + Na]^+$ (6), 559 $[M + Na]^+$ (5), 542 (100), 521 $[M - CH_3]^+$ (23), 519 $[M + H - H_2O]^+$ (2), 503 $[M - CH_3 - H_2O]^+$ (7), 501 $[M + H - 2H_2O]^+$ (23), 478 $[M -$ acetone$]^+$ (4), 445 (14), 413 (6), 314 (10), 304 (55); HRESIMS m/z 537.3418 $[M + H]^+$ (calcd for $C_{30}H_{48}O_8$, 537.3414).

Acknowledgment. This project was financially supported and sponsored by the grants of the Hungarian National Science and Research Fund (OTKA T046127). A.S. is grateful for a Varga/Rohr Fellowship.

References and Notes

(1) Lafont, R. *Russ. J. Plant Physiol.* **1998**, *45*, 276–295.
(2) Dinan, L. *Eur. J. Entomol.* **1995**, *92*, 271–283.
(3) Báthori, M.; Tóth, N.; Hunyadi, A.; Márki, Á.; Zádor, E. *Curr. Med. Chem* **2008**, *15*, 75–91.
(4) Lafont, R.; Dinan, L. *J. Insect Sci.* **2003**, *3*, 1–30.
(5) Lafont, R.; Harmatha, J., Marion-Poll, F.; Dinan, L.; Wilson, I. D. *The Ecdysone Handbook*, 2002 (http://ecdybase.org).
(6) Mamadalieva, N. Z.; Zibareva, L. N.; Saatov, Z.; Lafont, R. *Chem. Nat. Compd.* **2003**, *39*, 199–203.
(7) Mamadalieva, N. Z.; Zibareva, L. N.; Edvrard-Todeschi, N.; Girault, J. P.; Annick, M.; Ramazanov, N. S.; Saatov, Z.; Lafont, R. *Collect. Czech. Chem. Commun.* **2004**, *69*, 1675–1680.
(8) Tóth, N.; Báthori, M. *J. Chromatogr. Sci.* **2008**, *46*, 111–116.
(9) Hunyadi, A.; Gergely, A.; Simon, A.; Tóth, G.; Veress, G.; Báthori, M. *J. Chromatogr. Sci.* **2007**, *45*, 76–86.
(10) Jayasinghe, L.; Kumarihamy, B. M. M.; Arundathie, B. G. S.; Dissanayake, L.; Hara, N.; Fujimoto, Y. *Steroids* **2003**, *68*, 447–450.
(11) Simon, A.; Pongrácz, Z.; Tóth, G.; Mák, M.; Máthé, I.; Báthori, M. *Steroids* **2004**, *69*, 389–394.
(12) Liktor-Busa, E.; Simon, A.; Tóth, G.; Fekete, G.; Kele, Z.; Báthori, M. *J. Nat. Prod.* **2007**, *70*, 884–886.
(13) Pretsch, E.; Tóth, G.; Munk, M. E.; Badertscher, M. *Computer-Aided Structures Elucidation. Spectra Interpretation and Structure Generation*; Wiley-VCH Verlag: Weinheim, 2002.
(14) Duddeck, H.; Dietrich, W.; Tóth, G. *Structure Elucidation by Modern NMR: A Workbook*; Springer-Steinkopff: Darmstadt, 1998.

NP800139Q