Antiproliferative Triterpenoid Saponins of Dodonaea viscosa from the Madagascar Dry Forest

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Antiproliferative Triterpenoid Saponins of *Dodonaea viscosa* from the Madagascar Dry Forest[1]

Shugeng Cao[†,‡], **Peggy Brodie**[†], **Martin Callmander**[§], **Richard Randrianaivo**[§], **Jeremi Razafitsalama**[§], **Etienne Rakotobe**[⊥], **Vincent E. Rasamison**[⊥], **Karen TenDyke**[‖], **Yongchun Shen**[‖], **Edward M. Suh**[‖], and **David G. I. Kingston**[*,†]

Department of Chemistry, M/C 0212, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, Missouri Botanical Garden, P. O. Box 299, St. Louis, Missouri 63166-029 and B.P 3391, Antananarivo, Madagascar, Centre National d'Application des Recherches Pharmaceutiques, B. P. 702, Antananarivo 101, Madagascar, and Eisai Research Institute, 4, Corporate Drive, Andover, Massachusetts 01810

Abstract

Bioassay-guided fractionation of an EtOH extract obtained from the roots of the Madagascan plant *Dodonaea viscosa* led to the isolation of two new antiproliferative oleanane-type triterpenoid saponins, dodoneasides A and B (**1** and **2**). The structures of these two new compounds were elucidated using 1D and 2D NMR experiments and mass spectrometry. Compounds **1** and **2** showed antiproliferative activity against the A2780 human ovarian cancer cell line with IC_{50} values of 0.79 and 0.70 μM, respectively.

In our continuing search for bioactive molecules from the Madagascar rainforests as part of an International Cooperative Biodiversity Group (ICBG) program, we obtained an extract of the roots of *Dodonaea viscosa* (L.) Jacq. (Sapindaceae). This extract, designated MG 3397, showed reproducible cytotoxicity to the A2780 ovarian cancer cell line, with an IC_{50} value of 6.0 μg/mL. The extract was selected for bioassay-guided fractionation based on this activity. Previous work on *Dodonaea viscosa* revealed the presence of flavonoids, [2] fatty acids, [3] and cyanolipids. [4] Some *ent*-clerodane diterpenoids were obtained from *D. boroniaefolia*. [5] A southern Brazilian outbreak of acute hepatic insufficiency in which 14 dairy animals died after consumption of *Dodonaea viscosa* has been reported.[6] In this paper, we report the isolation, structure elucidation, and antiproliferative activity of two new triterpenoid saponins (**1** and **2**) obtained from the roots of *Dodonaea viscosa*.

Liquid-liquid partitioning of a portion of an EtOH extract of the roots of *Dodonaea viscosa* into hexane, CH_2Cl_2 and aqueous MeOH fractions indicated that the CH_2Cl_2 fraction (326.5 mg) was the most active fraction, with an IC_{50} value of 1.0 μg/mL. Purification of the CH_2Cl_2 fraction using a C_{18} open column, followed by preparative C_{18} HPLC, led to the isolation of antiproliferative compounds **1** and **2**.

[*]To whom correspondence should be addressed. Tel: (540) 231-6570. Fax: (540) 231-3255. dkingston@vt.edu.
[†]Virginia Polytechnic Institute and State University.
[‡]Current address: Department of Biological Chemistry and Molecular Pharmacology & Osher Research Center, Harvard Medical School, Harvard University, 240 Longwood Av., Boston MA 02115
[§]Missouri Botanical Garden
[⊥]Centre National d'Application des Recherches Pharmaceutiques.
[‖]Eisai Research Institute

Supporting Information Available: Spectroscopic data, consisting of ^{1}H NMR spectra of compounds **1** and **2**, are available as Supporting Information. This material is available free of charge via the internet at http://pubs.acs.org

Compound **1** was obtained as white amorphous solid. HRFABMS (positive-ion mode) analysis suggested that the molecular formula **1** was $C_{57}H_{88}O_{23}$. Its 1D NMR spectra revealed seven tertiary methyl groups between δ_H 0.87 and 1.42 and a double bond with typical ^{13}C NMR resonances at δ_C 127.0 and 143.6, indicating an olean-12-ene triterpene derivative since H_3-27 (δ_H 1.42, s) showed a ^3J HMBC correlation to C-13 (δ_C 143.6). The HMBC spectrum (Figure 1) also exhibited correlations between H_3-23/H_3-24 (δ_H 1.09, s/δ_H 0.87, s) and C-3 (δ_C 92.3), H_3-27 (δ_H 1.42, s) and C-15 (δ_C 68.5), H_2-28 (δ_H 3.00 & 3.20, d, J = 9.6 Hz) and C-16/C-22 (δ_C 74.5/δ_C 73.9), and H_3-29/H_3-30 (δ_H 0.89, s/δ_H 1.06, s) and C-21 (δ_C 79.7), indicating that the aglycone was 3,15,16,21,22,28-hexaoxygenated olean-12-ene. Signals for three anomeric protons [δ_H 5.24 (1H, d, J = 2.2 Hz, H-1‴), 4.72 (1H, d, J = 7.7 Hz, H-1″), 4.55 (1H, d, J = 8.0 Hz, H-1′)] were observed in the ^1H NMR spectrum. The ^1H and ^{13}C NMR data of the sugar moieties were completely assigned on the basis of the ^1H-^1H COSY, TOCSY, ROESY, HSQC, HSQC-TOCSY and HMBC spectra and by a comparison of their NMR data with those of aesculioside IIe.[7] These three sugar moieties were identified as *β*-glucuronopyranosyl [GlcA-1′–6′ (δ_H/δ_C): 4.55, d, J = 8.0 Hz/105.5; 3.78, dd, J = 8.0, 8.2 Hz/78.1; 3.69, dd, J = 8.2, 8.2 Hz/86.1; 3.58, dd, J = 8.2 Hz/72.4; 3.61, d, J = 8.2 Hz/76.8; 174 (C-6′)], *β*-glucopyranosyl [Glc-1″–6″ (δ_H/δ_C): 4.72, d, J = 7.7 Hz/103.7; 3.19, dd, J = 7.7, 8.8 Hz/76.0; 3.36, dd, J = 8.8, 8.8 Hz/77.9; 3.09, dd, J = 8.8, 8.8 Hz/72.0; 3.29, m/77.9; 3.25 & 3.80, m/63.8], and *α*-arabinofuranosyl [Ara-1‴–5‴ (δ_H/δ_C): 5.24, d, J = 2.2 Hz/110.7; 4.14, m/83.4; 3.86, m/78.6; 4.10, m/85.3; 3.64 & 3.76, m/62.8]. The relative stereochemistry of the arabinofuranosyl moiety was assigned based on a comparison of its ^{13}C NMR chemical shifts with those of the arabinofuranose ring of aesculioside IIe.[7,8] H-1′, H-1″ and H-1‴ showed ^3J HMBC correlations to C-3, C-2′ (δ_C 78.1) and C-3′ (δ_C 86.1), respectively, which established the connectivities between these sugar moieties and the aglycone. The ^1H NMR spectrum also had signals for two olefinic protons at δ_H 6.06 (2H, qq, J = 7,3, 1.4 Hz, H-A3 and H-A3′) and four olefinic methyl groups [δ_H 1.82 (3H, q, J = 1.4 Hz, H_3-A5′), 1.84 (3H, q, J = 1.4 Hz, H_3-A5), 1.91 (3H, dq, J = 7.3, 1.4 Hz, H_3-A4′), 1.91 (3H, dq, J = 7.3, 1.4 Hz, H_3-A4)]; these signals were attributed to two angeloyl moieties. The locations of these two angeloyl moieties were determined on the basis of HMBC correlations between C-A1 (δ_C 169.4) and H-A3/H_3-A4/H-21 (δ_H 5.94, d, J = 10.2 Hz), and C-A1′ (δ_C 169.2) and H-A3′/H_3-A4′/H-22 (δ_H 5.61, d, J = 10.2 Hz). The double bonds in the angeloyl moieties were determined as *E* by the ROESY correlations between H-A3 and H_3-A5, and H-A3′ and H_3-A5′, and also by a comparison of their NMR data with those of floratheasaponin B.[9] A ROESY correlation between H-3 and H-5 indicated the *α*-orientation of H-3. A 10.2 Hz coupling constant between H-21 and H-22 was compatible with a 21–22 diaxial orientation of the hydrogens. The *β*-axial orientation of H-15, and *β*-equatorial for H-16, were deduced by the ROESY correlations between H-16 and H-28b, and H-15 and H_3-26 (Figure 2). The above results were confirmed by comparing the ^{13}C NMR data of the aglycone of **1** with those of the floratheasaponin B (**3**) (Table 1).[8] The identity of these signals confirmed the structure of compound **1** as shown.[10]

Compound **2** was also obtained as a white amorphous solid. Comparison of the NMR data (Table 1) of **1** and **2** in CD_3OD indicated that there was no substituent at the C-15 position of **2** and that the angeloyl group at the C-21 position of **1** was replaced by an epoxyangeloyl group in **2**. The NMR spectra indicated that the other parts of **2** were identical to those of **1**. The NMR data of the aglycone and the two substitutents at both the C-21 and C-22 positions of **2** were compatible with those of 22-angeloyl-21-epoxyangeloylbarringtogenol.[11] Therefore, the structure of **2** was determined as shown.

Compounds **1** and **2** are oleanane-type triterpenoid saponins with a double bond at the 12-position, an OH group at the 16-position, and substituents at the 3-, 21-, and 28-positions, like gummiferaosides A-C. [12] The triterpenoid sapogenin portion of **1** and **2** is similar to 3β,15α, 21β,22α,28-pentahydroxy-16α-angeloyloxy-12-oleanene isolated from *Dodonaea viscosa*.[13]

Compounds **1** and **2** were evaluated for antiproliferative activity against the A2780 human ovarian cancer cell line, and compound **1** was also evaluated in the breast cancer BT-549, prostate cancer DU 145, NSCLC NCI-H460, and colon cancer HCC-2998 cell lines (Table 2). The activities against the A2780 cell line were similar to those shown by gummiferaosides AC,[12] which suggests that the structural features noted are important for their activity. This finding is similar to that of a recent study that showed that acylation with diangeloyl groups at the C-21 and C-22 positions in triterpenoid saponins is essential for cytotoxicity.[14]

Experimental Section

General Experimental Procedures

Optical rotations were recorded on a JASCO P-2000 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 for ^{1}H, ^{13}C, HMQC, and HMBC and an INOVA 400 spectrometer for TOCSY, COSY, ROESY, and HSQC-TOCSY. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectra were obtained on a JEOL JMS-HX-110 instrument, in the positive-ion mode. HPLC was performed on a Shimadzu LC-10AT instrument with a preparative C_{18} Varian Dynamax column (8 μm, 250 × 21.4 mm) and a semi-preparative C_{18} Varian Dynamax column (5 μm, 250 × 10 mm).

Antiproliferative Bioassays

Cytotoxicity measurements were performed at Virginia Polytechnic Institute and State University against the A2780 ovarian cancer cell line, as described previously.[15] The A2780 cell line is a drug-sensitive human ovarian cancer cell line.[16]

Plant Material

Dodonaea viscosa Jacq. (Sapindaceae) was collected on the eastern side of the Montagne des Français in littoral forest on sand at Ivovona, Antsiranana Province, Madagascar, elevation: ca. 5 m, co-ordinates: 12.21.40 S, 49.29.42 E, on July 19, 2005. Its assigned collection number is Randrianaivo et al. 1208. The collection was made from a shrub on the seashore. The genus *Dodonaea* Mill. consists of ca. 50 species, 2 of which occur in Madagascar. One is endemic (*Dodonaea madagascariensis* Radlk.) and the second one *Dodonaea viscosa* has a large distribution throughout the tropics near the sea. Voucher specimens have been deposited at herbaria of the Centre National d'Application des Recherches Pharmaceutiques, Madagascar (CNARP); the Parc Botanique et Zoologique de Tsimbazaza, Madagascar (TAN); the Missouri Botanical Garden, St. Louis, Missouri (MO); and the Muséum National d'Histoires Naturelles, Paris, France (P).

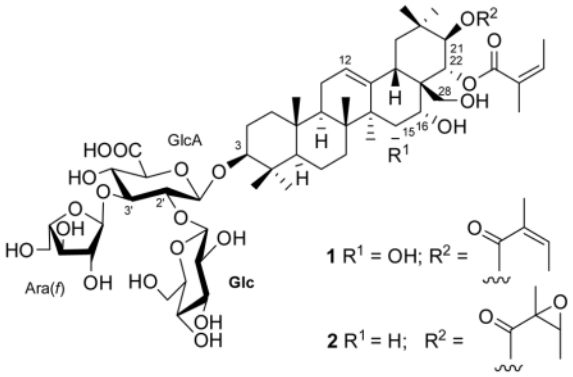
Extraction and Isolation

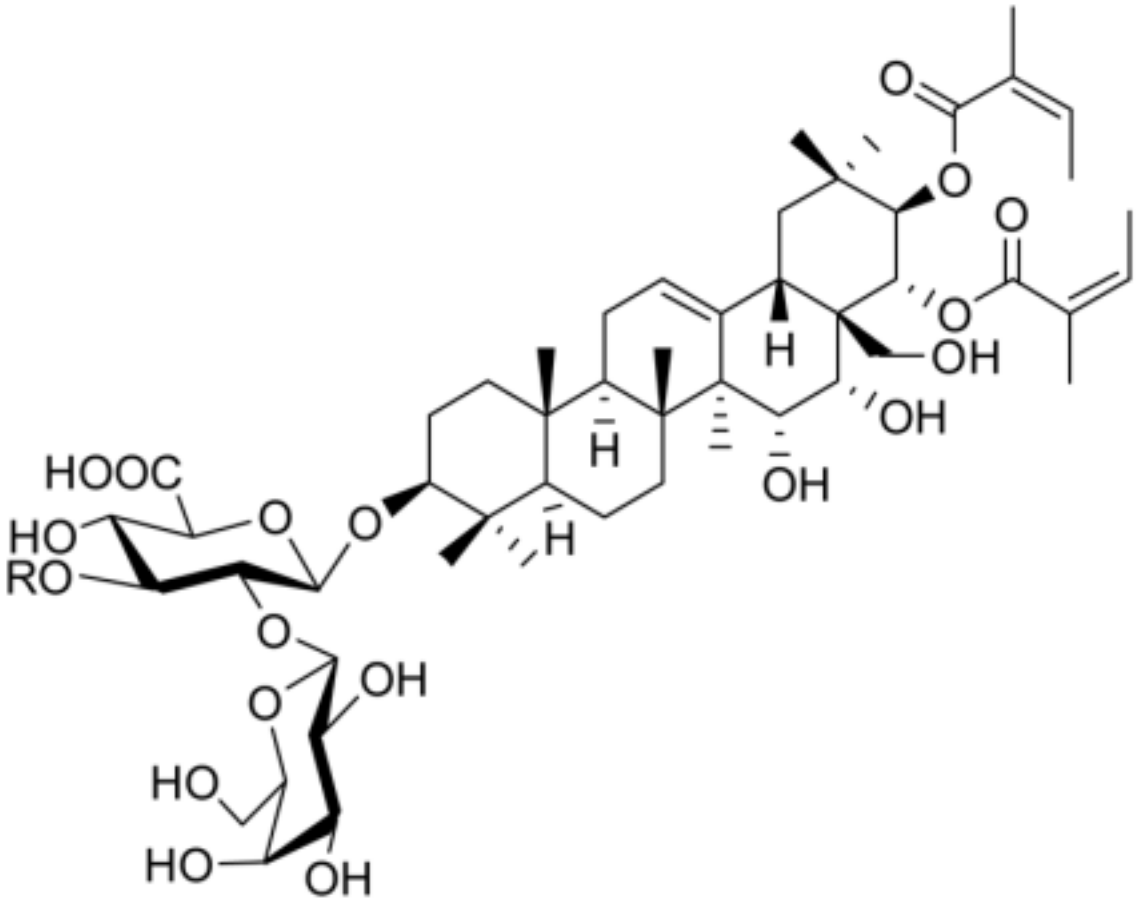
Dried roots of *Dodonaea viscosa* (253 g) were ground in a hammer mill, then extracted with EtOH by percolation for 24 h at rt to give the crude extract MG 3397 (8.9 g), of which 2.6 g was made available to Virginia Polytechnic Institute and State University (VPISU). Extract MG 3397 (2 g, IC_{50} 6.0 μg/mL) was suspended in aqueous MeOH (MeOH-H_2O, 9:1, 100 mL) and extracted with hexane (3 × 100 mL portions). The aqueous layer was then diluted to 70% MeOH with H_2O and extracted with CH_2Cl_2 (3 × 100 mL portions). The CH_2Cl_2 extract (326.5 mg) was active with an IC_{50} 1.0 μg/mL, while the hexane extract (153.5 mg) was inactive and the aqueous MeOH extract (1.5 g) was much less active than the CH_2Cl_2 extract. The CH_2Cl_2 extract was chromatographed on an open C_{18} column (50 × 10 mm) using H_2O-MeOH (80:20 to 20:80, then 0:100) to yield the three fractions A [40.2 mg (polar, inactive)], B [198.7 mg, IC_{50}: 0.5 μg/mL], and C [59.7 mg, IC_{50}: 4.3 μg/mL]. Fraction B furnished 19 subfractions

after HPLC separation on a C_{18} column (0-25-30-60-70 min:50-50-70-70-100% MeOH/H_2O, 10 mL/min). Subfraction 18 yielded compound **1** (t_R 66 min, 5.0 mg). Compound **2** (t_R 21 min, 0.9 mg) was obtained by HPLC of subfraction 16 using C_{18} HPLC (0-30-40 min:70-70-100% MeOH/H_2O, 2 mL/min).

Dodonaeaside A (1)—white solid; $[\alpha]^{25}_D$ -44.4 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.3) nm; IR (film) ν_{max} 3389, 1727, 1152, 1033 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table 1; HRFABMS *m/z* 1163.5595 (calcd for $C_{57}H_{88}O_{23}Na$, 1163.5614).

Dodonaeaside B (2)—white solid; $[\alpha]^{25}_D$ -80 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.1) nm; IR (film) ν_{max} 3390, 1698, 1076, 1033 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see Table 1; HRFABMS *m/z* 1163.5580 (calcd for $C_{57}H_{88}O_{23}Na$, 1163.5614).





3 R = β-D-xylopyranosyl(1->2)-α-L-arabinopyranosyl

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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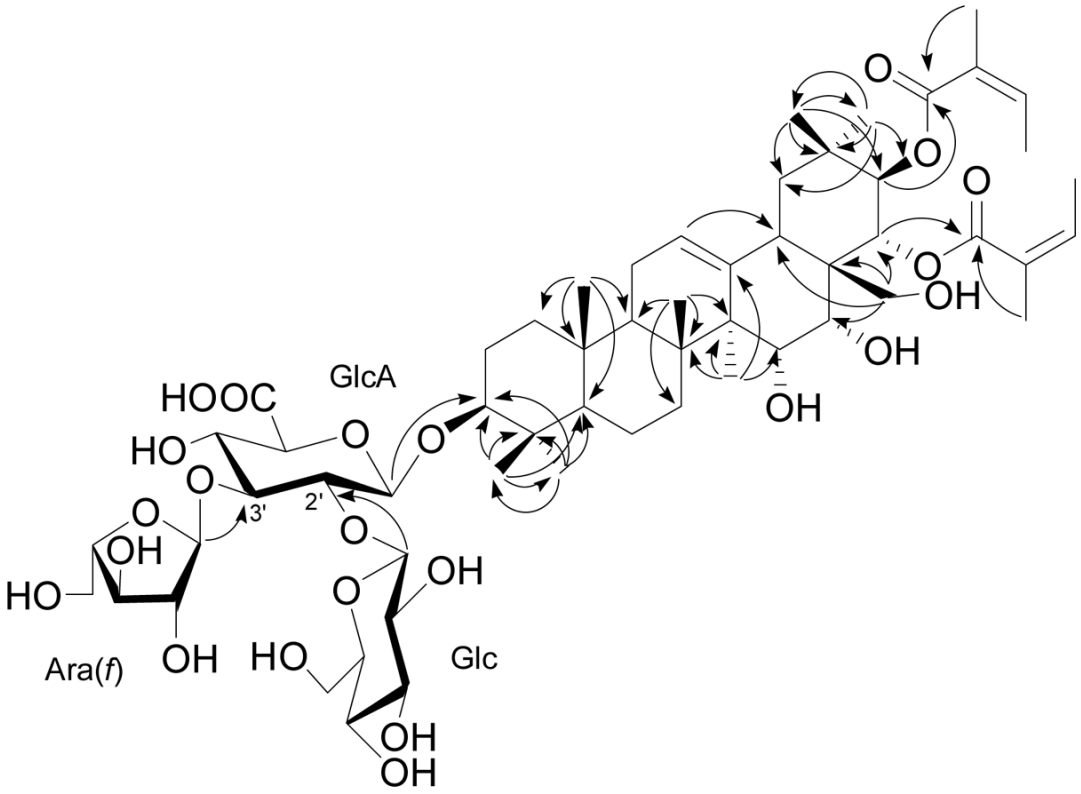

Figure 1.
Key HMBC correlations for compound **1**

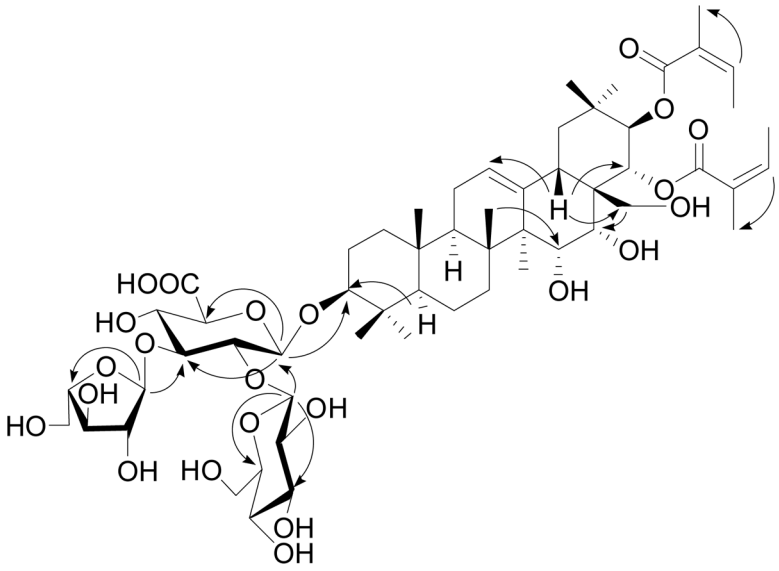

Figure 2.
Key ROESY correlations for compound **1**

Table 1

^{1}H and ^{13}C NMR Data of Compounds **1** - **3**[a,b,c]

position	1		2		3
	^{1}H	^{13}C	^{1}H	^{13}C	^{13}C
1	1.05; 1.70	40.0	1.10; 1.65	39.9	39.1
2	1.77; 1.85	27.1	1.77; 1.85	27.0	26.6
3	3.24	92.3	3.21	92.3	89.6
4		40.4		40.4	39.6
5	0.80 br d (11.0)	56.6	0.79 br d (11.0)	56.9	55.7
6	1.45; 1.55	19.5	1.45; 1.55	19.3	18.9
7	1.75	37.2	1.75	33.9	36.7
8		42.3		40.8	41.5
9	1.62	48.4	1.62	47.7	47.2
10		37.9		37.7	37.0
11	1.95	24.8	1.95	24.7	24.0
12	5.47 br t (3.5)	127.0	5.39 br t (3.5)	125.3	125.5
13		143.6		142.9	143.7
14		48.1		41.0	48.5
15	3.80	68.5	1.65	34.9	73.1
16	3.84	74.5	3.99	69.7	67.5
17		48.5		49.0	47.8
18	2.63	41.6	2.85	42.3	40.9
19	1.20; 2.54	47.5	1.20; 2.65	47.9	46.9
20		36.8		37.1	36.4
21	5.94 d (10.2)	79.7	6.01 d (10.2)	81.9	78.7
22	5.61 d (10.2)	73.9	5.60 d (10.2)	73.7	73.3
23	1.09 s	28.2	1.07 s	28.3	28.1
24	0.87 s	16.9	0.87 s	16.8	16.9
25	0.99 s	16.2	0.98 s	16.3	15.8
26	1.02 s	17.9	0.94 s	17.3	17.6
27	1.42 s	21.0	1.47 s	27.8	21.2
28	3.00 d (9.6)	63.6	2.92 d (9.6)	64.4	63.1

position	1		2		3	
	^{1}H	^{13}C	^{1}H	^{13}C	^{13}C
29	0.89 s	29.6	0.90 s	29.8	29.5
30	1.06 s	20.2	1.11 s	20.2	20.2
21-angeloyl					
1		169.4		171.34	167.6
2		129.3		61.2	128.7
3	6.06 qq (7,3, 1.4)	139.2	3.05 qq (5.5, 1.4)	61.1	138.4
4	1.91 dq (7.3, 1.4)	16.0	1.15 dq (5.5, 1.4)	13.9	16.0
5	1.84 q (1.4)	20.9	1.50 br s	19.8	21.0
22-angeloyl					
1		169.2		169.1	168.2
2		129.3		128.9	129.1
3	6.06 qq (7,3, 1.4)	139.1	6.18 qq (7,3, 1.4)	141.4	136.6
4	1.91 dq (7.3, 1.4)	15.9	2.02 dq (7.3, 1.4)	16.2	15.7
5	1.82 q (1.4)	20.8	1.86 q (1.4)	21.0	20.6
3-*β*-glcA					
1′	4.55 d (8.0)	105.5	4.48 d (8.0)	105.4	105.6
2′	3.78 dd (8.0, 8.2)	78.1	3.78 dd (8.0, 8.2)	78.1	79.1
3′	3.69 dd (8.2, 8.2)	86.1	3.69 dd (8.2, 8.2)	86.2	84.0
4′	3.58 dd (8.2, 8.2)	72.4	3.58 dd (8.2, 8.2)	72.4	71.1
5′	3.61 d (8.2)	76.8	3.61 d (8.2)	76.8	77.2
6′		172.0		172.0	172.0
2′-*β*-Glc					
1″	4.72 d (7.7)	103.7	4.72 d (7.7)	103.7	
2″	3.19 dd (7.7, 8.8)	76.0	3.19 dd (7.7, 8.8)	75.9	
3″	3.36 dd (8.8, 8.8)	77.9	3.36 dd (8.8, 8.8)	77.8	
4″	3.09 dd (8.8, 8.8)	72.0	3.09 dd (8.8, 8.8)	72.1	
5″	3.29 m	77.9	3.29 m	77.8	
6″	3.80 m	63.8	3.80 m	63.6	
3′-*α*-Ara(*f*)					
1‴	5.24 d (2.2)	110.7	5.27 br s	110.7	
2‴	4.14 m	83.4	4.14 m	83.3	

position	1		2		3
	^{1}H	^{13}C	^{1}H	^{13}C	^{13}C
3‴	3.86 m	78.6	3.86 m	78.7	
4‴	4.10 m	85.3	4.10 m	85.4	
5‴	3.64 m, 3.76 m	62.8	3.64 m, 3.76 m	62.8	

[a] δ (ppm) 500 MHz for ^{1}H and 125 MHz for ^{13}C; multiplicities; *J* values (Hz) in parentheses.

[b] The signals of the sugar carbons were assigned by HSQC-TOCSY and ^{13}C NMR.

[c] In CD_3OD.

Table 2

Antiproliferative activity of compounds **1** and **2**.

Cell line	Cancer type	IC_{50} (μM)	
		1	**2**
A2780	ovarian	0.79	0.70
BT-549	breast	>5	NT[a]
DU 145	prostate	>5	NT
NCI-H460	NSCLC	>5	NT
HCC-2998	colon	>5	NT

[a] NT = not tested