New Triterpenoid Saponins from *Maesa japonica*

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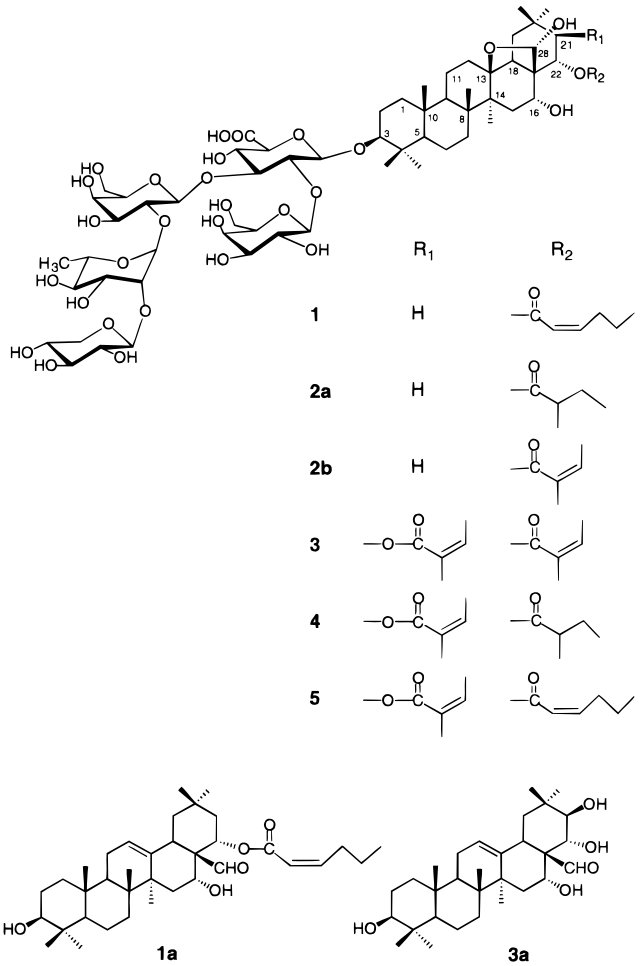
New triterpenoid saponins, maejaposides A, B, C, D, and E, were isolated from the roots of *Maesa japonica* and were, respectively, defined to be 3-*O*-[*β*-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→3)] [*β*-D-galactopyranosyl-(1→2)]*β*-D-glucuronopyranosides of 22α-[(*Z*)-2-hexenoyloxy]-13*β*,28-oxido-olean-16α,28α-diol (**1**); 22α-[2′-methylbutanoyl]-13*β*,28-oxido-olean-16α,28α-diol (**2a**) and 22α-angeloyloxy-13*β*,28-oxido-olean-16α,28α-diol (**2b**); 21*β*,22α-diangeloyloxy-13*β*,28-oxido-olean-16α,28α-diol (**3**); 21*β*-angeloyloxy,22α-(2′-methylbutanoyl)-13*β*,28-oxido-olean-16α,28α-diol (**4**), and 21*β*-angeloyloxy,22α-[(*Z*)-2′-hexenoyl]-13*β*,28-oxido-olean-16α,28α-diol (**5**). Their structures were established on the basis of extensive NMR (DEPT, COSY, HOHAHA, HETCOR, HMBC, and NOESY) and ESIMS/MS studies, along with chemical degradation.

Maesa japonica (Thunb) Morizi & Zoll. (Myrsinaceae) is an evergreen bush or small tree growing in the southern parts of Japan. In China the leaves and roots of this species have been well documented as folk medicine for curing symptoms associated with common cold.[1] Many other plants in the genus have also been used as a folk remedy in African and Asian countries.[2] Previous chemical investigations on *Maesa* ssp. demonstrated the presence of quinones[3,4] and of triterpenoid components.[5] Six new tetrasaccharide triterpenoid saponins were isolated from the leaves of *M. lanceolata*,[6] and quite recently a pentasaccharide triterpenoid saponin was isolated from the leaves of *M. ramentacea*.[7] Until now, no chemical study has been reported on the saponin fraction of *M. japonica*. Its medicinal importance and our continuing interest in the chemistry of triterpenoid saponins prompted us to initiate the chemical investigation on this plant. In this paper, we wish to report the isolation and structure elucidation of five novel triterpenoid saponins from the roots of this species.



Results and Discussion

A MeOH extract of the freshly collected roots of *M. japonica* was partitioned between *n*-BuOH and water. The *n*-BuOH-soluble fraction, on chromatographic purification over Diaion HP-20 and Si gel, followed by repeated MPLC and HPLC purifications, afforded the new triterpenoid saponins maejaposides A (**1**), B (**2a,b**), C (**3**), D (**4**), and E (**5**). Among them, compound **2** is a mixture of two inseparable compounds. All the compounds contained the same pentasaccharide sugar chain linked to C-3 of the aglycons. Compounds **1** and **2** are derivatives of anagalligenin A,[8,9] and **3**−**5** are derivatives of 21*β*-OH anagalligenin A with different acyl substituents at C_{21} and/or C_{22}.

Maejaposide A (**1**), an amorphous solid, had a molecular formula of $C_{65}H_{104}O_{30}$ determined from its negative ion ESIMS (*m*/*z* 1363 $[M - H]^-$) and ^{13}C DEPT NMR data. Its spectral features and physicochemical properties suggested **1** to be a triterpenoid saponin. The IR spectrum showed absorptions at 3416 cm^{-1} (−OH) and at 1681 cm^{-1} (conjugated ester carbonyl). Of the 65 carbons, 30 were assigned to the aglycon part, 29 to the oligosaccharide moiety, and the remaining six to an acyl group (Table 1).

Among the 30 carbons of the aglycon, seven were assigned to the methyl carbons at *δ* 16.4, 16.6, 18.6, 19.7, 25.6, and 33.4 ppm, and the correspondent methyl protons were identified by an HSQC experiment. Four methine carbons bearing oxygen were found at *δ* 69.9, 72.7, 89.8 (assigned to C-3), and 97.7 ppm (δ_H 5.27, s), attributed to

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Table 1. ^{1}H and ^{13}C NMR Data for the Aglycon Part and Sugar Moieties of Maejaposide A (**1**) (in pyridine-d_5)[a]

	aglycon part		
1	39.2	0.76, 1.49	38.9[b]
2	26.6	1.78, 2.14	28.1
3	89.8	3.24 dd (11.3, 3.9)	77.9
4	39.8		39.4
5	55.6	0.69	55.7
6	19.2	1.42, 1.79	18.6
7	34.3	1.24, 1.57	31.9
8	42.7		42.0
9	50.3	1.26	47.0
10	36.8		37.3
11	17.9	1.49	23.7
12	33.3	1.58	124.8
13	87.6		141.4
14	43.9		40.1
15	36.8	1.59, 2.20	33.3
16	69.9	4.84	67.0
17	51.5		56.3
18	47.4	2.22 m	40.6
19	38.3	1.37, 2.91	44.4
20	33.4		31.6
21	41.7	2.07, 2.84	45.9
22	72.7	6.12 m	70.3
23	28.1	1.26	28.7
24	16.6	1.15	16.5
25	16.4	0.81	15.6
26	18.6	1.33	17.4
27	19.7	1.63	27.0
28	97.7	5.27 s	204.4
29	33.4	1.10	33.7
30	25.6	1.17	25.1
1′	166.2		165.2
2′	121.2	5.94 dd (11.6, 1.5)	120.7
3′	149.3	6.12 m	151.4
4′	31.1	2.70 m, 2.78 m	31.1
5′	22.5	1.36 m (2H)	22.5
6′	13.9	0.83 t (7.3)	13.9

	sugar part	
glucuronic acid		
1	105.3	4.98 d (6.7 Hz)
2	79.9	4.69
3	83.0	4.72
4	71.1	4.46
5	77.3	4.58 d (9.4)
6	172.3	
galactose (terminal)		
1	103.9	5.69 d (7.7)
2	73.5	4.51
3	75.3	4.34
4	70.2	4.51
5	76.8	4.46
6	62.7	4.37, 4.51
galactose		
1	101.5	6.07 d (7.9)
2	77.4	4.64
3	75.7	4.46
4	71.2	4.42
5	77.0	4.20
6	62.0	4.31 (2H)
rhamnose		
1	101.5	6.29 s
2	82.2	4.78
3	72.7	4.74
4	74.4	4.15
5	69.5	4.82
6	18.1	1.41 d (6.1)
xylose		
1	107.5	5.07 d (7.3)
2	75.6	4.00
3	78.4	4.03
4	70.9	4.11
5	67.3	4.18 3.51 t (10.7),

[a] Assignments were based upon COSY, HOHAHA, DEPT, HSQC, NOESY, and HMBC experiments. [b] ^{13}C data in this column from the artificial aglycon **1a**.

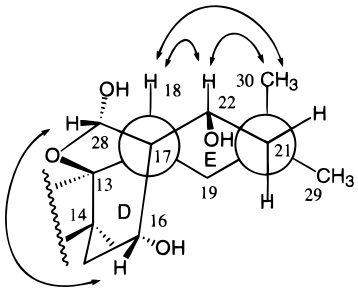

Figure 1. Key NOEs for the aglycon part of maejaposide A (**1**) observed in a phase-sensitive NOESY.

C-28 in the form of a hemiacetal function group. Structure assignment was initiated from the long-range coupling networks observed between the methyl protons and the adjacent carbons from an HMBC experiment. Extensive NMR analyses showed the aglycon was of an oleanane skeleton with an oxygen bridge between C-13 (δ 87.6 ppm) and C-28 (97.7 ppm).

Besides the two hydroxyls at C-3 and C-28, the other two groups were located at C-16 and C-22. Their configurations were determined using the NOE information from a phase-sensitive NOESY (Figure 1). The spatial proximity observed between H-3 and H-23 ($-CH_3$), H-3 and H-5, H-16 and H-26 ($-CH_3$) (weak), H-16 and H-28 indicated the β-orientation of the hydroxyl at C-3 and α-orientation at C-16, respectively. The NOEs observed between H-22 and H-18, H-22 and H-30 ($-CH_3$); between H-28 and H-26 ($-CH_3$), H-28 and H-16 indicated the α-orientation of the hydroxyls at C-22 and C-28, respectively. From the above evidences, the aglycon was identified as 13β,28-epoxyolean-3β,16α,22α,28α-tetraol, a compound named anagalligenin A, first isolated from *Anagallis arvensis*.[8] An acyl group was also mapped out from COSY, HOHAHA, and HSQC correlations and identified as (*Z*)-2-hexenoyl esterified to C-22 hydroxyl as established from the long-range HMBC coupling between H-22 (δ 6.12 ppm, m) and C-1 of the acyl group (166.2 ppm) (Figure 2) and confirmed by the lowfield signal of H-22, indicative for acylation. The *cis*-configuration of the acyl moiety was determined from the coupling constant (11.6 Hz) between the olefinic protons and was further confirmed by GLC analysis of the methanolysis product when compared to the standard methyl *(E)*-2-hexenoate (see Experimental Section). Structural information about the genuine aglycon came also from the spectral data of the artificial sapogenin (**1a**) obtained from acid hydrolysis, in which the original 13β,28-epoxy in **1** was broken, leading to the formation of a double bond between C-12 (δ_C 124.8, CH, δ_H, 5.53, 1H, br t) and C-13 (141.4 ppm) and an aldehyde group at C-17 (δ_C 204.4, δ_H 9.69 ppm).

Moreover, the presence of five sugar moieties was evidenced by the ^{1}H and ^{13}C NMR that displayed five sugar anomeric protons at δ 4.98 d (J = 6.7 Hz), 5.07 d (7.3), 5.69 d (7.7), 6.07 d (7.9), and 6.29 (s) and carbons at δ 105.3, 107.5, 103.9, and 101.5 (× 2) (Table 1), respectively. The lowfield chemical shifts of C-3 (δ 89.8) indicated that the pentasaccharide chain was connected to this position, whose sequence was determined by a combination of COSY, HOHAHA, DEPT, HSQC, HMBC, and phase-sensitive NOESY experiments. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were assigned by COSY and HOHAHA. Furthermore, a NOESY experiment, in addition to the NOEs across the glycosidic bonds, also revealed the 1,3 and 1,5-diaxial relationships for glucuronic acid, galactose, and xylose, thus greatly simplifying the mapping of these spin systems. On the basis of the assigned protons, the ^{13}C resonances of each

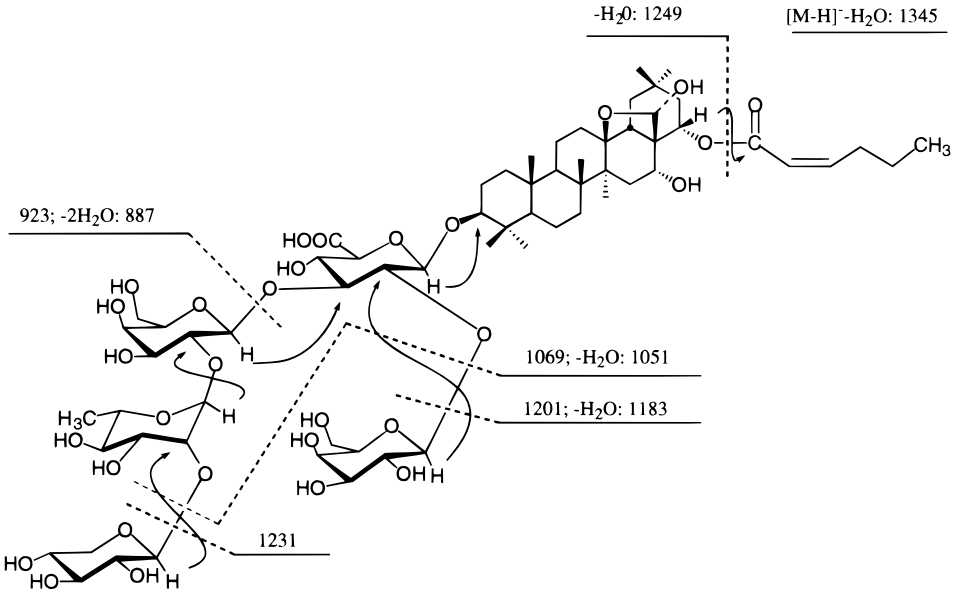

Figure 2. Fragmentation patterns of (**1**) observed from ESIMS/MS and key long-range correlations from an HMBC experiment.

sugar unit were identified by HSQC and further confirmed by HMBC. These data led to the identification of the five monosaccharide units as β-glucuronic acid, β-galactose ($\times$ 2), α-rhamnose, and β-xylose, which were further confirmed by the GLC of the acid hydrolysate of **1** (see Experimental Section). The inter-sugar linkages were established from the following HMBC correlations: H-1 of the xylose with C-2 of rhamnose; H-1 of rhamnose with C-2 of the galactose; H-1 of the galactose with C-3 of the glucuronic acid; H-1 of the remaining galactose (terminal) with C-2 of glucuronic acid, while the attachment of the pentasaccharide chain to C-3 of the aglycon was based on a correlation between H-1 of glucuronic acid and the C-3 of the aglycon (Figure 2).

The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiments, even if, due to the highly overlapped nature of the proton spectrum, NOEs should, therefore, not be used as the sole source of evidence for the inter-sugar linkage. The sugar sequence was also supported from the fragmentation patterns observed in the ESIMS/MS experiment (Figure 2). MS/MS analysis of the deprotonated molecular ion $[M - H]^-$ (*m*/*z* 1363) gave a daughter ion at *m*/*z* 1345 $[(M-H)^-$-18] by losing one molecule of water and at *m*/*z* 1249 $[(M - H)^- - 18 - 96]$ from further loss of the side chain. Relatively strong fragments were observed at *m*/*z* 1231 $[(M - H)^- - 132]$ by the loss of the terminal xylose and at *m*/*z* 1201 $[(M - H)^- - 162]$ with the loss of the terminal galactose from the deprotonated molecular ion. The most prominent fragment observed at *m*/*z* 1183 $[(M - H)^- - 18 - 162]$ was due to the losses of one mole of water and the terminal galactose from the molecule. Other ions at *m*/*z* 1069 and 1051, corresponding to the loss of both of the terminal xylose and galactose, and at *m*/*z* 887 resulted from losing the trisaccharide (xyl−rha−gal) fragment and two moles of water from $[M - H]^-$. All the monosaccharides were in the pyranose forms, as determined from their ^{13}C NMR data. The β anomeric configurations for the galactose, glucuronic acid, and xylose were based on their $^3J_{H1,\ H2}$ coupling constants (7−8 Hz). The ^1H nonsplitting pattern and the three-bond strong HMBC correlations from the anomeric proton to C-3 and C-5 (the dihedral angles between H-1 and C-3, H-1 and C-5 about 180°), indicating the anomeric proton was equatorial, thus possessed an α configuration. The absolute configurations of these sugars were chosen in keeping with those mostly encountered among other plant glycosides. Based upon the above evidence, maejaposide A (**1**) is established as 3-*O*-{[β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)] [β-D-galactopyranosyl-(1→2)]β-D-glucuronopyranosyl}-22α-[(*Z*)-2-hexenoyloxy]-13β,28-oxido-olean-16α,28α-diol (**1**).

Maejaposide B (**2**), an amorphous solid, was shown to be a mixture of two compounds (**2a** and **2b**), which, in the negative ESIMS, displayed two $[M - H]^-$ ions at *m*/*z* 1351 and 1349, corresponding to the molecular formulas of $C_{64}H_{104}O_{30}$ and $C_{64}H_{102}O_{30}$, respectively. Compounds **2a** and **2b** were an inseparable mixture by HPLC in a ratio of 1:1. ^1H and ^{13}C NMR spectra of **2** revealed that both the carbohydrate and the aglycon parts were superimposable with that of **1** but differed in the acyl group linked to C-22 of the aglycon (Table 2). NMR analyses established the acyl groups to be 2-methylbutanoyl for **2a** and angeloyl [(*E*)-2-methyl-2-butenoyl] for **2b**. Thus, the structures of **2a** and **2b** were elucidated to be 3-*O*-{[β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)] [β-D-galactopyranosyl-(1→2)]β-D-glucuronopyranosyl}-22α-[2′-methylbutanoyl]-13β,28-oxido-olean-16α,28α-diol and 3-*O*-{[β-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)] [β-D-galactopyranosyl-(1→2)]β-D-glucuronopyranosyl}-22α-angeloyloxy-13β,28-oxido-olean-16α,28α-diol, respectively.

Maejaposide C (**3**), amorphous solid, had a molecular formula of $C_{69}H_{108}O_{32}$, determined from its negative ESIMS and ^{13}C DEPT NMR data. ^1H and ^{13}C NMR spectra indicated that compound **3** had the same sugar arrangement as that of **1** but differed in the aglycon part and the acyl groups linked to the E-ring (Table 2). DEPT spectrum indicated **3** had eight *sp*3 methylene carbons, one fewer than those in **1**. Detailed NMR analyses showed an additional hydroxyl group at C-21 (δ 79.3 ppm) on the β face (equatorial). The trans−axial relationship of H-21 (δ 6.35, d, J = 10.0 Hz) and H-22 (δ 6.78, d, J = 10.0 Hz) was clearly indicated from their large ^3J coupling constant. Based upon extensive 2D NMR studies, the aglycon for **3** was established to be 13β,28-oxido-olean-3β,16α,21β,22α,-28α-pentaol (21β-OH anagalligenin A), and the acyl groups attached to $C_{21,22}$−OH were both angeloyl.

The structure assignments were mainly accomplished using a combination of COSY, HOHAHA, HETCOR, HMBC,

Table 2. ^{13}C and ^{1}H NMR Data for the Acyl Groups Linked to C-21 and C-22 of Maejaposides B (**2a,b**), C (**3**), D (**4**), and E (**5**)

acyl group	**2a**	**2b**	**3**		**4**		**5**	
C-21								
1′			167.7		167.6		167.7	
2′			128.9		128.7		128.9	
3′			137.5	5.99 m	138.5	6.03 m	137.0	5.89 m
4′			15.9	2.09 d (7.0)	16.0	2.15 d (7.4)	15.8	2.12 d (7.0)
5′			21.0	2.04 s	21.1	2.05 s	20.9	2.04 s
C-22								
1″	175.8	167.7	167.3		175.6		165.5	
2″	41.6	128.5	129.2		41.6	2.26 m	120.5	5.75 d (11.5)
3″	27.4	137.8	136.4	5.85 m	27.2	1.36 m, 1.61 m	149.8	6.10 m
4″	11.7	15.9	15.8	1.99 d (6.7)	11.7	0.80 t (7.5)	30.9	2.66 m, 2.74 m
5″	16.8	20.9	20.8	1.85 s	16.8	1.08 d (7.5)	22.4	1.36 m (2H)
6″							13.8	0.83 t (7.5)

and NOESY. The presence of five sugars in **3** was indicated from the five anomeric protons and carbons in their ^{1}H and ^{13}C NMR spectra. The exact linkage positions for the pentasaccharide unit and for the acyl groups were established using the HMBC correlations as depicted for **1** in Figure 2. The stereochemistry of each anomeric carbon was determined from the same observation as that of **1**. Thus, the structure of maejaposide C was established to be 3-*O*-{[*β*-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→3)] [*β*-D-galactopyranosyl-(1→2)]*β*-D-glucuronopyranosyl}-21*β*,22α-diangeloyloxy-13*β*,28-oxido-olean-16α,28α-diol (**3**).

Maejaposides D (**4**) and E (**5**), amorphous solids, had molecular formulas of $C_{69}H_{110}O_{32}$ and $C_{70}H_{110}O_{32}$, respectively, determined from their ESIMS and ^{13}C DEPT NMR data. ^{1}H and ^{13}C NMR spectra indicated that compounds **4** and **5** had the same sugar chain and aglycon moiety of **3** but differed in the acyl groups attached at C_{22} of the E-ring (Table 2). Detailed NMR analyses established the acyl group for compound **4** was 2-methylbutanoyl at C-22 and (*Z*)-2-hexenoyl for compound **5**. Under alkaline treatment, compounds **3**, **4**, and **5** furnished the same main product obtained from de-acylation of the groups linked to C-21 and 22 as revealed from their HPLC analyses. Further acid hydrolysis furnished the artificial aglycon **3a**. From the above evidence, the structures of maejaposides D and E were elucidated to be 3-*O*-{[*β*-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→3)] [*β*-D-galactopyranosyl-(1→2)]*β*-D-glucuronopyranosyl}-21*β*-angeloyloxy,22α-(2′-methylbutanoyl)-13*β*,28-oxido-olean-16α,28α-diol (**4**) and 3-*O*-{[*β*-D-xylopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→3)] [*β*-D-galactopyranosyl-(1→2)]*β*-D-glucuronopyranosyl}-21*β*-angeloyloxy,22α-[(*Z*)-2′-hexenoyloxy]-13*β*,28-oxido-olean-16α,28α-diol (**5**), respectively.

Experimental Section

General Experimental Procedures. IR spectra were determined using a JASCO D-300 FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. ESIMS were conducted using Finnigan MAT LCQ mass spectrometer. FABMS were conducted using JEOL DX-303 mass spectrometer. ^{1}H and ^{13}C NMR were recorded using a JEOL α-500 or a JEOL EX-400 FT-NMR spectrometer. All the NMR data were measured in pyridine-d_5, and chemical shifts were expressed in δ (ppm) referring to TMS. Diaion HP-20 (Mitsubishi Chemical), Si gel (Si gel 60, Merck), and ODS (Chromatorex, 100−200 mesh, Fujisylisia) were used for column chromatography. Preparative HPLC was performed using an ODS column (PEGASIL ODS, Senshu Pak, 10 mm i.d. × 250 mm; detector, UV 210 nm). GLC: Shimadzu GC-7A. Column: Silicone OV-17 on Uniport HP (80−100 mesh), 3 mm i.d. × 2.1 m; column temperature, 160 °C; carrier gas, N_2, flow rate 30 mL/min.

Plant Material. *Maesa japonica* was collected from Bousou Peninsula, Chiba prefecture, Japan, in April 1997, and was identified by Dr. T. Sakura (Faculty of Agriculture, The University of Tokyo). The specimen of the plant is kept in the herbarium of the pharmacognosy department of Toho University.

Extraction and Isolation. Finely cut roots (1.9 kg) were extracted with MeOH three times (3 L, each) under reflux for 2 h. The combined MeOH extract was concentrated under reduced pressure to give a dark-brown residue (141.7 g). Solvent partition between *n*-BuOH and H_2O afforded in the organic layer the crude saponin fraction (92.4 g), which was applied to a column of Diaion HP-20 (2000 mL) and washed with 30, 50, 70, and 100% MeOH. The fractions containing saponins (from 70 and 100% MeOH) were combined and repeatedly chromatographed over Si gel and ODS columns to give several saponin fractions. Further HPLC purification (65−70% MeOH−0.06% TFA in H_2O, 1.5 mL/min, UV detector, 210 nm) gave maejaposides A (51.2 mg), B (18.7 mg), C (31.2 mg), D (28.6 mg), and E (40.4 mg).

Maejaposide A (1): amorphous solid; $[\alpha]^{24}_D$ −24.0° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3416, 2938, 1681, 1371, 1087 cm^{-1}; NMR data, see Table 1; ESIMS (negative ion mode) *m/z* 1363 $[M - H]^-$.

Maejaposide B (2a and 2b): amorphous solid; $[\alpha]^{24}_D$ −23.2° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3414, 2930, 1701, 1637, 1385, 1255, 1078 cm^{-1}; ^{1}H and ^{13}C NMR data of the aglycon and the sugar moieties were the same as those reported for **1**; other NMR data see, Table 2; ESIMS (negative ion mode) *m/z*, 1351 $[M - H]^-$ (**2a**) and 1349 $[M - H]^-$ (**2b**).

Maejaposide C (3): amorphous solid; $[\alpha]^{24}_D$ −23.2° (MeOH, *c* 0.5); IR (KBr) ν_{max} 3414, 2930, 1681, 1382, 1242, 1079 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 6.78 (1H, d, J = 10.0 Hz, H-21), 6.35 (1H, d, J = 10.0 Hz, H-22), 6.29 (1H, s, H-1 of rha), 6.07 (1H, d, J = 7.7 Hz, H-1 of gal), 5.69 (1H, d, J = 7.7 Hz, H-1 of terminal gal), 5.20 (1H, s, H-28), 5.10 (1H, d, J = 7.0 Hz, H-1 of xyl), 4.89 (1H, d, J = 6.5 Hz, H-1 of glcA), 3.24 (1H, m, H-3), 2.37 (1H, dd, J = 14.7, 2.1 Hz, H-18), 1.41 (3H, d, J = 7.0 Hz, H-6 of rha), 1.61, 1.33, 1.27, 1.25, 1.15, 1.14, 0.81 (each 3H, s, Me of C-27, C-26, C-23, C-30, C-24, C-29, C-25); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 97.0 (d, C-28), 89.8 (d, C-3), 87.4 (s, C-13), 79.3 (d, C-21), 73.3 (d, C-22), 68.8 (d, C-16), 55.5 (d, C-5), 54.5 (s, C-17), 50.2 (d, C-9), 46.3 (d, C-18), 43.8 (s, C-14), 42.7 (s, C-8), 39.8 (s, C-4), 39.2 (t, C-1), 38.1 (t, C-19), 37.5 (s, C-20), 36.8 (s, C-10), 36.6 (t, C-15), 34.4 (t, C-7), 33.3 (t, C-11), 29.8 (q, C-29), 28.5 (q, C-23), 26.6 (t, C-2), 20.7 (q, C-30), 19.2 (t, C-6), 18.6 (q, C-26), 17.9 (t, C-12), 16.6 (q, C-24), 16.4 (q, C-25); ^{1}H and ^{13}C NMR data of the sugar parts were almost the same as those reported for **1**; for other NMR data, see Tables 2; ESIMS (negative ion mode) *m/z*, 1447 $[M - H]^-$.

Maejaposide D (4): amorphous solid; $[\alpha]^{24}_D$ −20.4° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3421, 2930, 1693, 1385, 1246, 1078 cm^{-1}; δ 6.71 (1H, d, J = 10.0 Hz, H-21), 6.25 (1H, d, J = 10.1 Hz, H-22), 5.16 (1H, s, H-28), 3.23 (1H, m, H-3), 2.34 (1H, dd, J = 14.4, 2.0 Hz, H-18), 1.59, 1.33, 1.27, 1.22, 1.13 (× 2), 0.82, (each

3H, s, Me of C-27, C-26, C-23, C-30, C-24, 29, C-25); ^{13}C NMR data of the aglycon and the NMR data for the sugar moieties were almost the same as those reported for **3** and **1**, respectively; for other NMR data, see Tables 2; ESIMS (negative ion mode) *m*/*z*, 1449 [M − H]$^-$.

Maejaposide E (5): amorphous solid; $[\alpha]^{24}_D$ −23.6° (*c* 0.5, MeOH); IR (KBr) ν_{max} 3421, 2932, 1695, 1385, 1246, 1078 cm^{-1}; δ 6.76 (1H, d, *J* = 10.0 Hz, H-21), 6.32 (1H, d, *J* = 10.0 Hz, H-22), 5.15 (1H, s, H-28), 3.24 (1H, m, H-3), 2.37 (1H, dd, *J* = 14.6, 2.0 Hz, H-18), 1.60, 1.32, 1.27, 1.25, 1.15 (× 2), 0.81 (each 3H, s, Me of C-27, C-26, C-23, C-30, C-24, 29, C-25);^{13}C NMR data of the aglycon and NMR data for the sugar moieties were almost the same as those reported for **3** and **1**, respectively; for other NMR data, see Tables 2; ESIMS (negative ion mode) *m*/*z*, 1461 [M − H]$^-$.

Acid Hydrolysis of Maejaposides A (1), B (2a,b), C (3), D (4), and E (5). Compound **1** (27 mg) was heated in 1 mL 1N HCl (dioxane−H_2O, 1:1) at 80 °C for 2 h in a water bath. After dioxane was removed, the solution was extracted with EtOAc (1 mL × 3). The organic layer was washed with H_2O and then concentrated to give an amorphous powder (12 mg), which was subjected to HPLC purification (90% MeOH in 0.06TFA% H_2O, 1 mL/min, UV 210 nm) obtaining **1a** (4 mg). The monosaccharide portion was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column, concentrated (dried overnight), then treated with 1-(trimethylsilyl)imidazole at room temperature for 2 h. After the excess reagent was decomposed with H_2O, the reaction product was extracted with hexane (1 mL × 2). The TMSi derivatives of the monosaccharides were identified to be galactose, rhamnose, and xylose (2:1:1) by co-GLC analyses with standard monosaccharides. The remaining monosaccharide was identified as glucuronic acid by co-TLC analysis (both **1** and the authentic sugar were applied to the TLC plate and then hydrolyzed under HCl vapor at 65 °C for 1 h, developing solvent: $CHCl_3$-MeOH−H_2O, 10:5:1). By the same method, the monosaccharides of **2**, **3**, **4**, and **5** were shown to be identical to those of **1**.

Aglycon 1a: amorphous solid; $[\alpha]^{24}_D$ −18.0° (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3430, 2928, 1725, 1246, 1187 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 9.69 (1H, s, H-28), 5.69 (1H, dd, *J* = 12.1, 5.5 Hz, H-22), 5.53 (1H, br t, H-12), 5.28 (1H, br s, H-16), 3.47 (1H, dd, *J* = 10.2, 5.3 Hz, H-3), 3.04 (1H, dd, *J* = 10.0, 3.3, H-18), 1.86, 1.25, 1.19, 1.06, 1.05, 0.95, 0.84 (each 3H, s, Me of C-27, C-23, C-30, C-29, C-24, C-25, C-26); for ^{13}C NMR data, see Table 1; FABMS (positive ion mode) *m*/*z*, 591 [M + Na]$^+$.

Methanolysis of Maejaposide A (1) and Identification of the Acyl Side Chain. Compound **1** (5 mg) was dissolved in 1.5 mL 1M NaOMe in dry MeOH and kept at room temperature for 4 h. After neutralization with dilute HCl (aqueous), the hydrolysate was extracted with hexane. Following GLC analysis (column temperature 80 °C, carrier gas N_2 20 mL/min) identified the acyl side chain to be *(Z)*-2-hexenoic acid in the form of methyl ester (t_R 6.0 min). The available standard methyl *(E)*-2-hexenoate had a retention time of 8.2 min.

Alkaline and Acid Hydrolyses of Maejaposides C (3), D (4), and E (5). Compound **3** (10 mg) in 1 mL of 1N KOH was heated at 80 °C for 2 h. After cooling, the reaction mixture was neutralized with 1N HCl and then extracted with *n*-BuOH (3 times). The organic layers were combined and then evaporated to dryness in a vacuum. By the same method, **4** and **5** (each 2 mg) afforded the same products as revealed by HPLC analysis. HPLC condition: ODS column, 1.0 × 30 cm, 63% MeOH−0.06% TFA in H_2O, 1.5 mL/min, UV detector, 210 nm. The resultants were combined and further subjected to acid hydrolysis (1N HCl in dioxane−H_2O at 80 °C for 2 h). Usual workup afforded the aglycon **3a** (1 mg).

Aglycon 3a: amorphous solid; $[\alpha]^{24}_D$ +44° (*c* 0.05, $CHCl_3$); IR (KBr) ν_{max} 3421, 2928, 1715, 1382, 1037 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz) δ 9.83 (1H, s, H-28), 6.51 (1H, br s, H-16), 5.57 (1H, br t, H-12), 4.89 (1H, d, *J* = 9.5 Hz, H-22), 4.26 (1H, d, *J* = 9.5 Hz, H-21), 3.47 (1H, dd, *J* = 9.5, 3.9 Hz, H-3), 3.13 (1H, dd, *J* = 10.0, 3.0, H-18), 1.83, 1.37, 1.34, 1.24, 1.06, 0.96, 0.90 (each 3H, s, Me of C-27, C-23, C-30, C-29, C-24, C-25, C-26); FABMS (positive ion mode) *m*/*z*, 511 [M + Na]$^+$.

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