Further Withaphysalin Derivatives from *Acnistus arborescens*

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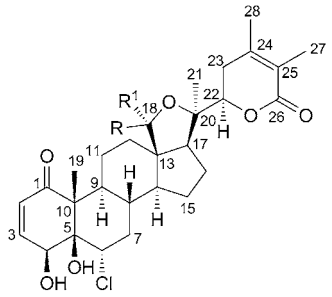
Two new epimeric chlorinated withaphysalins, *rel*-(4*β*,5*β*,6*α*,18*S*,22*R*)- and *rel*-(4*β*,5*β*,6*α*,18*R*,22*R*)-6-chloro-18,20-epoxy-18-ethoxy-4,5-dihydroxy-1-oxowitha-2,24-diene-26,22-lactone (**1** and **2** resp.), together with the new *rel*-(4*β*,5*β*,6*α*,18*R*,22*R*)-6-chloro-18,20-epoxy-4,5-dihydroxy-18-methoxy-1-oxowitha-2,24-diene-26,22-lactone (**3**) and *rel*-(3*β*,4*β*,5*β*,6*β*,18*R*,22*R*)-5,6:18,20-diepoxy-3,18-diethoxy-4-hydroxy-1-oxowith-24-ene-26,22-lactone (**4**) were isolated from the leaves of *Acnistus arborescens* and named withaphysalins T–W, respectively. The final structures and the complete ^{1}H- and ^{13}C-NMR assignments of the three chlorowithaphysalins **1**–**3** were performed by means of HR-ESI-MS and 1D- and 2D-NMR experiments, including COSY, HSQC, and HMBC, beside comparison with spectral data of analogous compounds from the literature. The structure of **4** was also confirmed by means of a single-crystal X-ray diffraction analysis.

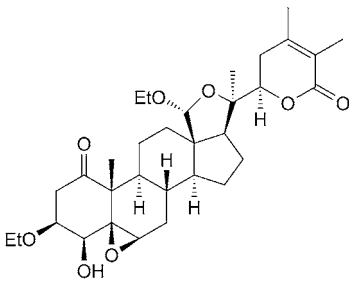
Introduction. – Plants of the genus *Acnistus* (Solanaceae) produce a complex group of natural C_{28} steroid lactones classified as acnistins, withanolides, jaborols, and withaphysalins [1]. As part of a chemical and pharmacological collaborative research program to identify novel naturally occurring anticancer agents, we investigated plants from the northeast of Brazil flora, among them one, *Acnistus arborescens*, is a medicinal plant used to treat cancerous growths [2]. Different research groups have previously studied this species reporting the isolation of several cytotoxic withanolides (=withan-26,22-lactones = 22-hydroxyergostan-26-oic acid *δ*-lactones) [2][3]. More recently, the isolation and characterization of several new withaphysalins, including their cytotoxic effects against several tumor cell lines have been reported by our group [4–6].

In this article, the isolation and the complete ^{1}H- and ^{13}C-NMR assignments of the three new chlorinated withaphysalins **1**–**3** and of the new diethoxylated withaphysalin **4** from the leaves of *A. arborescens* are described. Chlorinated withanolides have already been isolated from *Jaborosa magellanica* [7], *Withania somnifera* [8], *Physalis peruviana* [9], and *Acnistus breviflorus* [10], all Solanaceae, but so far no chlorinated withaphysalins have been reported for plants of that family. Unfortunately, these unknown chlorinated withaphysalin derivatives **1**–**3** did not show activity on any of the standard panels of cytotoxic cell lines used in our tests.

Results and Discussion. – The CH_2Cl_2-soluble fraction of the EtOH extract from the leaves of *A. arborescens* was submitted to different chromatographic methods,



1 R = EtO, R^1 = H
2 R = H, R^1 = EtO
3 R = H, R^1 = MeO



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including HPLC, to afford the four new withaphysalins T – W (**1** – **4**). Their structures were unequivocally determined by a combination of spectroscopic methods including ^{1}H- and ^{13}C-NMR (COSY, HSQC, HMBC, and NOESY) (*Tables 1 and 2*), IR, HR-ESI-MS, and for **4**, X-ray diffraction.

Compound **1** was isolated as colorless crystals. Its molecular formula $C_{30}H_{41}ClO_7$ was deduced by HR-ESI-MS which exhibited a quasi-molecular ion $[M+Na]^+$ at *m/z* 571.2444. The IR spectrum exhibited absorption bands for OH (3409 cm^{-1}) and CO (1689 cm^{-1}) groups. Comparative analysis of the ^{13}C-NMR and DEPT data (*Table 2*) showed the presence of five Me, seven CH_2 (one of which O-bearing at (δ(C) 63.6 (Me*C*H_2O–C(18))), and nine CH groups, including two olefinic (δ(C) 147.4 (C(3)) and 126.6 (C(2))), one hemiketal (δ(C) 108.9 (C(18))), and three other C-atoms linked to heteroatoms (δ(C) 82.0 (C(22)), 66.2 (C(6)), and 65.1 (C(4))). Conclusively, nine nonhydrogenated C-atoms were also observed. The signals at δ(C) 166.6 (C(26)), 149.3 (C(24)), and 122.2 (C(25)) indicated the presence of an α,β-unsaturated lactone moiety, while the signals at δ(C) 201.8 (C(1)), 147.4 (C(3)), and 126.6 (C(2)) were characteristic of an α,β-unsaturated ketone. The above data suggested that **1** was a withaphysalin containing as substituents a Cl-atom and an EtO group, the latter being assigned to C(18) (δ(C) 108.9) by the key HMBC cross-peaks from H–C(14) (δ(H) 1.37 – 1.43), H–C(17) (δ(H) 1.93 – 1.95), and MeCH_2O–C(18) (δ(H) 3.78 and 3.44 (2 *dd*, J = 9.8, 7.1 Hz)) to C(18). Comparison of the NMR data of **1** with those of withaphysalins previously isolated from *A. arborescens* [4][5] revealed significant changes on the chemical shifts related to rings *A* and *B*, indicating a different substitution pattern; to satisfy this fact, an OH group and a Cl atom were positioned at C(5) (δ(C) 79.9) and C(6) (δ(C) 66.2) of **1**, respectively, instead of an epoxy function like in those known compounds. The presence of the OH group at C(5) of **1** was confirmed by the ^{1}H-NMR spectrum of its acetylated derivative (*m/z* at 613.2499 $[M+Na]^+$) which exhibited only one additional Me signal (δ(H) 2.15 (*s*)) due to the presence of just one AcO group. This deduction was also supported by a comparative analysis of the NMR data of **1** with those published for analogous chlorowithanolides [10] and by the HMBC cross-peaks H–C(2) (δ(H) 6.21/C(4) (δ(C) 65.1), and H–C(3) (δ(H) 6.88) and H_a–C(7) (δ(H) 2.46/C(5) (δ(C) 79.9) (*Fig. 1*). The configurations at C(4), C(5), and C(6), *i.e.*, 4β-OH, 5β-OH, and 6α-Cl, were also deduced by comparison

Table 1. *^{1}H-NMR Data* ((D$_5$)pyridine, 500 MHz) *of Compounds* **1**–**4**[a]). δ in ppm, J in Hz.

H-Atom	**1**	**2**	**3**	**4**
H–(2) or CH_2(2)	6.21 (d, J = 10.2)	6.24 (d, J = 10.0)	6.25 (d, J = 10.25)	3.22 (dd, J = 15.6, 8.2), 3.04 (dd, J = 15.6, 3.3)
H–(3)	6.88 (dd, J = 10.2, 2.2)	6.92 (dd, J = 10.0, 5.0)	6.92 (dd, J = 10.25, 2.3)	4.09 (m)
H–(4)	5.38 (br. s)	5.43 (s)	5.43 (s)	3.95 (br. s)
H–(6)	4.71 (dd, J = 12.7, 4.5)	4.72 (dd, J = 10.0, 5.0)	4.71 (d, J = 2.5)	3.44 (br. s)
CH_2(7)	2.46 (dt, J = 13.2, 4.4), 1.86–1.87 (m)	2.36 (dt, J = 13.2, 3.9), 1.98–2.03 (m)	2.40 (dt, J = 13.0, 3.3), 1.99–2.02 (m)	2.33 (dd, J = 14.5, 2.7), 1.44–1.46 (m)
H–(8)	2.08–2.10 (m)	1.68–1.70 (m)	1.68–1.71 (m)	1.63–1.65 (m)
H–(9)	1.59–1.61 (m)	1.68–1.70 (m)	1.68–1.71 (m)	1.44–1.46 (m)
CH_2(11)	1.37–1.42, 1.21–1.24 (2m)	2.01–2.03, 1.21–1.24 (2m)	1.93–1.94, 1.21–1.25 (2m)	2.11–2.14, 1.63–1.65 (2m)
CH_2(12)	1.94–1.97, 1.15–1.18 (2m)	2.54 (d, J = 12.6)	2.52 (d, J = 12.4), 1.34–1.37 (m)	2.56 (d, J = 12.0), 1.36–1.39 (m)
H–(14)	1.37–1.43 (m)	1.28–1.31 (m)	1.93–1.94 (m)	1.87–1.91 (m)
CH_2(15)	1.94–1.97, 1.53–1.55 (2m)	1.28–1.31, 1.54–1.56 (2m)	1.21–1.25, 1.54–1.56 (2m)	1.68–1.69, 1.13–1.15 (2m)
CH_2(16)	1.86–1.87, 1.67–1.71 (2m)	1.41–1.43, 1.67–1.69 (2m)	1.38–1.41, 1.67–1.70 (2m)	1.60–1.61, 1.40–1.45 (2m)
H–(17)	1.93–1.95 (m)	1.31–1.34 (m)	1.21–1.25 (m)	1.16–1.18 (m)
H–(18)	4.91 (s)	4.86 (s)	4.75 (br. s)	4.79 (s)
Me(19)	1.56 (s)	1.63 (s)	1.63 (s)	1.75 (s)
Me(21)	1.34 (s)	1.48 (s)	1.48 (s)	1.49 (s)
H–C(22)	4.58 (dd, J = 13.0, 2.9)	4.50 (d, J = 10.0)	4.50 (d, J = 12.4)	4.50 (dd, J = 13.2, 2.0)
CH_2(23)	2.28 (t, J = 15.0), 2.06–2.08 (m)	2.26 (t, J = 16.7), 1.98–2.01 (m)	2.27 (t, J = 15.8), 1.99–2.02 (m)	2.26 (t, J = 15.0), 1.97–1.98 (m)
Me(27)	1.95 (s)	1.94 (s)	1.95 (s)	1.94 (s)
Me(28)	1.80 (s)	1.78 (s)	1.80 (s)	1.78 (s)
MeCH_2O–C(18) or MeO–C(18)	3.78, 3.44 (2dd, each J = 9.8, 7.1)	3.84–3.90, 3.47–3.53 (2m)	3.43 (s)	3.53–3.56 (m)
MeCH_2–C(18)	1.07 (t, J = 7.0)	1.14 (t, J = 7.05)	–	1.10 (t, J = 7.0)
MeCH_2–C(3)	–	–	–	3.80–3.84 (m), 3.35–3.38 (m)
MeCH_2–C(3)	–	–	–	1.22 (t, J = 7.0)

Table 2. *^{13}C-NMR Data* ((D$_5$)pyridine, 125 MHz) *of Compounds* **1**–**4**. δ in ppm.

C-Atom	**1**	**2**	**3**	**4**
C(1)	201.8	201.9	203.0	209.9
C(2)	126.6	126.6	126.9	42.0
C(3)	147.4	147.5	147.7	77.2
C(4)	65.1	65.2	65.4	75.2
C(5)	79.9	79.9	80.2	65.4
C(6)	66.2	66.0	66.2	58.5
C(7)	40.4	40.2	40.5	32.4
C(8)	36.4	36.7	37.0	31.5
C(9)	46.7	46.2	46.4	43.1
C(10)	58.4	58.5	58.7	51.4
C(11)	25.8	25.6	25.9	23.8
C(12)	37.2	34.8	35.1	35.2
C(13)	59.4	58.7	59.0	58.1
C(14)	53.1	55.2	56.9	56.9
C(15)	27.6	26.5	26.8	26.9
C(16)	25.6	25.9	26.2	26.3
C(17)	56.5	56.7	55.7	56.8
C(18)	108.9	107.2	109.0	107.1
C(19)	10.6	10.6	10.8	15.8
C(20)	85.2	85.3	85.8	85.4
C(21)	24.0	21.5	21.7	21.6
C(22)	82.0	81.2	81.4	81.3
C(23)	32.6	31.6	31.8	31.7
C(24)	149.3	149.3	149.3	149.1
C(25)	122.2	122.1	122.5	122.3
C(26)	166.6	166.4	166.5	166.3
C(27)	13.0	12.9	13.2	13.0
C(28)	20.4	20.3	20.6	20.4
Me*C*H$_2$O–C(18) or *Me*O–C(18)	63.6	64.3	56.2	64.4
*Me*CH$_2$O–C(18)	15.6	15.7	–	15.7
Me*C*H$_2$O–C(3)	–	–	–	64.2
*Me*CH$_2$O–C(3)	–	–	–	16.1

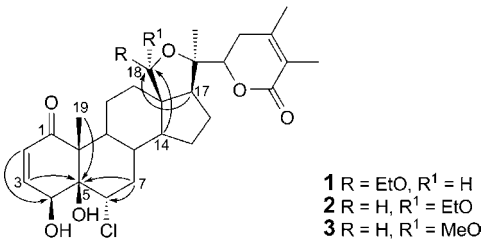

Fig. 1. *Important HMBCs* (H → C) *for* **1**–**3**

with the literature data [10] and supported by the NOESY experiment. The NOE H–C(4)/H–C(9) supported the β configuration of OH–C(4), while the α configuration for the Cl-atom was deducted from the NOE Me(19)/H–C(6). The aforementioned

spectroscopic data and functionalities suggested that the withasteroid **1** was a chlorowithaphysalin whose structure was established as the *rel*-(4*β*,5*β*,6*α*,18*S*,22*R*)-6-chloro-18,20-epoxy-18-ethoxy-4,5-dihydroxy-1-oxowitha-2,24-diene-26,22-lactone, named withaphysalin T.

Compound **2** was isolated as a colorless amorphous powder. The HR-ESI-MS showed a quasi-molecular ion $[M+Na]^+$ at *m*/*z* 571.2425 indicating the same molecular formula as **1** ($C_{30}H_{41}ClO_7$), suggesting that they were stereoisomers. A detailed analysis of the 1D- and 2D-NMR spectra revealed that **2** shared high structural similarity to **1**, the main difference being related to the chemical shift of the hemiketal C(18) that occurred at δ(C) 107.2 instead of 108.9 as for **1**. It is worth to mention the observed shielding of C(12) (δ(C) 34.8) in **2** relative to the same C-atom (δ(C) 37.2) in **1** what can be justified by the steric γ effect. The configuration ascribed to C(4), C(5), and C(6), *i.e.*, 4*β*-OH, 5*β*-OH, and 6*α*-Cl was also deduced by comparison with the literature data [10] and supported by the NOESY experiment as discussed previously for **1**. Thus, according to the above data and the detailed spectroscopic analysis (*Tables 1* and *2*) the structure of **2** was established as *rel*-(4*β*,5*β*,6*α*,18*R*,22*R*)-6-chloro-18,20-epoxy-18-ethoxy-4,5-dihydroxy-1-oxowitha-2,24-diene-26,22-lactone, an epimer of **1** at C(18). Compound **2** was named withaphysalin U.

Compound **3** was isolated as a colorless amorphous powder. Its HR-ESI-MS showed a quasi-molecular ion $[M+Na]^+$ at *m*/*z* 557.2288 indicating the molecular formula $C_{29}H_{39}ClO_7$. Analyses of the 1D- and 2D-NMR spectra showed that compound **3** presents close similarity to withaphysalins **1** and **2** (*Tables 1* and *2*). The main difference observed in the ^{1}H- and ^{13}C-NMR spectra of **3** when compared with those of **1** and **2** was due the presence of a MeO signal (δ(C) 56.2 and δ(H) 3.43) group in **3** instead of those of the EtO group. As expected, the chemical shifts of C(12) and C(21) of compounds **2** and **3**, both bearing an α-alkoxy group, were shielded compared to **1**, while the chemical shift of the C(14) of **2** and **3** were deshielded compared to **1**. These differences can be justified by the steric interactions of the substituents at C(18), changing their volumes with surrounding neighborhood. The relative configuration of **3** was determined to be identical to that of **2**, particularly by the NOEs H–C(18)/H–C(6) and H–C(6)/Me(19). Based on the aforementioned spectroscopic data the structure of **3** was determined as the new chlorowithaphysalin *rel*-(4*β*,5*β*,6*α*,18*R*,22*R*)-6-chloro-18,20-epoxy-4,5-dihydroxy-18-methoxy-1-oxowitha-2,24-diene-26,22-lactone, named withaphysalin V. This is the first example of a natural methoxywithaphysalin. Several chlorowithanolides have been isolated and, as was observed, especially by *Nittala et al.* [10], sizeable changes in the ^{13}C-NMR chemical shifts related to rings *A* and *B* of these compounds were observed when compared with those of the nonchlorinated parent epoxides. The same behavior was observed for the 6-chlorowithaphysalins relative to withaphysalins devoid of the Cl-atom, but possessing the 5,6-epoxy moiety.

Compound **4** also was isolated as colorless amorphous powder. The HR-ESI-MS showed a quasi-molecular ion $[M+Na]^+$ at *m*/*z* 581.3050 indicating the molecular formula $C_{32}H_{46}O_8$. The ^{1}H- and ^{13}C-NMR spectra revealed that compound **4** is a 2,3-dihydrowithaphysalin, with some additional structural differences when compared with **1**–**3** (*Tables 1* and *2*). For instance, instead of an OH group at C(5) (δ(C) 65.4) and a Cl-atom at C(6) (δ(C) 58.5), **4** was substituted by an epoxy moiety at these C-atoms,

like all withaphysalins previously isolated from *A. arborescens*. Moreover, an additional EtO group located at C(3) (δ(C) 77.2) was confirmed by the HMBC cross-peaks H–C(3)/C(1), C(4), and C(5) (*Fig. 2*). The relative configuration of **4** was deduced by a NOESY experiment, by analogy with the NMR data of (4β,5β,6β)-5,6-epoxy-4-hydroxywithaphysalins previously reported and by an X-ray crystal-structure analysis (*Fig. 3*)[1]. The presence of the NOEs H–C(3)/H–C(6) and H–C(6)/H–C(4) confirmed the β-orientation of the EtO group at C(3). Thus, the structure of **4** was determined as *rel*-(3β,4β,5β,6β,18*R*,22*R*)-5,6 : 18,20-diepoxy-3,18-diethoxy-4-hydroxy-1-oxowith-24-ene-26,22-lactone, named withaphysalin W.

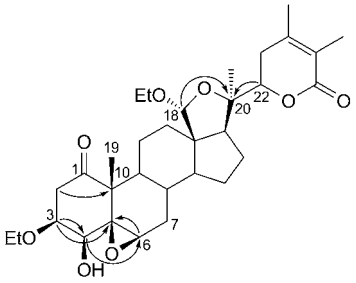

Fig. 2. *Important HMBCs* (H → C) *for* **4**

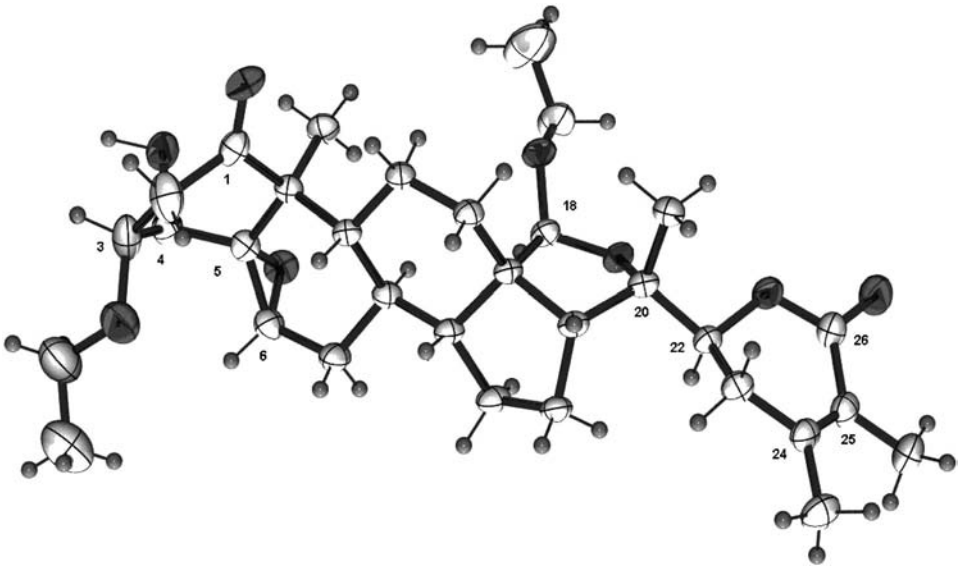

Fig. 3. *An ORTEP-3 projection of the molecule* **4** *showing the atom numbering and displacement ellipsoids at the 30% probability level*

[1]) CCDC-847318 contains the supplementary crystallographic data for compound **4**. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk.

Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2, 70–230 mesh; *VETEC*). TLC: precoated silica gel polyester sheets (Kieselgel *60* F_{254}, 0.20 mm; *Merck*); detection by immersion in a 5% molybdatophosphoric acid hydrate/EtOH soln. followed by heating at 120°. HPLC: *Shimadzu-UFLC* system equipped with a *SPD-M20A* diode-array UV/VIS detector; *Phenomenex*® reversed phase column (250 × 4.6 mm i.d.; 5 μm); gradient elution with H_2O (solvent *A*) and MeOH (solvent *B*) at 40°; injection volume ('loop') 200 μl, flow-rate 3.0 ml/min; the wavelength scan range of the photo-diode-array (PDA) detector was set to 190–400 nm, and the chromatograms were recorded at 230 nm; t_R in min. M.p.: digital *Mettler-Toledo-FP90* apparatus; uncorrected. X-Ray Analysis: *Enraf-Nonius-Kappa-CCD* diffractometer (95 mm CCD camera on κ-goniostat), with graphite-monochromated MoK_α radiation (0.71073 Å), at r.t. IR Spectra (KBr): *Perkin–Elmer-FT-IR-1000* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-DRX-500*-spectrometer at 500 (^{1}H) and 125 MHz (^{13}C); chemical shift δ in ppm rel. to residual ^{1}H-(D_5)pyridine (δ(H) 7.22, 7.58, and 8.74) and the central peak of ^{13}C t_s (δ(C) 127.87, 135.91, and 150.35), *J* in Hz. ESI-HR-MS: Quadrupole *Shimadzu-LCMS-IT-TOF* spectrometer equipped with an electrospray ionization source; in *m*/*z*.

Plant Material. *Acnistus arborescens* leaves were harvested in August 2006 from the Pico Alto locality (Guaramiranga Mountain, State of Ceará), at an elevation of 1000 m. The plant material was identified by Prof. *Edson P. Nunes*. A voucher specimen (No. 30.513) is deposited with the Herbário Prisco Bezera (EAC) of the Departamento de Biologia, Universidade Federal do Ceará.

Extraction and Isolation. The dry and powdered leaves (4.3 kg) of *A. arborescens* were defatted with hexane (2 × 72 h) and than extracted with EtOH (3 × 72 h), at r.t. The solvents were evaporated yielding the crude hexane (96.0 g) and EtOH (557.0 g) extracts. The EtOH extract was submitted to liquid–liquid fractionation with the solvents CH_2Cl_2, AcOEt, and BuOH. The CH_2Cl_2 fraction (370.0 g) was subjected to gravity CC (SiO_2, CH_2Cl_2/AcOEt of increasing polarity and MeOH). The eluate obtained with CH_2Cl_2 (105.0 g) was subjected to CC (SiO_2, CH_2Cl_2/MeOH 10:0, 9.5:0.5, 9:1, 8:2, 7:3, 5:5, and 0:10): *Fractions 1–7. Fr. 1* (63.0 g; eluted with CH_2Cl_2) was fractionated by CC (SiO_2; CH_2Cl_2/AcOEt 10:0, 9:1, 8:2, 7:3, 5:5, 3:7, and 0:10): *Frs. 1.1–1.7. Fr. 1.3* (2.6 g; eluted with CH_2Cl_2/AcOEt 8:2), after repeated CC with the same solvent system, yielded **1** (44.0 mg). *Fr. 1.5* (4.5 g; eluted with CH_2Cl_2/AcOEt 1:1), after successive CC, including HPLC separation (isocratic mode (80% *B* for 10 min. t_R 7.73), afforded **2** (40.0 mg) and **3** (12.4 mg). *Fr. 1.2* (1.2 g) was subjected to CC (SiO_2, $CHCl_3$/AcOEt 9.5:0.5, 9:1, and 8:2): **4** (39.1 mg; eluted with CH_2Cl_2/AcOEt 9.5:0.5).

rel-*(4β,5β,6α,18*S*,22*R*)-6-Chloro-18,20-epoxy-18-ethoxy-4,5-dihydroxy-1-oxowitha-2,24-diene-26,22-lactone* (= rel-*(4β,5β,6α,18*S*,22*R*)-6-Chloro-18,20-epoxy-18-ethoxy-4,5,22-trihydroxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **1**): Colorless crystals. M.p. 198.4–199.8°. $[\alpha]_D^{20} = +36$ ($c = 0.2$, CH_2Cl_2). IR (KBr): 3475, 3409, 2983, 2873, 1689, 1647, 1448, 1319, 1132, 1103, 1010. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 571.2444 ($[M + Na]^+$, $C_{30}H_{41}ClNaO_7^+$; calc. 571.2433).

rel-*(4β,5β,6α,18*R*,22*R*)-6-Chloro-18,20-epoxy-18-ethoxy-4,5-dihydroxy-1-oxowitha-2,24-diene-26,22-lactone* (= rel-*(4β,5β,6α,18*R*,22*R*)-6-Chloro-18,20-epoxy-18-ethoxy-4,5,22-trihydroxy-1-oxogosta-2,24-diene-26-oic Acid δ-Lactone*; **2**): Colorless amorphous powder. M.p. 197.8–199.1°. $[\alpha]_D^{20} = +35$ ($c = 0.2$, CH_2Cl_2). IR (KBr): 3496, 2968, 2948, 2871, 1774, 1699, 1552, 1456, 1380, 1130, 1103, 1076, 1004. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 571.2425 ($[M + Na]^+$, $C_{30}H_{41}ClNaO_7^+$; calc. 571.2433).

rel-*(4β,5β,6α,18*R*,22*R*)-6-Chloro-18,20-epoxy-4,5-dihydroxy-18-methoxy-1-oxowitha-2,24-diene-26,22-lactone* (= rel-*(4β,5β,6α,18*R*,22*R*)-6-Chloro-18,20-epoxy-4,5,22-trihydroxy-18-methoxy-1-oxoergosta-2,24-dien-26-oic Acid δ-Lactone*; **3**): Colorless amorphous powder. M.p. 182.3–183.5°. $[\alpha]_D^{20} = +19$ ($c = 0.1$, CH_2Cl_2). IR (KBr): 3446, 2931, 2873, 1706, 1683, 1637, 1456, 1379, 1103, 1010. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 557.2288 ($[M + Na]^+$, $C_{29}H_{39}ClNaO_7^+$; calc. 557.2282).

rel-*(3β,4β,5β,6β,18*R*,22*R*)-5,6:18,20-Diepoxy-3,18-diethoxy-4-hydroxy-1-oxowith-24-ene-26,22-lactone* (= rel-*(3β,4β,5β,6β,18*R*,22*R*)-5,6:18,20-Diepoxy-3,18-diethoxy-4,22-dihydroxy-1-oxoergost-24-en-26-oic Acid δ-Lactone*; **4**): Colorless amorphous powder. M.p. 258.1–259.9°. $[\alpha]_D^{20} = -7$ ($c = 0.1$, CH_2Cl_2). IR (KBr): 3376, 2974, 2913, 1707, 1686, 1452, 1320, 1207, 1077. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 581.3050 ($[M + Na]^+$, $C_{32}H_{46}NaO_8^+$; calc. 581.3090).

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