24. New Saponins and a Prosapogenin from *Polygala chamaebuxus* L.

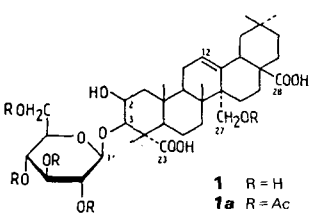
by **Matthias Hamburger** and **Kurt Hostettmann***

Institut de Pharmacognosie et Phytochimie, Université de Lausanne, 2, rue Vuillermet, CH-1005 Lausanne

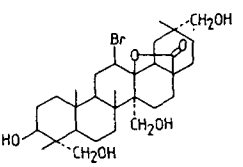
(11.XI.85)

Two glycosides **2** and **3** of the formerly undescribed triterpene 3β,23,27,29-tetrahydroxyolean-12-en-28-oic acid (**4**) have been isolated from the aerial parts of *Polygala chamaebuxus* L. In addition, a prosapogenin, obtained by basic hydrolysis of a mixture of bidesmosidic saponins, has been identified as tenuifolin (**1**). The structures have been established by spectroscopic (IR, ^{1}H- and ^{13}C-NMR, DCI- and FAB-MS) and chemical methods (acid, basic, and enzymatic hydrolysis, acetylation, bromination).

Introduction. – In spite of the increasing number of phytochemical investigations of the genus *Polygala,* very little is known about the constituents of the European species. As part of a study of some Polygalaceae species, we examined *P. chamaebuxus* L. which is widely distributed in the alpine and subalpine regions of Central Europe. Only the presence of ferulic and sinapic acids in a leaf hydrolysate [1] and the triterpene aglycone presenegenin [2] have been reported previously. We recently characterized some new hydroxycinnamoyl esters from the aerial parts of the plant [3], and we now describe the isolation and structure elucidation of the saponins **1**–**3**.



1 R = H
1a R = Ac



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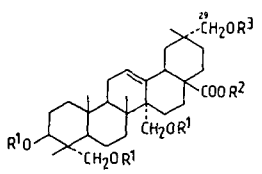

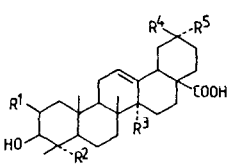

Compound	R^1	R^{2a})	R^{3b})
2	H	ara	H
3	H	H	glc
4	H	H	H
4a	Ac	H	Ac

a) ara: α-L-Arabinopyranosyl.
b) glc: β-D-Glucopyranosyl.

Compound	R^1	R^2	R^3	R^4	R^5
6	H	CH_3	CH_3	CH_3	CH_3
7	OH	COOH	CH_2OH	CH_3	CH_3
8	H	CH_3	CH_3	CH_2OH	CH_3
9	H	COOH	CH_3	CH_3	CH_2OH
10	H	CH_2OH	CH_3	CH_3	CH_3

Results. – A preliminary purification of the MeOH extract of the aerial parts was obtained by partition between BuOH and H_2O, followed by an Et_2O precipitation. The precipitate was further separated by open column chromatography on silica gel and *Sephadex LH 20* to obtain a purified fraction of bidesmosidic saponins. The HPLC analysis of this fraction revealed the presence of at least 15 components. The basic hydrolysis of the mixture afforded a common prosapogenin **1**, which was identified as *tenuifolin (presenegenin-3-O-β-D-glucopyranoside)* [4] [5] by the spectral data (IR, ^{1}H- and ^{13}C-NMR, FAB- and DCI-MS) of **1** and its pentaacetate **1a**, and by co-chromatography with an authentic sample isolated from *P. senega* L.

Saponins **2** and **3** were obtained from the Et_2O-soluble portion by combination of preparative liquid chromatography, centrifugal thin-layer [6] and low-pressure liquid chromatography on silica gel and *RP-8* (for details of the extraction and isolation: see *Exper. Part*).

Anisaldehyde/H_2SO_4 staining gave an intense pink coloration on the TLC of the compounds **1**–**3**. This reaction is considered to be characteristic for glycosides of presenegenin [7]. Acid hydrolysis of **2** and **3** afforded arabinose and glucose, respectively, whereas the aglycones decomposed under the applied conditions. Compound **3** resisted basic hydrolysis, but **2** yielded arabinose and the sapogenin **4**. Saponin **3**, upon treatment with β-glucuronidase, afforded an aglycone which was identical with **4** in regard to chromatographic behavior and staining.

The molecular weight of **4** was established by mass spectroscopy. The fast-atom-bombardment (FAB) MS [8] (thioglycerol matrix, negative-ion mode) showed quasimolecular ions at m/z 539 ($[M + Cl]^-$) and 503 ($[M - H]^-$). Elimination of a 30 amu fragment ($- CH_2O$) led to the signal at m/z 473 ($[(M - H) - 30]^-$), as shown by MS-MS experiments. In the desorption-chemical-ionization (DCI) MS [9] (NH_3, positive-ion mode), a quasimolecular peak was observed at m/z 522 ($[M + NH_4]^+$), together with fragment ions at m/z 492 ($[(M + NH_4) - 30]^+$) and 475 ($[(M + H) - 30]^+$).

The functional groups of **4**, identified by the ^{13}C-NMR data (broad-band-decoupling and distortionless-enhancement-by-polarization (DEPT) experiments [10]), were: one secondary (73.68 ppm) and three primary (68.75, 64.37, 73.99 ppm) OH groups, four CH_3 groups (12.85, 16.31, 18.82, 19.82 ppm), one COOH group (180.33 ppm). Furthermore, two olefinic C-atoms were observed at 127.7 and 140.07 ppm. The ^{1}H-NMR spectrum showed the singlets of four CH_3 groups at 0.96, 1.05, 1.06, and 1.21 ppm (3 H each). The overlapping signals of 3 *AB* quartets (6 H) between 3.7 and 4.2 ppm could be assigned to CH_2OH moieties. On the basis of the MS and the DEPT spectra, the molecular formula was found to be $C_{30}H_{48}O_6$.

The Δ-12-oleanene skeleton of the compound, strongly suggested by the signals for a double bond, eight exocyclic and seven quaternary C-atoms in the ^{13}C-NMR spectrum, was confirmed by bromination. It is well-established that olean-12-en-28-oic acids, upon bromination in acidic solution, are readily converted into the corresponding γ-bromolactones, whereas other types of triterpenic acids do not react [11]. Bromination of **4** in AcOH solution yielded γ-lactone **5** (IR: 1760 cm^{-1}). Acetylation of **4** afforded the tetraacetate **4a**. The complete acetylation was confirmed by the IR spectrum (no absorption band at 3450 cm^{-1}). The DCI-MS of **4a** showed a quasimolecular ion at m/z 690 ($[M + NH_4]^+$), and in the ^{1}H-NMR spectrum three singlets attributable to four Ac groups were observed at 2.02 ppm (3 H), 2.04 ppm (3 H) and 2.06 ppm (6 H).

Table 1. *^{13}C-NMR Spectral Data of Compounds* **4**, **4a**, *and* **6–10**

	4[a][b])	**6**[c])	**7**[d])	**8**[c])	**9**[d])	**10**[e])	**4a**[b][f])
C(1)	38.63 s	38.5	45.0	38.7	37.6	38.7	37.86 s
C(2)	27.59 s	27.1	71.6	27.0	26.2	27.4	23.29 s
C(3)	73.68 t	78.7	75.8	78.9	74.1	73.7	74.41 t
C(4)	42.74 q	38.7	54.0	38.7	53.0	42.6	40.60 q
C(5)	49.01 t[g])	55.2	52.3	55.3	50.4	48.7	47.87 t
C(6)	18.67 s	18.3	21.6	18.5	20.2	18.5	17.95 s
C(7)	33.40 s	32.6	34.1	32.8	31.6	32.7	32.77 s
C(8)	40.43 q	39.3	40.9	39.3	38.7	39.6	39.94 q
C(9)	48.80 t[g])	47.6	49.5	47.7	46.9	47.9	48.71 t
C(10)	37.40 q	37.0	37.1	37.2	35.2	37.1	37.00 q
C(11)	24.08 s	23.1	24.0[g])	23.4	22.4	23.7	22.89 s
C(12)	127.70 t	122.1	127.6	122.8	–	122.9	127.71 t
C(13)	140.07 q	143.4	139.7	143.2	143.6	144.1	136.64 q
C(14)	46.93 q	41.6	46.5	41.7	40.7	41.9	45.15 q[g])
C(15)	24.40 s	27.7	24.7[g])	27.7	26.9	28.0	22.81 s
C(16)	23.76 s	23.4	24.1[g])	23.4	22.4	23.3	23.82 s
C(17)	48.03 q	46.6	48.1	46.8	45.7	46.9	46.34 q[g])
C(18)	41.18 t	41.3	41.8	40.3	39.9	41.7	39.94 t
C(19)	40.37 s	45.8	45.5	40.3	39.9	46.0	39.13 s
C(20)	36.55 q	30.6	31.0	35.2	35.5	30.7	34.38 q
C(21)	28.93 s	33.8	33.5	32.8[g])	27.7	33.9	28.33 s
C(22)	32.66 s	32.3	33.2	32.1[g])	31.2	32.7	31.40 s
C(23)	68.75 s	28.1	180.8	28.1	179.7	68.4	65.24 s[h])
C(24)	12.85 p	15.6[g])	13.7	15.7	10.9	12.8	13.10 p
C(25)	16.31 p	15.3[g])	17.5	15.4	14.6	15.9	16.03 p
C(26)	18.82 p	16.8	18.8	16.9	15.9	17.1	18.27 p
C(27)	64.37 s	26.0	64.4	26.0	24.8	26.1	65.55 s[h])
C(28)	180.33 q	177.9	180.8	178.5	178.9	177.9	–
C(29)	73.99 s	33.1	33.2	28.9	72.4	33.1	74.06 s
C(30)	19.82 p	23.6	23.2	65.8	18.3	23.7	19.27 p
$CH_3OOC(28)$		51.3		50.6		51.4	
CH_3COO-							20.76 p
							20.84 p
							2×21.15 p
CH_3COO-							170.44–70

[a]) 90.5 MHz, (D_5)pyridine.
[b]) p: primary C-atom, s: secondary C-atom, t: tertiary C-atom, q: quaternary C-atom.
[c]) Measured as methyl ester (15.1 MHz, $CDCl_3$).
[d]) 25 MHz, (D_5)pyridine.
[e]) Measured as methyl ester, 22.6 MHz, (D_5)pyridine.
[f]) 90.5 MHz, $CDCl_3$.
[g])[h]) Signals in the vertical columns may be interchanged.

The positions of the OH groups were determined by the ^{13}C-NMR data of **4** and **4a** in comparison with the published data of related Δ-12-oleanene derivatives *(Table 1)*: the absence of CH_3 signals at fields lower than 20 ppm indicates that the three CH_2OH groups are attached to C(4), C(14), and C(20). The OH group at C(27) has a noticeable influence on the C(13) (140.07 ppm) and C(12) (127.70 ppm) signals. In comparison with oleanolic acid **6** [12], they are shifted by −4.3 and +5.6 ppm, respectively. The observed values for C(12), C(13), and C(27) are in excellent agreement with those reported for presenegenin **7**

[5]. After the acetylation of **4**, the C(13) signal appears upfield at 136.64 ppm (−3.4 ppm). The position of the substituents attached to C(20) are determined by comparison with the reported data of quercetaroic acid **8** (OH at C(30)) [12] and dianic acid **9** (OH at C(29)) [13]: while the E-ring of **4** differs from methylquercetaroate in the ^{13}C-NMR shifts for C(21), C(29), and C(30), the observed values are in good agreement with dianic acid. The substitution effect of the OH group shifts the C(29) signal downfield to 73.99 ppm, whereas, in comparison with oleanolic acid **6** the C(30) and C(21) signals are observed upfield at 19.82 ppm (−3.8 ppm) and 28.93 ppm (−3.9 ppm), respectively. The OH groups attached to C(3) and C(23) are identified by comparison of **4** and **4a** with the reported data of hederagenin **10** [14] and its peracetate [12]. The configuration at C(3) is further confirmed by the ^{1}H-NMR spectrum of the tetraacetate **4a**: the signal at 4.78 ppm (*dd*, J = 12, 4 Hz), corresponding to H_x–C(3), is in good agreement with data observed for H_{ax}–C(3) in analogous triterpenes [15]. Thus, the OH group has the β-configuration. The remaining signals of the ^{13}C-NMR spectrum of **4** were assigned by comparison with hederagenin (ring-A and ring-B C-atoms), presenegenin (ring-C and ring-D C-atoms) and dianic acid (ring-E C-atoms). On the basis of this chemical and spectral evidence, the sapogenin **4** is identified as *3β,23,27,29-tetrahydroxyolean-12-en-28-oic acid.*

The FAB-MS of **2** (thioglycerol matrix, negative-ion mode) shows a quasimolecular ion at m/z 671 ($[M + Cl]^-$). In the DCI-MS (NH_3, positive-ion mode), a quasimolecular peak is observed at m/z 654 ($[M + NH_4]^+$). The signal at m/z 522 ($[(M + NH_4) - 132]^+$) corresponds to the loss of an arabinosyl moiety. In the FAB-MS of **3**, quasimolecular ions appear at m/z 701 ($[M + Cl]^-$) and 665 ($[M - H]^-$), confirming the presence of one glucosyl moiety.

Table 2. *^{13}C-NMR Spectral Data of Saponins* **2a** *and* **3** (90.5 MHz, (D_5)pyridine)[b])

Saponin	C(28)	C(29)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C(6′)
2	176.72 q	73.54 s	95.87 t	71.29 t	74.08 t	67.83 t	65.94 s	
3		81.55s	105.37 t	75.18 t	78.50 t[c])	71.70 t	78.34 t[c])	62.84 s

[a]) Pertinent shifts only.
[b]) s: secondary C-atom, t: tertiary C-atom, q: quaternary C-atom.
[c]) Attributions may be interchanged.

The position of attachment of the sugars to the aglycone in saponins **2** and **3** were determined by ^{13}C-NMR spectroscopy. Pertinent shifts are summarized in *Table 2.* In saponin **2**, the sugar moiety is linked to the COOH group, the signal of which appears at 176.72 ppm. The other signals attributed to the aglycone remain unchanged. The α-conformation of the arabinopyranosyl moiety is indicated by the signal of the anomeric C-atom at 95.87 ppm. The shift is in good agreement with reported data of α-L-arabinopyranose [16] and usual esterification shifts [17]. Additional confirmation is given by the ^{1}H-NMR spectrum: the coupling constant of the anomeric proton at 6.31 ppm (J = 5.8 Hz) corresponds with reported data for α-L-arabinopyranosides [18]. Thus, the structure of **2** is established as *α-L-arabinopyranosyl-3β,23,27,29-tetrahydroxyolean-12-en-28-oate*. The glucosyl moiety of saponin **3** is attached to the aglycone at C(29). In the ^{13}C-NMR spectrum of **3**, this C-atom appears at 81.55 ppm, 7.5 ppm downfield from the corresponding signal in the spectrum of **4**. The β-conformation of the glucose is deduced from the ^{13}C-NMR (C(1′) at 105.37 ppm) and ^{1}H-NMR data (H–C(1′) at 4.75 ppm;

$J(1',2') = 8$ Hz), in comparison with reported data for β-D-glucopyranosides [5]. Saponin **3** is, therefore, identified as *29-[O-β-D-glucopyranosyloxy]3β,23,27-trihydroxyolean-12-en-28-oic acid.*

Discussion. – Saponins **2** and **3** are derived from a new triterpenoid sapogenin. This aglycone shows an unusual substitution pattern of the Δ-12 olenanene skeleton, since functional groups attached to C(27) and C(29) have rarely been found. However, the CH_2OH moiety at C(14) is not very surprising in the present case, because presenegenin, the major sapogenin found in species of the genus *Polygala*, bears the same group. An OH group attached to C(29) has, to our knowledge, only been found in mesembryanthemoidigenin [19] and dianic acid [13]. The positions of attachment of a single sugar chain at C(28) and C(29), as found in **2** and **3**, respectively, are uncommon. In monodesmosidic saponins of the Δ-12-oleanene type, the glucosyl moiety is most often linked to the C(3) carbon of the aglycone. Prosapogenin **1** (tenuifolin) has been previously isolated from *P. senega* L. and *P. tenuifolia* WILLD. [4]. The compounds **1–3** were inactive against the schistosomiasis-transmitting snail *Biomphalaria glabrata* [20] at a concentration of 50 ppm within 24 h. Tenuifolin **1** showed a spermicidal activity against human spermatozoids at a concentration of 4 mg/ml within 3 min [21].

Financial support has been provided by the *Swiss National Science Foundation*. We are grateful to Mr. *E. Anchisi, Alpine Botanical Garden*, Champex VS, for the collection and identification of the plant material, and to Prof. *J. Shoji*, Tokyo, for supplying a reference sample of tenuifolin. Thanks are also due to Prof. *J. Lauterwein* (^{1}H- and ^{13}C-NMR), Lausanne, to Prof. *W. J. Richter* and Mr. *M. Raschdorf* (FAB-MS), both *Ciba-Geigy AG*, Basel, and to Mr. *D. Chollet* (DCI-MS) *Zyma SA*, Nyon for spectral measurements.

Experimental Part

General. The plant material was collected near Champex VS and identified by Mr. *E. Anchisi, Alpine Botanical Garden*, Champex VS. M.p. were measured on a *Kofler* block and are uncorrected. IR spectra were obtained on a *Pye Unicam SP 3-200*. ^{1}H- and ^{13}C-NMR spectra were measured in (D_5)pyridine or $CDCl_3$ on a *Bruker WH-360* instrument at 360 and 90.5 MHz, respectively. The selective 10-mm ^{13}C probehead gave 90° pulses of 13.5 μsec for ^{13}C and 36 μsec for ^{1}H (10 W). For ^{1}H broad-band decoupling, 4 W were used. In the DEPT sequence, the interpulse delay was chosen to be equal to $(2\ J)^{-1}$ for an average ^1J of 135 Hz. The recycle time was 2 sec, considerably larger than the ^{1}H T_1's. In usual practice, the DEPT sequence with two different θ values ($\pi/2$ was $3\pi/4$) was sufficient to obtain the assignment of the CH, CH_2, and CH_3 multiplicities (and indirectly of the quaternary C-atoms from the broad-band decoupled spectrum). In case of overlapping of different carbon resonances or interference of a carbon resonance with the solvent (compound **4a**), fully edited spectra were recorded which need a two-fold recording time. Desorption-chemical-ionization (DCI) MS were recorded in the positive-ion mode on a *Ribermag-R10-10B* quadrupole instrument with NH_3 as reactant gas. Fast-atom-bombardment (FAB) MS in the negative-ion mode were obtained on a *ZAB-S1* spectrometer. The samples were suspended in thioglycerol, and the target was bombarded with 5-keV Xe-atoms.

Extraction and Isolation. The powdered aerial parts of *P. chamaebuxus* (500 g) were extracted at r.t. with $CHCl_3$, followed by MeOH. The MeOH extract (150 g) was dissolved in H_2O (3 l) and extracted 3× with BuOH, saturated with H_2O. The BuOH layers were combined and evaporated. The residue (70 g) was dissolved in MeOH (350 ml) and poured into Et_2O (1 l) under vigorous stirring. The precipitate was filtered off, dissolved in MeOH (100 ml), and precipitated again with Et_2O (200 ml). The solid residue (32 g) was chromatographed on a silica-gel column (i.d. 5.5 × 90 cm) with BuOH (sat. with H_2O) and BuOH (sat. with H_2O)/MeOH 90:10. The saponin fraction (7.8 g) was purified on a *Sephadex LH 20* column (i.d. 4.0 × 90 cm) (eluent: MeOH) to yield a mixture of bidesmosidic saponins (3.5 g). The Et_2O-soluble fraction (30 g) was subjected to prep. liquid chromatography (*Chromatospac Prep 10, Jobin-Yvon*, Longjumeaux) in 3 portions of 10 g each. The column (i.d. 4 × 50 cm) was packed with a slurry of 180 g of silica gel (15 μm, *Merck*, Darmstadt), or with 150 g of *LiChroprep RP-8* material (15–25 μm, *Merck*, Darmstadt). For centrifugal TLC, a *Chromatotron* model 7924 (*Harrison Research*, Palo Alto)

was used. The plates were coated with silica gel; layer thickness 2 mm. Saponin **3** was purified on a *Lobar LiChroprep RP-8* column (40–63 μm, i.d. 2.5 × 27 cm) (*Merck*, Darmstadt), equipped with a *Duramatic-80* pump (*Chemie und Filter*, Regensdorf). Final purification of all compounds was carried out on a *Sephadex LH 20* column with MeOH. TLC were carried out on silica-gel-precoated Al sheets (*Merck*, Darmstadt) with mixtures of $CHCl_3$/MeOH/H_2O of various proportions, or on *RP-8* nanoplates (*Merck*, Darmstadt) with MeOH/H_2 65:35 or 75:25. The purity of the saponins was checked by HPLC on a *Knauer LiChrosorb RP-8* column (i.d. 4.6 × 250 mm) with MeOH/H_2O 45:55 (2 ml/min); detection was at 204 nm. The conditions for the analysis of the mixture of bidesmosidic saponins were the following: *Knauer LiChrosorb RP-8* (i.d. 4.6 × 250 mm); MeCN/H_2O 15:85→35:65 in 40 min; flow rate: 2 ml/min; detection: 204 nm.

Acid Hydrolysis. The saponin (2 mg) was refluxed in 4N HCl (2 ml) for 2 h. The mixture was extracted with AcOEt. The org. layer was checked by TLC for the aglycones. The aq. phase was adjusted to pH 6 with $NaHCO_3$. After freeze drying, the sugars were extracted with pyridine from the residue and analyzed by TLC on silica gel with AcOEt/MeOH/H_2O/AcOH 65:15:15:20; detection with *p*-anisidine-phthalate.

Basic Hydrolysis. The saponin (2 mg) was refluxed in 0.5N KOH (2 ml) for 2 h. The mixture was adjusted to pH 4 with aq. HCl and extracted subsequently with AcOEt and BuOH. The org. layers were analyzed for aglycones or glycosides by TLC on silica gel with $CHCl_3$/MeOH/H_2O 80:20:2. The aq. phase was treated as described above.

Enzymatic Hydrolysis. The saponin (1 mg) and β-glucuronidase (1 mg) from *Helix pomatia (G-1512, Sigma Chemical*, St. Louis) were dissolved in acetate buffer, pH 5.5 (2 ml). The mixture was kept at 36° for 2 d. After acidification with aq. HCl to pH 4, the mixture was extracted with Et_2O. The org. layer was analyzed by co-chromatography with an authentic sample of **4**.

Acetylation. The compound was dissolved in a mixture of Ac_2O/pyridine 1:1 and stirred at r.t. for 36 h. The mixture was poured into ice water and the precipitate was filtered off. The crude peracetate was purified on a *Sephadex-LH-20* column (MeOH).

Tenuifolin (= *3β-(β-*D*-Glucopyranosyloxy-2β,27-dihydroxyolean-12-en-23,28-dioic acid*; **1**). A portion of the mixture of bidesmosidic saponins (400 mg) was refluxed with 1N KOH (25 ml) for 2 h. The suspension was extracted with BuOH. The org. layer was washed with H_2O and evaporated. The residue was purified on a silica-gel column with $CHCl_3$/MeOH/H_2O 80:20:2→65:35:10 to yield **1** (50 mg). Amorphous white powder, decomposes upon heating at 300° (298–300° dec. [4]). TLC (SiO_2, $CHCl_3$/MeOH/H_2O 70:30:3): R_f 0.27, TLC (*RP-8*, MeOH/H_2O 75:25): R_f 0.42. ^{1}H-NMR and ^{13}C-NMR: identical with reported data [5]. DCI-MS (NH_3, positive ions). 698 ($[M + NH_4]^+$); 668 ($[(M + NH_4) - 30]^+$). FAB-MS (thioglycerol, negative ions): 1359 ($[2M - H]^-$); 679 ($[M - H]^-$); 517 ($[(M - H) - 162]^-$).

Pentaacetyl Derivative **1a**. Acetylation of **1** (40 mg) gave the pentaacetate **1a** (32 mg). Amorphous white powder, m.p. 223–225°. TLC (SiO_2, toluene/AcOEt 1:1): R_f 0.42. IR (KBr): 3500, 2950, 1750, 1740, 1370, 1230, 1040. DCI-MS (NH_3, positive ions): 908 ($[M + NH_4]^+$).

α-L-Arabinopyranosyl 3β,23,27,29-Tetrahydroxyolean-12-en-28-oate (**2**). Amorphous, white powder, m.p. 197–201°. TLC (SiO_2, $CHCl_3$/MeOH/H_2O 70:30:3): R_f 0.56. ^{1}H-NMR (360 MHz, (D_5)pyridine): 0.87, 1.04, 1.09, 1.13 (4*s*, 4 CH_3); 3.85, 3.95, 4.14 (3*q AB*, 3 CH_2OH); 5.83 (*t*, *J* = 3, H–C(12)); 6.31 (*d*, *J* = 5.8, H–C(1′)). ^{13}C-NMR (90.52 MHz, (D_5)pyridine): 12.82 (C(24)); 16.42 (C(25)); 18.75 (C(6)); 18.98 (C(26)); 19.77 (C(30)); 23.42 (C(16)); 23.92 (C(11)); 24.43 (C(15)); 27.55 (C(2)); 28.84 (C(21)); 32.25 (C(22)); 33.43 (C(7)); 36.44 (C(20)); 37.45 (C(10)); 38.74 (C1)); 40.15 (C(19)); 40.65 (C(8)); 40.95 (C(18)); 42.74 (C(4)); 47.49, 47.98 (C(14), C(17)); 48.81, 49.06 (C(9), C(5)); 64.35 (C(27)); 68.82 (C(23)); 73.54 (C(29)); 73.75 (C(3)); 128.07 (C(12)); 139.54 (C(13)); 176.72 (C(28)); signals of the sugar moiety: see *Table 2*. DCI-MS (NH_3, positive ions): 654 ($[M + NH_4]^+$); 625 ($[(M + NH_4) - 30]^+$); 522 ($[(M + H) - 132]^+$). FAB-MS (thioglycerol, negative ions): 671 ($[M + Cl]^-$); 503 ($[M - H]^-$); 473 ($[(M - H) - 30]^-$).

*29-(β-*D*-Glucopyranosyloxy)-3β,23,27-trihydroxyolean-12-en-28-oic Acid* (**3**). Amorphous, white powder, m.p. 211–215°. TLC (SiO_2, $CHCl_3$/MeOH/H_2O 70:30:3): R_f 0.45. ^{1}H-NMR (360 MHz, (D_5)pyridine): 0.92, 1.00, 1.03, 1.20 (4*s*, 4 CH_3); 5.77 (br. *s*, H–C(12)). ^{13}C-NMR (90.5 MHz, (D_5)pyridine): 12.84 (C(24)); 16.36 (C(25)); 18.75 (C(6)); 18.99 (C(26)); 20.01 (C(30)); 23.94 (C(16)); 24.25 (C(11)); 24.43 (C(15)); 27.55 (C(2)); 29.35 (C(21)); 32.58 (C(22)); 33.41 (C(7)); 35.78 (C(20)); 37.45 (C(10)); 38.70 (C(1)); 40.44 (C(19)); 40.67 (C(18)); 41.14 (C(8)); 42.74 (C(4)); 47.00 (C(14)); 48.04 (C(17)); 48.85 (C(9)); 49.12 (C(5)); 64.35 (C(27)); 68.91 (C(23)); 74.12 (C(3)); 81.55 (C(29)); 127.50 (C(12)); 140.20 (C(13)); signals of the sugar moiety: see *Table 2*. DCI-MS (NH_3, positive ions): 684 ($[M + NH_4]^+$); 654 ($[(M + NH_4) - 30]^+$). FAB-MS (thioglycerol, negative ions): 701 ($[M + Cl]^-$); 665 ($[M - H]^-$); 635 ($[(M - H) - 30]^-$).

3β,23,27,29-Tetrahydroxyolean-12-en-28-oic Acid (**4**). Compound **2** (60 mg) was refluxed with 0.5N KOH for 2 h. The mixture was adjusted to pH 4 with aq. HCl and then extracted with Et_2O (3 × 25 ml). After evaporation, the residue was purified to yield **4** (40 mg). Amorphous, white powder, m.p. 206–210°. TLC (SiO_2, $CHCl_3$/MeOH/H_2O

90:10:1): R_f 0.26. ^{1}H-NMR (360 MHz, (D_5)pyridine): 0.96, 1.05, 1.06, 1.21 (4*s*, 4 CH_3); 3.70–4.20 (3*q AB*, 3 CH_2OH); 6.90 (*t*, *J* = 3, H–C(12)). ^{13}C-NMR (90.5 MHz, (D_5)pyridine): see *Table 1*. DCI-MS (NH_3, positive ions): 522 ($[M + NH_4]^+$); 493 ($[(M + NH_4) - 29]^+$). FAB-MS (thioglycerol, negative ions): 561 ($[(M - H) + NaCl]^-$); 539 ($[M + Cl]^-$); 503 ($[M - H]^-$); 473 ($[M - H) - 30]^-$).

Tetraacetate **4a.** Acetylation of **4** (20 mg) yielded **4a** (21 mg) as an amorphous, white powder, m.p. 104–107°. TLC (SiO_2, toluene/AcOEt 1:1): R_f 0.35. ^{1}H-NMR (360 MHz, $CDCl_3$): 0.73, 0.82, 0.97, 1.00 (4*s*, 3 CH_3); 2.02, 2.04 (2*s*, 2 CH_3COO); 2.06 (*s*, 2 CH_3COO); 3.74 (*qAB*, *J* = 10, CH_2OAc); 3.79 (*qAB*, *J* = 12, CH_2OAc); 4.10 (*qAB*, *J* = 13, CH_2OAc); 4.78 (*dd*, *J* = 12,4, H–C(3)); 5.62 (*t*, *J* = 3, H–C(12)). ^{13}C-NMR (90.5 MHz, $CDCl_3$): see *Table 1*. DCI-MS (NH_3, positive ions): 690 ($[M + NH_4]^+$).

Bromolactone **5**. The sapogenin **4** (6 mg) was dissolved in 90% AcOH (1 ml), containing AcONa · 3 H_2O (13 mg). A soln. of Br_2 (0.1 ml) in AcOH 100% (2 ml) was added dropwise. The mixture was poured into H_2O containing $NaHSO_3$. The aq. phase was extracted with $CHCl_3$. The org. layer was washed with H_2O and evaporated to dryness to yield **5** (4 mg). Amorphous, white powder, m.p. 176–180°. TLC (SiO_2, $CHCl_3$/MeOH/H_2O 90:10:1): R_f 0.28. IR (KBr): 3440; 2940, 2870, 1760. DCI-MS (NH_3, positive ions): 519 ($[(M + NH_4) - HBr]^+$); 502 ($[(M + H) - HBr]^+$).

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