Withanolide Compounds from the Flower of *Datura metel* L.

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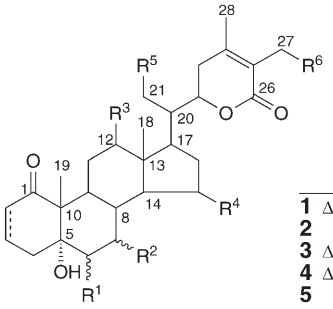
Three new withanolide compounds named baimantuoluoline A (**1**), B (**2**), and C (**3**) and the two known withanolides withafastuosin E (**4**) and withametelin C (**5**) were isolated from the fraction exhibiting activity for psoriasis in the flower of *Datura metel* L. The three new structures were determined as (5α,6α,7α,12β,15β,22*R*)-6,7-epoxy-5,12,15-trihydroxy-1-oxowitha-2,24-dienolide (**1**), (5α,6β,15β,22*R*)-5,6,15,21-tetrahydroxy-1-oxowith-24-enolide (**2**), and (5α,6β,12β,22*R*)-5,6,12,21-tetrahydroxy-27-methoxy-1-oxowitha-2,24-dienolide (**3**) on the basis of extensive spectroscopic data (HR-ESI-MS, ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY) (withanolide = 22-hydroxyergostan-26-oic acid δ-lactone).

Introduction. – Withanolides (= 22-hydroxyergostan-26-oic acid δ-lactones) are a group of C_{28} steroidal lactones isolated from several genera of Solananceae. A characteristic feature of their skeleton is the (mostly) α,β-unsaturated δ-lactone ring formed in the side chain. Biogenetic transformations, however, can produce highly modified compounds, both at the steroid polycycle and within the side chain. Such compounds have been described as withasteroids.

Flos daturae (baimantuoluo in Chinese) is the dry flower of *Datura metel* L., which belongs to the family of the Solananceae. It was originally recorded in a Chinese ancient book, 'Compendium of Materia Medica', and also described in the 'Chinese Pharmacopoeia'. This herb has the long history as a traditional Chinese medicine and is widely utilized to cure many diseases such as cough, asthma, convulsion, *etc.*, due to its strong and wide biological activities. It has been reported that the clinical use of *D. metel* L. showed a significant effect on the treatment of psoriasis. However, few reports on its active constituents and pharmacological effects related to the treatment of psoriasis were published. Our studies have shown that the nonalkaloid H_2O-soluble part of the flower of *D. metel* L. plays a significant role in treating psoriasis. Moreover, the 50% EtOH fraction of the extract has been determined by pharmalogical research as an active principle in healing psoriasis.

In this paper, we report that three new withanolides named baimantuoluolines **A**–**C** (**1**–**3**) were isolated from the 50% EtOH eluate fraction obtained from an extract of *D. metel* L. Also isolated from the same fraction were the two known withanolides withafastuosin E (**4**) and withametelin C (**5**). The chemical structures of **1**–**5** were fully elucidated by extensive spectroscopic methods including ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY experiments and HR-ESI-MS.



		R^1	R^2	R^3	R^4	R^5	R^6
1	Δ^2	–O–		OH	OH	H	H
2		OH	H	H	OH	OH	H
3	Δ^2	OH	H	OH	H	OH	MeO
4	Δ^2	OH	H	H	H	OH	OH
5		OH	H	H	H	OH	H

Results and Discussion. – Compound **1**, named baimantuoluoline A, was obtained as white powder. The molecular formula was determined as $C_{28}H_{38}O_7$ by analysis of the HR-ESI-MS, indicating 10 degrees of unsaturation. The UV spectrum showed an absorption maximum at 225 nm ($\log \varepsilon$ 4.3) in MeOH, which is characteristic for the overlapping of two chromophores, namely of an α,β-unsaturated C=O group and an unsaturated δ-lactone system [1–3]. In accord with the ^{1}H- and ^{13}C-NMR (*Table*), HSQC, ^{1}H,^{1}H-COSY, HMBC, and NOESY data (*Fig.*), and published data of similar compounds, the structure of baimantuoluoline (**1**) was established as (5α,6α,7α,15β,22*R*)-6,7-epoxy-5,12,15-trihydroxy-1-oxowitha-2,24-dienolide.

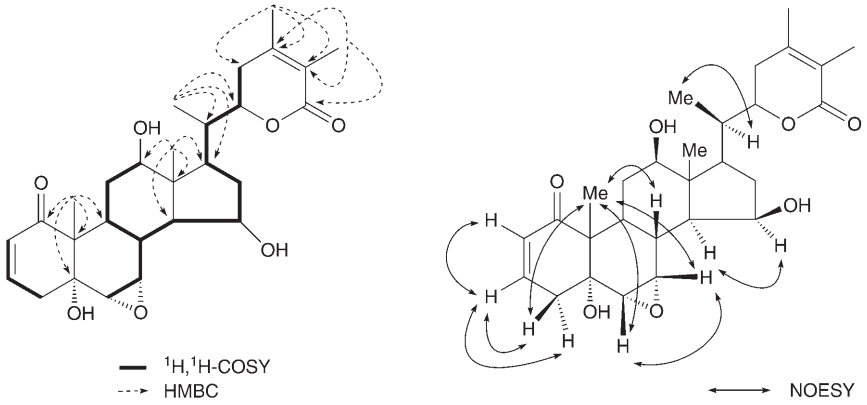


Figure. *Key ^{1}H,^{1}H-COSY and HMBC interactions* (left) *and key NOESY correlations* (right) *of* **1**

The ^{1}H-NMR spectrum of **1** included several features which are characteristic of withanolides. Four *s* at δ 1.03, 1.21, 1.85, and 1.98 were attributed to Me(18), Me(19), Me(27), and Me(28), respectively. The Me(21) signal appeared as a *d* at δ 1.17 (J = 6.8 Hz). The two olefinic protons at δ 5.74 (*dd*, J = 10.0, 2.4 Hz) and 6.64 (*ddd*, J = 10.0, 5.2, 2.0 Hz) were attributed to H–C(2) and H–C(3), respectively. The multiplicity of the H–C(3) signal indicated that position C(4) was unsubstituted. Thus, ring A was a 1-oxocyclohex-2-ene moiety. A *d* at δ 3.02 (J = 4.0 Hz) was due to a methine proton at C(6), and a *dd* at δ 3.62 (*dd*, J = 3.6, 1.6) was assigned to H–C(7). The chemical shifts and coupling constants of H–C(6) and H–C(7) indicated the presence of a 6α,7α-epoxy-5α-hydroxy-substituted steroid because their positions and multiplicities agreed with those of a similar substitution pattern [4]. There is a *dd* at δ 3.36

Table. *^{1}H- and ^{13}C-NMR Data of* **1**–**3**. At 400 and 100 MHz, resp., in CD_3OD. δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	205.8	–	217.0	–	207.3
H–C(2) or CH_2(2)	5.74 (*dd*, *J* = 10.0, 2.4)	129.4	2.71–2.80 (*m*), 1.96–2.04 (*m*)	38.2	5.78 (*dd*, *J* = 10.0, 2.4)	128.9
H–C(3) or CH_2(3)	6.64 (*ddd*, *J* = 10.0, 5.2, 2.0)	142.4	1.98–2.04 (*m*), 1.89–1.97 (*m*)	21.9	6.66 (*ddd*, *J* = 10.0, 5.2, 2.0)	144.0
CH_2(4)	2.78 (*dt*, *J* = 18.8, 2.4), 2.45 (*dd*, *J* = 18.8, 5.2)	38.0	2.62–2.70 (*m*), 1.30–1.34 (*m*)	31.2	3.26 (*dt*, *J* = 20.0, 2.4), 2.06 (*dd*, *J* = 20.0, 5.2)	36.5
C(5)	–	74.9	–	79.4	–	78.2
H–C(6)	3.02 (*d*, *J* = 4.0)	56.9	3.52 (*t*, *J* = 2.6)	76.6	3.53 (*t*, *J* = 2.0)	75.2
H–C(7) or CH_2(7)	3.62 (*dd*, *J* = 3.6, 1.6)	56.4	1.76–1.86 (*m*)	33.2	1.54–1.70 (*m*)	33.9
H–C(8)	2.00–2.05 (*m*)	33.2	2.00–2.05 (*m*)	27.4	1.68–1.73 (*m*)	30.4
H–C(9)	1.75–1.82 (*m*)	34.7	1.90–1.97 (*m*)	42.4	1.89–1.96 (*m*)	41.0
C(10)	–	52.3	–	55.7	–	52.8
CH_2(11)	2.89 (*dt*, *J* = 12.8, 4.0), 1.29–1.39 (*m*)	33.0	1.76–1.81 (*m*), 1.25–1.34 (*m*)	23.8	2.45 (*dt*, *J* = 12.4, 3.6), 1.35–1.44 (*m*)	33.6
H–C(12) or CH_2(12)	3.36 (*dd*, *J* = 11.2, 4.4)	78.9	1.90–1.95 (*m*), 1.34–1.42 (*m*)	41.8	3.58 (*dd*, *J* = 11.2, 4.0)	80.0
C(13)	–	49.6	–	43.8	–	49.6
H–C(14)	1.20–1.28 (*m*)	55.7	1.07 (*dd*, *J* = 10.9, 5.6)	61.2	1.17–1.25 (*m*)	55.3
H–C(15) or CH_2(15)	4.36 (*t*, *J* = 5.8)	69.9	4.23 (*t*, *J* = 5.6)	70.8	1.25–1.33 (*m*), 1.68–1.73 (*m*)	24.9
CH_2(16)	2.30–2.36 (*m*), 1.65–1.69 (*m*)	41.3	2.27–2.34 (*m*), 1.45–1.52 (*m*)	40.8	1.83–1.91(*m*), 1.49–1.57 (*m*)	28.8
H–C(17)	1.46–1.51 (*m*)	54.6	1.60–1.69 (*m*)	48.5	1.81–1.86 (*m*)	49.3
Me(18)	1.03 (*s*)	10.1	1.03 (*s*)	15.8	0.82 (*s*)	8.3
Me(19)	1.21 (*s*)	15.3	1.42 (*s*)	17.5	1.31 (*s*)	16.1
H–C(20)	2.00–2.09 (*m*)	39.0	1.90–1.95 (*m*)	46.7	1.89–1.96 (*m*)	47.5
Me(21) or CH_2(21)	1.17 (*d*, *J* = 6.8)	15.3	3.90 (*dd*, *J* = 11.2, 2.5), 3.74 (*dd*, *J* = 11.2, 4.4)	60.0	3.91 (*dd*, *J* = 11.6, 3.6), 3.85 (*dd*, *J* = 11.6, 3.2)	60.5
H–C(22)	4.55 (*dt*, *J* = 13.8, 4.5)	80.8	4.46 (*dt*, *J* = 13.5, 3.5)	79.5	4.56 (*dt*, *J* = 13.2, 3.6)	79.5
CH_2(23)	2.48 (*dd*, *J* = 18.8, 13.6), 2.15 (*dd*, *J* = 18.8, 2.0)	31.9	2.84 (*dd*, *J* = 18.0, 15.6), 2.21 (*dd*, *J* = 18.0, 2.4)	33.7	2.83 (*dd*, *J* = 18.4,13.6), 2.38 (*dd*, *J* = 18.4, 2.8)	33.6
C(24)	–	152.8	–	153.5	–	160.5
C(25)	–	122.1	–	122.0	–	123.7
C(26)	–	169.6	–	169.5	–	168.3
Me(27) or CH_2(27)	1.85 (*s*)	12.4	1.85 (*s*)	12.5	4.25 (*d*, *J* = 10.8), 4.17 (*d*, *J* = 10.8)	66.5
Me(28)	1.98 (*s*)	20.5	1.97 (*s*)	20.4	2.08 (*s*)	20.5
*Me*O–C(27)	–	–	–	–	3.34 (*s*)	58.4

($J = 11.2, 4.4$ Hz) in the ^{1}H-NMR spectrum, whose low coupling constants and splitting is characteristic of a (12β)-12-hydroxywithanolide [5][6]. Furthermore, a significant COSY interaction was observed between the protons at δ 4.36 (H–C(15)) and 1.24 (H–C(14)) (*Fig.*), suggesting the presence of an OH group at C(15). The ^{13}C-NMR spectrum of **1** indicated the presence of 28 C-atoms including five Me, four CH_2, and twelve CH groups, and seven quaternary C-atoms. The characteristic downfield signals at δ 205.8 and 169.6 were due to ketone and lactone C=O, respectively, and the characteristic *d* at δ 129.4 and 142.4 arose from the olefinic C(2) and C(3), respectively, in ring A. The *s* at δ 152.8 and 122.1 were attributed to the quaternary olefinic C(24) and C(25), respectively. The typical signals at δ 80.8, 78.9, 74.9, 69.9, 56.9, and 56.4 were assigned to the oxygenated atoms C(22), C(12), C(5), C(15), C(6), and C(7), respectively. The signals appearing at δ 20.5, 15.3, 15.3, 12.4, and 10.1 were assigned to Me(28), Me(19), Me(21), Me(27), and Me(18), respectively. In the ^{1}H,^{13}C-HMBC plot, long-range correlations were observed between Me(18) and C(12), C(13), C(14), and C(17), between Me(19) and C(1), C(5), C(9), and C(10), between Me(21) and C(17), C(20), and C(22), and between Me(28) and C(24), C(25), and C(26) (*Fig.*). Taking for granted the absolute configuration of the steroid polycycle, the configuration at C(22) can be determined by the multiplicity of the H–C(22) signal in the ^{1}H-NMR spectrum: in case of a broad *s* ($W_{1/2} \approx 5.0$ Hz), the configuration is (*S*), and in case of a *dd* ($J = 13.8$, 4.5 Hz), the configuration is (*R*) [7–11]. In the NOESY plot, no NOE correlation was observed between H–C(15) and Me(18) (*Fig.*). Thus, H–C(15) must be α-oriented.

Compound **2**, named baimantuoluoline B, was obtained as white powder. The molecular formula was determined as $C_{28}H_{42}O_7$ by analysis of the HR-ESI-MS, indicating 8 degrees of unsaturation. The assignments of all the functional groups of **2** were achieved by analysis of the ^{1}H- and ^{13}C-NMR (*Table*), DEPT, ^{1}H,^{1}H-COSY, HSQC, and HMBC data, allowing to elucidated the structure of **2** as (5α,6β,15β,22*R*)-5,6,15,21-tetrahydroxy-1-oxowith-24-enolide.

In the typical ^{1}H-NMR spectrum of **2**, no signals of olefinic protons were observed, *i.e.*, the α,β-unsaturated carbonyl system in ring A was absent. Four Me *s* appeared at δ 1.03, 1.42, 1.85, and 1.97. The downfield chemical shifts of Me(27) and Me(28) supported their location at a C=C bond. The missing Me(21) signal and the appearance of 2 *dd* as part of an *ABX* system at δ 3.90 and 3.74 strongly suggested that C(21) was present as a CH_2OH group [12]. The secondary OH group was assigned to C(6) in the β-configuration because of the small coupling constants of the *t* of H–C(6) ($J = 2.6$ Hz). The tertiary OH group was assigned to C(5) in the α-configuration as the resonances within ring A and B of **2** were in complete agreement with those of 5α,6β-dihydroxy-1-oxo-substituted withanolides [13–16]. A significant COSY interaction was observed between the protons at δ 4.23 (H–C(15)) and 1.07 (H–C(14)). In the NOESY plot, no NOE correlation was observed between H–C(15) and Me(18), establishing the α-orientation of H–C(15). The typical downfield methine *dt* at δ 4.46 ($J = 13.5, 3.5$ Hz) was assigned to the H–C(22) of the lactone moiety. The ^{13}C-NMR spectrum of **2** indicated the presence of 28 C-atoms including four Me, nine CH_2, and eight CH groups, and seven quaternary C-atoms. The characteristic downfield signals at δ 217.0 and 169.5 were due to the ketone and lactone C=O, respectively, and the characteristic *s* at δ 153.5 and 122.0 arose from the quaternary olefinic C(24) and C(25), respectively. The typical signals at δ 79.5, 79.4, 76.6, 70.8, and 60.0 were assigned to the oxygenated atoms C(22), C(5), C(6), C(15), and C(21), respectively, and the signals appearing at δ 20.4, 17.5, 15.8, 12.5 to Me(28), Me(19), Me(18), and Me(27), respectively.

Compound **3**, named baimantuoluoline C, was obtained as white powder. The UV spectrum showed absorption maxima at 225 nm (log ε 4.5), which is characteristic for the overlapping of two chromophores, the α,β-unsaturated C=O and the α,β-unsaturated δ-lactone system. The molecular formula was determined as $C_{29}H_{42}O_8$ by analysis of the HR-ESI-MS, indicating 9 degrees of unsaturation. The assignments of all the functional groups of **3** were achieved by analysis of the ^{1}H- and ^{13}C-NMR (*Table*),

DEPT, ^{1}H,^{1}H-COSY, HSQC, and HMBC data, allowing to elucidate the structure of **3** as (5α,6β,12β,22*R*)-5,6,12,21-tetrahydroxy-27-methoxy-1-oxowitha-2,24-dienolide.

The ^{1}H-NMR spectrum of **3** was characteristic of the steroidal structure of a withanolide. Three *s* at δ 0.82, 1.31, and 2.08 were attributed to Me(18), Me(19), and Me(28), respectively. The missing Me(21) signal and the appearance of 2 *dd* at δ 3.91 and 3.85 strongly suggested that C(21) was present as a CH_2OH group. The missing Me(27) signal and the appearance of 2 *dd* at δ 4.17 and 4.25 strongly suggested that C(27) was substituted by an MeO group. The signals of the olefinic protons at δ 5.78 (*dd*, $J = 10.0$, 2.4 Hz) and 6.66 (*ddd*, $J = 10.0$, 5.2, 2.0 Hz) were attributed to H–C(2) and H–C(3), respectively, in a steroidal 1-oxo-2-ene system. The multiplicity of the H–C(3) signal indicated that C(4) was unsubstituted. The secondary OH group was assigned to C(6) in the β-configuration because of the small coupling constants of the *t* of H–C(6) ($J = 2.0$ Hz). The tertiary OH group was assigned to C(5) in the α-configuration as the resonances of the protons of ring A and B were in complete agreement with those of withanolides having a 5α,6β-dihydroxy-1-oxo-2-ene moiety [13–17]. The *dd* at δ 3.58 ($J = 11.2$, 4.0 Hz) was characteristic of a (12β)-12-hydroxywithanolide. The ^{13}C-NMR spectra of **3** showed resonances for all 29 C-atoms. A notable feature was the appearance of a downfield quaternary-C-atom signal at δ 207.3 which was due to a C=O group. The three quaternary-C-atom signals at δ 160.5, 123.7, and 168.3 were due to two alkene C-atoms and to an ester C=O group. In the ^{1}H,^{13}C-HMBC plot, long-range correlations were observed between Me(18) and C(12), C(13), C(14), and C(17), between Me(19) and C(1), C(5), C(9), and C(10), and between Me(28) and C(24), C(25), and C(26).

Compounds **4** and **5** were elucidated as withafastuosin E (=(5α,6β,22*R*)-5,6,21,27-tetrahydroxy-1-oxowitha-2,24-dienolide) [17] and withametelin C (=5α,6β,22*R*)-5,6,21-trihydroxy-1-oxowith-24-enolide) [5], respectively, with the help of spectroscopic techniques. Their ^{1}H- and ^{13}C-NMR data were in agreement with the ones reported in the literature.

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Experimental Part

General. Ion exchange: macroporous absorption resin (*Styrene-DVB* 001 × 7 and *AB-8* cross-linked polystyrene; *Nan Kai*, Tian Jin, China). Column chromatography (CC): silica gel (200–300 mesh; *Yinhai*, Qing Dao, China); *ODS-A* (120A, 50 μm; *YMC Co.*); *Sephadex LH-20* (18–111 μm dry; 27–163 μm in MeOH; *Amersham Co.* Anal. HPLC: *Waters 2695-2996* instrument; *Hypersil ODS II* (5 μm 4.6 × 250 mm; *Yilite*, Da Lian, China). Prep. HPLC: *Waters Delta-600-2487* instrument; *Hypersil ODS II* (10 μm, 20 × 300 mm; *Yilite*, Da Lian, China) and *Pegasil ODS II* (5 μm, 10 × 250 mm; *Senshu Pak*, Japan). M.p.: *Kofler* micromelting-point apparatus; uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *Shimadzu UV-1601*; λ_{max} (log ε) in nm. CD Spectra: *Jasco J-715* spectrometer; λ ($\Delta\varepsilon$) in nm. NMR Spectra: *Bruker DPX-400* spectrometer; at 400 (^{1}H) and 100 MHz (^{13}C); chemical shifts δ in ppm rel. to $SiMe_4$ as internal standard, coupling constant *J* in Hz. HR-ESI-MS and ESI-MS: *IonSpec Ultima-7.0-T-FTICR* and *Finnigan MAT-LCQ* mass spectrometer, resp.; in *m*/*z*.

Plant Material. The dry flowers of *Datura metel* L. were collected from Jiangsu Province of China in 2002. The voucher specimen was deposited at the Heilongjiang University of Chinese Medicine, Harbin, China.

Extraction and Isolation. The dried flowers (30 kg) of *Datura metel* L. were extracted with hot 70% EtOH under reflux (2 × 100 l) for 2.5 h, and the combined soln. was filtered and concentrated to a syrup, which was suspended in H_2O. The suspension was acidified with 0.1% HCl soln., and then filtered and

subjected to ion exchange (*Styrene-DVB* 001 × 7). The exchanged soln. was again submitted to ion exchange (*AB-8* cross-linked polystyrene, sequentially H_2O, 50% EtOH/H_2O, and 95% EtOH/H_2O. The 50% EtOH/H_2O eluate was concentrated to yield a syrup (52 g), which was subjected to CC (silica gel, CH_3Cl_3/MeOH 10:1 → 1:1): *Fractions 1–10*. *Fr. 5* was subjected to CC (*Sephadex LH-20*, MeOH/H_2O 5:5; then *ODS*) to yield five white powders which were purified by prep. HPLC and semi-prep. HPLC: **1** (24 mg), **2** (38 mg), **3** (19 mg), **4** (27.5 mg), and **5** (25.5 mg).

Baimantuoluoline A (=*(5α,6α,7α,12β,15β,22*R*)-6,7-Epoxy-5,12,15,22-tetrahydroxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **1**): White powder. M.p. 257–260°. $[\alpha]_D^{20} = +26$ ($c = 0.18$, MeOH). UV (MeOH): 225 (4.3). CD (MeOH): 255 (+6.5; pos. max). ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 995 ($[2M + Na]^+$). HR-ESI-MS (pos.): 509.25164 ($C_{28}H_{38}O_7Na^+$, $[M + Na]^+$; calc. 509.25152).

Baimantuoluoline B (=*(5α,6β,15β,22*R*)-5,6,15,21,22-Pentahydroxy-1-oxoergost-24-en-26-oic Acid δ-Lactone*; **2**): White powder. M.p. 212–215°. $[\alpha]_D^{20} = +57.8$ ($c = 0.35$, MeOH). UV(MeOH): 223 (3.5). CD (MeOH): 249 (+0.71, pos. max). ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 513 ($[M + Na]^+$). HR-ESI-MS (pos.): 513.28199 ($C_{28}H_{42}O_7Na^+$, $[M + Na]^+$; calc. 513.28282).

Baimantuoluoline C (=*(5α,6β,12β,22*R*)-5,6,12,21,22-Pentahydro-27-methoxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **3**): White powder. M.p. 262–265°. $[\alpha]_D^{20} = +17.5$ ($c = 0.10$, MeOH). UV (MeOH): 225 (4.5). CD (MeOH): 256 (+5.3, pos. max). ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS: 519 ($[M + H]^+$). HR-ESI-MS (pos.): 541.27644 ($C_{29}H_{42}O_8Na^+$, $[M + Na]^+$; calc. 541.27774).

Withafastuosin E (=*(5α,6β,22*R*)-5,6,21,22,27-Pentahydroxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **4**): White powder. ^{1}H-NMR (CD_3OD, 400 MHz): 0.80 (*s*, 3 H); 1.30 (*s*, 3 H); 2.04 (*dd*, $J = 19.8$, 5.2, 1 H); 2.09 (*s*, 3 H); 2.29 (*dd*, $J = 18.0$, 3.2, 1 H); 2.94 (*dd*, $J = 18.0$, 13.5, 1 H); 3.25 (*dt*, $J = 19.8$, 2.5, 1 H); 3.77 (*dd*, $J = 11.2$, 4.4, 1 H); 3.92 (*dd*, $J = 11.2$, 2.5, 1 H); 4.30 (*d*, $J = 11.7$, 1 H); 4.38 (*d*, $J = 11.7$, 1 H); 4.52 (*dt*, $J = 13.4$, 3.4, 1 H), 5.76 (*dd*, $J = 10.0$, 2.4, 1 H); 6.64 (*ddd*, $J = 10.0$, 5.2, 2.0, 1 H). ^{13}C-NMR (CD_3OD, 100 MHz): 13.0 (C(18)); 16.3 (C(19)); 20.2 (C(28)); 24.5 (C(11)); 25.3 (C(15)); 28.1 (C(16)); 31.4 (C(8)); 33.9 (C(23)); 34.1 (C(7)); 36.6 (C(4)); 40.5 (C(9)); 42.6 (C(12)); 44.0 (C(13)); 46.9 (C(17)); 46.9 (C(20)); 53.0 (C(10)); 56.4 (C(27)); 57.0 (C(14)); 60.0 (C(21)); 75.2 (C(6)); 78.3 (C(5)); 79.4 (C(22)); 126.3 (C(25)); 129.0 (C(2)); 143.9 (C(3)); 158.6 (C(24)); 168.6 (C(26)); 207.6 (C(1)). ESI-MS (pos.): 999 ($[2M + Na]^+$).

Withametelin C (=*(5α,6β,22*R*)-5,6,21,22-Tetrahydroxy-1-oxoergost-24-en-26-oic Acid δ-Lactone*; **5**): White powder. ^{1}H-NMR (CD_3OD, 400 MHz): 0.68 (*s*, 3 H); 1.25 (*s*, 3 H); 1.75 (*s*, 3 H); 1.95 (*s*, 3 H); 2.16 (*t*, $J = 16.3$, 1 H); 2.77 (*t*, $J = 16.0$, 1 H); 3.36 (br. *s*); 4.34 (*dt*, $J = 13.5$, 3.5, 1 H). ^{13}C-NMR (CD_3OD, 100 MHz): 12.5 (C(27)); 12.6 (C(18)); 16.7 (C(19)); 20.3 (C(28)); 20.5 (C(3)); 22.6 (C(11)); 24.1 (C(15)); 26.6 (C(16)); 29.7 (C(8)); 30.2 (C(4)); 32.2 (C(23)); 33.8 (C(7)); 37.0 (C(2)); 38.9 (C(12)); 40.6 (C(9)); 42.7 (C(13)); 45.3 (C(20)); 47.0 (C(17)); 53.8 (C(10)); 55.5 (C(14)); 58.1 (C(21)); 74.2 (C(6)); 77.4 (C(22)); 77.8 (C(5)); 120.3 (C(25)); 151.4 (C(24)); 166.3 (C(26)); 213.2 (C(1)). ESI-MS (pos.): 971 ($[2M + Na]^+$).

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