Four Novel Cycloartane Glycosides from *Astragalus oleifolius*[1]

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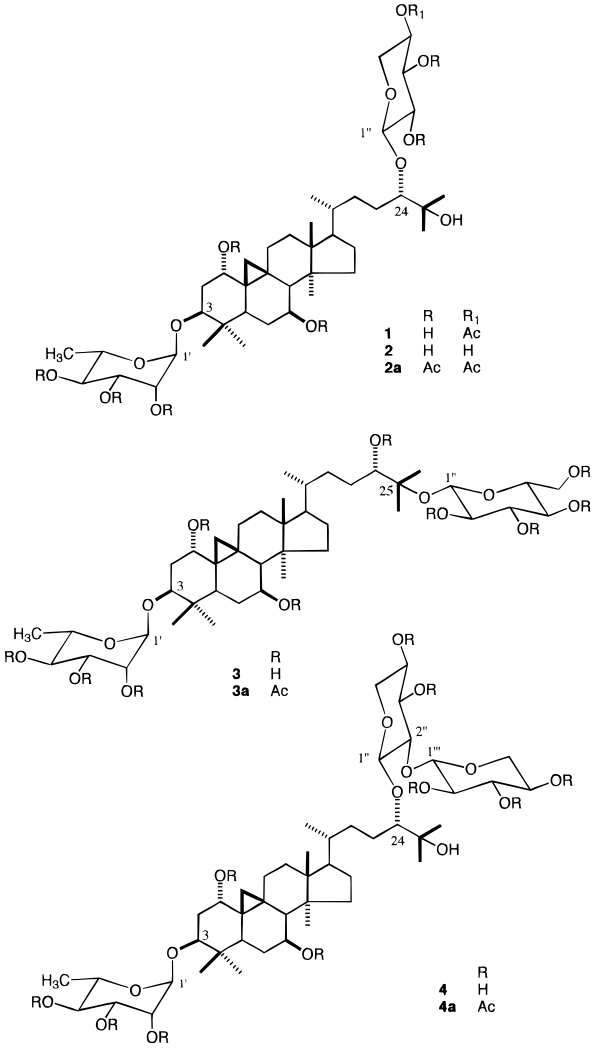
Four novel cycloartane-type triterpene glycosides, macrophyllosaponins A−D (**1**−**4**) were isolated from the roots of *Astragalus oleifolius*. By means of chemical (acetylation, alkaline hydrolysis) and spectroscopic methods (IR, 1D- and 2D-NMR, FABMS), their structures were established as 3-*O*-α-L-rhamnopyranosyl-24-*O*-(4″-*O*-acetyl)-*β*-D-xylopyranosyl-1α,3*β*,7*β*,24(*S*),-25-pentahydroxycycloartane (**1**), 3-*O*-α-L-rhamnopyranosyl-24-*O*-*β*-D-xylopyranosyl-1α,3*β*,7*β*,-24(*S*),25-pentahydroxycycloartane (**2**), 3-*O*-α-L-rhamnopyranosyl-25-*O*-*β*-D-glucoyranosyl-1α,3*β*,7*β*,24(*S*),25-pentahydroxycycloartane (**3**), and 3-*O*-α-L-rhamnopyranosyl-24-*O*-(2-*O*-*β*-D-xylopyranosyl)-*β*-D-xylopyranosyl-1α,3*β*,7*β*,24(*S*),25-pentahydroxycycloartane (**4**).

Astragalus L., the largest genus in the family Leguminosae, is represented by 380 species in the flora of Turkey.[2] The roots of various *Astragalus* species represent very old and well-known drugs in traditional medicine, used for antiperspirant, diuretic, and tonic purposes and for the treatment of nephritis, diabetes, leukemia, and uterine cancer.[3] Previous studies performed on this genus have resulted in the isolation of a series of cycloartane-type triterpenoid glycosides.[4−15] Also reported for *Astragalus* species are their antiinflammatory, analgesic, diuretic, hypotensive, sedative, and cardiotonic activities.[4] The current report describes the isolation and structure elucidation of four novel cycloartane-type triterpene glycosides named as macrophyllosaponins A (**1**), B (**2**), C (**3**), and D (**4**) from the roots of *Astragalus oleifolius* DC. (section of genus: *Macrophyllium*).



Results and Discussion

An aqueous EtOH extract of the air-dried roots of *Astragalus oleifolius* was separated using reversed-phase vacuum−liquid chromatography (VLC). Further purification of the resulting fractions by normal, and reversed-phase medium-pressure liquid chromatography (MPLC) and column chromatography led to the isolation of four compounds, **1**−**4**.

The IR spectra of compounds **1**−**4** showed hydroxy absorption bands in all cases, and additionally an ester carbonyl absorption band for **1**. The FABMS exhibited sodiated molecular ion peaks at *m*/*z* 835 $[M + Na]^+$ for **1**, 793 $[M + Na]^+$ for **2**, 823 $[M + Na]^+$ for **3**, and 925 $[M + Na]^+$ for **4**, which are compatible with the molecular formulas $C_{43}H_{72}O_{14}$, $C_{41}H_{70}O_{13}$, $C_{42}H_{72}O_{14}$, and $C_{46}H_{78}O_{17}$, respectively.

Compound **2** was the major compound isolated in this investigation. Its ^{1}H-NMR spectrum showed signals characteristic of cyclopropane methylene protons, six tertiary methyl and two secondary methyl groups, of which one was assigned to an α-L-rhamnose moiety. Additionally, the resonances for two anomeric protons (δ 4.65 d, J = 1.7 Hz, α-L-rhamnose; δ 4.19 d, J = 7.5 Hz, *β*-D-xylose) were observed. The ^{1}H-NMR signals could be assigned by means of 2D-^{1}H−^{1}H-shift correlation (COSY). Among the sugar protons, the remaining signals showed correlations with the resonances at highfield, indicating the presence of four protons geminal to oxygenated carbons of the sapogenol moiety. Seven molecular fragments (Scheme 1), in addition to methyl resonances, were evident, and corresponding

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Scheme 1. Fragments of **2** Deduced from 2D-NMR Measurements

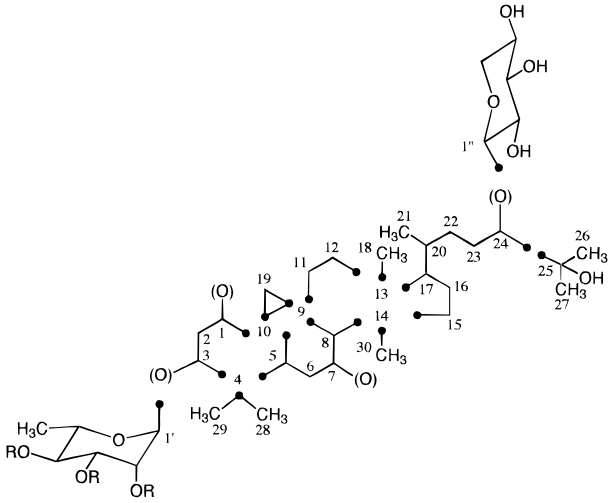

Table 1. $^{1}H-^{13}C$ Heteronuclear Multiple-Bond Correlations (HMBC) for Compound **2**

carbon	HMBC
1	H_2-2, H_2-19
2	
3	H_2-2, H-5, H_3-28, H_3-29, H-1′
4	H_2-2, H-5, H_3-28, H_3-29
5	H-6, H_3-28, H_3-29, H_2-19
6	H-5, H-8
7	H-5, H_2-6, H-8
8	H-6, H-8, H-11, H_3-30, H_2-19
9	H-8, H_2-11, H_2-12, H_2-19
10	H_2-2, H-5, H_2-11, H_2-19
11	H_2-12
12	H_2-11
13	H_2-16, H-17, H_3-18, H_3-30
14	H_2-15, H_2-16, H-17, H_3-30
15	H-8, H_3-30
16	H-17
17	H_2-15, H_2-16, H_3-18, H_3-21
18	H-17
19	H-5, H-8, H-11
20	H_2-16, H-17
21	H-17
22	H_3-21
23	H_2-22
24	H_2-23, H_3-26, H_3-27, H-1″
25	H_3-26, H_3-27
26	H-24, H_3-27
27	H_3-26
28	H-3, H_3-29
29	H-3, H-5
30	H_2-15
1′	H-3
1″	H-24

carbon resonances were assigned by a 2D $^{1}H-^{13}C$-heteronuclear correlation (HMQC). In order to establish the interfragment relationship, a heteronuclear multiple-bond correlation experiment (HMBC) was performed (Table 1), which not only showed connectivities but also resolved the interglycosidic linkages. Thus, the anomeric proton of α-L-rhamnose (δ 4.79, d, J = 1.7 Hz) showed a long-range correlation to C-3 (δ 86.1 d), while the anomeric proton of β-D-xylose (δ 4.33, d, J = 7.6 Hz), exhibited a long-range correlation to C-24 (δ 90.2 d), indicating the bidesmosidic structure of **2**. Similarly, long-range correlations between C-1′ (δ 105.4 d) and H-3 (δ 3.69) and C-1″ (δ 106.4 d) and H-24 (δ 3.44, dd, J = 9.2, 1.8 Hz) were observed. ^{1}H- and ^{13}C-NMR data for H-24 and C-24 are comparable to those reported for analogous compounds having a 24*S* configuration.[4,10]

Acetylation of **2** yielded an octaacetate, **2a**. From the FABMS of **2a**, which displayed a $[M - H_2O]^+$ ion at m/z 988, a molecular formula of $C_{57}H_{86}O_{21}$ was proposed for **2a**. The IR spectrum of **2a** still exhibited a free hydroxyl absorption band after acetylation, indicating the presence of a free tertiary hydroxyl group at C-25. Additionally, the peaks observed at m/z 273 and 259 corresponding to the triacetylrhamnoseoxonium and the triacetylxyloseoxonium ions, respectively, confirmed the terminal positions of these units. Thus, in the ^{1}H-NMR spectrum of **2a**, two of the eight acetoxyl resonances could be attributed to the sapogenol moiety. Indeed, the resonances due to the H-1 and H-7 protons were observed at δ 4.67 and 4.84, showing the expected downfield shifts in comparison to **2**. On the other hand, the relative configurations of the oxygenated carbon atoms were determined from the magnitude of the vicinal proton−proton coupling constants to be: C-1 (α-OH; δ 4.67 dd, J = 3.0, 2.9 Hz, H_{eq}-1), C-3 (β-OH; δ 3.47 dd, J = 12.1, 4.4 Hz, H_{ax}-3), C-7 (β-OH; δ 4.84 ddd, J = 10.9, 8.3, 5.0 Hz, H-7), and C-24 (δ 3.35 dd, J = 9.3, 1.8 Hz, H-24). Consequently, the structure of the saponin **2** was established as 3-*O*-α-L-rhamnopyranosyl-24-*O*-β-D-xylopyranosyl-1α,3β,7β,24(*S*),25-pentahydroxycycloartane.

The IR spectrum of **1** showed an ester carbonyl absorption band at 1735 cm^{-1}. In the FABMS of **1** a peak at m/z 835, $[M + Na]^+$, 42 mass units higher than in **2**, indicated the presence of an acetoxy group which was confirmed in the ^{1}H-NMR spectrum (δ 2.11 s, 3H, $COCH_3$). The site of acylation was clear from the downfield shift of H-4″ (δ 4.74 ddd, J = 10.3, 9.3, 5.4 Hz) of the xylose moiety. This conclusion was confirmed by the ^{13}C-NMR data as well as by a COSY experiment. The alkaline hydrolysis of **1** afforded **2** on TLC. Consequently, the structure of **1** was established as 3-*O*-α-L-rhamnopyranosyl-24-*O*-(4″-*O*-acetyl)-β-D-xylopyranosyl-1α,3β,7β,24(*S*),25-pentahydroxycycloartane.

The ^{1}H-NMR spectrum of **3** showed two anomeric proton resonances at δ 4.78 (d, J = 1.7 Hz) and 4.54 (d, J = 7.8 Hz), which were assigned to α-L-rhamnose and β-D-glucose moieties, respectively. The ^{13}C-NMR resonances arising from the sapogenol moiety were very similar to those of **1** and **2**, except for the signals of C-24 (δ 79.0 d), C-25 (δ 82.5 s), C-26 (δ 24.0 q), and C-27 (δ 23.6 q), and exhibited significant glycosidation shifts for C-3 (δ 86.1 d) and C-25 (δ 82.5 s). An HMBC experiment performed on **3** established the interglycosidic connectivities, showing correlations between C-1′ (δ 105.4 d) of the α-L-rhamnose unit and H-3 (δ 3.67) of the aglycon, C-3 (δ 86.1 d) of the aglycon and the anomeric proton (H-1′; δ 4.78 d, J = 1.7 Hz) of the α-L-rhamnose unit, and C-25 (δ 82.5 s) of the aglycon and the anomeric proton (H-1″; δ 4.54 d, J = 7.8 Hz) of β-D-glucose.

Acetylation of **3** yielded a decaacetate, **3a**. The FABMS of **3a** showed a peak at m/z 1244 $[M + Na + H]^+$, compatible with the molecular formula $C_{62}H_{92}O_{24}$. The peaks observed at m/z 273 and 331, corresponding to the (triacetylrhamnosyl)oxonium and the (tetracetylglucosyl)oxonium ions, respectively, showed that the rhamnose and glucose units were linked to the sapogenol moiety from different locations, supporting the

presence of a bidesmosidic structure. In the ^{1}H-NMR spectrum of **3a**, three of the ten acetoxyl resonances had thus to be attributed to the sapogenol moiety: H-1 (δ 4.69, dd, J = 2.9, 3.0 Hz), H-7 (δ 4.84, ddd, J = 10.9, 8.3, 5.0 Hz), and H-24 (δ 4.88, dd, J = 1.7, 10.7 Hz) were shifted downfield in comparison to those of **3**, indicating the site of acetylations on the sapogenol moiety. Thus, the structure of saponin **3** was established as 3-*O*-α-L-rhamnopyranosyl-25-*O*-*β*-D-glucopyranosyl-1α,3*β*,7*β*,24-(*S*),25-pentahydroxycycloartane (**3**).

Saponin **4** has a triglycosidic structure, consisting of a rhamnose unit (δ 4.78 d, J = 1.7 Hz) and two xylose units (δ 4.55 d, J = 7.4 Hz and δ 4.48 d, J = 7.1 Hz). Carbon and proton resonances attributed to the sapogenol moiety and the saccharidic units were similar to those of **2**. The position of the second xylose residue was deduced from a +8.1 ppm downfield shift for C-2″ (δ 84.3 d) of the xylose unit linked to C-24 (δ 89.7, d) of the aglycon, revealing the presence of a disaccharide unit at C-24. All connectivities, including the interglycosidic linkages, within **4** were also proved by an HMBC experiment, where correlations between H-1′ (δ 4.78 d, J = 1.7 Hz) of the rhamnose unit and C-3 (δ 86.1, d) of the aglycon moiety, H-1″ (δ 4.48 d, J = 7.1 Hz) of one of the xylose units and C-24 (δ 89.7 d) of the aglycon, moiety and H-1‴ (δ 4.55 d, J = 7.4 Hz) of the terminal xylose unit and C-2″ (δ 84.3, d) of the bridging xylose unit were indicated.

Acetylation of **4** yielded the decaacetate **4a**. In the ^{1}H-NMR spectrum of **4a**, the resonances of H-1 (δ 4.67) and H-7 (δ 4.86) were shifted downfield. As no downfield shift was observed for the signals of H-3 (δ 3.48), H-24 (δ 3.37), and H-2″ (δ 3.69) of one of the xylose units, the interglycosidic linkages must be present at C-3 and C-24 of the sapogenol moiety and C-2″ of the bridging xylose unit, confirming the presence of a bidesmosidic structure as those of **1** and **2**, and a disaccharide moiety, 2-*O*-*β*-D-xylosyl-D-xylose. The FABMS of **4a** exhibited a peak at m/z 1346 $[M + Na + H]^+$, compatible with the molecular formula $C_{66}H_{98}O_{27}$. Additional fragmentation peaks were observed at m/z 273, 259, and 475, which were attributed to the (triacetylrhamnosyl)oxonium, (triacetylxylosyl)oxonium, and ((pentaacetylxylopyranosyl)xylosyl)oxonium ions, respectively, supporting the proposed structure of **4**. Thus, the structure of **4** was elucidated as 3-*O*-α-L-rhamnopyranosyl-24-*O*-(2-*O*-*β*-D-xylopyranosyl)-*β*-D-xylopyranosyl-1α,3*β*,7*β*,24(*S*),25-pentahydroxycycloartane (**4**).

To the best of our knowledge, the sapogenol moiety of these saponins is a new one and so are the saponins. The lack of a hydroxyl group in ring D is very rare. For these compounds we propose the trivial names macrophyllosaponins A (**1**), B (**2**), C(**3**), and D (**4**), and macrophyllogenin for their aglycon [24(*S*)-cycloartane-1α,3*β*,7*β*,24,25-pentol].

Experimental Section

General Experimental Procedures. Optical rotations were recorded with a Perkin-Elmer 241 polarimeter using MeOH as solvent. IR spectra were measured on a Perkin-Elmer 2000 FT-IR spectrometer as pressed KBr disks. 1D- and 2D-NMR spectra were recorded using Bruker AMX-300, AMX-400, and AMX-500 instruments. FABMS were recorded using ZAB2-SEQ spectrometer. A Büchi MPLC instrument was used throughout this study for the separation. LiChroprep C_{18} (Merck) and Si gel 60 (70−230 mesh, Merck) were used as reversed and normal phases for chromatographic separations, respectively. Si gel 60 F_{254} precoated Al sheets (0.2 mm, Merck) were used for TLC.

Plant Material. *A. oleifolius* DC. (Leguminosae) was collected from Ahlatlibel, Ankara (Central Anatolia), in May 1994. Voucher specimens (94-004) have been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

Extraction and Isolation. Air-dried and powdered roots of the plant (250 g) were kept in EtOH−H_2O (4:1; 2 L) overnight, then refluxed for 2 h and filtered. The filtrate was concentrated to dryness *in vacuo* (65 g, yield 26%). An aliquot of the extract (10 g) was partitioned in H_2O (100 mL) and applied to VLC using reversed-phase material, employing H_2O (250 mL), H_2O−MeOH (4:1; 250 mL), H_2O−MeOH (7:3; 100 mL), and MeOH (400 mL) as the eluents. Fractions eluted with MeOH were combined to give the crude saponins (2.38 g). This mixture was further fractionated by normal-phase MPLC using $CHCl_3$−MeOH (4:1) and $CHCl_3$−MeOH−H_2O (80:20:1, 80:20:2, 70:30:2, 60:40:3, and 60:40:4) as eluents to yield nine main fractions (A, 84 mg; B, 174 mg; C, 134 mg; D, 109 mg; E, 177 mg; F, 200 mg; G, 50 mg; H, 91 mg; I, 555 mg).

Fraction A was chromatographed on a Si gel column eluted with $CHCl_3$−MeOH−H_2O (90:10:0.5, 80:20:1) to give compound **1** (41 mg). Fraction B was chromatographed on a Si gel column eluted with $CHCl_3$−MeOH−H_2O (80:20:1) to give compound **2** (112 mg). Fraction C consisted almost entirely of compound **2** (134 mg). Fraction D was subjected to MPLC using reversed-phase material as stationary phase. Elution with increasing amounts of MeOH in H_2O (50−80% MeOH) yielded compound **3** (25 mg) and **2** (20 mg). Fraction F was chromatographed on a Si gel column employing $CHCl_3$−MeOH−H_2O (80:20:2, 70:30:3) as the eluents, to give compound **4** (98 mg).

Macrophyllosaponin A (1): $[\alpha]^{20}_D$ −5.0° (*c* 0.28, MeOH); IR ν max (KBr) 3400 (OH), 1735 (ester carbonyl) cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 4.79 (1H, d, J = 1.7 Hz, H-1′), 4.74 (1H, ddd, J = 10.3, 9.3, 5.4 Hz, H-4″), 4.38 (1H, d, J = 7.6 Hz, H-1″), 4.00 (1H, dd, J = 11.3, 5.4 Hz, H-5″a), 3.88 (1H, dd, J = 3.4, 1.7 Hz, H-2′), 3.71 (1H, dq, J = 9.5, 6.3 Hz, H-5′), 3.70 (1H, m, H-3_{ax}), 3.68 (1H, dd, J = 3.4, 9.5 Hz, H-3′), 3.60 (1H, dd, J = 9.3, 9.2 Hz, H-3″), 3.56 (1H, m, H-1_{eq}), 3.56 (1H, m, H-7), 3.44 (1H, m, H-24), 3.41 (1H, t, J = 9.5 Hz, H-4′), 3.32 (1H, dd, J = 7.6, 9.2 Hz, H-2″), 3.22 (1H, dd, J = 11.3, 10.3 Hz, H-5″b), 2.11 (3H, s, $COCH_3$), 1.60 (1H, m, H-17), 1.28 (1H, d, J = 6.3 Hz, H_3-6′), 1.21 (3H, s, H_3-27), 1.19 (3H, s, H_3-26), 1.09 (3H, s, H_3-30), 1.07 (3H, s, H_3-18), 1.02 (3H, s, H_3-28), 0.97 (3H, d, J = 6.3 Hz, H_3-21), 0.87 (3H, s, H_3-29), 0.81 and 0.48 (1H each, AB system, J_{AB} = 4.6 Hz, H_2-19); ^{13}C NMR (125 MHz, CD_3OD) δ 173.2 (s, *C*OCH_3), 106.2 (d, C-1″), 105.3 (d, C-1′), 90.0 (d, C-24), 86.1 (d, C-3), 76.3 (d, C-3″), 75.1 (d, C-4′), 75.0 (d, C-2″), 74.7 (d, C-1), 74.5 (d, C-4″), 74.0 (s, C-25), 73.6 (d, C-3′), 73.5 (d, C-2′), 72.1 (d, C-7), 70.9 (d, C-5′), 64.6 (t, C-5″), 57.0 (d, C-8), 54.0 (d, C-17), 50.7 (s, C-14), 47.9 (s, C-13), 42.5 (s, C-4), 41.1 (d, C-5), 39.5 (t, C-15), 38.7 (d, C-20), 38.0 (t, C-2), 35.4 (t, C-22), 35.0 (t, C-12),

33.1 (t, C-6), 32.4 (s, C-10), 30.6 (t, C-16), 30.4 (t, C-23), 30.3 (t, C-19), 28.0 (t, C-11), 27.4 (q, C-27), 27.0 (q, C-28), 26.4 (q, C-26), 23.0 (s, C-9), 21.8 (q, $CO\underline{C}H_3$), 20.2 (q, C-30), 19.9 (q, C-21), 19.2 (q, C-18), 18.8 (q, C-6′), 15.5 (q, C-29); FABMS *m*/*z* $[M + Na]^+$ 835 (38) [(monoacetylxylosyl)oxonium ion]$^+$ 175 (15).

Macrophyllosaponin B (2): $[\alpha]^{20}_D$ +2.8° (*c* 0.58, MeOH); IR *ν* max (KBr) 3400 (OH) cm^{-1}; ^1H NMR (500 MHz, CD_3OD) sapogenol moiety: *δ* 4.79 (1H, d, *J* = 1.7 Hz, H-1′), 4.33 (1H, d, *J* = 7.6 Hz, H-1″), 3.90 (1H, dd, *J* = 11.4, 5.4 Hz, H-5″a), 3.88 (1H, dd, *J* = 3.4, 1.7 Hz, H-2′), 3.70 (1H, dq, *J* = 9.5, 6.3 Hz, H-5′), 3.69 (1H, m, $H\text{-}3_{ax}$), 3.68 (1H, dd, *J* = 9.5, 3.4 Hz, H-3′), 3.54 (1H, dd, *J* = 3.2, 2.7 Hz, $H\text{-}1_{eq}$), 3.54 (1H, m, H-7), 3.53 (1H, ddd, *J* = 10.1, 9.5, 5.4 Hz, H-4″), 3.44 (1H, dd, *J* = 9.2, 1.8 Hz, H-24), 3.40 (1H, t, *J* = 9.5 Hz, H-4′), 3.35 (1H, dd, *J* = 9.5, 9.2 Hz, H-3″), 3.25 (1H, dd, *J* = 9.2, 7.6 Hz, H-2″), 3.22 (1H, dd, *J* = 11.4, 10.1 Hz, H-5″b), 2.29 (1H, m, H-11a), 2.16 (1H, dd, *J* = 13.1, 4.4 Hz, H-5), 2.08 (1H, ddd, *J* = 12.7, 4.2, 3.2 Hz, H-2a), 2.04 (1H, m, H-16a), 1.88 (1H, td, *J* = 12.7, 2.7 Hz, H-2b), 1.77 (1H, m, H-6a), 1.74 (2H, m, H_2-12), 1.65 (1H, m, H-23a), 1.63 (1H, m, H-17), 1.59 (1H, m, H-8), 1.59 (2H, m, H_2-15), 1.54 (1H, m, H-23b), 1.54 (2H, m, H_2-22), 1.44 (1H, m, H-20), 1.36 (1H, m, H-11b), 1.35 (1H, m, H-16b), 1.26 (d, *J* = 6.3 Hz, H_3-6′), 1.21 (3H, s, H_3-27), 1.19 (3H, s, H_3-26), 1.09 (3H, s, H_3-30), 1.07 (1H, m, H-6b), 1.06 (3H, s, H_3-18), 1.01 (3H, s, H_3-28), 0.96 (3H, d, *J* = 6.3 Hz, H_3-21), 0.86 (3H, s, H_3-29), 0.80 and 0.48 (1H each, AB system, J_{AB} = 4.6 Hz, H_2-19); ^{13}C NMR (125 MHz, CD_3OD) *δ* 106.4 (d, C-1″), 105.4 (d, C-1′), 90.2 (d, C-24), 86.1 (d, C-3), 78.9 (d, C-3″), 76.2 (d, C-2″), 75.1 (d, C-4′), 74.7 (d, C-1), 74.5 (s, C-25), 73.6 (d, C-3′), 73.5 (d, C-2′), 72.1 (d, C-7), 72.1 (d, C-4″), 71.0 (d, C-5′), 68.0 (t, C-5″), 57.0 (d, C-8), 54.1 (d, C-17), 50.7 (s, C-14), 47.9 (s, C-13), 42.5 (s, C-4), 41.1 (d, C-5), 39.5 (t, C-15), 38.7 (d, C-20), 38.0 (t, C-2), 35.4 (t, C-22), 35.0 (t, C-12), 33.1 (t, C-6), 32.1 (s, C-10), 30.7 (t, C-16), 30.5 (t, C-23), 30.3 (t, C-19), 28.0 (t, C-11), 27.5 (q, C-27), 27.0 (q, C-28), 26.2 (q, C-26), 23.0 (s, C-9), 20.3 (q, C-30), 19.9 (q, C-21), 19.3 (q, C-18), 18.9 (q, C-6′), 15.5 (q, C-29); FABMS *m*/*z* $[M + Na]^+$ 793 (13).

Macrophyllosaponin C (3): $[\alpha]^{20}_D$ +15° (*c* 0.32, MeOH); IR *ν* max (KBr) 3400 (OH) cm^{-1}; ^1H NMR (500 MHz, CD_3OD) *δ* 4.78 (1H, d, *J* = 1.7 Hz, H-1′), 4.54 (1H, d, *J* = 7.8 Hz, H-1″), 3.91 (1H, dd, *J* = 3.3, 1.7 Hz, H-2′), 3.85 (1H, dd, *J* = 12.0, 2.2 Hz, H-6″a), 3.70 (1H, dq, *J* = 9.5, 6.3 Hz, H-5′), 3.67 (1H, m, $H\text{-}3_{ax}$), 3.67 (1H, m, H-6″b), 3.66 (1H, dd, *J* = 9.5, 3.3 Hz, H-3′), 3.58 (1H, m, H-7), 3.56 (1H, dd, *J* = 3.6, 2.7 Hz, $H\text{-}1_{eq}$), 3.49 (1H, dd, *J* = 6.1, 6.0 Hz, H-24), 3.40 (1H, dd, *J* = 9.2, 9.0 Hz, H-3″), 3.39 (1H, t, *J* = 9.5 Hz, H-4′), 3.31 (1H, t, *J* = 9.0 Hz, H-4″), 3.29 (1H, m, H-5″), 3.19 (1H, dd, *J* = 9.2, 7.8 Hz, H-2″), 2.27 (1H, m, H-11a), 2.16 (1H, dd, *J* = 13.1, 4.4 Hz, H-5), 2.07 (1H, ddd, *J* = 13.5, 4.2, 3.6 Hz, H-2a), 1.98 (1H, m, H-16a), 1.87 (1H, td, *J* = 13.5, 2.7 Hz, H-2b), 1.75 (1H, m, H-6a), 1.70 (2H, m, H_2-12), 1.61 (1H, m, H-17), 1.60 (2H, m, H_2-15), 1.59 (1H, m, H-8), 1.57 (2H, m, H-22a), 1.46 (1H, m, H-20), 1.46 (2H, m, H_2-23), 1.36 (1H, m, H-11b), 1.36 (1H, m, H-16b), 1.33 (1H, m, H-22b), 1.28 (1H, d, *J* = 6.3 Hz, H_3-6′), 1.26 (3H, s, H_3-26), 1.23 (3H, s, H_3-27), 1.08 (3H, s, H_3-30), 1.05 (1H, m, H-6b), 1.05 (3H, s, H_3-18), 1.00 (3H, s, H_3-28), 0.94 (3H, d, *J* = 6.4 Hz, H_3-21), 0.85 (3H, s, H_3-29), 0.80 and 0.45 (1H each, AB system, J_{AB} = 4.6 Hz, H_2-19); ^{13}C NMR (125 MHz, CD_3OD) *δ* 105.4 (d, C-1′), 99.0 (d, C-1″), 86.1 (d, C-3), 82.5 (s, C-25), 79.3 (d, C-3″), 79.0 (d, C-24), 78.8 (d, C-5″), 76.4 (d, C-2″), 75.1 (d, C-4′), 74.8 (d, C-1), 73.6 (d, C-3′), 73.5 (d, C-2′), 72.7 (d, C-4″), 72.1 (d, C-7), 71.0 (d, C-5′), 63.8 (t, C-6″), 57.0 (d, C-8), 54.2 (d, C-17), 50.7 (s, C-14), 47.9 (s, C-13), 42.5 (s, C-4), 41.1 (d, C-5), 39.5 (t, C-15), 38.3 (d, C-20), 38.0 (t, C-2), 35.7 (t, C-22), 35.0 (t, C-12), 33.1 (t, C-6), 32.4 (s, C-10), 30.6 (t, C-16), 30.3 (t, C-19), 30.0 (t, C-23), 28.0 (t, C-11), 27.0 (q, C-28), 24.0 (q, C-26), 23.6 (q, C-27), 23.0 (s, C-9), 20.2 (q, C-30), 19.9 (q, C-21), 19.3 (q, C-18), 18.8 (q, C-6′), 15.5 (q, C-29); FABMS *m*/*z* $[M + Na]^+$ 823 (6).

Macrophyllosaponin D (4): $[\alpha]^{20}_D$ −1.0° (*c* 0.32, MeOH); IR *ν* max (KBr) 3400 (OH) cm^{-1}; ^1H NMR (500 MHz, CD_3OD) *δ* 4.78 (1H, d, *J* = 1.7 Hz, H-1′), 4.55 (1H, d, *J* = 7.4 Hz, H-1‴), 4.48 (1H, d, *J* = 7.1 Hz, H-1″), 3.90 (1H, m, H-5″a), 3.88 (1H, m, H-5‴a), 3.87 (1H, dd, *J* = 3.4, 1.7 Hz, H-2′), 3.70 (1H, dq, *J* = 9.5, 6.3 Hz, H-5′), 3.68 (1H, m, $H\text{-}3_{ax}$), 3.67 (1H, dd, *J* = 9.5, 3.4 Hz, H-3′), 3.58 (1H, m, H-4″), 3.56 (1H, m, $H\text{-}1_{eq}$), 3.56 (1H, m, H-3″), 3.54 (1H, m, H-7), 3.51 (1H, ddd, *J* = 10.2, 9.5, 5.4 Hz, H-4‴), 3.43 (1H, m, H-2″), 3.41 (1H, dd, *J* = 9.3, 1.8 Hz, H-24), 3.39 (1H, t, *J* = 9.5 Hz, H-4′), 3.36 (1H, dd, *J* = 9.5, 9.1 Hz, H-3‴), 3.30 (1H, dd, *J* = 9.1, 7.4 Hz, H-2‴), 3.23 (1H, m, H-5″b), 3.20 (1H, m, H-5‴b), 2.27 (1H, m, H-11a), 2.16 (1H, dd, *J* = 13.1, 4.4 Hz, H-5), 2.07 (1H, ddd, *J* = 13.5, 4.3, 3.5 Hz, H-2a), 2.0 (1H, m, H-16a), 1.87 (1H, ddd, *J* = 13.5, 13.1, 2.8 Hz, H-2b), 1.76 (1H, m, H-6a), 1.73 (2H, m, H_2-12), 1.68 (1H, m, H-23a), 1.63 (1H, m, H-17), 1.60 (1H, m, H-8), 1.59 (2H, m, H_2-15), 1.54 (2H, m, H_2-22), 1.51 (1H, m, H-23b), 1.41 (1H, m, H-20), 1.36 (1H, m, H-11b), 1.33 (1H, m, H-16b), 1.26 (3H, d, *J* = 6.3 Hz, H_3-6′), 1.18 (3H, s, H_3-26), 1.17 (3H, s, H_3-27), 1.09 (3H, s, H_3-30), 1.06 (3H, s, H_3-18), 1.05 (1H, m, H-6b), 1.00 (3H, s, H_3-28), 0.95 (3H, d, *J* = 6.4 Hz, H_3-21), 0.86 (3H, s, H_3-29), 0.80 and 0.46 (1H each, AB system, J_{AB} = 4.6 Hz, H_2-19); ^{13}C NMR (125 MHz, CD_3OD) *δ* 107.5 (d, C-1‴), 105.4 (d, C-1′), 104.9 (d, C-1″), 89.7 (d, C-24), 86.1 (d, C-3), 84.3 (d, C-2″), 78.7 (d, C-3‴), 78.6 (d, C-3″), 76.8 (d, C-2‴), 75.1 (d, C-4′), 74.8 (d, C-1), 74.6 (s, C-25), 73.6 (d, C-3′), 73.5 (d, C-2′), 72.2 (d, C-4‴), 72.1 (d, C-7), 71.9 (d, C-4″), 71.0 (d, C-5′), 68.2 (t, C-5‴), 67.5 (t, C-5″), 57.0 (d, C-8), 54.0 (d, C-17), 50.7 (s, C-14), 47.9 (s, C-13), 42.5 (s, C-4), 41.1 (d, C-5), 39.5 (t, C-15), 38.9 (d, C-20), 38.0 (t, C-2), 35.6 (t, C-22), 35.0 (t, C-12), 33.1 (t, C-6), 32.4 (s, C-10), 30.7 (t, C-16), 30.3 (t, C-23), 30.1 (t, C-19), 28.0 (t, C-11), 27.3 (q, C-27), 27.0 (q, C-28), 26.3 (q, C-26), 23.0 (s, C-9), 20.4 (q, C-30), 19.9 (q, C-21), 19.3 (q, C-18), 18.8 (q, C-6′), 15.5 (q, C-29); FABMS *m*/*z* $[M + Na]^+$ 925 (19).

Alkaline Hydrolysis of 1. Compound **1** (2 mg) was heated in 5% aqueous KOH solution (0.5 mL) at 80 °C for 1 h. After neutralizing the solution with 5% aqueous HCl solution and evaporating it, the resulting residue was compared with compound **2** by TLC using $CHCl_3$−MeOH−H_2O (61:32:7 and 80:20:2) as solvent systems.

Acetylation of 2−4 to 2a−4a. Treatment of each compound (**2**−**4**; 10−20 mg) with Ac_2O (1 mL) and pyridine (1 mL) at room temperature overnight followed by the usual workup yielded compounds **2a**−**4a**.

Macrophyllosaponin B octaacetate (2a): IR *ν* max (KBr) 3400 (OH), 2972 (CH, aliphatic), 1750 (C═O, ester), 1372, 1225, 1050 (C−O−C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 5.25 (1H, dd, *J* = 9.9, 3.4 Hz, H-3′), 5.22 (1H, dd, *J* = 3.4, 1.7 Hz, H-2′), 5.18 (1H, dd, *J* = 9.5,

9.2 Hz, H-3″), 5.04 (1H, t, J = 9.9 Hz, H-4′), 4.98 (1H, ddd, J = 9.9, 9.2, 5.5 Hz, H-4″), 4.97 (1H, dd, J = 9.5, 7.5 Hz, H-2″), 4.84 (1H, ddd, J = 10.9, 8.3, 5.0 Hz, H-7), 4.76 (1H, d, J = 1.7 Hz, H-1′), 4.67 (1H, dd, J = 3.0, 2.9 Hz, H-1), 4.52 (1H, d, J = 7.5 Hz, H-1′), 4.12 (1H, dd, J = 11.6, 5.5 Hz, H-5″a), 3.89 (1H, dq, J = 9.9, 6.3 Hz, H-5′), 3.47 (1H, dd, J = 12.1, 4.4 Hz, H-3), 3.35 (1H, dd, J = 9.3, 1.8 Hz, H-24), 3.32 (1H, dd, J = 11.6, 9.9 Hz, H-5″b), 2.13 (3H, s, acetoxyl), 2.07 (2H, m, H-2b, H-5), 2.05, 2.04, 2.03, 2.03, 2.02, 2.01, 1.98 (3H each, s, acetoxyl × 7), 1.95 (1H, d, J = 8.3 Hz, H-8), 1.90 (1H, m, H-22a), 1.87 (1H, m, H-15a), 1.85 (2H, m, H-2a, H-6β), 1.67 (1H, m, H-11a), 1.55 (1H, m, H-12a), 1.50 (3H, m, H-12b, H_2-16), 1.48 (3H, m, H-17, H_2-23), 1.42 (1H, m, H-11b), 1.33 (1H, m, H-20), 1.27 (1H, m, H-15b), 1.24 (1H, m, H-22b), 1.15 (3H, d, J = 6.3 Hz, H_3-6′), 1.12, 1.11 (3H each, s, H_3-26 and H_3-27, respectively), 1.01 (1H, m, H-6α), 0.97 (3H, s, H_3-28), 0.94 (1H, d, J = 5.2 Hz, H-19a), 0.93, 0.92, 0.83 (3 H each, s, H_3-29, H_3-30, and H_3-18, respectively), 0.83 (3H, d, J = 6.8 Hz, H_3-21), 0.41 (1H, d, J = 5.2 Hz, H-19b); FABMS m/z [M − H_2O]$^+$ 988 (0.5), [(triacetylrhamnosyl)oxonium ion]$^+$ 273 (100), [(triacetylxylosyl)oxonium ion]$^+$ 259 (51).

Macrophyllosaponin C decaacetate (3a): IR ν max (KBr) 2960 (CH, aliphatic), 1757 (C═O, ester), 1372, 1246, 1050 (C−O−C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27 (1H, dd, J = 9.9, 3.4 Hz, H-3′), 5.24 (1H, dd, J = 3.4, 1.7 Hz, H-2′), 5.21 (1H, t, J = 9.5 Hz, H-3″), 5.06 (1H, t, J = 9.9 Hz, H-4′), 5.02 (1H, t, J = 9.7 Hz, H-4″), 4.97 (1H, dd, J = 9.7, 8.0 Hz, H-2″), 4.88 (1H, dd, J = 10.7, 1.7 Hz, H-24), 4.84 (1H, ddd, J = 10.9, 8.3, 5.0 Hz, H-7), 4.77 (1H, d, J = 1.7 Hz, H-1′), 4.71 (1H, d, J = 8.0 Hz, H-1′), 4.69 (1H, dd, J = 3.0, 2.9 Hz, H-1), 4.19 (1H, dd, J = 12.0, 5.7 Hz, H-6″a), 4.12 (1H, dd, J = 12.0, 2.4 Hz, H-6″b), 3.91 (1H, dq, J = 10.0, 6.3 Hz, H-5′), 3.69 (1H, m, H-5″), 3.48 (1H, dd, J = 12.1, 4.4 Hz, H-3), 2.15, 2.08, 2.07, 2.05, 2.04 (3H each, s, acetoxyl × 5), 2.03, 2.00 (6H each, s, acetoxyl × 4), 1.99 (3H, s, acetoxyl), 1.21, 1.17 (3H each, s, tertiary methyl × 2), 1.16 (3H, J = 6.3 Hz, H_3-6′), 0.97 (3H, s, tertiary methyl), 0.95 (1H, d, J = 5.1 Hz, H-19a), 0.93, 0.92, 0.84 (3H each, s, tertiary methyl × 3), 0.83 (3H, d, J = 6.3 Hz, H_3-21), 0.40 (1H, d, J = 5.1 Hz, H-19b); FABMS m/z [M + Na + H]$^+$ 1244 (5), [(tetracetylglucosyl)-oxonium ion]$^+$ 331 (14), [(triacetylrhamnosyl)oxonium ion]$^+$ 273 (35).

Macrophyllosaponin D decaacetate (4a): IR ν max (KBr) 3400 (OH), 2932 (CH, aliphatic), 1752 (C═O, ester), 1373, 1244, 1043 (C−O−C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.27 (1H, dd, J = 9.9, 3.4 Hz, H-3′), 5.23 (1H, dd, J = 3.4, 1.7 Hz, H-2′), 5.15 (1H, t, J = 9.1 Hz, H-3″), 5.11 (1H, t, J = 9.1 Hz, H-3‴), 5.05 (1H, t, J = 10 Hz, H-4′), 4.91 (1H, dd, J = 9.3, 7.4 Hz, H-2″), 4.86 (1H, m, H-7), 4.77 (1H, d, J = 1.7 Hz, H-1′), 4.67 (1H, dd, J = 3.0, 2.9 Hz, H-1), 4.63 (1H, d, J = 7.4 Hz, H-1″), 4.44 (1H, d, J = 7.1 Hz, H-1‴), 4.11 (1H, dd, J = 11.9, 5.4 Hz, H-5″a), 4.04 (1H, J = 11.9, 5.4 Hz, H-5‴a), 3.90 (1H, dq, J = 10, 6.3 Hz, H-5′), 3.69 (1H, dd, J = 9.1, 7.1 Hz, H-2″), 3.51 (1H, s, C(25)−OH), 3.48 (1H, dd, J = 12.1, 4.3 Hz, H-3), 3.37 (1H, dd, J = 9.3, 1.8 Hz, H-24), 3.34 (1H, J = 11.9, 9.9 Hz, H-5″b), 3.30 (1H, J = 11.9, 9.0 Hz, H-5‴b), 2.15, 2.11, 2.06, 2.04, 2.03, 2.02, 2.015, 2.01, 2.00, 1.99 (3H each, s, acetoxyl × 10), 1.17 (3H, d, J = 6.3 Hz, H_3-6′), 1.16, 1.15, 1.14, 0.97, 0.96 (3H each, s, tertiary methyl × 5), 0.95 (1H, d, J = 5.1 Hz, H-19a), 0.90 (3H, d, J = 5.9 Hz, H_3-21), 0.84 (3H, s, tertiary methyl), 0.43 (1H, d, J = 5.1 Hz, H-19b); FABMS m/z [M + Na + H]$^+$ 1346 (6), [((pentaacetylxylopyranosyl)-xylosyl)oxonium ion]$^+$ 475 (100), [(triacetylrhamnosyl)-oxonium ion]$^+$ 273 (90), [(triacetylxylosyl)oxonium ion]$^+$ 259 (95).

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References and Notes

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