Two New Triterpene Saponins from *Cyclamen africanum* BOISS. & REUTER

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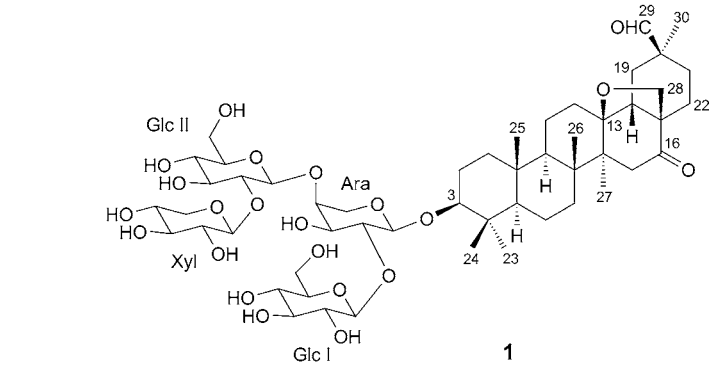
Two new oleanane-type triterpene saponins, afrocyclamins A and B (**1** and **2**, resp.), were isolated from a MeOH extract of the roots of *Cyclamen africanum* BOISS. & REUTER, together with three known triterpenoid saponins, lysikokianoside, deglucocyclamin I, and its dicrotalic acid derivative. The structures were elucidated, on the basis of 1D- and 2D-NMR experiments and mass spectrometry as (3*β*,20*β*)-13,28-epoxy-16-oxo-3-{*O*-*β*-D-xylopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranosyl}oxy}oleanan-29-al (**1**) and (3*β*,16*α*,20*β*)-16,28,29-trihydroxy-olean-12-en-3-yl *O*-4-*O*-(4-carboxy-3-hydroxy-3-methyl-1-oxobutyl)-*β*-D-xylopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*O*-[*β*-D-glucopyranosyl-(1→2)]-*α*-L-arabinopyranoside (**2**).

Introduction. – *Cyclamen* is a genus of 23 species of flowering plants belonging to the Primulaceae family [1], many of them being reported to contain pentacyclic triterpenoids [2], with a wide range of bioactivities, such as somatic embryogenesis [3], fungicidal, antitumor, and uterocontractile activities [4], and in the treatment against infertility [5]. As part of our continuing studies on saponins from the genus *Cyclamen* [6][7], we chose *Cyclamen africanum*, an endemic plant of northern Algeria (Bejaia), which was used in folk medicine against warts. A previous report revealed the presence of the sapogenin cyclamiretin A [8], but no systematic study on the saponins of this plant has been reported so far. We describe in this work the isolation and structure elucidation of two new triterpene saponins named afrocyclamins A and B (**1** and **2**, resp.) and of three known saponins from the rhizome MeOH extract of *C. africanum.*

Results and Discussion. – The dried and powdered rhizome parts of *C. africanum* BOISS. & REUTER were extracted with 70% MeOH. An aliquot of the MeOH extract was subjected to successive vacuum liquid chromatography (VLC) separations on silica gel and *RP-18* silica gel. Afrocyclamin A (**1**) and B (**2**), and the known saponins lysikokianoside 1, deglucocyclamin I, and its dicrotalic acyl derivative were obtained by separation of the saponin-containing fraction by medium-pressure liquid chromatography (MPLC) on normal and reversed-phase silica gel. The structures of **1** and **2** were



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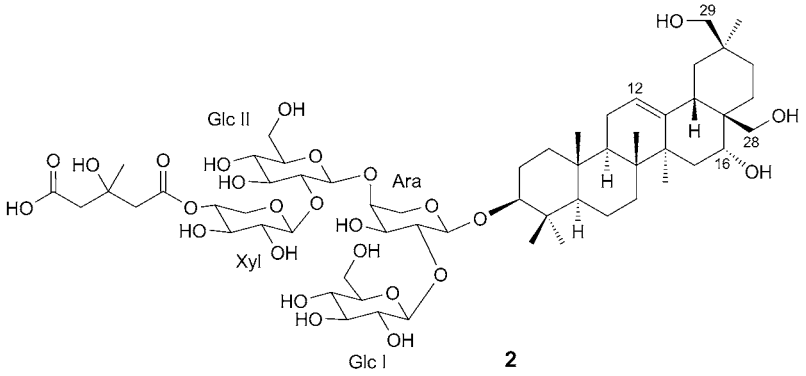

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established mainly by 1D- and 2D-NMR spectroscopic methods (^{1}H- and ^{13}C-NMR, DEPT, COSY, TOCSY, NOESY, HSQC, and HMBC; *Tables 1* and *2*), in combination with mass spectrometry (HR-ESI- and FAB-MS).

The high-resolution electrospray-ionization mass spectrum (HR-ESI-MS; positive-ion mode) of compound **1** exhibited a quasi-molecular ion at *m*/*z* 1081.5201 ($[M+Na]^+$) establishing the molecular formula of **1** as $C_{52}H_{84}O_{22}$. The FAB-MS (negative-ion mode) of **1** showed a quasi-molecular ion at *m*/*z* 1057 ($[M-H]^-$). Other fragment ions at *m*/*z* 925 ($[M-H-132]^-$), 763 ($[M-H-132-162]^-$), 601 ($[M-H-132-162-162]^-$), and 469 ($[M-H-132-162-162-132]^-$) indicated the loss of one pentosyl, two hexosyl, and one pentosyl units, respectively. The ^{13}C-NMR spectrum displayed 52 C-atom signals of which 30 were attributed to the aglycone and the remaining 22 to the sugar moieties. The HSQC spectrum of the aglycone of **1** exhibited six signals of tertiary Me groups at δ(H)/δ(C) 1.18(*s*)/18.0 (C(26)), 1.12(*s*)/27.3 (C(23)), 1.04(*s*)/21.9 (C(27)), 1.00(*s*)/16.0 (C(24)), 0.86(*s*)/23.3 (C(30)), and 0.70(*s*)/15.5 (C(25)), which are characteristic of a pentacyclic triterpenoid. In addition, one pair of geminal H-atoms at δ(H) 3.30 (*d*, $J=8.0$ Hz) and 3.82 (*d*, $J=8.0$ Hz) giving correlations with the downfield-shifted signal at δ(C) 74.6 corresponded to the oxymethylene function located at C(28). Another significant signal at δ(H)/δ(C) 9.56/207.1 corresponded to an aldehyde function located at C(29), whereas the carbonyl signal at δ(C) 212.4 corresponded to C(16). Based on the above findings and an

Table 1. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; C_5D_5N) *of the Aglycones of* **1** *and* **2** *from 1D- and 2D-NMR Experiments*[a]). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	0.74, 1.48	38.8	0.76, 1.53	38.7
CH_2(2)	1.76, 1.92	26.2	1.76, 1.92	26.2
H–C(3)	3.08–3.12 (*m*)	89.0	3.08–3.15 (*m*)	89.0
C(4)		39.1		39.2
H–C(5)	0.57 (*d*, *J* = 9.5)	55.0	0.62–0.64 (*m*)	55.5
CH_2(6)	1.25 (*d*, *J* = 9.2)[b])	17.0	1.34[b])	18.0
CH_2(7)	[b]), [b])	33.4	[b]), [b])	32.9
C(8)		43.0		40.0
H–C(9)	1.02	49.9	1.56–1.60 (*m*)	46.7
C(10)		36.3		36.0
CH_2(11)	[b]), [b])	18.3	0.96, 1.72	23.6
CH_2(12) or H–C(12)	1.52, 1.94	31.2	5.36	124.0
C(13)		86.0		144.2
C(14)		49.5		41.8
CH_2(15)	1.91[b])	45.4	[b]), [b])	33.9
C(16) or H–C(16)		212.4	4.58	73.0
C(17)		54.9		39.5
H–C(18)	1.78	55.6	2.40–2.44 (*m*)	42.0
CH_2(19)	[b]), [b])	33.5	2.50–2,54 (*m*), 1.72	42.7
C(20)		47.8		35.7
CH_2(21)	1.80, 1.92–1.96 (*m*)	29.1	2.12–2.16 (*m*), 1.74	31.4
CH_2(22)	[b]), [b])	33.2	1.52, 2.28	27.8
Me(23)	1.12 (*s*)	27.3	1.12 (*s*)	27.6
Me(24)	1.00 (*s*)	16.0	1.00 (*s*)	16.0
Me(25)	0.70 (*s*)	15.5	0.74 (*s*)	15.7
Me(26)	1.18 (*s*)	18.0	0.86 (*s*)	16.5
Me(27)	1.04 (*s*)	21.9	1.70 (*s*)	27.0
CH_2(28)	3.30, 3.82 (2*d*, each *J* = 8.0)	74.6	3.56, 3.65 (2*d*, each *J* = 11.0)	69.0
CHO(29) or CH_2(29)	9.56 (*s*)	207.1	3.82 (*d*, *J* = 10.4), 3.92	66.5
Me(30)	0.86 (*s*)	23.3	1.19 (*s*)	27.7

[a]) Overlapped signals are reported without designated multiplicity. [b]) Not determined.

extensive 1D- and 2D-NMR analysis, the aglycone of **1** was identified as cyclamigenin B (=(3β,20β)-13,28-epoxy-3-hydroxy-16-oxooleanan-29-al), previously reported from *C. europaeum* [9], *Ardisia gigantifolia* [10] and *A. mamillata* [11]. The ^{1}H-NMR spectrum of **1** showed four anomeric H-atom signals at δ(H) 5.38 (*d*, *J* = 8.0 Hz), 4.86 (*d*, *J* = 7.6 Hz), 4.82 (*d*, *J* = 8.6 Hz), and 4.72 (*d*, *J* = 5.9 Hz), correlated in the HSQC spectrum with four anomeric C-signals at δ(C) 103.8, 103.4, 106.8, and 104.2, respectively, indicating the presence of four sugar units. Evaluation of spin–spin couplings and chemical shifts from the 2D-NMR spectra allowed the identification of two β-glucopyranosyl (Glc I and Glc II), one β-xylopyranosyl (Xyl), and one α-arabinopyranosyl (Ara) unit. The β-orientation at the anomeric center of Glc and Xyl and the α-orientation at the anomeric center of Ara, in their pyranose form, was

Table 2. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; C_5D_5N) *of the Sugar Moieties of* **1** *and* **2** *from 1D- and 2D-NMR Experiments*[a]). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
3-*O*-Sugars:				
Ara				
H–C(1)	4.72 (*d*, *J* = 5.9)	104.2	4.72 (*d*, *J* = 5.9)	104.2
H–C(2)	4.43 (*t*-like, *J* = 9.0)	78.8	4.42 (*t*-like, *J* = 9.0)	78.8
H–C(3)	4.22	72.9	4.20	72.8
H–C(4)	4.18	78.2	4.18	78.8
CH_2(5)	4.60 (br. *d*, *J* = 10.4), 3.72	64.0	4.60, 3.70 (br. *d*, *J* = 11.2)	64.0
Glc I				
H–C(1)	5.38 (*d*, *J* = 8.0)	103.8	5.38 (*d*, *J* = 7.0)	103.8
H–C(2)	3.97 (*t*, *J* = 9.6)	75.5	3.97	75.2
H–C(3)	4.16	76.2	4.16	76.2
H–C(4)	4.04	71.0	4.04	71.0
H–C(5)	3.96	77.2	3.96	77.2
CH_2(6)	4.46, 4.24	62.2	4.46, 4.24	62.2
Glc II				
H–C(1)	4.86 (*d*, *J* = 7.6)	103.4	4.84 (*d*, *J* = 7.6)	104.1
H–C(2)	3.77 (*d*, *J* = 8.5)	84.2	3.78 (*t*, *J* = 8.5)	84.8
H–C(3)	4.10	76.5	4.10	76.5
H–C(4)	4.06	70.0	4.06	70.0
H–C(5)	3.75	77.3	3.75	77.2
CH_2(6)	4.34 (*d*, *J* = 10.2), 4.17	61.9	4.34 (br. *d*, *J* = 11.6), 4.17	61.7
Xyl				
H–C(1)	4.82 (*d*, *J* = 8.6)	106.8	4.82 (*d*, *J* = 8.6)	106.5
H–C(2)	3.90	75.5	3.90	75.5
H–C(3)	4.00	76.8	4.00	76.7
H–C(4)	4.06	70.3	5.18	72.0
CH_2(5)	4.47, 3.68	66.9	4.46, 3.68	66.9
Acid at Xyl C(4)				
C(1)				170.0
H–C(2)			3.02 (*d*, *J* = 12.3), 2.82 (*d*, *J* = 12.1)	46.8
C(3)				70.0
H–C(4)			[b]), [b])	47.0
C(5)				172.3
Me(6)			1.46	27.5

[a]) Overlapped signals are reported without designated multiplicity. [b]) Not determined.

supported by the relatively large J values of their anomeric H-atoms (J = 5.9–8.6 Hz). The absolute configuration was determined to be D for Glc and Xyl and L for Ara by GC analysis (see *Exper. Part*). HMBC Cross-peaks at δ(H)/δ(C) 4.72 (Ara H–C(1))/89.0 (Agly C(3)), 5.38 (Glc I H–C(1))/78.8 (Ara C(2)), 4.86 (Glc II H–C(1))/78.2 (Ara C(4)), and 4.82 (Xyl H–C(1))/84.2 (Glc II C(2)) allowed the oligosaccharide sequence Xyl-(1 → 2)-Glc II-(1 → 4)-[Glc I-(1 → 2)]-Ara to be linked at Agly C(3). These linkages were also confirmed by the following NOESY cross-peaks: 4.72 (Ara

H–C(1))/3.08–3.12 (Agly H–C(3)), 5.38 (Glc I H–C(1))/4.43 (Ara H–C(2)), 4.86 (Glc II H–C(1))/4.18 (Ara H–C(4)), and 4.82 (Xyl H–C(1))/3.77 (Glc II H–C(2)). From the above evidence, the structure of **1** was elucidated as (3*β*,20*β*)-13,28-epoxy-16-oxo-3-{{*O*-*β*-D-xylopyranosyl-(1 → 2)-*O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-[*β*-D-glucopyranosyl-(1 → 2)]-*α*-L-arabinopyranosyl}oxy}oleanan-29-al, a new compound named afrocyclamin A.

On the basis of HR-ESI-MS (positive-ion mode: *m*/*z* 1229.6780 ($[M+Na]^+$)) and ^{13}C-NMR data, the molecular formula of compound **2** was established as $C_{58}H_{94}O_{26}$. The negative-ion-mode FAB-MS exhibited a quasi-molecular ion at *m*/*z* 1205 ($[M-H]^-$). Other fragment ions at *m*/*z* 1061 ($[M-H-144]^-$), 929 ($[M-H-144-132]^-$), 767 ($[M-H-144-132-162]^-$), 605 ($[M-H-144-132-162-162]^-$), and 473 ($[M-H-144-132-162-162-132]^-$) corresponded to the successive loss of one unit of 144 ($C_6H_{12}O_5$) suggesting the presence of an acid, one pentosyl, two hexosyl, and one pentosyl moiety. The ^{13}C-NMR spectrum of **2** displayed 58 C-signals, of which 30 could be readily assigned to a triterpene skeleton and the 28 remaining to the saccharide and acid portion. The HSQC spectrum corresponding to the aglycone part of **2** revealed the presence of six tertiary Me groups as *s* at *δ*(H)/*δ*(C)1.70(*s*)/27.0 (Me(27)), 1.19(*s*)/27.7 (Me(30)), 1.12(*s*)/ 27.6 (Me(23)), 1.00(*s*)/16.0 (Me(24)), 0.86(*s*)/16.5 (Me(26)), and 0.74(*s*)/15.7 (Me(25)), together with the signals for an olefinic C-atom at *δ*(H)/*δ*(C) 5.36(*s*)/124.0 (C(12)) and a quaternary C-atom at *δ*(C) 144.2 (C(13)). These data suggested an olean-12-ene skeleton for the aglycone of **2**. Furthermore, two CH_2OH and one CHOH moieties were observed in the HSQC spectrum at *δ*(H)/*δ*(C) 3.82 and 3.92/66.5 (C(29)), 3.56 and 3.65/69.0 (C(28)), and 4.58/73.0 (C(16)). The HMBC cross-peaks of the aglycone part of **2** between *δ*(H) 1.19 (Me(30)) and *δ*(C) 42.7 (C(19)), 35.7 (C(20)), 31.4 (C(21)), and 66.5 (C(29)) indicated that one primary-alcohol function was located at C(29). A close comparison between the signals of the aglycones of **1** and **2** showed that the characteristic signals of the 13,28-epoxy bridge at *δ*(C) 86.0 (C(13)), 74.6 (C(28)), and 54.9 (C(17)), and of the keto group at *δ*(C) 212.4 (C(16)) in **1** were replaced by the signals of a C(12)=C(13) bond at *δ*(C) 124.0 (C(12)) and 144.2 (C(13)), of a primary-alcohol function at *δ*(C) 69.0 (C(28)), and of a secondary-alcohol function at *δ*(C) 73.0 (C(16)) in **2**. This data suggested the aglycone of **2** to be dihydrocyclamiretin D or pridentigenin E (=(3*β*,16*α*,20*β*)-olean-12-ene-3,16,28,29-tetrol) isolated from *Primula denticulata* [12]. The relative configuration of the aglycone of **2** was established by the NOESY data. Namely the cross-peaks *δ*(H)/*δ*(H) 1.19 (Me(30))/2.50–2.54 (*m*, H_{ax}–C(19)), 2.50–2.54 (*m*, H_{ax}–C(19))/1.70 (Me(27)), 1.19 (Me(30))/1.74 (H_{ax}–C(21)), and 2.50–2.54 (*m*, H_{ax}–C(19))/1.74 (H_{ax}–C(21)) confirmed the *α*-equatorial orientation of Me(30) and, consequently, the *β* axial orientation of the CH_2OH group at C(20). The NOESY cross-peaks *δ*(H)/*δ*(H) 4.58 (H–C(16))/3.65 (CH_2(28)) and 4.58 (H–C(16))/2.14 (H_{eq}–C(21)) confirmed the *β*-equatorial orientation of H–C(16) and, consequently, the *α*-axial orientation of OH–C(16). These data confirmed the identification of the aglycone of **2** as (3*β*,16*α*,20*β*)-olean-12-ene-3,16,28,29-tetrol (also named olean-12-ene-3*β*,16*α*,28,30-tetraol), which was recently reported as the aglycone of hederifolioside E isolated from *C. hederifolium* [13]. The HSQC spectrum of **2** showed four anomeric H-atom signals at *δ*(H) 5.38 (*d*, *J* = 7.0 Hz), 4.84 (*d*, *J* = 7.6 Hz), 4.82 (*d*, *J* = 8.61 Hz), and 4.72 (*d*, *J* = 5.9 Hz) which correlated with four anomeric C-signals at

δ(C) 103.8, 104.1, 106.5, and 104.2, indicating the presence of four sugar units. All the ^{1}H- and ^{13}C-NMR signals corresponding to the oligosaccharide part of **2** were almost superimposable to those of **1** (Xyl-(1 → 2)-Glc II-(1 → 4)-[Glc I-(1 → 2)]-Ara-), except for the deshielded signals of Xyl H–C(4) at δ(H) 5.18/δ(C) 72.0, indicating an acylation at this position. The presence of a dicrotalic acid (= 3-hydroxy-3-methylpentanedioic acid) acyl group was ascertained by the observation of a set of additional signals in the 1D- and 2D-NMR spectra corresponding to a 4-carboxy-3-hydroxy-3-methyl-1-oxobutyl moiety, which were in good agreement with literature data [6]. This was confirmed by the observation of a fragment ion at *m*/*z* 1061 ([*M* – H – 144]$^-$) in the FAB-MS (negative-ion mode). From the above evidence, **2** was elucidated as (3β,16α,20β)-16,28,29-trihydroxyolean-12-en-3-yl *O*-4-*O*-(4-carboxy-3-hydroxy-3-methyl-1-oxobutyl)-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside, which we called afrocyclamin B.

The three known compounds isolated besides **1** and **2** were identified by comparison of their 1D- and 2D-NMR and MS data with those reported in the literature as lysikokianoside 1 (= (3β,16α)-13,28-epoxy-16-hydroxyoleanan-3-yl *O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranoside), isolated previously from *C. persicum* [7] *and Lysimachia sikokiana* [14], deglucocyclamin I (= (3β,16α,20β)-13,28-epoxy-3-{{*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl}oxy}-16-hydroxyoleanan-29-al) [6][8], and its dicrotalic acyl derivative (3β,16α,20β)-3-{{*O*-4-*O*-(4-carboxy-3-hydroxy-3-methyl-1-oxobutyl)-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[β-D-glucopyranosyl-(1 → 2)]-α-L-arabinopyranosyl}oxy}-13,28-epoxy-16-hydroxyoleanan-29-al, isolated previously from *C. trocopteranthum* [6]. Deglucocyclamin I, isolated for the first time from *C. africanum*, has been obtained from several *Cyclamen* species (*C. mirabile* [4], *C. trocopteranthum* [6], *C. persicum* [7], *C. greacum* [8], *C. hederifolium* [13], *C. repandum* [15], *C. neapolitanum* [16], *C. europaeum* [17], and *C. coum var coum* [18]). This compound may represent a chemotaxonomic marker for the genus *Cyclamen*.

Experimental Part

General. Vacuum liquid chromatography (VLC)/medium-pressure liquid-chromatography (MPLC): SiO_2 *60* (*Merck*, 15–40 μm), *RP-18* (*Silicycle*; 75–200 μm). MPLC: *Gilson M 303* pump, *Büchi* glass column (460 × 15 mm and 230 × 15 mm), *Büchi* precolumn (110 × 15 mm). Anal. TLC: SiO_2 plates *60F$_{254}$* (*Silicycle*); HP-TLC on SiO_2 *60F$_{254}$* (*Merck*); detection of saponins with vanillin reagent (1% vanillin in EtOH/H_2SO_4 50 : 1). GC: *Termoquest* gas chromatograph, *DB-1701* cap. column (30 m × 0.25 mm, i.d.; *J&W Scientific*) [7]; FI detection; detector temp. 250°, injection temp. 230°, initial temp. 80° for 5 min and then increase to 270° at the rate of 15°/min; carrier gas He. Optical rotations: *AA-OR* automatic polarimeter. For 1D and 2D-NMR spectra (^{1}H,^{1}H-COSY, TOCSY, NOESY, HSQC, and HMBC), see [6]. MS: *Q-TOF 1*-micromass spectrometer (pos.-ion mode HR-ESI) and *Jeol-SX-102* mass spectrometer (neg.-ion mode FAB, glycerol matrix) in *m*/*z*.

Plant Material. The roots of *Cyclamen africanum* BOISS. & REUTER, were collected near Constantine (Algeria) in 2008 and identified by Prof. *Debellaire*, Faculty of Chemistry, University of Annaba. A voucher specimen (LOST ca05.08) has been deposited with the Phytochemistry Department, Mentouri University, Constantine, Algeria.

Extraction and Isolation. The air-dried powdered roots of *Cyclamen africanum* (5 kg) were extracted with 70% MeOH (3 × 1000 ml) for 3 h under reflux to give 345 g of a crude extract after evaporation. A

fraction (10 g) of this extract was submitted to VLC (SiO_2 *60* (15–40 μm), $CHCl_3$/MeOH/H_2O 70:23:4, 60:32:7, and 0:100:0 (each eluent 400 ml)): *Fractions 1–10*. *Fr. 1–3* (2.07 g; eluted with $CHCl_3$/MeOH/H_2O 70:23:4) were separated by VLC (*RP-18*, MeOH/H_2O 0:100 → 100:0): *Frs. 1–3.1*, *1–3.2*, and *1–3.3*. *Fr. 1–3.2* (860 mg; eluted with MeOH/H_2O 1:1) was submitted to successive MPLC (*RP-18*, MeOH/H_2O gradient) and MPLC (SiO_2 *60* (15–40 μm), $CHCl_3$/MeOH/H_2O 70:23:4, 60:32:7, and 0:100:0): **1** (10 mg). *Fr. 1–3.3* (640 mg eluted with MeOH) was fractionated by successive MPLC (*RP-18*, MeOH/H_2O gradient): **2** (6 mg), lysikokianoside 1 (23 mg), deglucocyclamin I (75 mg), and deglucocyclamin I dicrotalic acyl derivative (8 mg).

Afrocyclamin A (=*{(3β,20β)-13,28-Epoxy-16-oxo-3-{{*O*-β-*D*-xylopyranosyl-(1 → 2)*-O*-β-*D*-glucopyranosyl-(1 → 4)*-O*-[β-*D*-glucopyranosyl-(1 → 2)]-α-*L*-arabinopyranosyl}oxy}oleanan-29-al*; **1**): White amorphous powder. $[\alpha]_D^{25} = -2.5$ ($c = 0.50$, MeOH). ^{1}H- and ^{13}C-NMR (C_5D_5N): *Tables 1* and *2*. FAB-MS (neg.): 1057 ($[M-H]^-$), 925 ($[M-H-132]^-$), 763 ($[M-H-132-162]^-$), 601 ($[M-H-132-162-162]^-$), 469 ($[M-H-132-162-162-132]^-$). HR-ESI-MS (pos.): 1081.5201 ($[M+Na]^+$, $C_{52}H_{84}NaO_{22}^+$; calc. 1081.5195).

Afrocyclamin B (=*(3β,16α,20β)-16,28,29-Trihydroxyolean-12-en-3-yl* O*-4-*O*-(4-Carboxy-3-hydroxy-3-methyl-1-oxobutyl)-β-*D*-xylopyranosyl-(1 → 2)*-O*-β-*D*-glucopyranosyl-(1 → 4)*-O*-[β-*D*-glucopyranosyl-(1 → 2)]-α-*L*-arabinopyranoside*; **2**): White amorphous powder. $[\alpha]_D^{25} = -17.0$ ($c = 0.43$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS (neg.): 1205 ($[M-H]^-$), 1061 ($[M-H-144]^-$), 929 ($[M-H-144-132]^-$), 767 ($[M-H-144-132-162]^-$), 605 ($[M-H-144-132-162-162]^-$), 473 ($[M-H-144-132-162-162-132]^-$). HR-ESI-MS (pos.): 1229.6780 ($[M+Na]^+$, $C_{58}H_{94}NaO_{26}^+$; calc. 1229,6786).

Acid Hydrolysis and GC Analysis. Each compound (2 mg) was hydrolyzed with 2N aq. CF_3COOH (5 ml) for 3 h at 95°. After extraction with CH_2Cl_2 (3 × 5 ml), the aq. layer was repeatedly concentrated to dryness with MeOH until neutral, and then analyzed by TLC (SiO_2, $CHCl_3$/MeOH/H_2O 8:5:1) by comparison with authentic samples. The (trimethylsilyl)thiazolidine derivatives of the sugar residue of each compound were prepared and analyzed by GC [19]. The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (*Sigma–Aldrich*). The following sugars were detected: D-glucose, D-xylose, and L-arabinose for both **1** and **2**.

REFERENCES

[1] L. Affre, J. D. Thomson, M. Debussche, *Bot. J. Linn. Soc.* **1995**, *118*, 309.
[2] S. T. Bennett, J. M. Grimshaw, *Plant Syst. Evol*. **1991**, *176*, 135.
[3] A.-K. Pueschel, H.-G. Schwenkel, T. Winkelmann, *Plant Cell Tiss. Org.* **2003**, *72*, 43.
[4] İ. Çaliş, M. E. Şatana, A. Yürüker, P. Kelican, R. Demirdamar, R. Alaçam, N. Tanker, H. Rüegger, O. Sticher, *J. Nat. Prod.* **1997**, *60*, 315.
[5] Z. Jia, K. Koike, T. Ohmoto, M. Ni, *Phytochemistry* **1994**, *37*, 1389.
[6] G. Mihci-Gaidi, S. Ozbey, I. Orhan, B. Sener, T. Miyamoto, J.-F. Mirjolet, O. Duchamp, A.-C. Mitaine-Offer, M.-A. Lacaille-Dubois, *Planta Med*. **2010**, *76*, 218.
[7] G. Mihci-Gaidi, D. Pertuit, T. Miyamoto, J.-F. Mirjolet, O. Duchamp, A.-C. Mitaine-Offer, M.-A. Lacaille-Dubois, *Nat. Prod. Commun.* **2010**, *5*, 1023.
[8] G. Reznicek, J. Jurenitsch, W. Robien, W. Kubelka, *Phytochemistry* **1989**, *28*, 825.
[9] R. O. Dorchai, J. B. Thomson, *Tetrahedron Lett.* **1963**, *26*, 2223.
[10] Q. Q. Gong, L. H. Mu, P. Liu, S. L. Yang, B. Wang, Y. L. Feng, *Chin. Chem. Lett.* **2010**, *21*, 449.
[11] J. Huang, H. Zhang, N. Shimizu, T. Takeda, *Chem. Pharm. Bull*. **2003**, *51*, 875.
[12] A. Viqar Uddin, S. Qazi Najmus, K.Usmanghani, *Phytochemistry* **1980**, *9*, 1875.
[13] H. Altunkeyik, D. Gülcemal, M. Masullo, O. Alankus-Caliskan, S. Piacente, T. Karayildirim, *Phytochemistry* **2012**, *73*, 127.
[14] H. Kohda, O. Takeda, S. Tanaka, *Chem. Pharm. Bull*. **1989**, *37*, 3304.
[15] S. Dall'Acqua, I. Castagliuolo, P. Brun, F. Ditadi, G. Palu, G. Innocenti, *Carbohydr. Res.* **2010**, *345*, 709.

[16] A. Huseyin, *Doga* **1977**, *1*, 148.
[17] R. Tschesche, H. J. Mercker, G. Wuff, *Justus Liebigs Ann. Chem*. **1969**, *721*, 194.
[18] I. Çaliş, A. Yürüker, N. Tanker, A. D. Wright, O. Sticher, *Planta Med*. **1997**, *63*, 166.
[19] S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull*. **1987**, *35*, 501.

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