Antiproliferative Constituents from Umbelliferae Plants. IX.[1] New Triterpenoid Glycosides from the Fruits of *Bupleurum rotundifolium*

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The MeOH extract of the fruits of *Bupleurum rotundifolium* showed inhibitory activity against human gastric adenocarcinoma (MK-1) cell growth. Bioactivity-guided fractionation of the MeOH extract led to the isolation of four new triglycosides of 13β,28-epoxy oleanane-type triterpenes, named rotundiosides O, Q, S and T; 12 new glycosides of oleanane-type triterpenes, named rotundiosides J—N, P, R, U—Y, and others; echinocystic acid 3-*O*-sulfate; and three known oleanane-type triterpene glycosides, rotundiosides A, F and G. The structures of the new isolates were determined based on chemical and spectroscopic evidence. The GI_{50} of isolates against MK-1, HeLa and B16F10 cell lines are reported.

Key words antiproliferative constituent; *Bupleurum rotundifolium*; Umbelliferae 13β,28-epoxy oleanane-type triterpene glycoside

Our previous papers have reported the isolation and characterization of seven new triglycosides of 13β,28-epoxy ursane-type triterpenes, named rotundifoliosides A and E—J, and two new triglycosides of ursane-type triterpenes, named rotundifoliosides B and C, from active fractions of the fruits of *Bupleurum rotundifolium* (Umbelliferae). Among these compounds, rotundifoliosides H—J showed 50% growth inhibition (GI_{50}) at $<20\,\mu$M against MK-1 (human gastric adenocarcinoma) and B16F10 (mouse murine melanoma) cells, and at $<40\,\mu$M against HeLa (human uterus carcinoma) cells.[2]

Further detailed investigation of this active fraction has resulted in the isolation of four new triglycosides of 13β,28-epoxy oleanane-type triterpenes, named rotundiosides O (**1**), Q (**2**), S (**3**) and T (**4**); 12 new glycosides of oleanane-type triterpenes, named rotundiosides J (**5**), K (**6**), L (**7**), M (**8**), N (**9**), P (**10**), R (**11**), U (**12**), V (**13**), W (**14**), X (**15**), Y (**16**), and others; echinocystic acid 3-*O*-sulfate; and three known oleanane-type triterpene glycosides, rotundiosides A (**17**),[3] F (**18**)[4] and G (**19**).[5] This paper describes the isolation and characterization of these compounds and the evaluation of their antiproliferative activity against MK-1, HeLa and B16F10 cell lines.

Repeated chromatography of the previously obtained MeOH fraction, which exhibits antiproliferative activity against MK-1 cell lines, on silica gel, octadecyl silane (ODS), and the preparative HPLC (ODS), has led to the isolation of four new triglycosides of 13β,28-epoxy oleanane-type triterpenes, named rotundiosides O (**1**), Q (**2**), S (**3**) and T (**4**); 12 new triglycosides of oleanane-type triterpenes, named rotundiosides J (**5**), K (**6**), L (**7**), M (**8**), N (**9**), P (**10**), R (**11**), U (**12**), V (**13**), W (**14**), X (**15**), Y (**16**), and others; echinocystic acid 3-*O*-sulfate; and three known oleanane-type triterpene glycosides, rotundiosides A (**17**), F (**18**) and G (**19**).

Rotundioside O (**1**) was obtained as a white powder and the positive-ion high resolution (HR) FAB-MS gave the molecular formula $C_{48}H_{76}O_{17}$, which is 14 mass units (+O−2H) more than that ($C_{48}H_{78}O_{16}$) of rotundioside F (**18**). Compound **1** gave the same sugars, D-fucose, D-glucose and L-rhamnose, as those of **18** on acid hydrolysis. The ^{13}C-nuclear magnetic resonance (NMR) spectrum of **1** was analogous to that of **18**, concerning the signals arising from the sugar moiety. The structure of the sugar moiety of **1** was established to be the same as that of **18**.

The ^{1}H-NMR spectrum of **1** (Table 1) showed signals of seven tertiary methyl groups (δ 0.94, 1.10, 1.21, 1.31, 1.36, 1.36, 1.61); a secondary methyl group assignable to H-6 of the fucopyranosyl group (δ 1.50, d, J=6.0 Hz); a secondary methyl group assignable to H-6 of the rhamnopyranosyl group (δ 1.84, d, J=6.0 Hz); one di-substituted olefinic group (δ 5.57, dd, J=3.0, 10.5 Hz; δ 6.04, d, J=10.5 Hz); one hydroxymethyl group assignable to H-6 of the glucopyranosyl group (δ 4.20, dd, J=6.0, 12.0 Hz; δ 4.30, dd, J=3.0, 12.0 Hz); methylene protons next to oxygen (δ 3.53, d, J=7.0 Hz; δ 3.56, d, J=7.0 Hz); and three anomeric protons (δ 4.78, d, J=8.0 Hz; δ 5.38, d, J=7.5 Hz; δ 6.40, br s). The ^{13}C-NMR spectrum of **1** (Table 2) exhibited the signals of six C–C bonded quaternary carbons (δ 36.36, 39.87, 42.35, 43.57, 44.14, 50.85); disubstituted olefinic carbons (δ 131.22, 132.53); an oxygen-bearing quaternary carbon (δ 84.80); a hydroxymethyl group assignable to C-6 of the glucopyranosyl group (δ 63.31); a methylene carbon next to oxygen (δ 75.12); and three anomeric carbons (δ 101.85, 102.14, 105.20). From the above-mentioned results, the aglycone of **1** was presumed to be the oleanane-type triterpene analogous to rotundioside F (**18**). The ^{13}C-NMR spectrum of **1** showed a decrease of one methylene (δ 36.80), identified as C-21 from **18**, and addition of a ketone group (δ 213.50). When the ^{13}C-NMR spectra of **1** and **18** were compared, the spectrum of **1** showed upfield shifts of C-28 (Δ 2.65 ppm) and C-29 (Δ 7.51 ppm), and downfield shifts of C-17 (Δ 5.52 ppm), C-20 (Δ 12.29 ppm), C-22 (Δ 14.55 ppm) and C-30 (Δ 2.23 ppm) (Table 2). These spectral data indicate that the structure of rotundioside O (**1**) is 13β,28-epoxy-16α-hydroxyolean-11-en-21-one-3β-yl α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside, as shown in Fig. 1.

The heteronuclear multiple bond connectivity (HMBC) spectrum also clearly supported the above structure.

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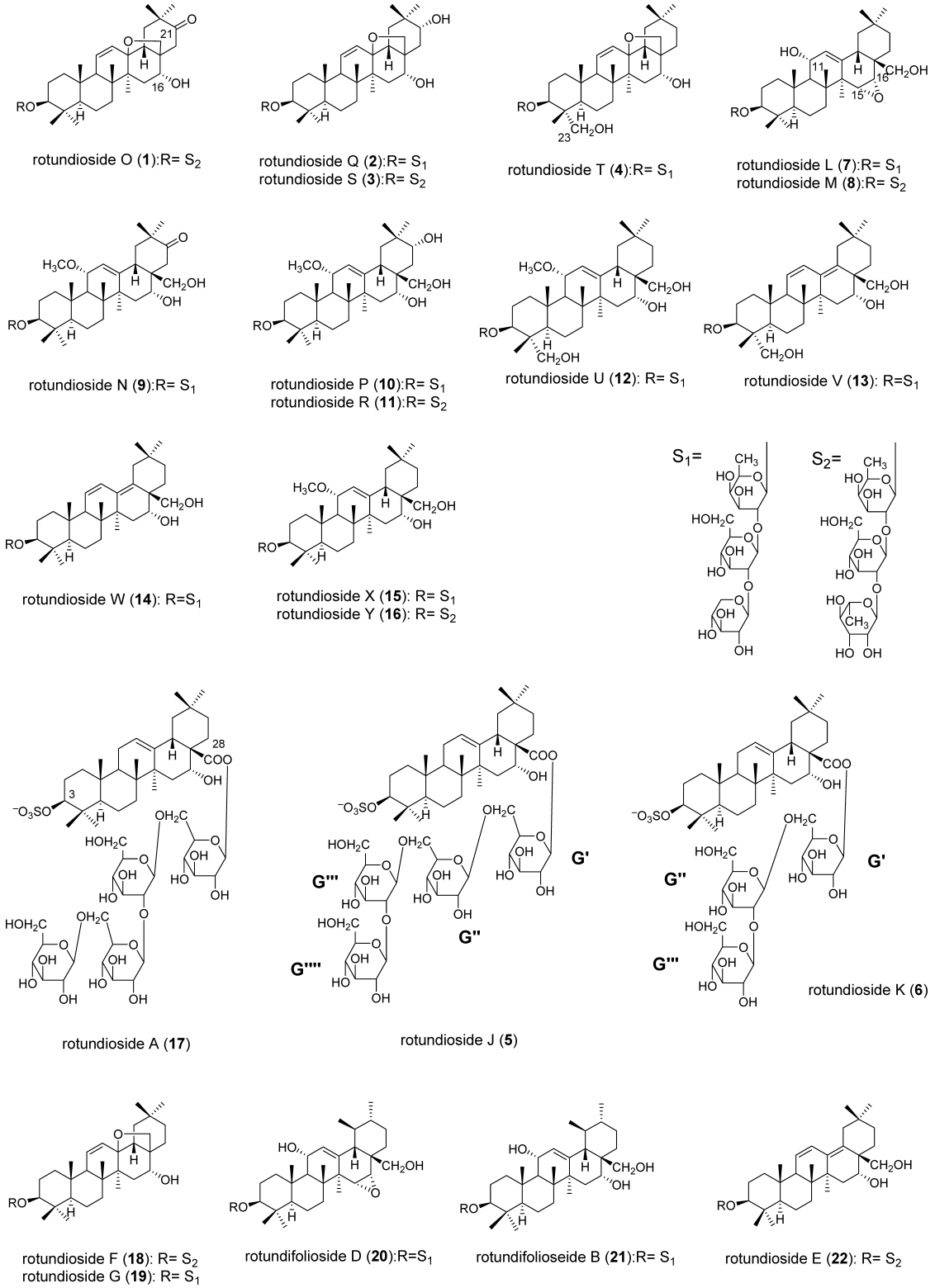

Fig. 1

Rotundioside Q ($C_{47}H_{76}O_{17}$) (**2**), a white powder, is 16 mass units heavier than rotundioside G (**19**). Compound **2** yielded the same sugars, D-fucose, D-glucose and D-xylose, as **19** on acid hydrolysis. The ^{13}C-NMR spectrum of **2** was analogous to that of **19** with regard to the signals arising from the sugar moieties (Table 2). The ^{13}C-NMR spectrum of **2** showed a decrease of one methylene (δ 36.79) identified as C-21 from **19**, and addition of a hydroxyl group (δ 72.64). When the ^{13}C-NMR spectra of **2** and **19** were compared, the spectrum of **2** showed upfield shifts of C-19 (Δ 4.35 ppm), and downfield shifts of C-20 (Δ 5.19 ppm) and C-22 (Δ 7.04 ppm). These spectral data indicate that the structure of rotundioside Q (**2**) is 13β,28-epoxy-16α,21-dihydroxy-olean-11-en-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyra-

Table 1. ^{1}H-NMR Data (δ, J in Hz) for Compounds **1**—**16**, **18** and **19** (Pyridine-d_5, 500 MHz)

	1	**2**	**3**	**4**	**7**
H-1	*ca.* 1.03	*ca.* 0.97	*ca.* 1.00	*ca.* 1.05	*ca.* 1.70
	ca. 1.85	1.83 (br d, 13.5)	*ca.* 1.83	*ca.* 1.88	2.44 (ddd, 3.0, 3.0, 14.0)
H-2	*ca.* 1.92	1.93 (br d, 13.5)	*ca.* 1.90	*ca.* 2.06	*ca.* 1.97
	ca. 2.22	*ca.* 2.23	*ca.* 2.20	*ca.* 2.30	*ca.* 2.30
H-3	3.31 (dd, 5.0, 11.5)	3.30 (dd, 4.5, 11.5)	3.30 (dd, 4.5, 11.5)	4.07 (dd, 4.5, 12.5)	3.37 (dd, 4.5, 12.0)
H-5	*ca.* 0.85	*ca.* 0.80	*ca.* 0.83	*ca.* 1.49	1.00 (br d, 10.0)
H-6	*ca.* 1.25, 1.52	*ca.* 1.50, 1.50	*ca.* 1.52	*ca.* 1.58, 1.79	*ca.* 1.47, 1.65
H-7	*ca.* 1.25, 1.52	*ca.* 1.25, 1.50	*ca.* 1.27, 1.52	*ca.* 1.24, 1.58	*ca.* 1.58, 1.72
H-9	*ca.* 1.60	*ca.* 2.05	*ca.* 2.07	*ca.* 2.13	1.93 (d, 8.0)
H-11	5.57 (dd, 3.0, 10.5)	5.68 (dd, 3.0, 10.5)	5.67 (dd, 3.0, 10.5)	5.68 (dd, 3.0, 10.0)	*ca.* 4.32
H-12	6.04 (d, 10.5)	6.02 (d, 10.5)	6.02 (d, 10.5)	6.02 (d, 10.0)	5.72 (d, 4.0)
H-15	*ca.* 1.60	1.65 (d, 14.5)	*ca.* 1.67	*ca.* 1.47	3.09 (d, 3.5)
	ca. 2.09	2.13 (dd, 4.5, 14.5)	2.13 (dd, 4.5, 14.0)		
H-16	4.13 (br s)	4.09 (br s)	4.09 (br d, 4.5)	*ca.* 4.20	3.33 (d, 3.5)
H-18	2.45 (dd, 4.0, 14.5)	2.02 (dd, 2.5, 14.0)	2.01 (dd, 2.5, 14.0)	1.98 (dd, 3.0, 14.0)	2.50 (dd, 4.5, 13.5)
H-19	1.70 (dd, 4.0, 13.0)	1.34 (dd, 2.5, 13.0)	*ca.* 1.33	1.40 (br d, 12.5)	*ca.* 1.10
	3.29 (dd, 13.0, 14.5)	2.79 (dd, 13.0, 14.0)	2.78 (dd, 14.0, 14.0)	2.66 (dd, 12.5, 14.0)	2.34 (dd, 13.5, 13.5)
H-21		3.63 (br s)	3.62 (br s)	*ca.* 1.24	*ca.* 1.37
				2.50 (ddd, 5.0, 13.5, 13.5)	1.83 (ddd, 4.0, 13.5, 13.5)
H-22	2.63 (d, 15.0)	*ca.* 2.08 (2H)	*ca.* 2.07 (2H)	*ca.* 1.58	2.06 (ddd, 4.0, 4.0, 13.5)
	2.67 (d, 15.0)			*ca.* 1.91	2.30 (ddd, 4.0, 13.5, 13.5)
H-23	1.36 (s)	1.30 (s)	1.34 (s)	3.76 (d, 11.5)	1.35 (s)
				ca. 4.28	
H-24	1.10 (s)	1.12 (s)	1.09 (s)	1.10 (s)	1.17 (s)
H-25	0.94 (s)	0.95 (s)	0.93 (s)	1.03 (s)	1.06 (s)
H-26	1.36 (s)	1.33 (s)	1.32 (s)	1.35 (s)	1.07 (s)
H-27	1.61 (s)	1.68 (s)	1.68 (s)	1.61 (s)	1.63 (s)
H-28	3.53 (d, 7.0)	3.35 (d, 7.0)	3.34 (d, 7.0)	3.33 (d, 7.0)	*ca.* 3.80
	3.56 (d, 7.0)	3.66 (d, 7.0)	3.65 (d, 7.0)	3.58 (d, 7.0)	*ca.* 3.80
H-29	1.31 (s)[a)]	1.24 (s)[b)]	1.24 (s)[c)]	1.02 (s)[d)]	0.94 (s)[e)]
H-30	1.21 (s)[a)]	0.98 (s)[b)]	0.97 (s)[c)]	0.97 (s)[d)]	0.97 (s)[e)]
Fuc 1	4.78 (d, 8.0)	4.78 (d, 8.0)	4.76 (d, 8.0)	4.92 (d, 8.0)	4.78 (d, 7.5)
2	4.62 (dd, 8.0, 9.5)	4.52 (dd, 8.0, 9.0)	4.61 (dd, 8.0, 9.5)	4.47 (dd, 8.0, 9.0)	4.49 (dd, 7.5, 9.0)
3	4.39 (dd, 3.0, 9.5)	*ca.* 4.24	4.38 (dd, 3.0, 9.5)	4.15 (dd, 3.0, 9.0)	4.23 (dd, 3.5, 9.0)
4	3.83 (br d, 3.0)	4.01 (br d, 3.0)	3.82 (br d, 3.0)	3.96 (br d, 3.0)	4.00 (br d, 3.5)
5	3.77 (br q, 6.0)	3.83 (br q, 6.5)	3.76 (br q, 6.5)	3.71 (br q, 6.5)	3.80 (br q, 6.5)
6	1.50 (d, 6.0)	1.55 (d, 6.5)	1.49 (d, 6.5)	1.50 (d, 6.5)	1.52 (d, 6.5)
Glc 1	5.38 (d, 7.5)	5.42 (d, 7.5)	5.66 (d, 7.5)	5.40 (d, 7.5)	5.42 (d, 7.5)
2	4.30 (dd, 7.5, 9.0)	*ca.* 4.17	4.28 (dd, 7.5, 9.0)	*ca.* 4.15	4.18 (dd, 7.5, 9.0)
3	4.21 (t, 9.0)	*ca.* 4.17	4.20 (t, 9.0)	*ca.* 4.15	*ca.* 4.20
4	4.10 (t, 9.0)	*ca.* 4.17	4.04 (t, 9.0)	*ca.* 4.22	*ca.* 4.20
5	3.62 (ddd, 3.0, 6.0, 9.0)	3.68 (ddd, 3.5, 5.0, 9.0)	*ca.* 3.61	3.62 (ddd, 3.5, 3.5, 9.0)	3.68 (ddd, 3.5, 4.5, 9.0)
6	4.20 (dd, 6.0, 12.0)	4.29 (dd, 5.0, 11.0)	4.20 (dd, 6.0, 11.5)	*ca.* 4.31	4.30 (dd, 4.5, 11.5)
	4.30 (dd, 3.0, 12.0)	4.36 (dd, 3.5, 11.0)	4.29 (dd, 3.0, 11.5)		4.36 (dd, 3.5, 11.5)
Xyl 1		5.39 (d, 7.0)		5.36 (d, 7.0)	5.39 (d, 7.0)
2		4.13 (dd, 7.0, 9.0)		4.10 (dd, 6.0, 7.0)	4.10 (dd, 7.0, 8.0)
3		*ca.* 4.22		*ca.* 4.17	*ca.* 4.14
4		*ca.* 4.20		*ca.* 4.17	*ca.* 4.20
5		3.75 (dd, 9.5, 11.5)		3.75 (dd, 11.0, 11.0)	3.74 (dd, 9.5, 11.5)
		4.45 (dd, 5.0, 11.5)		4.43 (dd, 5.0, 11.0)	4.44 (dd, 5.0,11.5)
Rha 1	6.40 (br s)		6.40 (br s)		
2	*ca.* 4.78		*ca.* 4.76		
3	*ca.* 4.78		*ca.* 4.76		
4	4.36 (t, 9.5)		4.35 (t, 9.5)		
5	5.07 (dq, 9.5, 6.0)		5.06 (dq, 9.5, 6.0)		
6	1.84 (d, 6.0)		1.84 (d, 6.0)		

a—*p*) Signals may be interchangeable in each vertical column. Abbreviations: Fuc, D-fucose; Glc, D-glucose; Xyl, D-xylose; Rha, L-rhamnose, all in a pyranose form.

nosyl(1→2)-β-D-fucopyranoside. The signal of H-21 (δ 3.63, br s) indicates that its configuration is β. Thus, the configuration of the hydroxyl group at C-21 was determined to be α, and the structure of **2** was established as 13β,28-epoxy-16α,21α-dihydroxyolean-11-en-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside, as shown in Fig. 1.

Rotundioside S ($C_{48}H_{78}O_{17}$) (**3**), a white powder, is 2 mass units (2 H) heavier than **1**. Compound **3** gave the same sugars as those of **1** on acid hydrolysis. The ^{13}C-NMR spectrum of the sugar moiety of **3** was analogous to that of **1** (Table 2). The ^{13}C-NMR spectrum of the aglycone moiety of **3** was according to that of **2**. From the above-mentioned results, rotundioside S (**3**) was established as 13β,28-epoxy-16α,21α-dihydroxyolean-11-en-3β-yl α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

Table 1. (Continued)

	8	9	10	11
H-1	*ca.* 1.73	*ca.* 1.47	*ca.* 1.47	*ca.* 1.49
	2.44 (ddd, 3.0, 3.0, 14.0)	*ca.* 2.02	*ca.* 2.05	*ca.* 2.06
H-2	*ca.* 1.96	*ca.* 2.00	*ca.* 1.96	*ca.* 1.94
	ca. 2.26	*ca.* 2.25	*ca.* 2.25	*ca.* 2.22
H-3	3.38 (dd, 4.5, 11.5)	3.33 (dd, 4.5, 11.5)	3.33 (dd, 4.5, 12.0)	3.34 (dd, 4.5, 11.5)
H-5	*ca.* 1.03	*ca.* 0.86	*ca.* 0.87	*ca.* 0.90
H-6	*ca.* 1.45, 1.65	*ca.* 1.25, 1.33	*ca.* 1.30, 1.52	*ca.* 1.30, 1.58
H-7	*ca.* 1.60, 1.73	*ca.* 1.25, 1.53	*ca.* 1.30, 1.52	*ca.* 1.30, 1.58
H-9	1.96 (d, 8.0)	*ca.* 2.00	*ca.* 2.01	*ca.* 2.05
H-11	*ca.* 4.32	3.81 (dd, 3.5, 8.5)	3.81 (dd, 3.5, 9.0)	*ca.* 3.81
H-12	5.74 (d, 4.0)	5.74 (d, 3.5)	5.61 (d, 3.5)	5.61 (br d, 3.5)
H-15	3.09 (d, 3.5)	1.64 (dd, 2.0, 15.0)	1.72 (dd, 1.5, 14.0)	1.72 (dd, 2.0, 14.0)
		1.93 (dd, 4.0, 15.0)	*ca.* 2.01	*ca.* 2.04
H-16	3.35 (d, 3.5)	4.31 (br s)	4.31 (br s)	4.32 (br s)
H-18	2.51 (dd, 4.5, 14.0)	3.17 (dd, 4.5, 13.5)	2.79 (dd, 4.0, 14.0)	2.79 (dd, 4.0, 14.0)
H-19	*ca.* 1.10	1.75 (dd, 4.5, 13.0)	*ca.* 1.30	*ca.* 1.30
	2.34 (dd, 13.0, 13.0)	3.28 (dd, 13.0, 13.5)	2.88 (dd, 14.0, 14.0)	2.89 (dd, 14.0, 14.0)
H-21	*ca.* 1.38		3.78 (br s)	3.75 (br s)
	ca. 1.84			
H-22	2.07 (ddd, 3.5, 3.5, 14.0)	2.83 (d, 15.5)	2.21 (dd, 1.5, 15.0)	2.22 (dd, 2.0, 15.5)
	2.30 (ddd, 3.5, 13.5, 14.0)	3.34 (d, 15.5)	2.86 (dd, 5.0, 15.0)	2.86 (dd, 4.5, 15.5)
H-23	1.40 (s)	1.33 (s)	1.33 (s)	1.38 (s)
H-24	1.16 (s)	1.16 (s)	1.15 (s)	1.14 (s)
H-25	1.02 (s)	1.04 (s)	1.05 (s)	1.04 (s)
H-26	1.07 (s)	0.96 (s)	0.96 (s)	0.96 (s)
H-27	1.65 (s)	1.88 (s)	1.93 (s)	1.94 (s)
H-28	*ca.* 3.80	3.56 (d, 10.5)	3.61 (d, 8.5)	3.61 (d, 9.5)
	ca. 3.80	3.78 (d, 10.5)	3.66 (d, 8.5)	3.67 (d, 9.5)
H-29	0.94 (s)[f]	1.36 (s)[g]	1.22 (s)[h]	1.23 (s)[i]
H-30	0.97 (s)[f]	1.28 (s)[g]	1.18 (s)[h]	1.19 (s)[i]
OCH_3		3.28 (s)	3.26 (s)	3.26 (s)
Fuc 1	4.78 (d, 8.0)	4.89 (d, 8.0)	4.78 (d, 8.0)	4.79 (d, 8.0)
2	4.62 (dd, 8.0, 9.0)	4.53 (dd, 8.0, 9.0)	4.51 (dd, 8.0, 9.0)	4.61 (dd, 8.0, 9.0)
3	4.39 (dd, 3.5, 9.0)	*ca.* 4.25	4.24 (dd, 3.0, 9.0)	4.40 (dd, 3.0, 9.0)
4	3.81 (br d, 3.5)	4.02 (br d, 3.0)	4.00 (br d, 3.0)	*ca.* 3.81
5	3.75 (br q, 6.5)	3.84 (br q, 6.5)	3.83 (br q, 6.0)	3.78 (br q, 6.5)
6	1.47 (d, 6.5)	1.52 (d, 6.5)	1.52 (d, 6.0)	1.48 (d, 6.5)
Glc 1	5.67 (d, 8.0)	5.42 (d, 7.5)	5.43 (d, 7.5)	5.68 (d, 8.0)
2	4.30 (dd, 8.0, 9.0)	*ca.* 4.15	*ca.* 4.16	4.30 (dd, 8.0, 9.0)
3	4.21 (t, 9.0)	*ca.* 4.15	4.23 (t, 9.0)	4.21 (t, 9.0)
4	4.05 (t, 9.0)	*ca.* 4.15	*ca.* 4.16	4.06 (t, 9.0)
5	3.63 (ddd, 3.5, 5.5, 9.0)	3.70 (ddd, 3.0, 5.0, 8.5)	3.68 (ddd, 3.0, 5.0, 9.0)	3.61 (ddd, 3.0, 5.0, 9.0)
6	4.21 (dd, 5.5, 11.5)	4.29 (dd, 5.0, 11.5)	4.29 (dd, 5.0, 11.5)	4.21 (dd, 5.0, 11.5)
	4.32 (dd, 3.5, 11.5)	*ca.* 4.39	4.36 (dd, 3.0, 11.5)	*ca.* 4.30
Xyl 1		5.39 (d, 7.0)	5.38 (d, 7.0)	
2		*ca.* 4.12	4.10 (dd, 7.0, 9.0)	
3		*ca.* 4.23	*ca.* 4.16	
4		*ca.* 4.23	*ca.* 4.20	
5		3.74 (dd, 10.0, 11.0)	3.74 (dd, 9.5, 11.5)	
		4.46 (dd, 5.0, 11.0)	4.44 (dd, 5.0, 11.5)	
Rha 1	6.40 (br s)			6.42 (br s)
2	*ca.* 4.77			*ca.* 4.77
3	*ca.* 4.77			*ca.* 4.77
4	4.35 (t, 9.0)			4.35 (t-like, 9.0)
5	5.08 (dq, 9.0, 6.0)			5.07 (dq, 9.0, 6.0)
6	1.83 (d, 6.0)			1.83 (d, 6.0)

Rotundioside T ($C_{47}H_{76}O_{17}$) (**4**), a white powder, is 16 mass units (1 O) heavier than **19**. The ^{13}C-NMR spectrum of **4** was analogous to that of **19** with regard to the signals arising from the sugar moieties (Table 2). A comparison of the ^{13}C-NMR spectrum of **4** with that of **19** in relation to the aglycone moiety showed the absence of signals due to a tertiary methyl group (δ 27.85) assignable to C-23 from **19**, and the appearance of signals due to a hydroxymethyl group (δ 65.59). When the ^{13}C-NMR spectra of **4** and **19** were compared, the spectrum of **4** showed upfield shifts of C-3 (Δ 5.68 ppm), C-5 (Δ 7.03 ppm) and C-24 (Δ 3.50 ppm), and a downfield shift of C-4 (Δ 3.87 ppm). These spectral data indicate that the hydroxymethyl group in **4** is located at C-23, and is therefore 13β,28-epoxy-16α,23-dihydroxyolean-11-en-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside, as shown in Fig. 1.

Rotundioside J (**5**) was obtained as a white powder. The negative-ion HR-FAB-MS of **5** gave the molecular formula

Table 1. (Continued)

	12	13	14	15
H-1	*ca.* 1.53	*ca.* 1.12	*ca.* 1.02	*ca.* 1.49
	ca. 2.13	*ca.* 1.88	*ca.* 1.83	*ca.* 2.07
H-2	*ca.* 2.09	*ca.* 2.07	*ca.* 1.94	*ca.* 1.96
	ca. 2.29	*ca.* 2.32	*ca.* 2.24	*ca.* 2.25
H-3	*ca.* 4.06	*ca.* 4.10	3.32 (dd, 4.5, 12.0)	3.33 (dd, 4.5, 11.5)
H-5	*ca.* 1.60	*ca.* 1.57	*ca.* 0.87	0.87 (br d, 12.0)
H-6	*ca.* 1.40, 1.74	*ca.* 1.48, 1.80	*ca.* 1.40, 1.57	*ca.* 1.32, 1.52
H-7	*ca.* 1.25, 1.68	*ca.* 1.32, 1.48	*ca.* 1.38, 1.38	*ca.* 1.26, 1.55
H-9	2.10 (d, 9.0)	*ca.* 2.22	2.12 (br s)	2.01 (d, 8.5)
H-11	3.89 (dd, 3.5, 9.0)	6.70 (dd, 3.0, 10.5)	6.72 (dd, 3.0, 11.0)	3.84 (dd, 3.5, 8.5)
H-12	5.61 (d, 3.5)	5.72 (br d, 10.5)	5.70 (br d, 11.0)	5.62 (d, 3.5)
H-15	*ca.* 1.58	*ca.* 1.69	*ca.* 1.73	*ca.* 1.64
	ca. 2.10	*ca.* 2.19	*ca.* 2.23	*ca.* 2.12
H-16	4.58 (br s)	4.79 (br d, 3.5)	4.83 (br s)	4.64 (br s)
H-18	2.57 (dd, 4.0, 13.5)			2.61 (dd, 4.0, 13.5)
H-19	*ca.* 1.40	1.93 (d, 14.5)	1.94 (d, 14.0)	*ca.* 1.43
	2.72 (dd, 13.5, 13.5)	2.66 (d, 14.5)	2.68 (d, 14.0)	2.77 (dd, 13.5, 13.5)
H-21	*ca.* 1.40	*ca.* 1.48	*ca.* 1.45	*ca.* 1.43
	2.41 (ddd, 6.0, 12.0, 12.0)	*ca.* 1.78	*ca.* 1.80	2.47 (ddd, 6.5, 12.0, 12.0)
H-22	*ca.* 2.23	*ca.* 2.23	*ca.* 2.27	*ca.* 2.25
		ca. 2.70	*ca.* 2.70	
H-23	*ca.* 3.76	*ca.* 3.78	1.31 (s)	1.32 (s)
	ca. 4.28	*ca.* 4.23		
H-24	1.13 (s)	1.10 (s)	1.11 (s)	1.15 (s)
H-25	1.14 (s)	1.01 (s)	0.93 (s)	1.05 (s)
H-26	1.01 (s)	0.89 (s)	0.87 (s)	0.98 (s)
H-27	1.86 (s)	1.67 (s)	1.73 (s)	1.95 (s)
H-28	*ca.* 3.60	*ca.* 3.75	3.77 (d, 10.0)	3.62 (d, 10.5)
	ca. 3.73	*ca.* 4.29	4.23 (d, 10.0)	3.72 (d, 10.5)
H-29	1.01 (s)[j]	1.04 (s)[k]	1.06 (s)[l]	1.05 (s)[m]
H-30	1.13 (s)[j]	1.00 (s)[k]	1.01 (s)[l]	1.15 (s)[m]
OCH_3	3.28 (s)			3.27 (s)
Fuc 1	4.91 (d, 7.5)	4.95 (d, 7.5)	4.78 (d, 8.0)	4.80 (d, 7.5)
2	4.47 (dd, 7.5, 9.5)	4.50 (dd, 7.5, 9.5)	4.51 (dd, 8.0, 9.0)	4.50 (dd, 7.5, 9.5)
3	*ca.* 4.18	*ca.* 4.19	4.25 (dd, 3.5, 9.0)	*ca.* 4.27
4	*ca.* 3.96	3.98 (br d, 3.0)	4.02 (br d, 3.5)	4.02 (br d, 3.0)
5	*ca.* 3.72	*ca.* 3.76	3.84 (br q, 6.0)	3.84 (br q, 6.0)
6	1.47 (d, 6.5)	1.53 (d, 6.5)	1.57 (d, 6.0)	1.53 (d, 6.0)
Glc 1	5.40 (d, 7.5)	5.41 (d, 7.5)	5.43 (d, 8.0)	5.46 (d, 7.5)
2	*ca.* 4.18	*ca.* 4.19	*ca.* 4.18	*ca.* 4.18
3	*ca.* 4.18	*ca.* 4.10	*ca.* 4.15	*ca.* 4.15
4	4.21 (t, 8.5)	*ca.* 4.24	*ca.* 4.21	*ca.* 4.22
5	*ca.* 3.62	3.64 (ddd, 3.5, 3.5, 9.0)	3.68 (ddd, 3.5, 5.0, 9.0)	3.69 (ddd, 3.5, 5.0, 9.0)
6	*ca.* 4.32	*ca.* 4.32	4.30 (dd, 5.0, 11.5)	4.32 (dd, 5.0, 11.5)
			4.35 (dd, 3.5, 11.5)	4.38 (dd, 3.5, 11.5)
Xyl 1	5.35 (d, 7.0)	5.37 (d, 7.0)	5.40 (d, 7.0)	5.42 (d, 7.0)
2	*ca.* 4.10	*ca.* 4.10	4.11 (dd, 7.0, 9.0)	*ca.* 4.14
3	*ca.* 4.17	*ca.* 4.22	*ca.* 4.25	*ca.* 4.25
4	*ca.* 4.17	*ca.* 4.19	*ca.* 4.20	*ca.* 4.23
5	*ca.* 3.75	*ca.* 3.74	3.74 (dd, 9.5, 11.5)	3.75 (dd, 9.5, 11.5)
	4.43 (dd, 5.0, 11.5)	4.43 (dd, 5.0, 11.0)	4.45 (dd, 5.0, 11.5)	4.46 (dd, 5.0, 11.5)
Rha 1				
2				
3				
4				
5				
6				

$C_{54}H_{87}O_{27}S$. Compound **5** and rotundioside A (**17**) yielded only D-glucose as a component sugar on acid hydrolysis. The ^{13}C-NMR spectrum of **5** was analogous to that of **17** with regard to the signals arising from the aglycone moiety. The structure of the aglycone moiety of **5** was established to be the same as that of **17** (echinocystic acid).

The ^{1}H-NMR spectrum of **5** (Table 1) showed signals from seven tertiary methyl groups (δ 0.91, 0.97, 0.98, 1.03, 1.09, 1.32, 1.78); one trisubstituted olefinic group (δ 5.56, br s); and four anomeric protons (δ 4.95, d, J=7.5 Hz; δ 5.00, d, J=7.5 Hz; δ 5.30, d, J=7.5 Hz; δ 6.16, d, J=8.0 Hz). The ^{13}C-NMR spectrum of **5** (Table 2) exhibited signals from six C–C-bonded quaternary carbons (δ 30.74, 37.21, 38.88, 40.08, 42.05, 49.19); trisubstituted olefinic carbons (δ 122.64, 144.45); an ester carbon (δ 176.10); and four anomeric carbons (δ 95.76, 103.03, 105.29, 106.02). From the above-mentioned results, **5** was presumed to be a 3β-sulfate ester of a 16α-hydroxyolean-12-ene-28-oyl 28-*O*-

Table 1. (Continued)

	16	18	19
H-1	*ca.* 1.47	*ca.* 1.03	*ca.* 1.00
	ca. 2.09	*ca.* 1.84	*ca.* 1.83
H-2	*ca.* 1.92	*ca.* 1.92	*ca.* 1.93
	ca. 2.20	*ca.* 2.19	*ca.* 2.20
H-3	3.33 (dd, 4.5, 11.5)	3.29 (dd, 4.5, 11.5)	3.29 (dd, 4.5, 11.5)
H-5	0.92 (br d, 12.0)	*ca.* 0.83	*ca.* 0.80
H-6	*ca.* 1.35, 1.55	*ca.* 1.52	*ca.* 1.51
H-7	*ca.* 1.30, 1.61	*ca.* 1.28, 1.53	*ca.* 1.27, 1.48
H-9	2.02 (d, 8.5)	*ca.* 2.08	2.05 (br s)
H-11	4.85 (dd, 3.5, 8.5)	5.69 (dd, 3.5, 10.5)	5.69 (dd, 3.0, 10.5)
H-12	5.61 (d, 3.5)	6.01 (d, 10.5)	6.01 (d, 10.5)
H-15	*ca.* 1.61	*ca.* 1.52	*ca.* 1.52
	ca. 2.09	*ca.* 2.17	2.17 (dd, 5.0, 14.5)
H-16	4.59 (br s)	*ca.* 4.20	*ca.* 4.23
H-18	2.59 (dd, 4.0, 13.5)	*ca.* 2.00	2.00 (dd, 3.0, 14.0)
H-19	*ca.* 1.40	*ca.* 1.41	1.43 (br d, 12.5)
	2.74 (dd, 13.5, 13.5)	2.69 (dd, 12.5, 14.0)	2.70 (dd, 12.5, 14.0)
H-21	*ca.* 1.42	*ca.* 1.28	*ca.* 1.27
	2.44 (ddd, 6.5, 12.0, 12.0)	2.53 (ddd, 5.5, 13.5, 13.5)	2.53 (ddd, 5.5, 13.5, 13.5)
H-22	*ca.* 2.25	*ca.* 1.62	1.63 (ddd, 5.5, 13.5, 13.5)
		ca. 1.92	*ca.* 1.93
H-23	1.37 (s)	1.35 (s)	1.29 (s)
H-24	1.12 (s)	1.09 (s)	1.11 (s)
H-25	1.05 (s)	0.92 (s)	0.95 (s)
H-26	0.99 (s)	1.33 (s)	1.33 (s)
H-27	1.92 (s)	1.68 (s)	1.67 (s)
H-28	3.60 (d, 10.0)	3.33 (d, 7.0)	3.30 (d, 7.5)
	3.69 (d, 10.0)	3.59 (d, 7.0)	3.59 (d, 7.5)
H-29	1.04 (s)[n)]	1.06 (s)[o)]	1.06 (s)[p)]
H-30	1.14 (s)[n)]	0.98 (s)[o)]	0.98 (s)[p)]
OCH_3	3.27 (s)		
Fuc 1	4.77 (d, 7.5)	4.77 (d, 7.5)	4.76 (d, 7.5)
2	4.59 (dd, 7.5, 9.0)	4.61 (dd, 7.5, 9.5)	4.50 (dd, 7.5, 9.0)
3	4.37 (dd, 3.0, 9.0)	*ca.* 4.38	*ca.* 4.23
4	3.80 (br d, 3.0)	3.82 (br d, 3.0)	4.01 (br d, 3.0)
5	3.76 (br q, 6.5)	3.75 (br q, 6.5)	3.81 (br q, 6.5)
6	1.47 (d, 6.5)	1.49 (d, 6.5)	1.55 (d, 6.5)
Glc 1	5.65 (d, 8.0)	5.67 (d, 7.5)	5.42 (d, 7.5)
2	4.27 (dd, 8.0, 9.0)	4.29 (dd, 7.5, 9.5)	*ca.* 4.16
3	4.18 (t, 9.0)	4.22 (t, 9.5)	*ca.* 4.13
4	4.04 (t, 9.0)	4.05 (t, 9.5)	*ca.* 4.20
5	3.60 (ddd, 4.0, 5.5, 9.0)	3.61 (ddd, 3.0, 5.5, 9.5)	*ca.* 3.68
6	4.18 (dd, 5.5, 12.0)	4.18 (dd, 5.5, 11.5)	4.29 (dd, 4.5, 11.5)
	4.27 (dd, 4.0, 12.0)	4.29 (dd, 3.0, 11.5)	4.35 (dd, 3.0, 11.5)
Xyl 1			5.39 (d, 7.0)
2			*ca.* 4.11
3			*ca.* 4.21
4			*ca.* 4.15
5			3.74 (dd, 10.0, 11.5)
			4.45 (dd, 5.0, 11.5)
Rha 1	6.39 (br s)	6.41 (br s)	
2	*ca.* 4.74	*ca.* 4.76	
3	*ca.* 4.74	*ca.* 4.76	
4	4.33 (br t, 9.5)	4.35 (br t, 9.5)	
5	5.00 (dq, 9.5, 6.0)	5.06 (dq, 9.5, 6.0)	
6	1.81 (d, 6.0)	1.84 (d, 6.0)	

Table 1. (Continued)

	5	6
H-1	*ca.* 0.94	*ca.* 0.93
	ca. 1.51	*ca.* 1.51
H-2	*ca.* 1.91	*ca.* 1.91
	ca. 2.65	*ca.* 2.64
H-3	*ca.* 4.50	*ca.* 4.47
H-5	0.84 (br d, 12.0)	0.83 (br d, 12.0)
H-6	*ca.* 1.30, 1.46	*ca.* 1.30, 1.46
H-7	*ca.* 1.37, 1.51	*ca.* 1.35, 1.51
H-9	*ca.* 1.70	*ca.* 1.70
H-11	*ca.* 1.96	*ca.* 1.93
H-12	5.56 (br s)	5.57 (br s)
H-15	*ca.* 1.76	*ca.* 1.76
	ca. 2.47	*ca.* 2.48
H-16	5.26 (br s)	5.26 (br s)
H-18	3.47 (dd, 4.0, 14.0)	3.46 (dd, 4.0, 14.0)
H-19	*ca.* 1.34	*ca.* 1.34
	2.76 (dd, 13.0, 14.0)	2.75 (dd, 13.5, 13.5)
H-21	*ca.* 1.27	*ca.* 1.20
	ca. 2.38	*ca.* 2.33
H-22	2.14 (ddd, 4.5, 14.5, 14.5)	*ca.* 2.04
	ca. 2.38	*ca.* 2.23
H-23	1.32 (s)	1.31 (s)
H-24	0.98 (s)	0.97 (s)
H-25	0.91 (s)	0.91 (s)
H-26	1.09 (s)	1.09 (s)
H-27	1.78 (s)	1.78 (s)
H-28		
H-29	0.97 (s)	0.96 (s)
H-30	1.03 (s)	1.01 (s)
Glc 1	6.16 (d, 8.0)	6.19 (d, 8.0)
2	*ca.* 4.03	*ca.* 4.18
3	*ca.* 4.13	*ca.* 4.18
4	*ca.* 4.27	*ca.* 4.47
5	*ca.* 4.03	*ca.* 4.05
6	*ca.* 4.37	*ca.* 4.30
	4.65 (br d, 9.5)	*ca.* 4.56
Glc 1	4.95 (d, 7.5)	4.93 (d, 7.5)
2	*ca.* 3.98	*ca.* 4.01
3	*ca.* 4.10	*ca.* 4.22
4	*ca.* 4.37	*ca.* 4.14
5	*ca.* 3.94	*ca.* 3.92
6	*ca.* 4.27	*ca.* 4.33
	4.60 (br d, 9.0)	*ca.* 4.52
Glc 1	5.00 (d, 7.5)	5.26 (d, 7.5)
2	*ca.* 4.10	*ca.* 4.03
3	*ca.* 4.22	*ca.* 4.14
4	*ca.* 4.16	*ca.* 4.18
5	3.80 (ddd, 2.5, 5.0, 9.5)	3.76 (ddd, 2.5, 5.0, 9.0)
6	*ca.* 4.31	4.28 (dd, 5.0, 12.0)
	4.45 (dd, 2.5, 12.0)	4.37 (dd, 2.5, 12.0)
Glc 1	5.30 (d, 7.5)	
2	*ca.* 4.06	
3	*ca.* 4.10	
4	*ca.* 4.10	
5	*ca.* 3.90	
6	*ca.* 4.27	
	ca. 4.36	

tetraglucoside, such as rotundioside A (**17**). The last problem concerning the structure of **5** is the position of the glucopyranosyl group. This problem was solved by NMR spectroscopy. All proton signals from the sugar moieties of **5** were assigned as summarized in Table 1, using ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), nuclear Overhauser effect (NOE) difference spectroscopy (NOEDS), decoupling difference spectroscopy, and homonuclear Hartmann–Hahn (HOHAHA) spectroscopy techniques. Thus, the NOE between the anomeric proton of the terminal glucopyranosyl group (G‴-1) and the H-2 of the third glucopyranosyl group (G‴-2), the anomeric proton of the third glucopyranosyl group (G‴-1) and the H-6 of the second glucopyranosyl group (G″-6), and the anomeric proton of the second glucopyranosyl group (G″-1) and the H-6 of the inner glucopyranosyl group (G′-6) were observed. These spectral data indicate that the structure of the sugar moiety of **5** is β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1→6)-β-D-glucopyra-

Table 2. ^{13}C-NMR Data (δ) for Compounds **1**—**16**, **18** and **19** (Pyridine-d_5, 125 MHz)

	1	**2**	**3**	**4**	**7**	**8**	**9**	**10**	**11**
1	38.58	38.61	38.60	38.56	40.85	40.89	40.22	40.13	40.10
2	26.48	26.53	26.50	25.82	26.90	26.86	26.98	26.96	26.93
3	89.36	88.70	89.38	83.00	88.78	89.56	88.86	88.69	89.33
4	39.87	39.79	39.87	43.64	39.89	40.00	40.01	39.95	40.02
5	55.46	55.43	55.50	48.41	56.01	56.12	56.02	56.04	56.09
6	17.86	17.86	17.88	17.74	18.79	18.83	18.63	18.57	18.57
7	31.77	31.85	31.84	31.61	33.26	33.29	33.55	33.67	33.70
8	42.35	41.84	41.86	41.86	43.68	43.74	43.71	43.39	43.38
9	52.87	52.85	52.86	52.97	56.78	56.79	51.97	51.68	51.64
10	36.36	36.33	36.37	36.30	38.51	38.58	38.25	38.26	38.28
11	131.22	131.54	131.54	131.98	65.77	65.87	76.07	75.98	75.98
12	132.53	132.22	132.26	131.91	128.17	128.21	123.13	122.96	122.98
13	84.80	85.00	85.01	84.87	142.39	142.41	147.57	148.95	148.95
14	43.57	43.45	43.45	43.58	42.26	42.29	41.66	41.77	41.77
15	34.35	33.41	33.44	35.43	55.66	55.74	34.47	33.76	33.74
16	75.52	74.67	74.66	77.14	62.95	62.96	73.04	72.82	72.36
17	50.85	46.14	46.13	45.32	37.52	37.55	48.16	42.26	42.24
18	49.68	50.48	50.48	51.32	42.59	42.68	40.92	40.57	40.57
19	38.27	34.07	34.08	38.39	44.75	44.78	48.78	43.16	43.16
20	44.14	37.04	37.06	31.85	30.46	30.46	44.83	36.33	36.33
21	213.50	72.64	72.65	36.80	35.58	35.60	215.99	72.58	72.81
22	45.80	38.29	38.29	31.25	29.74	29.76	44.51	37.40	37.38
23	28.01	27.85	28.02	65.59	28.14	28.34	28.25	28.24	28.42
24	16.12	16.23	16.13	12.73	16.77	16.69	16.88	16.87	16.73
25	18.13	18.28	18.22	18.74	17.23	17.26	17.36	17.34	17.27
26	19.67	19.38	19.39	19.50	20.58	20.58	18.44	18.37	18.36
27	18.89	17.70	17.68	18.08	22.62	22.59	27.11	26.45	26.42
28	75.12	78.21	78.22	77.77	67.17	67.24	67.36	69.41	69.40
29	26.22[a)]	25.59[b)]	25.60[c)]	33.70[d)]	33.15[e)]	33.13[f)]	25.90[g)]	25.20[h)]	25.18[i)]
30	26.61[a)]	29.06[b)]	29.07[c)]	24.38[d)]	23.81[e)]	23.85[f)]	26.10[g)]	28.72[h)]	28.72[i)]
OCH_3							54.18	53.90	53.85
Fuc 1	105.20	104.87	105.19	103.57	104.92	105.23	104.97	104.90	105.22
2	78.09	80.48	77.32	81.12	80.61	77.31	80.26	80.48	77.39
3	76.17	75.47	76.17	75.49	75.45	76.16	75.55	75.47	76.17
4	72.91	72.55	72.91	72.52	72.51	72.92	72.64	72.36	72.90
5	70.83	71.11	70.82	71.01	71.10	70.79	71.20	71.11	70.79
6	17.30	17.22	17.31	17.22	17.35	17.26	17.26	17.21	17.25
Glc 1	102.14	103.09	102.15	103.31	103.14	102.17	103.06	103.10	102.18
2	79.43	84.66	78.06	84.54	84.71	78.16	84.72	84.69	78.01
3	77.28	77.61	79.44	77.63	77.64	79.43	77.61	77.59	79.44
4	72.82	71.82	72.83	71.14	71.75	72.84	71.87	71.84	72.81
5	77.08	77.37	77.03	77.51	77.37	77.06	77.57	77.37	76.99
6	63.31	62.84	63.51	62.28	62.79	63.34	62.93	62.88	63.30
Xyl 1		106.48		106.45	106.53		106.49	106.48	
2		75.79		75.81	75.83		75.80	75.78	
3		77.93		77.88	77.93		77.97	77.93	
4		70.73		70.70	70.70		70.78	70.73	
5		67.35		67.40	67.38		67.43	67.33	
Rha 1	101.85		101.83			101.88			101.81
2	72.73		72.72			72.73			72.71
3	72.46		72.45			72.46			72.45
4	74.33		74.32			74.33			74.31
5	69.44		69.46			69.46			69.47
6	18.95		18.94			18.93			18.92

a—*q*) Signals may be interchangeable in each vertical column.

nosyl(1→6)-β-D-glucopyranoside, as shown in Fig. 1.

Rotundioside K (**6**) was obtained as a white powder. The negative-ion HR-FAB-MS of **6** gave the molecular formula $C_{48}H_{77}O_{22}S$. Compound **6** and rotundioside A (**17**) only yielded D-glucose as a component sugar on acid hydrolysis. The ^{13}C-NMR spectrum of **6** was analogous to that of **17** with regard to the signals arising from the aglycone moiety. The ^{1}H-NMR spectrum of **6** (Table 1) showed signals from seven tertiary methyl groups (δ 0.91, 0.96, 0.97, 1.01, 1.09, 1.31, 1.78); one trisubstituted olefinic group (δ 5.57, br s); and three anomeric protons (δ 4.93, d, J=7.5 Hz; δ 5.26, d, J=7.5 Hz; δ 6.19, d, J=8.0 Hz). The ^{13}C-NMR spectrum of **6** (Table 2) exhibited signals from six C–C-bonded quaternary carbons (δ 30.75, 37.21, 38.87, 40.08, 42.05, 49.18); trisubstituted olefinic carbons (δ 122.61, 144.48) an ester carbon (δ 176.11); and three anomeric carbons (δ 95.74, 102.65, 105.94). From the above-mentioned results, **6** was presumed to be a 3β-sulfate ester of a 16α-hydroxyolean-12-ene-28-oyl 28-*O*-triglucoside. A comparison of the ^{13}C-NMR spectrum of **6** with that of **17** in relation to the sugar moiety

Table 2. (continued)

	12	13	14	15	16	18	19		5	6
1	40.05	38.34	38.41	40.10	40.15	38.61	38.61		38.82	38.83
2	26.20	25.89	26.57	26.96	26.94	26.49	26.52		24.95	24.94
3	83.33	82.99	88.76	88.63	89.40	89.40	88.68		85.05	85.11
4	43.75	43.62	39.76	39.92	40.03	39.87	39.77		38.88	38.87
5	48.83	48.39	55.49	55.96	56.13	55.52	55.44		56.36	56.34
6	18.46	18.42	18.56	18.56	18.63	17.89	17.87		18.73	18.73
7	33.52	32.37	32.61	33.66	33.75	31.82	31.85		33.42	33.42
8	43.43	41.06	41.85	43.32	43.39	41.87	41.85		40.08	40.08
9	51.74	53.94	53.78	51.68	51.64	52.84	52.81		47.10	47.10
10	38.24	36.51	36.55	38.21	38.29	36.37	36.33		37.21	37.21
11	76.07	126.22	126.23	75.98	76.06	131.92	131.92		23.81	23.82
12	122.35	126.18	126.11	122.29	122.38	131.95	131.90		122.64	122.61
13	149.75	136.06	136.00	149.65	149.66	84.88	84.85		144.45	144.48
14	41.98	41.85	41.04	41.91	41.97	43.60	43.58		42.05	42.05
15	34.99	31.90	31.89	34.94	34.98	35.42	35.42		36.08	36.07
16	74.13	67.72	67.73	74.13	74.17	77.15	77.14		74.28	74.26
17	40.75	45.25	45.28	40.72	40.76	45.33	45.33		49.19	49.18
18	41.98	132.99	133.06	41.87	41.97	51.33	51.32		41.24	41.27
19	48.35	39.00	39.00	48.37	48.38	38.43	38.42		47.19	47.21
20	31.29	32.51	32.57	31.30	31.29	31.85	31.85		30.74	30.75
21	37.23	35.46	35.45	37.22	37.23	36.80	36.79		35.89	35.88
22	30.75	24.43	24.43	30.86	30.74	31.25	31.25		32.08	31.92
23	66.02	64.77	27.90	28.22	28.45	28.03	27.85		28.72	28.70
24	13.32	12.80	17.19	16.86	16.77	16.13	16.23		17.16	17.14
25	17.89	18.78	18.30	17.34	17.29	18.17	18.23		15.72	15.72
26	18.49	17.22	16.32	18.35	18.42	19.45	19.43		17.57	17.55
27	26.38	21.87	21.88	26.40	26.39	18.13	18.13		27.30	27.29
28	70.06	65.57	64.78	70.02	70.03	77.77	77.75		176.10	176.11
29	33.36[j]	25.08[k]	25.07[l]	33.40[m]	33.38[n]	33.73[o]	33.72[p]		33.14	33.15
30	24.66[j]	32.56[k]	32.50[l]	24.57[m]	24.66[n]	24.38[o]	24.36[p]		24.71	24.67
OCH_3	53.78			53.87	53.81					
Fuc 1	103.54	103.66	104.91	104.91	105.22	105.18	104.84	Glc 1	95.76	95.74
2	81.08	81.12	80.57	80.48	77.38	77.30	80.51	2	73.80	74.00
3	75.52	75.48	75.44	75.48	76.17	76.13	75.45	3	77.96[q]	78.44
4	72.56	72.53	72.54	72.53	72.92	72.91	72.52	4	70.73	70.47
5	70.96	71.05	71.11	71.11	70.80	70.81	71.10	5	77.76	77.12
6	17.21	17.25	17.22	17.25	17.26	17.30	17.20	6	69.72	69.29
Glc 1	103.30	103.32	103.13	103.08	102.17	102.13	103.09	Glc 1	105.29	102.65
2	84.59	84.55	84.66	84.72	78.01	78.05	84.63	2	75.10	84.09
3	77.62	77.62	77.60	77.65	79.43	79.44	77.60	3	78.06[q]	78.07
4	71.16	71.15	71.82	71.69	72.82	72.82	70.70	4	70.88	71.07
5	77.56	77.49	77.32	77.42	76.96	77.03	77.33	5	76.30	78.65
6	62.28	62.32	62.84	62.74	63.31	63.29	62.82	6	69.87	62.12
Xyl 1	106.48	106.45	106.47	106.52			106.46	Glc 1	103.03	105.94
2	75.84	75.82	75.78	75.84			75.76	2	84.14	76.14
3	77.87	77.88	77.93	77.94			77.93	3	78.19[q]	78.09
4	70.72	70.70	70.71	70.71			71.81	4	70.79	70.70
5	67.42	67.40	67.33	67.37			67.32	5	78.48	78.26
								6	62.17	62.12
Rha 1					101.80	101.83		Glc 1	106.02	
2					72.71	72.72		2	76.19	
3					72.44	72.45		3	78.39[q]	
4					74.30	74.33		4	71.14	
5					69.46	69.44		5	78.66	
6					18.94	18.94		6	62.09	

showed an absence of signals due to the terminal glucosyl moiety from **17**. When the ^{13}C-NMR spectra of **6** and **17** were compared, the spectrum of **6** showed upfield shift of the C-6 of the third glucopyranosyl group (G‴-6, Δ 7.48 ppm), and downfield shift of the C-5 of the third glucopyranosyl group (G‴-5, Δ 1.46 ppm). These spectral data indicate that **6** has the structure shown in Fig. 1.

Rotundioside L ($C_{47}H_{76}O_{17}$) (**7**), a white powder, showed the same molecular formula of rotundifolioside D (**20**).[2] The ^{13}C-NMR spectrum of **7** was analogous to that of **20** with regard to the signals arising from the sugar moieties (Table 2). A comparison of the ^{1}H-NMR (Table 1) and ^{13}C-NMR spectra of **7** with those of **20** showed the absence of two secondary methyl groups designated as C-29 and C-30 in **20**, as well as the appearance of two tertiary methyl groups and one C–C-bonded quaternary carbon. Furthermore, the ^{13}C-NMR spectrum of **7** was analogous to that of **20**, except for the upfield shifts of C-17 (Δ 1.51 ppm), C-19 (Δ 5.59 ppm), C-20 (Δ 9.15 ppm) and C-22 (Δ 5.07 ppm), and the downfield shift of C-18 (Δ 11.09 ppm). These spectroscopic data indicate

that the aglycone of **7** is presumed to be an oleanane-type triterpene corresponding to that of the ursane-type triterpene glycoside, rotundifolioside D (**20**). From the above-mentioned results, rotundioside L (**7**) was established to be 15α,16α-epoxy-11α,28-dihydroxyolean-12-en-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

The ^{13}C-NMR spectrum of rotundioside M ($C_{48}H_{78}O_{17}$) (**8**), a white powder, was analogous to that of **7** in relation to the signals arising from the aglycone moiety (Table 2). The ^{13}C-NMR spectrum of **8** was also analogous to that of **1** with regard to the signals arising from the sugar moiety. From the above-mentioned results, rotundioside M (**8**) was established to be 15α,16α-epoxy-11α,28-dihydroxyolean-12-en-3β-yl α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

Rotundioside N ($C_{48}H_{78}O_{18}$) (**9**), a white powder, is 28 mass units (1 O and 1 C) heavier than rotundifolioside B (**21**).[2] The ^{13}C-NMR spectrum of **9** was analogous to that of **21** with regard to the signals arising from the sugar moiety (Table 2). A comparison of the ^{1}H-NMR (Table 1) and ^{13}C-NMR spectra of **9** with those of **21** showed the absence of two secondary methyl groups designated C-29 and C-30 in **21**, as well as the appearance of two tertiary methyl groups, one C–C-bonded quaternary carbon, a ketone (δ 215.99), and a methoxy group (δ 54.18). Furthermore, the ^{13}C-NMR spectrum of **9** was analogous to that of **21**, except for the upfield shifts of C-12 (Δ 8.2 ppm), C-18 (Δ 13.04 ppm) and C-28 (Δ 3.61 ppm), and the downfield shifts of C-11 (Δ 8.64 ppm), C-13 (Δ 7.25 ppm), C-17 (Δ 6.16 ppm), C-19 (Δ 9.40 ppm), C-20 (Δ 4.93 ppm), C-22 (Δ 10.34 ppm), C-29 (Δ 7.50 ppm) and C-30 (Δ 4.40 ppm). These spectroscopic data indicate that the aglycone of **9** is presumed to be an oleanane-type triterpene, corresponding to that of the ursane-type triterpene glycoside, rotundifolioside B (**21**). These spectral data also indicated the presence of a ketone at C-21 and a methoxy group at C-11. The HMBC spectrum of **9** also supported the presence of a methoxy group at C-11. Its configuration was determined to be a from $J_{H9,H11}$ (8.5 Hz). From the above-mentioned results, rotundioside N (**9**) was established to be 11α-methoxy-16α,28-dihydroxyolean-12-en-21-one-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside..

Rotundioside P ($C_{48}H_{80}O_{18}$) (**10**), a white powder, is 2 mass units (2 H) heavier than **9**. The ^{13}C-NMR spectrum of **10** was analogous to that of **9** with regard to the signals arising from the sugar moiety (Table 2). A comparison of the ^{1}H-NMR (Table 1) and ^{13}C-NMR spectra of **10** with those of **9** showed the absence of a ketone (δ 215.99) designated as C-21 in **9**, as well as the appearance of a hydroxy group (δ 72.58).

Furthermore, the ^{13}C-NMR spectrum of **10** was analogous to that of **9**, except for the upfield shift of C-17 (Δ 5.90 ppm), C-19 (Δ 5.62 ppm), C-20 (Δ 8.50 ppm) and C-22 (Δ 7.11 ppm). From the above-mentioned results, rotundioside P (**10**) was established to be 11α-methoxy-16α,21,28-trihydroxyolean-12-en-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside. The signal of H-22 (δ 2.21 dd, J=1.5, 15.0 Hz; δ 2.86, dd, J=5.0, 15.0 Hz) indicates the configuration of H-21 to be β. Thus, the configuration of the hydroxyl group at C-21 was determined to be α, and the structure of **10** was established as 11α-methoxy-16α,21α,28-trihydroxyolean-12-en-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

Rotundioside R ($C_{49}H_{82}O_{18}$) (**11**), a white powder, is 14 mass units (1 C and 2 H) heavier than **10**. The ^{13}C-NMR spectrum of **11** was analogous to that of **10** in relation to the signals arising from the aglycone moiety (Table 2). A comparison of the ^{1}H-NMR (Table 1) and ^{13}C-NMR spectra of **11** with those of **10** showed the absence of the terminal xylopyranosyl group, as well as the appearance of a rhamnopyranosyl group. From the above-mentioned results, rotundioside R (**11**) was established to be 11α-methoxy-16α,21α,28-trihydroxyolean-12-en-3β-yl α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

Rotundioside U ($C_{48}H_{80}O_{18}$) (**12**), a white powder, is the same mass as **10**. The ^{13}C-NMR spectrum of **12** was analogous to that of **10** with regard to the signals arising from the sugar moiety (Table 2). A comparison of the ^{1}H-NMR (Table 1) and ^{13}C-NMR spectra of **12** with those of **10** showed the absence of the hydroxy group designated C-21 (δ 72.58) and the tertiary methyl group (δ 28.24) designated C-23 in **10**, as well as the appearance of a methylene group and a hydroxymethyl group (δ 66.02).

Furthermore, the ^{13}C-NMR spectrum of **12** was analogous to that of **10**, except for the upfield shifts of C-3 (Δ 5.36 ppm), C-5 (Δ 7.21 ppm), C-20 (Δ 5.04 ppm), C-22 (Δ 6.65 ppm) and C-24 (Δ 3.55 ppm), and the downfield shifts of C-4 (Δ 3.8 ppm) and C-19 (Δ 5.19 ppm). From the above-mentioned results, rotundioside U (**12**) was established to be 11α-methoxy-16α,23,28-trihydroxyolean-12-en-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

The ^{13}C-NMR spectrum of rotundioside V ($C_{47}H_{76}O_{17}$) (**13**), a white powder, was analogous to that of **12** in relation to the signals arising from the sugar moiety (Table 2). The ^{1}H-NMR spectrum of **13** (Table 1) showed signals from six tertiary methyl groups (δ 0.89, 1.00, 1.01, 1.04, 1.10, 1.67); a secondary methyl group assignable to H-6 of the fucopyranosyl group (δ 1.53, d, J=6.5 Hz); one disubstituted olefinic group (δ 5.72, d, J=10.5 Hz; δ 6.70, dd, J=3.0, 10.5 Hz); and three anomeric protons (δ 4.95, d, J=7.5 Hz; δ 5.37, d, J=7.0 Hz; δ 5.41, d, J=7.5 Hz). The ^{13}C-NMR spectrum of **13** (Table 2) exhibited signals from six C–C-bonded quaternary carbons (δ 32.51, 36.51, 41.06, 41.85, 43.62, 45.25); disubstituted olefinic carbons (δ 126.18, 126.22); tetrasubstituted olefinic carbons (δ 132.99, 136.06); and three anomeric carbons (δ 103.32, 103.66, 106.45). From the above-mentioned results, the aglycone of **13** was presumed to be an oleanane-type triterpene corresponding to that of the oleanane-type triterpene glycoside, rotundioside E (**22**).[4] The ^{13}C-NMR spectrum of **13** showed a decrease of one tertiary methyl group (δ 28.2) designated as C-23 in **22**, and addition of a hydroxymethyl group (δ 64.77). When the ^{13}C-NMR spectra of **13** and **22** were compared, the spectrum of **13** showed upfield shifts of C-3 (Δ 6.61 ppm), C-5 (Δ 7.21 ppm) and C-24 (Δ 3.50 ppm), and downfield shift of C-4 (Δ 3.72 ppm), indicating the presence of a hydroxymethyl group at C-23 of **13**. These spectral data indicate that the structure of rotundioside V (**13**) is 16α,23,28-trihydroxyolean-11,13(18)-dien-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside, as shown in Fig.

Table 3. Antiproliferative Activity (GI_{50}, μM) against MK-1, HeLa and B16F10 Cell Lines

Compounds	MK-1	HeLa	B16F10
1	30.3	36.8	6.9
2	34.0	37.3	12.1
3	29.2	34.0	8.9
4	13.2	11.5	7.7
5	>100	>100	>100
6	>100	>100	>100
7	>100	>100	>100
8	>100	>100	>100
9	>100	>100	78.6
10	>100	>100	94.3
11	>100	>100	>100
12	80.5	>100	45.6
13	>100	>100	>100
14	48.0	>100	58.0
15	75.4	81.9	85.1
16	91.3	>100	70.1
17	>100	>100	>100
18	17.0	18.7	6.6
19	7.8	15.1	17.3

1.

Rotundioside W ($C_{47}H_{76}O_{16}$) (**14**), a white powder, is 16 mass units (1 O) lighter than **13**. The ^{13}C-NMR spectrum of **14** was analogous to that of **13** with regard to the signals arising from the sugar moiety (Table 2). The ^{13}C-NMR spectrum of **14** was analogous to that of **22** in relation to the signals arising from the aglycone moiety. From the above-mentioned results, rotundioside W (**14**) was established to be 16α,28-dihydroxyolean-11,13(18)-dien-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside, as shown in Fig. 1.

Rotundioside X ($C_{48}H_{80}O_{17}$) (**15**), a white powder, is 16 mass units (1 O) lighter than **12**. The ^{13}C-NMR spectrum of **15** was analogous to that of **12** with regard to the signals arising from the sugar moiety (Table 2). A comparison of the ^{1}H- and ^{13}C-NMR spectra of **15** with those of **12** showed the absence of the hydroxymethyl group (δ 66.02) designated as C-23 in **12**, as well as the appearance of a tertiary methyl group. From the above-mentioned results, rotundioside X (**15**) was established to be 11α-methoxy-16α,28-dihydroxyolean-12-en-3β-yl β-D-xylopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

Rotundioside Y ($C_{49}H_{82}O_{17}$) (**16**), a white powder, is 14 mass units (1 C and 2 H) heavier than **15**. The ^{13}C-NMR spectrum of **16** was analogous to that of **15** in relation to the signals arising from the aglycone moiety (Table 2). A comparison of the ^{1}H- and ^{13}C-NMR spectra of **16** with those of **15** showed the absence of the terminal xylopyranosyl group, as well as the appearance of a rhamnopyranosyl group. From the above-mentioned results, rotundioside Y (**16**) was established to be 11α-methoxy-16α,28-dihydroxyolean-12-en-3β-yl α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

The antiproliferative activities of isolates against MK-1, HeLa and B16F10 cell lines were examined by MTT assay. GI_{50} values are summarized in Table 3. Almost all of these compounds showed 50% growth inhibition at >40 μM except for **1**—**4**, **18** and **19**. Generally, triterpene glycosides showed no or weak antiproliferative activity, as seen for **5**—**8**, **11**, **13** and **17**. Since only **1**—**4**, **18** and **19** have epoxy ring at C-13 and C-28, it might be considered that the epoxy ring at C-13 and C-28 plays an important role in presenting the antiproliferative activity. Compound **7** and **8**, which have epoxy ring at C-15 and C-16, showed no antiproliferative activity against MK-1, HeLa and B16F10 cells. Since **7** and **8** have epoxy ring at C-15 and C-16, but not at C-13 and C-28, it might be considered that the size of an epoxy ring plays an important role in presenting the antiproliferative activity.

Experimental

The instruments and materials used in this work were the same as those shown in Part III.[6] ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR spectra were measured in pyridine-d_5. Chemical shifts are expressed on the δ scale, with tetramethylsilane as an internal standard. The signal assignment was based on comparison with data reported for compounds having similar structures, and confirmed with the aid of NMR spectral techniques (decoupling difference spectrum, NOEDS, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HMQC, long-range ^{1}H–^{13}C COSY, and HMBC).

Extraction, Fractionation and Isolation of the Constituents Extraction, fractionation and isolation of the constituents are described in a previous paper.[2] All compounds were obtained as white powders, rotations ($[\alpha]_D^{24}$ in pyridine), HR-FAB-MS data (*m/z*) of rotundiosides are shown below. ^{1}H- and ^{13}C-NMR data are listed in Tables 1 and 2, respectively.

Rotundioside O (**1**): −148.3° (*c*=0.14). $C_{48}H_{76}O_{17}Na$, calcd.: 947.4980, observed: 947.4966 ($[M+Na]^+$).

Rotundioside Q (**2**): −42.2° (*c*=0.60). $C_{47}H_{76}O_{17}Na$, calcd.: 935.4980, observed: 935.4985 ($[M+Na]^+$).

Rotundioside S (**3**): −47.8° (*c*=1.30). $C_{48}H_{78}O_{17}Na$, calcd.: 949.5137, observed: 949.5144 ($[M+Na]^+$).

Rotundioside T (**4**): +13.1° (*c*=0.99). $C_{47}H_{76}O_{17}Na$, calcd.: 935.4980, observed: 935.4988 ($[M+Na]^+$).

Rotundioside J (**5**): −39.4° (*c*=0.73). $C_{54}H_{87}O_{27}S$, calcd.: 1199.5156, observed: 1199.5177 ($[M-H]^-$).

Rotundioside K (**6**): −14.1° (*c*=0.93). $C_{48}H_{77}O_{22}S$, calcd.: 1037.4627, observed: 1037.4615 ($[M-H]^-$)

Rotundioside L (**7**): −45.2° (*c*=0.80). $C_{47}H_{76}O_{17}Na$, calcd.: 935.4980, observed: 935.4979 ($[M+Na]^+$).

Rotundioside M (**8**): −33.6° (*c*=0.82). $C_{48}H_{78}O_{17}Na$, calcd.: 949.5136, observed: 949.5121 ($[M+Na]^+$).

Rotundioside N (**9**): −103.5° (*c*=0.45). $C_{48}H_{78}O_{18}Na$, calcd.: 965.5086, observed: 965.5062 ($[M+Na]^+$).

Rotundioside P (**10**): −63.3° (*c*=2.70). $C_{48}H_{80}O_{18}Na$, calcd.: 967.5242, observed: 967.5244 ($[M+Na]^+$).

Rotundioside R (**11**): −73.5° (*c*=0.80). $C_{49}H_{82}O_{18}Na$, calcd.: 981.5399, observed: 981.5404 ($[M+Na]^+$).

Rotundioside U (**12**): −40.2° (*c*=0.73). $C_{48}H_{80}O_{18}Na$, calcd.: 967.5242, observed: 867.5251 ($[M+Na]^+$).

Rotundioside V (**13**): −76.0° (*c*=0.79). $C_{47}H_{76}O_{17}Na$, calcd.: 935.4980, observed: 935.4988 ($[M+Na]^+$).

Rotundioside W (**14**): −90.2° (*c*=0.66). $C_{47}H_{76}O_{16}Na$, calcd.: 919.5031, observed: 919.5056 ($[M+Na]^+$).

Rotundioside X (**15**): −76.1° (*c*=0.04). $C_{48}H_{80}O_{17}Na$, calcd.: 951.5293, observed: 951.5291 ($[M+Na]^+$).

Rotundioside Y (**16**): −70.3° (*c*=1.19). $C_{49}H_{82}O_{17}Na$, calcd.: 965.5450, observed: 965.5459 ($[M+Na]^+$).

Identification of Component Monosaccharides of the Glycosides A glycoside (5 mg) was dissolved in 1 N HCl/MeOH (0.5 ml) and heated at 90 °C for 1 h. The acidic solution was neutralized with an ion exchange resin (Amberlite IR-410) and concentrated *in vacuo*. The residue was trimethylsilylated and checked by gas–liquid chromatography (GLC). Authentic sugar samples were treated in the same manner and t_R values were compared with those of the tetramethylsilyl derivatives of the methanolysate of the glycoside.

The absolute configurations of the component monosaccharides were determined according to the method reported by Hara *et al.*[7] Thus, glycosides (5 mg) were hydrolyzed with 1 N HCl. After neutralization with Amberlite IR-410, the free sugars in the hydrolysate were converted into thiazolidine derivatives and checked by GLC after trimethylsilylation. Authentic sugar samples were treated in the same manner, and the unknown sugar was identified by comparison of its t_R value with those of the authentic sugar derivatives.

Measurement of Antiproliferative Activity against Tumor Cell Lines MK-1, HeLa and B16F10 were used as tumor cell lines. Cell growth was evaluated using the MTT-microculture tetrazolium assay described by Mosmann.[8] MK-1 cells were provided by Prof. M. Katano, Faculty of Medicine, Kyushu University, Japan, and HeLa and B16F10 cells were supplied by the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University, Japan.

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