Novel Acylated Saponins from Montbretia (*Crocosmia crocosmiiflora*). Isolation of Saponins and the Structures of Crocosmiosides A, B and H

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Nine novel triterpenoid saponins, named crocosmiosides A—I, were isolated from the corms of montbretia (*Crocosmia crocosmiiflora* N. E. BR., Iridaceae). Among those saponins, the structures of crocosmiosides A (1), B (2) and H (3) were determined on the basis of spectral and chemical evidence. They are unique acylated saponins which have 2,9,16-trihydroxypalmitic acid glycoside as the acyl moiety, and their structures were elucidated as polygalacic acid-3-{α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosido}-28-{2-*O*-[β-D-apio-D-furanosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-*O*-(9-hydroxy-16-α-L-rhamnopyranosyloxy-2-β-D-xylopyranosyloxyhexadecanoyl)- β-D-fucopyranoside} (1), polygalacic acid-3-{α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosido}-28-{2-*O*-[β-D-apio-D-furanosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-*O*-(9,16-dihydroxy-2-β-D-xylopyranosyloxyhexadecanoyl)-β-D-fucopyranoside} (2) and polygalacic acid-3-{α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosido}-28-{2-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-*O*-(9-hydroxy-16-α-L-rhamnopyranosyloxy-2-β-D-xylopyranosyloxyhexadecanoyl)-β-D-fucopyranoside} (3), respectively.

Keywords *Crocosmia crocosmiiflora*; montbretia; Iridaceae; crocosmioside A; crocosmioside B; crocosmioside H; acylated saponin; 2,9,16-trihydroxypalmitic acid; polygalacic acid; triterpenoid saponin

Montbretia (*Crocosmia crocosmiiflora* N. E. BR., Iridaceae) is widely cultivated as a garden plant. Several phytochemical investigations of this plant have been undertaken. An antimicrobial naphtazarin derivative has been isolated by Masuda *et al.*[1] The hot water extract of its corms has been found to have antitumor activity, and Nagamoto *et al.* reported that the antitumor active principle is a mixture of saponins composed of medicagenic acid and polygalacic acid as major sapogenins.[2] In our previous papers, we reported the structures of two acylated flavonol glycosides[3] and two acylated saponins.[4] The present paper describes the isolation of nine saponins named crocosmiosides A—I, and the structure determination of crocosmiosides A (**1**), B (**2**) and H (**3**).

The nine novel crocosmiosides were isolated from the methanolic extract of commercial corms of montbretia as shown in Chart 1.

Crocosmioside A (**1**) revealed the $(M-H)^-$ ion peak at *m/z* 1917 in the negative ion fast atom bombardment mass spectrum (FAB-MS). The infrared (IR) spectrum showed absorptions at 3420 cm^{-1} (OH) and 1735 cm^{-1} (ester). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed signals of six tertiary methyl groups at δ 0.79, 0.88, 0.93, 0.97, 1.28, 1.39 and three secondary methyl groups at δ 1.09, 1.25, 1.32. It also showed eight anomeric proton signals at δ 4.25 (1H, d, J=6.5 Hz), 4.33 (1H, d, J=7.0 Hz), 4.43 (1H, d, J=7.8 Hz), 4.49 (1H, d, J=7.8 Hz), 4.65 (1H, d, J=1.5 Hz), 5.05 (1H, d, J=3.0 Hz), 5.35 (1H, d, J=1.5 Hz) and 5.38 (1H, d, J=8.0 Hz). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum showed eight anomeric carbon signals at δ 96.2, 101.8, 101.9, 105.0, 105.2, 105.6, 107.2, 109.6 and two ester carbon signals at δ 174.7 and 177.7. Also, methylene carbon signals due to hydroxylated fatty acid were observed at δ 20—40. Acidic hydrolysis of **1** yielded an aglycone (**4**). The ^{1}H-NMR spectrum of **4** exhibited signals due to six tertiary methyl groups, a hydroxymethyl group, three carbinyl protons, an olefinic proton and an assignable allylic proton. The identification of **4** as polygalacic acid [5] was established by comparison of the ^{13}C-NMR spectrum with reference data[6] and of the IR spectrum of its methyl ester with that of an authentic sample.

The negative ion FAB-MS of crocosmioside B (**2**) revealed the $(M-H)^-$ ion peak at *m/z* 1771 which is 146 mass units (deoxyhexose) less than that of **1**. The ^{1}H-NMR spectrum of **2** showed signals of six tertiary methyl groups at δ 0.79, 0.88, 0.93, 0.97, 1.28, 1.39 and two secondary methyl groups at δ 1.09, 1.32. It also showed seven anomeric proton signals at δ 4.25 (1H, d, J=6.5 Hz), 4.32 (1H, d, J=7.0 Hz), 4.43 (1H, d, J=7.8 Hz), 4.49 (1H, d, J=7.8 Hz), 5.05 (1H, d, J=3.0 Hz), 5.35 (1H, d, J=1.5 Hz) and 5.38 (1H, d, J=8.0 Hz). In the ^{13}C-NMR spectrum, all of the aglycone carbon signals of **2** were almost superimposable on those of **1**, indicating that **2** has the same aglycone as **1**. It also showed seven anomeric carbon signals at δ 95.2, 101.7, 105.0, 105.2, 105.6, 107.2 and 109.6. Detailed comparisons of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **1** have shown that those of **2** lack the signals due to a rhamnosyl moiety.

Crocosmioside H (**3**) revealed the pseudo-molecular ion peak at *m/z* 1809 $(M+Na)^+$ in the positive ion FAB-MS. The ^{1}H-NMR spectrum of **3** showed signals of six tertiary methyl groups and three secondary methyl groups in analogy with those of **1**. It also showed seven anomeric proton signals at δ 4.24 (1H, d, J=6.5 Hz), 4.32 (1H, d, J=7.0 Hz), 4.43 (1H, d, J=7.8 Hz), 4.48 (1H, d, J=7.8 Hz), 4.65 (1H, d, J=1.5 Hz), 5.36 (1H, d, J=1.5 Hz) and 5.38 (1H, d, J=8.0 Hz). Detailed comparisons of the ^{1}H- and ^{13}C-NMR spectra of **3** with those of **1** have shown that those of **3** lack the signals due to an apiosyl moiety.

The results of methylation analyses[7] of **1**, **2** and **3** by gas chromatography–mass spectrometry (GC-MS) supported the above spectral evidence. Namely, **1**, **2** and **3** were each subjected to methylation analyses as follows; acid hydrolyses of permethylation products obtained by Hakomori's method, followed by $NaBH_4$ reduction and subsequent acetylation afforded a partially methylated alditol acetate mixture. These methyl alditol acetate mixtures were subjected to GC-MS analyses. The analytical result in the case of **1** suggested the presence of 6-linked glucopy-

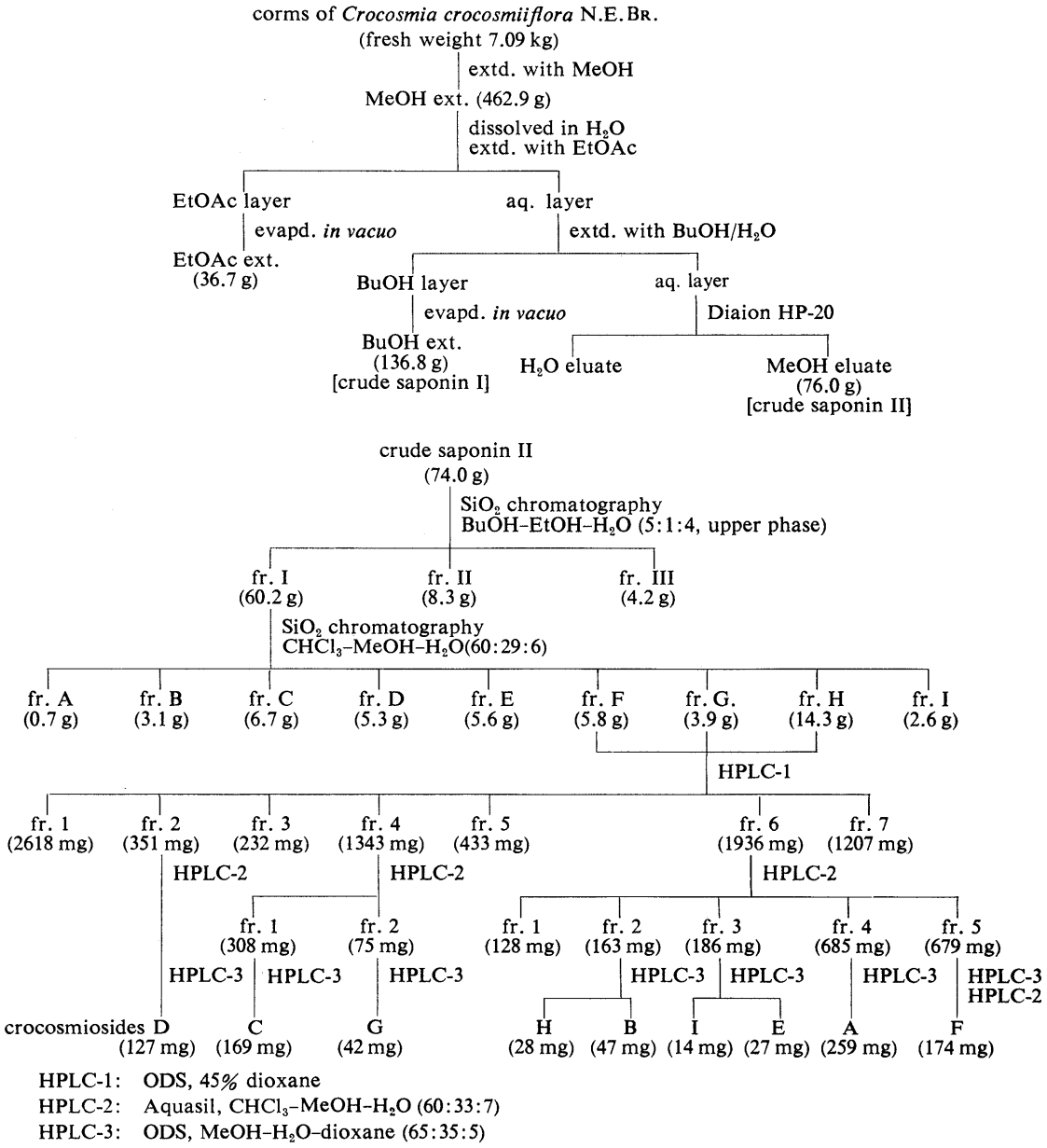

Chart 1. Extraction and Isolation of Crocosmiosides

ranose, terminal arabinopyranose and/or xylopyranose, 2-linked fucopyranose, 4-linked rhamnopyranose, 2- or 4-linked xylopyranose, terminal apiofuranose and terminal rhamnopyranose. To distinguish 4-linked xylopyranose from 2-linked xylopyranose, $NaBD_4$ was used instead of $NaBH_4$ for reduction. The mass fragments at *m/z* 118 and 189 arising from 4-linked xylopyranose can be distinguished from those at *m/z* 117 and 190 arising from 2-linked xylopyranose.[8] Similarly, the analytical result in the case of **2** suggested the presence of 6-linked glucopyranose, terminal arabinopyranose or xylopyranose, 2-linked fucopyranose, 4-linked rhamnopyranose, 4-linked xylopyranose and terminal apiofuranose, and that in the case of **3** suggested the presence of 6-linked glucopyranose, terminal arabinopyranose and/or xylopyranose, 2-linked fucopyranose, 4-linked rhamnopyranose and terminal rhamnopyranose.

As the IR absorption at 1735 cm^{-1} and the signals at δ 174.7 and 177.7 in the ^{13}C-NMR spectrum suggested the presence of two kinds of ester groups in **1**, weak alkaline treatment was examined to hydrolyze one of them. On hydrolysis with 0.5% $NaHCO_3$, **1** afforded a carboxylic acid glycoside (**5**) and desacylcrocosmioside A (**6**). The ^{13}C-NMR spectrum of **5**, MS *m/z* 605 $(M+Na)^+$, showed signals due to rhamnoside and xyloside moieties and signals at δ 68.8 (t), 72.7 (d), 79.4 (d) which suggested the presence of a primary hydroxy and two secondary hydroxy groups in palmitic acid. The positions of the OH groups were determined to be C-2, C-9, and C-16 from the ^1H- and ^{13}C-NMR spectra of **5** and the MS fragmentation of the methyl ester trimethylsilyl ether (**7**) derived from **5** (Chart 2). On the other hand, the result of enzymatic hydrolysis of **1** with naringinase, which afforded **2** and rhamnose, suggested that the rhamnoside moiety is attached to the 16-OH group in the carboxylic acid. Namely, comparing the signals at C-16 and C-15 of carboxylic acid in **1** with those in **2**, glycosylation shifts of +5.6 ppm (C-16) and −3.1 ppm (C-15) were observed. Furthermore, the location of the xyloside

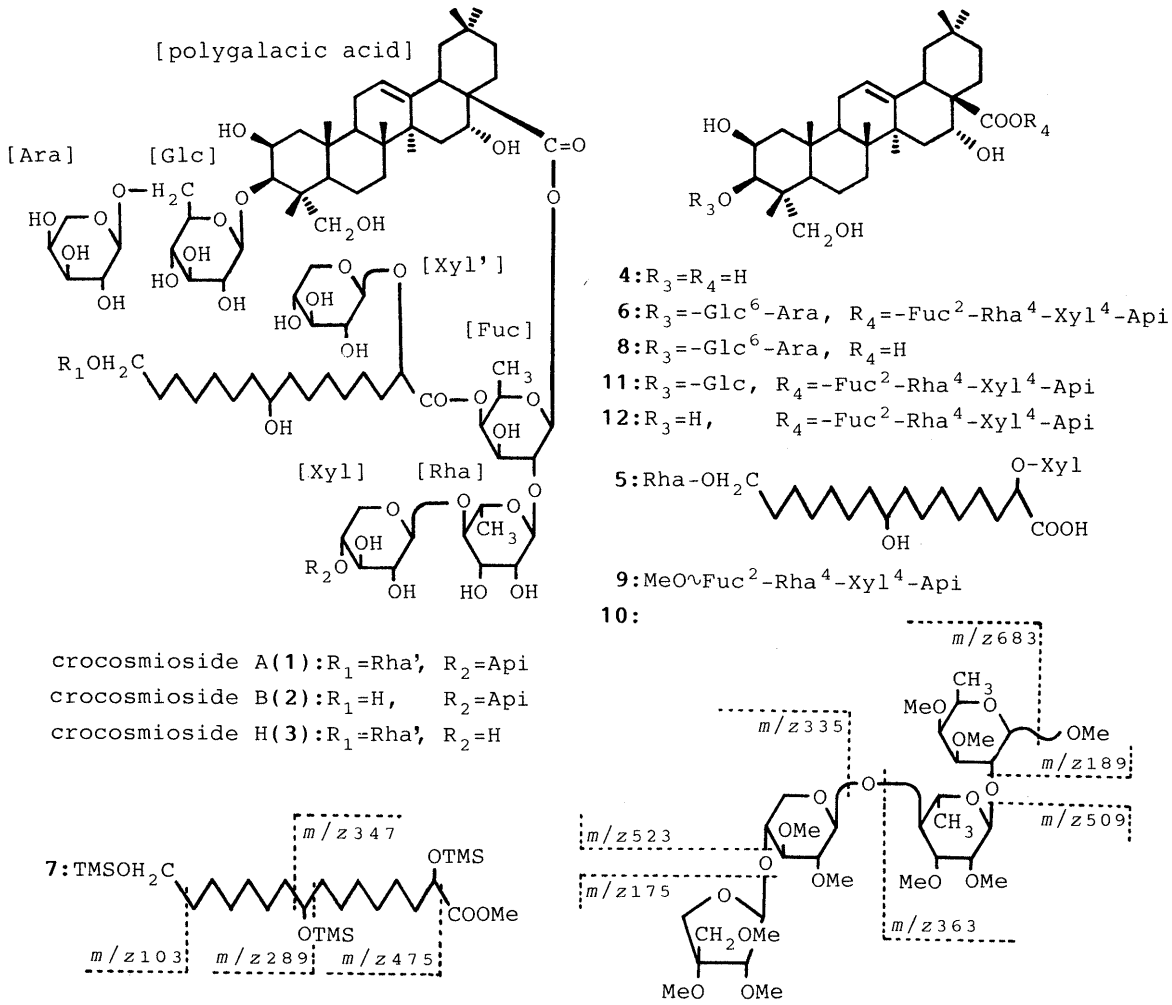

Chart 2. Structures of Crocosmiosides A, B, H and Related Compounds

moiety was determined to be C-2 in the carboxylic acid (**5**) on the basis of the ^{13}C–^{1}H heteronuclear shift correlation 2D spectrum (CH–COSY) of **5**, which indicated a correlation of the glycosylation-shifted methine carbon signal at δ 79.4 with the C-2 proton signal at δ 4.08 (1H, dd, J= 6.5, 6.5 Hz). These findings led us to formulate the carboxylic acid glycoside as **5**, except for the C-2 and C-9 configurations. The ^{13}C-NMR spectrum of **6** showed six anomeric carbon signals at δ 95.3, 101.4, 104.9, 105.1, 107.2 and 109.5. Its signal at δ 177.3 and the IR absorption at 1735 cm^{-1} suggested the presence of an ester-type glycoside linkage.

Treatment of **6** with anhydrous LiI, 2,6-lutidine and anhydrous methanol,[9] afforded a hydrolyzate (**8**) and a methyl glycoside (**9**). The positive ion FAB-MS of **8** revealed the pseudo-molecular ion peak at m/z 821 $(M+Na)^+$. The ^{1}H-NMR spectrum of **8** showed two anomeric proton signals at δ4.71 (1H, d, J=6.5 Hz) and 4.92 (1H, d, J=7.8 Hz). In the ^{13}C-NMR spectrum, signals due to the glucoside and arabinoside moieties were observed with glycosylation shifts at the C-3 carbon of the sapogenin moiety (δ 84.3, downfield shift by 10.4 ppm compared with that of **4**) and at the C-6 methylene carbon of the glucoside moiety (δ 70.1). The anomeric configurations of the glucopyranoside and arabinopyranoside linkages were considered to be β and α from the J values of its anomeric proton signals in the ^{1}H-NMR spectrum. Thus, **8** was regarded as polygalacic acid 3-*O*-vicianoside (=α-arabinopyranosyl-(1→6)-β-glucopyranoside). The methyl glycoside (**9**), an α and β anomeric mixture at the methyl glycoside linkage, revealed two methoxyl and seven anomeric proton (carbon) signals in the $^{1}H(^{13}C)$-NMR spectra. The anomeric ratio was determined from the intensities of anomeric proton signals in the ^{1}H-NMR spectrum ($\alpha:\beta = ca.$ 2:1). Also, the carbon signals due to α- and β-methylglycosides were observed in an intensity ratio of 2:1 in the ^{13}C-NMR spectrum. In the ^{1}H- and ^{13}C-NMR spectra, the differences of chemical shifts between each proton and carbon, induced by α and β methoxyl groups, decreased in the order of fucoside, rhamnoside and xyloside moieties, but no difference was observed in the apioside moiety (Tables IV and V). The data suggest that the tetrasaccharide in **6** is attached to the C-28 position of the aglycone in the above order. This was further supported by the result of methylation analysis of **9** and MS fragmentation of the permethylate (**10**) (Chart 2).

On the other hand, enzymatic hydrolysis of **6** with hesperidinase gave two hydrolyzates (**11** and **12**), and their structures were elucidated from the ^{1}H- and ^{13}C-NMR spectra. The ^{13}C-NMR spectrum of **11** showed five anomeric carbon signals at δ95.0, 101.2, 105.8, 106.4 and 109.1, and also the disappearance of the signals due to the arabinoside moiety. The C-6 methylene carbon of the glucoside moiety in **11** was shifted upfield by 7.0 ppm,

TABLE I. ^{13}C-NMR Chemical Shifts of Aglycone Moieties of Crocosmiosides A (**1**), B (**2**), H (**3**) and Related Compounds[a)]

Carbon No.	1	2	3	4	6	8	11	12
1	44.7	44.7	44.7	45.6 (45.1)	44.7 (44.3)	44.5 (44.3)	44.4	45.3
2	70.9	70.9	70.9	72.6 (71.8)	70.9 (70.0)	70.9 (70.2)	70.7	71.8
3	84.4	84.4	84.3	73.9 (73.2)	84.3 (84.5)	84.3 (84.2)	84.3	73.5
4	43.4	43.4	43.4	43.2 (42.6)	43.4 (43.0)	43.3 (43.0)	42.9	42.6
5	48.4	48.3	48.4	48.2 (48.4)	48.4 (47.9)	48.3 (47.8)	48.1	48.7
6	19.2	19.1	19.0	19.2 (18.5)	19.2 (18.6)	18.9 (18.4)	18.3	18.7
7	34.1	34.1	34.1	34.1 (33.5)	34.1 (33.4)	34.0 (33.4)	33.4	33.5
8	41.3	41.2	41.2	41.0 (40.2)	41.2 (40.4)	41.0 (40.2)	40.3	40.4
9	48.7	48.7	48.7	48.5 (47.8)	48.7 (47.7)	48.7 (47.7)	47.7	47.8
10	37.9	37.8	37.8	38.1 (37.4)	37.9 (37.2)	37.8 (37.2)	37.2	37.5
11	25.1	25.1	25.1	24.9 (24.2)	25.0 (24.2)	25.0 (24.2)	24.2	24.2
12	124.0	123.9	123.9	123.8 (122.8)	123.7 (122.8)	123.8 (122.8)	122.7	122.8
13	144.9	144.9	144.9	145.5 (145.3)	144.8 (144.6)	145.4 (145.2)	144.6	144.7
14	43.3	43.3	43.3	43.0 (42.4)	43.3 (42.5)	43.2 (42.4)	42.4	42.5
15	36.7	36.7	36.7	36.5 (36.3)	36.8 (36.4)	36.4 (36.3)	36.4	36.4
16	75.1	75.1	75.1	75.6 (74.9)	74.9 (74.3)	75.5 (74.9)	74.2	74.2
17	50.5	50.5	50.4	48.8 (49.0)	50.4 (49.4)	48.9 (49.0)	49.4	49.5
18	42.8	42.7	42.8	42.4 (41.6)	42.7 (41.9)	42.4 (41.6)	41.8	41.8
19	48.4	48.3	48.3	48.0 (47.4)	48.4 (47.7)	48.0 (47.4)	47.6	47.7
20	31.7	31.7	31.7	31.7 (31.2)	31.7 (30.9)	31.7 (31.2)	30.9	30.9
21	36.9	36.9	36.8	36.9 (36.3)	36.9 (36.2)	36.9 (36.4)	36.2	36.2
22	32.3	32.3	32.3	33.0 (33.1)	32.4 (32.1)	33.1 (33.0)	32.1	32.1
23	65.9	65.9	65.8	67.9 (67.8)	65.9 (66.3)	65.9 (66.0)	65.9	68.3
24	15.3	15.2	15.2	14.4 (14.8)	15.2 (15.4)	15.1 (15.4)	15.2	14.8
25	18.4	18.3	18.4	18.2 (17.7)	18.1 (17.7)	18.1 (17.7)	17.6	17.7
26	18.1	18.1	18.1	17.8 (17.5)	18.0 (17.7)	17.8 (17.6)	17.6	17.7
27	27.6	27.5	27.6	27.7 (27.4)	27.6 (27.3)	27.7 (27.4)	27.3	27.3
28	177.7	177.7	177.6	181.6 (180.2)	177.3 (176.2)	181.5 (181.2)	176.2	176.2
29	33.8	33.7	33.8	33.8 (33.5)	33.7 (33.4)	33.8 (33.5)	33.3	33.3
30	25.5	25.4	25.4	25.3 (24.8)	25.2 (24.6)	25.2 (24.9)	24.6	24.6

a) The spectra of **1**—**4**, **6** and **8** were measured in CD_3OD, and those of **4** (in parenthesis), **6** (in parenthesis), **8** (in parenthesis), **11** and **12** in C_5D_5N.

compared with that of **6**. This observation suggests that the arabinoside moiety is linked to C-6 of glucose. In the ^{13}C-NMR spectrum of **12**, the disappearance of the signals due to the glucoside moiety was observed, compared with that of **11**, resulting in an upfield shift (δ 73.5, -10.8 ppm) of the C-3 carbon of the sapogenin moiety. Assignments of carbon signals in **12** were achieved by analyses of HH- and CH-COSY spectra and a comparison of the spectral data with those of onjisaponin E.[11)] The result of this hydrolysis indicates that the vicianoside moiety is attached to the C-3 OH group of polygalacic acid, as described previously in **8**.

The carbon signals due to the 3-*O*-glycosyl moiety of **1** are similar to those of **8**, suggesting that the carboxylic acid glycoside (**5**) is attached to the 28-*O*-glycosyl moiety of **1**. In comparing the ^{1}H-NMR spectra of **1** and **6**, a characteristic doublet signal was observed at δ 5.09 (1H, d, J=3.5 Hz) in **1**,[12)] and it was assigned to the C-4 proton of fucose on the basis of the HH-COSY spectrum of **1**; δ 5.38 (1H, d, J= 8.0 Hz, Fuc-1), 3.74 (1H, dd, J=9.5, 8.0 Hz, Fuc-2), 3.90 (1H, dd, J=9.5, 3.5 Hz, Fuc-3) and 5.09 (1H, d, J=3.5 Hz, Fuc-4), while the C-4 proton signal of fucose of **6** was observed at δ 3.55 (1H, d, J=3.5 Hz). The assignment of the fucopyranosyl moiety in **6** was achieved by analysis of the HH-COSY spectrum and a proton decoupling experiment. Furthermore, in comparing the ^{13}C-NMR spectra of **1** and **6**, acylation shifts were observed for the signals due to C-3 (-1.8 ppm), C-4 ($+2.4$ ppm) and C-5 (-1.6 ppm). Based on the above evidence, the linkage site of the carboxylic acid glycoside (**5**) in **1** was determined to be the C-4 position of fucose.

As described previously, enzymatic hydrolysis of crocosmioside A (**1**) with naringinase yielded crocosmioside B (**2**). On the other hand, acid hydrolysis of **1** with 2 N HCl afforded crocosmioside H(**3**) and D-apiose, $[\alpha]_D$ $+6°$ (H_2O). The absolute configurations of component monosaccharides in **3** were confirmed by the procedure reported by Oshima *et al.*[13)] From the high performance liquid chromatography (HPLC) analysis of 1-deoxy-1-(*N*-acetyl-α-methylbenzylamino) alditol acetates, which were derived from component sugars of **3** and chiral α-methylbenzylamine in the presence of $NaBH_3CN$, fucose, glucose, xylose, rhamnose and arabinose in **3** were determined to be D, D, D, L and L, respectively. On the other hand, the anomeric configurations of glycoside linkages in **1** were determined from the J values of its anomeric proton signals in the ^{1}H-NMR spectrum. In the ^{1}H-NMR spectrum of **1**, eight anomeric proton signals, which were assigned by HH-COSY, CH-COSY and the comparison of the ^{1}H-NMR spectra of **1**, **2**, **3**, **6** and **8**, were observed at δ 4.25 (1H, d, J=6.5 Hz, Ara), 4.33 (1H, d, J=7.0 Hz, Xyl′), 4.43 (1H, d, J=7.8 Hz, Glc), 4.49 (1H, d, J=7.8 Hz, Xyl), 4.65 (1H, d, J=1.5 Hz, Rha′), 5.05 (1H, d, J=3.0 Hz, Api), 5.35 (1H, d, J=1.5 Hz, Rha) and 5.38 (1H, d, J=8.0 Hz, Fuc). From the J values of these anomeric proton signals, the configurations of the D-fucopyranose, D-glucopyranose, D-xylopyranose and L-arabinopyranose units were concluded to be β, β, β and α, respectively. The configuration of the L-rhamnose unit was considered to be α from the chemical shifts at C-1 (δ 101.8, Rha; δ 101.9, Rha′), C-3 (δ 72.6, Rha; δ72.8, Rha′) and C-5 (δ 69.2, Rha; δ 70.1, Rha′), and the $J_{C_1-H_1}$ values (d, J=173 Hz, Rha; d, J=168 Hz, Rha′) of its anomeric carbon signals in the ^{13}C-NMR spectrum.[14)]

TABLE II. ^{13}C-NMR Chemical Shifts of Sugar Moieties of Crocosmiosides A (**1**), B (**2**), H (**3**) and Related Compounds[a]

		1	2	3	6	8	11	12
C-3 sugars								
Glc	1	105.0	105.0	105.0	104.9 (105.6)	105.0 (105.7)	105.8	
	2	75.6	75.6	75.5	75.5 (75.5)	75.6 (75.5)	75.6	
	3	78.6	78.6	78.5	78.5 (78.7)	78.6 (78.7)	78.7[b]	
	4	72.3	72.3	72.2	72.2 (72.2)	72.2 (72.2)	71.7	
	5	76.8	76.8	76.8	76.8 (76.6)	76.8 (76.7)	78.4[b]	
	6	70.1	70.1	70.1	70.1 (69.8)	70.1 (69.7)	62.8	
Ara	1	105.2	105.2	105.2	105.1 (105.1)	105.2 (105.1)		
	2	72.8	72.8	72.8	72.8 (72.6)	72.8 (72.6)		
	3	74.4	74.4	74.3	74.3 (74.3)	74.4 (74.4)		
	4	69.8	69.8	69.8	69.8 (69.2)	69.8 (69.2)		
	5	67.1	67.0	67.1	67.0 (66.6)	67.1 (66.5)		
C-28 sugars								
Fuc	1	95.2	95.2	95.2	95.3 (95.1)		95.0	95.0
	2	75.4	75.3	75.1	74.6 (73.6)		73.5	73.7
	3	75.2	75.2	75.1	77.0 (76.9)		76.9	76.9
	4	76.2	76.2	76.1	73.8 (73.4)		73.4	73.4
	5	71.3	71.3	71.3	72.9 (73.6)		72.7	72.7
	6	17.0	17.0	17.0	16.9 (17.1)		17.0	17.1
Rha	1	101.8	101.7	101.7	101.4 (101.2)		101.2	101.3
	2	72.3	72.3	72.2	72.2 (72.1)		72.1	72.1
	3	72.6	72.5	72.5	72.5 (72.6)		72.6	72.7
	4	84.7	84.7	84.6	84.7 (83.5)		83.4	83.8
	5	69.2	69.2	69.2	69.0 (68.3)		68.3	68.3
	6	18.8	18.8	18.8	18.7 (18.6)		18.6	18.6
Xyl	1	107.2	107.2	107.4	107.2 (106.5)		106.4	106.6
	2	76.3	76.3	76.4	76.2 (76.1)		76.1	76.1
	3	76.8	76.8	78.5	76.8 (76.4)		76.4	76.4
	4	77.6	77.6	71.4	77.5 (76.7)		76.6	76.6
	5	65.1	65.0	67.6	65.0 (64.5)		64.5	64.5
Api	1	109.6	109.6		109.5 (109.1)		109.1	109.2
	2	78.3	78.3		78.2 (77.7)		77.7	77.7
	3	80.7	80.7		80.6 (80.4)		80.4	80.4
	4	65.4	65.3		65.3 (65.3)		65.3	65.3
	5	75.4	75.4		75.3 (75.4)		75.4	75.4

a) The spectra of **1**—**3**, **6** and **8** were measured in CD_3OD, and those of **6** (in parenthesis), **8** (in parenthesis), **11** and **12** in C_5D_5N. *b*) Assignments may be interchangeable within the same column. Glc, β-D-glucopyranosyl; Ara, α-L-arabinopyranosyl; Fuc, β-D-fucopyranosyl; Rha, α-L-rhamnopyranosyl; Xyl, β-D-xylopyranosyl; Api, β-D-apio-D-furanosyl.

The configuration of the D-apio-D-furanose unit was considered to be β on the basis of the $[M]_D$ comparison: $[M]_D$ of **1**$-[M]_D$ of **3**$=-278.3°$; $[M]_D$ of methyl-α-D-apio-D-furanoside$=+221°$; $[M]_D$ of methyl-β-D-apio-D-furanoside$=-167°$.[15]

Based on the above evidence, the structures of crocosmiosides A (**1**), B (**2**) and H (**3**), except for the absolute configurations of the 2- and 9-OH groups in the carboxylic acid moiety, were concluded to be polygalacic acid-3-{α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosido}-28-{2-*O*-[β-D-apio-D-furanosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-*O*-(9-hydroxy-16-α-L-rhamnopyranosyloxy-2-β-D-xylopyranosyloxyhexadecanoyl)-β-D-fucopyranoside} (**1**), polygalacic acid-3-{α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosido}-28-{2-*O*-[β-D-apio-D-furanosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-*O*-(9,16-dihydroxy-2-β-D-xylopyranosyloxyhexadecanoyl)-β-D-fucopyranoside} (**2**) and polygalacic acid-3-{α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosido}-28-{2-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl]-4-*O*-(9-hydroxy-16-α-L-rhamnopyranosyloxy-2-β-D-xylopyranosyloxyhexadecanoyl)-β-D-fucopyranoside} (**3**), respectively.

The sapogenin, polygalacic acid, was previously characterized as the aglycone part of polygalacins D and D_2,[16] while the sugar moieties are similar to those of onjisaponins[11] and senegins.[17] As most saponins so far obtained from monocotyledons are steroid saponins, it is interesting that triterpenoid saponins were obtained from montbretia, a monocotyledon. The chemical structures of crocosmiosides C—I and the biological activities of all the crocosmiosides will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto micro hot-stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 polarimeter in a 0.5 dm tube. IR spectra were taken on a JASCO IRA-I spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian XL-400 spectrometer. Chemical shifts are given on the δ scale (ppm). The following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet and br=broad. Coupling constants (*J* values) are given in Hz. HH-COSY, CH-COSY and distortionless enhancement by polarization transfer (DEPT) measurements were carried out to verify the assignments in **1**—**6**, **8**, **9**, **11** and **12**. Electron impact mass spectrum (EI-MS) and positive ion FAB-MS were taken on a JEOL JMS DX-300 and negative ion FAB-MS were taken on a JEOL JMS DX-303HF. For gas liquid chromatography (GLC), a Shimadzu GC-9A gas chromatograph was used. For column chromatography, Kieselgel 60 (230—400 mesh, Merck) and Wako-gel C-200 (Wako Pure Chemical Industries, Ltd.) were used. Thin layer chromatography was performed on precoated Silica gel 60 F_{254} plates (Merck) and RP-18 F_{254S} plates (Merck).

Extraction and Isolation of Crocosmiosides As shown in Chart 1, commercial corms of montbretia (*Crocosmia crocosmiiflora* N. E. BR.,

Table III. ^{13}C-NMR Chemical Shifts of Carboxylic Acid Moieties in CD_3OD

Carbon No.		1	2	3	5
1		174.7	174.7	174.7	177.0
2		79.9	79.9	79.9	79.4
3		34.1	34.1	34.1	33.8
4		26.1	26.1	26.1	26.0
5		30.9[a]	30.9[a]	31.0[a]	30.9[a]
6		31.0[a]	31.0[a]	30.9[a]	30.8[a]
7		27.1	27.0[c]	27.1	27.0
8		38.7[b]	38.7[b]	38.7	38.7
9		72.8	72.8	72.8	72.7
10		38.8[b]	38.8[b]	38.7	38.7
11		27.1	27.1[c]	27.1	27.1
12		31.2[a]	31.2[a]	31.1[a]	30.7[a]
13		30.9[a]	30.9[a]	30.9[a]	31.1[a]
14		27.6	27.2	27.6	27.6
15		30.9	34.0	30.9	30.9
16		68.9	63.3	68.9	68.8
Sugar moieties					
Xyl	1	105.6	105.6	105.6	105.4
	2	75.0	75.0	75.0	74.9
	3	77.9	77.9	77.9	77.9
	4	71.4	71.4	71.4	71.4
	5	67.4	67.3	67.4	67.3
Rha	1	101.9		101.9	102.0
	2	72.7		72.7	72.7
	3	72.8		72.8	72.8
	4	74.3		74.3	74.3
	5	70.1		70.0	70.0
	6	18.4		18.4	18.4

a—*c*) Assignments may be interchangeable within the same column.

Table IV. ^{1}H-NMR Chemical Shifts and Coupling Constants of Anomeric Protons in the Methyl Glycoside (**9**)

	α-Glycoside	β-Glycoside
Fuc	5.12 (2/3H, d, J=3.5 Hz)	4.41 (1/3H, d, J=9.5 Hz)
Rha	5.51 (2/3H, br s)	6.00 (1/3H, d, J=1.0 Hz)
Xyl	4.95 (2/3H, d, J=7.5 Hz)	4.97 (1/3H, d, J=7.5 Hz)
Api	5.64 (2/3H, d, J=3.0 Hz)	5.64 (1/3H, d, J=3.0 Hz)
OMe	3.24 (2/3×3H, s)	3.39 (1/3×3H, s)

fresh weight, 7.09 kg) (Yamato-noen Co., Ltd.; purchased in 1985) were extracted with MeOH to give a MeOH extract (462.9 g), which was dissolved in H_2O and extracted with EtOAc and *n*-BuOH successively. The aqueous layer was passed through a Diaion HP-20 column and eluted with H_2O and MeOH. Crude saponin II (76.0 g) eluted with MeOH was chromatographed twice on silica gel, eluting with BuOH–EtOH–H_2O (5 : 1 : 4, top layer) and $CHCl_3$–MeOH–H_2O (60 : 29 : 6). Fractions F, G and H were subjected to normal- and reversed-phase HPLC to give crocosmiosides A—I. The conditions of HPLC were as follows; HPLC-1: column, Senshu Pak NP-118 7 μm (reversed-phase) 10×300 mm; solvent, 45% dioxane; flow rate, 3 ml/min; detection, refraction index (RI). HPLC-2: column, Senshu Pak Aquasil 5 μm (normal-phase) 10×300 mm; solvent, $CHCl_3$–MeOH–H_2O (60 : 33 : 7); flow rate, 3 ml/min; detection, RI. HPLC-3: column, Senshu Pak NP-118 (reversed-phase); solvent, MeOH–H_2O–dioxane (65 : 35 : 5); flow rate, 3 ml/min; detection, RI. The yields of crocosmiosides were 0.0037% (A), 0.0006% (B), 0.0024% (C), 0.0018% (D), 0.0004% (E), 0.0025% (F), 0.0006% (G), 0.0004% (H) and 0.0002% (I).

Crocosmioside A (1) White powder, $[\alpha]_D^{25}$ −33.6° (c=0.89, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3420, 2940, 1735, 1635. *Anal.* Calcd for $C_{90}H_{150}O_{43}\cdot 4H_2O$: C, 54.26; H, 7.99. Found: C, 54.02; H, 7.75. Negative ion FAB-MS m/z: 1917 $(M-H)^-$, 1353, 1221, 1089, 797, 665, 503, 449. ^{1}H-NMR (CD_3OD) δ: 0.79, 0.88, 0.93, 0.97, 1.28, 1.39 (each 3H, s), 1.09 (3H, d, J=6.5 Hz, Fuc-6), 1.25 (3H, d, J=6.0 Hz, Rha′-6), 1.32 (3H, d, J=6.0 Hz, Rha-6), 2.31 (1H, dd, J=13.5, 13.5 Hz, 19-H_{ax}), 2.95 (1H, dd, J=13.5, 3.5 Hz, 18-H), 3.57 (2H, s, Api-4), 3.74 (1H, dd, J=9.5, 8.0 Hz, Fuc-2), 3.78 (1H, d, J=10.0 Hz, Api-5H$_a$), 3.89 (1H, d, J=3.0 Hz, Api-2), 3.90 (1H, dd, J=9.5, 3.5 Hz, Fuc-3), 3.92 (1H, dd, J=3.0, 1.5 Hz, Rha-2), 4.10 (1H, d, J=10.0 Hz, Api-5H$_b$), 4.25 (1H, d, J=6.5 Hz, Ara-1), 4.33 (1H, d, J=7.0 Hz, Xyl′-1), 4.43 (1H, d, J=7.8 Hz, Glc-1), 4.46 (1H, br s, 16-H), 4.49 (1H, d, J=7.8 Hz, Xyl-1), 4.65 (1H, d, J=1.5 Hz, Rha′-1), 5.05 (1H, d, J=3.0 Hz, Api-1), 5.09 (1H, d, J=3.5 Hz, Fuc-4), 5.34 (1H, dd, J=3.0, 3.0 Hz, 12-H), 5.35 (1H, d, J=1.5 Hz, Rha-1), 5.38 (1H, d, J=8.0 Hz, Fuc-1).

Crocosmioside B (2) White powder, $[\alpha]_D^{25}$ −31.2° (c=0.52, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1735, 1635. *Anal.* Calcd for $C_{84}H_{140}O_{39}\cdot 6H_2O$: C, 53.60; H, 8.14. Found: C, 53.78, H, 8.01. Negative ion FAB-MS m/z: 1771 $(M-H)^-$, 1353, 1221, 1089, 797, 665, 503, 303. ^{1}H-NMR (CD_3OD) δ: 0.79, 0.88, 0.93, 0.97, 1.28, 1.39 (each 3H, s), 1.09 (3H, d, J=6.5 Hz, Fuc-6), 1.32 (3H, d, J=6.0 Hz, Rha-6), 2.31 (1H, dd, J=13.5, 13.5 Hz, 19-H_{ax}), 2.95 (1H, dd, J=13.5, 3.5 Hz, 18-H), 3.56 (2H, s, Api-4), 3.74 (1H, dd, J=9.5, 8.0 Hz, Fuc-2), 3.79 (1H, d, J=10.0 Hz, Api-5H$_a$), 3.89 (1H, d, J=3.0 Hz, Api-2), 3.90 (1H, dd, J=9.5, 3.5 Hz, Fuc-3), 3.91 (1H, dd, J=3.0, 1.5 Hz, Rha-2), 4.10 (1H, d, J=10.0 Hz, Api-5H$_b$), 4.25 (1H, d, J=6.5 Hz, Ara-1), 4.32 (1H, d, J=7.0 Hz, Xyl′-1), 4.43 (1H, d, J=7.8 Hz, Glc-1), 4.46 (1H, br s, 16-H), 4.49 (1H, d, J=7.8 Hz, Xyl-1), 5.05 (1H, d, J=3.0 Hz, Api-1), 5.08 (1H, d, J=3.5 Hz, Fuc-4), 5.34 (1H, dd, J=3.0, 3.0 Hz, 12-H), 5.35 (1H, d, J=1.5 Hz, Rha-1), 5.38 (1H, d, J=8.0 Hz, Fuc-1).

Crocosmioside H (3) White powder, $[\alpha]_D^{25}$ −20° (c=0.53, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 1740, 1635. *Anal.* Calcd for $C_{85}H_{142}O_{39}\cdot 4H_2O$: C, 54.88; H, 8.13. Found: C, 54.85; H, 7.85. Positive ion FAB-MS m/z: 1809 $(M+Na)^+$, 821. ^{1}H-NMR (CD_3OD) δ: 0.79, 0.88, 0.92, 0.97, 1.27, 1.39 (each 3H, s), 1.09 (3H, d, J=6.5 Hz, Fuc-6), 1.25 (3H, d, J=6.0 Hz, Rha′-6), 1.32 (3H, d, J=6.0 Hz, Rha-6), 2.30 (1H, dd, J=13.5, 13.5 Hz, 19-H_{ax}), 2.95 (1H, dd, J=13.5, 3.5 Hz, 18-H), 3.74 (1H, dd, J=9.5, 8.0 Hz, Fuc-2), 3.78 (1H, dd, J=3.5, 1.5 Hz, Rha′-2), 3.90 (1H, dd, J=9.5, 3.5 Hz, Fuc-3), 3.91 (1H, dd, J=3.0, 1.5 Hz, Rha-2), 4.24 (1H, d, J=6.5 Hz, Ara-1), 4.32 (1H, d, J=7.0 Hz, Xyl′-1), 4.43 (1H, d, J=7.8 Hz, Glc-1), 4.46 (1H, br s, 16-H), 4.48 (1H, d, J=7.8 Hz, Xyl-1), 4.65 (1H, d, J=1.5 Hz, Rha′-1), 5.09 (1H, d, J=3.5 Hz, Fuc-4), 5.34 (1H, dd, J=3.0, 3.0 Hz, 12-H), 5.36 (1H, d, J=1.5 Hz, Rha-1), 5.38 (1H, d, J=8.0 Hz, Fuc-1).

Table V. ^{13}C-NMR Chemical Shifts of the Methyl Glycoside (**9**) in C_5D_5N[a]

		α-Glycoside	β-Glycoside
Sugar moieties			
Fuc	1	100.7	103.9
	2	78.6	76.5[b]
	3	70.2	76.2
	4	73.6	73.3
	5	66.8	71.4
	6	17.2	17.3
	OMe	55.1	56.3
Rha	1	104.4	102.3
	2	71.8	72.1
	3	72.9	73.0
	4	84.9	85.4
	5	68.2	67.8
	6	18.6	18.3
Xyl	1	107.0	107.2
	2	76.1	76.2
	3	76.5[b]	76.5[b]
	4	76.6[b]	76.5[b]
	5	64.6	64.6
Api	1	109.2	109.2
	2	77.7	77.7
	3	80.5	80.5
	4	65.3	65.3
	5	75.4	75.4

a) Assignments of signals were achieved by analyses of HH- and CH-COSY spectra and by comparison with reference data.[10] *b*) Assignments may be interchangeable.

Hydrolysis of 1 with 2 N HCl–Dioxane A solution of **1** (25.5 mg) in 2 N HCl–dioxane (1 : 1, 5 ml) was heated under Ar gas at 100 °C for 1 h. The reaction mixture was diluted with H_2O and evaporated to remove dioxane. The solution was extracted with EtOAc and evaporated to dryness. The residue was subjected to HPLC to give polygalacic acid (**4**, 3.8 mg). Conditions for HPLC: column, Senshu Pak NP-118; solvent, 70% MeOH;

flow rate, 3 ml/min. **4**: Colorless needles (from MeOH–H_2O), mp 315—318 °C, $[\alpha]_D^{22}$ +56.7° (c=0.12, C_5H_5N). IR ν_{max}^{KBr} cm^{-1}: 3480, 2940, 1675. EI-MS m/z (%): 504 (M^+, 3), 264 (34), 246 (100), 240 (5), 222 (13), 219 (9). ^{1}H-NMR (CD_3OD) δ: 0.80, 0.87, 0.90, 0.97, 1.28, 1.38 (each 3H, s), 1.02 (1H, dd, J=13.0, 4.0 Hz, 19-H_{eq}), 1.14 (1H, dd, J=14.0, 3.5 Hz, 1-H_{ax}), 2.06 (1H, dd, J=14.0, 3.5 Hz, 1-H_{eq}), 2.28 (1H, dd, J=14.0, 13.0 Hz, 19-H_{ax}), 3.00 (1H, dd, J=14.0, 4.0 Hz, 18-H), 3.24 (1H, d, J=11.0 Hz, 23-H_a), 3.49 (1H, d, J=11.0 Hz, 23-H_b), 3.58 (1H, d, J=4.0 Hz, 3-H), 4.07 (1H, ddd, J=4.0, 3.5, 3.5 Hz, 2-H), 4.45 (1H, dd, J=2.5, 2.5 Hz, 16-H), 5.30 (1H, dd, J=3.0, 3.0 Hz, 12-H). The aqueous layer was neutralized by Amberlite IRA-45 (OH^- form) and concentrated to about 2 ml. To this solution, $NaBH_4$ (40 mg) was added. The reaction mixture was stirred at room temperature for 2 h, acidified by the use of Amberlite IR-120 (H^+ form) and concentrated to dryness. Boric acid in the residue was removed by repeated co-distillation with MeOH. The resulting alditol mixture was acetylated with Ac_2O–C_5H_5N (1:1, each 1 ml) at room temperature overnight. The reagent was removed by co-distillation with toluene. The alditol acetate mixture obtained in this way was subjected to GLC: glass column (2.3 mm × 2 m) packed with 3% ECNSS-M on Gas-chrom Q; detector, hydrogen flame ionization detector (FID); injection temperature, 220 °C; column temperature, 195 °C; carrier gas, N_2 (50 ml/min). Retention times (min) of alditol acetates: rhamnitol acetate 9.7, fucitol acetate 10.6, arabinitol acetate 16.1, xylitol acetate 22.1 and glucitol acetate 54.3.

Methylation Analysis by GC-MS According to Hakomori's method, NaH (1.5 g) was stirred with dimethylsulfoxide (DMSO, 15 ml) at 65 °C for 1 h under N_2 gas flow. This reagent (methylsulfinyl carbanion, 0.5 ml) was added to a solution of saponin (2 mg) in DMSO (1 ml) and the mixture was sonicated at room temperature for 1 h. To this solution, CH_3I (1 ml) was added under cooling and the mixture was further sonicated at room temperature for 1 h. Then CH_3I was removed by blowing N_2 gas under heating. The solution was diluted with H_2O under cooling and was passed through a SEP-PAK C_{18} cartridge (Waters) and eluted with H_2O and MeOH. The MeOH eluate was hydrolyzed with 2 M trifluoroacetic acid (TFA) at 120 °C for 1 h in a sealed tube. The reaction mixture was neutralized with Amberlite IRA-93 (OH^- form) and concentrated to about 2 ml. To this solution, $NaBH_4$ ($NaBD_4$) (25 mg) was added. After being stirred at room temperature for 2 h, the solution was acidified by use of Dowex 50W-X8 (H^+ form) and concentrated to dryness. Boric acid in the residue was removed by repeated co-distillation with MeOH. The resulting methylated alditol mixture was acetylated with Ac_2O–C_5H_5N (1:1, each 1 ml) at room temperature overnight. The reagent was removed by co-distillation with toluene. The partially methylated alditol acetate mixture obtained in this way was analyzed by GC-MS. GC-MS was performed on a JEOL JMS DX-300 spectrometer equipped with a glass column (2.3 mm × 2 m) packed with 3% ECNSS-M on Gas-chrom Q, and with 1% OV-225 on Uniport HP at 170 °C, and operated at an ionization voltage of 70 eV. Relative retention times (rt_R) of partially methylated alditol acetates were recorded on the basis of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol (rt_R=1.00) as the standard. The following abbreviations are used: t=terminal, 2=2-linked, 4=4-linked, 6=6-linked. rt_R values of partially methylated alditol acetates (3% ECNSS-M, 1% OV-225, respectively) were as follows. Crocosmioside A (**1**): t-Rha (0.46, 0.44), t-Api (0.51, 0.49), t-Xyl and/or t-Ara (0.67, 0.55), 4-Rha (0.98, 0.91), 4-Xyl (1.48, 1.16), 2-Fuc (1.29, 1.04), 6-Glc (2.25, 2.23). Crocosmioside B (**2**): t-Api (0.51, 0.49), t-Xyl and/or t-Ara (0.67, 0.55), 4-Rha (0.98, 0.91), 4-Xyl (1.48, 1.16), 2-Fuc (1.29, 1.04), 6-Glc (2.25, 2.23). Crocosmioside H (**3**): t-Rha (0.46, 0.44), t-Xyl and/or t-Ara (0.67, 0.55), 4-Rha (0.98, 0.91), 4-Xyl (1.48, 1.16), 2-Fuc (1.29, 1.04), 6-Glc (2.25, 2.23). EI-MS of partially methylated alditol acetates (mass fragments containing deuterium after $NaBD_4$ reduction are given in parentheses), m/z: 6-Glc; 223, 189, 161 (162), 117 (118), t-Ara; 161 (162), 117 (118), 2-Fuc; 189 (190), 131, 4-Rha; 203, 161 (162), 117 (118), 4-Xyl; 189, 117 (118), t-Api; 233 (234), 205 (206), 161, 117 (118), t-Xyl; 161 (162), 117 (118), t-Rha; 175, 161 (162), 117 (118), 131.

Enzymatic Hydrolysis of 1 with Naringinase Naringinase (40.1 mg, Sigma Co., Ltd.) was added to a solution of **1** (25.3 mg) in H_2O (5 ml), and the mixture was incubated at 37 °C for 6 h. The reaction mixture was passed through a Diaion HP-20 column and eluted with H_2O and MeOH. The MeOH eluate was subjected to HPLC to give **2** (13 mg). Conditions of HPLC: column, Senshu Pak Aquasil; solvent, $CHCl_3$–MeOH–H_2O (60:33:7); flow rate, 3 ml/min. **2**: White powder, $[\alpha]_D^{22}$ −29.3° (c=1.21, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1735, 1635. Positive ion FAB-MS m/z: 1795 $(M+Na)^+$. The hydrolyzate (**2**) was identical with a naturally occurring specimen on the basis of $[\alpha]_D$, FAB-MS, ^{1}H- and ^{13}C-NMR spectral data. The H_2O eluate was converted into the alditol acetate and analyzed by GLC [glass column (2.3 mm × 2 m) packed with 3% ECNSS-M on Gas-chrom Q; detector, FID; injection temperature, 220 °C; column temperature, 195 °C; carrier gas, N_2 (50 ml/min)]. Retention time (min) of alditol acetate: rhamnitol acetate 9.1.

Hydrolysis of 1 with 0.5% $NaHCO_3$ A solution of **1** (30.7 mg) in 1% $NaHCO_3$–EtOH (1:1, 6 ml) was refluxed for 45 min. The reaction mixture was neutralized with Dowex 50W-X8 (H^+ form), and evaporated to remove EtOH. The solution was extracted successively with EtOAc and *n*-BuOH saturated with water. The aqueous layer was passed through a Diaion HP-20 column and eluted with H_2O and MeOH. The combined reaction products obtained from the *n*-BuOH fraction and the MeOH eluate of a Diaion HP-20 column were subjected to HPLC to give **5** (7.6 mg) and **6** (19.6 mg). Conditions of HPLC: column, Senshu Pak NP-118; solvent, MeOH–H_2O–dioxane (60:40:5); flow rate, 3 ml/min. **5**: White powder, $[\alpha]_D^{27}$ −46.6° (c=0.58, C_5H_5N). IR ν_{max}^{KBr} cm^{-1}: 3390, 2940, 1735. Positive ion FAB-MS m/z: 605 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 1.25 (3H, d, J=6.0 Hz, Rha-6), 3.16 (1H, dd, J=11.5, 10.0 Hz, Xyl-5H_{ax}), 3.35 (1H, dd, J=9.5, 9.5 Hz, Rha-4), 3.39 (1H, dt, J=9.5, 6.5 Hz, 16-H_a), 3.55 (1H, dq, J=9.5, 6.0 Hz, Rha-5), 3.62 (1H, dd, J=9.5, 3.5 Hz, Rha-3), 3.66 (1H, dt, J=9.5, 6.5 Hz, 16-H_b), 3.77 (1H, dd, J=3.5, 1.5 Hz, Rha-2), 3.82 (1H, dd, J=11.5, 5.5 Hz, Xyl-5H_{eq}), 4.08 (1H, dd, J=6.5, 6.5 Hz, 2-H), 4.26 (1H, d, J=7.0 Hz, Xyl-1), 4.64 (1H, d, J=1.5 Hz, Rha-1). **6**: White powder, $[\alpha]_D^{22}$ −31.8° (c=1.87, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3430, 1735. Positive ion FAB-MS m/z: 1377 $(M+Na)^+$, 821, 579. ^{1}H-NMR (CD_3OD) δ: 0.78, 0.87, 0.92, 0.94, 1.27, 1.38 (each 3H, s), 1.21 (3H, d, J=6.5 Hz, Fuc-6), 1.32 (3H, d, J=6.0 Hz, Rha-6), 2.30 (1H, dd, J=13.5, 13.5 Hz, 19-Hax), 2.94 (1H, dd, J=13.5, 4.0 Hz, 18-H), 3.55 (1H, d, J=3.5 Hz, Fuc-4), 3.67 (1H, dd, J=9.5, 3.5 Hz, Fuc-3), 3.67 (1H, q, J=6.5 Hz, Fuc-5), 3.79 (1H, d, J=10.0 Hz, Api-5H_a), 3.80 (1H, dd, J=9.5, 8.0 Hz, Fuc-2), 3.83 (1H, dd, J=8.5, 3.0 Hz, Rha-3), 3.89 (1H, d, J=3.0 Hz, Api-2), 3.92 (1H, dd, J=3.0, 1.8 Hz, Rha-2), 4.10 (1H, d, J=10.0 Hz, Api-5H_b), 4.24 (1H, d, J=6.5 Hz, Ara-1), 4.43 (1H, d, J=8.0 Hz, Glc-1), 4.47 (1H, br s, 16-H), 4.49 (1H, d, J=7.8 Hz, Xyl-1), 5.05 (1H, d, J=3.0 Hz, Api-1), 5.30 (1H, d, J=8.0 Hz, Fuc-1), 5.32 (1H, dd, J=3.0, 3.0 Hz, 12-H), 5.40 (1H, d, J=1.8 Hz, Rha-1). δ (C_5D_5N): 0.82, 0.84, 1.05, 1.20, 1.43, 1.59 (each 3H, s), 1.35 (3H, d, J=6.5 Hz, Fuc-6), 1.46 (3H, d, J=6.0 Hz, Rha-6), 2.62 (1H, dd, J=13.5, 13.5 Hz, 19-H_{ax}), 3.26 (1H, dd, J=13.5, 3.5 Hz, 18-H), 4.19 (1H, d, J=9.5 Hz, Api-5H_a), 4.55 (1H, dd, J=9.5, 8.5 Hz, Fuc-2), 4.56 (1H, d, J=3.0 Hz, Api-2), 4.56 (1H, d, J=9.5 Hz, Api-5H_b), 4.69 (1H, d, J=6.5 Hz, Ara-1), 4.90 (1H, d, J=7.8 Hz, Glc-1), 5.04 (1H, d, J=7.8 Hz, Xyl-1), 5.09 (1H, br s, 16-H), 5.47 (1H, dd, J=3.0, 3.0 Hz, 12-H), 5.59 (1H, d, J=3.0 Hz, Api-1), 5.86 (1H, d, J=8.0 Hz, Fuc-1), 6.34 (1H, br s, Rha-1).

Preparation of Trimethylsilyl Ether (7) Hesperidinase (4.8 mg) was added to a solution of **5** (1 mg) in H_2O (0.3 mg). The reaction mixture was incubated at 37 °C for 2 d, applied to a SEP-PAK C_{18} cartridge and eluted with H_2O and MeOH. The MeOH eluate was esterified by treatment with excess diazomethane in ether–MeOH. The methyl ester in pyridine (0.2 ml) was treated with *N*,*O*-bis(trimethylsilyl)acetamide (50 μl) to give the trimethylsilyl (TMS) ether (**7**). Compound **7** was subjected to GC-MS on a JEOL JMS DX-300 spectrometer equipped with a glass column 2.3 mm × 2 m packed with 1% SE-30 on Gas-chrom Q, at 230 °C, and operated at an ionization voltage of 70 eV. Retention time of **7** (min): 22.5. **7**: EI-MS m/z (%): 519 (M-15, 5), 475 (4), 347 (100), 289 (68), 103 (23).

Cleavage of Ester–Glycoside Linkage of 6 A solution of **6** (16.5 mg), anhydrous LiI (62.3 mg), and 2,6-lutidine (1.5 ml) in anhydrous MeOH (1.5 ml) was refluxed for 9 h. The solution was deionized by passing it through a column of Amberlite MB-3 and concentrated to dryness. The residue was subjected to HPLC to give **8** (5.9 mg) and crude **9**. Conditions of HPLC, column: Senshu Pak NP-118; solvent, 60% MeOH; flow rate, 3 ml/min. Crude **9** was further purified by normal- and reversed-phase HPLC to give **9** (4.2 mg). **8**: White powder, $[\alpha]_D^{24}$ +12.9° (c=0.59, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1690. Positive ion FAB-MS m/z: 821 $(M+Na)^+$. ^{1}H-NMR (CD_3OD) δ: 0.80, 0.88, 0.92, 0.97, 1.26, 1.38 (each, 3H, s), 2.28 (1H, dd, J=14.0, 14.0 Hz, 19-H_{ax}), 3.00 (1H, dd, J=14.0, 4.0 Hz, 18-H), 3.21 (1H, d, J=11.5 Hz, 23-H_a), 3.27 (1H, dd, J=9.0, 7.8 Hz, Glc-2), 3.37 (1H, dd, J=9.0, 9.0 Hz, Glc-3), 3.80 (1H, ddd, J=3.0, 3.0, 1.5 Hz, Ara-4), 3.85 (1H, dd, J=12.5, 3.0 Hz, Ara-5H_a), 4.14 (1H, dd, J=10.5, 2.0 Hz, Glc-6H_a), 4.24 (1H, d, J=6.5 Hz, Ara-1), 4.43 (1H, d, J=7.8 Hz, Glc-1), 4.46 (1H, br s, 16-H), 5.30 (1H, dd, J=3.0, 3.0 Hz, 12-H). δ (C_5D_5N): 0.92, 0.98, 1.05, 1.22, 1.45, 1.66 (each 3H, s), 2.70 (1H, dd, J=14.0, 13.5 Hz, 19-H_{ax}), 3.50 (1H, dd, J=14.0, 3.5 Hz, 18-H), 3.53 (1H, d, J=10.5 Hz, 23-H_a), 3.63 (1H, dd, J=12.0, 1.0 Hz, Ara-5H_{ax}), 4.06 (1H, dd, J=8.5, 3.0 Hz, Ara-3), 4.33 (1H, dd, J=8.0, 6.5 Hz, Ara-2), 4.66 (1H, d, J=9.0 Hz, Glc-

6H$_a$), 4.71 (1H, d, J=6.5 Hz, Ara-1), 4.92 (1H, d, J=7.8 Hz, Glc-1), 5.12 (1H, br s, 16-H), 5.50 (1H, dd, J=3.0, 3.0 Hz, 12-H), 6.21 (1H, br s, 16-OH). **9**: White powder, $[\alpha]_D^{22}$ −47.4° (c=0.42, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3430. Positive ion FAB-MS m/z: 611 (M+Na)$^+$. ^{1}H-NMR spectral data are summarized in Table IV.

Permethylation and Methylation Analysis of 9 Compound **9** (1 mg) was methylated by Hakomori's method as described above. The product was chromatographed on silica gel with benzene–acetone (2:1) to give a deca-*O*-methyltetrasaccharide (**10**). **10**: Colorless oil, EI-MS m/z (%): 683 (2), 583 (12), 523 (1), 509 (1), 423 (9), 363 (12), 335 (4), 249 (51), 189 (36), 175 (40), 143 (100). Compound **10** was hydrolyzed with 2 M TFA and the hydrolyzate was converted into alditol acetates by the same procedure as described above. The partially methylated alditol acetate mixture obtained in this way was analyzed by GC-MS in the same manner as above.

Enzymatic Hydrolysis of 6 with Hesperidinase Hesperidinase (90 mg) was added to a solution of **6** (30.3 mg) in H_2O (6 ml), and the mixture was incubated at 37 °C for 6 d. The reaction mixture was passed through a SEP-PAK C_{18} cartridge and eluted with H_2O and MeOH. The MeOH eluate was subjected to HPLC to give **11** (5.3 mg) and **12** (9.4 mg). Conditions of HPLC: Column, Senshu Pak Aquasil; solvent, $CHCl_3$–MeOH–H_2O (60:29:6); flow rate, 3 ml/min. **11**: White powder, $[\alpha]_D^{30}$ −31.7° (c=0.53, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 1735, 1635. Positive ion FAB-MS m/z: 1245 (M+Na)$^+$, 689, 579. ^{1}H-NMR (C_5D_5N) δ: 0.81, 0.84, 1.06, 1.22, 1.46, 1.62 (each 3H, s), 1.35 (3H, d, J=6.5 Hz, Fuc-6), 1.47 (3H, d, J=6.0 Hz, Rha-6), 2.61 (1H, dd, J=13.5, 13.5 Hz, 19-H$_{ax}$), 3.27 (1H, dd, J=13.5, 4.0 Hz, 18-H), 3.60 (1H, d, J=10.0 Hz, 23-H$_a$), 4.19 (1H, d, J=9.5 Hz, Api-5H$_a$), 4.55 (1H, dd, J=9.5, 8.0 Hz, Fuc-2), 4.55 (1H, d, J=3.0 Hz, Api-2), 4.56 (1H, d, J=9.5 Hz, Api-5H$_b$), 4.60 (1H, dd, J=9.5, 2.5 Hz, Rha-3), 4.66 (1H, br d, J=2.5 Hz, Rha-2), 5.05 (1H, d, J=8.0 Hz, Glc-1), 5.05 (1H, d, J=8.0 Hz, Xyl-1), 5.10 (1H, br s, 16-H), 5.50 (1H, dd, J=3.0, 3.0 Hz, 12-H), 5.59 (1H, d, J=3.0 Hz, Api-1), 5.86 (1H, d, J=8.0 Hz, Fuc-1), 6.33 (1H, br s, Rha-1). **12**: White powder, $[\alpha]_D^{30}$ −23.6° (c=0.94, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3420, 2940, 1740, 1635. Positive ion FAB-MS m/z: 1083 (M+Na)$^+$, 579. ^{1}H-NMR (C_5D_5N) δ: 0.81, 0.85, 1.10, 1.24, 1.51, 1.64 (each 3H, s), 1.35 (3H, d, J=6.5 Hz, Fuc-6), 1.48 (3H, d, J=6.0 Hz, Rha-6), 2.63 (1H, dd, J=13.5, 13.5 Hz, 19-H$_{ax}$), 3.22 (1H, dd, J=11.0, 11.0 Hz, Xyl-5H$_{ax}$), 3.29 (1H, dd, J=13.5, 4.0 Hz, 18-H), 3.63 (1H, d, J=10.5 Hz, 23-H$_a$), 3.80 (1H, q, J=6.5 Hz, Fuc-5), 3.83 (1H, d, J=3.0 Hz, Fuc-4), 4.03 (1H, d, J=10.5 Hz, 23-H$_b$), 4.05 (1H, dd, J=9.5, 3.0 Hz, Fuc-3), 4.19 (1H, d, J=9.5 Hz, Api-5H$_a$), 4.25 (1H, dd, J=9.0, 9.0 Hz, Rha-4), 4.37 (1H, dq, J=9.0, 6.0 Hz, Rha-5), 4.55 (1H, dd, J=9.5, 8.0 Hz, Fuc-2), 4.55 (1H, d, J=3.0 Hz, Api-2), 4.56 (1H, d, J=9.5 Hz, Api-5H$_b$), 4.61 (1H, dd, J=9.0, 3.0 Hz, Rha-3), 4.68 (1H, dd, J=3.0, 1.0 Hz, Rha-2), 5.02 (1H, d, J=7.0 Hz, Xyl-1), 5.11 (1H, br s, 16-H), 5.53 (1H, dd, J=3.0, 3.0 Hz, 12-H), 5.59 (1H, d, J=3.0 Hz, Api-1), 5.85 (1H, d, J=8.0 Hz, Fuc-1), 6.33 (1H, d, J=1.0 Hz, Rha-1).

Hydrolysis of 1 with 2 N HCl A solution of **1** (25.0 mg) in 2 N HCl (3 ml) was left for 26 h at room temperature. After being diluted with H_2O, the reaction mixture was passed through a SEP-PAK C_{18} cartridge (Waters) and eluted with H_2O and MeOH. The MeOH eluate was purified by HPLC to give **3** (13.6 mg). Conditions of HPLC: column, Senshu Pak NP-118; solvent, MeOH–H_2O–dioxane (65:35:5); flow rate, 3 ml/min. **3**: $[\alpha]_D^{25}$ −20.5° (c=0.41, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2935, 1740, 1635. The hydrolyzate (**3**) was identical with a naturally occurring specimen on the basis of $[\alpha]_D$, FAB-MS, ^{1}H- and ^{13}C-NMR spectral data. The H_2O eluate was neutralized by the use of Amberlite IRA-93 (OH^- form) and concentrated to dryness. The residue was subjected to HPLC to give D-apiose (1 mg). Conditions of HPLC: column, ERC-NH-1171 (6×200 mm); solvent, 85% CH_3CN; flow rate, 2 ml/min. Retention time (min) of apiose: 3.6, $[\alpha]_D^{25}$ +6° (c=0.1, H_2O).

Determination of D-L Series of Component Sugars by HPLC A solution of **3** (0.8 mg) in 2 N HCl–dioxane (1:1, 2 ml) was heated under N_2 gas at 100 °C for 1 h. The reaction mixture was diluted with H_2O and evaporated to remove dioxane. The solution was neutralized with Amberlite IRA-93 (OH^- form) and passed through a SEP-PAK C_{18} cartridge to give a sugar fraction. A solution of the sugar fraction in 1 ml of H_2O was treated with a solution of L(−)-α-methylbenzylamine (150 μl) and $NaBH_3CN$ (8 mg) in 1 ml of EtOH, and the mixture was kept at 40°C for 3 h. Then several drops of acetic acid were added, and the mixture was concentrated to dryness. The residue was acetylated with Ac_2O–C_5H_5N (1:1, each 1 ml) in a sealed microtube at 100°C for 1 h. The acetate mixture was passed through a SEP-PAK C_{18} cartridge and eluted with 20% CH_3CN (7 ml) and 100% CH_3CN. The latter eluate was analyzed by normal- and reversed-phase HPLC. Conditions of normal-phase HPLC: column, Senshu Pak Silica-4301-N 5 μm (10×300 mm); solvent, hexane–EtOH (92:8); flow rate, 4 ml/min; detection, UV (230 nm). Retention times (min) of 1-deoxy-1-(L(−)-*N*-acetyl-α-methylbenzylamino)alditol acetates: L-rhamnose 18.6, D-fucose 20.4, L-arabinose 25.4, D-xylose 28.3, D-glucose 28.3, (reference: D-rhamnose 17.0, L-fucose 17.7, D-arabinose 22.8, L-xylose 26.5, L-glucose 27.1). Conditions of reversed-phase HPLC: column, Senshu Pak NP-118; solvent, 40% CH_3CN; flow rate, 3 ml/min; detection, UV (230 nm). Retention times (min) of 1-deoxy-1-(L(−)-*N*-acetyl-α-methylbenzylamino)alditol acetates: L-arabinose 22.0, D-xylose 23.1, D-fucose 26.0, D-glucose 29.4, L-rhamnose 33.4, (reference: D-arabinose 23.0, L-xylose 22.4, L-fucose 28.0, L-glucose 28.0, D-rhamnose 33.4).

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