New Triterpenoid Saponins from *Maesa tenera*

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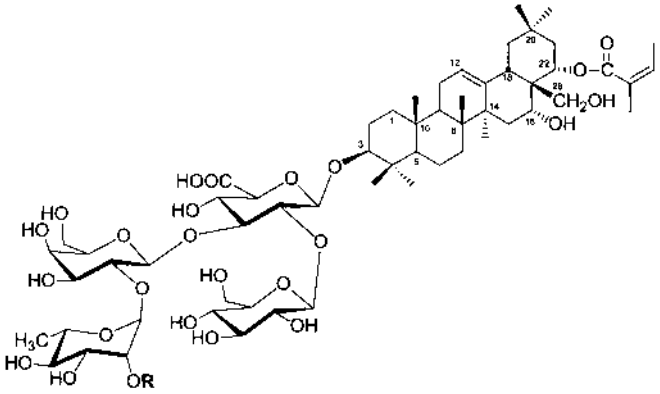
Two new triterpenoid saponins, maetenosides A and B were isolated from the aerial parts of *Maesa tenera* and were respectively defined as 3-*O*-[β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)][β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl camelliagenin A 22-*O*-angelate (1) and 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)][β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl camelliagenin A 22-*O*-angelate (2). Their structures were established on the basis of chemical and spectroscopic methods.

Key words *Maesa tenera*; Myrsinaceae; triterpenoid saponin; maetenoside A; maetenoside B

The *Maesa genus* (family Myrsinaceae) consists of *ca.* 200 species in the world. In Japan, this genus is represented by 2 species, *M. japonica* (THUNB.) MORITZI & ZOLL. and *M. tenera* MEZ. Many plants in the genus have been used as a folk remedy in African and Asian countries.[1] Previous chemical investigations on *Maesa* spp demonstrated the presence of phenol,[2] quinones,[3—8] triterpenoids,[9] and triterpenoid saponins.[10—16] Quite recently we reported the isolation and structure elucidation of 6 new triterpenoid saponins from *M. japonica.*[14] Until now no chemical study has been reported on *M. tenera.* Our continuing interest in the chemistry of triterpenoid saponins[17—19] prompted us to initiate a chemical investigation of this plant. In this paper, we report the isolation and structural elucidation of two novel triterpenoid saponins from the aerial parts of this species.



1 R = β-D-Glc
2 R = H

Chart 1

Results and Discussion

A MeOH extract of the freshly collected aerial parts of *M. tenera* was chromatographed over Diaion HP-20 and silica gel, followed by repeated octadecyl silica (ODS) MPLC and HPLC purifications, and afforded the new triterpenoid saponins maetenosides A (**1**) and B (**2**). Both compounds contained the same aglycon, camelliagenin A 22-*O*-angelate, but with different sugar structure linked to C-3 of the aglycon.

Maetenoside A (**1**), an amorphous solid, had a molecular formula of $C_{65}H_{104}O_{30}$ determined from its positive matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS (*m/z* 1387 $[M+Na]^+$, 1403 $[M+K]^+$) and ^{13}C distortionless enhancement polarization transfer (DEPT) NMR data. Its spectral features and physicochemical properties suggested that **1** is a triterpenoid saponin. The IR spectrum showed absorptions at 3399 cm^{-1} (–OH) and at 1680 cm^{-1} (conjugated ester carbonyl). Of the 65 carbons, 30 were assigned to the aglycon part, 30 to the oligosaccharide moiety, and the remaining 5 to an acyl group (Table 1). Among the 30 carbons of the aglycon, seven were assigned to the methyl carbons at δ 15.7, 16.7, 16.8, 25.7, 27.6, 28.0, and 33.5 and the correspondent methyl protons were identified by a ^{13}C–^1H heteronuclear shift correlation spectroscopy (HETCOR) experiment. Structural assignment was initiated from the long-range coupling networks observed between the methyl protons and the adjacent carbons from a heteronuclear multiple bond correlation spectroscopy (HMBC) experiment. Extensive NMR analyses showed the aglycon was of an olean-12-ene skeleton. Four carbons bearing oxygen were found at δ 63.6 (C-28), 70.2, 73.0, and 89.5 (C-3). 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 nuclear Overhauser effect (NOE) information from phase-sensitive homo nuclear Overhauser enhancement spectroscopy (NOESY). 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 (–CH_2OH) indicated the β-orientation of the hydroxyl group 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) indicated the α-orientation of the hydroxyl group at C-22. From the above evidence, the aglycon was identified as olean-12-en-3β,16α,22α-triol (camelliagenin A).[20—22] An acyl group was also mapped out from double-quantum filter homonuclear shift correlation spectroscopy (DQF-COSY), homonuclear Hartman–Hahn spectroscopy (HOHAHA) and HETCOR correlations and identified as an angeloyl group esterified to C-22 hydroxyl; this was established from the long-range HMBC coupling between H-22 (δ 6.22) and C-1 of the acyl group (168.0) and confirmed by the low field signal of H-22, indicative of acylation.

Moreover, the presence of five sugar moieties was evidenced by the ^1H- and ^{13}C-NMR that displayed five sugar

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Table 1. ^{13}C- and ^{1}H-NMR Data for the Aglycon Moieties of Maetenoside A (**1**) and B (**2**) (in Pyridine-d_5)[a,b]

	1		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
1	38.7 t		38.7 t	
2	26.4 t		26.4 t	
3	89.5 d	3.22 dd (11.7, 4.4)	89.7 d	3.21 dd (11.9, 4.6)
4	39.6 s		39.7 s	
5	55.7 d		55.7 d	
6	18.4 t		18.5 t	
7	33.1 t		33.1 t	
8	41.7 s		41.8 s	
9	46.9 d		46.9 d	
10	36.7 s		36.8 s	
11	23.8 t		23.8 t	
12	123.3 d	5.38 br s	123.3 d	5.38 br s
13	143.7 s		143.7 s	
14	40.1 s		40.1 s	
15	35.1 t		35.2 t	
16	70.2 d	4.64 br s	70.2 d	4.65 br s
17	44.9 s		44.9 s	
18	40.9 d	3.05 dd (14.0, 3.1)	41.7 t	3.05 dd (14.0, 3.4)
19	47.5 t	1.28,[c] 2.91 t (14.0)	47.5 t	1.28,[c] 2.91 t (14.0)
20	33.5 s		33.4 s	
21	41.7 t	2.05,[c] 2.84 t (11.7)	41.7 t	2.03,[c] 2.84 t (11.9)
22	73.0 d	6.22 dd (11.7, 5.7)	73.0 d	6.21 dd (11.9, 5.4)
23	28.0 q	1.18 s	28.0 q	1.18 s
24	16.7 q	1.06 s	16.7 q	1.06 s
25	15.7 q	0.78 s	15.7 q	0.78 s
26	16.8 q	0.86 s	16.8 q	0.86 s
27	27.6 q	1.87 s	27.6 q	1.87 s
28	63.6 t	3.70, 3.76 (each d, 10.6)	63.6 t	3.70, 3.76 (each d, 10.3)
29	33.5 q	1.06 s	33.5 q	1.05 s
30	25.7 q	1.29 s	25.2 q	1.29 s
1′	168.0 s		168.0 s	
2′	129.5 s		129.6 s	
3′	136.5 d	5.92 m	136.5 d	5.92 m
4′	15.9 q	2.09 dd (7.1, 1.4)	15.9 q	2.09 dd (7.1, 1.4)
5′	20.9 q	1.96 br s	21.0 q	1.96 br s

a) Assignments were based upon COSY, HOHAHA, DEPT, HETCOR, NOESY and HMBC experiments. *b*) *J* values (in Hz) in parentheses. *c*) Overlapping with other signals.

anomeric protons at δ 5.01 (1H, d, J=7.2 Hz), 5.06 (1H, d, J=7.6 Hz), 5.79 (1H, d, J=7.5 Hz), 6.07 (1H, d, J=7.7 Hz), 6.36 (1H, s) and carbons at δ 101.3, 101.5, 103.1, 105.2, 107.3 (Table 1), respectively. The low-field chemical shifts of C-3 (δ 89.5) indicated that the pentasaccharide chain was connected to this position, whose sequence was determined by a combination of COSY, HOHAHA, DEPT, HETCOR, 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, in addition to the NOEs across the glycosidic bonds, a NOESY experiment also revealed the 1,3 and 1,5-diaxial relationships for glucuronic acid, galactose and glucose, thus greatly simplifying the mapping of these spin systems. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit were identified by HETCOR and further confirmed by HMBC. These data led to the identification of the five monosaccharide units as β-glucuronic acid, β-galactose, α-rhamnose, and β-glucose (×2). Acid hydrolysis of **1** with 1 M hydrochloric acid in dioxane–water gave D-glucuronic acid, D-galactose, L-rhamnose, and D-glucose as the carbohydrate components. The inter-sugar linkages were established from the following HMBC correlations: H-1 of the glucose with C-2 of the rhamnose; H-1 of the rhamnose with C-2 of the galactose; H-1 of the galactose with C-3 of the glucuronic acid; H-1 of the remaining glucose with C-2 of the 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 (Fig. 1). The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiments. 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 glucose were based on their $^{3}J_{H1,H2}$ coupling constants (7—8 Hz). The ^{1}H non-splitting 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°),[23] indicating the anomeric proton was equatorial, thus possessed an α configuration for rhamnose. Based upon the above evidence, maetenoside A is established as 3-*O*-[β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→2)-β-D-

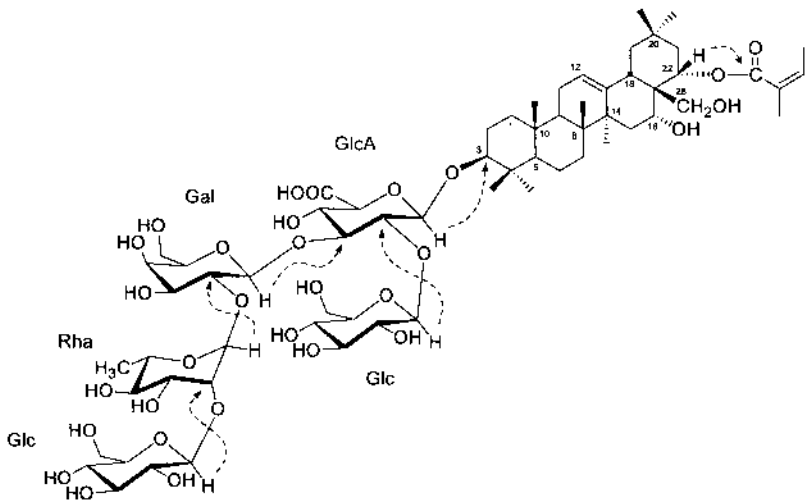

Fig. 1. Key Long-Range HMBC Correlations for Maetenoside A (**1**)

Table 2. ^{13}C- and ^{1}H-NMR for the Sugar Moieties of Maetenosides A (**1**) and B (**2**)[a,b]

Sugar unit	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
Glucuronic acid				
1	105.2	5.01 d (7.2)	105.4	4.89 d (6.4)
2	79.5	4.69 dd (7.2, 7.7)	79.5	4.75
3	82.7	4.77	82.7	4.78
4	71.3	4.62	71.2	4.66
5	77.4	4.60	77.5	4.59 d (9.8)
6	172.2	—	172.2	—
Glucose				
1	103.1	5.79 d (7.5)	102.7	5.92 d (7.6)
2	76.4	4.14	76.5	4.15 dd (7.6, 8.8)
3	78.1	4.43	78.2	4.43 dd (8.8, 9.1)
4	72.5	4.10	72.6	4.09 dd (9.1, 9.0)
5	78.4	4.42	78.5	4.43 m
6	63.5	4.32, 4.61	63.6	4.36, 4.70
Galactose				
1	101.3	6.07 d (7.7)	102.4	6.19 d (8.2)
2	78.0	4.62	76.4	4.72
3	75.9	4.41	76.0	4.50
4	70.9	4.41	71.2	4.49
5	76.9	4.16	77.0	4.31
6	61.8	4.33	61.9	4.35
Rhamnose				
1	101.5	6.36 s	101.4	6.24 s
2	82.6	4.78	72.6	4.79
3	72.5	4.73	72.6	4.73
4	74.4	4.13	73.9	4.22 dd (9.4, 9.2)
5	69.6	4.81 m	69.8	4.90 m
6	18.2	1.45 d (6.1)	18.2	1.44 d (6.1)
Glucose				
1′	107.3	5.06 d (7.6)		
2′	75.9	4.01 dd (7.6, 8.1)		
3′	78.4	4.13		
4′	71.7	4.07 t (9.2)		
5′	78.6	3.83 m		
6′	62.9	4.45, 4.17		

a) Assignments were based upon COSY, HOHAHA, DEPT, HETCOR, NOESY and HMBC experiments. *b*) *J* values (in Hz) in parentheses.

galactopyranosyl-(1→3)][β-D-glucopyranosyl-(1→2)]-β-D-glucuronopyranosyl camelliagenin A 22-*O*-angelate (**1**).

Maetenoside B (**2**), an amorphous solid, had a molecular formula of $C_{59}H_{94}O_{25}$, determined from its MALDI-TOF MS (*m/z* 1225 $[M+Na]^+$, 1241 $[M+K]^+$) and ^{13}C DEPT NMR data. ^{1}H- and ^{13}C-NMR spectra indicated that compound **2** had the same aglycon, camelliagenin A and the same acyl group (angeloyl) attached to C-22 as that of **1** but differed in the sugar part (Table 2). The presence of four sugars in **2** was indicated from the four anomeric protons at δ 4.89 (1H, d, *J*=6.4 Hz), 5.92 (1H, d, *J*=7.6 Hz), 6.19 (1H, d, *J*=8.2 Hz), 6.24 (1H, s) and carbons at δ 101.4, 102.4, 102.7, 105.4, respectively (Table 2). The overall structural assignment was accomplished using the same protocol as in **1**. Extensive NMR (DQF-COSY, HOHAHA, HETCOR, NOESY, and HMBC) studies permitted the full assignment of the proton and carbons. The exact linkage positions for the sugar unit were established using the HMBC and NOESY correlations as depicted for **1**. The stereochemistry of each anomeric carbon was determined from the same observation as that of **1**. Thus, maetenoside B was established as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)][β-D-glucopyranosyl-(1→2)]β-D-glucuronopyranosyl camelliagenin A 22-*O*-angelate (**2**).

Experimental

General IR spectra were determined using a JASCO 300E FTIR spectrometer. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. MALDI-TOF MS were conducted using a PerSpective Biosystems Voyager DE-STR 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 tetramethylsilane (TMS). Diaion HP-20 (Mitsubishi Chemical), silica gel (Silica 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).

Plant Material *Maesa tenera* Mez. was collected from Yakushima island, Kagoshima prefecture, Japan, in February 1998, and was identified by one of the authors (T. Nikaido). A specimen of the plant is kept in the herbarium of the School of Pharmaceutical Sciences, Toho University.

Extraction and Isolation The finely cut, fresh aerial parts of *M. tenera* (1.2 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 (60.0 g). The MeOH extract 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 silica gel and ODS columns to give several saponin fractions. Further HPLC purification (70—75% MeOH–0.06% trifluoroacetic acid (TFA) in H_2O, 1.0 ml/min, UV detector, 210 nm) gave maetenosides A (14.0 mg) and B (10.0 mg).

Maetenoside A (**1**): An amorphous solid; $[\alpha]_D^{22}$ −15.0° (*c*=0.7, MeOH); IR ν_{max}^{KBr}: 3399, 2935, 1680, 1378, 1076 cm^{-1}; NMR data: see Tables 1 and 2; MALDI-TOF MS (positive ion mode) *m/z* 1387 $[M+Na]^+$, 1403 $[M+K]^+$.

Maetenoside B (**2**): An amorphous solid; $[\alpha]_D^{22}$ −21.3° (*c*=0.6, MeOH); IR ν_{max}^{KBr}: 3422, 2932, 1680, 1379, 1242, 1076 cm^{-1}; ^{1}H- and ^{13}C-NMR data of the aglycon part were the same as those reported for **1**; for other NMR data, see Table 2; MALDI-TOF MS (*m/z* 1225 $[M+Na]^+$, 1241 $[M+K]^+$).

Determination of the Absolute Configuration of the Carbohydrate Subunits[24,25] A solution of **1** (5 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was heated at 100 °C for 2 h. After extracting with EtOAc, the H_2O layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column and concentrated to furnish the mono saccharide residue. After dissolving in H_2O (1 ml), the solutions of L-(−)-α-methylbenzylamine (5 mg) and $Na(BH_3CN)$ (8 mg) in EtOH (1 ml) were added. The mixture was allowed to stand overnight, then was acidified by addition of glacial acetic acid (0.2 ml) and evaporated to dryness. The resulting solid was acetylated with Ac_2O anhydride (0.3 ml) in pyridine (0.3 ml) at 100 °C for 1 h. After co-distillation with toluene, H_2O (1 ml) was added to the residue, and the crude mixture was passed through a Sep-pak C_{18} cartridge (Waters) and washed with H_2O–MeCN (4 : 1, 1 : 1, each 5 ml). The H_2O–MeCN (1 : 1) eluate contained a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which were identified by co-HPLC analysis with standard sugars prepared under the same conditions. HPLC conditions: column, PEGASIL ODS, 4.6×150 mm; solvent, MeOH–H_2O (33 : 67); flow rate, 0.8 ml/min; detection, UV 230 nm. The derivatives of D-glucuronic acid, D-glucose, D-galactose, and L-rhamnose were detected with t_R (min) of 23.2, 31.9, 39.1, and 44.9, respectively. By the same method, the monosaccharides of **2** were shown to be D-glucuronic acid D-galactose, D-glucose, and L-rhamnose.

Acknowledgements We are grateful to Mr. K. Tezuka of Yakushima Island for his help in collecting the plant materials.

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