# Additional Minor Phytoecdysteroids of *Serratula wolffii*

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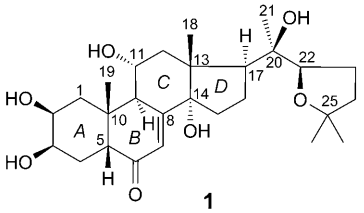
Three new and one known ecdysteroids were identified in the MeOH extract of the roots of *Serratula wolffii*. The new compounds isolated were (11$\alpha$)-11-hydroxyshidasterone (**1**), (2$\beta$,3$\alpha$,5$\beta$,14$\beta$,22*R*)-2,3,20,22,25-pentahydroxycholest-7-en-6-one (**2**), and (2$\beta$,3$\alpha$,5$\beta$,22*R*)-2,3,20,22,25-pentahydroxycholest-7-en-6-one (**3**), together with the known ponasterone A (**4**). This latter compound was now better characterized than earlier. The structures of compounds **1**–**4** were established by extensive spectroscopic techniques, including one- and two-dimensional NMR methods.

**Introduction.** – Ecdysteroids were discovered as steroid hormones of arthropods. They regulate the moulting, metamorphosis, reproduction, and diapause of insects [1]. The functional analogues of ecdysteroids have been used as selective pest control agents [2]. Phytoecdysteroids are structurally related to the main insect hormone ecdysone. Plant species synthesize ecdysteroids with diverse structural variations [3].

Pharmacological studies have revealed that ecdysteroids influence many physiological functions in a positive way, and that they are not toxic to mammals. Their most pronounced effect on mammals is a stimulation of protein synthesis without adverse androgenic, antigonadotropic, or thymolytic side-effects. Ecdysteroids have hypocholesterolaemic effects, through a reduction of cholesterol biosynthesis and an increase of its catabolism. Antidiabetic effects are also known for ecdysteroid-containing plants (*e.g.*, *Morus* sp.) used in traditional medicine. A new biomedical application is the ecdysteroid-inducible gene-expression system [4].

In our continuing phytochemical studies on the roots of *S. wolffii*, we isolated three novel ecdysteroids: (11$\alpha$)-11-hydroxyshidasterone (**1**), (2$\beta$,3$\alpha$,5$\beta$,14$\beta$,22*R*)-2,3,20,22,25-pentahydroxycholest-7-en-6-one (**2**), and (2$\beta$,3$\alpha$,5$\beta$,22*R*)-2,3,20,22,25-pentahydroxycholest-7-en-6-one (**3**), together with the known ponasterone A (**4**). This article describes the isolation and structure elucidation of compounds **1**–**4** on the basis of their spectroscopic analysis.

**Results and Discussion.** – The isolation of compounds **1**–**4** from the purified plant extract was based on the optimized sequence of combined chromatographic techniques: column chromatography on octadecyl-silica, RPC, and prep. HPLC.




The structures of compounds **1**–**4** were identified *via* NMR (*Tables 1* and *2*), UV, and MS measurements. A pseudomolecular-ion peak at *m*/*z* 478.2925 ($[M+H]^+$) in the HR-ESI-MS of compound **1** indicated a molecular formula $C_{27}H_{42}O_7$, in accordance with the $^1$H- and $^{13}$C-NMR data. The molecular formula of **2** was determined as $C_{27}H_{44}O_6$ by HR-ESI-MS of the protonated-molecular-ion peak at *m*/*z* 464.3120. The characteristic fragment ions were formed from the intact parent compound by loss of $H_2O$: *m*/*z* 447 ($[M+H-H_2O]^+$), 429 ($[M+H-2\,H_2O]^+$), and 411 ($[M+H-3\,H_2O]^+$). On the basis of the molecular-ion peak observed by HR-ESI-MS, compound **3** was assigned the molecular formula $C_{27}H_{44}O_6$. ESI-MS indicated pseudomolecular ions at *m*/*z* 503 ($[M+K]^+$), 487 ($[M+Na]^+$), and 465 ($[M+H]^+$). The UV spectra also indicated the 7-en-6-one chromophore group of these ecdysteroids. The previously known compound **4** was identified *via* its UV, $^1$H- and $^{13}$C-NMR, and mass spectra, these spectral data being compared with the data reported earlier [5].

The $^1$H- and $^{13}$C-NMR chemical shifts, multiplicities, and coupling constants of **1**–**4** are summarized in *Table 1* and *2*. The *s* Me signals in the $^1$H-NMR spectrum aided their assignments by using their characteristic correlations over two and three bonds in the HMBC plot. Identifications of the geminal Me(26) and Me(27) groups were straightforward due to their mutual HMBCs. The multiplicities of the Me $^1$H-NMR signals and the $\delta$(C) of C(25) verified the presence (in **1**–**3**) or the absence (in **4**) of an attached O-atom at C(25). Me(21) correlated with two C-atoms exhibiting strong deshielding ($\delta$(C) 77–86). Differentiation between the H-atoms of Me(19) and Me(18) was achieved by considering the coupling of the latter with C(17), which is also coupled to Me(21). We found that Me(18) of **2** and **3** gave HMBC cross-peaks with two methine C-atoms verifying the presence of a CH unit at position 14. In accordance with a 7-en-6-one moiety, the olefinic H–C(7) correlated with C(14) and two CH units. The HMBC cross-peaks of the latter methine H-atoms with the C-atom of the oxo group and the quaternary C-atom in the sp$^2$ hybrid state justified their assignments. The H-atoms of ring *A* form a common spin system, which was analyzed by $^1$H,$^1$H-COSY and HMQC-TOCSY experiments. The chemical shift and the coupling pattern of H–C(3) showed its axial (in **2** and **3**) or equatorial (in **1** and **4**) arrangement. The $^1$H-NMR signal assignments of rings *B*, *C*, and *D*, as well as of the side chain attached to C(17), were obtained in an analogous way. The high $\delta$(H) value and the multiplicity of H–C(11) of **1** indicated the presence of an equatorial OH–C(11). The chemical shifts of C(22) and C(25) of **1** ($\delta$(C) 85.7 and 82.0, resp.) and the H–C(22)/Me(26) NOESY correlation established the presence of a five-membered-ring unit in the side chain. Moreover, the $\delta$(H) and $\delta$(C) of the side chain of **1** are in good agreement with those of shidasterone ($^1$H-NMR ((D$_4$)MeOH): $\delta$(H) 3.94 (H–C(22)); $\delta$(C) 85.4 (C(22)) and 81.7 (C(25)) [6].

The $H_\alpha$–C(9)/$H_\alpha$–C(2) and Me(19)/$H_\beta$–C(5) correlations in the NOESY spectra of **1**–**3** established a *cis*-type junction of rings *A* and *B*. The NOESY cross-peaks of **2** (Me(18)/H–C(14)) and **3** ($H_\alpha$–C(9)/H–C(14), $H_\alpha$–C(17)/H–C(14)) showed the $\beta$- and $\alpha$-position of H–C(14) (*cis*- and *trans*-type junction of rings *C* and *D*), resp. In **1**, the $H_\beta$–C(12)/Me(18), $H_\beta$–C(12)/Me(21), and

Table 1. *$^1$H-NMR Data* ($(D_4)$MeOH) *of Compounds* **1**–**4**[a]). $\delta$ in ppm, $J$ in Hz.

| | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| $H_\alpha$–C(1) | 2.58 (*ddd*, *J* = 12.8, 4.5, 0.7) | 2.10 (*dd*, *J* = 13.9, 4.7) | 2.09 (*dd*, *J* = 13.3, 3.3) | 1.79 (*dd*, 13.6, 4.5) |
| $H_\beta$–C(1) | 1.34–1.40 (*m*) | 1.09 (*dd*, *J* = 13.9, 11.9) | 1.09 (*dd*, *J* = 13.9, 11.8) | 1.43 (*dd*, *J* = 13.2, 12.4) |
| $H_\alpha$–C(2) | 4.01 (*ddd*, *J* = 12.1, 4.2, 3.3) | 3.57–3.66 (*m*) | 3.62( *ddd*, *J* = 11.3, 8.7, 5.1) | 3.84 (*ddd*, *J* = 12.2, 4.3, 3.2) |
| $H_\alpha$–C(3) | 3.95 (*q*, *J* = 2.7) | – | – | 3.95 (*q*, *J* = 2.8) |
| $H_\beta$–C(3) | – | 3.33–3.38 (*m*) | 3.34 (*ddd*, *J*= 11.7, 8.7, 4.5) | – |
| $H_\alpha$–C(4) | 1.75–1.81 (*m*) | 1.37–1.46 (*m*) | 1.47 (*td*, *J* = 13.1, 11.3) | 1.68–1.77 (*m*)[b]) |
| $H_\beta$–C(4) | 1.69 (*dt*, *J* = 14.2, 3.6) | 1.74–1.82 (*m*) | 1.74–1.81 (*m*) | 1.68–1.77 (*m*)[b]) |
| $H_\beta$–C(5) | 2.33 (*dd*, *J* = 13.1, 3.7) | 2.07 (*dd*, *J* = 13.4, 3.8) | 2.08 (*dd*, *J* = 13.8, 4.3) | 2.385 (*dd*, *J* = 13.1, 4.4) |
| H–C(7) | 5.80 (*dd*, *J* = 2.7, 0.7) | 5.84 (*d*, *J* = 2.5) | 5.65 (*t*, *J* = 2.1) | 5.81 (*d*, *J* = 2.7) |
| $H_\alpha$–C(9) | 3.145 (*dd*, *J* = 8.8, 2.7) | 2.81 (*ddd*, *J* = 9.7, 6.1, 2.2) | 2.66 (*ddd*, *J* = 11.6, 6.9, 2.4) | 3.155 (*ddd*, *J* = 11.3, 7.2, 2.6) |
| $H_\alpha$–C(11) | – | 1.80–1.88 (*m*) | 1.88–1.95 (*m*) | 1.68–1.74 (*m*)[b]) |
| $H_\beta$–C(11) | 4.05–4.13 (*m*) | 1.58–1.68 (*m*) | 1.75–1.80 (*m*) | 1.78–1.85 (*m*)[b]) |
| $H_\alpha$–C(12) | 2.23 (*dd*, *J* = 12.5, 10.4) | 1.56–1.61 (*m*) | 1.56–1.64 (*m*) | 2.12 (*td*, *J* = 13.1, 4.4) |
| $H_\beta$–C(12) | 2.13 (*dd*, *J* = 12.4, 6.0) | 1.79–1.82 (*m*) | 2.31 (*ddd*, *J* = 13.1, 4.0, 3.0) | 1.88 (*ddd*, *J* = 12.8, 4.9, 2.1) |
| $H_\alpha$–C(14) | – | – | 2.22 (*ddd*; *J* = 11.7, 6.2, 1.6) | – |
| $H_\beta$–C(14) | – | 2.46 (*dd*, *J* = 11.7, 7.2 | – | – |
| $H_\alpha$–C(15) | 1.56–1.62 (*m*) | 1.68–1.74 (*m*) | 1.56–1.63 (*m*) | 1.57–1.64 (*m*) |
| $H_\beta$–C(15) | 1.97–2.00 (*m*) | 1.74–1.81 (*m*) | 1.68–1.72 (*m*) | 1.9–2.01 (*m*) |
| $H_\alpha$–C(16) | 1.80–1.83 (*m*) | 1.80–1.85 (*m*) | 1.96–2.02 (*m*) | [c]) |
| $H_\beta$–C(16) | 1.96–1.98 (*m*) | 1.80–1.85 (*m*) | 1.69–1.73 (*m*) | 1.97–2.00 (*m*) |
| $H_\alpha$–C(17) | 2.40 (*dd*, *J* = 9.6, 8.6) | 1.98 (*t*, *J* = 8.9) | 1.84 (*t*, *J* = 9.2) | 2.375 (*t*, *J* = 8.4) |
| $Me_\beta$(18) | 0.83 (*s*) | 1.27 (*s*) | 0.83 (*s*) | 0.89 (*s*) |
| $Me_\beta$(19) | 1.05 (*s*) | 0.91 (*s*) | 0.96 (*s*) | 0.97 (*s*) |
| Me(21) | 1.235 (*s*) | 1.23 (*s*) | 1.23 (*s*) | 1.18 (*s*) |
| H–C(22) | 3.92 (*dd*, *J* = 8.2, 6.1) | 3.41 (*dd*, *J* = 10.4, 1.6) | 3.29–3.34 (*m*) | 3.33 (*d*, *J* ≈ 10) |
| $H_a$–C(23) | 1.74–1.78 (*m*) | 1.28–1.40 (*m*) | 1.27–1.35 (*m*) | 1.21–1.25 (*m*) |
| $H_b$–C(23) | 1.89–1.93 (*m*) | 1.59–1.66 (*m*) | 1.65 (*ddd*, *J* = 12.0, 4.3, 1.6) | 1.56–1.59 (*m*) |
| $H_a$–C(24) | 1.71–1.78 (*m*) | 1.43–1.45 (*m*) | 1.38–1.47 (*m*) | 1.21–1.25 (*m*) |
| $H_b$–C(24) | 1.71–1.78 (*m*) | 1.77–1.81 (*m*) | 1.75–1.84 (*m*) | 1.45–1.49 (*m*) |
| H–C(25) | – | – | – | 1.54–1.59 (*m*) |
| Me(26) | 1.248 (*s*) | 1.19 (*s*) | 1.19 (*s*) | 0.914 (*d*, *J* = 6.6) |
| Me(27) | 1.252 (*s*) | 1.22 (*s*) | 1.21 (*s*) | 0.923 (*d*, *J* = 6.6) |

[a]) Ranges of (overlapping) *m* are given from HMQC and COSY spectra. [b]) Tentative. [c]) Under the noise level.

$H_\alpha$–C(12)/$H_\alpha$–C(17) cross-peaks and the absence of an $H_\alpha$–C(9)/$H_\alpha$–C(15) correlation verified the *trans*-type junction of rings *C* and *D*.

The $^1$H- and $^{13}$C-NMR data of compounds **3** and **4** and the Me(21)/$H_\beta$–C(12), H–C(22)/$H_\alpha$–C(16), and H–C(22)/$H_\beta$–C(16) NOESY correlations of **3** were in

Table 2. *$^{13}$C-NMR Data* ($(D_4)$MeOH) *of Compounds* **1**–**4**

| | **1** | **2** | **3** | **4** | | **1** | **2** | **3** | **4** |
|---|---|---|---|---|---|---|---|---|---|
| C(1) | 39.3 | 42.7 | 43.1 | 37.5 | C(15) | 32.0 | 34.1 | 23.6 | 31.9 |
| C(2) | 69.1 | 72.2 | 72.2 | 68.9 | C(16) | 21.9 | 28.2 | 22.8 | 21.5 |
| C(3) | 68.7 | 75.5 | 75.4 | 68.7 | C(17) | 51.8 | 56.3 | 56.4 | 50.6 |
| C(4) | 33.5 | 34.1 | 34.1 | 33.0 | C(18) | 19.1 | 24.9 | 14.8 | 18.2 |
| C(5) | 52.9 | 57.2 | 57.5 | 51.9 | C(19) | 24.8 | 23.9 | 24.3 | 24.5 |
| C(6) | [a]) | [a]) | 204.2 | 206.6 | C(20) | 77.4 | 78.1 | 77.8 | 78.0 |
| C(7) | 122.9 | 127.2 | 122.3 | 122.3 | C(21) | 20.9 | 20.4 | 21.1 | 21.1 |
| C(8) | 165.9 | 170.1 | 168.5 | 168.2 | C(22) | 85.7 | 78.8 | 78.4 | 78.1 |
| C(9) | 43.1 | 37.7 | 40.3 | 35.2 | C(23) | 28.63 | 27.4 | 27.4 | 30.6 |
| C(10) | [a]) | 40.2 | 39.6 | 39.5 | C(24) | 39.8 | 42.5 | 42.5 | 37.8 |
| C(11) | 69.7 | 21.9 | 23.1 | 21.7 | C(25) | 82.0 | 71.5 | 71.5 | 29.4 |
| C(12) | 43.8 | 39.9 | 40.6 | 32.7 | C(26) | 28.54 | 29.0 | 29.0 | 22.9 |
| C(13) | 48.5 | 45.8 | 47.0 | 48.8 | C(27) | 29.1 | 30.0 | 30.0 | 23.5 |
| C(14) | 85.0 | 59.2 | 57.2 | 85.4 | | | | | |

[a]) Under the noise level.

accordance with both literature data [6] and our results (unpublished) obtained for 20-hydroxyecdysone. The free rotation of the side chain does not result in a fully averaged conformational mixture. Due to the different energy of the possible conformers, one preferred conformation of 20-hydroxyecdysone was found on the basis of the NOESY data. We assume that the conformations of the side chain in compounds **3** and **4** are analogous to that of 20-hydroxyecdysone. Based on this precondition, we assigned the absolute configuration at C(20) and C(22) with quite high probability, by a procedure which was described earlier [7]. For compound **1**, the above-mentioned NOESY correlations were sufficient to identify the configuration at C(20), but they were insufficient to determine the configuration at C(22). Fortunately, the absolute configuration (22*R*) of shidasterone has been established [8].

The NOESY plots of compound **2** did not allow to identify the configurations at C(20) and C(22) because of the overlapping of the $H_\beta$–C(12), $CH_2$(16), and $H_b$–C(24) signals. *Harmatha* and co-workers reported the structure determination and the $^1$H- and $^{13}$C-NMR signal assignments of 20-hydroxy-14-epiecdysone [9]. The $\delta$(H) and $\delta$(C) of the side chain of compound **2** were identical to those measured by *Harmatha* and co-workers within an acceptable margin of error. This fact supports that the configurations at C(20) and C(22) are the same in these compounds.

**Conclusions.** – Compound **2** merits special attention as regards the *cis*-fused *C*/*D* ring junction in the steroid skeleton. The structurally related compounds 20-hydroxy-14-epiecdysone and 14-epiponasterone A 22-glucoside have been identified from *Serratula wolffii* and *Leuzea chartamoides*, respectively [10][11]. To date, the *trans* fusion of the rings *C* and *D* was one of the important characteristics of ecdysteroids. The surprising discovery that there are 14-epiecdysteroids, including compound **2**, appears to disprove the classical chemical definition of ecdysteroids. Compounds **2** and **3** are 3-epiecdysteroids. These ecdysteroids are mainly synthesized in insects but also occur in plants, especially as minor constituents in *Serratula* and *Ajuga* species. Compound **1** is

11$\alpha$-hydroxy-substituted shidasterone. The side-chain in shidasterone and its derivatives comprises a five-membered ether ring. These ecdysteroid derivatives are rather unusual in plants, but it is noteworthy that they are characteristic for *Serratula wolffii.* Besides shidasterone itself, two shidasterone derivatives have been discovered in this plant [7]. Compound **1** with its 11$\alpha$-OH group may also be important with respect to a structure–activity relationship in view of its anabolic activity [12]. The known ponasterone A (**4**) is one of the most potent inducers in the gene-expression system [13].

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## Experimental Part

*General.* Column chromatography (CC): *C-Gel* octadecyl-silica (0.06–0.02 μm; *Chemie Uetikon*, Uetikon, Switzerland). HPLC: *Jasco-PU-2080* pump and *Jasco UV-2070/2075* detector; *Zorbax-SIL* column (5 μm, 4.6 mm × 250 mm; *DuPont*, Paris, France) for normal-phase HPLC and *Zorbax-SB-C18* column (5 μm, 4.6 mm × 250 mm; *DuPont*, Paris, France) for reversed-phase HPLC. Rotation planar chromatography (RPC): *Harrison 8924-Chromatotron* instrument (*Harrison Research*, Palo Alto, CA); stationary phase, silica gel *60 $GF_{254}$* (*E. Merck*). Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-2101 PC* spectrophotometer; MeOH solns.; $\lambda_{max}$ (log $\varepsilon$) in nm. NMR Spectra: *Bruker Avance-DRX-500* and *Varian Unity-Inova-600* spectrometer; ($D_4$)MeOH solns. in *Shigemi* sample tubes at r.t.; assignments by 1D and 2D methods and widely accepted strategies [14]; chemical shifts $\delta$ in ppm referenced to the solvent ($D_4$)MeOH ($\delta$(C) 49.15 and $\delta$(H) 3.31); $^1$H, $^{13}$C, and DEPT-135, 1D measurements with 64K data points for the FID; 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 600 ms), and 1D gs-NOESY (mixing time 300 ms) with the pulse programs from the *Bruker* and *Varian* software library. MS: *Finnigan TSQ-7000* tandem mass spectrometer (*Finnigan MAT*, San Jose, CA, USA) equipped with a laboratory-built nanoelectrospray ion source (high voltage of *ca.* 1000 V in the ion source), scanning over the mass range 10–1500, with a scan time of 2 s; in *m/z* (rel.%). HR-ESI-MS: *Finnigan MAT-95SQ* tandem mass spectrometer (*Finnigan MAT*, Bremen, Germany).

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

*Extraction and Isolation.* Fresh roots of *Serratula wolffii* (4.7 kg) were extracted with MeOH, and the extract was purified by fractionated precipitation with acetone. The dry residue (137.5 g) of the purified extract was applied to a MN-polyamide-SC-6 column (*Woelm*, Eschwege, Germany). The fraction eluted with $H_2O$ (24.4 g) was subjected to low-pressure reversed-phase CC (octadecyl-silica). The fractions eluted with 45% MeOH/$H_2O$ (1.9 g) were further purified by RPC. The fractions eluted with $CH_2Cl_2$/MeOH/$C_6H_6$ 50:5:3 (100 mg), were fractionated again by RPC (AcOEt/EtOH/$H_2O$ 80:5:2). These fractions were further purified by normal-phase HPLC (*cyclo*-$C_6H_{12}$/i-PrOH/$H_2O$ 100:40:3, 1 ml/min; detection at 245 nm): **1** (0.5 mg), **2** (0.8 mg), and **3** (1 mg). The reversed-phase CC gave a fraction (390 mg) which was separated by a combination of RPC and reversed-phase HPLC. From the RPC fraction eluted with $CH_2Cl_2$/MeOH/$C_6H_6$ 50:3:2, **4** (1 mg) was obtained by reversed-phase HPLC (MeCN/$H_2O$ 35:65, 0.8 ml/min; detection at 245 nm).

*(11α)-11-Hydroxyshidasterone* (= *(2β,3β,5β,11α,22*R*)-22,25-Epoxy-2,3,11,14,20-pentahydroxycholest-7-en-6-one*; **1**): $[\alpha]_D^{25.5} = +7$ ($c = 0.1$, MeOH). UV (MeOH): 249 (3.543). $^1$H- and $^{13}$C-NMR (($D_4$)MeOH): *Tables 1* and *2*. ESI-MS: 517 (15, $[M+K]^+$), 501 (25, $[M+Na]^+$), 479 (15, $[M+H]^+$), 461 (5, $[M+H-H_2O]^+$), 443 (20, $[M+H-2\,H_2O]^+$), 425 (20, $[M+H-3\,H_2O]^+$), 407 (100, $[M+H-4\,H_2O]^+$). HR-ESI-MS: 478.2925 ($C_{27}H_{42}O_7^+$; calc. 478.2919).

*(2β,3α,5β,14β,22*R*)-2,3,20,22,25-Pentahydroxycholest-7-en-6-one* (**2**): $[\alpha]_D^{25.5} = +5$ ($c = 0.1$, MeOH), UV (MeOH): 248 (3.611). $^1$H- and $^{13}$C-NMR (($D_4$)MeOH): *Tables 1* and *2*. ESI-MS: 503 (100, [*M* +

K]$^+$), 487 (19, [$M$ + Na]$^+$), 465 (46, [$M$ + H]$^+$), 447 (21.5, [$M$ + H−$H_2O$]$^+$), 429 (14.5, [$M$ + H−2 $H_2O$]$^+$), 411 (23, [$M$ + H−3 $H_2O$]$^+$). HR-ESI-MS: 464.3120 ($C_{27}H_{44}O_6^+$; calc. 464.3126).

*(2β,3α,5β,22*R*)-2,3,20,22,25-Pentahydroxycholest-7-en-6-one* (**3**): $[\alpha]_D^{25} = +14$ ($c = 0.05$, MeOH). UV (MeOH): 246 (3.744). $^1$H- and $^{13}$C-NMR ((D$_4$)MeOH): *Tables 1* and *2*. ESI-MS: 503 (25, [$M$ + K]$^+$), 487 (100, [$M$ + Na]$^+$), 465 (45, [$M$ + H]$^+$), 447 (46, [$M$ + H−$H_2O$]$^+$), 429 (17, [$M$ + H−2 $H_2O$]$^+$), 411 (24, [$M$ + H−3 $H_2O$]$^+$). HR-ESI-MS: 464.3129 ($C_{27}H_{44}O_6^+$; calc. 464.3126).

*Ponasterone A* (= *(2β,3β,5β,22*R*)-2,3,14,20,22-Pentahydroxycholest-7-en-6-one*; **4**). *Table 1* and *2* show the $^1$H- and $^{13}$C-NMR data. The other spectroscopic data are in accordance with the reported structure [5].

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