Ecdysteroids from *Silene viridiflora*

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Two new ecdysteroid acetonide derivatives, 5α-2-deoxy-20-hydroxyecdysone 20,22-acetonide (**6**) and makisterone C 2,3;20,22-diacetonide (**8**), were isolated from the dried herb of *Silene viridiflora*. The already known 5β-2-deoxy-20-hydroxyecdysone 20,22-acetonide (**7**) is additionally reported here as a new constituent of *S. viridiflora*. Five earlier described *S. viridiflora* ecdysteroids, integristerone A (**1**), 5,20,26-trihydroxyecdysone (26-hydroxypolipodine B; **2**), 20,26-dihydroxyecdysone (**3**), 2-deoxy-20-hydroxyecdysone (**4**), 2-deoxyintegristerone A (**5**), are also included because of their improved characterization. The structures were established *via* spectroscopic analyses, including one- and two-dimensional NMR and mass spectrometry.

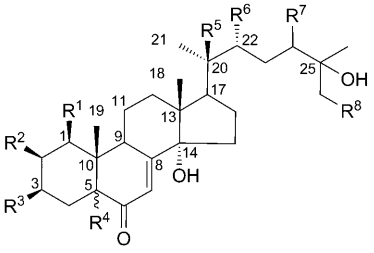
Introduction. – Phytoecdysteroids belong in the large group of polyhydroxylated steroids. They are structural analogs of the zooecdysteroids [1], which play an important role in the molting and metamorphosis of the arthropods [2]. The ecdysteroids in arthropods bind to the EcR protein, which is a member of the nuclear receptor superfamily [3]. After dimerization and the binding of the specific ligand, the nuclear receptors are able to translocate to the nucleus and regulate gene expression. Although the ecdysteroids exert a number of beneficial pharmacological effects on mammals (reviewed in [4]), the anabolic effect is the most widely examined one. While their mechanism of action is still unknown, it has already been demonstrated by radioligand binding assay that they are not able to bind to mammalian nuclear receptors [5]. This may explain the absence of their hormonal side-effects [6], which makes them a promising alternative to the anabolic steroids used in medical practice.

The distribution of phytoecdysteroids in the *Silene* genus is wide; the *Silene* species are characterized by a high accumulation of ecdysteroids even with unusual structures. More than 70 ecdysteroids (*ca.* 25% of the known phytoecdysteroids) have been detected in plants belonging in the *Silene* genus [7]. Several ecdysteroids with unusual hydroxylation profiles were isolated from *Silene* species, such as nusilsterone, which contains a 24-OH group [8], 2-deoxy-21-hydroxyecdysone [9], 20-hydroxy-22-epiecdysone [10], and 9α,20- [11] and 9β,20-dihydroxyecdysone [12]. Beside this, various acetate, benzoate, glucoside, sulfate, acetonide, crotonate, and tiglinate conjugations add the structural variety of the ecdysteroids from the *Silene* species [7]. Accordingly, the genus may be considered the most abundant natural source of these compounds.

Silene viridiflora L. contains a series of ecdysteroids characteristically hydroxylated at C(26). Four 26-hydroxylated ecdysteroids [13–15], two 26-hydroxylated ecdysteroid

diacetates [16], and three 26-hydroxylated ecdysteroid acetonides [15], as well as six further ecdysteroids [13] have been described from this plant.

We report now the isolation and structure elucidation of eight ecdysteroids from the *S. viridiflora* (*Fig. 1*): integristerone A (**1**), 5,20,26-trihydroxyecdysone (26-hydroxypolypodine B; **2**), 20,26-dihydroxyecdysone (**3**), 2-deoxy-20-hydroxyecdysone (**4**), 2-deoxyintegristerone A (**5**), the 5α- and 5β-isomer pair, **6** and **7**, respectively, of 2-deoxy-5,20-dihydroxyecdysone 20,22-acetonide, and the 2,3;20,22-diacetonide of makisterone C (**8**). Compounds **1**–**5** are already known, while compounds **6** and **8** are new natural compounds, and the known **7** is reported for the first time from *S. viridiflora*.



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8
1	OH	OH	OH	β-H	OH	OH	H	H
2	H	OH	OH	β-OH	OH	OH	H	OH
3	H	H	OH	β-H	OH	OH	H	OH
4	H	H	OH	β-H	OH	OH	H	H
5	OH	H	OH	β-H	OH	OH	H	H
6	H	H	OH	α-H			H	H
7	H	H	OH	β-H	O	O	H	H
8	H			β-H			Et	H

Fig. 1. *Structures of the isolated ecdysteroids* **1**–**8**

Results and Discussion. – The procedure for the isolation of compounds **1**–**8** from the MeOH extract includes liquid-liquid extraction, fractionated precipitation, and combined chromatographic procedures [14], such as column chromatography on polyamide, octadecyl-silica gel, silica gel, and a cyano-phase, rotation planar chromatography (RPC) on silica gel, and preparative NP-HPLC. The known compounds were identified by comparing their NP-HPLC retention times with those of authentic samples and their spectroscopic data with those published earlier [7]. The NMR data on **1**–**5** and **7** were supplemented through the use of advanced NMR methods. The structures of the new ecdysteroids **6** and **8** were elucidated by using NMR, UV, and MS measurements. *Fig. 1* shows the structures of compounds **1**–**8**.

The UV spectrum of compound **6** verified the presence of a conjugated C=C bond system. The molecular formula of **6**, $C_{30}H_{48}O_6$, was established on the basis of the molecular-ion peak observed in the HR-ESI-MS. ESI-MS gave a *pseudo*-molecular-ion peak at *m*/*z* 527 $[M+Na]^+$, which corresponds to the suggested structure. The UV spectrum of compound **8** is in accordance with the presence of the 7-en-6-one chromophore of ecdysteroids. The molecular formula of **8**, $C_{35}H_{56}O_7$, was established *via* the HR-ESI-MS molecular-ion peak, and the ESI-MS revealed a peak at *m*/*z* 611 for $[M+Na]^+$. The suggested formulas of compound **6** and **8** coincided with the ^{1}H- and ^{13}C-NMR data (see *Tables 1* and *2*). ^{1}H-NMR Data are given only for these two novel compounds.

Table 1. *^1H Chemical Shifts, Multiplicities, and Coupling Constants (J) of Compounds* **6** *and* **8**

Position		**6**	**8**
1	α	1.38–1.43 (*m*)	1.99 (*d*, *J* ~ 15.0[a]))
	β	1.87 (*d*, *J* ~ 12.5[a]))	1.23 (*t*, *J* ~ 14.5[a]))
2	α	1.76–1.82 (*m*)	4.27 (*dt*, *J* = 9.4, 5.0)
	β	1.37–1.41 (*m*)	–
3	α	3.55 (*tt*, *J* = 11.0, 4.4)	4.30 (*q*, *J* = 4.0)
4	α	2.07–2.14 (*m*)	1.95–2.01 (*m*)
	β	1.365 (*q*, *J* = 12.0[b]))	1.95–2.01 (*m*)
5	α	2.355 (*dd*, *J* = 12.2, 3.6)	2.245 (*dd*, *J* = 9.3, 8.2)
7		5.83 (*d*, *J* = 2.8)	5.80 (*d*, *J* = 2.5)
9	α	2.75 (*ddd*, *J*= 11.7, 7.2, 2.7)	2.935 (*ddd*, *J* = 11.7, 7.0, 2.5)
11	α	1.75–1.82 (*m*)	1.74–1.81 (*m*)
	β	1.60–1.66 (*m*)	1.65–1.70 (*m*)
12	α	2.07–2.16 (*m*)	2.105 (*td*, *J* = 13.0, 4.9)
	β	1.78–1.86 (*m*)	1.81–1.89 (*m*)
15	α	1.615 (*t*, *J* ~ 13.0[a]))	1.58–1.67 (*m*)
	β	1.93–1.98 (*m*)	1.96–2.02 (*m*)
16	α	1.83–1.89 (*m*)	1.83–1.90 (*m*)
	β	1.99–2.07 (*m*)	2.03–2.10 (*m*)
17	α	2.30 (*dd*, *J* = 9.1, 8.6)	2.304 (*t*, *J* = 8.9)
18	β	0.826 (*s*)	0.825 (*s*)
19	β	0.854 (*s*)	0.96 (*s*)
21		1.175 (*s*)	1.173 (*s*)
22		3.66–3.71 (*m*)	3.834 (*d*, *J* = 10.0)
23	a	1.47–1.58 (*m*)	1.16–1.25 (*m*)
	b	1.47–1.58 (*m*)	1.71–1.79 (*m*)
24	a	1.44–1.53 (*m*)	1.45 (*ddd*, *J* = 12.5, 6.6, 3.0)
	b	1.69–1.78 (*m*)	–
26		1.196 (*s*)	1.16 (*s*)
27		1.205 (*s*)	1.18 (*s*)
29		1.32 (*s*)	1.325 (*s*)
30		1.39 (*s*)	1.39 (*s*)
32			1.32 (*s*)
33			1.47 (*s*)
34	a		1.18–1.24 (*m*)
	b		1.63–1.72 (*m*)
35			1.006 (*t*, *J* = 7.5)

[a]) Coupling constant determined by HMQC. [b]) Coupling constant determined by COSY.

In the NMR investigation of the ecdysteroids **1**–**8**, the characteristic HMBC correlations of the Me signals over two and three bonds were utilized in their assignments. The HMBC correlations allowed the identification of the geminal Me(26)/Me(27) and the ketal-type (Me(29)/Me(30), Me(32)/Me(33)) Me groups. In compound **8**, the Me(35)/C(24) HMBC correlation verified the connection of an Et group to C(24). Differentiation between Me(19) and Me(18) was achieved by consideration of the coupling of the latter with C(17), which is also coupled to Me(21). In accordance with a 7,8-didehydro-6-oxo moiety, H–C(7) correlates *via* ^3J(C,H) couplings with C(5), C(9), and C(14). The H-atoms of ring *A* form a common spin system analyzed by

Table 2. *^{13}C Chemical Shifts of Compounds* **1**–**8**

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	76.5	34.4	29.8	29.9	72.6	38.0	29.9	38.9
2	68.6	68.6	29.1	29.0		31.5	29.4	73.8
3	71.1	70.4	65.6	65.6	68.7	71.31	65.5	73.3
4	33.6	36.3	33.3	33.5		31.0	33.3	27.8
5	46.9	80.4	52.5	52.5	47.8	54.7	52.4	52.6
6	205.7	202.5	206.7	206.7		203.3	206.5	205.8
7	122.3	120.7	122.1	122.1	122.3	123.6	122.0	122.0
8	167.4	167.7	168.6	168.6	168.0	166.5	168.3	167.0
9	35.7	39.1	34.4	34.9		47.6	34.6	35.9
10	43.9	45.6	37.8	37.8	42.3	39.7	37.6	39.0
11	22.0	22.6	22.0	22.0	21.7	21.7	21.9	21.8
12	32.6	32.7	32.9	32.9	32.5	32.4	32.6	32.5
13	48.7	48.9	49.3	49.4	49.1	48.4	49.2	49.0
14	85.2	85.2	85.6	85.6	85.5	85.3	85.6	85.4
15	31.9	31.9	31.8	31.8		31.9	31.7	31.8
16	21.5	21.6	21.7	21.7	21.7	22.5	22.6	22.7
17	50.7	50.6	50.7	50.7	50.7	50.7	50.6	50.4
18	18.1	18.2	18.2	18.2	18.2	17.8	17.8	17.8
19	20.1	17.1	24.5	24.5	20.0	13.4	24.5	24.2
20	78.0	78.0	78.1	78.1	78.1	86.0	85.9	86.1
21	21.1	21.2	21.2	21.2	21.1	22.7	22.7	22.6
22	78.5	78.6	78.6	78.6	78.6	83.5	83.4	81.5
23	27.5	26.7	26.7	27.5	27.5	24.9	24.8	30.9
24	42.5	37.3	37.3	42.5	42.5	42.4	42.3	50.0
25	71.4	73.8	73.8	71.5	71.5	71.27	71.2	74.4
26	29.1	70.9	70.9	29.1	29.1	29.1	29.1	26.8
27	29.8	23.7	23.7	29.8	29.8	29.6	29.6	27.9
28						108.2	108.1	108.1
29						27.3	27.3	27.2
30						29.5	29.5	29.5
31								109.6
32								26.8
33								29.0
34								25.3
35								14.9

^{1}H,^{1}H-COSY and HMQC-TOCSY experiments. The signals of rings *C* and *D*, as well as those of the side-chain at C(17), were assigned in an analogous way. The high chemical shifts of C(2) (in **8**), C(3) (in **8**), C(20) (in **6** and **8**), C(22) (in **6** and **8**), C(28) (108.2 ppm in **6** and **8**), and the C(31) (109.6 ppm in **8**), the H−C(22)/Me(29), H−C(1)/Me(33), and H−C(3)/Me(32) NOESY correlations proved the presence of ketal-type five-membered rings.

From the H_α−C(9)/H_α−C(2) and Me(19)/H_β−C(5) NOESY correlations in **8**, the *cis* junction of rings *A*/*B* is adequate. The H_α−C(1)/H_α−C(9), H_α−C(3)/H_α−C(5), H_α−C(5)/H_α−C(9), and Me(19)/H_β−C(4) NOESY correlations in **6** justified the *trans*-junction of rings *A*/*B*. The H_β−C(12)/Me(18), H_β−C(12)/Me(21), H_α−C(12)/H_α−C(17), Me(18)/H_β−C(15), and Me(18)/H_β−C(16) NOESY cross-peaks of both

compounds confirmed the *trans*-junction of rings *C*/*D*. It should be mentioned that in case of 14-epi steroids the steric interactions cause a characteristic 9 ppm shift of C(12) and C(15), but this is not observed in compounds **6** and **8** (see the ^{13}C chemical shifts of 20-hydroxyecdysone and 20-hydroxy-14-epiecdysone in [7]). The H_β–C(12)/Me(21), Me(21)/Me(30), H–C(22)/Me(29), and H–C(22)/CH_2(16) NOESY correlations verified the configurations of C(20) and C(22), respectively, and the conformation of the skeleton and the side chain of compounds **6** and **8** are depicted in *Fig. 2*, *a* and *2*, *b*. It should be mentioned that the NMR data can not prove the absolute configuration of the stereogenic centres in compound **8** and C(25) in compounds **2** and **3**.

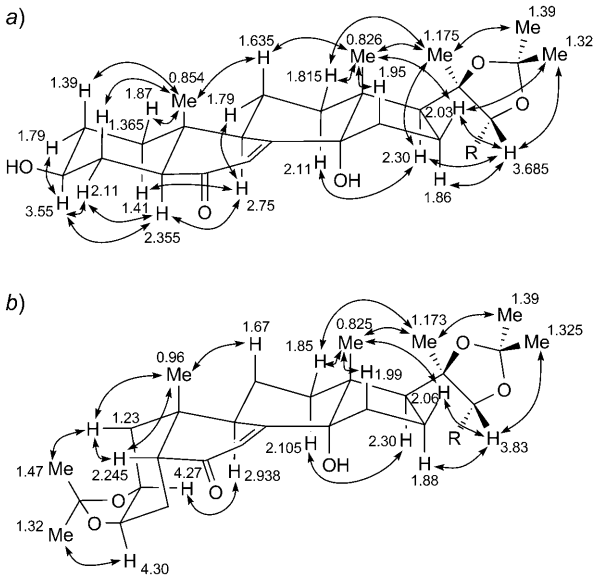
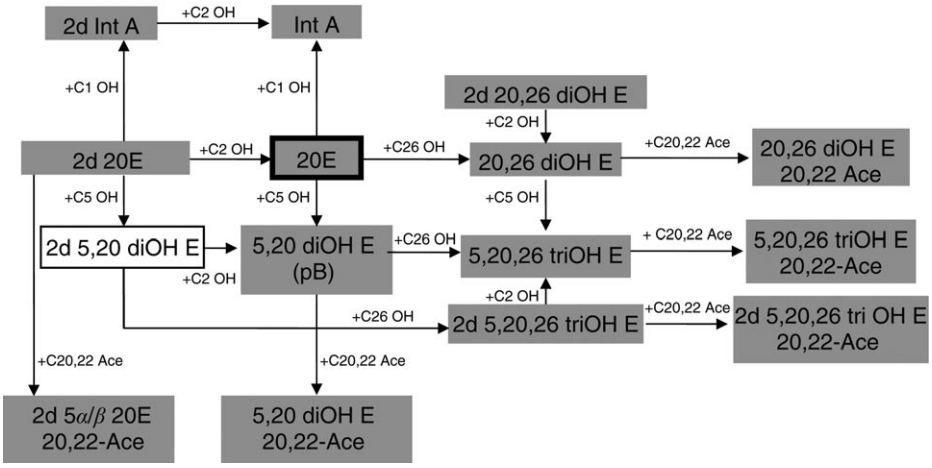


Fig. 2. *Structure of compounds a*) **6** *and b*) **8**. Arrows indicate the observed NOESY interactions. R: rest of the side-chain on C(17).

Scheme shows biosynthetic correlations between the ecdysteroids isolated from *S. viridiflora* by our team. The compound 2-deoxy-5,20-dihydroxyecdysone in the white box in the *Scheme* has not been isolated from *S. viridiflora*, although we could isolate its acetonide derivative, and the compound itself has been described from *S. italica* ssp. *nemoralis*. This is the missing link in the biogenetical correlations of *S. viridiflora* ecdysteroids, and the presence of this compound in the plant can therefore be expected. *Scheme* shows that the hydroxylation of the ecdysteroid skeleton is frequently found at C(1), C(2), C(5), and C(26).

Conclusions. – In *S. viridiflora*, a series of 2-deoxyecdysteroids have been found. They are common compounds in the *Silene* genus and intermediates of the biosynthesis of the main biologically important ecdysteroid, 20-hydroxyecdysone, and other, highly hydroxylated phytoecdysteroids. In several cases, the ecdysteroid biosynthesis in plants passes through 20-hydroxyecdysone and continues toward ecdysteroids containing seven or even a maximum of eight OH groups. *S. viridiflora* contains three

Scheme. *The Biosynthetic Correlation among the Ecdysteroids Isolated from S. viridiflora by Our Team*. The white box indicates a compound which has not been isolated yet from the *S. viridiflora*, but its presence can be expected in the plant.



Abbreviations: 20,26 diOH E 20,22-Ace, 20,26-dihydroxyecdysone 20,22-acetonide; 20,26 diOH E, 20,26-dihydroxyecdysone (**3**); 20E, 20-hydroxyecdysone; 2d 20,26 diOH E, 2-deoxy-20,26-trihydroxyecdysone; 2d 5,20 diOH E, 2-deoxy-5,20-dihydroxyecdysone; 2d 5,20,26 tri OH E 20,22-Ace, 2-deoxy-5,20,26-trihydroxyecdysone 20,22-acetonide; 2d 5,20,26 tri OH E, 2-deoxy-5,20,26-trihydroxyecdysone; 2d 5α/β 20E 20,22-Ace, 5α/β-2-deoxy-20-hydroxyecdysone 20,22-acetonide (**6**/**7**); 2d 5β,20E, 5β-2-deoxy-20-hydroxyecdysone (**4**); 2d Int A, 2-deoxyintegristerone A (**5**); 5,20 diOH E (pB), 5,20-dihydroxyecdysone (Polipodine B); 5,20 diOH E 20,22-Ace, 5,20-dihydroxyecdysone 20,22-acetonide; 5,20,26 triOH E 20,22-Ace, 5,20,26-trihydroxyecdysone 20,22-acetonide; 5,20,26 triOH E, 5,20,26-trihydroxyecdysone (**2**); Int A, Integristerone A (**1**).

heptahydroxylated ecdysteroids, integristerone A (**1**), 20,26-dihydroxyecdysone (**3**), and 5,20-dihydroxyecdysone (polypodine B) [13], and one octahydroxylated ecdysteroid, 5,20,26-trihydroxyecdysone (26-hydroxypolypodine B) (**2**). Only four octahydroxylated ecdysteroids are known [7].

Makisterone C has been found in several plant species, such as *Lemmaphyllum microphyllum*, *Podocarpus elatus*, *Podocarpus macrophyllus*, and *Leuzea carthamoides* [7]. Its 2,3;20,22-diacetonide derivative is a new natural compound, whereas the parent molecule is a commonly-occurring plant ecdysteroid.

The previously [15] and presently isolated ecdysteroid acetonides (compounds **6**–**8**) show the high derivative-synthesizing ability of the *S. viridiflora* growing in Hungary, so we might conclude that these components are characteristic to the plant. Ecdysteroid acetonides have previously been described from several other plant species [7] as natural components, and their genuine occurrence have also been proved experimentally [17].

Experimental Part

General. The stationary phases for column chromatography (CC) were: octadecyl-silica gel for vacuum reversed-phase chromatography (SiO_2; 0.06 – 0.02 μm, *Chemie Uetikon*, Uetikon, Switzerland); MN-polyamide (*SC 6*, *Woelm*, Eshwege, Germany) for polyamide column; CN-phase (0.063 – 0.200 mm, *Chemie Uetikon* C-gel, Uetikon, Switzerland) for cyano-phase chromatography, and silica gel *60* (*E. Merck*, Darmstadt, Germany) for normal phase CC. 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*). The HPLC work was performed on an *Agilent 1100 Series* Isocratic Pump (*Agilent Technologies Inc.*, Palo Alto, United States) coupled with a *Jasco UV-2075 Plus* detector (*Jasco Corporation*, Tokyo, Japan). *Zorbax RX-Sil* column (5 μm, *Agilent Technologies Inc.*, Palo Alto, United States) at 1 ml/min flow was used for NP-HPLC. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV 2101 PC* spectrophotometer; in MeOH. NMR Spectra: in (D_4)MeOH in a *Shigemi* sample tube at r.t. 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 [18][19]. Chemical shifts are given on the δ-scale and are referenced to the solvent ((D_4)MeOH: δ(C) 49.15 and δ(H) 3.31). In the 1D measurements (^{1}H, ^{13}C, APT, DEPT-135), 64-K 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 software library. MS Spectra: *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 used in the normal MS mode over the mass range 10 – 1500 amu, with a scan time of 2 s. HR-ESI-MS: *Finnigan MAT 95SQ tandem* mass spectrometer (*Finnigan MAT*, Bremen, Germany).

Plant Material. The aerial parts of the cultivated *S. viridiflora* were collected in June 2002 in Vácrátót, Hungary. A voucher specimen was deposited with the Department of Pharmacognosy, University of Szeged, Szeged, Hungary (specimen No.: SV-020612).

Extraction and Isolation. The dried herb (1.2 kg) was extracted with MeOH (13 l) and purified by liquid-liquid extraction and fractionated precipitation [14]. The dry residue (60.03 g) of the purified extract was subjected to reversed-phase CC on octadecyl-silica gel. The fraction eluted with 40% MeOH/H_2O was further purified by crystallization, and the mother liquid was separated by normal phase CC on SiO_2 and eluted with CH_2Cl_2/EtOH (10 : 0 to 7 : 3, *v*/*v*). The fractions eluted from the SiO_2 column with CH_2Cl_2/EtOH 8 : 2 (*v*/*v*) were subjected to RPC (AcOEt/EtOH/H_2O 160 : 40 : 20, *v*/*v*/*v*), and from the fractions containing compounds **1**, **2**, and **3** (0.15 g), the compounds were purified by NP-HPLC (CH_2Cl_2/i-PrOH/H_2O (125 : 40 : 3, *v*/*v*/*v*), 1 ml/min, UV detection 245 nm) to yield **1** (7 mg), **2** (2 mg), and **3** (1.8 mg). The fraction eluted with 50% MeOH (2.94 g) from the reversed-phase column was subjected to repeated CN-phase CC, and compound **4** (8 mg) was crystallized from the fraction eluted with 80% hexane/acetone (0.5 g), while compound **5** (6 mg) was obtained by NP-HPLC (CH_2Cl_2/i-PrOH/H_2O (125 : 40 : 2, *v*/*v*/*v*), 1 ml/min, UV detection at 245 nm) from fraction eluted with 70% hexane/acetone (0.02 g). The fraction eluted with 60% MeOH/H_2O (2.62 g) from the reversed-phase column was subjected to polyamide CC. The fraction eluted with 90% H_2O/MeOH (1.38 g) was separated by CN-phase CC, and the fractions eluted with 90% hexane/acetone were further purified by vacuum reversed-phase CC on octadecyl-silica gel. Compounds **6** (3.42 mg) and **7** (13.34 mg) were separated from the fractions eluted with 70% MeOH/H_2O (76.2 mg) by NP-HPLC on SiO_2 (CH_2Cl_2/i-PrOH/H_2O (125 : 40 : 2 *v*/*v*/*v*), 1 ml/min, UV detection at 245 nm). Compound **8** (3.35 mg) was purified from the fractions eluted with 75% MeOH/H_2O (34.2 mg) by NP-HPLC on SiO_2 (CH_2Cl_2/i-PrOH/H_2O (125 : 40 : 3, *v*/*v*/*v*), 1 ml/min, UV detection at 245 nm).

Integristerone A (=*(1β,5β)-1,14,20,25-Tetrahydroxycholest-7-en-6-one*; **1**). Colorless crystals. Physical and spectroscopic data determined for the isolated compound agree with those in the literature [7]. *Table 2* shows the ^{13}C-NMR data.

5,20,26-Trihydroxyecdysone (=*26-Hydroxypolipodine B*; *(2β,3β,5β,22*R*)-2,3,5,14,20,22,25,26-Octahydroxycholest-7-en-6-one*; **2**). Colorless crystals. Physical and spectroscopic data are identical with the data reported [13]. *Table 2* shows the ^{13}C-NMR data.

20,26-Dihydroxyecdysone (=*(3β,5β,22*R*)-3,14,20,22,25,26-Hexahydroxycholest-7-en-6-one*; **3**). Colorless crystals. Our spectroscopic data are in accordance with those reported in the literature [7].

2-Deoxy-20-hydroxyecdysone (=*(3β,5β,22*R*)-3,14,20,22,25-Pentahydroxycholest-7-en-6-one*; **4**). Colorless crystals. The physical and spectroscopic data obtained agree with the characteristic data reported earlier on the compound [7][13]. ^{13}C-NMR Data are shown in *Table 2*.

2-Deoxyintegristerone A (=*(1β,3β,5β,22*R*)-1,3,14,20,22,25-Hexahydroxycholest-7-en-6-one*; **5**). Colorless crystals. The spectroscopic data are in accordance with those reported in [7][20]. *Table 2* lists the ^{13}C-NMR data.

5α-2-Deoxy-20-hydroxyecdysone 20,22-Acetonide (=*(3β,5α,17β)-3,14-Dihydroxy-17-[(4*R*,5*R*)-5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]androst-7-en-6-one*; **6**). Colorless crystals. $[\alpha]_D^{25} = +57$ ($c = 0.05$, MeOH). UV (MeOH): 238 (3.4). ^{1}H- and ^{13}C-NMR ((D$_4$)MeOH): *Tables 1* and *2*. ESI-MS: 1031 (9, $[2M + Na]^+$), 544 (9, $[M + H + K]^+$), 528 (14, $[M + H + Na]^+$), 527 (41, $[M + Na]^+$), 504 (10, M^+), 487 (39, $[M + H - H_2O]^+$), 429 (100, $[M + H - H_2O - Me_2CO]^+$), 413 (23), 391 (60), 333 (21). HR-ESI-MS: 527.3336 ($[M + Na]^+$, $C_{30}H_{48}NaO_6^+$; calc. 527.3349).

5β-2-Deoxy-20-hydroxyecdysone 20,22-Acetonide (=*(3β,5β,17β)-3,14-Dihydroxy-17-[(4*R*,5*R*)-5-(3-hydroxy-3-methylbutyl)-2,2,4-trimethyl-1,3-dioxolan-4-yl]androst-7-en-6-one*; **7**). Colorless crystals. $[\alpha]_D^{25} = +14$ ($c = 0.05$, MeOH). UV (MeOH): 243 (3.7). ^{13}C-NMR ((D$_4$)MeOH): *Table 2*. The other spectroscopic data are in accordance with those reported in [7].

Makisterone C 2,3;20,22-Diacetonide (=rel-*(1*S*,3a*S*,5a*R*,6a*R*,9a*S*,10a*R*,10b*R*,12a*R*)-1-{(4*R*,5*R*)-5-[(2*R*)-2-Ethyl-3-hydroxy-3-methylbutyl]-2,2,4-trimethyl-1,3-dioxolan-4-yl}-1,2,3a,5a,6,6a,9a,10,10a,10-b,11,12,12a-tetradecahydro-3a-hydroxy-8,8,10a,12a-tetramethyl-5*H*-cyclopenta[7,8]phenanthro[2,3-*d*][1,3]dioxol-5-one*; **8**). Colorless crystals. $[\alpha]_D^{25} = +65$ ($c = 0.005$, MeOH). UV (MeOH): 240 (3.4). ^{1}H- and ^{13}C-NMR ((D$_4$)MeOH): *Tables 1* and *2*. ESI-MS: 627 (42, $[M + K]^+$), 612 (41, $[M + H + Na]^+$), 611 (100, $[M + Na]^+$), 588 (6.4, M^+), 576 (55, $[M + H + Na - 2\,H_2O]^+$), 563 (41), 545 (55), 531 (40, $[M + H - Me_2CO]^+$), 513 (42, $[M + H - H_2O - Me_2CO]^+$), 495 (20, $[M + H - 2\,H_2O - Me_2CO]^+$), 473 (17, $[M + H - 2\,Me_2CO]^+$), 455 (24, $[M + H - H_2O - 2\,Me_2CO]^+$), 437 (25, $[M + H - 2\,H_2O - 2\,Me_2CO]^+$), 419 (56, $[M + H - 3\,H_2O - 2\,Me_2CO]^+$), 391 (86), 381 (28), 338 (25), 333 (29). HR-ESI-MS: 611.3913 ($[M + Na]^+$, $C_{35}H_{56}NaO_7^+$; calc. 611.3924).

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