Triterpene Glycosides from the Roots of *Codonopsis lanceolata*

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In the course of the development of new designer foods using the roots of *Codonopsis lanceolata*, we found that hot-water extracts of *C. lanceolata* recovered decreased testosterone levels in the blood and accelerated the restoration of reproductive dysfunction induced by hyperthermic treatment in male mice. Thus we studied the constituents of the polar fraction of the roots of *C. lanceolata* and identified six new triterpene saponins, lancemasides B (2), C (3), D (4), E (5), F (6), and G (7), along with the known saponin lancemasaide A (1) and phenylpropanoid glycosides 8—10. The structures of the new compounds 2—7 were determined by means of spectral data including 2D-NMR studies and chemical reactions to be oleanan-type bisdesmoside with sugars at C-3 and C-28. Compounds 2—6 have echinocystic acid as an aglycone, and compound 7 has asterogenic acid as an aglycone. Identification of the sugars and determination of their D,L-chiralities were carried out by application of the exciton chirality method to the per-*O*-*p*-bromobenzoylmethyl sugar derived from saponins.

Key words *Codonopsis lanceolata*; Campanulaceae; triterpene saponin; oleanane; bisdesmoside; exciton chirality method

Some herbal medicines have been shown to ameliorate male sterility and impotence since ancient times. However, the scientific evaluation of the effects of these crude drugs remains insufficient. Partial androgen deficiency of the aging male (PADAM)-like symptoms[1] in middle-aged men result from aging and various stresses of daily life in contemporary society and now present a severe social problem. The ability of the hot-water extract of *Codonopsis lanceolata* Trautv (Campanulaceae) to accelerate recovery from dysfunctional spermatogenesis and sexual behavior induced by local acute hyperthermia (42 °C for 30 min) were investigated. The extract and its fraction allowed the recovery of spermatogenesis and improved sexual function (unreported data). The hot-water extract of *C. lanceolata* also showed improvement of PADAM-like symptoms.[2] Thus we produced a new designer food, P-EX, containing *C. lanceolata*, *Butea superba,* and *Apocynum venetum* extracts with the aim of ameliorating PADAM-like symptoms in middle-aged men. In the course of developing this new designer food, we confirmed that the saponin lancemaside A isolated from the roots of *C. lanceolata* ameliorated PADAM-like symptoms in animal experiments. The roots of *C. laceolata* have been used as an antitussive, expectorant, and detoxicant in folk medicine in China, Korea, and Japan, and as food for Csrizen dishes in Korea and China. In Korea, *C. lanceolata* has been used as a crude drug with a tonic action similar to that of *Panax ginseng*, which has been reported to be effective for the treatment of male sterility.[3] It was determined that lancemaside A contributed to the recovery of decreased testosterone levels in the blood.[4] Oleanan-type saponins have been isolated from *C. lanceolata*.[5,6] We therefore attempted the isolation and structural elucidation of constituents from the polar fraction of the roots of *C. lanceolata* imported from Korea and identified six new saponins along with a known saponin and phenylpropanoid glycosides.

Results and Discussion

The roots of *C. lanceolata* purchased in a Korean market were extracted with boiling water, which was then passed through a Diaion HP20 column, washed thoroughly with water, and eluted with methanol to give a methanol eluate. The methanol eluate improved spermatogenesis and was separated using various chromatographic techniques as described in Experimental to give six new saponins: lancemasides B (**2**), C (**3**), D (**4**), E (**5**), F (**6**), and G (**7**), along with the known saponin lancemasaide A (**1**), phenylpropanoid glycosides **8**—**10**, and amino acid **11** (as shown in Fig. 1).

Structures of the known compounds **8**—**11** were determined based on spectral data such as MS and NMR including 2D-NMR and identified by comparing the spectral data with their authentic data. The structure of compound **1** was determined to be 3-*O*-β-D-glucuronopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester. Compound **1** was reported to be an aster saponin Hc isolated from *Aster tataricus* as a methyl ester.[7] Compound **1** was hydrolyzed under acidic conditions in methanol to give methyl sugars and aglycone (**1a**), which was identified to be echinocystic acid from the MS and NMR data, and under alkaline conditions to give prosapogenin (**1b**). The structure of **1b** was determined to be 3-*O*-β-D-glucuronopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid from the MS and NMR data. Phenylpropanoid glycosides were identified as tangshenoside I (**8**), tangshenoside II (**9**), and syringin (**10**). Compound **11** was identified as tryptophan. Compounds **8**, **9**, and **10** were previously reported from *Codonopsis tangshen*.[8] We found that tangshenoside I (**8**) accelerated the restoration of reproductive dysfunction induced by hyperthermic treatment in male mice.

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was determined to be $C_{63}H_{100}O_{31}$

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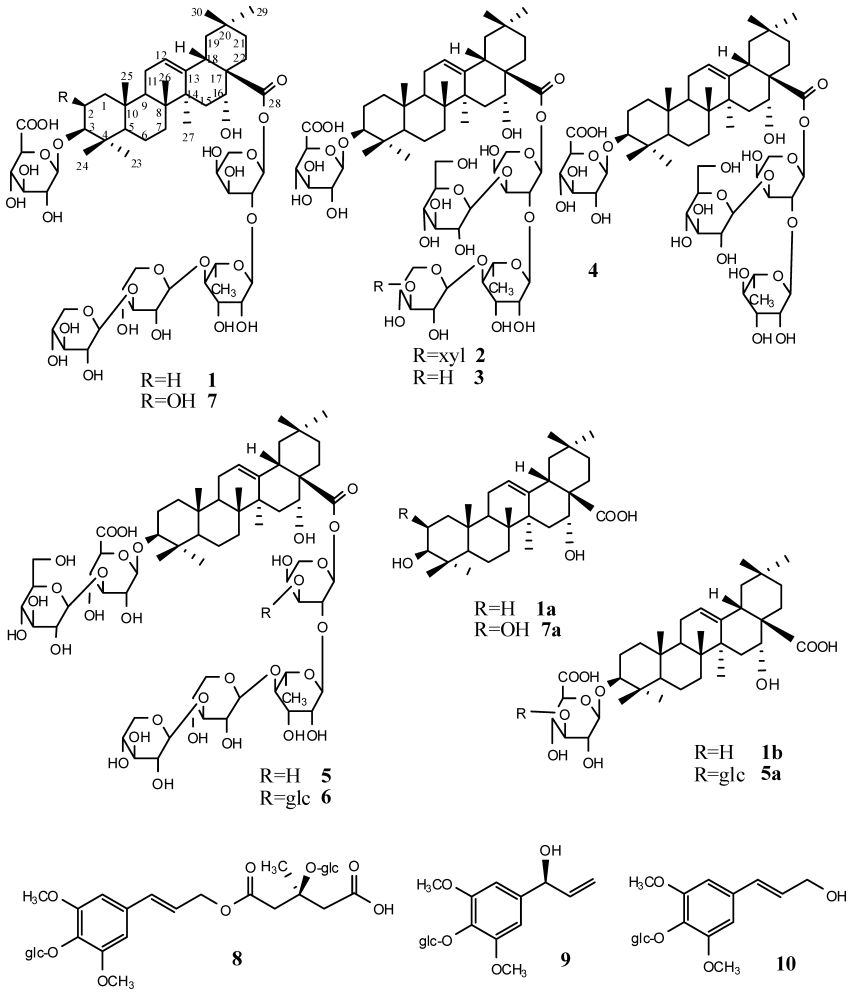

Fig. 1. Structures of Constituents of *C. lanceolata* and Related Compounds

from the pseudo molecular ion $[M-H]^-$ at *m/z* 1351.6162 by HR-ESI-MS (negative mode). The ^{1}H-NMR spectrum of **2** showed the presence of seven singlet methyl groups [δ 0.80 (3H, s), 0.95 (3H, s), 0.98 (3H, s), 1.07 (3H, s), 1.12 (3H, 3H, s), 1.27 (3H, s), 1.83 (3H, s)], one doublet methyl group [δ 1.71 (3H, d, J=5.8 Hz)], and six anomeric protons [δ 5.03 (1H, d, J=7.6 Hz), 5.13 (1H, d, J=7.6 Hz), 5.16 (3H, d, J=7.3 Hz), 5.22 (3H, d, J=7.6 Hz), 5.84 (1H, br s), 6.40 (1H, d, J=2.0 Hz)]. The aglycone portion of the ^{13}C-NMR spectrum of **2** showed an identical chemical shift to that of a reported saponin with echinocystic acid as an aglycone[9,10] The ^{13}C-NMR spectrum of **2** also showed the presence of six anomeric carbons (δ 93.2, 100.7, 104.3, 106.0, 106.2, 107.2), which were correlated with each anomeric proton in the HMQC spectrum. These data indicate that **2** is an analogous triterpene glycoside of **1**. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of **1** indicate that **2** has an extra glucopyranosyl group. Compound **2** was hydrolyzed under alkaline conditions to give prosapogenin, which was identified as the prosapogenin (**1b**) obtained from **1** by alkaline hydrolysis. Thus the existence of a glucopyranosyl group in the sugar residue connected to C-28 was detected. The position of the glucopyranosyl group in the C-28 glycosyl moiety was deduced from the HMBC analysis. The HMBC correlations of **2**, as shown in Fig. 2, revealed the following sugar connectivities: correlations between the H-1 (δ_H 5.03, d, J=7.6 Hz)

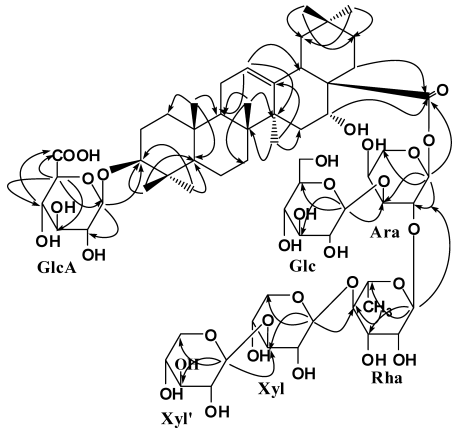

Fig. 2. Important HMBC Correlations for **2**

of GlcA and C-3 (δ_C 89.0); the H-1 (δ_H 6.40, d, J=2.0 Hz) of Ara and C-28 (δ_C 175.8); H-1 (δ_H 5.84, br s) of Rha and C-2 (δ_C 73.8) of Ara; H-1 (δ_H 5.16, d, J=7.3 Hz) of Xyl and C-4 (δ_C 83.2) of Rha; H-1 (δ_H 5.22, d, J=7.6 Hz) of Xyl′ and C-3 (δ_C 87.0) of Xyl; and H-1 (δ_H 5 13, d, J=7.6 Hz) of Glc and C-3 (δ_C 75.5) of Ara. Acid hydrolysis of **2** in methanol gave an aglycone (**1a**) and a methyl sugar fraction. The aglycone (**1a**) was identified as the echinocystic acid obtained from **1** by TLC and HPLC analysis. The methyl sugar fraction

was *p*-bromobenzoylated to give per-*O*-*p*-bromomenzoyl-methyl sugars, which were identified as per-*O*-*p*-bromobenzoyl-1-*O*-α-methylrhamnopyranoside (αMRB), per-*O*-*p*-bromobenzoyl-1-*O*-α-methylarabinopyranoside (αMAB), per-*O*-*p*-bromobenzoyl-1-*O*-α-methylxylopyranoside (αMXB), per-*O*-*p*-bromobenzoyl-1-*O*-β-methylxylopyranoside (βMXB), and per-*O*-*p*-bromobenzoyl-1-*O*-α-methylglucopyranoside (αMGB) using HPLC. The peaks of these benzoates were collected in an analytical scale and their CD and UV spectra were measured at the same concentration. The CD data were applied to the CD exciton chirality method,[11] and their *A*-values were calculated to be +102.1 (αMRB), +98.1 (αMAB), −4.2 (αMXB), +7.4 (βMXB), and +23.9 (αMGB), which were similar to the reported data.[12,13] From these results, the presence of D-glucose, D-xylose, L-rhamnose, and L-arabinose in **2** was clarified. Prosapogenin **1b** was hydrolyzed under acidic methanol conditions to give a methyl sugar. The methyl sugar was *p*-bromobenzoylated to give the per-*O*-*p*-bromobenzoate of 1-*O*-methylglucuronopyranoside methyl ester, of which the retention time in HPLC and *A*-value were identified as those of the *p*-bromobenzoate derived from D-glucuronic acid. This indicated that the glucuronic acid in **2** is in the D-configuration. The glucuronopyranosyl, glucopyranosyl, and xylopyranosyl linkages were identified to be β from the coupling constants (as shown above) of the anomeric protons. The arabinopyranosyl and rhamnopyranosyl linkages were also identified to be α from comparing the ^{1}H- and ^{13}C-NMR data of **2** with those of lancemaside A. Thus the structure of **2** was determined to be 3-*O*-β-D-glucuronopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester. Compound **2** was named lancemaside B.

Compound **3** was obtained as a white amorphous powder. The molecular formula of **3** was determined to be $C_{58}H_{92}O_{27}$ from the pseudomolecular ion $[M-H]^-$ at *m/z* 1219.5739 in HR-ESI-MS (negative mode). The ^{1}H-NMR spectrum of **3** showed the presence of seven singlet methyl groups [δ_H 0.81 (3H, s), 0.94 (3H, s), 0.98 (3H, s), 1.08 (3H, s), 1.09 (3H, s), 1.26 (3H, s), 1.81 (3H, s)], one doublet methyl group [δ_H 1.71 (3H, d, *J*=5.1 Hz)], and five anomeric protons [δ_H 4.99 (1H, d, *J*=7.6 Hz), 5.11 (1H, d, *J*=7.8 Hz), 5.13 (3H, d, *J*=7.6 Hz), 5.93 (1H, br s), 6.32 (1H, d, *J*=3.4 Hz)]. The ^{13}C-NMR spectrum of **3** showed the presence of five anomeric carbons (δ_C 93.5, 100.8, 104.6, 106.8, 107.2). The above data indicate that **3** is the de-xylose variant of **2**. This structure was confirmed based on the HMBC data of **3**. Alkaline hydrolysis of **3** gave prosapogenin (**1b**). Acid hydrolysis of **3** in methanol gave an aglycone (**1a**). From the ^{1}H- and ^{13}C-NMR (Table 1) data, **3** has glucuronopyranosyl, glucopyranosyl, xylopyranosyl, rhamnopyranosyl, and arabinopyranosyl moieties and the glycosyl linkages were β in the case of glucuronopyranosyl, glucopyranosyl, and xylopyranosyl and α in the case of rhamnopyranosyl and arabinopyranosyl based on the coupling constants of the anomeric protons. Thus the structure of **3** was determined to be 3-*O*-β-D-glucuronopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester. Compound **3** was named lancemaside C.

Compound **4** was obtained as a white amorphous powder. The molecular formula of **4** was determined to be $C_{53}H_{84}O_{23}$ from the pseudomolecular ion $[M-H]^-$ at *m/z* 1087.5316 in HR-ESI-MS (negative mode). The ^{1}H-NMR spectrum of **4** showed the presence of seven singlet methyl groups [δ_H 0.86 (3H, s), 0.96 (3H, s), 1.02 (3H, s), 1.14×2 (6H, s), 1.27 (3H, s), 1.87 (3H, s)], one doublet methyl group [δ_H 1.71 (3H, d, *J*=4.5 Hz)], and four anomeric protons [δ_H 5.07 (1H, d, *J*=7.6 Hz), 5.19 (1H, d, *J*=7.6 Hz), 6.01 (1H, br s), 6.41 (br s)]. The ^{13}C-NMR spectrum of **4** showed the presence of four anomeric carbons (δ_C 93.5, 101.2, 104.5, 107.3). Compound **4** gave prosapogenin (**1b**) by alkaline hydrolysis and an aglycone (**1a**) by acid hydrolysis. The ^{1}H- and ^{13}C-NMR (Table 1) data showed the absence of a xylose moiety in **4**. This was confirmed from the HMQC and HMBC spectra. Thus the structure of **4** was determined to be 3-*O*-β-D-glucuronopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-α-L-arabinopyranosyl ester. Compound **4** was named lancemaside D.

Compound **5** was obtained as a white amorphous powder. The molecular formula of **5** was determined to be $C_{63}H_{100}O_{31}$ from the pseudomolecular ion $[M-H]^-$ at *m/z* 1351.6189 in HR-ESI-MS (negative mode). The ^{1}H-NMR spectrum of **5** showed the presence of seven singlet methyl groups [δ_H 0.80 (3H, s), 0.97 (3H, s), 1.00 (3H, s), 1.05 (3H, s), 1.14 (3H, 3H, s), 1.26 (3H, s), 1.83 (3H, s)], one doublet methyl group [δ_H 1.71 (3H, d, *J*=5.5 Hz)], and six anomeric protons [δ_H 4.99 (1H, d, *J*=7.8 Hz), 5.19 (1H, d, *J*=7.8 Hz), 5.24 (1H, d, *J*=7.8 Hz), 5.40 (1H, d, *J*=7.8 Hz), 5.72 (1H, s), 6.51 (1H, d, *J*=2.3 Hz)]. The ^{13}C-NMR spectrum of **5** showed the presence of six anomeric carbons (δ_C 93.4, 100.9, 105.9, 106.0, 106.1, 106.7). Compound **5** gave a prosapogenin (**5a**), of which the structure was determined to be 3-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucurono-pyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid from the ESI-MS, ^{1}H-NMR, ^{13}C-NMR (Table 1), and 2D-NMR spectral data. Acid hydrolysis of **5** gave **1a**. The ^{13}C-NMR chemical shifts of the aglycone portion and sugar moiety linked at C-28 showed almost the same chemical shifts as **1** (Table 1). These data indicate that **5** is a saponin with an extra glucosyl group at C-3 of GlcA when compared with **1**. The ^{1}H- and ^{13}C-NMR spectral data were assigned, and the structure of **5** was confirmed. Thus the structure of **5** was determined to be β-D-glucopyranosyl-(1→3)-β-D-glucuronopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid 28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl ester. Compound **5** was named lancemaside E.

Compound **6** was obtained as a white amorphous powder. The molecular formula of **6** was determined to be $C_{69}H_{110}O_{36}$ from the pseudomolecular ion $[M-H]^-$ at *m/z* 1513.6685 in HR-ESI-MS (negative mode). The ^{1}H-NMR spectrum of **6** showed the presence of seven singlet methyl groups [δ_H 0.79 (3H, s), 0.95 (3H, s), 0.99 (3H, s), 1.08 (3H, s), 1.12 (3H, 3H, s), 1.25 (3H, s), 1.84 (3H, s)], one doublet methyl group [δ_H 1.71 (3H, d, *J*=5.5 Hz)], and seven anomeric protons [δ_H 4.99 (1H, d, *J*=7.3 Hz), 5.13 (1H, d, *J*=7.8 Hz), 5.16 (1H, d, *J*=7.3 Hz), 5.23 (1H, d, *J*=7.3 Hz), 5.40 (1H, d, *J*=7.8 Hz), 5.86 (1H, s), 6.40 (1H, br s)]. The ^{13}C-NMR spectrum of **6** showed the presence of seven anomeric carbons (δ_C 93.2, 100.7, 104.3, 105.9, 106.0, 106.2, 106.7). Compound **6** gave

Table 1. ^{1}H-NMR Spectral Data of Saponines, Prosapogenins and an Aglycon (Pyridine-d_5)[a)]

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**1b**	**5a**	**7a**
H-1	1.39 (overlap)	1.40 (overlap)	1.37 (overlap)	1.42 (overlap)	1.37 (overlap)	1.36 (overlap)	2.23 (overlap)	1.42 (overlap)	1.38 (overlap)	2.34 (overlap)
	0.87 (overlap)	0.88 (t, 12.7)	0.85 (overlap)	0.89 (overlap)	0.85 (overlap)	0.85 (overlap)	1.15 (overlap)	0.89 (dt, 3.6, 13.1)	0.87 (br t, 13.1)	1.26 (overlap)
2	2.22 (overlap)	2.23 (overlap)	2.17 (overlap)	2.22 (overlap)	2.18 (overlap)	2.17 (overlap)	4.72 (br s)	2.24 (overlap)	2.19 (overlap)	4.40 (q-like)
	1.86 (overlap)	1.86 (overlap)	1.81 (overlap)	1.88 (overlap)	1.81 (overlap)	1.86 (overlap)		1.86 (overlap)	1.81 (overlap)	
3	3.38 (dd, 11.9, 3.0)	3.37 (overlap)	3.37 (dd, 11.4, 4.1)	3.41 (br d, 9.0)	3.36 (dd, 11.9, 4.1)	3.35 (dd, 10.6, 3.7)	3.43 (overlap)	3.40 (dd, 11.7, 4.3)	3.36 (dd, 11.4, 4.1)	3.43 (d, 3.9)
5	0.79 (d, 12.0)	0.77 (d, 12.0)	0.77 (d, 12.2)	0.81 (d, 11.4)	0.78 (d, 12.3)	0.78 (overlap)	0.96 (d, 11.0)	0.80 (overlap)	0.78 (overlap)	1.01 (overlap)
9	1.73 (overlap)	1.74 (t, 8.5)	1.74 (overlap)	1.78 (t, 8.0)	1.72 (t, 7.8)	1.73 (overlap)	1.74 (overlap)	1.77 (overlap)	1.78 (overlap)	1.80 (overlap)
12	5.57 (br s)	5.64 (t-like)	5.62 (t-like)	5.67 (br s)	5.57 (t-like)	5.64 (t-like)	5.58 (br s)	5.60 (t-like)	5.60 (t-like)	5.66 (t-like)
16	5.27 (br s)	5.27 (br s)	5.26 (br s)	5.34 (br s)	5.28 (br s)	5.29 (br s)	5.27 (br s)	5.23 (br s)	5.23 (br s)	5.24 (br s)
18	3.57 (overlap)	3.51 (dd, 13.7, 3.7)	3.49 (dd, 13.5, 3.9)	3.54 (br d, 13.0)	3.57 (dd, 13.7, 4.1)	3.51 (dd, 13.7, 4.0)	3.58 (br d, 13.8)	3.59 (dd, 14.9, 3.4)	3.59 (overlap)	3.63 (dd, 13.4, 4.1)
19	2.74 (t, 13.2)	2.78 (t, 13.4)	2.76 (t, 13.7)	2.82 (t, 13.8)	2.78 (t, 13.8)	2.78 (t, 13.3)	2.78 (t, 13.3)	2.81 (t, 12.9)	2.82 (t, 12.9)	2.81 (t, 12.9)
	1.33 (br d, 14.2)	1.33 (overlap)	1.34 (overlap)	1.34 (overlap)	1.34 (overlap)	1.35 (overlap)	1.35 (overlap)	1.35 (overlap)	1.36 (overlap)	1.39 (overlap)
21	2.42 (br t, 12.4)	2.42 (dt, 4.0, 12.5)	2.41 (dt, 4.6, 12.9)	2.47 (br t, 10.2)	2.43 (dt, 4.6, 14.8)	2.44 (t-like)	2.44 (t-like)	2.49 (dt, 4.6, 12.9)	2.49 (dt, 4.8, 12.9)	2.49 (dt, 4.6, 12.9)
	1.29 (overlap)	1.31 (overlap)	1.30 (overlap)	1.31 (overlap)	1.28 (overlap)	1.29 (overlap)	1.28 (overlap)	1.28 (overlap)	1.35 (overlap)	1.36 (overlap)
22	2.31 (overlap)	2.31 (overlap)	2.31 (overlap)	2.36 (overlap)	2.32 (overlap)	2.30 (overlap)	2.31 (overlap)	2.41 (overlap)	2.41 (overlap)	2.41 (overlap)
	2.19 (overlap)	2.17 (dt, 3.5, 13.0)	2.14 (dt, 4.9, 13.4)	2.18 (t-like)	2.19 (dt, 4.2, 12.8)	2.17 (overlap)	2.22 (overlap)	2.24 (overlap)	2.26 (overlap)	2.24 (overlap)
23	1.28 (3H, s)	1.27 (3H, s)	1.26 (3H, s)	1.27 (3H, s)	1.26 (3H, s)	1.25 (3H, s)	1.33 (3H, s)	1.28 (3H, s)	1.25 (3H, s)	1.23 (3H, s)
24	0.98 (3H, s)	0.95 (3H, s)	0.94 (3H, s)	0.96 (3H, s)	0.97 (3H, s)	0.95 (3H, s)	1.39 (3H, s)	0.96 (3H, s)	0.95 (3H, s)	1.35 (3H, s)
25	0.81 (3H, s)	0.80 (3H, s)	0.81 (3H, s)	0.86 (3H, s)	0.80 (3H, s)	0.79 (3H, s)	1.50 (3H, s)	0.82 (3H, s)	0.80 (3H, s)	1.54 (3H, s)
26	1.05 (3H, s)	1.07 (3H, s)	1.08 (3H, s)	1.14 (3H, s)	1.05 (3H, s)	1.08 (3H, s)	1.12 (3H, s)	1.00 (3H, s)	0.99 (3H, s)	1.09 (3H, s)
27	1.83 (3H, s)	1.83 (3H, s)	1.81 (3H, s)	1.87 (3H, s)	1.83 (3H, s)	1.84 (3H, s)	1.83 (3H, s)	1.85 (3H, s)	1.86 (3H, s)	1.84 (3H, s)
29	1.00 (3H, s)	0.98 (3H, s)	0.98 (3H, s)	1.02 (3H, s)	1.00 (3H, s)	0.99 (3H, s)	1.00 (3H, s)	1.04 (3H, s)	1.04 (3H, s)	1.04 (3H, s)
30	1.14 (3H, s)	1.12 (3H, s)	1.09 (3H, s)	1.14 (3H, s)	1.14 (3H, s)	1.12 (3H, s)	1.15 (3H, s)	1.15 (3H, s)	1.15 (3H, s)	1.16 (3H, s)
glcA-1	5.04 (d, 7.3)	5.03 (d, 7.6)	4.99 (d, 7.6)	5.07 (d, 7.0)	4.99 (d, 7.8)	4.99 (d, 7.3)	5.06 (d, 7.0)	5.02 (d, 7.8)	4.96 (d, 7.5)	
5	4.73 (d, 9.2)	4.71 (d, 9.6)	4.64 (d, 9.5)	4.66 (overlap)	4.65 (d, 9.6)	4.65 (overlap)	4.65 (d, 9.6)	4.44 (d, 9.5)	4.60 (d, 9.2)	
ara-1	6.51 (br s)	6.40 (d, 2.0)	6.32 (d, 3.4)	6.41 (br s)	6.51 (d, 2.3)	6.40 (br s)	6.52 (br s)			
rha-1	5.72 (br s)	5.84 (br s)	5.93 (br s)	6.01 (br s)	5.72 (br s)	5.86 (br s)	5.71 (br s)			
Rha-6	1.71 (br s)	1.71 (d, 5.8)	1.71 (d, 5.1)	1.71 (d, 4.5)	1.71 (d, 5.5)	1.71 (d, 5.5)	1.71 (br s)			
xyl-1	5.19 (d, 7.4)	5.16 (d, 7.3)	5.13 (d, 7.6)		5.19 (d, 7.8)	5.16 (d, 7.3)	5.19 (d, 7.8)			
xyl-1′	5.24 (d, 7.8)	5.22 (d, 7.6)			5.24 (d, .7.8)	5.23 (d, 7.3)	5.24 (d, 7.8)			
glc-1		5.13 (d, 7.6)	5.11 (d. 7.8)	5.19 (d, 6.0)		5.13 (d, 7.8)				
glc-1′					5.40 (d, 7.8)	5.40 (d, 7.8)			5.34 (d, 8.0)	

a) Assignments have been confirmed by HMQC, HMBC and H–H COSY experiments.

prosapogenin (**5a**) by alkaline hydrolysis. The ^{1}H- and ^{13}C-NMR data of **6** showed the presence of two terminal glucosyl moieties. The ^{1}H- and ^{13}C-NMR spectral patterns of the sugar moiety linked at C-3 of **6** were similar to thosw of **5**, and the ^{1}H- and ^{13}C-NMR spectral patterns of the sugar moiety linked at C-28 were similar with that of **2**. These findings indicate that two glucosyl moieties are linked to C-3 of the glucuronopyranosyl moiety and to C-3 of the arabinopyranosyl moiety. This was confirmed in the HMBC spectrum. Thus the structure of **6** was determined to be 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranosyl-3*β*,16*α*-dihydroxy-olean-12-en-28-oic acid 28-*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-arabinopyranosyl ester. Compound **6** was named lancemaside F.

Compound **7** was obtained as a white amorphous powder. The molecular formula of **7** was determined to be $C_{57}H_{90}O_{27}$ from the pseudomolecular ion $[M-H]^-$ at *m/z* 1205.5548 in HR-ESI-MS (negative mode). The ^{1}H-NMR spectrum of **7** showed the presence of seven singlet methyl groups [δ_H 1.00 (3H, s), 1.12 (3H, s), 1.15 (3H, s), 1.33 (3H, s), 1.39 (3H, 3H, s), 1.50 (3H, s), 1.83 (3H, s)], one doublet methyl group [δ_H 1.71 (3H, br s)], and five anomeric protons [δ_H 5.06 (1H, d, *J*=7.0 Hz), 5.19 (1H, d, *J*=7.8 Hz), 5.24 (3H, d, *J*=7.8 Hz), 5.71 (3H, br s), 5.71 (1H, br s)]. The ^{13}C-NMR spectrum of **7** showed the presence of five anomeric carbons (δ_C 93.4, 101.0, 106.0, 106.1, 106.5). The ^{13}C-NMR profile of the sugar portion of **7** is approximately identical to those of **1** (Table 1), but the ^{13}C-NMR signals of the A-ring part of the aglycone are different from that of **1**. The molecular formula of **7** is 16 mass units higher than that of **1**. The aglycone portion of the ^{13}C-NMR spectrum of **7** showed identical chemical shifts to those of reported saponins with asterogenic acid as an aglycone.[14—16] This indicates that **7** has an additional hydroxyl group in the aglycone portion of **1**. Acid hydrolysis of compound **7** gave an aglycone (**7a**) and methyl sugars. The structure of the aglycone (**7a**) was identified to be 2*β*,3*β*,16*α*-trihydroxyolean-28-oic acid (asterogenic acid) based on the ESI-MS, ^{1}H- and ^{13}C-NMR spectra of **7a**. The methyl sugar portion of **7** was *p*-bromobenzoylated to give *A*-values of +114.2 (*α*MRB), +98.0 (*α*MAB), −4.7 (*α*MXB), and +8.9 (*β*MXB), from which the presence of L-rhamnose, L-arabinose, and D-xylose in **7** was confirmed. The location of the sugar moieties at C-3 and C-28 were determined from the HMBC data. Thus the structure of **7** was determined to be 3-*O*-*β*-D-glucuronopyranosyl-2*β*,3*β*,16*α*-trihydroxyolean-12-en-28-oic acid 28-*O*-*β*-D-xylopyrano-syl-(1→3)-*β*-D-xylpyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl ester. Compound **7** was named lancemaside G.

Experimental

Melting points were observed on a Yanagimoto micromelting-point apparatus and are uncorrected. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured on a Varian Inova 500 NMR spectrometer and on a JEOL *α*-500 NMR spectrometer. Chemical shifts are shown as *δ*-values (ppm) with tetramethyl silane (TMS) as an internal standard, including NOE, HMQC, and HMBC. HR-ESI-MS data were measured on a Waters Micromass Q-Tof micromass spectrometer. $[\alpha]_D$ values were measured on a JASCO P-1010 polarimeter at 25 °C. Analytical HPLC and preparative HPLC were carried out using a reverse-phase column (Mightysil RP-18, Kanto Chemical Co., Ltd.) with a CH_3CN–H_2O solvent system. TLC was performed using precoated silica gel $60F_{254}$ (Merck) 200×200×0.25-mm plates for the analysis and 200×200×0.5-mm plates for the purification of constituents.

Plant Materials Roots of *C. lanceolata* were purchased from Korea on

Table 2. ^{13}C-NMR of Lancemasides A—H and Prosapogenins (Aglycone Portions) (Pyridine-d_5)

	1	1b	2	3	4	5	5a	6	7
C-1	38.7	38.6	38.7	38.7	38.7	38.6	38.6	38.6	44.4
2	26.6	26.5	26.6	26.6	26.6	26.5	26.5	26.5	70.2
3	89.0	88.9	89.0	89.0	89.0	89.1	89.1	89.1	89.5
4	39.5	39.4	39.5	39.5	39.5	39.5	39.5	39.5	38.9
5	55.8	55.8	55.8	55.8	55.8	55.7	55.7	55.7	56.2
6	18.3	18.4	18.3	18.4	18.4	18.4	18.4	18.5	18.3
7	33.4	33.4	33.4	33.4	33.5	33.4	33.4	33.4	33.5
8	39.9	39.8	40.0	39.9	40.0	39.9	39.8	40.0	40.1
9	47.0	47.0	47.1	47.0	47.1	47.0	47.1	47.1	47.5
10	36.9	36.9	36.9	36.9	36.9	36.9	36.9	36.9	37.0
11	23.7	23.7	23.7	23.7	23.8	23.7	23.7	23.7	23.9
12	122.8	122.3	122.8	122.7	122.8	123.3	122.3	123.3	123.2
13	144.3	145.0	144.3	144.3	144.5	144.3	145.0	144.3	144.3
14	42.0	42.0	42.1	42.0	42.1	42.0	42.0	42.1	42.2
15	36.1	36.1	36.0	36.0	36.1	36.1	36.2	36.0	36.0
16	74.0	74.7	73.9	73.9	73.9	74.0	74.7	73.9	74.0
17	49.5	48.8	49.4	49.4	49.4	49.5	48.8	49.4	49.5
18	41.2	41.3	41.3	41.3	41.3	41.2	41.4	41.3	41.2
19	47.1	47.2	47.1	47.1	47.2	47.0	47.2	47.1	47.0
20	30.9	31.0	30.9	30.8	30.9	30.9	31.0	30.9	30.9
21	35.9	36.1	36.0	36.0	36.0	35.9	36.1	36.0	35.9
22	32.1	32.8	32.2	32.1	32.2	32.1	32.8	32.2	32.1
23	28.1	28.1	28.1	28.1	28.1	28.0	28.0	28.0	29.6
24	16.9	16.9	16.9	16.9	17.0	16.9	16.9	16.9	18.6
25	15.6	15.5	15.6	15.6	15.6	15.6	15.5	15.6	16.6
26	17.5	17.4	17.5	17.5	17.5	17.5	17.4	17.5	17.6
27	27.1	27.2	27.1	27.0	27.1	27.1	27.2	27.1	27.2
28	175.9	179.9	175.8	175.7	175.8	175.9	179.9	175.8	175.9
29	33.2	33.3	33.2	33.1	33.2	33.2	33.3	33.2	33.2
30	24.7	24.6	24.6	24.5	24.6	24.7	24.6	24.6	24.7

Table 3. ^{13}C-NMR Data of Lancemasides and Prosapogenins (Sugar Portions) (Pyridine-d_5)

	1	1b	2	3	4	5	5a	6	7
GlcA-1	107.2	107.1	107.2	107.1	107.3	106.7	106.6	106.7	106.5
2	75.3	75.5	75.2	75.5	75.5	74.3	74.3	74.3	75.2
3	78.2	78.1	78.1	78.1	78.1	87.4	87.5	87.5	78.0
4	73.4	73.4	73.4	73.3	73.3	71.7	71.7	71.7	73.4
5	77.9	77.6	77.8	77.7	77.9	77.3	77.1	77.3	77.2
6	172.8	172.8	172.8	173.7	n.o.	172.4	172.4	172.0	173.0
Ara-1	93.4		93.2	93.5	93.5	93.4		93.2	93.4
2	75.1		73.8	73.4	73.7	75.1		73.8	74.9
3	69.4		75.5	76.3	75.5	69.4		75.6	69.5
4	65.8		65.3	65.8	65.9	65.8		65.3	65.8
5	62.7		63.5	64.1	64.1	62.7		63.5	62.7
Rha-1	100.9		100.7	100.7	101.2	100.9		100.7	101.0
2	71.8		71.6	71.7	72.2	71.8		71.6	71.8
3	72.7		72.5	72.4	72.5	72.7		72.5	72.7
4	83.3		83.2	83.3	73.7	83.3		83.2	83.3
5	68.4		68.6	68.6	70.5	68.4		68.6	68.5
6	18.4		18.3	18.3	18.6	18.3		18.3	18.3
Xyl-1	106.0		106.0	106.7		106.0		106.0	106.1
2	74.9		74.8	75.9		74.9		74.8	74.9
3	87.0		87.0	78.5		87.0		87.0	87.0
4	68.9		69.0	70.9		69.0		69.0	69.0
5	66.8		66.8	67.3		66.9		66.8	66.8
Xyl-1′	106.1		106.2			106.0		106.2	106.0
2′	75.5		75.2			75.3		75.2	75.3
3′	78.1		78.1			78.2		78.1	78.1
4′	70.9		70.9			70.9		70.9	70.9
5′	67.3		67.3			67.3		67.3	67.3
Glc-1			104.3	104.5	104.5			104.3	
2			73.1	73.4	73.5			73.1	
3			78.0	78.1	78.1			78.0	
4			71.1	71.7	71.1			71.1	
5			78.7	78.6	78.7			78.7	
6			62.3	62.3	62.3			62.4	
Glc-1′						105.9	105.9	105.9	
2′						75.3	75.5	75.2	
3′						78.2	78.1	78.2	
4′						71.5	71.5	71.5	
5′						78.7	78.6	78.7	
6′						62.3	62.3	62.3	

n.o.=not observed.

October 2003. A voucher specimen was deposited in the Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima.

Extraction and Isolation The powdered roots (1.4 kg) of *C. lanceolata* cultivated in Korea were extracted with boiling water. The water solution was passed through a Diaion HP-20 column (Mitsubishi Chemical Ind., Japan) and thoroughly washed with water and eluted with methanol (MeOH) to give a MeOH eluate (11 g). The MeOH eluate was chromatographed on a silica gel column with a gradient $CHCl_3$–MeOH solvent system to give eight fractions, fr. 1 (520 mg), 2 (840 mg), 3 (480 mg), 4 (680 mg), 5 (1.12 g), 6 (1.96 g), 7 (1.41 g), and 8 (870 mg). Fr. 2 (800 mg) was purified on HPLC using an ODS column and 25% CH_3CN to give tangshenoside II (**9**, 100 mg) and syringin (**10**, 140 mg). Fr. 5 (1.10 g) was purified on a HPLC using a ODS column and 20% CH_3CN solution to give tangshenoside I (**8**, 48 mg) and tryptophan (**11**, 86 mg). Fr. 7 (1.40 g) was repeatedly purified on HPLC using 20—30% CH_3CN [containing 0.1% trifluoroacetic acid (TFA)] and fractions obtained were passed through a Diaion HP-20 column for removal of TFA to give compounds **1** (250 mg), **2** (72 mg), **3** (18 mg), **4** (14 mg), **5** (32 mg), **6** (8 mg), and **7** (9 mg).

Lancemaside A (**1**): A white amorphous powder. HR-ESI-MS (negative mode): *m/z* 1189.5588 $[M-H]^-$ (Calcd for $C_{57}H_{89}O_{26}$, 1189.5642). $[\alpha]_D^{25}$ −62.9° (*c*=0.03, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Lancemaside B (**2**): A white amorphous powder. HR-ESI-MS (negative mode): *m/z* 1351.6162 $[M-H]^-$ (Calcd for $C_{63}H_{99}O_{31}$, 1351.6170). $[\alpha]_D^{25}$ −50.5° (*c*=0.03, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Lancemaside C (**3**): A white amorphous powder. HR-ESI-MS (negative mode): *m/z* 1219.5739 $[M-H]^-$ (Calcd for $C_{58}H_{91}O_{27}$, 1219.5748). $[\alpha]_D^{25}$ −85.2° (*c*=0.02, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Lancemaside D (**4**): A white amorphous powder. HR-ESI-MS (negative mode): *m/z* 1087.5316 $[M-H]^-$ (Calcd for $C_{53}H_{83}O_{23}$, 1087.5325). $[\alpha]_D^{25}$ −43.7° (*c*=0.02, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Lancemaside E (**5**): A white amorphous powder. HR-ESI-MS (negative mode): *m/z* 1351.6189 $[M-H]^-$ (Calcd for $C_{63}H_{99}O_{31}$, 1351.6170). $[\alpha]_D^{25}$ −51.5° (*c*=0.02, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Lancemaside F (**6**): A white amorphous powder. HR-ESI-MS (negative mode): *m/z* 1513.6685 $[M-H]^-$ (Calcd for $C_{69}H_{109}O_{36}$, 1513.6699). $[\alpha]_D^{25}$ −46.4° (*c*=0.02, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Lancemaside G (**7**): A white amorphous powder. HR-ESI-MS (negative mode): *m/z* 1205.5548 $[M-H]^-$ (Calcd for $C_{57}H_{89}O_{27}$, 1205.5591). $[\alpha]_D^{25}$ −55.7° (*c*=0.03, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Acid Hydrolysis of Lancemaside A (1) A solution of **1** (25 mg) in 30 ml of MeOH and 3 ml of concentrated HCl was refluxed for 2 h. The reaction solution was poured into ice water and extracted with ethyl acetate to give an ethyl acetate solution and a water fraction. The ethyl acetate solution was evaporated to give a crude aglycone, which was purified using preparative TLC to give pure **1a** (7 mg).

Acid Hydrolysis of 7 Ten milligrams of **7** was hydrolyzed with 3% HCl in MeOH as same as **1** to give aglycone **7a** (3 mg).

7a: White amorphous powder. ESI-MS: *m/z* 489 $[M+Na]^+$. ^{1}H-NMR data, see Table 1.

Alkaline Hydrolysis of 1 A solution of **1** (50 mg) in 3% KOH–MeOH (50 ml) was refluxed for 30 min. The reaction solution was poured into ice water and extracted with $CHCl_3$ to give a $CHCl_3$ solution, which was washed with water and evaporated to give a crude product. The crude prosapogenin was purified on HPLC to give pure **1b** (18 mg).

Prosapogenin (**1b**): An amorphous solid. HR-ESI-MS (negative mode): *m/z* 647.3781 $[M-H]^-$ (Calcd for $C_{36}H_{55}O_{10}$, 647.3795). $[\alpha]_D^{25}$ −11.1° (*c*=0.03, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Alkaline Hydrolysis of 5 A solution of **5** (12 mg) in 3% KOH–MeOH (20 ml) was refluxed for 30 min. The reaction solution was poured into ice water and extracted with chloroform to give a $CHCl_3$ solution, which was washed with water and evaporated under reduced pressure to give a crude product. The crude product was purified on preparative TLC to give pure prosapogenin **5a** (5 mg).

Prosapogenin (**5a**): An amorphous solid. HR-ESI-MS (negative mode): *m/z* 809.4322 $[M-H]^-$ (Calcd for $C_{42}H_{65}O_{15}$, 809.4323). $[\alpha]_D^{25}$ −1.85° (*c*=0.04, MeOH). ^{1}H-NMR data, see Table 1, and ^{13}C-NMR data, see Tables 2 and 3.

Identification and Determination of D,L-Configurations of Sugars in the Saponins The saponins (2 mg each) were hydrolyzed in 3% HCl–MeOH solution under refluxing for 2 h. The reaction solutions were evaporated under reduced pressure to give reaction products, which were purified on TLC to give the methyl sugar portions. The methyl sugar portions were benzoylated with *p*-bromobenzoylchloride and pyridine. The benzoylated reaction solutions were poured into ice water and extracted with ethyl acetate to give ethyl acetate solutions, which were washed with diluted aqueous HCl and saturated aqueous $NaHCO_3$ solution successively and evaporated under reduced pressure to give methyl sugar per-*O*-*p*-bromobenzoates. The benzoate mixtures were analyzed and identified with the authentic methyl sugar benzoates on HPLC (C8 Mightysil, 85% CH_3CN at 245 nm), and the corresponding peaks were collected in an analytical scale. The collected solutions were measured under CD and UV at the same concentration and *A*-values ($=\Delta\varepsilon_1-\Delta\varepsilon_2$) were calculated. Prosapogenin **1b** was treated as described above to give per-*O*-*p*-bromobenzoate of methylglucuronopyranoside methyl ester and identified with that derived from D-glucuronic acid.

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