Studies on the Constituents of *Luffa acutangula* ROXB. I. Structures of Acutosides A—G, Oleanane-Type Triterpene Saponins Isolated from the Herb

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From the herb of *Luffa acutangula* ROXB. (Cucurbitaceae), seven oleanane-type triterpene saponins, acutosides A—G, were isolated and their structures were determined.

Acutoside A is oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside. Acutosides B, D, E, F and G have a common prosapogenin structure, acutoside A, and only differ in the structures of the ester-linked sugar moieties. Acutoside B is a 28-*O*-[*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, D is a 28-*O*-[*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, E is a 28-*O*-[*O*-α-L-arabinopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, F is a 28-*O*-[*O*-β-D-xylopyranosyl-(1→3)-[*O*-β-D-xylopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, and G is a 28-*O*-[*O*-β-D-xylopyranosyl-(1→3)-[*O*-α-L-arabinopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester. Acutoside C is a machaelinic acid (=21β-hydroxyoleanolic acid) saponin having the same sugar moiety as that of acutoside B.

Keywords *Luffa acutangula*; Cucurbitaceae; triterpene saponin; 3,28-*O*-bisdesmoside; acutoside; oleanolic acid saponin; machaelinic acid; herb saponin

Luffa acutangula ROXB. (Cucurbitaceae) is a vine which is cultivated in the tropical and subtropical Asian region from India to the southern islands of Japan. The young fruits are eaten as a vegetable. The fruits are used in Ayurvedic medicine as an anthelmintic, stomachic and antipyretic, and the seeds are also used as an emetic and an expectorant.[1] The chemical constituents of this plant were first investigated by Barua *et al.*,[2] and they reported the isolation of cucurbitacin B and an oleanolic acid saponin from the seeds. However, the structure of the saponin has not been characterized. Takemoto *et al.* isolated tens of saponins from the herb of *Luffa cylindrica* ROEM.,[3] and we also isolated twelve triterpenoid saponins from the herb of *Luffa operculata* COGN.[4] The herb of *Luffa acutangula* was also expected to contain saponins, and our preliminary investigation indicated the presence of several kinds of triterpenoid saponins in the herb.

This paper deals with the isolation of seven saponins, named acutosides A—G, from the herb, and determination of their structures.

The dried herb was percolated with 50% MeOH and the aqueous solution after evaporation of MeOH was passed through a highly porous polymer Diaion HP 20 column. The saponin fraction was obtained by elution with MeOH after washing the column with water. The saponin fraction was repeatedly chromatographed on normal-phase and reversed-phase materials and seven saponins were isolated in a homogeneous state. They were designated as acutosides A—G in the order of increasing polarity. The physical data and the nuclear magnetic resonance (NMR) data are summarized in Tables I, II and III.

Acutoside A (I) showed an $[M+Na]^+$ ion at m/z 803 in the positive fast-atom bombardment mass spectrum (FAB-MS), and the molecular formula $C_{42}H_{68}O_{13}$ was obtained by high-resolution FAB-MS. Compound I gave D-glucose and oleanolic acid on acid hydrolysis, and the structure of I was determined to be oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside by examination of the ^{1}H- and ^{13}C-NMR spectra of the methyl ester (II) of I. The spectral basis for determination of the structure of the sugar moiety is shown in Table IV.

Acutoside B (III), $C_{58}H_{94}O_{25}$, gave oleanolic acid, D-glucose, L-rhamnose, L-arabinose and D-xylose on acid hydrolysis, and the molecular weight (1190) and formula indicated that III is an oleanolic acid glycoside having 2 mol of D-glucose and 1 mol each of L-rhamnose, L-arabinose and D-xylose.

The ^{1}H-NMR signal of III at δ 6.45 (d, J=3 Hz) and the ^{13}C-NMR signals at δ 93.5 (the signals of the anomeric H and C of the ester-linked sugar) and δ 89.0 (C_3 of oleanolic

TABLE I. Physical Constants and MS Data of Acutosides

Acutoside		mp	$[\alpha]_D^{24}$	FAB-MS		Formula[c]
				Negative[a]	Positive[b]	
A (I)	Amorphous powder	265—270 °C (dec.)	36.5° (c=1.0, MeOH)	779	803.4543	$C_{42}H_{68}O_{13}$
B (III)	Amorphous powder	225—250 °C (dec.)	−18.3° (c=1.0, 80% MeOH)	1189	1213.5980	$C_{58}H_{94}O_{25}$
C (VI)	Amorphous powder	220—225 °C (dec.)	−15.5° (c=0.5, 80% MeOH)	1205	1229.5920	$C_{58}H_{94}O_{26}$
D (VII)	Amorphous powder	260—265 °C (dec.)	−21.4° (c=1.0, 80% MeOH)	1321	1345.6400	$C_{63}H_{102}O_{29}$
E (X)	Colorless needles	246—251 °C	−14.2° (c=1.0, 80% MeOH)	1321	1345.6390	$C_{63}H_{102}O_{29}$
F (XIII)	Amorphous powder	215—223 °C (dec.)	−25.3° (c=1.0, 80% MeOH)	1321	1345.6410	$C_{63}H_{102}O_{29}$
G (XVI)	Amorphous powder	250—252 °C (dec.)	−22.5° (c=1.0, H_2O)	1453	1477.6810	$C_{68}H_{110}O_{33}$

a) $[M-H]^-$ ion. b) $[M+Na]^+$ ion. Errors are within 2 mmu. c) Calculated by high-resolution FAB-MS measured by adding NaI.

Table II. NMR Chemical Shifts of Acutosides (Aglycone Moieties)

	Acut. A (I)		Acut. B (III)		Acut. C (VI)	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	*ca.* 0.85, 1.45	38.7	*ca.* 0.85, 1.40	38.8	*ca.* 0.85, 1.45	38.8
2	*ca.* 1.80, 2.19	26.6	*ca.* 1.80, 2.19	26.6	*ca.* 1.80, 2.19	26.6
3	3.29 (dd, 4, 12)	89.0	*ca.* 3.27	89.0	3.35 (dd, 4, 12)	89.0
4	—	39.5	—	39.5	—	39.5
5	0.75 (d, 11)	55.8	0.73 (d, 11)	55.8	0.75 (d, 12)	55.9
6	*ca.* 1.50	18.5	*ca.* 1.50	18.5	*ca.* 1.52	18.6
7		33.2		33.2	*ca.* 1.45	33.2
8	—	39.7	—	39.9	—	39.8
9	1.62 (t, 9)	48.0	*ca.* 1.60	48.0	*ca.* 1.60	48.0
10	—	36.9	—	36.9	—	37.0
11	1.91 (dd, 3, 9)	23.7	*ca.* 1.87	23.8	*ca.* 1.89	23.8
12	5.48 (t, 3)	122.5	5.45 (t, 4)	122.9	5.50 (t, 3)	122.9
13	—	144.9	—	144.2	—	143.4
14	—	42.0	—	42.1	—	42.2
15	*ca.* 1.20, 2.16	28.3		28.3	*ca.* 1.28, 2.03	28.5
16		23.7		23.2	*ca.* 2.19	24.6
17	—	46.7	—	47.3	—	49.2
18	3.29 (dd, 4, 12)	42.2	*ca.* 3.27	41.7	3.43 (dd, 4, 14)	41.4
19	*ca.* 1.30, 1.80	46.5	*ca.* 1.23, 1.80	46.3	*ca.* 1.44, 2.02	47.1
20	—	30.9	—	30.9	—	36.8
21	*ca.* 1.22, 1.45	34.2	*ca.* 1.17, 1.35	34.1	*ca.* 3.90	72.4
22		33.2		33.2	2.31 (d, 8)	41.1
23	1.30 s	28.2	1.29 s	28.2	1.29 s	28.2
24	1.10 s	16.8	1.10 s	16.8	1.10 s	16.8
25	0.84 s	15.4	0.84 s	15.5	0.85 s	15.5
26	1.00 s	17.4	1.06 s	17.4	1.25 s	17.7
27	1.30 s	26.2	1.27 s	26.0	1.29 s	25.9
28	—	180.2	—	176.2	—	175.4
29	0.96 s	33.2	0.93 s	33.1	1.25 s	29.7
30	1.02 s	23.7	0.99 s	23.7	1.06 s	17.4

The NMR chemical shifts of acutosides D (VII), E (X), F (XIII) and G (XVI) are almost the same as those of III and the chemical shifts are not shown.

acid) indicated that III is a 3,28-*O*-bisdesmoside of oleanolic acid. The selective cleavage of the ester-linked sugar moiety according to Ohtani's method[5] provided an anomeric mixture of a methyl glycoside and a prosapogenin, which was proved to be identical with I. The anomers of the methyl glycoside were separated by high performance liquid chromatography (HPLC). The faster-eluting anomer (IV) showed an $[M-H]^-$ ion at *m/z* 441, fragment ions at *m/z* 309 (441 − pentose) and *m/z* 163 (309 − rhamnose) in the negative FAB-MS, indicating that IV is a methyl pentosyl–rhamnosyl–pentoside. By the close examination of the NMR spectra, IV was presumed to be methyl *O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside and the other anomer (V), to be the *β*-anomer. The identity was established by comparison of the NMR data with those of a sample obtained from foetidissimoside isolated from the herb of *Aster tataricus* L.f. (Compositae).[6] The assignments of the ^1H- and ^{13}C-NMR signals are shown in Table V.

The structure of III is, therefore, 3-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]-oleanolic acid 28-[*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl] ester. The configuration and conformation of the ester-linked arabinopyranosyl group in III were presumed to be *α*- and $^1C_4$ based on the $J_{H1,H2}$ (3 Hz) and $J_{C1,H1}$ (169 Hz) values[7] and splitting pattern (dd, $J=3$, 5 Hz) of the C_2-H signal (δ 4.55) which was observed by using the decoupling difference spectroscopy technique, irradiating at the frequency (δ 6.45) of the anomeric proton signal.

Table III. The NMR Signal Assignments of the Ester-linked Sugar Moieties of Acutosides

Sugar		B (III)		C (VI)		D (VII)		E (X)		F (XIII)		G (XVI)	
		^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
28-*O*-Ara[1]	1	6.45 (d, 3)	93.5	6.45 (d, 3)	93.6	6.48 br s	93.3	6.48 br s	93.3	6.52 (d, 2)	93.3	6.53 (d, 2)	93.2
	2	4.55 (dd, 3, 5)	75.1	*ca.* 4.55	75.1	*ca.* 4.53	75.2	*ca.* 4.54	75.2	*ca.* 4.55		*ca.* 4.52	75.3
	5	*ca.* 3.90, 4.50	63.1	*ca.* 3.90, 4.50	63.2	*ca.* 3.90, 4.50	62.8		62.8	*ca.* 3.90, 4.50	62.5	*ca.* 3.90, 4.50	62.3
Rha(1—2A[1])	1	5.78 s	101.0	5.78 s	101.0	5.71 (d, 1)	100.9	5.73 br s	100.9	5.71 (d, 1)	100.6	5.66 br s	100.7
	2	*ca.* 4.57				*ca.* 4.51		*ca.* 4.54		4.77 (dd, 1, 3)	71.5	4.76 (dd, 1, 2)	71.4
	3									4.59 (dd, 3, 10)	82.3	*ca.* 4.57	82.3
	4	*ca.* 4.35	84.3	*ca.* 4.35	84.2	*ca.* 4.35	83.8	*ca.* 4.35	83.9	*ca.* 4.51	78.3	*ca.* 4.50	
	5	*ca.* 4.38	68.5	*ca.* 4.39	68.5	*ca.* 4.32	68.4	*ca.* 4.40	68.4	*ca.* 4.44	68.7	*ca.* 4.40	68.6
	6	1.78 (d, 6)	18.3	1.78 (d, 6)	18.3	1.74 (d, 5)	18.3	1.74 (d, 5)	18.3	1.79 (d, 6)	18.6	1.74 (d, 6)	18.6
Xyl[1](1—4R)	1	5.10 (d, 7)	107.2	5.10 (d, 7)	107.1	5.13 (d, 7)	106.4	5.09 (d, 8)	106.4	5.14 (d, 7)[a]	105.8	5.11 (d, 7)[a]	105.7
	2	*ca.* 4.03		*ca.* 4.03		*ca.* 4.02		*ca.* 3.90		*ca.* 3.90			
	3					*ca.* 4.03	87.0	*ca.* 4.05	86.3			*ca.* 4.05	86.7
	5	3.50 (t, 11), *ca.* 4.21	67.4	3.50 (t, 11), *ca.* 4.20	67.4	*ca.* 3.45, *ca.* 4.20 [a]	66.8[a]	3.43 (t, 11), *ca.* 4.20	66.8	3.40 (t, 10), *ca.* 4.14 [b]	67.1[b]	3.43 (t, 12), *ca.* 4.08 [b]	67.0[b]
Xyl[2](1—3X[1])	1					5.20 (d, 8)	105.9	—	—	—	—	—	—
	2					*ca.* 4.02		—	—	—	—	—	—
	5					3.66 (t, 11), *ca.* 4.30 [a]	67.2[a]	—	—	—	—	—	—
Ara[2](1—3X[1])	1							5.21 (d, 7)	105.5	—	—	—	—
	2							*ca.* 4.50		—	—	—	—
	5							3.77 (t, 11), *ca.* 4.30	67.1	—	—	—	—
Xyl[3](1—3R)	1									5.41 (d, 8)[a]	105.2[a]	5.45 (d, 8)[a]	104.6[a]
	2									*ca.* 3.90		*ca.* 3.90	
	5									3.44 (t, 11), *ca.* 4.06 [b]	67.0[b]	3.38 (t, 12), *ca.* 4.12 [b]	66.5[b]
Ara[3](1—3X[1])	1											5.17 (d, 7)	105.5
	5											3.51 (dd, 1, 12), *ca.* 4.20	66.7

The chemical shifts of the signals which were difficult to assign or ambiguous are not shown. *a*, *b*) The signals with the same superscripts in each column may be interchanged.

Acutoside C (VI) showed an $[M+Na]^+$ ion at *m/z* 1229, and an $[M-H]^-$ ion at *m/z* 1205 in the FAB-MS and the high-resolution FAB-MS gave the molecular formula $C_{58}H_{94}O_{26}$ for VI, having one more oxygen atom than III. On acid hydrolysis, VI gave D-glucose, L-rhamnose, L-arabinose and D-xylose, and the thin layer chromatography (TLC) showed a spot of an aglycone which is a little more polar than oleanolic acid. The ^{1}H- and ^{13}C-NMR spectra of the sugar moiety were almost superimposable on those of III, suggesting that VI is a glycoside of a hydroxy-oleanolic acid which has the same sugar moiety as that of III. The ^{1}H- and ^{13}C-NMR spectra of the aglycone moiety of VI were examined, and the data were compared with those of the reported compounds. The ^{13}C-NMR chemical shifts of the aglycone moiety were almost the same as those of lucyoside C (21β-hydroxyoleanolic acid 3,28-*O*-bis-β-D-glucopyranoside) isolated from the herb of *Luffa cylindrica* ROEM. by Takemoto *et al.*[3a] Therefore, acutoside C was concluded to be 3-*O*-[*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]machaelinic acid 28-[*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.

Acutoside D (VII), $C_{63}H_{102}O_{29}$, gave oleanolic acid, D-glucose, L-rhamnose, L-arabinose and D-xylose on acid hydrolysis. The molecular weight (1322) indicated that the sugar moiety is composed of 2 mol of D-glucose, 1 mol of L-rhamnose and 3 mol of pentose, and the ^{1}H- and ^{13}C-NMR spectra suggested that VII is an oleanolic acid 3,28-*O*-bisdesmoside. On selective cleavage of the ester-linked sugar moiety, a prosapogenin and an anomeric mixture of a methyl glycoside were obtained. The former was proved to be identical with acutoside A (I). The latter was separated by HPLC on a reversed-phase material to give two anomers (VIII and IX) of the methyl glycoside. The faster-eluting anomer (VIII) showed an $[M+Na]^+$ ion at *m/z* 597 in the positive FAB-MS and the negative FAB-MS showed ions at *m/z* 573 ($[M-H]^-$), 441 (573−pentose), 309 (441−pentose) and 163 (309−rhamnose). This fragmentation pattern indicated that the sugar sequence in VIII is either a linear CH_3O–pentose(1)–rhamnose–pentose(2)–pentose(3) or a branched chain CH_3O–pentose(1)–rhamnose(pentose)$_2$.

In order to determine the number of the xylose and arabinose units in VIII, the ^{1}H-NMR spectrum was carefully examined. The ^{1}H-NMR spectrum of VIII showed the signals of the C_5-H (axial) of the pentosyl groups at δ 3.48 (dd, J=10, 11 Hz), 3.67 (dd, J=10, 11 Hz) and 3.68 (dd, J=3, 11 Hz), and the other anomer (IX) showed the

TABLE IV. The NMR Signal Assignments of the Sugar Moiety of II

	Inner glucose ^{1}H	Inner glucose ^{13}C	Outer glucose ^{1}H	Outer glucose ^{13}C
1	4.90 (d, 8)	105.0	5.35 (d, 8)	106.0
2	4.20 (dd, 8, 9)	83.5	4.09 (dd, 8, 9)	77.0
3	4.30 (t, 9)	78.3	4.21 (t, 9)	77.9
4	4.29 (t, 9)	71.7	4.13 (t, 9)	71.6
5	3.9 (m)	78.1[a]	3.9 (m)	77.9[a]
6	4.33 (dd, 6, 12)[b] / 4.52 (dd, 3, 12)[b]	62.9[c]	4.43 (dd, 4, 12)[b] / 4.47 (dd, 3, 12)[b]	62.8[c]

a—*c*) Assignments may be interchanged. The assignment of the anomeric proton signals was performed by examination of the NOE difference spectra. On irradiation at the frequency of δ 4.90, the NOE was observed at the signal of C_3-H of the aglycone.

TABLE V. The NMR Signal Assignments of Methyl Glycosides IV, V, VIII, IX, XI and XII

		IV ^{1}H	IV ^{13}C	V ^{1}H	V ^{13}C	VIII ^{1}H	VIII ^{13}C	IX ^{1}H	IX ^{13}C	XI ^{1}H	XI ^{13}C	XII[c] ^{1}H	XII[c] ^{13}C
OCH_3		3.49 s	56.0	3.39 s	55.1	3.50 s	56.0	3.40 s	55.1	3.50 s	55.9	3.39 s	55.3
Ara[1]	1	4.55 (d, 7)	103.6	5.29 (d, 3)	104.1	4.55 (d, 6)	103.6	5.27 (d, 4)	101.1	4.55 (d, 6)	103.6	5.26 (d, 4)	101.2
	2	4.45 (dd, 7, 8)	76.9	4.56 (dd, 3, 10)	78.6	4.45 (dd, 6, 7)	76.9	4.55 (dd, 4, 9)	78.7	4.44 (dd, 6, 8)	76.8	4.54 (dd, 4, 10)	78.8
	3	4.14 (dd, 8, 3)	69.2	4.46 (dd, 3, 10)	69.1	*ca.* 4.15		4.46 (dd, 4, 9)	69.1	*ca.* 4.14	69.0	4.44 (dd, 4, 10)	69.1
	4	*ca.* 4.17	74.2	4.28 m	70.7			*ca.* 4.29	70.6	*ca.* 4.17	74.4	*ca.* 4.30	70.6
	5	3.67 (d, 10) / 4.20 (dd, 10, 4)	65.9	3.95 (2H, d, 2)	63.5	3.68 (dd, 2, 12) / *ca.* 4.20	65.9	3.96 (2H, d, 2)	63.5	3.67 (d, 10) / *ca.* 4.20	65.9	3.96 (2H, br s)	63.7
Rha(1—2A[1])	1	5.94 (d, 1)	102.1	5.61 s	101.1	5.93 (d, 2)	102.1	5.60 br s	101.1	5.94 (d, 1)	102.1	5.59 (d, 2)	104.3
	2	4.67 (dd, 1, 3)	72.0	4.63 br s	71.7	4.67 (dd, 2, 4)	72.0	4.55	71.7	4.66 (dd, 1, 3)	72.0	4.64 (dd, 2, 3)	71.7
	3	4.64 (dd, 3, 9)	72.8	4.63 br s	72.7	4.61 (dd, 4, 9)	72.7	4.55	72.6	4.60 (dd, 3, 10)	72.7	4.58 (dd, 3, 9)	72.8
	4	4.34 (t, 9)	84.8	4.32 (t, 9)	84.5	4.34 (t, 9)	84.3	*ca.* 4.35	84.0	4.33 (t, 10)	84.4	*ca.* 4.30	84.0
	5	*ca.* 4.56	67.9	*ca.* 4.40 m	68.1	*ca.* 4.54	67.8	*ca.* 4.35	68.0	*ca.* 4.44	67.8	*ca.* 4.30	68.2
	6	1.69 (d, 6)	18.2	1.67 (d, 6)	18.4	1.64 (d, 6)	18.2	1.63 (d, 6)	18.4	1.64 (d, 6)	18.2	1.60 (d, 5)	18.6
Xyl[1](1—4R)	1	5.12 (d, 7)	107.2	5.11 (d, 7)	107.1	5.20 (d, 8)[a]	105.8[a]	5.20 (d, 8)[a]	105.9[a]	5.13 (d, 8)	106.4	5.11 (d, 8)	106.2
	2	4.04 (t, 8)	76.1	4.02 (t, 9)	76.1	*ca.* 4.05		*ca.* 4.03		4.02 (t, 8)	75.0	*ca.* 4.00	75.0
	3	4.07 (t, 8)	78.6	4.06 (t, 9)	78.7	*ca.* 4.03	87.1	*ca.* 4.02	87.0	4.05 (t, 8)	86.4	*ca.* 4.05	86.6
	4	*ca.* 4.14	71.0	4.14 m	71.0	*ca.* 4.10				*ca.* 4.04	69.0	*ca.* 4.02	69.1
	5	3.52 (t, 11) / 4.25 (dd, 5, 11)	67.5	3.51 (t, 10) / 4.23 (dd, 10, 5)	67.5	3.48 (dd, 10, 11)[b] / *ca.* 4.25	66.7[b]	3.48 (dd, 9, 11)[b] / 4.23 (dd, 4, 11)[b]	66.8[b]	3.46 (dd, 9, 11) / *ca.* 4.21	66.8	3.45 (dd, 10, 11) / 4.20 (dd, 5, 11)	66.8
Xyl[2](1—3X[1])	1	—	—	—	—	5.16 (d, 7)[a]	106.3[a]	5.14 (d, 7)[a]	106.3[a]				
	2	—	—	—	—	*ca.* 4.05		*ca.* 4.03		—	—	—	—
	3	—	—	—	—					—	—	—	—
	4	—	—	—	—					—	—	—	—
	5	—	—	—	—	3.67 (dd, 10, 11)[b] / *ca.* 4.27	67.3[b]	3.67 (dd, 10, 11)[b] / *ca.* 4.29	67.2[b]	—	—	—	—
Ara[2](1—3X[1])	1	—	—	—	—	—	—	—	—	5.20 (d, 7)	105.5	5.17 (d, 7)	105.5
	2	—	—	—	—	—	—	—	—	4.49 (dd, 7, 9)	72.7	4.50 (dd, 7, 9)	72.6
	3	—	—	—	—	—	—	—	—	4.16 (dd, 9, 3)		4.16 (dd, 3, 9)	74.4
	4	—	—	—	—	—	—	—	—	*ca.* 4.27	69.2	*ca.* 4.30	69.3
	5	—	—	—	—	—	—	—	—	3.77 (dd, 2, 12) / 4.32 (dd, 3, 12)	67.1	3.77 (d, 11) / *ca.* 4.32	67.2

a, b) The signals with the same superscripts in each column may be interchanged. *c*) The spectra were measured in pyridine-d_5–D_2O mixture. Abbreviations: A; arabinopyranosyl, R; rhamnopyranosyl, X; xylopyranosyl. Ara[2](1—3X[1]) means the second arabinopyranosyl group which is linked to the C_3-OH group of the first xylopyranosyl group.

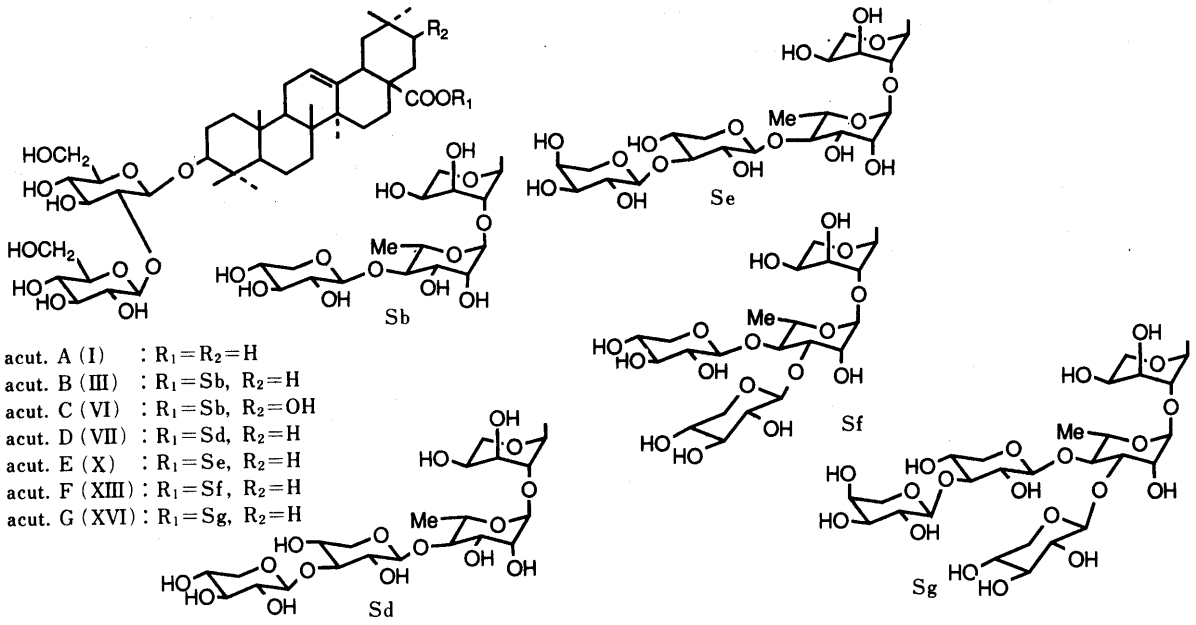


Chart 1

C_5-H signals at δ 3.48 (dd, axial, $J=9$, 11 Hz), 3.67 (dd, axial, $J=10$, 11 Hz) and δ 3.96 (2H, C_5-H_a, C_5-H_e, d, $J=2$ Hz). These signal patterns clearly indicate that VIII and IX have 1 mol of arabinose and 2 mol of xylose as the pentose units.

The identification of the pentose(1) and the determination of the position of the rhamnose linkage were performed by the examination of the ^{1}H- and ^{13}C-NMR spectra of VIII and IX. The ^{1}H-NMR signals of the C_1-H and C_2-H of the pentose (1) were observed at δ 4.55 (d, $J=6$ Hz) and δ 4.45 (dd, $J=6$, 7 Hz), respectively, but the signal of the C_3-H was overlapped by the signals of other sugar units and the splitting pattern could not be observed. On the other hand, the signal of the C_3-H of IX was fortunately separated from others and observed at δ 4.46 as a double doublet ($J=4$, 9 Hz). The splitting pattern of the C_3-H signal of IX clearly indicates that the pentose(1) is L-arabinose.

The chemical shifts of C_2 of the arabinosyl units in VIII and IX were 76.9 and 78.7, respectively, and these chemical shifts are in good agreement with the data for methyl *O*-α-L-rhamnopyranosyl-(1→2)-α- and β-L-arabinopyranosides reported by Mizutani *et al.*[8] Therefore, it is apparent that the rhamnopyranosyl group is linked to the C_2-hydroxyl group of the arabinosyl unit.

The position of the xylose linkage to the rhamnose unit was also determined by examination of the ^{1}H- and ^{13}C-NMR spectra of the rhamnosyl moieties in VIII and IX. Assignments of the ^{1}H- and ^{13}C-NMR signals are shown in Table V.

The ^{1}H-NMR signals of the rhamnosyl group overlapped and clear assignment could not be done in the case of IX. However, signals of the rhamnosyl group in VIII were clearly separated and could be easily assigned. The ^{13}C-NMR chemical shifts were determined from the C–H correlation spectroscopy (COSY) spectrum. As shown in Table V, only the C_4 signals were shifted to lower magnetic field, thus indicating that the xylobiose is linked to the C_4-OH group of the rhamnopyranosyl unit. The last problem on the structure of VIII is the position of the linkage of the terminal xylose unit. The signals of the C_2-H and C_3-H of the xylose units appeared at almost the same region, overlapped by the other sugar signals, and it was difficult to assign the ^{13}C-NMR signal at δ 87.1 to C_2 or C_3. The position of the terminal xylose linkage was unambiguously determined as C_3-OH by gas chromatography-chemical ionization-mass spectrometry (GC-CI-MS) identification of the methyl glycosides of 2,3,4-tri-*O*-methyl D-xylose, 2,4-di-*O*-methyl D-xylose, 3,4-di-*O*-methyl L-arabinose and 2,3-di-*O*-methyl L-rhamnose after methanolysis of the permethylate of IX. From the above-mentioned data, the structure of VIII is concluded to be methyl *O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and IX, to be its β-anomer.

Therefore, the structure of acutoside D (VII) was determined to be as shown in the chart. The configuration and conformation of the esterified L-arabinopyranosyl group were presumed to be α- and $^1C_4$ because the arabinosyl group showed almost the same NMR chemical shifts as those of III.

Acutoside E (X) showed an $[M+Na]^+$ ion at *m/z* 1345 and the high-resolution FAB-MS indicated the molecular formula $C_{63}H_{102}O_{29}$, the same formula as that of VII. The NMR spectra of X suggested that it is also an oleanolic acid 3,28-*O*-bisdesmoside. Compound X gave oleanolic acid, D-glucose, L-rhamnose, L-arabinose and D-xylose on acid hydrolysis, and the selective cleavage of the ester-linked sugar moiety provided acutoside A (I) along with an anomeric mixture of a methyl glycoside. The anomers (XI and XII) were preparatively separated by HPLC. The faster-eluting anomer (XI) showed an $[M-H]^-$ ion at *m/z* 573 and fragment ions at *m/z* 441 (573−pentose), 309 (441−pentose) and 163 (309−rhamnose) in the negative FAB-MS. From this fragmentation pattern, the sugar sequence of XI and the other anomer (XII) is either a linear CH_3O–pentose(1)–rhamnose–pentose(2)–pentose(3) or a branched chain CH_3O–pentose(1)–rhamnose–$(pentose)_2$.

The numbers of arabinose and xylose in XI were

determined by examination of the signal patterns of C_5-H of the pentosyl groups. Compound XI showed a doublet (1H, $J=10$ Hz) at δ 3.67, a double doublet (1H, $J=9$, 11 Hz) at δ 3.46 and a double doublet (1H, $J=2$, 12 Hz) at δ 3.77. These signals are those of the axial hydrogens at C_5 and the splitting patterns showed that XI has 1 mol of D-xylose and 2 mol of L-arabinose as the pentose units.

The pentose(1) was identified as L-arabinose by examination of the ^{1}H-NMR spectrum of XII measured in pyridine-d_5–D_2O mixture. The signal of the anomeric H appeared at δ 5.26 (d, $J=4$ Hz), C_2-H at δ 4.54 (dd, $J=4$, 10 Hz) and C_3-H at δ 4.44 (dd, $J=10$, 4 Hz). The site of rhamnose linkage was determined by examination of the ^{13}C-NMR chemical shifts of the arabinosyl unit. The C_2 signal of XI appeared at δ 76.8 and that of XII appeared at δ 78.8, and these data indicated that the rhamnopyranosyl group is linked to the C_2-OH group of the arabinopyranosyl group.

The position of the linkage of the pentose(2) to the rhamnosyl unit was determined from the ^{13}C-NMR glycosylation shifts of the carbons of the rhamnosyl unit after assignments of the ^{1}H-NMR signals. The results of assignments of ^{1}H- and ^{13}C-NMR signals are shown in Table V.

Only the signal of the C_4 was shifted downfield and this shift showed that the pentobiose is attached to the C_4-OH group of the rhamnosyl unit to form a linear methyl tetraglycoside.

The identification of the pentose(2) and the determination of the position to which the pentose(3) is linked were performed as follows. The ^{13}C-NMR signal at δ 86.4 in the spectrum of XI is correlated to the H signal at δ 4.05 (t, $J=8$ Hz). This proton signal showed in a ^{1}H–^{1}H COSY spectrum a cross peak with the signal at δ 4.02 (t, $J=8$ Hz), which showed a cross peak with the anomeric proton signal at δ 5.13 (d, $J=8$ Hz). These chemical shifts and splitting patterns of the C_1-H, C_2-H and C_3-H unambiguously indicated that the inner pentosyl unit is xylopyranose and, thus, the terminal one is arabinose, which is linked to the C_3-OH group of the xylopyranosyl group. From all the spectral evidence, the structure of XI was indicated to be methyl *O*-α-L-arabinopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, and XII, to be the β-anomer of XI. The GC-CI-MS analysis of the methanolysis product of the permethylate of XII supported the above structure.

The structure of acutoside E (X) is, therefore, elucidated as shown in the chart.

Acutoside F (XIII), $C_{63}H_{102}O_{29}$, gave oleanolic acid, D-glucose, L-rhamnose, L-arabinose and D-xylose on acid hydrolysis, and acutoside A (I) was obtained together with an anomeric mixture of a methyl glycoside on heating XIII in 2,6-lutidine–MeOH mixture with LiI. The methyl glycoside fraction was separated by HPLC to give two anomers (XIV and XV). The faster-eluting anomer (XIV) showed an $[M-H]^-$ ion at m/z 573 and fragment ions at m/z 441, 309 and 163, suggesting that XIV is either a linear methyl tetraglycoside CH_3O–pentose(1)–rhamnose–pentose(2)–pentose(3) or a branched chain methyl tetraglycoside which has two terminal pentose units at the rhamnosyl unit. The ^{1}H-NMR spectrum (Table VI) of XIV showed the signals of the axial hydrogens on the C_5 of pentose units at δ 3.40 (2H, two triplets overlapped, $J=$ *ca.* 9 Hz) and at δ 3.63 (dd, $J=2$, 12 Hz). The ^{1}H-NMR spectrum of XV showed one broad singlet (δ 3.95) equivalent to two protons at C_5, and two triplets at δ 3.18 ($J=11$ Hz) and 3.41 ($J=10$ Hz) as the signals of the axial C_5-H. These signals indicated that XIV has 1 mol of L-arabinose and 2 mol of D-xylose as the pentose units.

In order to identify the pentose(1), the ^{1}H-NMR spectrum of XIV was examined. The anomeric proton signal appeared at δ 4.55 (d, $J=6$ Hz) and the C_2-H signal was assigned at δ 4.47 (dd, $J=6$, 8 Hz) from the ^{1}H–^{1}H COSY spectrum. However, the signal of C_3-H appeared at around δ 4.12 overlapped by signals of other sugar protons and the splitting pattern was not clearly observed. The decoupling difference spectrum was measured by irradiation at the frequency (δ 4.47) of the C_2-H, and the signal of the C_3-H was observed at δ 4.12 as a double doublet with coupling constants of 4 and 8 Hz. This splitting pattern indicates that the pentose(1) is L-arabinose.

The position of the rhamnose linkage was determined as the C_2-OH of the L-arabinosyl unit on the same basis as in the cases of the other methyl glycosides.

In order to determine the position(s) of the linkage(s) of a xylobiose or two xylose units, the NMR spectra of the rhamnosyl group were carefully examined. The assignments of the ^{1}H- and ^{13}C-NMR signals of XIV are summarized in Table VI. The down-field shifts of the C_3 and C_4 signals indicate that the two xylose units are linked to the C_3- and C_4-OH groups to form a branched-chain methyl tetraglycoside. The structures of XIV and XV are, therefore, methyl *O*-β-D-xylopyranosyl-(1→3)-[*O*-β-D-xylopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and its β-anomer, respectively, and the structure of XIII was elucidated as shown in the chart.

Acutoside G (XVI) showed an $[M+Na]^+$ ion at m/z 1477 in the positive FAB-MS, and the high-resolution FAB-MS gave the molecular formula $C_{68}H_{110}O_{33}$. The ^{1}H-NMR spectrum suggested that XVI is also an oleanolic acid 3,28-*O*-bisdesmoside, and it gave oleanolic acid, D-glucose, L-rhamnose, L-arabinose and D-xylose on acid hydrolysis. On selective cleavage of the ester-linked sugar moiety, XVI gave acutoside A(I) and an anomeric mixture of a methyl glycoside. The anomers (XVII and XVIII) were separated by HPLC. The faster-eluting anomer (XVII) showed in the negative FAB-MS an $[M-H]^-$ ion at m/z 705 and fragment ions at m/z 573 (705 − pentose), 441 (573 − pentose), 309 (441 − pentose) and 163 (309 − rhamnose), indicating that XVII is a methyl pentaglycoside and has a similar sugar sequence to those of XI and XIV, having one more pentose unit than XI and XIV.

The numbers of xylose and arabinose units were determined by checking the ^{1}H-NMR signals of C_5-H of the pentose units. Compound XVII showed in its ^{1}H-NMR spectrum the signals of C_5-H at δ 3.40 (2H, t, $J=9$ Hz), δ 3.56 (1H, dd, $J=1$, 12 Hz) and δ 3.63 (1H, dd, $J=1$, 12 Hz). The ^{1}H-NMR spectrum of the other anomer (XVIII) showed two triplets at δ 3.18 ($J=11$ Hz) and δ 3.41 ($J=11$ Hz), one double doublet at δ 3.56 ($J=1$, 12 Hz) and one broad singlet equivalent to two protons at δ 3.94. These signal patterns of C_5-H indicated that XVII and XVIII have 2 mol each of L-arabinose and D-xylose as the pentose units.

Identification of the methyl glycosylated pentose was

performed as follows. The ^{1}H-NMR spectrum of XVII showed an anomeric proton signal at δ 4.54 (d, J=6 Hz) and the C_2-H signal appeared at δ 4.47 (dd, J=6, 8 Hz). The C_2-H signal is overlapped with the signal of the C_2-H of the other pentose, of which the anomeric H appeared at δ 5.16. The decoupling difference spectrum was measured to see the chemical shifts and splitting patterns of the C_3-H, irradiating at the frequency of the C_2-H signal (δ 4.47). The signals of the C_3-H of two pentose units appeared at δ 4.04 and 4.12 as double doublets with coupling constants of 3 and 8 Hz. The splitting pattern of the C_3-H signals indicated that the two pentoses in question are both arabinose.

The position of the rhamnose linkage was determined as the C_2-OH of the methyl arabinosyl unit by the fact that the signal (δ 4.57, dd, J=4, 10 Hz) of C_2-H of the arabinosyl group in XVIII was observed in the nuclear Overhauser effect (NOE) difference spectrum obtained by irradiation at the frequency (δ 5.62) of the anomeric proton of the rhamnosyl group. The chemical shifts of the C_2 signals of arabinosyl groups in XVII and XVIII were in good agreement with Mizutani's data.[8]

Determination of the positions in rhamnose to which the pentose is linked was accomplished by examination of the ^{1}H- and ^{13}C-NMR signals of the rhamnopyranosyl units of XVII and XVIII. The assignments of the ^{1}H- and ^{13}C-NMR signals are shown in Table VI.

The signals of C_3 and C_4 were shifted downfield as seen in the case of XIV. These glycosylation shifts indicate that a pentose unit and a pentobiose unit are linked to the C_3- and C_4-OH groups of the rhamnosyl unit to form a branched sugar chain.

In order to determine the positions of the linkages of one arabinose and two xylose units, the NOE difference spectra of XVIII were measured, irradiating at the frequencies at δ 5.45 [xylose(1)], 5.16 (arabinose) and 4.91 [xylose(2)]. When the signal at δ 5.45 was irradiated, the NOE was observed at a triplet at δ 4.45, and this proton signal was assigned to be that of C_4-H of the rhamnosyl group. On irradiation at the frequency (δ 4.91) of the other xylose anomeric proton, NOE was observed at the signal (δ 4.63, dd, J=3, 9 Hz) which was assigned as the signal of C_3-H of the rhamnosyl group. Therefore, xylose(1) and xylose(2) are linked to the respective C_4-OH and C_3-OH groups of the rhamnosyl unit, and the arabinosyl group is linked to one of the xylosyl units.

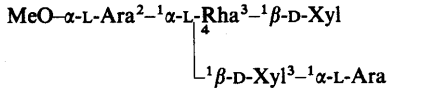
On irradiation at the signal (δ 5.16) of the anomeric proton of the arabinosyl group, the NOE difference spectrum showed a triplet (J=8 Hz) at δ 4.14 which was assigned to one of two C_3-H of xylosyl units. The assignment of the signal could not be done because the two C_2-H signals of the xylose units were very close and there was a risk of miss-assignment. Therefore, the HOHAHA spectra were measured, irradiating at the two xylose anomeric protons. The HOHAHA spectrum obtained by irradiation at the anomeric proton (δ 4.91) of xylose(2), showed the C_2-H signal at δ 3.90 (t, J=8 Hz), the C_3-H signal at δ 3.95 (t,

Table VI. The NMR Signal Assignments of Methyl Glycosides XIV, XV, XVII and XVIII

		XIV		XV		XVII		XVIII	
		^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
MeO		3.50 s	55.8	3.39 s	55.0	3.51 s	55.8	3.40 s	55.0
Ara^1(1—OMe)	1	4.55 (d, 6)	103.5	5.30 (d, 3)	101.1	4.54 (d, 6)	103.5	5.29 (d, 4)	101.0
	2	4.47 (dd, 6, 8)	76.9	4.58 (dd, 3, 10)	78.8	4.47 (dd, 6, 8)	76.9	4.57 (dd, 4, 10)	78.8
	3	4.12 (dd, 4, 8)	70.9	*ca.* 4.45	69.0	4.12 (dd, 3, 8)[a]	70.9	*ca.* 4.48	
	4	*ca.* 4.18		*ca.* 4.26 br s	70.6	*ca.* 4.18		*ca.* 4.27	
	5	3.63 (dd, 2, 12) *ca.* 4.18	66.1	3.95 (2H, br s)	63.5	3.63 (dd, 1, 12)[b] *ca.* 4.18	66.1[b]	3.94 (2H, br s)	63.5
Rha(1—2A^1)	1	5.98 (d, 2)	102.0	5.63 (d, 2)	104.0	5.97 (d, 1)	102.0	5.62 (d, 1)	104.0
	2	4.91 br s	71.8	*ca.* 4.82	71.3	4.90 (dd, 1, 3)	71.8	4.82 (dd, 1, 3)	71.3
	3	4.61 (dd, 3. 9)	82.5	4.65 (dd, 3, 9)	82.4	4.69 (dd, 3, 9)	82.4	4.63 (dd, 3, 9)	82.4
	4	4.54 (t, 9)	78.8	*ca.* 4.46	78.6	4.53 (t, 9)	78.4	4.45 (t, 9)	78.2
	5	*ca.* 4.64 m	68.3	*ca.* 4.48	68.2	*ca.* 4.60	68.0	*ca.* 4.43	68.0
	6	1.69 (d, 6)	18.4	1.66 (d, 6)	18.6	1.64 (d, 6)	18.4	1.61 (d, 6)	18.6
Xyl^1(1—4R)	1	5.45 (d, 8)[a]	105.2[a]	5.41 (d, 8)[a]	105.2[a]	5.51 (d, 8)	104.5	5.45 (d, 8)	104.5
	2							3.95 (t, 8)	
	3						86.8	4.14 (t, 8)	86.8
	4							4.05 m	
	5	3.40 (t, 9) *ca.* 4.10	67.1	3.41 (t, 10) 4.18 (dd, 5, 10)[b]	66.9[b]	3.40 (t, 9)[c] *ca.* 4.15	66.4[c]	3.41 (t, 11) 4.16 (dd, 5, 11)	66.4
Xyl^2(1—3R)	1	5.31 (d, 8)[a]	105.8[a]	4.94 (d, 7)[a]	105.5[a]	5.18 (d, 7)	105.8	4.91 (d, 8)	105.5
	2							3.90 (t, 8)	75.3
	3							3.95 (t, 8)	
	4							*ca.* 4.05 m	
	5	3.40 (t, 9) *ca.* 4.10	67.1	3.18 (t, 11)[b] *ca.* 3.88	67.1[b]	3.40 (t, 9)[c] *ca.* 4.10	67.0[c]	3.18 (t, 11) *ca.* 3.95	66.8
Ara^2(1—3X^1)	1	—	—	—	—	5.16 (d, 7)	105.5	5.16 (d, 6)	105.4
	2	—	—	—	—	4.47 (dd, 7, 8)	72.7	*ca.* 4.48	
	3	—	—	—	—	4.04 (dd, 3, 8)[a]			
	4	—	—	—	—	*ca.* 4.21		*ca.* 4.20	
	5	—	—	—	—	3.56 (dd, 1, 12)[b] *ca.* 4.21	66.8[b]	3.56 (dd, 1, 12) *ca.* 4.20	66.8

The NMR spectra were taken in pyridine-d_5 and the chemical shifts of the signals which were difficult to assign or ambiguous are not shown. a—c) The signals with the same superscripts in each column may be interchanged. Abbreviations: A, arabinopyranosyl; R, rhamnopyranosyl; X, xylopyranosyl. Ara^2(1—3X^1) means the second arabonopyranosyl group which is linked to the C_3-OH group of the first xylopyranosyl group.

$J=8$ Hz) and the C_4-H signal at δ 4.05 (m), and on irradiation at δ 5.45 [the anomeric proton signal of xylose(1)], the signals of the C_2-H, C_3-H and C_4-H appeared at δ 3.95 (t, $J=8$ Hz), δ 4.14 (t, $J=8$ Hz) and δ 4.05 (m), respectively. The triplet at δ 4.14 is the signal of C_3-H of the xylose (1) which is linked to the C_4-OH group of the rhamnosyl unit, and from all the above-mentioned spectral evidence, the structure of XVIII was determined to be



and XVII is the β-anomer of XVIII. The GC-CI-MS analysis of the component methylated sugars of the permethylates of XVII supported the above sugar sequence.

Thus, the structure of XVI was elucidated as shown in the chart.

Our preliminary examination of the saponin constituents in the seeds of this plant has indicated the presence of some oleanolic acid glucuronide saponins. The isolation and structures of these saponins will be reported in the next paper.

Experimental[9]

Extraction, Fractionation and Isolation of Acutosides from the Herb *Luffa acutangula* Roxb. was cultivated in the herbal garden of this Faculty and the herb was harvested in August, 1988. The dried and powdered herb (1 kg) was percolated with 50% MeOH (15 l). MeOH was evaporated off *in vacuo*, and the aqueous solution was filtered. The filtrate was passed through a column (1 l) of Diaion HP 20. After being washed with water (10 l), the column was washed with MeOH (10 l). The MeOH solution was concentrated *in vacuo* to give a saponin-containing fraction (fr. I, 29 g). Fraction I (29 g) was repeatedly chromatographed on silica gel using the solvent systems $CHCl_3$–MeOH–H_2O (15:4:0.5, 15:6:0.5, 15:6:1) and AcOEt–MeOH–H_2O (8:1:0.5, 8:2:0.5, 8:2:1, 6:2:1) and fractionated into seven fractions (fr. Ia—Ig).

Fraction Ia (820 mg) was chromatographed on an octadecyl silica (ODS) column (Fuji gel, 25 cm × 3 cm i.d.) using 85% MeOH as an eluent to give acutoside A (I) (365 mg). Fraction Ib (2.1 g) gave acutoside B (III, 1.56 g) by the same chromatography. Fraction Ic (258 mg) was chromatographed on the same Fuji gel column using 60% MeOH to give acutoside C (VI) (90 mg). Fraction Id (514 mg) was chromatographed on silica gel using AcOEt–MeOH–H_2O (8:2:0.3) to give acutoside D (VII) (311 mg). Fraction Ie (7 g) was almost pure acutoside E (X). Fraction If (3 g) contained a major amount of X and a minor amount of acutoside F (XIII). This fraction was chromatographed on an ODS column using 65% MeOH and 67.5% MeOH to give another crop of X (1.31 g) and XIII (308 mg). Fraction Ig (1.5 g) was almost pure acutoside G (XVI) with a small amount of other contaminants. This fraction was chromatographed on an ODS column using 65% MeOH as an eluant to give XVI (1.30 g). Acutoside E was obtained as colorless needles, but the others could not be crystallized and were obtained as amorphous white powders. The physical and analytical data are shown in Table I. The NMR data are summarized in Tables II and III.

Acid Hydrolysis of Acutosides, Identification of the Aglycone and Sugars The saponin (2 mg) was dissolved in 1 N HCl–MeOH and the solution was refluxed for 2 h. After neutralization with Ag_2CO_3 and filtration, the filtrate was bubbled through with H_2S, and the solvent was evaporated off. The residue was checked by TLC (silica gel, $CHCl_3$–MeOH, 95:5) for the aglycone. The remainder of the methanolysis product was treated with trimethylsilylimidazole and checked by gas-liquid chromatography (GLC) for the component sugars. Methyl glycosides were identified by comparison of the t_R values with those of the methanolysates of authentic sugar samples.

The absolute configurations of the sugars were determined in the same way as described in the previous paper from this laboratory.[10] The GLC conditions are as follows: column, Shimadzu capillary column HiCap (0.25 mm i.d. × 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 sugars identified for all acutosides are described in the text.

Selective Cleavage of the Ester-linked Sugar Moiety Acutoside B (III) (509 mg) and LiI (566 mg) were added to a mixture of 2,6-lutidine (5 ml) and dry MeOH (3 ml). The mixture was heated at 160 °C for 10 h, and allowed to cool. Then 50% MeOH (10 ml) was added and the diluted solution was passed through a column of Amberlite MB-3 (20 ml). The eluate was concentrated. The residue was suspended in water and chromatographed on a column of Diaion HP 20 (20 ml). The methyl glycoside fraction (278 mg) was eluted first with 30% MeOH (30 ml) and the prosapogenin fraction (385 mg) was eluted next with MeOH. The prosapogenin fraction was purified by chromatography on silica gel using $CHCl_3$–MeOH–H_2O (32:8:1) to give a thin-layer-chromatographically homogeneous prosapogenin (174 mg). The ^{1}H- and ^{13}C-NMR spectra were the same with those of acutoside A (I). The methyl glycoside fraction (278 mg) was chromatographed on an ODS column using 10% MeOH as an eluent to give 122 mg of a mixture of anomers, which were separated by HPLC [Shiseido Capcell Pak C18, 10% MeOH] to give an α-anomer (IV) (40 mg) and a β-anomer (V) (72 mg).

The same treatment of acutosides D (VII), E (X), F (XIII) and G (XVI) gave I as the prosapogenin, and VIII (11 mg) and IX (25 mg) (from 129 mg of VII), XI (36 mg) and XII (64 mg) (from 500 mg of X), XIV (9 mg) and XV (19 mg) (from 103 mg of XIII) and XVII (15 mg) and XVIII (28 mg) (from 524 mg of XVI) as the methyl glycosides.

IV: Amorphous powder. $[\alpha]_D^{26}$ −50.5° ($c=1.00$, MeOH). Negative FAB-MS *m/z*: 441 ($[M-H]^-$), 309 and 163.

V: Amorphous powder. $[\alpha]_D^{26}$ +39.3° ($c=0.90$, MeOH).

VIII: Amorphous powder. $[\alpha]_D^{26}$ −48.9° ($c=0.55$, MeOH). Negative FAB-MS *m/z*: 573 ($[M-H]^-$), 441, 309 and 163.

IX: Amorphous powder. $[\alpha]_D^{26}$ +13.9° ($c=0.95$, MeOH).

XI: Amorphous powder. $[\alpha]_D^{26}$ −38.3° ($c=1.10$, MeOH). Negative FAB-MS *m/z*: 573 ($[M-H]^-$), 441, 309 and 163.

XII: Amorphous powder. $[\alpha]_D^{26}$ +29.6° ($c=1.15$, MeOH).

XIV: Amorphous powder. $[\alpha]_D^{26}$ −58.0° ($c=0.45$, MeOH). Negative FAB-MS *m/z*: 573 ($[M-H]^-$), 441, 309 and 163.

XV: Amorphous powder. $[\alpha]_D^{26}$ +12.5° ($c=0.80$, MeOH).

XVII: Amorphous powder. $[\alpha]_D^{26}$ −41.7° ($c=0.65$, MeOH). Negative FAB-MS *m/z*: 705 ($[M-H]^-$), 573, 441, 309 and 163.

XVIII: Amorphous powder. $[\alpha]_D^{26}$ +10.3° ($c=1.45$, MeOH).

Methylation of Methyl Glycosides and Identification of the Component Methylated Sugars The major one of two anomers was used for analysis. Methylation was performed according to the method reported by Hakomori.[11]

The dimethyl sulfinyl carbanion solution (0.5 ml) (prepared from 30 mg of NaH in 1 ml of dimethylsulfoxide (DMSO) was added to the methyl glycoside (*ca.* 10 mg) and stirred at room temperature for 10 min. CH_3I (1 ml) was added to the solution and the mixture was stirred for 24 h. The reaction mixture was diluted with $CHCl_3$ (3 ml) and washed twice with water (3 ml). The organic solvent layer was dried over Na_2SO_4 and filtered. After evaporation of the solvent, the residue was chromatographed on silica gel (benzene–acetone, 4:1) to give a thin-layer-chromatographically homogeneous methylation product (*ca.* 10 mg). The product was dissolved in 1 N HCl–MeOH (0.5 ml) and the solution was refluxed for 1 h. The acid was neutralized with Ag_2CO_3, the precipitate was filtered off, and the filtrate was bubbled through with H_2S and concentrated to dryness. The methanolysate was acetylated in a usual manner and the acetylation product was analyzed by GC-CI-MS. Conditions were as follows.

Conditions for GC: Column, 2% OV-17 on Chromosorb AW DMCS glass column (1 m × 3 mm i.d.); column temperature, 130→190 °C (3 °C/min); carrier gas, He 20 ml/min.

Conditions for CI-MS: Reagent gas, isobutane; scan number, 1—300 (t_R 1.00—20.00); mass range, *m/z* 100—400; scan speed, 4 s (*m/z* 100—400); ion source temperature, 270 °C; ionization voltage, 150 eV; box current, 200 μA; ion accelerating voltage, 3.5 kV.

The permethylated and partially methylated and acetylated methyl glycosides identified by GC-CI-MS were as follows. Numbers in parentheses are retention times in minutes.

V: 2,3,4-Tri-*O*-methyl-β-D-xylopyranoside (2,3,4-M-β-Xyl) (1.2), 2,3,4-M-α-Xyl (1.6), 2,3-M-4-A-α-Rha (4.9), 3,4-M-2-A-α-Ara (6.0).

IX: 2,3,4-M-β-Xyl (1.2), 2,3,4-M-α-Xyl (1.6), 2,4-M-3-A-β-Xyl (4.5), 2,3-M-4-A-α-Rha (4.9), 2,4-M-3-A-α-Xyl (5.8), 3,4-M-2-A-α-Ara (6.0).

XII: 2,3,4-M-α-Ara (2.6), 2,4-M-3-A-β-Xyl (4.5), 2,3-M-4-A-α-Rha (4.9), 2,4-M-3-A-α-Xyl (5.8), 3,4-M-2A-α-Ara (6.0).

XV: 2,3,4-M-β-Xyl (1.2), 2,3,4-M-α-Xyl (1.6), 3,4-M-2-A-α-Ara (6.0), 2-M-3,4-A-α-Rha (7.5).

XVIII: 2,3,4-M-β-Xyl (1.2), 2,3,4-M-α-Xyl (1.6), 2,3,4-M-α-Ara (2.6), 2,4-M-3-A-β-Xyl (4.5), 2,4-M-3-A-α-Xyl (5.8), 3,4-M-2-A-α-Ara (6.0), 2-M-3,4-A-α-Rha (7.5).

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References and Notes

1) K. S. Grewal and B. D. Kochhar, *Indian J. Med. Research*, **31**, 63 (1943).
2) A. K. Barua, S. K. Chakraborti and A. K. Ray, *J. Indian Chem. Soc.*, **35**, 480 (1958).
3) *a*) T. Takemoto, S. Arihara, K. Yoshikawa, K. Kusumoto, T. Yano and T. Hayashi, *Yakugaku Zasshi*, **104**, 246 (1984); *b*) T. Takemoto, S. Arihara, K. Yoshikawa, R. Tanaka and T. Hayashi, *ibid.*, **105**, 834 (1985).
4) K. Kusumoto, T. Nagao, H. Okabe and T. Yamauchi, *Chem. Pharm. Bull.*, **37**, 18 (1989); H. Okabe, T. Nagao, S. Hachiyama and T. Yamauchi, *ibid.*, **37**, 895 (1989).
5) K. Ohtani, K. Mizutani, R. Kasai and O. Tanaka, *Tetrahedron Lett.*, **25**, 4537 (1984).
6) R. Tanaka, T. Nagao, H. Okabe and T. Yamauchi, *Chem. Pharm. Bull.*, **38**, 1153 (1990).
7) K. Bock and C. Pederson, *J. Chem. Soc., Perkin Trans. 2*, **1974**, 293.
8) K. Mizutani, A. Hayashi, R. Kasai, O. Tanaka, N. Yoshida and T. Nakajima, *Carbohydr. Res.*, **126**, 177 (1984).
9) The instruments and materials used in this work were as follows. JASCO DIP-360 digital polarimeter (rotations), JEOL JNM GX-400 spectrometer (100 MHz for ^{13}C-NMR spectra and 400 MHz for ^{1}H-NMR spectra), JEOL DX-300 and HX-110 mass spectrometer (MS), Shimadzu gas chromatograph GC-8A (GC of sugar derivatives), Shimadzu Auto GCMS-6020 with GC-MSPAC 500 FDG data analyzer (GC-CI-MS of the methylated sugar derivatives), Kieselgel 60 (63—210 μm, E. Merck), Diaion HP 20 (Mitsubishi Chemical Industries Ltd.), Fuji gel prepacked ODS column (30 cm × 2.5 cm i.d.) (Wako Pure Chemical Industries, Ltd.), YMC Gel (ODS, 230/70 mesh, Yamamura Chemical Laboratories Co., Ltd.), precoated Kieselgel 60 F_{254} plate, RP-18 F_{254}S plate (E. Merck), Capcell Pak C18 column (25 cm × 1 cm i.d.) (Shiseido Co., Ltd.). ^{1}H- and ^{13}C-NMR spectra were measured in pyridine-d_5 or pyridine-d_5 containing D_2O and chemical shifts were expressed on the δ scale using tetramethylsilane as an internal standard. The FAB-MS were obtained in a glycerol and/or thioglycerol matrix.
10) S. Hara, H. Okabe and K. Mihashi, *Chem. Pharm. Bull.*, **35**, 501 (1987).
11) S. Hakomori, *J. Biochem.*, **55**, 255 (1964).