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Spectral Assignments and Reference Data

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Structure elucidation of new oleanane-type glycosides from three species of *Acanthophyllum*

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From the roots of three species of *Acanthophyllum* (Caryophyllaceae), two new gypsogenic acid glycosides, 1 and 2, were isolated, 1 from *A. sordidum* and *A. lilacinum*, 2 from A. *elatius* and *A. lilacinum*, together with three known saponins, glandulosides B and C, and SAPO50. The structures of 1 and 2 were established mainly by 2D NMR techniques as 23-*O*-β-D-galactopyranosylgypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-galactopyranoside (1) and gypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-galactopyranoside (2). The cytotoxicity of several of these saponins was evaluated against two human colon cancer cell lines (HT-29 and HCT 116).

Keywords: NMR; ^{1}H; ^{13}C; 2D NMR; triterpene saponins; gypsogenic acid; Caryophyllaceae; *Acanthophyllum*

Introduction

In a continuation of our studies of the chemotaxonomy of the Caryophyllaceae family, especially the *Acanthophyllum* species,[1–4] and saponin constituents thereof, we have examined the saponin fraction of the roots of three species of *Acanthophyllum, A. sordidum* Bunge ex Boiss, *A. elatius* Bunge, and *A. lilacinum* Schischk (Caryophyllaceae). All species of *Acanthophyllum* are used as soup in Khorasan province, and the aqueous extract of their roots are added to make a special type of candy.[4] No previous phytochemical study has been reported on saponins of these plants. In this article, we describe the isolation and structure elucidation of two new triterpene saponins, **1** and **2**, from these species, along with three known saponins. Compound **1**, 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3,4-di-*O*-acetyl-β-D-fucopyranoside (glanduloside C),[3] and 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranoside (SAPO50),[5] was isolated from *A. sordidum*. Compound **2**, glanduloside C[3] and SAPO50,[5] was isolated from *A. elatius*, whereas **1, 2**, 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin-28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-3-*O*-acetyl-β-D-fucopyranoside (glanduloside B),[3] glanduloside C,[3] and SAPO50[5] were obtained from *A. lilacinum*. The structures of these five saponins were elucidated mainly by 600 MHz NMR analysis, including 1D and 2D NMR (^{1}H-^{1}H COSY, HSQC, HMBC, TOCSY, and NOESY) spectroscopy and mass spectrometry, and compared with literature data for the known compounds. The cytotoxicity of several of these saponins was evaluated against two human colon cancer cell lines (HT-29 and HCT 116).

Results and Discussion

Compound **1**, a white amorphous powder, exhibited in high-resolution electrospray ionization mass spectrometry (HR-ESIMS) (positive-ion mode) a pseudo-molecular ion peak at m/z = 1157.5350 $[M + Na]^+$ (calculated 1157.5356), consistent with a molecular formula of $C_{54}H_{86}O_{25}Na$. Its fast-atom bombardment mass spectrum (FABMS) (negative-ion mode) showed a quasi-molecular ion peak at m/z = 1133 $[M - H]^-$, indicating a molecular weight of 1134. Other significant fragment ion peaks were observed at m/z = 971 $[(M - H) - 162]^-$, 809 $[(M - H) - 162 - 162]^-$, 647 $[(M - H) - 162 - 162 - 162]^-$, and 485 $[(M - H) - 162 - 162 - 162 - 162]^-$, corresponding to the successive

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Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of the aglycons of **1** and **2** in pyridine-d_5 (δ in ppm, J in Hz)

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	38.5	0.94, 1.44	39.0	nd
2	26.8	1.82, nd	26.8	1.46, nd
3	74.8	4.51	75.7	4.14
4	54.7	–	54.8	–
5	51.4	1.75	51.2	1.68
6	23.3	1.76, 1.82	23.0	1.76, 1.80
7	32.0	1.64, 1.77	32.0	1.60, 1.70
8	39.7	–	39.2	–
9	48.0	1.60	47.3	1.57
10	36.3	–	36.2	–
11	24.0	1.79, 1.85	24.0	1.85, 1.79
12	122.1	5.31 br t (2.9)	123.1	5.32 br t (3.0)
13	143.8	–	143.6	–
14	41.7	–	42.2	–
15	27.8	0.98, 2.03	27.6	1.08, 2.02
16	20.9	1.45, nd	20.3	nd
17	46.7	–	46.7	–
18	41.7	3.03 dd (12.6, 3.0)	41.2	3.01 dd (12.6, 3.0)
19	46.0	1.08, 1.61	45.8	1.06, 1.57
20	30.3	–	30.1	–
21	33.5	1.01, 1.21	33.4	1.01, 1.17
22	32.3	1.21, 1.50	31.8	1.17, 1.48
23	177.8	–	183.8	–
24	11.5	1.45 s	11.6	nd
25	15.6	0.81 s	15.6	0.81 s
26	16.9	0.92 s	17.1	1.02 s
27	25.8	1.03 s	25.5	1.05 s
28	176.2	–	176.6	–
29	32.5	0.76 s	32.6	0.76 s
30	23.1	0.78 s	23.1	0.78 s

Overlapped proton NMR signals are reported without designated multiplicity.
nd, not determined.

Table 2. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) data of the sugar moieties of **1** and **2** in pyridine-d_5 (δ in ppm, J in Hz)

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
28-*O*-				
Gal I-1	94.5	6.07 d (8.3)	94.3	6.01 d (8.3)
2	72.4	4.11	72.2	4.07
3	87.0	4.23 dd (8.1, 3.1)	86.4	4.19 dd (9.0, 3.3)
4	68.5	4.17	68.1	4.10
5	76.9	4.01	76.5	3.95
6	68.9	4.19, 4.53 dd (11.2, 4.0)	68.1	4.10, 4.44 br d (11.4)
Glc I-1	104.7	5.23 d (7.3)	104.1	5.18 d (7.1)
2	74.8	3.95	74.5	3.89 t (8.1)
3	77.3	4.07	77.0	4.02
4	70.8	3.94	70.7	3.86
5	77.8	3.90	77.5	3.83
6	62.0	4.09, 4.44 br d (11.7)	61.6	4.02, 4.35 br d (11.7)
Glc II-1	104.5	4.84 d (7.6)	103.9	4.78 d (7.6)
2	74.7	3.88 t (8.1)	74.1	3.80 t (7.8)
3	77.3	4.09	77.0	4.05
4	70.8	3.92	70.6	3.93 t (9.0)
5	77.8	3.91	77.2	3.72 m
6	62.0	4.15, 4.28 br d (11.7)	61.6	4.07, 4.26 br d (11.7)
23-*O*-				
Gal II-1	95.8	6.22 d (7.8)		
2	73.4	4.10		
3	77.6	4.18		
4	70.8	4.12		
5	78.8	3.92		
6	61.9	4.17, 4.29 br d (11.7)		

Overlapped proton NMR signals are reported without designated multiplicity.

loss of four hexosyl moieties. The fragment ion peak at *m/z* 485 corresponded to the aglycon.

The ^{1}H NMR spectrum of **1,** in pyridine-d_5, showed signals of an aglycon part and an oligosaccharidic part characteristic of a saponin structure. For the aglycon moiety, the ^{1}H NMR spectrum displayed signals for six angular methyl groups as singlets at δ_H 0.76, 0.78, 0.81, 0.92, 1.03, and 1.45, one olefinic proton at δ_H 5.31 (br t, J = 2.9 Hz, H-12), and one oxygen-bearing methine proton at δ_H 4.51 (H-3). In the ^{13}C NMR spectrum, the chemical shifts at δ_C 74.8 (C-3), δ_C 177.8 (C-23), and δ_C 176.2 (C-28) suggested one free hydroxyl group at C-3 and two ester functions at C-23 and C-28, respectively. The structure of the aglycon of **1** was thus recognized to be the triterpene gypsogenic acid using the correlations observed mainly in the 2D NMR spectra (NOESY, HSQC, and HMBC) (Table 1) and was in full agreement with literature data.[3,4] For the structure of the oligosaccharidic chain of **1**, the ^{1}H NMR spectrum showed four anomeric signals at δ_H 6.22 (d, J = 7.8 Hz), 6.07 (d, J = 8.3 Hz), 5.23 (d, J = 7.3 Hz), and 4.84 (d, J = 7.6 Hz). In the HSQC spectrum, these four anomeric protons correlated with four anomeric carbon signals at δ_C 95.8, 94.5, 104.7 and 104.5, respectively. The ring protons of the monosaccharide residues were assigned starting from the anomeric proton by means of the ^{1}H-^{1}H-COSY, TOCSY, HSQC, and HMBC experiments (Table 2), and the sequence of the oligosaccharide chains was obtained from the HMBC and NOESY experiments. Units of two β-galactopyranosyl (Gal I and II) and two β-glucopyranosyl (Glc I and II) were identified. The relatively large $^{3}J_{H-1,H-2}$ values of Gal I and II (8.3 and 7.8 Hz, respectively) and Glc I and II (7.3 and 7.6 Hz, respectively) indicated a β-anomeric orientation for these four sugars.[6] The D configuration of Gal I and II, and Glc I and II was determined by GC analysis.[7] A correlation observed in a HMBC spectrum between an anomeric signal at δ_H 6.22 (d, J = 7.8 Hz, Gal II-1) and δ_C 177.8 (C-23) revealed an ester linkage between C-23 position and Gal II. Moreover, a correlation between another anomeric signal at δ_H 6.07 (d, J = 8.3 Hz, Gal I-1) and δ_C 176.2 (C-28) showed a second ester linkage between C-28 position and Gal I. The ^{1}H and ^{13}C NMR data of Gal I obtained from 2D NMR data (Table 2) suggested a 1, 3, 6 trisubstituted galactopyranosyl moiety. The sequence of the 28-*O*-oligosaccharidic moiety was assigned by analysis of the HMBC and NOESY spectra. The HMBC correlation between δ_H 5.23 (d, J = 7.3 Hz, Glc I-1) and δ_C 87.0 (Gal I-3) indicated that Glc I was linked to C-3 of Gal I, which was confirmed by a NOESY cross-peak between δ_H 5.23 (d, J = 7.3 Hz, Glc I-1) and δ_H 4.23 (dd, J = 8.1, 3.1 Hz, Gal I-3). The NOESY cross-peak between δ_H 4.84 (d, J = 7.6 Hz, Glc II-1) and δ_H 4.19 (Gal I-6), and the deshielded signal of Gal I-6 at δ_C 68.9/δ_H 4.19, 4.53 (dd,

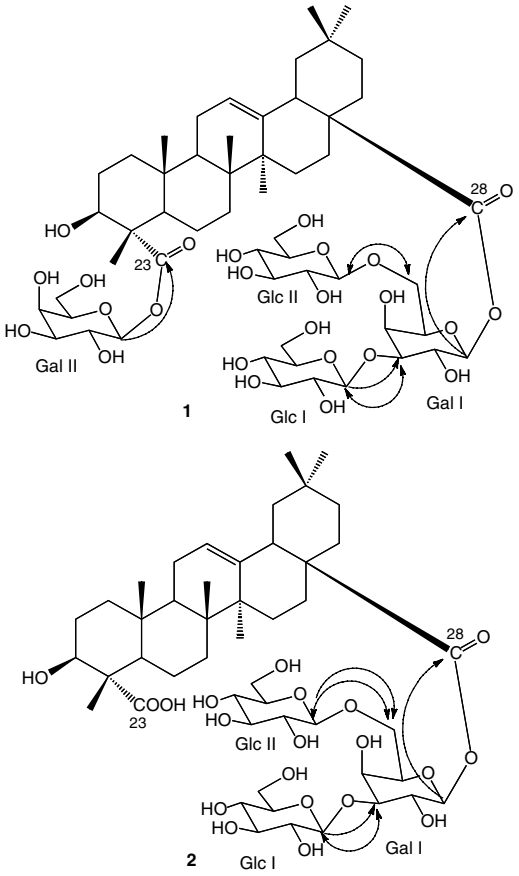

Figure 1. Important HMBC ( ) and NOESY ( ) correlations for **1** and **2**.

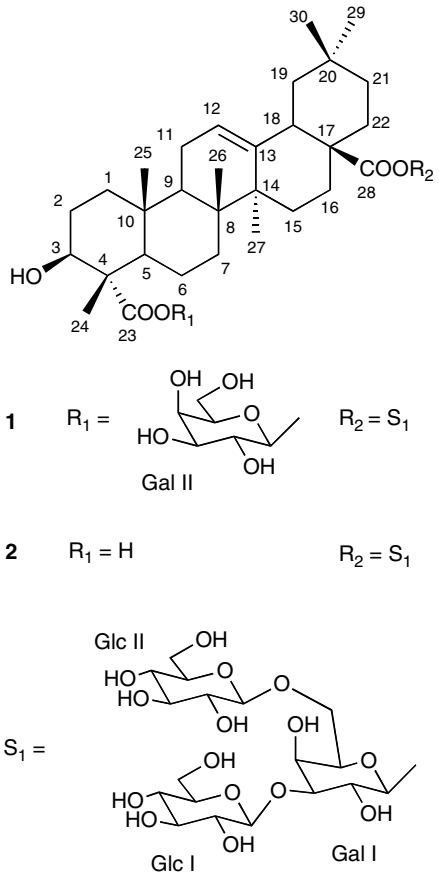

Figure 2. Structures of **1** and **2**.

$J = 11.2, 4.0$ Hz), indicated that Glc II was linked to Gal I by a (1→6) linkage (Fig. 1).

Based on the above results, the structure of **1** was concluded to be 23-*O*-β-D-galactopyranosylgypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-galactopyranoside (Fig. 2).

Compound **2**, a white amorphous powder, exhibited in high-resolution electrospray ionization mass spectrometry (HR-ESIMS) (positive-ion mode) a pseudo-molecular ion peak at $m/z = 995.4824$ $[M + Na]^+$ (calculated for 995.4828), consistent with a molecular formula of $C_{48}H_{76}O_{20}Na$. Its FABMS (negative-ion mode) showed a quasi-molecular ion peak at $m/z = 971$ $[M - H]^-$, indicating a molecular weight of 972, 162 mass units lower than that of **1**. All the ^1H and ^{13}C NMR signals corresponding to the aglycon part of **2** (Table 1) were in accordance with signals of gypsogenic acid with a free carboxylic function at C-23 at δ_C 183.8.[4] For the position of the oligosaccharidic chain, a correlation in the HMBC spectrum between δ_H 6.01 (d, $J = 8.3$ Hz, Gal I-1) and δ_C 176.6 (Agly C-28) proved that the Gal I residue was linked to the carboxyl group of the aglycon at C-28 by an ester linkage. Moreover, as in compound **1**, ^1H and ^{13}C NMR signals of a 1, 3, 6 trisubstituted galactopyranosyl moiety remained. The substitution of this sugar by two glucopyranosyl moieties was confirmed by HMBC correlations between δ_H 5.18 (d, $J = 7.1$ Hz, Glc I-1) and δ_C 86.4 (Gal I-3), and between δ_H 4.78 (d, $J = 7.6$ Hz, Glc II-1) and δ_C 68.1 (Gal I-6), together with NOESY cross-peaks between δ_H 5.18 (d, $J = 7.1$ Hz, Glc I-1) and δ_H 4.19 (dd, $J = 9.0$, 3.3 Hz, Gal I-3), and between δ_H 4.78 (d, $J = 7.6$ Hz, Glc II-1) and δ_H 4.10 (Gal I-6) (Fig. 1). The absolute configuration of Glc and Gal was determined to be D as described above. On the basis of the above results, the structure of compound **2** was established as gypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-galactopyranoside (Fig. 2).

In a previous publication of our laboratory, saponins isolated from *Acanthophyllum squarrosum* (Caryophyllaceae) were tested for their concentration-dependent immunomodulatory effect in an *in vitro* lymphocyte proliferation assay.[1] Only one gypsogenin glycoside displayed immunostimulant activity at low concentrations and a cytotoxic activity at higher concentrations. That is why three saponins were selected, compound **1**, glanduloside C, and SAPO50 (Fig. 3), and tested for their cytotoxicity against two human colon cancer cell lines (HT-29 and HCT 116) by a colorimetric [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay.[8] The choice was based on their structure: the aglycon is gypsogenic acid for **1**, and gypsogenin for glanduloside C and SAPO50. Moreover, the hydroxyl function at C-3 of the aglycon is free in the case of **1**, and substituted by a branched oligosaccharidic chain in the case of SAPO50 and glanduloside C. The latter two structures are very close, and their IC_{50} are comparable with values at 6.5 (HCT 116) and 12.0 µM (HT-29) for SAPO50, and 4.8 (HCT 116) and 11.9 µM (HT-29) for glanduloside C. These values are better than those of compound **1** ($IC_{50} = 15.0$ and 17.1 µM for HCT 116 and HT-29, respectively), but showed a moderate cytotoxicity when compared with paclitaxel used as a positive control (1.1 and 3.6 nM for HCT 116 and HT-29, respectively). These results suggested the importance of the formyl group at the C-23

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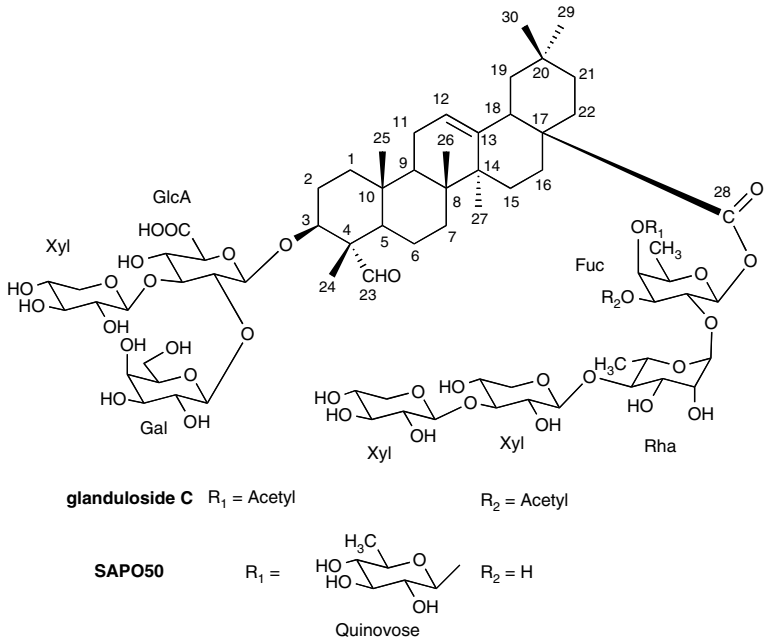

Figure 3. Structures of glanduloside C and SAPO50.

position of gypsogenin and/or the substitution of the C-3 position, for the cytotoxic activity.

Experimental

General

Optical rotations values were recorded on an AA-OR automatic polarimeter. HR-ESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer. FABMS were conducted in the negative-ion mode on a JEOL SX-102 mass spectrometer. Medium-pressure liquid chromatography (MPLC) was performed on a Gilson pump M 305, with Büchi glass column (460 × 15 mm and 250 × 15 mm), a Büchi precolumn (110 × 15 mm), using silica gel 60 (Merck, 15–40 μm), and reversed-phase RP-18 silica gel (Silicycle, 75–200 μm). Vacuum liquid chromatography (VLC) was carried out using reversed-phase RP-18 silica gel (Silicycle, 75–200 μm). Flash chromatography was performed using a CombiFlash RETRIEVE instrument (column silica gel 15–40 μm, 40 g, Teledyne Isco). TLC (Silicycle) and HPTLC (Merck) were achieved on precoated silica gel plates $60F_{254}$ and the solvent system $CHCl_3$–MeOH–AcOH–H_2O (15 : 8 : 3 : 2) were used. The spray reagent used for saponins was Komarowsky reagent, which is a mixture (5 : 1) of *p*-hydroxybenzaldehyde (2% in MeOH) and ethanolic H_2SO_4 (50%).

NMR spectroscopy

The NMR spectra were recorded on a Varian UNITY Inova 600 spectrometer equipped with 5-mm probes. Samples were dissolved in C_5D_5N-D_2O (8 : 1) and transferred into 5-mm NMR tubes (Shigemi). The ^1H and ^{13}C NMR spectra (at 600 and 150 MHz, respectively) were measured at 303 K. Chemical shifts are given on the δ scale and referenced to the residual solvent signals (δ_H = 7.19, δ_C = 123.5). Coupling constants (*J*) are in Hz. For two-dimensional experiments, Varian software using pulse field gradient was applied. The pulse conditions in C_5D_5N were as follows: for the ^1H NMR spectrum, observation frequency (OF) = 599.88 MHz, acquisition time (AQ) = 4.202 s, relaxation delay (RD) = 5.0 s, 90 pulse width = 10.0 ms, spectral width (SW) = 7798.8 Hz, Fourier transform (FT) size = 65536; for the ^{13}C NMR spectrum, OF = 150.854 MHz, AQ = 0.453 s, RD = 1.547 s, 90 pulse = 15.8 ms, SW = 36182.7 Hz, line broadening (LB) = 1.0 Hz, FT size = 65536; for the COSY spectrum, AQ = 0.131, F_2 = 2048, F_1 = 256, RD = 0.369, SW = 7798.8 Hz; for the NOESY spectrum, AQ = 0.131, F_2 = 2048, F_1 = 256, RD = 0.369, SW = 7798.8 Hz, mixing time = 500 ms; for the TOCSY spectrum, AQ = 0.131, F_2 = 2048, F_1 = 256, RD = 0.369, SW = 7798.8 Hz, mixing time = 60 ms; for the HSQC spectrum, AQ = 0.131, RD = 0.369, F_1 = 36182.7 Hz, F_1 = 7798.8 Hz; and for the HMBC spectrum, AQ = 0.131, SF = 599.880 MHz, RD = 0.369, DE = 50 ms, F_1 = 36182.7 Hz, F_1 = 7798.8 Hz.

Plant material

The roots of *A. sordidum* Bunge ex Boiss, *A. elatius* Bunge and *A. lilacinum* Schischk were collected from the northeastern part of Iran, Khorasan province, and identified by Mr Joharchi. Voucher specimens under the reference nos 220309, 230309, and 240309, respectively, were deposited in the Herbarium of the Laboratory of the school of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

Extraction and isolation

The dried, powered roots of *A. sordidum* (600 g) were defatted with 3 l of petroleum ether for 24 h. The air-dried plant was extracted with 5 l of MeOH for 48 h yielding, after evaporation, a brown residue (40 g). The MeOH extract was dissolved in 400 ml of H_2O and partitioned with H_2O-saturated *n*-BuOH (3 × 300 ml) to give, after evaporation, the *n*-BuOH fraction (15 g). The latter was solubilized in MeOH (10 ml) and precipitated in Et_2O (3 × 250 ml) yielding 9 g of crude saponin mixture (CSM). An aliquot of 600 mg of CSM was then submitted to Flash chromatography on silica gel

($CHCl_3$–MeOH–H_2O, 8 : 5 : 1), followed by successive MPLC on RP-18 silica gel (MeOH–H_2O linear gradient 40 : 60→70 : 30) to give compound **1** (3.2 mg). Final purification was carried out by MPLC on silica gel ($CHCl_3$–MeOH–H_2O, 8 : 5 : 1) yielding glanduloside C (5.6 mg) and SAPO50 (3.7 mg).

For *A. elatius* and *A. lilacinum*, a CSM was obtained according to the method described above. For *A. elatius*, 2 g of the CSM was submitted to VLC on RP-18 silica gel (H_2O containing increasing amounts of MeOH) yielding seven fractions. The fractions 3, 4, and 5, rich in saponins, eluted with MeOH–H_2O 3 : 7, 5 : 5, and 7 : 3, were fractionated by successive MPLC on silica gel ($CHCl_3$–MeOH–H_2O, 8 : 5 : 1 and 65 : 40 : 8), giving compound **2** (7.0 mg), glanduloside C (10.0 mg), and SAPO50 (10.0 mg). For *A. lilacinum*, the same protocol was applied, starting from 2 g of CSM. Further separations were performed by successive MPLC on silica gel using only $CHCl_3$–MeOH–H_2O (8 : 5 : 1), to give compounds **1** and **2**, together with glanduloside B (9.0 mg), glanduloside C (6.0 mg), and SAPO50 (7.9 mg).

Compound 1: White amorphous powder. $[\alpha]_D{}^{25} -7.1$ ($c = 0.10$, MeOH). For ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2. HR-ESIMS $m/z = 1157.5350$ $[M + Na]^+$ (calculated 1157.5356**).** FABMS m/z 1133 $[M - H]^-$, 971 $[(M - H) - 162]^-$, 809 $[(M - H) - 162 - 162]^-$, 647 $[(M - H) - 162 - 162 - 162]^-$, 485 $[(M - H) - 162 - 162 - 162 - 162]^-$.

Compound 2: White amorphous powder. $[\alpha]_D{}^{25} - 15.6$ ($c = 0.05$, MeOH). For ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2. HR-ESIMS m/z 995.4824 $[M + Na]^+$ (calculated 995.4828). FABMS m/z 971 $[M - H]^-$.

Acid hydrolysis

Each compound (3 mg) was hydrolyzed with 2N aq. CF_3COOH (5 ml) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 × 5 ml), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral, and then analyzed by TLC over silica gel ($CHCl_3$–MeOH–H_2O, 8 : 5 : 1) by comparison with authentic samples. The D configuration of glucose and galactose was determined by GC analysis using a method described earlier.[7]

MTT cytotoxicity assay

The bioassay was carried out according to the method described in ref. [8] with two human colorectal cancer cell lines HCT 116 and HT-29. Paclitaxel was used as a positive control, and exhibited IC_{50} values of 1.1 and 3.6 nM against HCT 116 and HT-29, respectively.

Acknowledgement

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References

[1] G. Gaidi, T. Miyamoto, A. Rustaiyan, V. Laurens, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2000**, *63*, 1497.
[2] G. Gaidi, T. Miyamoto, A. Rustaiyan, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2001**, *64*, 920.
[3] G. Gaidi, T. Miyamoto, M. Ramezani, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2004**, *67*, 1114.
[4] M. Haddad, T. Miyamoto, M. Ramezani, M.-A. Lacaille-Dubois, *Helv. Chim. Acta.* **2004**, *87*, 73.
[5] C. Delay, J. A. Gavin, A. Aumelas, P.-A. Bonnet, C. Roumestand, *Carbohydr. Res.* **1997**, *302*, 67.
[6] Y. Mimaki, A. Yokosuka, M. Hamanaka, C. Sakuma, T. Yamori, Y. Sashida, *J. Nat. Prod.* **2004**, *67*, 1511.
[7] M. Haddad, T. Miyamoto, V. Laurens, M.-A. Lacaille-Dubois, *J. Nat. Prod.* **2003**, *66*, 372.
[8] J. Carmichael, W. G. DeGraff, A. F. Gazdar, J. D. Minna, J. B. Mitchell, *Cancer Res.* **1987**, *47*, 936.