Studies on the Constituents of *Actinostemma lobatum* MAXIM. VI.[1c] Structures of Lobatosides I, J and K, Oleanolic Acid and Gypsogenin Glycosides Isolated from the Seed

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Two new gypsogenin glycosides, named lobatosides I and J, and one oleanolic acid glycoside, lobatoside K, were isolated from the seed of *Actinostemma lobatum* MAXIM. (Cucurbitaceae), and thier structures were elucidated to be as follows based on chemical and spectral evidence.

Lobatoside I: 3-*O*-[*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranosyluronic acid]gypsogenin 28-{*O*-*β*-D-glucopyranosyl-(1→3)-[*O*-*β*-D-xylopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl}ester.

Lobatoside J: 3-*O*-[*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranosyluronic acid]gypsogenin 28-{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-[*O*-*β*-D-glucopyranosyl-(1→3)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-fucopyranosyl}ester.

Lobatoside K is an oleanolic acid glycoside. The structure of the sugar moiety is the same as that of lobatoside I.

Keywords *Actinostemma lobatum*; Cucurbitaceae; lobatoside; triterpene saponin; gypsogenin; oleanolic acid; 3,28-*O*-bisdesmoside; glucuronide saponin

In the preceding papers[1] of this series, we reported the isolation and structures of six dammarane-type triterpene glycosides, actinostemmosides A—D, G and H,[1a] two baccharane-type triterpene glycosides, actinostemmosides E and F,[1b] and eight oleanane-type triterpene glycosides, lobatosides A—H[1c] from the herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae). As a continuation of the phytochemical investigation of the title plant, the saponin constituents of the seed were investigated. Two new gypsogenin glycosides, lobatosides I (I) and J (II), and one oleanolic acid glycoside, lobatoside K (III), were isolated. This paper deals with the isolation and the structures of these saponins.

The powder of the dried seed was percolated with 50% MeOH, and the extract was suspended in water and washed with hexane to remove the oily materials. The water solution was extracted with BuOH. The BuOH extract was roughly chromatographed on Sephadex LH-20, and the saponin fraction was chromatographed repeatedly on silica gel, a reversed-phase material, and finally purified by high-performance liquid chromatography (HPLC) on a reversed-phase material to give lobatosides I (0.02% of the dried seed), J (0.005%) and K (0.001%).

Lobatoside I (I) was obtained as a white amorphous powder from aqueous MeOH. The positive ion fast atom bombardment mass spectrum (FAB-MS) showed an $[M+Na]^+$ ion at *m/z* 1417 and the negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 1393, indicating the molecular weight to be 1394. The result of the high-resolution FAB-MS was consistent with the molecular formula $C_{65}H_{102}O_{32}$. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum revealed six tertiary methyl signals (δ 0.85, 0.88, 0.91, 1.07, 1.26 and 1.45), two secondary methyl signals (δ 1.46, d, J=6 Hz; δ 1.68, d, J=6 Hz), an olefinic proton signal (δ 5.38, br s), an aldehydic proton signal (δ 9.94, s) and six anomeric proton signals (δ 4.90, d, J=8 Hz; δ 5.22, d, J=8 Hz; δ 5.40, d, J=8 Hz; δ 5.46, d, J=8 Hz; δ 5.90, br s; and δ 5.98, d, J=8 Hz) (Table I).

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum showed signals of six C–C bonded quaternary carbons (δ 30.8, 36.3, 40.3, 42.3, 47.1 and 55.1), trisubstituted olefinic carbons (δ 122.5 and 144.2), an ester carbon (δ 176.6), an aldehydic carbon (δ 209.9) and six anomeric carbons (δ 95.1, 102.1, 103.5, 105.2, 105.4 and 106.2) (Tables II and III). From these spectral data and by comparison of the spectral data with those of luperoside I,[2] I was indicated to be a 3,28-*O*-bisdesmosidic hexaglycoside of gypsogenin.

Compound I gave D-glucuronic acid, D-galactose, D-glucose, D-xylose, D-fucose and L-rhamnose on acid hydrolysis.[3] The positive ion FAB-MS showed a fragment ion derived from the ester-linked sugar moiety at *m/z* 609, of which the ion composition is $C_{23}H_{38}NaO_{17}$, and the negative ion FAB-MS showed fragment ions at *m/z* 807, 645 and 469 which originated from the prosapogenin moiety. These data indicated that a hexosyl-glucuronic acid group is linked to C_3-OH of gypsogenin and the ester-linked sugar moiety is composed of D-xylose, D-glucose (or D-galactose), D-fucose and L-rhamnose. On selective cleavage of the ester glycoside linkage according to the method reported by Ohtani *et al*,[4] I provided a prosapogenin (IV) and an anomeric mixture (V) of a methyl oligoglycoside. Compound IV gave D-galactose and D-glucuronic acid on acid hydrolysis and it showed an $[M+Na]^+$ ion at *m/z* 831 in the positive ion FAB-MS. The negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 807 and fragment ions at *m/z* 645 ($[M-H-\text{galactose}]^-$) and *m/z* 469 ($[M-H-\text{galactose}-\text{glucuronic acid}]^-$). The ^{1}H-NMR spectrum of IV was examined carefully by using ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), ^{1}H nuclear Overhauser enhancement and exchange spectroscopy (NOESY), rotating frame nuclear Overhauser enhancement and exchange spectroscopy (ROESY), nuclear Overhauser effect (NOE) difference spectroscopy and decoupling difference spectroscopy techniques, and all proton signals of IV were assigned. The corresponding ^{13}C-NMR signals were assigned using the ^{1}H–^{13}C COSY. The assignments are summarized in Tables I, II and III. NOE were observed between the anomeric proton of the glucuronic acid group and C_3-H of the aglycone, and between the anomeric proton of the galactopyranosyl group and C_2-H of the glucuronic acid group. Therefore, IV was determined to be gypsogenin 3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranosiduronic acid. The mode of the sugar linkage in IV

TABLE I. ^{1}H-NMR Chemical Shifts for Lobatosides and the Degradation Products

H number	I	IV	Va	Vb	II	III
H- 3	*ca.* 4.05	*ca.* 4.05			*ca.* 4.05	3.31 dd (4, 12)
H-12	5.38 br s	5.42 br s			5.40 br s	5.41 br s
H-18	3.11 dd (4, 14)	3.24 dd (4, 14)			3.10 dd (4, 14)	3.12 dd (4, 14)
H-23	9.94 s	9.92 s			9.92 s	1.30 s[a]
H-24	1.45 s	1.41 s			1.45 s	1.15 s
H-25	0.85 s	0.79 s			0.87 s	0.88 s
H-26	1.07 s	0.92 s			1.07 s	1.12 s
H-27	1.26 s	1.27 s			1.27 s	1.31 s[a]
H-29	0.88 s	0.95 s			0.90 s	0.89 s[b]
H-30	0.91 s	1.00 s			0.90 s	0.90 s[b]
3Glc.U.A-1	4.90 d (8)	4.90 d (8)			4.81 d (8)	5.00 d (8)
3Glc.U.A-2	4.21 dd (8, 9)	4.19 dd (8, 9)			4.19 dd (8, 9)	4.31 dd (8, 9)
3Glc.U.A-3	4.31 dd (9, 9)	4.29 dd (9, 9)			4.29 dd (9, 9)	4.38 dd (9, 9)
3Glc.U.A-4	*ca.* 4.45	*ca.* 4.45			*ca.* 4.40	*ca.* 4.55
3Glc.U.A-5						
3Gal-1	5.22 d (8)	5.00 d (8)			5.19 d (8)	5.23 d (8)
3Gal-2	*ca.* 4.53	4.53 dd (8, 9)			4.47 dd (8, 8)	4.59 dd (8, 9)
3Gal-3	*ca.* 4.12	4.12 dd (9, 3)			*ca.* 4.08	4.16 dd (9, 3)
3Gal-4	*ca.* 4.56	4.56 br d (3)			4.50 br d (3)	4.68 br d (3)
3Gal-5	*ca.* 4.12	*ca.* 4.12			4.04 br t-like (6)	*ca.* 4.58
3Gal-6	*ca.* 4.60	*ca.* 4.50			*ca.* 4.43	*ca.* 4.58
28Fuc-1	5.98 d (8)		4.51 d (8)	5.21 d (3)	5.94 d (8)	6.01 d (8)
28Fuc-2	4.46 dd (8, 8)		4.37 dd (8, 9)	4.48 dd (3, 9)	4.42 dd (8, 9)	4.49 dd (8, 9)
28Fuc-3	*ca.* 4.11		4.07 dd (9, 3)	*ca.* 4.45	4.09 dd (9, 3)	4.13 dd (9, 2)
28Fuc-4	*ca.* 3.98		3.92 br d (3)	*ca.* 4.08	4.00 br d (3)	3.99 br s
28Fuc-5	3.87 br q (6)		3.69 br q (6)	4.05 br q (6)	3.88 br q (6)	3.78 br q (6)
28Fuc-6	1.46 d (6)		1.46 d (6)	1.48 d (6)	1.45 d (6)	1.48 d (6)
28Rha-1	5.90 br s		6.00 br s	5.54 d (2)	5.86 br s	5.96 br s
28Rha-2	5.22 br s		5.07 br s	5.02 dd (2, 3)	5.20 br s	5.28 br s
28Rha-3	4.90 dd (3, 9)		4.80 dd (3, 9)	4.75 dd (3, 9)	4.82 dd (3, 9)	4.92 dd (3, 9)
28Rha-4	*ca.* 4.51		4.50 dd (9, 9)	*ca.* 4.45	4.47 dd (9, 9)	4.54 dd (9, 9)
28Rha-5	*ca.* 4.48		4.66 dq (9, 6)	*ca.* 4.45	4.42 dq (9, 6)	4.52 dq (9, 6)
28Rha-6	1.68 d (6)		1.66 d (6)	1.63 d (6)	1.63 d (6)	1.73 d (6)
28Glc-1	5.40 d (8)		5.27 d (8)	4.99 d (8)	5.29 d (8)	5.43 d (8)
28Glc-2	4.02 dd (8, 8)		4.02 dd (8, 8)	3.97 dd (8, 8)	4.03 dd (8, 9)	4.07 dd (8, 9)
28Glc-3	*ca.* 4.15		*ca.* 4.15	*ca.* 4.06	4.10 dd (9, 9)	4.19 dd (9, 9)
28Glc-4	4.05 dd (9, 9)		4.12 dd (9, 9)	*ca.* 4.06	4.00 dd (9, 9)	*ca.* 4.05
28Glc-5	*ca.* 3.94		3.74 ddd (9, 6, 3)	3.59 m	3.92 ddd (9, 6, 2)	*ca.* 3.97
28Glc-6	*ca.* 4.19		4.19 dd (6, 12)	4.14 dd (6, 12)	*ca.* 4.16	*ca.* 4.20
	ca. 4.57		4.37 dd (3, 12)	4.31 dd (2, 12)	*ca.* 4.43	*ca.* 4.50
28Xyl-1	5.46 d (8)		5.44 d (8)	5.38 d (8)	5.43 d (8)	5.48 d (8)
28Xyl-2	3.93 dd (8, 8)		3.97 dd (8, 8)	3.97 dd (8, 8)	3.94 dd (8, 9)	3.97 dd (8, 9)
28Xyl-3	*ca.* 4.10		*ca.* 4.08	*ca.* 4.10	4.08 dd (9, 9)	4.12 dd (9, 9)
28Xyl-4	*ca.* 4.18		*ca.* 4.15	*ca.* 4.15	4.02 br dd (9, 9)	*ca.* 4.19
28Xyl-5	3.40 t-like (12)		3.45 t-like (12)	3.46 t-like (12)	3.42 t-like (12)	3.40 t-like (12)
	ca. 4.18		*ca.* 4.18	*ca.* 4.18	*ca.* 4.16	*ca.* 4.19
28Xyl'-1					5.13 d (8)	
28Xyl'-2					3.99 dd (8, 9)	
28Xyl'-3					4.08 dd (9, 9)	
28Xyl'-4					*ca.* 4.11	
28Xyl'-5					3.55 t-like (12)	
					ca. 4.19	
MeO–			3.52 s	3.39 s		

The spectra were measured in pyridine-d_5 containing D_2O. The figures in parentheses are coupling constants in Hz. Abbreviations: Glc.U.A, glucuronic acid; Gal, galactose; Fuc, fucose; Rha, rhamnose; Glc, glucose; Xyl, xylose; Xyl', terminal xylose in II, all in a pyranose form. 3Gal-1 means the anomeric H of the galactopyranosyl group in the sugar moiety which is linked to C_3 of the aglycone. Chemical shifts and splitting patterns were determined by normal measurement, and by COSY, NOESY, ROESY and ^{13}C–^{1}H COSY spectra. In some cases, nuclear Overhauser effect difference spectra (NOEDS), rotating frame nuclear Overhauser effect difference spectra (ROEDS), homonuclear Hartmann–Hahn (HOHAHA) spectra, ^{1}H–^{1}H HOHAHA and ^{1}H–^{13}C HOHAHA spectra were measured to determine the chemical shifts. The signal of C_5-H of the glucuronic acid moiety could not be assigned. *a, b*) Assignments may be interchanged.

was confirmed by the gas chromatography-chemical ionization mass spectrometric (GC-CI-MS) analysis of the component methylated sugars of the permethylate of the reduction product of IV.

The anomeric mixture of the methyl oligoglycoside obtained by the selective cleavage of the ester-glycoside linkage gave D-fucose, D-glucose, D-xylose and L-rhamnose on acid hydrolysis, and it showed an $[M-H]^-$ ion at *m/z* 617 and fragment ions at *m/z* 485 ($[M-H-\text{xylose}]^-$), *m/z* 455 ($[M-H-\text{glucose}]^-$) and *m/z* 322 ($[M-H-\text{xylose}-\text{glucose}]^-$), indicating that V is a branched-chain methyl tetraglycoside which has D-xylose and D-glucose both linked to the second methylpentosyl group. The anomeric glycoside mixture was fully methylated according to Hakomori's method,[5] and the permethylate was methanolyzed. The methanolyzate was analyzed by GC-CI-MS after

TABLE II. ^{13}C-NMR Chemical Shifts[a] for the Aglycone Moieties of Lobatosides and the Prosapogenin (IV)

C No.	I	IV	II	III
1	38.2	38.1	38.2	38.9
2	25.1	25.0	25.1	26.8
3	83.7	83.8	83.7	89.4
4	55.1	55.1	55.2	39.7
5	48.6	48.4	48.5	55.9
6	20.6	20.5	20.6	18.7
7	32.5	32.5	32.4	33.3
8	40.3	40.0	40.3	40.1
9	47.9	47.9	47.9	48.1
10	36.3	36.3	36.3	37.0
11	23.8[b]	23.7[b]	23.8	23.6
12	122.5	122.4	122.6	122.8
13	144.2	145.1	144.2	144.1
14	42.3	42.2	42.3	42.3
15	28.2	28.3	28.2	28.3
16	23.6[b]	23.8[b]	23.6	23.9
17	47.1	46.7	47.1	47.1
18	42.0	42.0	42.0	42.0
19	46.4	46.5	46.4	46.5
20	30.8	31.0	30.9	30.9
21	34.0	34.2	34.0	34.1
22	32.6	33.2	32.6	32.5
23	209.9	209.9	210.0	28.3
24	11.1	11.0	11.1	16.9
25	15.8	15.7	15.9	15.8
26	17.4	17.4	17.4	17.5
27	26.1	26.2	26.1	26.1
28	176.6	180.4	176.6	176.7
29	33.2	33.3	33.2	33.3
30	23.9	23.8	23.8	23.9

a) The spectra were measured in pyridine-d_5 containing D_2O and chemical shifts were expressed in δ values. *b*) The values having the same superscripts in each column may be interchanged.

TABLE III. ^{13}C-NMR Chemical Shifts[a] for the Sugar Moieties of Lobatosides and the Degradation Products

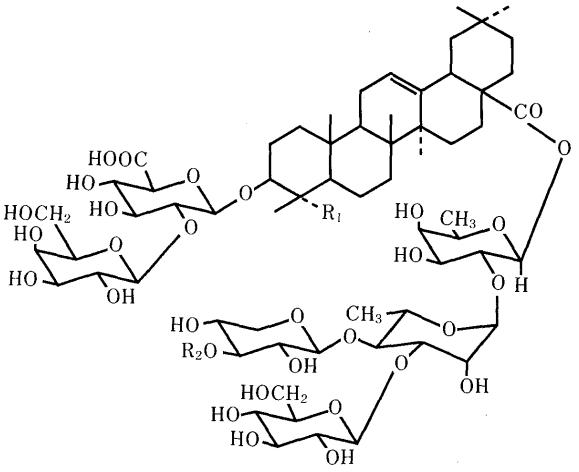
Sugars	I	IV	Va	Vb	II	III
3Glc.U.A-1	103.5 (157)	103.5			103.5 (159)	105.4 (157)
3Glc.U.A-2	82.2	82.3			82.0	83.7
3Glc.U.A-3		77.7			77.9	
3Glc.U.A-4		72.9			73.1	
3Glc.U.A-5						
3Glc.U.A-6						172.9
3Gal-1	106.2 (159)	106.2			105.9 (162)	107.0 (157)
3Gal-2		74.3			74.2	
3Gal-3		74.8			74.9[a]	
3Gal-4		70.0			70.2	69.5
3Gal-5		77.1			77.1	
3Gal-6	62.1	62.1			62.2	61.3
28Fuc-1	95.1 (168)		103.8	100.6	95.3 (171)	95.1 (159)
28Fuc-2			77.0	78.8	76.0	75.6
28Fuc-3			75.6	69.9	75.3	
28Fuc-4			73.0	73.3	72.9	
28Fuc-5	72.3		71.2	66.7	72.5	72.3
28Fuc-6	17.0		17.1	17.1	17.1	17.0
28Rha-1	102.1 (171)		102.1	104.0	102.3 (171)	102.2 (168)
28Rha-2	70.9		71.4	71.0	70.9	71.0
28Rha-3	82.4		82.9	82.8	82.7	82.5
28Rha-4			78.9	78.7[a]	78.7	
28Rha-5			68.1	68.3	69.1	
28Rha-6	19.0		18.5	18.8	19.1	19.1
28Glc-1	105.4 (159)		105.1	104.8	105.4 (159)	105.4 (157)
28Glc-2			75.4	75.3[b]	75.5	
28Glc-3			78.2[a]	78.0	78.4[b]	
28Glc-4			71.5	71.4[c]	71.8	
28Glc-5			78.1	77.9	78.3[b]	
28Glc-6	62.8		62.5	62.3	62.8	62.8
28Xyl-1	105.2 (160)		105.3	105.3	104.6 (163)	105.2 (160)
28Xyl-2			75.6	75.5[b]	75.0[a]	
28Xyl-3			79.0[a]	78.8[a]	88.4	
28Xyl-4			71.2	71.2[c]	69.6	
28Xyl-5	67.1		67.2	67.2	66.5	67.1
28Xyl'-1					106.0 (162)	
28Xyl'-2					75.4	
28Xyl'-3						
28Xyl'-4					70.9	
28Xyl'-5					67.1	
MeO			56.1	55.0		

The spectra were measured in pyridine-d_5 containing D_2O and chemical shifts were expressed in δ values. Signals of II were assigned by using ^{13}C–^{1}H COSY and ^{1}H–^{13}C HOHAHA spectra, and signals of other samples were assigned by using ^{13}C–^{1}H COSY spectra. The chemical shifts of the signals which were not readily assigned are not shown. Figures in parentheses are $^1J_{C_1H_1}$ values in Hz. 28Xyl-1 means the anomeric carbon of the xylopyranosyl group in the ester-linked sugar moiety, and 28Xyl' is the additional xylopyranosyl group. *a*—*c*) Values having the same superscripts in each column may be interchanged.

acetylation. Methyl glycosides of 3,4-di-*O*-methyl-2-*O*-acetyl-D-fucopyranose, 2-*O*-methyl-3,4-di-*O*-acetyl-L-rhamnopyranose, 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,4-tri-*O*-methyl-D-xylose were identified, indicating that the methyl tetraglycoside is either methyl *O*-D-xylosyl-(1→3)-[*O*-D-glucosyl-(1→4)]-*O*-L-rhamnosyl-(1→2)-D-fucoside or methyl *O*-D-xylosyl-(1→4)-[*O*-D-glucosyl-(1→3)]-*O*-L-rhamnosyl-(1→2)-D-fucoside. The mixture was subjected to HPLC and anomers (Va and Vb) were separated. The ^{1}H-NMR spectra of the two anomers were examined carefully, and all proton signals were assigned. The results are summarized in Table I. The assignment of the ^{13}C-NMR signals was performed by examination of the ^{1}H–^{13}C COSY spectra, and the results are shown in Table III. NOE was observed between the anomeric proton of the fucopyranosyl group and the methoxyl protons, the anomeric proton of the rhamnopyranosyl group and C_2-H of the fucopyranosyl group, the anomeric proton of the glucopyranosyl group and C_3-H of the rhamnopyranosyl group, and the anomeric proton of the xylopyranosyl group and C_4-H of the rhamnopyranosyl group, thus indicating that Va is methyl *O*-β-D-xylopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-(1→3)]-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside and Vb, its α-anomer.

Consequently, the structure of I is formulated to be as shown in the chart. The configurations and conformations of the component monosaccharides were determined from the $J_{C_1H_1}$ values[6] and the coupling constants of the oxymethine protons. The $J_{C_1H_1}$ value (168 Hz) of the ester-linked fucopyranosyl group rather suggests the β-configuration in the $^1C_4$ conformation, while the coupling constants of C_1-H, C_2-H and C_3-H unequivocally indicate the β-configuration and the $^4C_1$ conformation of the fucopyranosyl group.

Lobatoside J (II) was obtained as a white amorphous powder. The positive ion FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1549 and the negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 1525. The molecular formula obtained from the high-resolution positive ion FAB-MS is $C_{70}H_{110}O_{36}$. Compound II gave D-glucuronic acid, D-glucose, D-galactose, D-xylose, D-fucose and L-rhamnose on acid hydrolysis. The general features of the NMR spectra strongly suggested that II is also a 3,28-*O*-bisdesmoside of gypsogenin having an additional xylosyl group attached to lobatoside I (I). The positive ion FAB-MS showed the fragment ion derived from the ester-linked sugar moiety at *m/z* 741, and the negative ion FAB-MS showed the fragment ions at *m/z* 807 and 645 which are derived from the



lobatoside I (I) : R_1=CHO, R_2=H
lobatoside J (II) : R_1=CHO, R_2=β-D-Xyl
lobatoside K (III) : R_1=CH_3, R_2=H

prosapogenin moiety. These data indicated that II has the same prosapogenin structure as that of lobatoside I and the additional xylosyl group is linked to the ester-linked sugar moiety of lobatoside I.

The ^{1}H-NMR spectrum was examined by using ^{1}H–^{1}H COSY, NOESY, NOE difference spectroscopy and decoupling difference spectroscopy techniques. NOEs were observed between the anomeric proton of the rhamnopyranosyl group and C_2-H of the fucopyranosyl group, the anomeric proton of the glucopyranosyl group and C_3-H of the rhamnopyranosyl group, the anomeric proton (δ 5.43) of one xylopyranosyl group and C_4-H of the rhamnopyranosyl group, the anomeric proton of the glucuronic acid group and C_3-H of the aglycone, and the anomeric proton of the galactopyranosyl group and C_2-H of the glucuronic acid group. The anomeric proton of the additional xylopyranosyl group (δ 5.13) showed NOE at the signal at around δ 4.0 ppm, but the signal could not be identified because of overlapping of several proton signals. The ^{13}C-NMR spectrum of II showed a signal at δ 88.4 which is not observed in the spectrum of I, and this signal seems to be that of the carbon to which the xylosyl group in question is attached. Considering the chemical shift and the ordinary glycosylation shift value, the signal can be assigned to C_3 of the xylopyranosyl group or the glucopyranosyl group.

The aldehydic group on II was reduced with $NaBH_4$ and the reduction product was fully methylated. The product was again treated with $NaBH_4$ to reduce $-COOCH_3$ to $-CH_2OH$ and methylated. The permethylate was methanolyzed and the product was examined by GC-CI-MS after acetylation. The methyl glycosides of 2,3,4,6-tetra-*O*-methyl galactopyranose, 2,3,4,6-tetra-*O*-methyl glucopyranose, 3,4,6-tri-*O*-methyl-2-*O*-acetyl glucopyranose, 2,3,4-tri-*O*-methyl xylopyranose, 2,4-di-*O*-methyl-3-*O*-acetyl xylopyranose, 2-*O*-methyl-3,4-di-*O*-acetyl rhamnopyranose and 3,4-di-*O*-methyl-2-*O*-acetyl fucopyranose were identified. Therefore, it became clear that the additional xylopyranosyl group is linked to C_3-OH of the xylopyranosyl group of lobatoside I, and II was concluded to have the structure shown in the chart. The configurations and conformations of the component sugars were determined on the same basis as described for I.

Lobatoside K (III) was obtained as a white amorphous powder. The positive ion FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1403 and the negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 1379, 14 mass unit less than I, while the high resolution FAB-MS gave the molecular formula $C_{65}H_{104}O_{31}$.

The ^{1}H-NMR spectrum revealed the signals of seven tertiary methyl groups (δ 0.88, 0.89, 0.90, 1.12, 1.15, 1.30 and 1.31), two secondary methyl groups (δ 1.48, d, J=6 Hz; δ 1.73, d, J=6 Hz), one olefinic proton (δ 5.41, br s) and six anomeric protons (δ 5.00, d, J=8 Hz; δ 5.23, d, J=8 Hz; δ 5.43, d, J=8 Hz; δ 5.48, d, J=8 Hz; δ 5.96, br s; δ 6.01, d, J=8 Hz). The ^{13}C-NMR spectrum showed signals of six C–C bonded quaternary carbons (δ 30.9, 37.0, 39.7, 40.1, 42.3 and 47.1), trisubstituted olefinic carbons (δ 122.8 and 144.1), an ester carbon (δ 176.7) and six anomeric carbons (δ 95.1, 102.2, 105.2, 105.4, 105.4 and 107.0). The ^{13}C-NMR chemical shifts of the aglycone moiety were almost the same as those reported[7] for oleanolic acid glycosides, and the ^{1}H-NMR and ^{13}C-NMR spectra of the sugar moieties were similar to those of I, although some deviations were observed in the chemical shifts of the sugar moiety which is linked to C_3-OH of the aglycone. The carboxyl group was methylated and reduced with $NaBH_4$, and the reduction product was fully methylated. The component methylated sugars of the permethylate were analyzed by GC-CI-MS. The methylated sugars identified were the same as those derived from lobatoside I. From these data, III was concluded to be an oleanolic acid glycoside having the same sugar moieties as those of I.

Experimental[8]

Extraction and Isolation of Lobatosides I (I), J (II) and K (III) Dried seeds (1.76 kg) of *Actinostemma lobatum* MAXIM. collected in the suburbs of Fukuoka City in October, 1988 were powdered and percolated with 50% MeOH (16 l). MeOH was evaporated off and the aqueous solution was extracted with *n*-hexane (10 l) and 1-BuOH (4 l), successively. The BuOH solution was cencentrated *in vacuo* to dryness to give a dark resin (16 g), which was dissolved in MeOH, and the solution was passed through a Sephadex LH-20 column (MeOH) to give a crude glycoside fraction (12.7 g). This fraction was subjected to silica gel column chromatography, eluted first with $CHCl_3$–MeOH–H_2O (9:1:0.1) and then with $CHCl_3$–MeOH–H_2O (25:17:3) to obtain 8 fractions. Lobatosides were contained in the 7th fraction. Fraction 7 (2.1 g) was chromatographed on silica gel ($CHCl_3$–MeOH–AcOH–H_2O, 60:40:1:9) and on Lichroprep RP-18 (70% MeOH containing AcOH (1%)) and then purified by HPLC on a reversed-phase material (Inertsil PREP-ODS, 10 μm, 250 mm × 10.7 mm i.d., and Capcell pak C18, 5 μm, 250 mm × 10 mm i.d. solvent, 70% MeOH containing AcOH (1%)) to give lobatosides I (I) (396 mg), J (II) (90 mg) and K (III) (20 mg).

Lobatoside I (I): A white amorphous powder from aqueous MeOH, mp 222—227 °C (dec.), $[\alpha]_D^{25}$ +1.7° (c=2.2, pyridine). Positive ion FAB-MS *m/z*: 1417 ($[M+Na]^+$), 609. Negative ion FAB-MS *m/z*: 1393 ($[M-H]^-$), 807, 645, 469. High-resolution positive ion FAB-MS *m/z*: 1417.622. $C_{65}H_{102}NaO_{32}$ requires *m/z* 1417.625.

Lobatoside J (II): A white amorphous powder, mp 229—234 °C (dec.), $[\alpha]_D^{25}$ +3.8° (c=3.2, pyridine). Positive ion FAB-MS *m/z*: 1549 ($[M+Na]^+$), 741. Negative ion FAB-MS *m/z*: 1525 ($[M-H]^-$), 807.645. High-resolution positive ion FAB-MS *m/z*: 1549.666. $C_{70}H_{110}NaO_{36}$ requires *m/z* 1549.668.

Labatoside K (III): A white amorphous powder, mp 235—239 °C (dec.), $[\alpha]_D^{25}$ −3.4° (c=1.0, pyridine). Positive ion FAB-MS *m/z*: 1403 ($[M+Na]^+$), 595. Negative ion FAB-MS *m/z*: 1379 ($[M-H]^-$), 807, 645. High-resolution positive ion FAB-MS *m/z*: 1403.649. $C_{65}H_{104}NaO_{31}$ requires *m/z* 1403.646.

The NMR chemical shift are summarized in Tables I, II and III.

Identification of the Component Monosaccharides A glycoside (*ca.* 5 mg) was dissolved in 10% methanolic HCl (2 ml) and heated at 70 °C for 2 h. The acid was neutralized with Ag_2CO_3 and the mixture was filtered. One half of the filtrate was concentrated *in vacuo*. The residue was trimethylsilylated with trimethylsilylimidazole and checked by GC. GC conditions were as follows: column, ULBON HR-101 (25 m × 0.3 mm i.d.); column bath temperature, 200 °C (for pentose and methylpentose derivatives) or 230 °C (for hexose and glucuronic acid derivatives); injection port temperature, 270 °C; carrier gas, He 1 ml/min; split ratio, 1/50; make-up gas, He 50 ml/min.

The component sugars of the three lobatosides were the same and the monosaccharides identified and shown in the text. The t_R values are as follows.

The figure marked with an asterisk is the t_R value of the main anomer of each sugar. 200 °C: 7.3 (Rha), 8.0*, 8.7 (Fuc), 9.1*, 9.7 (Xyl). 230 °C: 6.9, 7.8*, 8.5 (Gal), 9.2*, 10.4 (Glc. U.A), 9.6*, 10.0 (Glc).

The other half of the filtrate was used for determination of the absolute configuration (D/L) of the component sugars according to Hara *et al.*[3] Thus, the filtrate was concentrated *in vacuo* and the residue was dissolved in 1 N HCl (1 ml). The solution was heated at 90 °C for 1 h. The acid was neutralized with Ag_2CO_3 and the precipitates were filtered off. The sugar was converted into the polyhydroxyalkylthiazólidine derivative, trimethylsilylated and subjected to GC. By comparison of the t_R values with those of the authentic sugar samples, the absolute configurations of the component sugars were determined. The absolute configuration of glucuronic acid was determined as D after conversion to glucose. The GC conditions were the same as above except for the column bath temperature (250 °C) and the injection port temperature (290 °C). The t_R values are as follows. 11.8 (D-Xyl), 14.1 (L-Rha), 14.6 (D-Fuc), 20.1 (D-Glc) and 21.1 (D-Gal). The t_R values of the thiazolidine derivatives of L-Xyl, D-Rha, L-Fuc, L-Glc and L-Gal were 12.6, 12.8, 13.4, 15.6, 21.3 and 23.7, respectively.

Selective Cleavage of the Ester Glycoside Linkage of I Compound I (100 mg) and LiI (100 mg) were dissolved in a mixture of 2,6-lutidine (2 ml) and dry MeOH (1 ml) and the solution was heated at 180 °C for 5 h. After the solution had been cooled, 50% MeOH was added and the solution was passed through a column of Amberlite MB-3. The eluate was concentrated *in vacuo*. The residue was chromatographed on Diaion CHP-20P. The anomeric mixture (V) of the methyl glycoside was eluted with 20% MeOH and a prosapogenin (IV) was eluted afterward with MeOH. The methyl glycoside fraction was subjected to HPLC (Inertsil PREP-ODS, 20% MeOH) to give Va (18 mg) and Vb (14 mg). The prosapogenin fraction was purified by HPLC (Inertsil PREP-ODS, 70% MeOH containing AcOH (1%)) to give IV (11 mg).

IV: A white amorphous powder. EI-MS *m/z*: 248, 203, 163. Positive ion FAB-MS *m/z*: 831 ($[M+Na]^+$). Negative ion FAB-MS *m/z*: 807 ($[M-H]^-$), 645 ($[M-H-162]^-$), 469 ($[M-H-162-176]^-$). NMR chemical shifts are shown in Tables I, II and III.

Va: A colorless syrup. Positive ion FAB-MS *m/z*: 641 ($[M+Na]^+$). Negative ion FAB-MS *m/z*: 617 ($[M-H]^-$), 485 ($[M-H-132]^-$), 455 ($[M-H-162]^-$), 322 ($[M-H-132-162]^-$). NMR chemical shifts are summarized in Tables I and III.

Vb: A colorless syrup. Positive ion FAB-MS *m/z*: 641 ($[M+Na]^+$). Negative ion FAB-MS *m/z*: 617 ($[M-H]^-$), 485 ($[M-H-132]^-$), 455 ($[M-H-162]^-$), 322 ($[M-H-132-162]^-$). NMR chemical shifts are summarized in Tables I and III.

$NaBH_4$ Reduction of I, II and IV A glycoside (*ca.* 10 mg) was dissolved in 50% MeOH (1 ml). $NaBH_4$ (*ca.* 20 mg) was added and the mixture was stirred for 2 h. The reaction mixture was diluted with H_2O (2 ml), neutralized with AcOH and then passed through a column of Diaion CHP-20P. The column was washed with H_2O and then eluted with MeOH. The MeOH eluate was concentrated *in vacuo* and the residue was subjected to HPLC (Inertsil PREP-ODS, 70% MeOH containing AcOH (1%)). The product did not show the signal of an aldehydic group in the ^{1}H-NMR spectrum and the positive ion FAB-MS showed an $[M+Na]^+$ ion heavier by 2 mass units than that of the original glycoside.

Identification of the Component Methylated Monosaccharides by GC-CI-MS The $NaBH_4$ reduction product (30 mg) derived from I was fully methylated according to the method reported by Hakomori.[5] The product was dissolved in MeOH (3 ml) and $NaBH_4$ (50 mg) was added, then the mixture was stirred for 2 h to reduce $-COOCH_3$ to $-CH_2OH$. After neutralization with AcOH, the reaction mixture was diluted with H_2O and concentrated *in vacuo*. The residue was extracted with $CHCl_3$ and the $CHCl_3$ extract was again methylated by Hakomori's method. The methylation product was purified by HPLC (Inertsil PREP-ODS, MeOH) to give a permethylate (8 mg). The positive ion FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1657.

The $NaBH_4$ reduction product derived from lobatoside J (II) (10 mg) was methylated, reduced with $NaBH_4$ and again methylated in the same manner as described above, and a permethylate (4 mg) was obtained. The positive ion FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1817.

Lobatoside K (III) (20 mg) was methylated by Hakomori's method and the methylation product was reduced in MeOH (3 ml) with $NaBH_4$ (50 mg) at room temperature. The reduction product was again methylated by Hakomori's method. The methylation product was purified by HPLC (Inertsil PREP-ODS, MeOH) to give a permethylate (7 mg, positive ion FAB-MS *m/z*: 1627 ($[M+Na]^+$)).

Compound IV (5 mg) was treated in the same manner as described above and a permethylate (3 mg) was obtained.

The anomeric mixture (V) (10 mg) of the methyl glycoside obtained from lobatoside I (I) was methylated by Hakomori's method and a permethylate (8 mg) was obtained.

The permethylate of a glycoside was dissolved in 10% methanolic HCl and the solution was refluxed for 3 h. After neutralization with Ag_2CO_3 and filtration, the filtrate was concentrated *in vacuo*. The residue was acetylated with a mixture (1 : 1) of pyridine and Ac_2O, and checked by GC-CI-MS. Methylated sugars were identified by comparison of t_R values and CI-MS patterns with those of authentic samples. GC-CI-MS conditions were as follows: GC part: column, 2% OV-17 on Uniport HP (80—100 mesh) packed in a glass column (1.0 m × 3 mm i.d.); column oven temperature, 130→190 °C (elevation rate, 3 °C/min); carrier gas, He (20 ml/min); injection port temperature, 250 °C. CI-MS part: reagent gas, isobutane (pressure, less than 1.5×10^{-5} Torr); ionization source temperature, 270 °C; ionization energy, 150 eV; scan range; *m/z* 100→400; scan interval, 4 s. The methylated sugars identified are shown in the text. The t_R values were as follows. Methyl 2,3,4-tri-*O*-methyl-α-D-xylopyranoside (234M-α-Xyl), 1.70; 234 M-β-Xyl, 1.30; methyl 2,4-di-*O*-methyl-3-*O*-acetyl-α-D-xylopyranoside (24M3A-α-Xyl), 5.90; 2M34A-α-Rha, 7.60; 34M2A-α-Fuc, 5.10; 34M2A-β-Fuc, 6.80; 2346M-α-Glc, 4.70; 2346M-β-Glc, 3.50; 346M2A-α-Glc, 7.90; 346M2A-β-Glc, 9.10; 2346 M-α-Gal, 5.50.

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References and Notes

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8) The instruments and materials used in this work were as follows: Yanaco micromelting point apparatus (melting points), JASCO DIP-4 digital polarimeter (specific rotations), JEOL JNM GX-400 spectrometer (^{1}H- and ^{13}C-NMR spectra), JEOL JMS HX-110 mass spectrometer (MS), Shimadzu GC-mini 2 gas chromatograph (GC), Shimadzu Auto GCMS-6020 gas chromatograph-mass spectrometer with a GC-MSPAC 500 FDG data analyzer (GC-CI-MS). HPLC was performed on Inertsil PREP-ODS (Gasukuro Kogyo Inc.) and Capcell pak C18 (Shiseido Company Ltd.). The NMR spectra were obtained in pyridine-d_5–D_2O solution.