Studies on the Constituents of *Luffa operculata* COGN. II.[1] Isolation and Structure Elucidation of Saponins in the Herb

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Four oleanane-type triterpene saponins named luperosides I, J, K and L were isolated as their methyl esters from the MeOH extract of the herb of *Luffa operculata* COGN., and their structures were elucidated on the basis of chemical and spectral evidence. Luperosides I and J are 3-*O*-[β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyluronic acid]-28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-quinovopyranosyl] gypsogenin (I) and 3-*O*-[β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyluronic acid]-28-*O*-[β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-quinovopyranosyl] gypsogenin (J), respectively. Luperosides K and L are quillaic acid glycosides corresponding to luperosides I and J, respectively.

Keywords *Luffa operculata*; cucurbitaceae; luperoside; triterpene saponin; bisdesmoside; glucuronide saponin; gypsogenin; quillaic acid; D-quinovose

In the preceding paper[2] of this series, we reported the isolation and structure elucidation of luperosides A—H, dammarane-type triterpene glycosides isolated from the less polar glycoside fraction of the MeOH extract of the herb of *Luffa operculata* COGN. (Cucurbitaceae). In the course of fractionation of the glycoside fraction, a saponin mixture was obtained from the more polar fraction as a powder which is sparingly soluble in MeOH. This fraction showed on thin-layer chromatography (TLC) two major spots which stained bluish green when the TLC plate was sprayed with sulfuric acid then heated, and each of them yielded two spots on reversed-phase (LiChroprep RP-18) TLC. This paper deals with the isolation of four major saponins as their methyl esters and elucidation of their structures.

The saponin fraction was passed through a column of ion exchange resin (Amberlite IRC 84) and the acidic eluate was converted to the methyl ester by CH_2N_2 treatment. The methyl ester was chromatographed on silica gel to separate into four fractions (frs. I—IV). Fractions II and III were further chromatographed on the reversed-phase material to give luperoside I methyl ester (I) and luperoside J methyl ester (II) from fr. II, and luperoside K methyl ester (III) and luperoside L methyl ester (IV) from fr. III.

Compound I showed in the fast atom bombardment mass spectrum (FAB-MS) an $[M+Na]^+$ ion at m/z 1547 and an $[M-H]^-$ ion at m/z 1523, and the analysis data were consistent with $C_{71}H_{112}O_{35}\cdot 2H_2O$. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum revealed six tertiary methyl signals (δ 0.79, 0.90, 0.95, 0.98, 1.17 and 1.42), three secondary methyl signals [δ 1.54 (J=6 Hz), 1.62 (J=6 Hz) and 1.73 (J=5 Hz)], a carbomethoxyl signal (δ 3.74), seven anomeric proton signals [δ 4.82 (d, J=7 Hz), 5.07 (d, J=7 Hz), 5.17 (d, J=8 Hz), 5.47 (d, J=8 Hz), 5.67 (br s), 5.76 (br s) and 6.29 (d, J=6 Hz)], an olefinic proton signal [δ 5.37 (t-like)] and an aldehydic proton signal (δ 9.92 s).

The ^{13}C-NMR spectrum (Table I) showed signals of six C–C bonded quaternary carbons (δ 30.8, 36.2, 40.1, 41.9, 47.0 and 54.9), seven anomeric carbons (δ 93.7, 100.8, 101.6, 103.9, 104.2, 105.0 and 107.1), trisubstituted olefinic carbons (δ 122.5 and 144.0), two ester carbons (δ 169.8 and 176.1) and an aldehydic carbon (δ 210.1). These spectral data, coupled with the results of the FAB-MS and elemental analysis, suggested I to be a methyl ester of a bisdesmosidic gypsogenin heptaglycoside.

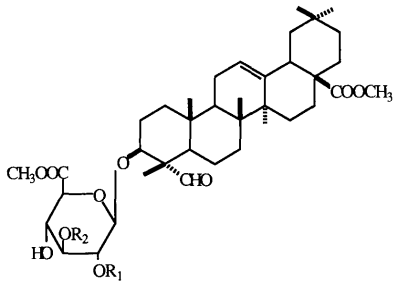
On acid hydrolysis, I gave D-glucuronic acid, D-galactose, L-arabinose, D-quinovose, L-rhamnose and D-xylose.

When I was heated with LiI in a mixture of anhydrous MeOH and 2,6-lutidine, the method for the selective cleavage of the ester-glycoside linkage reported by Tanaka *et al.*,[3] it gave a prosapogenin and methyl glycosides. The prosapogenin methyl ester (V) was hydrolyzed to give D-

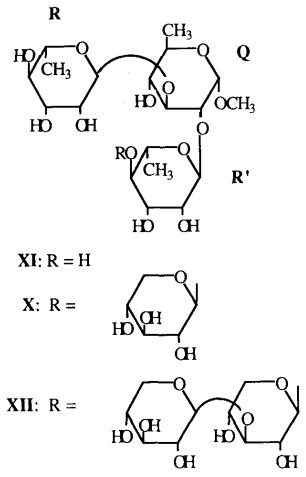
TABLE I. ^{13}C-NMR Chemical Shifts of the Aglycone Moieties of Luperosides and Their Degradation Products[a]

C	IX	VII	VIII	V	XIII	I	III
1	38.5	38.0	37.9	38.0	38.0	38.0	38.1
2	27.0	25.1	24.8	25.1	25.2	25.2	25.2
3	71.7	82.2	83.5	84.5	84.4	84.3	84.3
4	56.2	55.3	54.9	54.9	55.0	54.9	55.0
5	48.9	47.8	47.7[b]	48.6	48.6	48.8	48.8
6	21.0	20.3	20.3	20.3	20.4	20.5	20.5
7	32.7	32.7	32.7	33.9	32.4	32.5	32.8
8	40.0	39.9	39.9	39.8	40.0	40.1	40.3
9	47.7	47.8	48.3[b]	47.7	46.9	47.7	46.9
10	36.1	36.1	36.2	36.2	36.2	36.2	36.2
11	23.3	23.3	23.3	23.3	23.6	23.2	23.7
12	122.6	122.5	122.5	122.5	122.3	122.5	122.2
13	144.2	144.1	144.2	144.1	144.5	144.0	144.4
14	42.0	42.0	41.9	41.9	41.9	41.9	42.0
15	28.0	28.0	28.0	28.0	35.7[b]	28.1	35.8[b]
16	23.6	23.6	23.6	23.6	74.2	23.6	?[c]
17	46.9	46.9	46.8	46.9	49.0	47.0	49.2
18	41.8	41.8	41.8	41.8	41.2	42.0	41.5
19	46.1	46.0	46.0	46.0	46.9	46.2	47.2
20	30.8	30.7	30.7	30.7	30.8	30.8	30.8
21	33.9	33.9	33.9	32.7[b]	35.9[b]	33.9	36.0[b]
22	32.4	32.3	32.3	32.3[b]	32.6	32.3	31.8
23	207.2	206.7	209.4	209.9	209.7	210.1	209.8
24	9.6	10.4	10.9	11.0	11.0	11.1	11.1
25	15.7	15.5	15.6	15.5	15.7	15.6	15.7
26	17.1	17.0	17.0	17.0	17.1	17.5	17.5
27	26.1	26.1	26.0	26.0	27.0	25.9	27.0
28	177.9	177.8	177.8	177.9	177.7	176.1	175.9
29	33.1	33.1	33.0	33.0	33.1	33.1	33.1
30	23.7	23.6	23.6	23.6	24.7	23.8	24.7
$COOCH_3$	51.5	51.5	51.5	51.5	51.7		

a) Assignments of the signals are based on the reports by Tori *et al.*[5] and Takemoto *et al.*[8] *b*) The signals with the same superscripts in each column may be interchanged. *c*) The signal could not be differentiated from the sugar carbon signals.



VII : $R_1 = R_2 = H$
VIII : R_1 = β-D-Gal., $R_2 = H$
V : R_1 = β-D-Gal.
R_2 = α-L-Ara



XI: R = H

X: R =

XII: R =

glucuronic acid, D-galactose and L-arabinose as the component monosaccharides. Compound V was reduced with $NaBH_4$ and the reduction product (VI) was fully methylated. The permethylate gave, on methanolysis, methyl glycosides of 2,3,4-tri-*O*-methyl-L-arabinopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 4,6-di-*O*-methyl-D-glucopyranose [identified by comparison of the gas chromatographic retention time and the fragment pattern in the chemical ionization-mass spectrum (CI-MS) with those of authentic samples].

On heating in the 2 N MeOH solution of trifluoroacetic acid, V gave a dimethylester (VII) of gypsogenin-3-*O*-yl β-D-glucopyranosiduronic acid and a dimethyl ester (VIII) of gypsogenin-3-*O*-yl galactosyl-glucopyranosiduronic acid together with gypsogenin methyl ester (IX). The ^{1}H-NMR signals of the methyl glucopyranosiduronate moiety of VIII were assigned with the aid of the ^{1}H–^{1}H COSY spectrum and the corresponding carbon signals were specified by the ^{1}H–^{13}C COSY spectrum. The chemical shift of C_2 is δ 83.5, indicating that the β-D-galactopyranosyl group is linked to the C_2-hydroxyl group of glucuronic acid.

Consequently, the structure of V was determined as a dimethyl ester of 3-*O*-[β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyluronic acid]-gypsogenin.

The glycoside fraction obtained by the selective cleavage of the ester-glycoside linkage was an anomeric mixture, the major component (X) of which was purified by column chromatography on a reversed-phase material. Acid hydrolysis of X gave D-quinovose, L-rhamnose and D-xylose. Methanolysis of the permethylate of X gave methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylopyranose, 2,3,4-tri-*O*-methyl-L-rhamnopyranose, 2,3-di-*O*-methyl-L-rhamnopyranose and 4-*O*-methyl-D-quinovopyranose, indicating that X is a methyl tetraglycoside branched at the D-quinovose unit and having D-xylose and L-rhamnose at two terminals.

Enzymatic hydrolysis of X with cellulase furnished a methyl trioside (XI) composed of 1 mol of D-quinovose and 2 mol of L-rhamnose. In order to determine the sugar sequence and positions of sugar linkages, assignment of all protons of the three sugar units was attempted by checking the ^{1}H–^{1}H COSY spectrum. All proton signals were separated from each other and unambiguous assignments were performed as shown in the Fig. 1A and Table II. The nuclear Overhauser effect (NOE) difference spectra obtained by

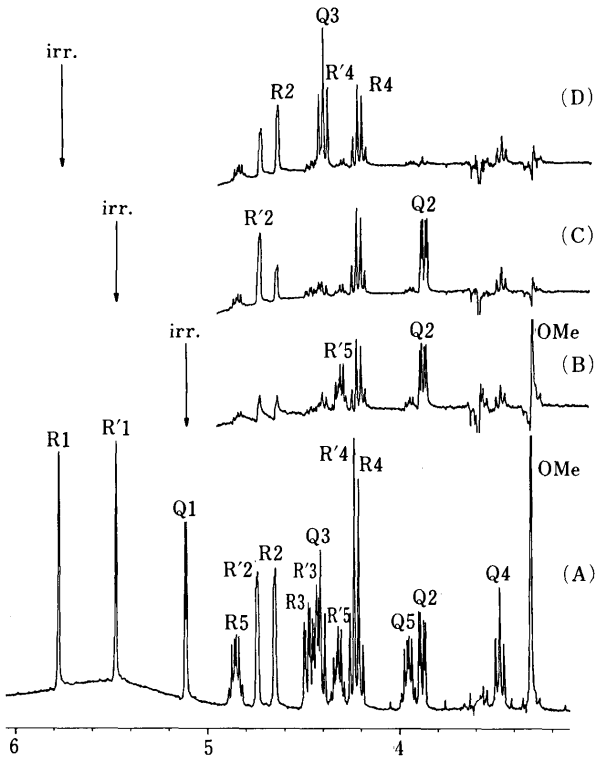


Fig. 1. ^{1}H-NMR (A) and NOE Difference Spectra (B—D) of VII

irradiation at the frequencies of the three anomeric protons are shown in the Fig. 1B—D. When the signal (R_1) at δ 5.78 was irradiated, NOE was observed at the signals of R'_2, R_2, Q_3, R'_4, and R_4. Irradiation at the frequency (δ 5.48) of R'_1 yielded NOE at the signals of R'_2, R_2, R_4, R'_4 and Q_2, and irradiation at the anomeric proton signal (δ 5.12) resulted in NOE at R'_5, R_4, R'_4, Q_2 and the methoxyl signal.

The ^{13}C-NMR signals of C_2 and C_3 of the quinovosyl group appeared at δ 82.4 and 80.0, respectively. Accordingly, XI is methyl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-α-D-quinovopyranoside. The α-configuration of D-quinovopyranose was determined from the coupling constant (J=3 Hz) of the anomeric proton and the α-configuration of L-rhamnopyranosyl linkages was determined from the J_{CH} values (J=172 Hz).[4]

The structure of X is, therefore, confined to the one of the

TABLE II. NMR Chemical Shifts of X, XI and XII

	XI		X		XII	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
Quinovosyl (Q)-1	5.11 d (3)	99.9	5.09 d (3)	99.8	5.08 d (3)	99.8
-2	3.89 dd (3, 9)	82.4	3.87 dd (3, 10)	82.5	3.86 dd (3, 10)	82.5
-3	4.42 dd (9, 9)	80.0	4.40 dd (10, 9)	79.9	4.40 dd (10, 10)	79.9
-4	3.48 dd (9, 9)	75.6	3.47 dd (9, 9)	75.5	*ca.* 3.48	75.5
-5	3.97 dq (9, 6)	68.3	3.96 dq (9, 6)	68.2	3.96 dq (10, 6)	68.2
-6	1.52 d (6)	18.4	1.51 d (6)	18.4	1.52 d (6)	18.4
Q2-Rhamnosyl (R′)-1	5.48 brs	104.6	5.45 d (2)	104.4	5.43 brs	104.4
-2	4.74 d (3)	72.4	4.71 dd (2, 3)	72.0	4.68 brs	72.0
-3	4.44 dd (3, 9)	72.6	4.52 dd (3, 9)	72.5	4.49 dd (3, 9)	72.4
-4	4.22 dd (9, 9)	73.7	*ca.* 4.3	83.9	*ca.* 4.3	83.4
-5	4.33 dq (9, 6)	70.1	*ca.* 4.3	68.2	*ca.* 4.3	68.1
-6	1.60 d (6)	18.5	1.67 d (5)	18.4	1.62 d (6)	18.4
Q3-Rhamnosyl (R)-1	5.78 brs	102.9	5.74 d (1)	103.0	5.72 brs	103.0
-2	4.65 d (3)	72.5	4.62 dd (1, 3)	72.4	4.60 brs	72.5
-3	4.49 dd (3, 9)	72.6	4.47 dd (3, 9)	72.7	4.46 dd (3, 9)	72.7
-4	4.24 dd (9, 9)	73.8	4.24 dd (9, 9)	73.9	*ca.* 4.24	73.9
-5	4.81 dq (9, 6)	69.9	*ca.* 4.86	69.9	4.86 dq (9, 6)	69.9
-6	1.60 d (6)	18.5	1.62 d (6)	18.4	1.63 d (6)	18.4
Xylosyl (inner)-1			5.12 d (7)	107.0	5.16 d (7)	106.2
-2			4.01 dd (9, 9)	76.0	*ca.* 4.01	74.9[a]
-3			4.07 dd (9, 9)	78.6	*ca.* 4.06	87.0
-4			4.15 ddd (11, 9, 5)	70.9	*ca.* 4.15	69.0[b]
-5			3.53 dd (11, 11)	67.4	3.63 dd (10, 10)	67.3
			4.25 dd (5, 11)		*ca.* 4.3	
Xylosyl (outer)-1					5.22 d (7)	105.9
-2					*ca.* 4.04	75.3[a]
-3					*ca.* 4.14	78.1
-4					*ca.* 4.06	70.9[b]
-5					*ca.* 3.48	66.7
					ca. 4.24	
OCH_3	3.31	54.7	3.29	54.6	3.30	54.7

a, b) The chemical shifts with the same superscripts may be reversed. Numbers in the parentheses are coupling constants in Hz.

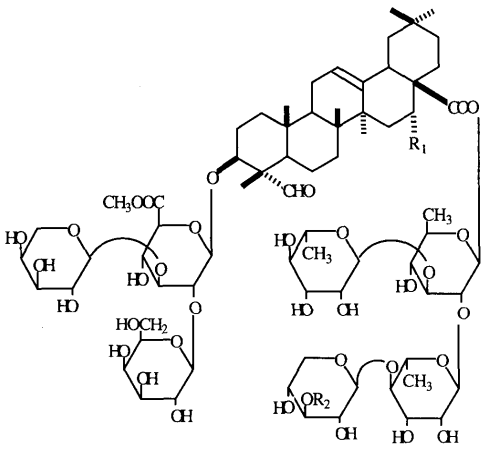
following two structures.

Xa MeO-α-D-Qui.pyr.^{2}-^{1}α-L-Rha.pyr.^{4}-^{1}β-D-Xyl.pyr.
^{3}
^{1}α-L-Rha.pyr.

Xb MeO-α-D-Qui.pyr.^{2}-^{1}α-L-Rha.pyr.
^{3}
^{1}α-L-Rha.pyr.^{4}-^{1}β-D-Xyl.pyr.

The ^{1}H-NMR spectrum of X showed the signals of anomeric protons at δ 5.09 (d, J=3 Hz), 5.12 (d, J=7 Hz), 5.45 (d, J=2 Hz) and 5.74 (d, J=1 Hz). The second signal should be that of the anomeric proton of the β-D-xylopyranosyl group. The other proton signals were assigned with the aid of the ^{1}H–^{1}H COSY spectrum as shown in the Table II. The ^{13}C-NMR signals were also correlated in the ^{1}H–^{13}C COSY spectrum. The carbon signals correlated to the two-proton signals at *ca.* δ 4.30 (assigned at R′$_4$ and R′$_5$ in the ^{1}H–^{1}H COSY spectrum) appeared at δ 68.2 and 83.9, the former of which should be C_5 and the latter C_4. The chemical shift of C_4 indicates that the xylopyranosyl unit is linked to the C_4-OH of the rhamnopyranosyl unit (**R′**), which is further linked to the C_2-OH of the quinovopyranosyl group. The NOE difference spectra showed the presence of NOEs between the anomeric proton of the xylose unit and C_4-H (R′$_4$) of the rhamnosyl unit (**R′**), and between the anomeric proton (R′$_1$) of the rhamnosyl unit and the C_2-H (Q_2) of the quinovosyl unit. Therefore the structure of X is concluded to be methyl β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-α-D-quinovopyranoside (Xa).

From all the above evidence, the structure of I was elucidated as a methyl ester of 3-*O*-[β-D- galactopyransoyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyl-uronic acid]-28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-quinovopyranosyl] gypsogenin.



I : R_1 = R_2 = H
II : R_1 = H, R_2 = β-D-Xyl.
III : R_1 = OH, R_2 = H
IV : R_1 = OH, R_2 = β-D-Xyl.

Compound II was obtained as colorless fine needles and showed in the FAB-MS an $[M+Na]^+$ ion at *m/z* 1679 and an $[M-H]^-$ ion at *m/z* 1655 indicating its molecular weight to be 1656. It gave analysis data consistent with $C_{76}H_{120}O_{39}\cdot 4H_2O$. The ^{1}H- and ^{13}C-NMR spectra of II showed the similar patterns as those of I except for the region of hydroxymethine signals. The ^{1}H-NMR spectrum revealed anomeric proton signals at δ 4.88 (d, $J=ca.$ 8 Hz), 5.08 (d, $J=7$ Hz), 5.20 (2H, d, $J=7$ Hz), 5.48 (d, $J=8$ Hz), 5.66 (br s), 5.76 (br s) and 6.27 (d, $J=6$ Hz). The ^{13}C-NMR spectrum showed the corresponding anomeric carbon signals at δ 103.9, 106.4, 105.0, 105.7, 104.1, 101.7, 100.8 and 93.7, respectively. These spectral data suggested that II is a bisdesmosidic gypsogenin glycoside having one more pentosyl unit than I. Acid hydrolysis of II gave D-glucuronic acid, D-galactose, L-arabinose, D-quinovose, L-rhamnose and D-xylose.

The selective cleavage of the ester-glycoside linkage of II followed by methylation with CH_2N_2 gave an anomeric mixture of methyl glycosides and a prosapogenin methyl ester which was proved to be identical with V (by NMR spectroscopy).

The methyl glycoside fraction was purified by column chromatography on a reversed-phase column and the major glycoside (XII) was obtained in a homogeneous state. It gave D-quinovose, L-rhamnose and D-xylose on acid hydrolysis. The ^{1}H-NMR spectrum (Table II) showed five anomeric proton signals [δ 5.08 (d, $J=3$ Hz), 5.16 (d, $J=7$ Hz), 5.22 (d, $J=7$ Hz), 5.43 (br s) and 5.72 (br s)], and the corresponding anomeric carbon signals were detected at δ 99.8, 106.2, 105.9, 104.4 and 103.0. Enzymatic hydrolysis of XII gave a methyl trioside which was identical with XI. On methanolysis of the permethylate of XII, methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylopyranose, 2,3,4-tri-*O*-methyl-L-rhamnopyranose, 2,4-di-*O*-methyl-D-xylopyranose, 3,4-di-*O*-methyl-L-rhamnopyranose and 4-*O*-methyl-D-quinovopyranose were obtained. These data indicated that the β-D-xylopyranosyl-(1→3)-β-D-xylopyranose unit is linked to the C_2-OH group of one of the two rhamnose units. In order to determine the rhamnosyl unit to which the xylobiose is linked, the ^{1}H-NMR spectrum of XII was examined in detail. The signals of oxymethine protons are so crowded in the region from δ 3.4 to δ 5.0 that there was some difficulty in assigning signals and establishing the splitting patterns, but the signals due to the quinovopyranosyl group, the rhamnopyranosyl group (**R'**) which is linked to the C_2-OH of the quinovosyl unit and the rhamnopyranosyl group (**R**) connected to the C_3-OH of the quinovosyl unit could be assigned with the aid of the ^{1}H–^{1}H COSY spectrum (Table II). The signal of C_4-H (R'_4) of **R'** appeared at δ 4.3 overlapped by the signal of C_5-H (R'_5) of **R'** and C_5-H (X_5) of the inner xylose unit. The C_4-H (R_4) of **R** was observed at δ 4.24 overlapped with the signal of the C_5-H (X_5) of the outer xylose unit. The corresponding carbon signals were assigned by ^{13}C–^{1}H COSY at δ 67.3 (C_5 of inner Xyl), 68.1 (C_5 of **R'**), 83.4 (C_4 of **R'**), 66.7 (C_5 of outer Xyl.) and 73.9 (C_4 of **R**).

Accordingly, the structure of XII was elucidated as methyl β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L- rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-α-D-quinovopyranoside. Thus, the structure of II is a methyl ester of 3-*O*-[β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyluronic acid]-28-*O*-[β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-quinovopyranosyl] gypsogenin.

Compound III is an amorphous powder and showed in the FAB-MS an $[M+Na]^+$ ion at *m/z* 1563 indicating the molecular weight to be 1540. The analysis data were consistent with $C_{71}H_{112}O_{36}\cdot 3.5H_2O$. Its NMR spectra were similar to those of I. The signals of the sugar moiety were almost superimposable on those of I, although the signals of the aglycone moiety were a little different. Compound III gave D-glucuronic acid, D-galactose, L-arabinose, D-quinovose, L-rhamnose and D-xylose on acid hydrolysis. The selective cleavage of the ester-glycoside linkage and esterification with CH_2N_2 gave a prosapogenin dimethyl ester (XIII) and an anomeric mixture of methyl glycosides. The latter was separated and purified by column chromatography on reversed-phase material to give X as a major methyl glycoside. Compound XIII showed in the FAB-MS an $[M+Na]^+$ ion at *m/z* 1007. It gave D-glucuronic acid, D-galactose and L-arabinose on acid hydrolysis. The aglycone fraction showed several spots on TLC and the aglycone could not be identified. The molecular weight (984) of XIII, 16 more than that of V, and the NMR spectral features suggested the aglycone to be a hydroxy derivative of gypsogenin. The electron impact-mass spectrum (EI-MS) of XIII showed fragment ions at *m/z* 278 and 260, the former having originated from the *retro*-Diels–Alder fission at ring C of the oleanene-type triterpene and the latter being the dehydration product of the former. These fragment ions showed that the hydroxyl group is located at either one of rings D and E. The hydroxymethine proton appeared at δ 5.00 as a broad singlet and the ^{1}H–^{1}H COSY spectrum indicated that this hydroxymethine group is adjacent to a quaternary carbon and methylene group. Therefore, the aglycone is either the C_{16}-OH, C_{21}-OH or the C_{22}-OH derivative of gypsogenin. The ^{13}C-NMR spectrum of XIII was compared with that of quillaic acid methyl ester (16-hydroxy-gypsogenin methyl ester).[5] The signals of the aglycone moiety showed almost identical chemical shifts although there were some deviations in the chemical shifts of carbons in ring A.

The chemical shifts of the sugar moiety of XIII are identical with those of IV, indicating the same sugar structure, *viz.* 3-*O*-β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosiduronic acid methyl ester.

Accordingly, the structure of III is a methyl ester of 3-*O*-[β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucopyranosyluronic acid]-28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-quinovopyranosyl] quillaic acid.

Compound IV, FAB-MS *m/z*: 1695 ($[M+Na]^+$), gave analysis data consistent with $C_{76}H_{120}O_{40}\cdot 5H_2O$. The NMR signals due to the aglycone moiety are almost superimposable on those of III, and the signals of the sugar moiety have the almost identical chemical shifts with those of II. Acid hydrolysis of IV gave D-glucuronic acid, D-galactose, L-arabinose, D-quinovose, L-rhamnose and D-xylose. The ^{1}H-NMR spectrum revealed eight anomeric proton signals at δ 4.83 (d, $J=7$ Hz), 5.15—5.22 (3H), 5.47 (d, $J=7$ Hz), 5.66 (br s), 5.73 (br s) and 6.22 (d, $J=5$ Hz), and the ^{13}C-

NMR spectrum showed the corresponding carbon signals at δ 103.8, 102.5, 105.0, 105.8, 104.1, 101.1, 101.8 and 94.1, respectively. The selective cleavage of the ester-glycoside linkage followed by methylation with CH_2N_2 furnished a prosapogenin dimethyl ester and an anomeric mixture of methyl glycosides. The former was identified as XIII (by NMR spectroscopy). The latter was chromatographed on a reversed-phase column to give a major methyl glycoside which proved to be identical with XII. Therefore IV is a methyl ester of C_{16}-hydroxyl-luperoside J, *viz.*, a methyl ester of 3-*O*-[β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]- β-D-glucopyranosyluronic acid]-28- *O*-[β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-quinovopyranosyl] quillaic acid.

Experimental[6]

Isolation of Methyl Esters of Luperosides I, J, K and L (I—IV) The saponin fraction (fr. 1 described in the preceding paper,[2] 14 g) was dissolved in 50% MeOH (100 ml) and the solution was passed through a column of Amberlite IRC-84 (100 ml). The acidic eluate was concentrated *in vacuo*. The residue was dissolved in MeOH (200 ml) and the CH_2N_2 etherate was added until the solution became neutral. Evaporation of the solvent gave the methyl ester as an amorphous powder (12 g). The methyl ester was repeatedly chromatographed on silica gel (100—200 times the weight of the material to be chromatographed) using $CHCl_3$–MeOH–H_2O (15 : 7 : 1) and separated into four fractions (frs. I—IV). Fractions II (3.5 g) and III (1.5 g) each showed a single spot on silica gel TLC [$CHCl_3$–MeOH–H_2O–AcOH (21 : 15 : 3 : 1)], but each fraction showed two spots on reversed-phase TLC (LiChroprep RP-18 WF_{254} S, 40% MeOH). Column chromatography of fr. II on ODS YMC Gel (200 ml for 1 g of the material) using 70% MeOH gave I (1.5 g, 0.15% from the dried herb) and II (970 mg, 0.1%).

Similar treatment of fr. III gave III (500 mg, 0.06%) and IV (410 mg, 0.05%).

Luperoside I Methyl Ester (I): Fine needles from MeOH, mp 242—245 °C (dec.). $[\alpha]_D^{27}$ −6.4° (c=1.30, 80% MeOH). *Anal.* Calcd for $C_{71}H_{112}O_{35}\cdot 2H_2O$: C, 54.61; H, 7.49. Found: C, 54.53; H, 7.66. FAB-MS m/z: 1547 $[(M+Na)^+]$, 1523 $[(M-H)^-]$. ^{1}H-NMR δ: aglycone moiety: angular CH_3; 0.79 (C_{25}-H), 0.90 (C_{29}-H), 0.95 (C_{26}-H), 0.98 (C_{30}-H), 1.17 (C_{27}-H), 1.42 (C_{24}-H). olefinic H; 5.37 (t-like). aldehydic H; 9.92 s. sugar moiety: anomeric H; 4.82 d (7 Hz), 5.07 d (7 Hz), 5.17 d (8 Hz), 5.47 d (8 Hz), 5.67 br s, 5.76 br s, 6.29 d (6 Hz). $C_6OOC\underline{H}_3$; 3.74 s, C_5-$C\underline{H}_3$; 1.54 d (6 Hz), 1.62 d (6 Hz), 1.73 d (6 Hz). ^{13}C-NMR δ: aglycone moiety: shown in Table I; sugar moiety: anomeric C; 103.9, 107.1, 105.0, 104.2, 101.6, 100.8, 93.7. C_5-$\underline{C}H_3$; 18.9, 18.5, 18.4. $\underline{C}_6OOCH_3$; 169.8. $C_6OO\underline{C}H_3$; 52.1.

Luperoside J Methyl Ester (II): Fine needles from MeOH, mp 237—240 °C. $[\alpha]_D^{28}$ −9.8° (c=1.10, 80% MeOH). *Anal.* Calcd for $C_{76}H_{120}O_{39}\cdot 4H_2O$: C, 52.77; H, 7.46. Found: C, 52.59; H, 7.19. FAB-MS m/z: 1679 $[(M+Na)^+]$, 1655 $[(M-H)^-]$. ^{1}H-NMR δ: aglycone moiety: angular CH_3; 0.79 (C_{25}-H), 0.90 (C_{29}-H), 0.96 (C_{26}-H), 0.98 (C_{30}-H), 1.18 (C_{27}-H), 1.42 (C_{24}-H); olefinic H; 5.38 (t-like). aldehydic H; 9.88 s. sugar moiety: anomeric H; 4.88 d(*ca.* 8 Hz), 5.08 d (7 Hz), 5.20 d (2H, 7 Hz), 5.48 d (8 Hz), 5.66 (br s, 5.76 br s, 6.27 d (6 Hz). $C_6OOC\underline{H}_3$; 3.75 s. C_5-$C\underline{H}_3$; 1.54 d (6 Hz), 1.62 d (6 Hz), 1.69 d (5 Hz). ^{13}C-NMR δ: aglycone moiety: chemical shifts of the carbon signals are the same as those of I. sugar moiety: anomeric C; 103.9, 106.4, 105.0, 105.7, 104.1, 101.7, 100.8, 93.7. C_5-$\underline{C}H_3$; 18.9, 18.4, 18.4. $\underline{C}_6OOCH_3$; 169.8. $C_6OO\underline{C}H_3$; 52.1.

Luperoside K Methyl Ester (III): Amorphous powder from EtOH, mp 242—244 °C. $[\alpha]_D^{28}$ −18.2° (c=1.30, 80% MeOH). *Anal.* Calcd for $C_{71}H_{112}O_{36}\cdot 3.5H_2O$: C, 53.14; H, 7.48. Found: C, 53.04; H, 7.60. FAB-MS m/z: 1563 $[(M+Na)^+]$. ^{1}H-NMR δ: aglycone moiety: angular CH_3; 0.82 (C_{25}-H), 0.98 (C_{29}-H), 1.01 (C_{26}-H), 1.12 (C_{30}-H), 1.42 (C_{24}-H), 1.71 (C_{24}-H). olfeinic H; 5.53 (t-like). aldehydic H; 9.88 s. sugar moiety: anomeric H; 4.84 d (7 Hz), 5.14 d (8 Hz), 5.18 d (7 Hz), 5.47 d (7 Hz), 5.67 br s, 5.77 br s, 6.22 d (6 Hz). $C_6OOC\underline{H}_3$; 3.74 s. C_5-$C\underline{H}_3$; 1.54 d (6 Hz), 1.61 d (6 Hz), 1.69 d (5 Hz). ^{13}C-NMR δ: aglycone moiety: shown in Table I; sugar moiety: anomeric C; 103.8, 106.8, 105.0, 104.2, 101.9, 100.9, 94.1. C_5-$\underline{C}H_3$; 18.7, 18.4, 18.4. $\underline{C}_6OOCH_3$; 169.8. $C_6OO\underline{C}H_3$; 52.1.

Luperoside L Methyl Ester (IV): Amorphous powder from EtOH, mp 246—248 °C (dec.). $[\alpha]_D^{28}$ −21.6° (c=1.40, 70% MeOH). *Anal.* Calcd for $C_{76}H_{120}O_{40}\cdot 5H_2O$: C, 51.75; H, 7.43. Found: C, 51.90; H, 7.31. FAB-MS m/z: 1695 $[(M+Na)^+]$. ^{1}H-NMR δ: aglycone moiety: angular CH_3; 0.83 (C_{25}-H), 0.98 (C_{29}-H), 1.01 (C_{26}-H), 1.13 (C_{30}-H), 1.42 (C_{24}-H), 1.72 (C_{27}-H). olefinic H; 5.54 (t-like). aldehydic H; 9.89 s. sugar moiety: anomeric H; 4.83 d (7 Hz), 5.15—5.22 (3H), 5.47 d (7 Hz), 5.66 br s, 5.73 br s, 6.22 d (5 Hz). $C_6OOC\underline{H}_3$; 3.74 s. C_5-$C\underline{H}_3$; 1.54 d (6 Hz), 1.61 d (6 Hz), 1.65 d (6 Hz). ^{13}C-NMR δ: aglycone moiety: same as those of III. sugar moiety: anomeric C; 103.8, 105.0, 105.8, 106.2, 104.1, 101.8, 101.1, 94.1. C_5-$\underline{C}H_3$; 18.7, 18.4, 18.4; $\underline{C}_6OOCH_3$; 169.8. $C_6OO\underline{C}H_3$; 52.1.

Determination of Component Monosaccharides A glycoside (3 mg) was dissolved in 1 N HCl–MeOH (0.5 ml) and the solution was heated at 100 °C for 30 min. The acid was neutralized in the usual manner to give a methanolysate, which was trimethylsilylated with trimethylsilyl imidazole and analyzed by gas liquid chromatography (GLC) [column, Shimadzu Hi-Cap (0.2 mm i.d. × 50 m); liquid phase, CB-P1, 0.25 μm; column bath temperature, 190 °C or 210 °C; injection port temperature, 270 °C; carrier gas, He (0.7 ml/min); split ratio, 1/115; make-up gas, He (50 ml/min)]. The results are summarized in Table III.

Another 4 mg of glycoside was hydrolyzed by heating in 1 N HCl (0.5 ml) at 100 °C for 1 h. The reaction solution was treated in a usual manner to give a hydrolysate, which was analyzed by GLC after converting the sugar sample to a thiazolidine derivative followed by trimethylsilylation.[7] The GLC conditions were the same as for analysis of the methanolysates except for the column bath temperature (250 °C) and the injection port temperature (290 °C). The results are given in Table IV. The absolute configuration of glucuronic acid was determined after $NaBH_4$ reduction of the methyl ester to glucose.

Selective Cleavage of the Ester-Glycoside Linkage Compound I (1.30 g) and LiI (1.30 g) were dissolved in anhydrous 2,6-lutidine (8 ml), then MeOH (4 ml) was added, and the mixture was heated in a sealed test tube at 130 °C for 12 h. The reaction mixture was diluted with water and passed through the column of Amberlite MB-3 (100 ml). The eluate was concentrated *in vacuo* to dryness. The residue was chromatographed on

Table III. GLC of TMS Ethers of Methanolysates of Luperosides and Their Degradation Products

Column bath temp.	t_R (min) 190 °C	210 °C
Methanolysate of		
I, II, III, IV	11.1, 11.4, 12.1, 12.8, 15.2, 16.2, 17.5, 18.4	13.5, 15.1, 16.5, 18.0, 18.6
V, XIII	11.1, 11.4	13.5, 15.0, 16.5, 18.0, 18.6
X, XII	12.1, 12.8	
XI	12.1, 12.8, 17.5, 18.4	
D-Xylose	15.2, 16.2	
L-Arabinose		11.0, 11.4
L-Rhamnose		12.1, 12.8
D-Quinovose	17.5, 18.4	
D-Galactose		13.5, 15.0, 16.5
D-Glucuronic acid		18.0, 18.6

TMS: tetramethylsilyl.

Table IV. GLC of TMS Ethers of Thiazolidine Derivatives of the Hydrolysates of the Degradation Products of Luperosides

	t_R (min)	
Hydrolysate of		
V	15.2, 26.2	
XIII	15.2, 26.2	
VI	15.2, 26.2, 25.2	
X	15.4, 17.7, 18.2	
XII	15.4, 17.7, 18.3	
Arabinose	15.4 (L-)	16.4 (D-)
Xylose	15.6 (D-)	16.3 (L-)
Quinovose	17.7 (D-)	
Rhamnose	18.2 (L-)	18.6 (D-)
Galactose	26.6 (D-)	28.0 (L-)
Glucose	25.2 (D-)	25.9 (L-)

polystyrene polymer Diaion HP-20 (90 ml). The 35% MeOH eluate gave methyl glycosides (448 mg) and the 70% MeOH eluate contained a prosapogenin (900 mg). The prosapogenin fraction was treated with CH_2N_2 etherate and purified by column chromatography on silica gel [solvent: $CHCl_3$–MeOH–H_2O (15:3.5:0.3)] to give V: colorless prisms from MeOH, mp 210—213 °C (dec.). $[\alpha]_D^{29}$ 30.3° (c=1.50, MeOH). FAB-MS m/z: 991 $[(M+Na)^+]$, 967 $[(M-H)^-]$. ^{1}H-NMR: aglycone moiety: angular CH_3; 0.77 (C_{26}-H), 0.82 (C_{25}-H), 0.92 (C_{30}-H), 0.93 (C_{29}-H), 1.17 (C_{27}-H), 1.44 (C_{24}-H). olefinic H; 5.34 (t-like). aldehydic H; 9.93 s. $C_{28}OOCH_3$; 3.69 s. sugar moiety: anomeric H; 4.84 d (7 Hz), 5.18 d (8 Hz), 5.48 d (8 Hz). C_6OOCH_3; 3.74 s. ^{13}C-NMR δ: aglycone moiety: shown in Table I. sugar moiety: anomeric C; 103.8, 105.0, 104.2. C_6OOCH_3; 52.1. C_6OOCH_3; 169.8. The glycoside fraction showed two spots on reversed-phase TLC (LiChroprep RP-18 $WF_{254}S$, 40% MeOH). It was chromatographed on LiChroprep RP-18 (90 ml) using 25% MeOH containing tetrahydrofuran (1%) to give X: amorphous powder, $[\alpha]_D^{30}$ −17.7° (c=1.65, MeOH). FAB-MS m/z: 625 $[(M+Na)^+]$, 601 $[(M-H)^-]$. Calcd for $C_{24}H_{42}NaO_{17}$: 625.232, Found: 625.231. NMR: shown in Table II.

Treatment of II (838 mg) in the same way as described for I gave V (382 mg) and a methyl glycoside (XII) (200 mg): amorphous powder, $[\alpha]_D^{30}$ −25.7° (c=1.75, MeOH). FAB-MS m/z: 757 $[(M+Na)^+]$, 733 $[(M-H)^-]$. NMR: shown in Table II. Compound III (374 mg) gave a methyl glycoside (X) (63 mg) and a prosapogenin methyl ester (XIII) (93 mg): amorphous powder, $[\alpha]_D^{29}$ 22.0° (c=1.65, MeOH). FAB-MS m/z: 1007 $[(M+Na)^+]$. ^{1}H-NMR δ: aglycone moiety: angular CH_3; 0.81 (C_{26}-H), 0.85 (C_{27}-H), 1.02 (C_{29}-H), 1.09 (C_{30}-H), 1.44 (C_{24}-H), 1.71 (C_{27}-H). olefinic H; 5.50 (t-like). $C_{28}OOCH_3$; 3.68 s. sugar moeity: anomeric H; 4.84 d (7 Hz), 5.17 d (8 Hz), 5.47 d (8 Hz). C_6OOCH_3; 3.74 s. ^{13}C-NMR δ: aglycone moiety: shown in Table I. sugar moiety: anomeric C; 103.8, 105.0, 104.2. C_6OOCH_3; 52.1. C_6OOCH_3; 169.8.

Compound IV (371 mg) gave XIII (54 mg) and XII (67 mg).

$NaBH_4$ Reduction of V, Permethylation and Methanolysis Compound V (74 mg) was dissolved in MeOH (1 ml) and $NaBH_4$ (100 mg) was added. The reaction mixture was stirred at room temperature for 16 h. The reaction solution was neutralized with acetic acid and evaporated. The residue was purified by column chromatography on silica gel [15 g, $CHCl_3$–MeOH–H_2O (15:3:0.5)] to give VI (42 mg): an amorphous powder, FAB-MS m/z: 965 $[(M+Na)^+]$. The ^{1}H-NMR spectrum showed no carbomethoxyl and aldehydic proton signals. Compound VI (20 mg) was permethylated according to Hakomori's method. The product was purified by chromatography on silica gel [2 g, benzene–acetone (2:1)] to give the permethylate (21 mg), which was methanolyzed in 1 N HCl–MeOH under reflux for 2 h. The methanolysate was acetylated with Ac_2O–pyridine (1:1). The acetylated product was analyzed by gas chromatography–chemical ionization-mass spectrum (GC-CI-MS). Methyl glycosides of 2,3,4-tri-*O*-methyl-L-arabinopyranose, 2,3,4,6-tetra-*O*-methyl-D-galactopyranose and 2,3-di-*O*-acetyl-4,6-di-*O*-methyl-D-glucopyranose were identified by comparison of t_R values and CI-MS patterns with those of authentic samples.

Partial Hydrolysis of V with Trifluoroacetic Acid Compound V (156 mg) was dissolved in 2 N CF_3-COOH in MeOH (5 ml) and heated in a screw-capped test tube at 90 °C for 3 h. After evaporation of the solvent, the residue was chromatographed on silica gel using 2.5% acetone in benzene, 1.5% MeOH in $CHCl_3$, 5% MeOH in $CHCl_3$ and $CHCl_3$–MeOH–H_2O (15:3:0.1) to give IX (14 mg), VII (24 mg) and VIII (66 mg).

VII: Amorphous powder. $[\alpha]_D^{30}$ 29.0° (c=1.15, MeOH). FAB-MS m/z: 697 $[(M+Na)^+]$. ^{1}H-NMR δ: aglycone moiety: angular CH_3; 0.78 (C_{26}-H), 0.86 (C_{25}-H), 0.93 (C_{30}-H), 0.94 (C_{29}-H), 1.21 (C_{27}-H), 1.30 (C_{24}-H). olefinic H; 5.36 t (3 Hz). aldehydic H; 9.75 s. $C_{28}OOCH_3$; sugar moiety: 4.86 d [8 Hz, methyl glucosiduronate (GM-1)], 4.16 dd (8, 10 Hz, GM-2), 4.51 dd (10 Hz, GM-3), 4.43 dd (10 Hz, GM-4), 4.51 d (10 Hz, GM-5). C_6OOCH_3; 3.74 s. ^{13}C-NMR δ: aglycone moiety: shown in Table I. sugar moiety: 105.2 (GM-1), 74.9 (GM-2), 77.7 (GM-3), 72.9 (GM-4), 77.2 (GM-5), 170.5 (GM-6), 51.9 (C_6OOCH_3).

VIII: Amorphous powder. $[\alpha]_D^{30}$ 30.0° (c=0.75, MeOH). FAB-MS m/z: 859 $[(M+Na)^+]$. ^{1}H-NMR δ: aglycone moiety: angular CH_3; 0.78 (C_{26}-H), 0.84 (C_{25}-H), 0.92 (C_{29}-H), 0.94 (C_{30}-H), 1.20 (C_{27}-H), 1.43 (C_{24}-H). olefinic H; 5.35 (t-like). aldehydic H; 9.95 s. $C_{28}OOCH_3$; 3.70 s. sugar moiety: 4.89 d (7 Hz, GM-1), 4.15 dd (7, 9 Hz, GM-2), 4.22 dd (9 Hz, GM-3), 4.36 dd (9 Hz, GM-4), 4.43 d (9 Hz, GM-5), 5.21 d [8 Hz, galactose (Gal)-1], *ca.* 4.53 (4H, Gal-2, 4, 6), *ca.* 4.12 (2H, Gal-3, 5). 3.71 s (C_6OOCH_3). ^{13}C-NMR δ: aglycone moiety: shown in Table I. sugar moiety[9]: 103.2, (GM-1), 83.5 (GM-2), 77.4 (GM-3), 72.5 (GM-4), 76.7 (GM-5), 170.2 (GM-6), 106.3 (Gal-1), 74.3 (Gal-2), 75.3 (Gal-3), 70.1 (Gal-4), 77.1 (Gal-5), 62.2 (Gal-6), 52.0 (C_6OOCH_3).

IX: Amorphous powder. EI-MS m/z: 484 (M^+), 262, 203. ^{1}H-NMR δ: angular CH_3; 0.83, 0.92, 0.95, 0.95, 1.22, 1.36. olefinic H; 5.40 t (3 Hz). aldehydic H; 9.63 s. $C_{28}OOCH_3$; 3.70 s. ^{13}C-NMR: shown in Table I.

Compound VIII was methanolyzed in the same manner as described above to give methyl glucuronopyranosiduronic acids and methyl galactosides.

Permethylation of X and XII Methylation of X was performed according to Hakomori's method. Thus, the sugar sample (28 mg) was dissolved in dimethylsulfoxide (0.5 ml). A solution (0.5 ml) of dimethyl sulfinyl carbanion was added to the solution and the mixture was stirred for 10 min. CH_3I (0.5 ml) was added, and the reaction mixture was stirred at room temperature for 10 h. The reaction mixture was poured into water and extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on silica gel (2 g, hexane–AcOEt (1:1)] to give a permethylate (29 mg). The same treatment of XII (29 mg) gave 28 mg of the permethylate.

Methanolysis of the Permethylate and Identification of the Methylated Monosaccharides The permethylate of X or XII (8 mg) was dissolved in 1 N HCl–MeOH and heated at 80 °C for 3 h. After work-up in a usual manner, the methanolysate was acetylated and analyzed by GC-CI-MS. The methylated sugars identified were as follows.

From the permethylate of X: methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α- and β-D-quinovopyranosides, methyl 4-*O*-acetyl-2,3-di-*O*-methyl-α-L-rhamnopyranoside, methyl 2,3,4-tri-*O*-methyl-α- and -β-D-xylopyranosides and methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside.

From the permethylate of XII: methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α- and -β-D-quinovopyranosides, methyl 2,3-di-*o*-methyl-4-*O*-acetyl-α-L-rhamnopyranoside, methyl 2,4-di-*O*-methyl-3-*O*-acetyl-α- and -β-D-xylopyranosides, methyl 2,3,4-tri-*O*-methyl-α- and -β-D-xylopyranosides and methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside.

Enzymatic Hydrolysis of X and XII Compound X (70 mg) and cellulase (270 mg) were dissolved in H_2O (4 ml) and stirred at room temperature for 2 d. After evaporation of the solvent, the residue was suspended in MeOH and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by chromatography [silica gel, 10 g; solvent, $CHCl_3$–MeOH–H_2O (15:4:0.5)] to give a trioside fraction (47 mg), which was again chromatographed on LiChroprep RP-18 (40 ml) using 10% MeOH as the eluant to give XI (35 mg): amorphous powder. $[\alpha]_D^{30}$ −6.2° (c=1.70, MeOH). FAB-MS m/z: 493 $[(M+Na)^+]$.

Treatment of XII with cellulase gave the same compound (identified by TLC and NMR).

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