Triterpene Saponins from *Eryngium campestre*

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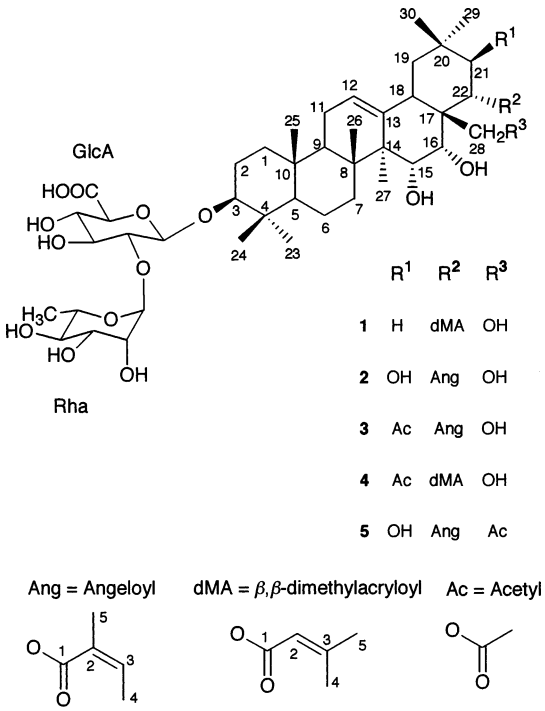
Five new triterpene saponins, 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-β,β-dimethylacryloyl-A1-barrigenol (**1**), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-angeloyl-R1-barrigenol (**2**), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-21-*O*-acetyl-22-*O*-angeloyl-R1-barrigenol (**3**), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-21-*O*-acetyl-22-*O*-β,β-dimethylacryloyl-R1-barrigenol (**4**), and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-angeloyl-28-*O*-acetyl-R1-barrigenol (**5**), were isolated from the roots of *Eryngium campestre*. Their structures were established mainly by 2D NMR techniques and mass spectrometry. Compounds **1**−**4** and 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucuronopyranosyl-22-*O*-β,β-dimethylacryloyl-A1-barrigenol, previously isolated from the same plant, showed a weak cytotoxicity when tested against HCT 116 and HT 29 human colon cancer cells.

Eryngium campestre L. is a well-known plant of the Apiaceae family and is used in Turkish folk medicine. Infusions of the aerial and root parts are used as an antitussive, diuretic, appetizer, stimulant, and aphrodisiac.[1] Only small amounts of saponins were isolated from the roots, mainly R1- and A1-barrigenol glycosides.[2,3] We describe in this paper the further phytochemical investigation of a *n*-BuOH-soluble fraction of the MeOH extract of the roots of *E. campestre*. Five new triterpene saponins (**1**−**5**) were isolated by successive chromatographic steps, and their structures were elucidated mainly by 400 MHz NMR analysis, including 1D and 2D NMR (^{1}H−^{1}H COSY, TOCSY, NOESY, HSQC, HMBC), and mass spectrometry.

Compound **1** exhibited in the HRESIMS (positive-ion mode) a pseudo-molecular ion peak at *m*/*z* 917.4922 $[M + Na]^+$ (calcd 917.4875), consistent with a molecular formula of $C_{47}H_{74}O_{16}Na$. Its FABMS (negative-ion mode) showed a quasi-molecular ion peak at *m*/*z* 893 $[M - H]^-$, indicating a molecular weight of 894. Another significant fragment ion peak was observed at *m*/*z* 747 $[(M - H) - 146]^-$, which revealed the elimination of one 6-desoxyhexosyl moiety.

For all the isolated compounds **1**−**5**, the oligosaccharidic chain linked to C-3 of the aglycon is the same and the differences between them were located at the aglycon. The ^{1}H NMR spectrum of **1** displayed signals for two anomeric protons at δ_H 4.76 (d, J = 7.2 Hz) and 5.83 (br s), which gave correlations, in the HSQC spectrum, with anomeric carbon signals at δ_C 106.2 and 101.4, respectively. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of COSY, TOCSY, HSQC, and HMBC spectroscopic experiments (Table 2). One β-D-glucuronopyranosyl (GlcA) unit and one α-L-rhamnopyranosyl (Rha) unit were identified. The relatively large $^3J_{H-1,H-2}$ values of the GlcA (7.2 Hz) moiety indicated a β anomeric proton for this group, and the multiplicity of the anomeric proton of the Rha unit as a broad singlet indicated an α-orientation.[4] The monosaccharides obtained by acid hydrolysis of the crude extract were identified as D-glucuronic acid and L-rhamnose by TLC and by measurement of their optical rotation after purification. Correlations observed in the HMBC spectrum between an anomeric signal at δ_H 4.76 (d, J = 7.2 Hz) (GlcA-1) and δ_C 89.1 (C-3), and in the NOESY spectrum between δ_H 4.76 (GlcA-1) and δ_H 3.18 (dd, J = 10.5, 5.5 Hz) (H-3), confirmed the substitution at C-3 of the aglycon by a 3-*O*-β-D-glucuronopyranosyl moiety. Moreover, a cross-peak in the NOESY spectrum between δ_H 4.49 (GlcA-2) and an anomeric signal at δ_H 5.83 (br s) (Rha-1) revealed the attachment of the Rha moiety to the GlcA unit by a (1→2) linkage. The structure of the oligosaccharidic chain of **1** and the other saponins **2**−**5** was thus determined as a 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranoside unit.



The ^{1}H NMR spectrum of the aglycon part of **1** showed signals for seven angular methyl groups as singlets, one olefinic proton at

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Table 1. ^{13}C NMR and ^{1}H NMR Data of the Aglycons of Compounds **1**−**5** in Pyridine-d_5 (δ ppm)[a]

		1		**2**		**3**		**4**		**5**	
no.	mult.[a]	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	CH_2	38.5	0.70, 1.27	38.5	0.70, 1.27	38.8	0.96, [b]	38.8	0.96, [b]	39.0	0.92, 1.52
2	CH	25.8	1.70, 2.08	25.8	1.70, 2.08	26.3	1.80, 2.04	26.3	1.80, 2.04	26.4	2.17,[b]
3	CH	89.1	3.18 dd (10.5, 5.5)	89.1	3.18 dd (10.5, 5.5)	89.2	3.36	89.2	3.36	89.2	3.34 dd (9.8, 4.2)
4	C	39.0		39.0		39.1		39.1		39.4	
5	CH	55.1	0.70	55.1	0.70	55.5	0.88	55.5	0.88	55.6	0.88
6	CH_2	18.0	1.20, 1.42	17.9	1.46, 1.60	18.8	1.40, 1.52	18.8	1.40, 1.52	18.8	1.40, 1.58
7	CH_2	35.8	1.93, 2.02	35.8	1.93, 2.02	36.7	2.09, 2.15	36.7	2.09, 2.15	36.7	2.09, 2.15
8	C	41.2		41.2		41.4		41.4		41.4	
9	CH	46.7	1.58	47.4	[b]	47.2	1.80	47.2	1.80	47.2	1.80
10	C	36.5		36.0		37.1		37.1		37.1	
11	CH_2	23.6	1.76, 1.82	23.6	1.76, 1.82	24.0	1.80, 1.88	24.0	1.80, 1.88	24.0	1.83, 1.86
12	CH	125.2	5.50 br t (3.0)	126.0	5.48 br t (3.0)	125.7	5.52 br t (3.0)	125.7	5.52 br t (3.0)	126.2	5.50 br t (3.2)
13	C	143.9		140.3		142.0		142.0		141.1	
14	C	47.6		47.9		48.0		48.0		48.1	
15	CH	68.2	4.20 d (3.8)	68.3	4.26 d (3.8)	67.6	4.40 d (3.6)	67.6	4.40 d (3.6)	67.6	4.40 d (3.5)
16	CH	74.0	4.46 d (3.8)	72.9	4.45 d (3.8)	72.5	4.58 d (3.6)	72.5	4.58 d (3.6)	72.5	4.58 d (3.5)
17	C	44.9		47.0		[b]		[b]		[b]	
18	CH	40.8	2.95 dd (13.6, 3.0)	54.0	3.00	54.4	3.12	54.4	3.12	54.5	3.10 dd (13.0, 3.2)
19	CH_2	46.7	1.28, 2.78 t (13.6)	34.9	2.22, 2.85	36.7	2.09, 2.16	36.7	2.09, 2.16	37.5	2.26, 2.98 t (13.0)
20	C	31.5		[b]		[b]		[b]		[b]	
21	CH_2 CH	41.0	1.91, 2.66 t (10.9)	71.3	4.23	71.5	4.88 d (7.0)	71.5	4.88 d (7.0)	71.2	4.22
22	CH	71.3	6.00 dd (10.9, 4.0)	80.0	6.10 d (7.6)	81.4	6.31 d (7.0)	81.4	6.31 d (7.0)	81.5	6.24 d (4.8)
23	CH_3	27.6	1.13 s	27.6	1.13 s	28.2	1.31 s	28.2	1.31 s	28.2	1.29 s
24	CH_3	16.6	0.87 s	16.6	0.91 s	16.8	1.01 s	16.8	1.01 s	17.0	0.98 s
25	CH_3	15.4	0.72 s	15.4	0.90 s	15.9	0.88 s	15.9	0.88 s	15.8	0.90 s
26	CH_3	17.0	0.99 s	17.0	1.08 s	17.1	1.07 s	17.1	1.07 s	17.9	1.04 s
27	CH_3	21.0	1.76 s	21.3	1.82 s	21.3	1.96 s	21.3	1.96 s	21.4	1.97 s
28	CH_2	62.8	3.50 d (10.9), 3.69 d (10.9)	64.9	3.71 d (13.5), 3.73 d (13.5)	65.5	3.81 d (12.6), 3.83 d (12.6)	65.5	3.81 d (12.6), 3.83 d (12.6)	68.9	4.28 d (13.0), 4.38 d (13.0)
29	CH_3	33.1	0.97 s	28.5	1.40 s	29.1	1.50 s	29.1	1.50 s	29.3	1.46 s
30	CH_3	24.7	1.20 s	27.0	1.43 s	28.4	1.52 s	28.4	1.52 s	28.2	1.50 s

[a] Multiplicities were assigned from DEPT spectra. Overlapped proton signals are reported without designated multiplicity. [b] Not determined.

Table 2. ^{13}C NMR and ^{1}H NMR Data of the Sugar Moieties and Acyl Groups of Compounds **1**−**5** in Pyridine-d_5 (δ ppm)[a,b]

	1		**2**		**3**		**4**		**5**	
no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
GlcA										
1	*106.2*	*4.76 d (7.2)*	*106.2*	*4.76 d (7.2)*	*106.8*	*4.89 d (7.4)*	*106.8*	*4.89 d (7.4)*	*106.8*	*4.90 d (7.3)*
2	*79.9*	*4.49*	*79.9*	*4.49*	*81.0*	*4.52*	*81.0*	*4.52*	*81.0*	*4.50*
3	75.1	4.02 t (8.0)	75.1	4.02 t (8.0)	75.8	4.02 t (8.2)	75.8	4.02 t (8.2)	75.7	4.01 t (9.7)
4	76.1	4.10	76.1	4.10	76.6	4.15	76.6	4.15	76.6	4.16 t (9.7)
5	78.0	4.37 t (8.7)	78.0	4.37 t (8.7)	78.2	4.50	78.2	4.50	78.2	4.49
6	[c]		[c]		[c]		[c]		[c]	
Rha										
1	101.4	5.83 br s	101.4	5.83 br s	102.0	5.83 br s	102.0	5.83 br s	102.2	5.91 br s
2	71.4	4.85 br s	71.4	4.85 br s	72.2	4.88 br s	72.2	4.88 br s	72.2	4.88 br s
3	71.6	4.53	71.6	4.53	72.5	4.58	72.5	4.58	72.5	4.58
4	73.2	4.15	73.2	4.15	73.4	4.20	73.4	4.20	73.8	4.22
5	69.7	4.82	69.7	4.82	70.2	4.83	70.2	4.83	70.1	4.85
6	17.9	1.51 d (5.8)	17.9	1.51 d (5.8)	18.4	1.61 d (6.0)	18.4	1.61 d (6.0)	18.5	1.63 d (6.5)
Ang										
1			168.0		169.3				168.0	
2			128.3		129.4				129.3	
3			137.0	5.82	136.2	5.85 qd (6.6 1.0)			136.5	5.93 qd (6.8, 1.0)
4			15.5	2.02 br dd (7.0, 1.0)	15.7	2.04 br dd (6.6, 1.0)			15.7	2.07 br dd (6.8, 1.0)
5			20.5	1.82 br s	20.8	1.96 br s			20.8	2.00 br s
dMA										
1	167.0						168.2			
2	117.0	5.55 br s					116.5	5.38 br s		
3	156.0						156.2			
4	19.7	2.11 s					20.8	2.19 s		
5	26.7	1.60 s					28.2	1.59 s		
Ac										
1					170.7		170.7		170.6	
2					21.0	2.09 s	21.0	2.09 s	20.8	1.97 s

[a] Multiplicities were assigned from DEPT spectra. Overlapped proton signals are reported without designated multiplicity. Ang: angelic acid, dMA: β,β-dimethylacrylic acid, Ac: acetyl. [b] Chemical shifts of substituted residues are italicized. [c] Not determined.

δ_H 5.50 (br t, J = 3.0 Hz) (H-12), four oxygen-bearing methine protons at δ_H 3.18 (dd, J = 10.5, 5.5 Hz) (H-3), 4.20 (d, J = 3.8 Hz) (H-15), 4.46 (d, J = 3.8 Hz) (H-16), and 6.00 (dd, J = 10.9, 4.0 Hz) (H-22), and one primary alcoholic function at δ_H 3.50, 3.69 (d, J = 10.9) (H_2-28). In the NOESY spectrum, cross-peaks between δ_H 4.20 (d, J = 3.8 Hz) (H-15) and δ_H 4.46 (d, J = 3.8 Hz) (H-16), and between δ_H 4.46 (H-16) and δ_H 3.50, 3.69 (d, J = 10.9) (H_2-28), allowed the location of two secondary alcoholic functions at C-15 and C-16. This was confirmed by a correlation between δ_H 1.76 (s) (H-27) and δ_C 68.2 (C-15) in the HMBC

spectrum. Moreover, in this spectrum, the two methyl group signals at δ_H 0.97 (s) (H-29) and 1.20 (s) (H-30) showed correlations with a methylene carbon at δ_C 41.0, which was assigned at the position 21. The cross-peak between the deshielded signal at δ_H 6.00 (dd, J = 10.9, 4.0 Hz) and the H_2-21 δ_H 1.91, 2.66 (t, J = 10.9 Hz) in the COSY spectrum indicated its location at C-22. The structure of the aglycon of **1** was thus recognized to be the triterpene A1-barrigenol and was in full agreement with literature data.[3,5] A correlation in the HMBC spectrum between H-22 at δ_H 6.00 and a carboxyl group at δ_C 167.0 suggested that C-22 was acylated. Two vinylic methyl groups at δ_H 1.60 (s) and 2.11 (s), which correlated in the HMBC spectrum with one ethylene quaternary carbon at δ_C 156.0 and an ethylene methine carbon at δ_C 117.0, revealed the presence of a β,β-dimethylacrylic acid acylating the C-22 position.

The relative configurations of C-3, C-15, C-16, and C-22 of the A1-barrigenol unit were determined by the multiplicity and the coupling constants of carbinol protons and from the connectivities observed in the NOESY spectrum between H-3 and H-5 and H-23, between H-15 and H-26, between H-16 and H-26 and H-28, and between H-22 and H-30. On the basis of the above results, the structure of **1** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-β,β-dimethylacryloyl-A1-barrigenol, a new triterpene glycoside.

Compound **2** exhibited in the HRESIMS (positive-ion mode) a pseudo-molecular ion peak at *m*/*z* 933.4871 $[M + Na]^+$ (calcd 933.4824), consistent with a molecular formula of $C_{47}H_{74}O_{17}Na$. Its FABMS (negative-ion mode) displayed a quasi-molecular ion peak at *m*/*z* 909 $[M - H]^-$, indicating a molecular weight of 910, differing from **1** by 16 amu. Another significant fragment ion peak was observed at *m*/*z* 763 $[(M - H) - 146]^-$, which revealed the elimination of one 6-desoxyhexosyl moiety. As the sugar part of **2** was the same as that of **1**, the difference between them was thus evident in the aglycon moiety and was represented by the presence of an additional hydroxyl group. In the HMBC spectrum, the two methyl protons at δ_H 1.40 (s) (H-29) and 1.43 (s) (H-30) and the deshielded signal at δ_H 6.10 (d, J = 7.6 Hz) (H-22) showed correlations with a oxygen-bearing methine carbon at δ_C 71.3 that was assigned at position 21. The aglycon of **2** was thus identified as R1-barrigenol.[3,6] The acylation at C-22 by an acid unit was suggested by the cross-peak observed in the HMBC spectrum between H-22 at δ_H 6.10 and a carboxyl group at δ_C 168.0. Two vinylic methyl groups at δ_H 1.82 (br s) and 2.02 (br dd, J = 7.0, 1.0 Hz), correlating in the COSY spectrum with one ethylene methine at δ_H 5.82, revealed the presence of an angeloyl residue. The structure of the new compound **2** was thus established as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-angeloyl-R1-barrigenol.

Compound **3** exhibited in the HRESIMS (positive-ion mode) a pseudo-molecular ion peak at *m*/*z* 975.4979 $[M + Na]^+$ (calcd 975.4929), consistent with a molecular formula of $C_{49}H_{76}O_{18}Na$. Its ESIMS (negative-ion mode) showed a quasi-molecular ion peak at *m*/*z* 951 $[M - H]^-$, indicating a molecular weight of 952. The ^1H and ^{13}C NMR signals of **3** assigned from 2D-NMR spectra were almost superimposable on those of **2**, except for the aglycon part, especially at positions 21 and 22. Signals of acylation at C-22 by an angeloyl residue were apparent as in **2**. The downfield shifts to δ_H 6.31 (d, J = 7.0 Hz) (H-22), δ_C 81.4 (C-22), and δ_H 4.88 (d, J = 7.0 Hz) (H-21) and the characteristic signals of an acetyl group suggested acetylation at position 21. These observations were used to assign the structure of **3** as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-21-*O*-acetyl-22-*O*-angeloyl-R1-barrigenol (**3**), a new saponin.

Compond **4** exhibited the same ESIMS (positive- and negative-ion mode) data as **3**. After examination of the NMR spectra of **4**, only one difference was detected in comparison with **3**, namely, in the nature of the acid at the C-22 position. Signals of a β,β-dimethylacryloyl group were found instead of those of an angeloyl residue. The structure of the new compound **4** was thus elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-21-*O*-acetyl-22-*O*-β,β-dimethylacryloyl-R1-barrigenol.

Compound **5** showed the same ESIMS (positive- and negative-ion mode) data as **3** and **4**. Signals due to acylation at C-22 by an angeloyl residue together with those of an acetyl group still remained. The downfield shift to δ_H 6.24 (d, J = 4.8 Hz) (H-22) and δ_C 81.5 (C-22) and the values at δ_H 4.28, 4.38 (d, J = 13.0 Hz) (H_2-28) and δ_C 68.9 indicated that the acetyl group was located at C-28. The structure of **5** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl-22-*O*-angeloyl-28-*O*-acetyl-R1-barrigenol, a new triterpene glycoside.

The cytotoxic activity of compounds **1**−**5**, and the previously isolated saponins 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucuronopyranosyl-22-*O*-angeloyl-R1-barrigenol and 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucuronopyranosyl-22-*O*-β,β-dimethylacryloyl-A1-barrigenol,[3] was determined against HCT 116 and HT-29 human tumor cell lines, by MTT assay.[7] No significant effect could be found for **5** and 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucuronopyranosyl-22-*O*-angeloyl-R1-barrigenol (IC_{50} > 100 μg/mL), while the saponins **1**−**4** and 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucuronopyranosyl-22-*O*-β,β-dimethylacryloyl-A1-barrigenol showed a weak activity, with IC_{50} between 40 and 100 μg/mL in the two cell lines. The IC_{50} value of the positive control, paclitaxel, was 8.0 ± 6.0 and 5.3 ± 1.2 ng/mL against HCT 116 and HT-29, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a AA-OR automatic polarimeter. The 1D and 2D NMR spectra (^1H−^1H COSY, TOCSY, NOESY, HSQC, and HMBC) were performed using a Bruker AV-400 instrument equipped with a inverse broadband probehead with z-gradient operating at 400.13 and 100.62 MHz, respectively. Compounds were analyzed in pyridine-d_5 at T = 308 K using the solvent signals as internal standard (pyridine-d_5: H-2 at 7.21 ppm, C-2 at 123.5 ppm). Standard 1D and 2D pulse sequences from the Bruker library were used. HRESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer, ESIMS (positive- and negative-ion mode) on a Finnigan LCQ Deca, and FABMS (negative-ion mode, glycerol matrix) on a JEOL SX 102 mass spectrometer. TLC precoated silica gel plates $60F_{254}$ (Merck) and the solvent system $CHCl_3$−MeOH−H_2O (61:32:7) were used. The spray reagent for saponins was Komarowsky reagent (2% 4-hydroxybenzaldehyde in MeOH−50% H_2SO_4, 5:1). Isolations were carried out using a medium-pressure liquid chromatographic (MPLC) system [Gilson pump M 303, Büchi glass column (460 × 15 mm and 250 × 15 mm), Büchi precolumn (110 × 15 mm), silica gel 60 (Merck, 15−40 μm), LiChroprep RP-18 (25−40 μm)].

Plant Material. In August 2003, *Eryngium campestre* was collected at Balíkesir-Edremit, Turkey, and identified by Prof. Dr. Hayri Duman (Department of Biology, Faculty of Sciences, Gazi University, Ankara, Turkey). A voucher specimen (AEF 22964) was deposited in the herbarium of the Faculty of Pharmacy, Ankara University, Ankara, Turkey.

Extraction and Isolation. The dried, powdered roots (150 g) were extracted and fractionated according to a previously described protocol yielding 13 main fractions.[3] The fraction (378 mg) eluted with CH_2Cl_2−MeOH−H_2O (70:30:3) was purified by MPLC on silica gel eluted with $CHCl_3$−MeOH−H_2O (64:40:8), then on LiChroprep RP-18 eluted with MeOH−H_2O mixtures (50:50 to MeOH) to give **1** (3 mg), **2** (4 mg), **3** (6 mg), **4** (4 mg), and **5** (5 mg).

Acid Hydrolysis. A 200 mg amount of the *n*-BuOH layer was refluxed with 2 N CF_3COOH for 2 h. After extraction with $CHCl_3$, the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral. Two sugars were identified as glucuronic acid and rhamnose, by comparison with authentic samples on TLC in $CHCl_3$−MeOH−H_2O (64:40:8). After preparative TLC of the sugar mixture in this solvent, the optical rotation of each purified sugar was measured.

MTT Cytotoxicity Assay. The bioassay was carried out according to the method described in ref 7 with two human colorectal cancer cells (HCT 116 and HT-29). Paclitaxel was used as a positive control and exhibited IC_{50} values of 8.0 ± 6.0 and 5.3 ± 1.2 ng/mL against HCT 116 and HT-29, respectively.

Compound 1: white, amorphous powder; $[\alpha]^{25}_D$ −29.3 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz), see Tables 1 and 2; positive HRESIMS *m*/*z* 917.4922 [M + Na]$^+$ (calcd 917.4875); negative FABMS *m*/*z* 893 [M − H]$^-$, 747 [(M − H) − 146]$^-$.

Compound 2: white, amorphous powder; $[\alpha]^{25}_D$ −10.2 (*c* 0.06, MeOH); ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz), see Tables 1 and 2; positive HRESIMS *m*/*z* 933.4871 [M + Na]$^+$ (calcd 933.4824); negative FABMS *m*/*z* 909 [M − H]$^-$, 763 [(M − H) − 146]$^-$.

Compound 3: white, amorphous powder; $[\alpha]^{25}_D$ −30.0 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz), see Tables 1 and 2; positive HRESIMS *m*/*z* 975.4979 [M + Na]$^+$ (calcd 975.4929); negative ESIMS *m*/*z* 951 [(M − H)]$^-$.

Compound 4: white, amorphous powder; $[\alpha]^{25}_D$ −30.1 (*c* 0.05, MeOH); ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz), see Tables 1 and 2; positive HRESIMS *m*/*z* 975.4978 [M + Na]$^+$ (calcd 975.4929); negative ESIMS *m*/*z* 951 [(M − H)]$^-$.

Compound 5: white, amorphous powder; $[\alpha]^{25}_D$ −6.5 (*c* 0.06, MeOH); ^{1}H NMR (pyridine-d_5, 400 MHz) and ^{13}C NMR (pyridine-d_5, 100 MHz), see Tables 1 and 2; positive HRESIMS *m*/*z* 975.4974 [M + Na]$^+$ (calcd 975.4929); negative ESIMS *m*/*z* 951 [(M − H)]$^-$.

References and Notes

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