Studies on the Constituents of *Luffa acutangula* ROXB. II.¹⁾ Structures of Acutosides H and I, Oleanolic Acid Saponins Isolated from the Seed

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From the seeds of *Luffa acutangula* ROXB. (Cucurbitaceae), two 3,28-*O*-bisdesmosidic heptaglycosides of oleanolic acid, named acutosides H and I, were isolated and their structures were elucidated. Acutosides H and I have the same prosapogenin structure, oleanolic acid 3-*O*-[*O*- α -L-arabinopyranosyl-(1 $\rightarrow$ 3)- β -D-glucopyranosyluronic acid] and differ in the structures of the ester-linked sugar moieties. Acutoside H is a 28-*O*-[*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-[*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl] ester, and acutoside I is a 28-*O*-[*O*- α -L-arabinopyranosyl-(1 $\rightarrow$ 3)-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-[*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl] ester.

Keywords *Luffa acutangula*; Cucurbitaceae; oleanolic acid saponin; glucuronide saponin; 3,28-*O*-bisdesmoside; rotating flame Overhauser effect difference spectrum; homodecoupling difference spectrum; one dimensional-homonuclear Hartmann–Hahn

In the preceding paper¹⁾ of this series, we reported the isolation and structure elucidation of seven oleanane-type triterpene saponins, acutosides A–G, from the herb of *Luffa acutangula* ROXB. (Cucurbitaceae). The seed kernels of this plant have been used in traditional Indian medicine as an emetic and an expectorant,²⁾ and the constituents of the seeds have been investigated by several groups. Cucurbitacin B and a saponin of oleanolic acid were isolated,³⁾ but the structure of the saponin has not been characterized.

The preliminary investigation of the constituents of the seeds suggested the presence of four acidic saponins. This paper deals with the isolation of the saponins and the structure elucidation of the two major, less polar saponins named acutosides H and I.

The defatted seed powder was extracted successively with MeOH and 50% MeOH. The 50% MeOH extract was passed through a column of Diaion HP-20 and the column was washed with H₂O and 50% MeOH. The saponin fraction was eluted with MeOH. The MeOH eluate showed spots of two major less polar saponins and two minor polar saponins on thin layer chromatography (TLC). They were named acutosides H, I, J and K in order of increasing polarity. The saponin fraction was treated with an ion-exchange resin IRC-84 and the acidic solution was methylated with diazomethane. The methylated product was repeatedly chromatographed on silica gel, reversed-phase material, and acutosides were isolated as their methyl esters.

The methyl ester (I) of acutoside H, obtained as colorless needles, showed in its fast-atom bombardment mass spectrum (FAB-MS) an [M+Na]⁺ ion at *m/z* 1475 and an [M–H][–] ion at *m/z* 1451, indicating the molecular weight to be 1452. The result of elemental analysis by high-resolution FAB-MS was consistent with the molecular formula C₆₈H₁₀₈O₃₃, and I gave L-arabinose, D-xylose, L-rhamnose and D-glucuronic acid on acid hydrolysis.

The ¹H-nuclear magnetic resonance (¹H-NMR) spectrum of I showed signals of seven tertiary methyl groups (δ 0.83, 0.93, 0.97, 1.01, 1.06, 1.28 and 1.29), one secondary methyl group [δ 1.74 (d, *J*=6 Hz)], one trisubstituted olefinic proton (δ 5.44, triplet-like) and seven anomeric protons [δ 4.91 (d, *J*=8 Hz), 5.09 (d, *J*=7 Hz), 5.15 (d, *J*=7 Hz), 5.28 (d, *J*=7 Hz), 5.45 (d, *J*=8 Hz), 5.67 s and 6.53 (d, *J*=2 Hz)].

The ¹³C-nuclear magnetic resonance (¹³C-NMR) spec-

trum revealed signals of six C–C bonded quaternary carbons (δ 30.9, 36.9, 39.5, 39.9, 42.1 and 47.3), two ester carbonyl carbons (δ 170.2 and 176.2), a pair of olefinic carbons (δ 122.8 and 144.1) (Table I) and seven anomeric carbons (δ 93.2, 100.7, 104.5, 105.7, 105.85, 105.9 and 106.7). The nuclear magnetic resonance (NMR) signals of the aglycone moiety were quite similar to those of lucyoside H, oleanolic acid 3,28-*O*-bis- β -D-glucoside, isolated from the herb of *Luffa cylindrica* ROEM. (Cucurbitaceae).⁴⁾ These spectral data indicated that I is a 3,28-*O*-bisdesmosidic heptaglycoside of oleanolic acid.

On selective cleavage of the ester–glycoside linkage,⁵⁾ I

TABLE I. ¹³C-NMR Chemical Shifts of I and II (Aglycone Moieties)

	Oleanolic acid ^{a)}	I	II
1	39.1	38.6	38.6
2	28.1	26.5	26.5
3	78.2	89.3	89.3
4	39.4	39.5	39.4
5	55.9	55.8	55.7
6	18.9	18.5	18.4
7	33.3	32.7	32.8
8	39.9	39.9	39.8
9	48.2	48.0	47.9
10	37.5	36.9	36.9
11	23.8	23.7	23.6
12	122.6	122.8	122.8
13	144.9	144.1	144.1
14	42.3	42.1	41.9
15	28.4	28.1	28.0
16	23.8	23.7	23.4
17	46.8	47.3	46.9
18	42.1	41.7	41.8
19	46.6	46.3	46.1
20	31.0	30.9	30.8
21	34.4	34.1	33.9
22	33.3	33.1	33.0
23	28.8	28.1	28.0
24	16.5	16.9	16.8
25	15.6	15.5	15.4
26	17.5	17.5	17.1
27	26.2	26.0	26.1
28	180.1	176.2	177.9
29	33.3	33.1	33.1
30	23.8	23.7	23.6
OMe	—	—	51.5

a) NMR data reported by Takemoto *et al.*⁴⁾

gave a prosapogenin and an anomeric mixture of a methyl oligoglycoside.

The methyl ester (II) of the prosapogenin showed an $[M+Na]^+$ ion at m/z 815 in its FAB-MS, and it gave L-arabinose and D-glucuronic acid on acid hydrolysis. From the mass number and NMR signals of anomeric protons [δ 4.93 (d, $J=8$ Hz) and 5.27 (d, $J=7$ Hz)] and anomeric carbons (δ 105.9 and 106.7), it is apparent that II is a glycoside of methyl oleanolate, which has 1 mol each of L-arabinose and D-glucuronic acid methyl ester linked to the C₃-hydroxyl group of the aglycone. The structure of II was determined to be oleanolic acid 3-*O*-[*O*- α -L-arabinopyranosyl-(1 $\rightarrow$ 3)- β -D-glucopyranosyluronic acid] dimethyl ester by examination of the NMR spectra. The assignment of the NMR signals of the sugar moiety is shown in Table II.

The anomeric mixture of the methyl oligoglycoside gave L-arabinose, L-rhamnose and D-xylose on acid hydrolysis. The positive FAB-MS showed an $[M+Na]^+$ ion at m/z 729, and its negative FAB-MS showed an $[M-H]^-$ ion at m/z 705 and fragment ions at m/z 573, 441, 309 and 163. These mass data indicate that the methyl oligoglycoside is composed of 4 mol of pentose and 1 mol of L-rhamnose and that it is either a linear MeO-pentose(1)-rhamnose-pentose(2)-pentose(3)-pentose(4) or one of two branched-chain pentaglycosides, one of which has a pentosyl group and a pentobiosyl group at the rhamnosyl unit and the other has three pentosyl groups at the rhamnosyl unit. The

anomers were separated by high performance liquid chromatography (HPLC) on a reversed-phase material to give a methyl pentaglycoside (III) and its anomer (IV).

Compound III showed in the 1H -NMR spectrum the signals of five anomeric protons [δ 4.54 (d, $J=7$ Hz), 5.15 (2H, d, $J=7$ Hz), 5.51 (d, $J=8$ Hz) and 5.97 (d, $J=2$ Hz)], while the other anomer (IV) showed the signals at δ 4.88 (d, $J=7$ Hz), 5.15 (d, $J=7$ Hz), 5.29 (d, $J=3$ Hz), 5.46 (d, $J=8$ Hz) and 5.62 (d, $J=1$ Hz). Compound III showed the signals of the axial C₅-protons of the pentosyl units at δ 3.39 (t, $J=10$ Hz), 3.42 (t, $J=10$ Hz), 3.47 (t, $J=10$ Hz) and at 3.63 (dd, $J=2, 12$ Hz), and IV showed the C₅-H signals at δ 3.17 (t, $J=11$ Hz, C₅-Ha), 3.42 (t, $J=10$ Hz, C₅-Ha), 3.47 (t, $J=10$ Hz, C₅-Ha) and 3.95 (2H, br s, C₅-Ha, e). From the coupling constants of the C₅-protons, III and IV were proved to have 1 mol of L-arabinose and 3 mol of D-xylose as the pentosyl units.

In order to identify the pentose(1), the NMR spectra of III and IV were compared and fully examined, and the 1H - and ^{13}C -NMR signals were assigned as shown in Table III by the 1H - 1H correlation spectroscopy (COSY) and 1H - ^{13}C COSY techniques. The coupling constants of the C₁-H (7 Hz in III, 3 Hz in IV), C₂-H (7, 8 Hz in III, 3, 10 Hz in IV) and C₃-H (8, 4 Hz in III, 10, 4 Hz in IV) showed that the methyl glycosylated sugar is L-arabinose. The ^{13}C -NMR chemical shifts of the C₂ of the arabinosyl groups (δ 76.9 in III and 78.8 in IV) indicate that the rhamnosyl group is linked to C₂-OH of the arabinosyl group.

The NMR chemical shifts of the rhamnosyl group were assigned as shown in Table III. The chemical shifts of the arabinosyl group and rhamnosyl group in III and IV were in good agreement with those of methyl *O*- α -L-arabinopyranosyl-(1 $\rightarrow$ 3)-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-[*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside and its β -anomer, respectively, which were derived from the ester-linked sugar moiety of acutoside G isolated from the herb,¹⁾ suggesting that III and IV are branched-chain pentaglycosides having a xylopyranosyl group and a xylobiosyl group at the C₃-OH and C₄-OH groups of the rhamnopyranosyl unit.

In order to determine the positions of the xylosyl and the xylobiosyl groups at the rhamnosyl unit and the position of the terminal xylosyl group linkage, the assignments of the anomeric protons and carbons of xylosyl units were performed by using the rotating-frame Overhauser effect (ROE) difference spectroscopy (ROEDS) technique (Fig. 1).

On irradiation at the frequency (δ 4.88) of the anomeric proton of the first xylosyl unit (Xa) of IV, NOE was observed on the signals at δ 4.82 (br s, C₂-H), δ 4.63 (dd, $J=3, 9$ Hz, C₃-H), and δ 4.48 (t, $J=9$ Hz, C₄-H) of the rhamnosyl group (Fig. 1C). The intensities of the signals clearly showed that Xa is linked to the C₃-OH group of the rhamnopyranosyl group. Irradiation at the anomeric signal (δ 5.46) of the third xylosyl group (Xc) brought NOE on the signals at δ 4.82 (br s, C₂-H), δ 4.63 (dd, $J=3, 9$ Hz, C₃-H) and δ 4.48 (t, $J=9$ Hz, C₄-H) of the rhamnosyl group. There was also NOE on the signals at δ 4.11 (t, $J=9$ Hz, C₃-H of Xc) and δ 3.42 (t, $J=10$ Hz, C₅-Ha of Xc) (Fig. 1D). The intensities of the NOE signals indicated that Xc is linked to the C₄-OH group of the rhamnosyl unit. Irradiation at the anomeric proton signal (δ 5.15) of the second xylosyl unit (Xb) caused NOE on the triplet signal at δ 4.11 (t, $J=9$ Hz) (Fig. 1E),

TABLE II. NMR Chemical Shifts of the Sugar Moiety of II

	Methyl glucuronate		Arabinosyl	
	1H	^{13}C	1H	^{13}C
1	4.93 (d, 8)	106.7	5.27 (d, 7)	105.9
2	4.06 (dd, 8, 9)	74.5	4.47 (dd, 7, 9)	72.8
3	4.28 (t, 9)	85.7	4.15 (dd, 3, 9)	74.4
4	4.35 (t, 9)	71.1	ca. 4.27	69.2
5	4.52 (d, 9)	76.7	3.78 (dd, 2, 12)	67.1
			4.34 (dd, 3, 12)	
6	—	170.1		
OCH ₃	3.74 (s)	52.0		

TABLE III. NMR Chemical Shifts of III and IV

		III		IV	
		1H	^{13}C	1H	^{13}C
MeO		3.52 (s)	55.8	3.42 (s)	55.0
Ara(1-OMe)	1	4.54 (d, 7)	103.5	5.29 (d, 3)	101.1
	2	4.46 (dd, 7, 8)	76.9	4.58 (dd, 3, 10)	78.8
	3	4.12 (dd, 8, 4)	74.2	4.47 (dd, 10, 4)	69.0
	4	ca. 4.20	69.3	4.27 (br s)	70.6
	5	3.63 (dd, 2, 12)	66.1	3.95 (2H, br s)	63.5
		ca. 4.19			
Rha(1-2Ara)	1	5.97 (d, 2)	102.0	5.62 (d, 1)	104.0
	2	4.90 (dd, 2, 3)	71.8	4.82 (br s)	71.3
	3	4.68 (dd, 3, 9)	82.5	4.63 (dd, 3, 9)	82.4
	4	4.53 (t, 9)	78.4	4.48 (t, 9)	78.2
	5	ca. 4.60	68.0	ca. 4.42	68.0
	6	1.64 (d, 6)	18.4	1.62	18.6
Xyl(1-3Rha)	1	5.15 (d, 7)	105.8 ^{a)}	4.88 (d, 7)	105.5
Xyl(1-4Rha)	1	5.15 (d, 7)	105.9 ^{a)}	5.15 (d, 7)	105.9
Xyl(1-3Xyl)	1	5.51 (d, 8)	104.5	5.46 (d, 8)	104.4

a) Assignments are interchangeable. Xyl(1-3Rha) means the xylopyranosyl group which is linked to the C₃-OH group of the rhamnopyranosyl group.

Fig. 1. The ^1H -NMR Spectrum (A) and ROESD Spectra (B—E) of IV
Rha-1 means the $\text{C}_1\text{-H}$ signal of the rhamnopyranosyl group.

Chart 1

which was proved to be the $\text{C}_3\text{-H}$ of the Xc by the homonuclear Hartmann–Hahn (HOHAHA) technique. From these ROE difference spectroscopic and HOHAHA techniques, the structure of IV was determined to be methyl $O\text{-}\beta\text{-D}$ -xylopyranosyl(Xb)-(1 $\rightarrow$ 3)- $O\text{-}\beta\text{-D}$ -xylopyranosyl-(Xc)-(1 $\rightarrow$ 4)-[$O\text{-}\beta\text{-D}$ -xylopyranosyl(Xa)-(1 $\rightarrow$ 3)] $O\text{-}\alpha\text{-L}$ -rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta\text{-L}$ -arabinopyranoside.

Compound IV was fully methylated and methanolized. The methanolysis product was acetylated and analyzed by gas chromatography-chemical ionization mass spectrometry (GC-CI-MS). Methyl glycosides of 2,3,4-tri- O -methyl-D-xylopyranose, 2,4-di- O -methyl-3- O -acetyl-D-xylopyranose, 2- O -methyl-3,4-di- O -acetyl-L-rhamnopyranose and 3,4-di- O -methyl-2- O -acetyl-L-arabinopyranose were identified, and these results were consistent with the above structure.

The configuration and conformation of the ester-linked L-arabinopyranosyl group in I were determined to be α and $^1\text{C}_4$ from the $J_{\text{C}_1\text{H}_1}$ value (172 Hz)⁶ and the coupling constants of the $\text{C}_1\text{-H}$ ($J=2$ Hz) and the $\text{C}_2\text{-H}$ (dd, $J=2, 4$ Hz) determined by the homodecoupling difference spectroscopy technique. The structure of I is, therefore, concluded to be as shown in the chart.

The methyl ester (V) of acutoside I was obtained as colorless needles, and its FAB-MS showed an $[\text{M} + \text{Na}]^+$ ion at m/z 1475 and an $[\text{M} - \text{H}]^-$ ion at m/z 1451. Elemental analysis by high-resolution FAB-MS gave the molecular formula $\text{C}_{68}\text{H}_{108}\text{O}_{33}$.

Compound V gave L-arabinose, L-rhamnose, D-xylose and D-glucuronic acid on acid hydrolysis. The NMR spectra indicated that V is also a 3,28- O -bisdesmosidic heptaglyco-

side of oleanolic acid, and V gave a prosapogenin and an anomeric mixture of a methyl pentaglycoside which is composed of L-arabinose, L-rhamnose and D-xylose. The methyl ester of the prosapogenin was proved to be identical with II. The anomers of the methyl glycoside were separated by HPLC on a reversed-phase material. Both methyl glycosides gave L-arabinose, L-rhamnose and D-xylose on acid hydrolysis. The NMR spectra of the faster-eluting anomer (VI) and the other one (VII) were identical with methyl *O*- α -L-arabinopyranosyl-(1 $\rightarrow$ 3)-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-[*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside and its β -anomer, respectively, which were derived from acutoside G isolated from the herb.¹⁾

The configuration and conformation of the ester-linked L-arabinopyranosyl group in V were determined to be α and 1C_4 from the J_{C1H1} value (171 Hz)⁶⁾ and the coupling constants of C₁-H ($J=3$ Hz) and the C₂-H (dd, $J=3, 4$ Hz) determined by the homodecoupling difference spectroscopy technique. Therefore, the structure of V was elucidated to be as shown in the chart. Acutosides H and I are contained in the seed as the carboxylate forms.

Acutosides J and K are more polar than H and I, and the NMR data and the molecular weights (1584) suggested that they are 3,28-*O*-bisdesmosidic octaglycosides of oleanolic acid, but their structures could not be determined owing to the small yields of the glycosides.

Experimental⁷⁾

Extraction and Fractionation The seeds (1 kg) of *Luffa acutangula* cultivated in the herbal garden of this university were powdered and extracted first with hexane (15 l). The residue was then percolated with MeOH (10 l) and 50% MeOH (10 l). The 50% MeOH eluate was concentrated to half volume and passed through a column of Diaion HP 20 (1 l). After washing of the column with H₂O (2 l) and 50% MeOH (2 l), MeOH (2 l) was passed through a column to give a crude saponin fraction (9.94 g). The saponin fraction was dissolved in H₂O (100 ml) and passed through the column of Amberlite IRC-84 (100 ml). The acidic solution was concentrated *in vacuo*. The residue was dissolved in 50% MeOH and treated with an ether solution of CH₂N₂ to give a methyl ester of the saponin fraction.

Isolation of Methyl Esters of Acutosides H, I, J and K The methylation product was repeatedly chromatographed on silica gel using CHCl₃-MeOH-H₂O (15:6:0.5, 15:6:1) and AcOEt-MeOH-H₂O (8:2:1) and divided into four fractions (fr. I-IV), based on monitoring by TLC (solvent: CHCl₃-MeOH-H₂O-AcOH, 21:15:3:1). Fraction I was almost pure acutoside H methyl ester (I) and this fraction was purified by column chromatography on YMC gel (ODS) using 75% MeOH to give a thin-layer-chromatographically homogeneous white powder (2.765 g). Fractions II, III and IV were purified in a same manner to give acutoside I methyl ester (V) (1.892 g), acutoside J methyl ester (337 mg) and acutoside K methyl ester (198 mg), respectively.

Acutoside H Methyl Ester (I): Colorless fine needles from 80% MeOH, mp 235–238 °C, $[\alpha]_D^{24} -53.1^\circ$ ($c=0.58$, 50% MeOH). Positive FAB-MS m/z : 1475.6700 ($[C_{68}H_{108}O_{33}Na]^+$). Negative FAB-MS m/z : 1451 ($[M-H]^-$). 1H -NMR δ : aglycone moiety: δ -CH₃, 0.83, 0.93, 0.97, 1.01, 1.06, 1.28, 1.29; δ -CH-, 5.44 (t-like). Sugar moiety: anomeric H, 4.91 (d, $J=8$ Hz), 5.09 (d, $J=7$ Hz), 5.15 (d, $J=7$ Hz), 5.28 (d, $J=7$ Hz), 5.45 (d, $J=8$ Hz), 5.67 (br s), 6.53 (d, $J=2$ Hz). ^{13}C -NMR δ : aglycone moiety: shown in Table I. Sugar moiety: anomeric C, 93.2, 100.7, 104.5, 105.7, 105.85, 105.9, 106.7.

Acutoside I Methyl Ester (V): Colorless needles from 80% MeOH, mp 234–237 °C, $[\alpha]_D^{24} -28.7^\circ$ ($c=2.0$, 50% MeOH). Positive FAB-MS m/z : 1475.6660 ($[C_{68}H_{108}O_{33}Na]^+$). 1H -NMR δ : aglycone moiety: almost the same as those of I. Sugar moiety: anomeric H, 4.92 (d, $J=8$ Hz), 5.11 (d, $J=8$ Hz), 5.17 (d, $J=7$ Hz), 5.28 (d, $J=7$ Hz), 5.44 (d, $J=8$ Hz), 5.67 (br s), 6.52 (d, $J=2$ Hz). ^{13}C -NMR δ : aglycone moiety: almost the same as those of I. Sugar moiety: anomeric C, 93.2, 100.7, 104.6, 105.5, 105.6, 105.9, 106.7.

Identification of the Sugar Species The component sugar species and

their absolute configurations were determined in the same manner as described in the preceding paper¹⁾ of this series. Thus, the glycoside or a saponin (*ca.* 3 mg) was dissolved in 2N HCl-MeOH (0.5 ml) and the solution was refluxed for 2 h. The acid was neutralized with Ag₂CO₃ and the mixture was filtered. The filtrate was bubbled through with H₂S and concentrated to dryness. Trimethylsilylimidazole (25 μ l) was added to the residue. Hexane (0.5 ml) was added to the mixture and washed with water (0.5 ml). The hexane solution was injected into the gas chromatography (GC) column. Authentic sugar samples were treated in the same manner and the retention times were compared with those of the test samples.

Determination of the absolute configurations of the sugars was performed according to the method reported from this faculty.⁸⁾ A glycoside (*ca.* 5 mg) was dissolved in 1N HCl and heated at 90 °C for 1 h. The acid was neutralized in the same manner described above to give the hydrolyzate. A pyridine solution (100 μ l) of L-cysteine methyl ester hydrochloride (0.06 mol/l) was added to the hydrolyzate and the mixture was warmed at 60 °C for 1 h. Pyridine was blown off with N₂ and trimethylsilylimidazole (25 μ l) was added to the residue. Hexane (0.5 ml) and water (0.5 ml) were added and the mixture was shaken. The hexane solution was injected into the GC column. Authentic sugar samples were treated in the same way and the retention times were compared.

The absolute configuration of the component glucuronic acid was determined as glucose after NaBH₄ reduction of the prosapogenin methyl ester followed by hydrolysis.

The GC conditions were as follows: column, Shimadzu capillary column HiCap (0.25 mm i.d. $\times$ 50 m); liquid phase, CBP-1; carrier gas, He at 0.7 ml/min (20 cm/s). Temperature: 190 °C for pentose and methylpentose derivatives, and 210 °C for hexose derivatives and for the determination of the absolute configuration. The sugar species identified are cited in the text.

Selective Cleavage of the Ester-Glycoside Linkage Selective cleavage of the ester-linked sugar moiety was performed according to the method reported by Ohtani, *et al.*⁵⁾ Acutoside H methyl ester (I) (640 mg) and LiI (815 mg) were added to the mixture of 2,6-lutidine (8 ml) and MeOH (6 ml), and the mixture was heated at 140 °C for 24 h. Then 50% MeOH (35 ml) was added to the reaction mixture and the solution was passed through a column of Amberlite MB-3 (60 ml). The effluent was concentrated to dryness and the residue was dissolved in MeOH and treated with CH₂N₂. After evaporation of the solvent, the residue was redissolved in 50% MeOH and chromatographed on a Diaion HP-20 column eluted with 50% MeOH (100 ml) and MeOH (100 ml). The 50% MeOH eluate (263 mg) was chromatographed on a prepacked Fuji gel (ODS) column (30 cm $\times$ 2.5 cm i.d.) using 13% MeOH and finally purified by HPLC on a Shiseido Capcell Pak C18 column (25 cm $\times$ 10 mm i.d.) using 15% MeOH (flow rate, 2 ml/min) to give III (33 mg) and IV (52 mg). The MeOH eluate (300 mg) was chromatographed on a silica gel (50 g) column (AcOEt-MeOH-H₂O, 8:1:0.1) to give a prosapogenin methyl ester (II) (184 mg).

Acutoside I methyl ester (V) (800 mg) gave II (300 mg) and an anomeric mixture (299 mg) of a methyl glycoside. The latter was chromatographed on Fuji gel and purified by HPLC in the same manner as described above to give VI (52 mg) and VII (87 mg). The 1H - and ^{13}C -NMR spectra of VI and VII were superimposable on those of methyl *O*- α -L-arabinopyranosyl-(1 $\rightarrow$ 3)-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-[*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 3)]-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside and its β -anomer, respectively.

Prosapogenin Methyl Ester (II): Colorless needles from MeOH, mp 203–207 °C, $[\alpha]_D^{24} 23.5^\circ$ ($c=1.0$, MeOH). Positive FAB-MS m/z : 815 ($[M+Na]^+$). 1H -NMR δ : aglycone moiety: δ -CH₃, 0.82, 0.85, 0.93, 0.95, 0.97, 1.25, 1.30; δ -CH-, 5.37 (t-like); COOCH₃, 3.71. Sugar moiety: shown in Table II. ^{13}C -NMR: shown in Tables I and II.

Compound III: An amorphous powder, $[\alpha]_D^{24} -58.2^\circ$ ($c=1.35$, MeOH). Positive FAB-MS m/z : 729 ($[M+Na]^+$). Negative FAB-MS m/z : 725 ($[M-H]^-$), 573 (725-pentose), 441 (573-pentose), 309 (441-pentose), 163 (309-rhamnose). NMR: shown in Table III.

Compound IV: An amorphous powder, $[\alpha]_D^{24} -1.6^\circ$ ($c=1.6$, MeOH). FAB-MS: same as those of III. NMR: shown in Table III.

Permethylation of IV and Determination of the Component Methylated Sugars of the Permethylate Compound IV (17 mg) was added to dimethylsulfinyl carbanion solution (1 ml) prepared by heating a dimethyl sulfoxide (DMSO) solution of NaH (50 mg) at 80 °C for 20 min. After the mixture had been stirred for 10 min, MeI (1 ml) was added, and the whole was stirred at room temperature for 24 h. Water (5 ml) was added to the reaction mixture and the methylation product was extracted with CHCl₃ (5 ml $\times$ 3). The CHCl₃ layer was separated and dried over Na₂SO₄. After evaporation of CHCl₃, the residue was chromatographed on silica gel

(15 g) (solvent: benzene–acetone, 4:1) to give the thin-layer-chromatographically homogeneous product (20 mg). The methylation product was heated in 1 N HCl–MeOH (0.5 ml) for 1 h, neutralized with Ag_2CO_3 and filtered. The filtrate was bubbled through with H_2S and concentrated to dryness. The methanolysate was acetylated with Ac_2O –pyridine (1:1) (1 ml) at room temperature and checked by GC–CI–MS.

The following methyl glycosides were identified. The numbers in parentheses are retention times. Methyl 2,3,4-tri-*O*-methyl- β -D-xylopyranoside (1.2) and its α -anomer (1.5), methyl 2,4-di-*O*-methyl-3-*O*-acetyl- β -D-xylopyranoside (4.3) and its α -anomer (5.3), methyl 3,4-di-*O*-methyl-2-*O*-acetyl- α -L-arabinopyranoside (5.6) and 2-*O*-methyl-3,4-di-*O*-acetyl- α -L-rhamnopyranoside (7.4).

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References and Notes

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