Studies on the Constituents of *Aster tataricus* L. f. IV.[1] Structures of Aster Saponins Isolated from the Herb

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Five echinocystic acid glucuronide saponins, aster saponins Ha, Hb, Hc, Hd and foetidissimoside A (=kirengeshomasaponin I), were isolated from the ground part of *Aster tataricus* L. f. (Compositae) as their methyl esters, and their structures were determined based on spectral evidence and chemical degradation. These saponins have a common prosapogenin structure, echinocystic acid-3-*O*-glucopyranosiduronic acid, and differ in the structures of the 28-*O*-linked sugar moieties.

Aster saponin Ha is a 28-[α-L-arabinopyranosyl]ester, Hb, a 28-[*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester Hc, a 28-[*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester and Hd, a 28-[*O*-β-D-xylopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-[*O*-β-D-apiofuranosyl-(1→3)]-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester of the prosapogenin.

Keywords *Aster tataricus*; Compositae; aster saponin; triterpene glycoside; echinocystic acid; 3β,16α-dihydroxyolean-12-en-28-oic acid; glucuronide saponin; foetidissimoside A; kirengeshomasaponin I

As a continuation of the chemical investigation of the saponin constituents of *Aster tataricus* L. f. (Compositae), the ground part of the plant was investigated. The glycoside fraction of the MeOH and 50% MeOH extracts showed five spots on a thin-layer chromatography (TLC) plate which stained reddish violet on heating after spraying of 10% sulfuric acid. The saponin fraction was treated with an ion-exchange resin, Amberlite IRC-84, and the acidic product was converted into the methyl ester with CH_2N_2. The methyl ester was chromatographed on silica gel and a reversed-phase material to give the saponin methyl esters (I—V, in order of increasing polarity). This paper deals with their structures.

Compound III was obtained as colorless needles, and it showed in the fast atom bombardment mass spectrum (FAB-MS) an $[M+Na]^+$ ion at *m/z* 1095. The results of elemental analysis were consistent with the molecular formula $C_{53}H_{84}O_{22}\cdot 2H_2O$. Compound III gave L-arabinose, L-rhamnose, D-xylose and D-glucuronic acid on acid hydrolysis.[2]

The ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectrum of III showed signals of seven tertiary methyl groups (δ 0.86, 0.97, 1.02, 1.08, 1.14, 1.28 and 1.80), one secondary methyl group [δ 1.74 (d, *J*=6 Hz)], one trisubstituted olefinic proton (δ 5.61, br dd) and four anomeric protons [δ 6.44 (d, *J*=3 Hz), 5.78 (br s), 5.15 (d, *J*=7 Hz), 4.97 (d, *J*=8 Hz)]. The ^{13}C-NMR spectrum (Table I) showed signals of six C–C bonded quaternary carbons (δ 30.8, 37.0, 39.5, 40.0, 42.0 and 49.5), two ester carbonyl carbons (δ 170.7 and 175.8), a pair of olefinic carbons (δ 122.7 and 144.4) and four anomeric carbons (δ 93.6, 101.0, 106.8 and 107.1).

The numbers and chemical shifts of the tertiary methyl groups and quaternary carbons suggested that the aglycone is an oleanane-type triterpene and the ^{1}H-NMR signal at δ 6.44 and the ^{13}C-NMR signal at δ 93.6 indicated the presence of an ester-linked sugar moiety.

On selective cleavage of the ester–glycoside linkage according to the method reported by Ohtani *et al.*,[3] III gave a prosapogenin and methyl glycosides. The methyl ester (VI) of the prosapogenin showed an $[M+Na]^+$ ion at *m/z* 699 in the FAB-MS and the NMR spectra showed that VI is a methyl glucosiduronate of a triterpene having a carbomethoxyl group. On treatment with $NaBH_4$, VI gave a monomethyl ester (VII), which gave D-glucose and an aglycone, $C_{31}H_{50}O_4$ (VIII) on acid hydrolysis. Compound VIII showed in the electron impact mass spectrum (EI-MS) an $[M]^+$ ion at *m/z* 486 and fragment ions at *m/z* 468, 278, 260, 219 and 201. The ^{1}H-NMR spectrum (Table II) of VIII showed the signals of two hydroxymethine groups [δ 3.46 (ddd, *J*=5, 5, 10 Hz) and 5.03 (br s)], a carbomethoxyl

TABLE I. ^{13}C-NMR Chemical Shifts (δ) of the Aglycone Moiety of Foetidissimoside A Methyl Ester (III) and Related Compounds[a]

No.	VIII	VI	XIV	III
1	39.0	38.7	39.1	38.8
2	28.1	26.5	28.1	26.6
3	78.1	89.1	78.1	89.1
4	39.3	39.5	39.3	39.5
5	55.9	55.8	55.9	55.9
6	18.8	18.5	18.8	18.5
7	33.4	33.3	33.5	33.4
8	39.8	39.8	40.0	40.0
9	47.2	47.1	47.2	47.1
10	37.4	37.0	37.4	37.0
11	23.8	23.7	23.8	23.8
12	122.7	122.6	122.8	122.7
13	144.5	144.4	144.4	144.4
14	41.9	41.9	42.1	42.0
15	36.0	35.9	36.2	36.0
16	74.4	74.3	74.0	74.0
17	49.1	49.0	49.6	49.5
18	41.3	41.2	41.3	41.3
19	47.0	46.9	47.2	47.1
20	30.8	30.8	30.8	30.8
21	35.9	35.8	35.9	36.0
22	32.4	32.4	31.9	32.0
23	28.7	28.1	28.7	28.1
24	16.5	16.9	16.5	16.9
25	15.6	15.5	15.7	15.6
26	17.3	17.2	17.5	17.5
27	27.1	27.1	27.1	27.1
28	177.7	177.7	175.8	175.8
29	33.1	33.2	33.2	33.2
30	24.6	24.6	24.8	24.7
OMe	51.7	51.7	—	—

a) Spectra were measured in pyridine-d_5 and assignment of the signals was performed with reference to the reported data.[5,9]

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group (δ 3.70, s) and a trisubstituted olefinic proton [δ 5.57 (dd, $J=3$, 3 Hz)]. The fragment ions at m/z 278 and 260 ($278-H_2O$) suggested that VIII is an olean-12-ene derivative having one hydroxyl group at ring A or B, and one hydroxyl group and a carbomethoxyl group at ring C, D or E.[4] By comparison of the ^{13}C-NMR chemical shifts (Table I) with the reported values,[5] VIII was identified as echinocystic acid methyl ester.

The position of the sugar linkage in VI was determined as the C_3-hydroxyl group based on the fact that the C_3 signal in VI appeared at lower field in comparison with that of VIII, and therefore, VI is a 3-*O*-glucopyranosiduronyl-echinocystic acid dimethyl ester. The anomers of the methyl glycosides derived from the ester-linked sugar moiety of III were preparatively separated by high-performance liquid chromatography (HPLC) using a reversed-phase column to give an α-anomer (IX) ($[\alpha]_D$ $-45.3°$) and a β-anomer (X) ($[\alpha]_D$ $+29.3°$). Compound IX gave L-arabinose, L-rhamnose and D-xylose on acid hydrolysis, and it showed an $[M+Na]^+$ ion at m/z 465 in the positive FAB-MS, while the negative FAB-MS showed an $[M-H]^-$ ion at m/z 441 and fragment ions at m/z 309 and 163, indicating that the sugars IX and X are methyl pentosyl-L-rhamnosyl-pentosides. The permethylate of IX gave methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylopyranose, 2,3-di-*O*-methyl-L-rhamnopyranose and, 3,4-di-*O*-methyl-L-arabinopyranose on methanolysis. Compounds IX and X were identified as methyl *O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and its β-anomer by comparison of the NMR data with those of the methyl glycosides obtained by the selective cleavage of the ester-linked sugar moiety of dubioside E, a quillaic acid 3,28-*O*-bisdesmoside, which was isolated from the tuber of *Thladiantha dubia* BUNGE (Cucurbitaceae).[6] 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 $J_{C1,H1}$ (171 Hz)[7] values and also from the fact that the chemical shifts and splitting patterns of the protons of the arabinopyranosyl group

TABLE II. ^{1}H-NMR Chemical Shifts (δ) of VIII in Pyridine-d_5[a]

1	*ca.* 1.06	18	3.41 dd (5, 14)
	ca. 1.62	19	*ca.* 1.35
2	1.8—1.95		2.77 dd (14, 14)
3	3.46 ddd (5, 5, 10)[b]	21	*ca.* 1.30
5	0.90 d (11)		2.43 ddd (5, 14, 14)
6	*ca.* 1.60	22	*ca.* 2.00
7	*ca.* 1.40		2.22 ddd (3, 5, 14)
	ca. 1.60	23	1.23 s
9	*ca.* 1.82	24	1.05 s
11	*ca.* 2.00	25	0.99 s
12	5.57 dd (3, 3)	26	0.93 s
15	1.68 dd (2, 15)	27	1.75 s
	2.07 dd (4, 15)	29	1.03 s
16	5.03 br s	30	1.11
		OMe	3.70 s

a) Numbers in parentheses are coupling constants in Hz. *b*) The C_3-H is coupled with two C_2 protons and a hydroxyl proton.

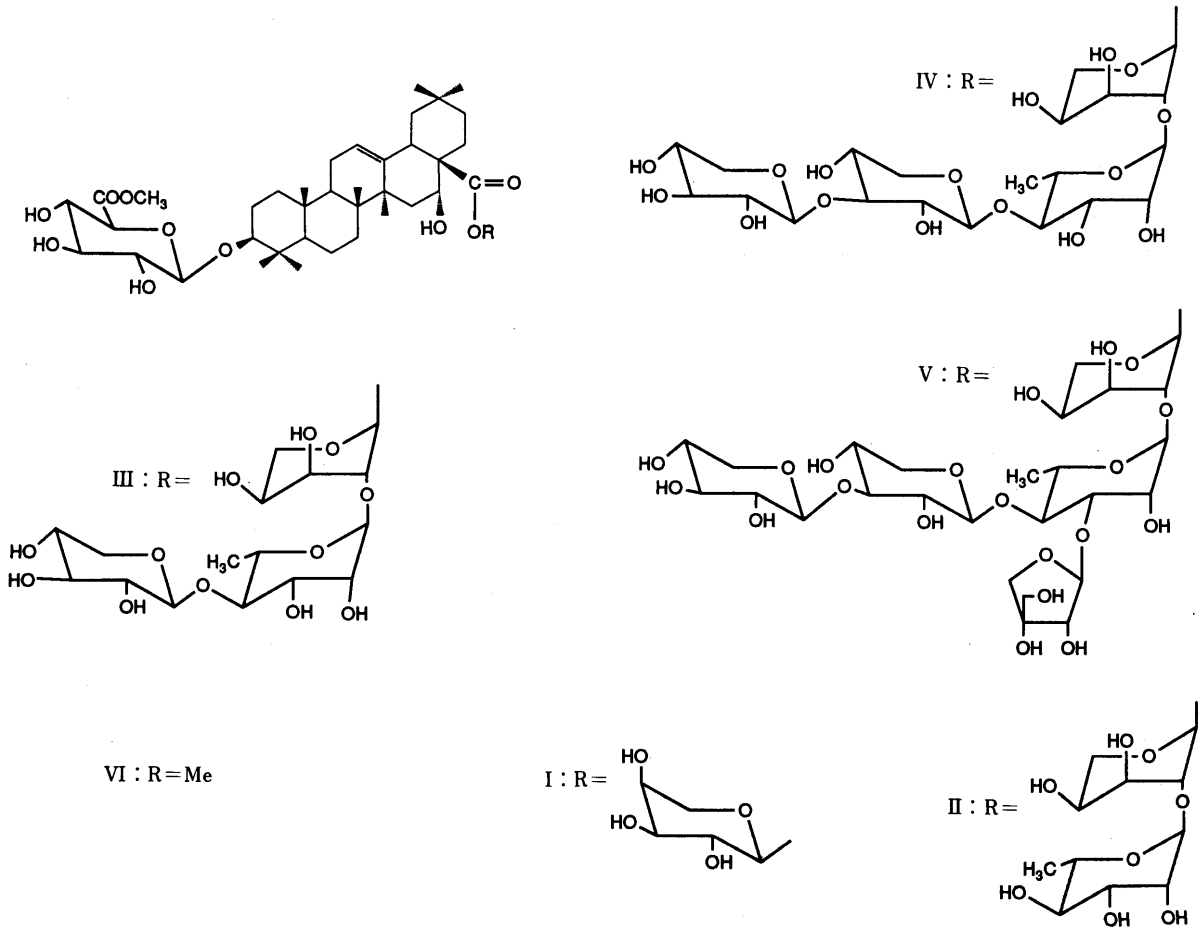

Chart 1

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were quite similar to those of desacyl-lobatoside B (3-*O*-[*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]-bayogenin 28-[*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester).[8]

The structure of III was, therefore, concluded to be as shown in the chart. Compound III seemed to be a methyl ester of foetidissimoside A isolated by Dubois *et al.* from the root of buffalo gourd (*Cucurbita foetidissima* H. B. K.)[9] and kirengeshomasaponin I isolated from the underground part of *Kirengeshoma palmata* YATABE (Saxifragaceae) by Fujimoto *et al.*[10] Identity was established by comparison of the NMR data of the saponification product with those of foetidissimoside A.

Compound IV was obtained as colorless needles. The molecular formula $C_{58}H_{92}O_{26}\cdot H_2O$ was determined by mass [FAB-MS *m/z*: 1227 ($[M+Na]^+$)] and elemental analyses. The general features of the ^{1}H-NMR and ^{13}C-NMR spectra similar to those of III and the molecular formula suggested that IV is an echinocystic acid 3,28-*O*-bisdesmoside having one more pentosyl unit than III.

On selective cleavage of the ester–glycoside linkage, IV gave a prosapogenin and an anomeric mixture of methyl glycosides. The prosapogenin was converted to a methyl ester, which was identified as VI.

The methyl glycosides showed an $[M+Na]^+$ ion at *m/z* 597 in the positive FAB-MS, and the negative FAB-MS showed an $[M-H]^-$ ion at *m/z* 573 and fragment ions at *m/z* 441, 309 and 163. Methyl glycosides gave L-arabinose, L-rhamnose and D-xylose on acid hydrolysis. The fragmentation pattern in the negative FAB-MS indicated that the methyl glycosides are methyl pentosyl-pentosyl-rhamnosyl-pentosides. The permethylates of the methyl glycosides gave methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylopyranose, 2,4-di-*O*-methyl-D-xylopyranose, 2,3-di-*O*-methyl-L-rhamnopyranose and 3,4-di-*O*-methyl-L-arabinopyranose on methanolysis, indicating that the methyl glycoside retains a linear sugar linkage and has a xylopyranosyl group at the terminal. The anomers were separated by HPLC to give an α-anomer (XI) ($[\alpha]_D$ −52.5°) and a β-anomer (XII) ($[\alpha]_D$ +12.1°). Compounds XI and XII were identified as 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, by comparison of the NMR data with those of methyl tetraglycosides obtained from dubioside F.[6] Therefore, the structure of IV was determined to be as shown in the chart.

Compound V was obtained as colorless needles and the molecular formula $C_{63}H_{100}O_{30}\cdot 2H_2O$ was determined by mass and elemental analyses. The NMR spectra suggested that V is also an echinocystic acid 3,28-*O*-bisdesmoside similar to III and IV. Compound V gave D-glucuronic acid, L-arabinose, L-rhamnose, D-xylose and D-apiose on acid hydrolysis. The prosapogenin methyl ester (VI) and an anomeric mixture (XIII) of methyl glycosides were obtained by the selective cleavage of the ester–glycoside linkage followed by treatment with CH_2N_2. The permethylate of XIII gave methyl glycosides of 2,3,5-tri-*O*-methyl-D-apiofuranose, 2,3,4-tri-*O*-methyl-D-xylopyranose, 2,4-di-*O*-methyl-D-xylopyranose, 2-*O*-methyl-L-rhamnopyranose and 3,4-di-*O*-methyl-L-arabinopyranose on methanolysis, indicating that XIII is a sugar which is branched at the rhamnopyranosyl moiety and has D-xylopyranose and D-apiofuranose at the terminals.

When V was treated with CF_3COOH–MeOH at 60 °C, a desapiofuranosyl compound was obtained, and it was identified as IV. Therefore, it was clarified that the D-apiofuranosyl group is attached to the C_3-hydroxyl group of the α-L-rhamnopyranosyl unit. The configuration of the apiofuranosyl group was determined to be β from the difference ($\Delta[M]_D$ −170°) between the molecular rotation ($[M]_D$ −839°) of V and that ($[M]_D$ −569°) of IV.[11] From the above-mentioned data, the structure of V was determined to be as shown in the Chart 1.

Compound I was obtained as an amorphous powder and the FAB-MS showed an $[M+Na]^+$ ion at *m/z* 817. It gave D-glucuronic acid and L-arabinose on acid hydrolysis. The NMR spectra showed anomeric proton signals at δ 4.98 (d, *J*=8 Hz) and δ 6.32 (d, *J*=5 Hz), and the corresponding anomeric carbon signals were seen at δ 107.2 and 95.8. The general features of the NMR spectra indicated that I is also an echinocystic acid 3,28-*O*-bisdesmoside and the structure of I was determined to be as shown in the chart by comparison of the ^{1}H-NMR and ^{13}C-NMR spectra of I with those of VI.

The configuration of the sugar linkage of the ester-linked L-arabinopyranosyl group in I was determined as α from the difference ($\Delta[M]_D$ −78°) of the molecular rotation ($[M]_D$ −153°) of I and that ($[M]_D$ −75°) of VI. The conformation of the arabinopyranosyl group seems to be an equilibrium mixture of $^4C_1$ and $^1C_4$ with the former predominating, judging from the splitting patterns of 1-H (d, *J*=5 Hz), 2-H (dd, *J*=5, 6 Hz) and 3-H (dd, *J*=3, 6 Hz) and also from the similarity of the ^{13}C-NMR chemical shifts of the arabinopyranosyl group to those of 3-*O*-acetyloleanolic acid 28-α-L-arabinopyranosyl ester, even though the $J_{C1,H1}$ value (167 Hz) implies the predominance of the $^1C_4$ conformer.[7]

The yield of II was too small to allow chemical degradation, so that the structure was determined by comparison of the NMR spectra with those of known compounds. Compound II [FAB-MS *m/z*: 963 ($[M+Na]^+$)] showed in its ^{1}H-NMR spectrum signals of the anomeric protons at δ 4.97 (d, *J*=8 Hz), 5.77 (br s) and 6.50 (d, *J*=2 Hz), and the ^{13}C-NMR spectrum showed the corresponding anomeric carbon signals at δ 107.2, 101.4 and 93.5. The presence of a >CH–CH_3 group and the similarity of the general features of the NMR spectra to those of I suggested that II is a saponin in which one rhamnopyranosyl group is linked to an ester-linked arabinopyranosyl group of I, and the structure was presumed to be as shown in the chart based on a comparison of the NMR spectra with those of I and 3-*O*-acetyloleanolic acid 28-[*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.[12]

Enzymatic hydrolysis of IV with cellulase followed by methylation with CH_2N_2 furnished II, III, VI and a new compound (XIV). Compound XIV [FAB-MS *m/z*: 773 ($[M+Na]^+$)] gave L-arabinose and L-rhamnose on acid hydrolysis and glucuronic acid was not detected. The structure of XIV was determined as echinocystic acid 28-[*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester by comparison of the ^{13}C-NMR spectrum with that of 3-*O*-acetyl oleanolic acid 28-[*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.[12]

The saponin fraction is neutral and the aster saponins are

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contained as carboxylates in the plant. The original saponins (acid form) corresponding to I, II, IV and V were named aster saponins Ha, Hb, Hc and Hd, respectively.

Experimental[13]

Extraction and Isolation The leaves and stems of *Aster tataricus* L. f. were air-dried and powdered. The powder (6.2 kg) was percolated with MeOH (100 l) and then with 50% MeOH (100 l). The MeOH eluate was concentrated *in vacuo*, suspended in 50% MeOH (2 l), and then extracted with benzene (1 l × 2). The 50% MeOH layer was concentrated to half volume and subjected to column chromatography on a styrene polymer (Diaion HP-20) column (500 ml). After being washed with H_2O and 50% MeOH, the column was eluted with MeOH. The MeOH eluate was concentrated *in vacuo* to give a glycoside fraction (31 g). The 50% MeOH percolate was concentrated to half volume and the insoluble materials were filtered off. The filtrate was subjected to Diaion HP-20 column chromatography, and treated in the same manner to give another crop (19 g) of the glycoside fraction. The two glycoside fractions were combined and again subjected to Diaion HP-20 column chromatography. The glycoside fraction was dissolved in 50% MeOH and applied to the column, which was washed with 50% MeOH. The glycosides were eluted with 70% and 85% MeOH. Both eluates were combined and concentrated *in vacuo* to give a brown powder (fr. I; 39 g). Fraction I was chromatographed on silica gel (solvent: EtOAc–MeOH–H_2O; 8:1:0.5→8:2:1→7:2:1→6:2:1) to give saponin fractions (fr. I-3; 1.23 g, fr. I-4; 14.5 g). Fraction I-3 was dissolved in 50% MeOH and passed through a column of Amberlite IRC-84 (100 ml). The acidic eluate was methylated with CH_2N_2 and chromatographed on silica gel (solvent: EtOAc–MeOH–H_2O, 8:1:0.5 and then $CHCl_3$–MeOH, 15:2, 15:3) and finally purified by HPLC (column, Capcell Pak C18, 250 mm × 10 mm i.d., Shiseido Company Ltd.; eluant, 80% MeOH) to give I (40 mg) and II (14 mg). Fraction I-4 was dissolved in 50% MeOH and passed through a column of Amberlite IRC-84 (200 ml). The acidic eluate was concentrated and the residue was dissolved in MeOH, and methylated with CH_2N_2. The solution was evaporated, and the residue was repeatedly chromatographed on silica gel (solvent: EtOAc–MeOH–H_2O, 8:1:0.5) and YMC gel ODS (75% MeOH) with monitoring by TLC to give III (1.07 g), IV (2.32 g) and V (0.65 g).

Aster Saponin Ha Methyl Ester (I): An amorphous powder. $[\alpha]_D^{27}$ −19.3° (*c*=1.9, MeOH). FAB-MS *m/z*: 817 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; $\gtrless CH_3$; 0.88, 0.98, 1.02, 1.05, 1.13, 1.28, 1.81. $\gtrless C=CH$; 5.61 (br dd). sugar moiety; arabinopyranosyl group; 6.32 (d, *J*=5 Hz, 1-H), 4.55 (dd, *J*=5, 6 Hz, 2-H), 4.39 (dd, *J*=3, 6 Hz, 3-H), *ca.* 4.47 (4-H), 3.92 (dd, *J*=3, 11 Hz, 5-H), *ca.* 4.43 (5-H). methyl glucosiduronate (Glc.U.Me) group; 4.98 (d, *J*=8 Hz, 1-H), 4.07 (dd, *J*=8, 9 Hz, 2-H), 4.24 (dd, *J*=9, 9 Hz, 3-H), *ca.* 4.45 (4-H), 4.57 (d, *J*=10 Hz, 5-H), 3.74 (s, $COOCH_3$). ^{13}C-NMR δ: aglycone moiety; almost identical with those of III. The difference of the chemical shifts of the corresponding carbons was within 0.2 ppm. sugar moiety; arabinopyranosyl group; 95.8 (C_1), 71.3 (C_2), 73.6 (C_3), 67.5 (C_4), 65.6 (C_5). Glc.U.Me. group; 107.2 (C_1), 75.3 (C_2), 77.9 (C_3), 73.1 (C_4), 77.1 (C_5), 170.7 (C_6), 52.0 ($COOCH_3$).

Aster Saponin Hb Methyl Ester (II): An amorphous powder. $[\alpha]_D^{29}$ −54.3° (*c*=2.0, MeOH). FAB-MS *m/z*: 963 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; $\gtrless CH_3$; 0.88, 0.96, 1.03, 1.08, 1.16, 1.27, 1.80. $\gtrless C=CH$; 5.61 (dd, *J*=3, 3 Hz). sugar moiety; arabinopyranosyl group; 6.50 (d, *J*=2 Hz, 1-H), 3.93 (dd, *J*=4, 11 Hz, 5-H). rhamnopyranosyl group; 5.77 (br s, 1-H), 1.68 (d, *J*=6 Hz, 6-H). Glc.U.Me group; 4.97 (d, *J*=8 Hz, 1-H), 4.07 (dd, *J*=8, 8 Hz, 2-H), 4.24 (dd, *J*=8, 9 Hz, 3-H), 3.74 (s, $COOCH_3$). ^{13}C-NMR δ: aglycone moiety; almost identical with those of III. sugar moiety; arabinopyranosyl group; 93.5 (C_1), 75.4 (C_2), 70.0 (C_3), 66.0 (C_4), 62.9 (C_5). rhamnopyranosyl group; 101.4 (C_1), 72.3 (C_2), 72.6 (C_3), 73.8 (C_4), 70.4 (C_5), 18.5 (C_6). Glc.U.Me group; 107.2 (C_1), 75.3 (C_2), 77.9 (C_3), 73.1 (C_4), 77.2 (C_5), 170.7 (C_6), 51.9 ($COOCH_3$).

Foetidissimoside A Methyl Ester (III): Colorless needles from aqueous MeOH. mp 220—222 °C. $[\alpha]_D^{26}$ −47.7° (*c*=2.2, MeOH). FAB-MS *m/z*: 1095 ($[M+Na]^+$). *Anal.* Calcd for $C_{53}H_{84}O_{22}\cdot 2H_2O$: C, 57.39; H, 8.00. Found: C, 57.52; H, 8.04. ^{1}H-NMR δ: aglycone moiety; $\gtrless CH_3$; 0.86, 0.97, 1.02, 1.08, 1.14, 1.28, 1.80. $\gtrless C=CH$–; 5.61 (br dd). sugar moiety; anomeric H; 4.97 (d, *J*=8 Hz, Glc.U.Me), 5.15 (d, *J*=7 Hz, Xyl), 5.78 (br s, Rha), 6.44 (d, *J*=3 Hz, Ara). $\gtrless CH-CH_3$; 1.74 (d, *J*=6 Hz, Rha-6). $COOCH_3$; 3.73 (s). ^{13}C-NMR δ: aglycone moiety; shown in Table I. sugar moiety; anomeric C; 93.6 (Ara), 101.0 (Rha), 106.8 (Xyl), 107.1 (Glc.U.Me), $\gtrless CH-CH_3$; 18.3. $COOCH_3$; 52.0.

Aster Saponin Hc Methyl Ester (IV): Colorless needles from aqueous MeOH, mp 227—228 °C. $[\alpha]_D^{27}$ −47.3° (*c*=2.0, MeOH). FAB-MS *m/z*: 1227 ($[M+Na]^+$). *Anal.* Calcd for $C_{58}H_{92}O_{26}\cdot H_2O$: C, 56.94; H, 7.75. Found: C, 56.80; H, 7.96. ^{1}H-NMR δ: aglycone moiety; $\gtrless CH_3$; 0.86, 0.97, 1.02, 1.07, 1.16, 1.28, 1.81. $\gtrless C=CH$–; 5.61 (br dd). sugar moiety; anomeric H; 4.97 (d, *J*=8 Hz, Glc.U.Me), 5.15 (d, *J*=7 Hz, Xyl), 5.19 (d, *J*=8 Hz, Xyl), 5.68 (d, *J*=3 Hz, Rha), 6.48 (d, *J*=3 Hz, Ara). $\gtrless CH-CH_3$; 1.71 (d, *J*=6 Hz). $COOCH_3$; 3.74 (s). ^{13}C-NMR δ: aglycone moiety; chemical shifts are almost the same as those of III. The difference of the chemical shifts of the corresponding carbon signals is within 0.2 ppm. sugar moiety; anomeric C; 93.4 (Ara), 101.0 (Rha), 105.9 (Xyl), 106.2 (Xyl), 107.2 (Glc.U.Me). $\gtrless CH-CH_3$; 18.3 (Rha-6). $COOCH_3$; 51.9.

Aster Saponin Hd Methyl Ester (V): Colorless needles from aqueous MeOH. mp 235—237 °C. $[\alpha]_D^{26}$ −62.8° (*c*=1.8, MeOH). FAB-MS *m/z*: 1359 ($[M+Na]^+$). *Anal.* Calcd for $C_{63}H_{100}O_{30}\cdot 2H_2O$: C, 55.09; H, 7.63. Found: C, 54.99; H, 7.84. ^{1}H-NMR δ: aglycone moiety; $\gtrless CH_3$; 0.87, 0.98, 1.03, 1.07, 1.18, 1.28, 1.80. $\gtrless C=CH$–; 5.61 (br dd). sugar moiety; anomeric H; 4.97 (d, *J*=8 Hz, Glc.U.Me), 5.22 (d, *J*=8 Hz, Xyl), 5.31 (d, *J*=8 Hz, Xyl), 5.56 (s, Rha), 5.92 (d, *J*=4 Hz, Api), 6.55 (br s, Ara). $\gtrless CH-CH_3$; 1.69 (d, *J*=6 Hz). $COOCH_3$; 3.74 (s). ^{13}C-NMR δ: aglycone moiety; chemical shifts are almost the same as those of III. sugar moiety; anomeric C; 93.1 (Ara), 101.0 (Rha), 104.7 (Xyl), 105.8 (Xyl), 107.2 (Glc.U.Me), 111.9 (Api). $\gtrless CH-CH_3$; 18.5. $COOCH_3$; 51.9. C_3 of Api; 79.7.

Determination of the Sugar Species and Their Absolute Configurations A glycoside (*ca.* 5 mg) was dissolved in 2 N HCl–MeOH (1 ml) and heated at 95 °C for 2 h. The acid was neutralized with Ag_2CO_3 and the precipitates were centrifuged off. The supernatant was treated with H_2S and concentrated *in vacuo*. The residue was trimethylsilylated with trimethylsilylimidazole reagent and checked by gas liquid chromatography (GLC).

Determination of the absolute configuration was performed according to the method reported by Hara *et al.*[14] Thus, a glycoside (*ca.* 20 mg) was hydrolyzed at 90 °C in 1 N HCl (0.7 ml) for 2 h. The acid was neutralized in the same manner as described above. The hydrolysate was suspended in H_2O (2 ml) and extracted with $CHCl_3$ to remove the aglycone. The aqueous layer was concentrated *in vacuo* and the residue was dissolved in pyridine (0.2 ml). After addition of a pyridine solution (0.4 ml) of L-cysteine methyl ester hydrochloride (0.06 mol/l), the mixture was warmed at 60 °C for 1 h. The solvent was blown off by an N_2 stream, and the residue was trimethylsilylated and checked by GLC. GLC conditions were reported in the previous paper[15] from this laboratory. The absolute configuration of glucuronic acid was determined in the same way after $NaBH_4$ reduction of VI into methyl echinocystate 3-*O*-β-glucopyranoside (VII). The results are shown 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.*[3] The details of the procedure were described in the previous paper.[15] Compound III (634 mg) gave a prosapogenin and methyl glycosides. The prosapogenin was converted into a methyl ester by CH_2N_2 and repeatedly chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O, 15:2:0.1; $CHCl_3$–MeOH, 100:2) and then purified by HPLC (85% MeOH) to give VI (249 mg). The methyl glycoside fraction (254 mg) was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O, 15:10:1) and then subjected to HPLC (10% MeOH) to give IX (34 mg) and X (61 mg).

VI: An amorphous powder. $[\alpha]_D^{28}$ −11.1° (*c*=3.6, MeOH). FAB-MS *m/z*: 699 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; $\gtrless CH_3$; 0.86, 0.89, 0.98, 1.02, 1.10, 1.30, 1.78. $\gtrless C=CH$–; 5.52 (br dd). $COOCH_3$; 3.68 (s). C_3-H; *ca.* 3.4 (overlapped by the signal of C_{18}-H). C_{16}-H; 5.00 (br s). sugar moiety; 4.97 (d, *J*=8 Hz, 1-H), 4.05 (dd, *J*=8, 8 Hz, 2-H), 4.22 (dd, *J*=8, 8 Hz, 3-H). 4.33 (dd, *J*=8, 8 Hz, 4-H), 4.55 (d, *J*=8 Hz, 5-H). $COOCH_3$; 3.73 (s). ^{13}C-NMR δ: aglycone moiety; shown in Table I. sugar moiety; 107.1 (C_1), 75.3 (C_2), 77.9 (C_3), 73.1 (C_4), 77.1 (C_5), 170.7 (C_6). $COOCH_3$; 51.7.

IX: An amorphous powder. $[\alpha]_D^{26}$ −45.3° (*c*=2.05, MeOH). Positive FAB-MS *m/z*: 465 ($[M+Na]^+$). Negative FAB-MS *m/z*: 441 ($[M-H]^-$), 309 (441 − Xyl), 163 (309 − Rha). ^{1}H-NMR δ: arabinopyranosyl group; 4.55 (d, *J*=7 Hz, 1-H), 4.45 (dd, *J*=7, 8 Hz, 2-H), 4.14 (dd, *J*=8, 3 Hz, 3-H), 3.67 (dd, *J*=2, 13 Hz, 5-H), 4.20 (dd, *J*=4, 13 Hz, 5-H). rhamnopyranosyl group; 5.94 (br s, 1-H), 4.67 (dd, *J*=2, 3 Hz, 2-H), 4.64 (dd, *J*=3, 9 Hz, 3-H), 4.34 (dd, *J*=9, 9 Hz, 4-H), 1.68 (d, *J*=6 Hz, 6-H). xylopyranosyl group; 5.12 (d, *J*=8 Hz, 1-H), 4.04 (dd, *J*=8, 9 Hz, 2-H), 4.07 (dd, *J*=9, 9 Hz, 3-H), 3.52 (dd, *J*=10, 11 Hz, 5-H), 4.25 (dd, *J*=5, 11 Hz, 5-H). OCH_3; 3.49 (s). ^{13}C-NMR δ: arabinopyranosyl group; 103.6 (C_1), 76.9 (C_2), 74.2 (C_3), 65.9 (C_5). rhamnopyranosyl group; 102.1 (C_1), 72.0 (C_2), 72.8 (C_3), 84.8 (C_4), 18.2 (C_6). xylopyranosyl group; 107.2 (C_1), 76.1 (C_2), 78.6 (C_3), 67.5 (C_5). OCH_3; 56.0.

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X: An amorphous powder. $[\alpha]_D^{27}$ +29.3° (c=1.75, MeOH). FAB-MS m/z: 465 ($[M+Na]^+$). ^{1}H-NMR δ: arabinopyranosyl group; 5.28 (br s, 1-H), 4.55 (br d, J=10 Hz, 2-H), 4.46 (br d, J=10 Hz, 3-H), 3.95 (2H, br s, 5-H). rhamnopyranosyl group; 5.61 (br s, 1-H), 4.62 (2H, br s, 2,3-H), 4.32 (br d, J=10 Hz, 4-H), 1.66 (d, J=6 Hz, 6-H). xylopyranosyl group; 5.10 (d, J=8 Hz, 1-H), 4.01 (dd, J=8, 8 Hz, 2-H), 4.06 (dd, J=8, 8 Hz, 3-H), 3.51 (dd, J=10, 10 Hz, 5-H), 4.23 (dd, J=5, 10 Hz, 5-H). OC$\underline{H}_3$; 3.39 (s). ^{13}C-NMR δ: arabinopyranosyl group; 101.1 (C_1), 78.7 (C_2), 69.1 (C_3), 63.5 (C_5). rhamnopyranosyl group; 104.1 (C_1), 71.7 (C_2), 72.7 (C_3), 84.5 (C_4), 18.5 (C_6). xylopyranosyl group; 107.1 (C_1), 76.1 (C_2), 78.6 (C_3), 67.5 (C_5). O$\underline{C}H_3$; 55.1.

Aster saponin Hc methyl ester (IV) (1 g) gave VI (275 mg) and methyl glycosides (325 mg). Methyl glycosides (229 mg) were separated by HPLC (10% MeOH) to give XI (66 mg) and XII (99 mg).

XI: An amorphous powder. $[\alpha]_D^{28}$ −52.5° (c=2.25, MeOH). Positive FAB-MS m/z: 597 ($[M+Na]^+$). Negative FAB-MS m/z: 573 ($[M-H]^-$), 441 (573−Xyl), 309 (441−Xyl), 163 (309−Rha). ^{1}H-NMR δ: arabinopyranosyl group; 4.55 (d, J=7 Hz, 1-H), 4.44 (dd, J=7, 8 Hz, 2-H), 3.66 (dd, J=6, 10 Hz, 5-H), 4.20 (dd, J=4, 10 Hz, 5-H). rhamnopyranosyl group; 5.93 (br s, 1-H), 4.66 (br s, 2-H), 4.61 (dd, J=3, 9 Hz, 3-H), 4.34 (dd, J=9, 9 Hz, 4-H), 1.65 (d, J=7 Hz, 6-H). xylopyranosyl groups; 5.21 (d, J=8 Hz, 1-H), 5.16 (d, J=7 Hz, 1-H). OC$\underline{H}_3$; 3.51 (s). ^{13}C-NMR δ: arabinopyranosyl group; 103.6 (C_1), 76.8 (C_2), 65.9 (C_5). rhamnopyranosyl group; 102.1 (C_1), 72.0 (C_2), 72.8 (C_3), 84.3 (C_4), 18.2 (C_6). xylopyranosyl groups; 105.9 (C_1), 106.4 (C_1), 87.0 (C_3). O$\underline{C}H_3$; 56.0.

XII: An amorphous powder. $[\alpha]_D^{28}$ +12.1° (c=2.60, MeOH). Positive FAB-MS m/z: 597 ($[M+Na]^+$). ^{1}H-NMR δ: arabinopyranosyl group; 5.28 (d, J=3 Hz, 1-H), 4.54 (dd, J=4, 10 Hz, 2-H), 4.45 (dd, J=2, 10 Hz, 3-H), 3.95 (2H, br s, 5-H). rhamnopyranosyl group; 5.59 (br s, 1-H), 4.59 (2H, br s, 2,3-H), 4.31 (dd, J=9, 11 Hz, 4-H), 1.63 (d, J=6 Hz, 6-H). xylopyranosyl groups; 5.20 (d, J=8 Hz, 1-H), 5.14 (d, J=7 Hz, 1-H). OC$\underline{H}_3$; 3.40 (s). ^{13}C-NMR δ: arabinopyranosyl group ; 101.1 (C_1), 78.7 (C_2), 69.1 (C_3), 63.5 (C_5). rhamnopyranosyl group; 104.1 (C_1), 71.7, 72.7 (C_2, C_3), 84.0 (C_4), 18.4 (C_6). xylopyranosyl groups; 105.9 (C_1), 106.3 (C_1), 87.0 (C_3). O$\underline{C}H_3$; 55.1.

Aster saponin Hd methyl ester (V) (349 mg) gave VI (155 mg) and methyl glycosides (152 mg). The methyl glycoside mixture was subjected to HPLC (15% MeOH) to give XIII (37 mg) and a mixture (22 mg) of XI and XII. XIII: FAB-MS m/z: 729 ($[M+Na]^+$).

$NaBH_4$ Reduction of VI Compound VI (200 mg) was dissolved in MeOH (5 ml), and $NaBH_4$ (200 mg) was added to the solution. The mixture was stirred at room temperature for 24 h. The reaction mixture was neutralized with 20% CH_3COOH–MeOH and evaporated. The residue (197 mg) was chromatographed on silica gel (solvent: $CHCl_3$–MeOH, 95 : 5) to give VII (163 mg).

VII: An amorphous powder: $[\alpha]_D^{28}$ +4.1° (c=1.5, MeOH). FAB-MS m/z: 671 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; >C$\underline{H}_3$; 0.86, 0.91, 1.02 (×2), 1.10, 1.30, 1.79. >C=C$\underline{H}$–; 5.55 (br dd). COOC$\underline{H}_3$; 3.69 (s). sugar moiety; 4.93 (d, J=8 Hz, 1-H), *ca.* 3.9 (2-H), *ca.* 4.2 (3,4-H), *ca.* 4.0 (5-H), 4.38 (dd, J=6, 12 Hz, 6-H), 4.60 (dd, J=3, 12 Hz, 6-H). ^{13}C-NMR δ: aglycone moiety; chemical shifts are almost the same as those of VI. sugar moiety; 106.8 (C_1), 75.8 (C_2), 78.7 (C_3), 71.9 (C_4), 78.2 (C_5), 63.1 (C_6).

Acid Hydrolysis of VII, Preparation of VIII Compound VII (112 mg) was dissolved in 1 N HCl–MeOH and heated at 90 °C for 2.5 h. The reaction mixture was neutralized in a usual manner, and concentrated to dryness. The residue was extracted with $CHCl_3$ and the extract was chromatographed on silica gel (solvent: $CHCl_3$–MeOH, 98 : 2) to give VIII (63 mg).

VIII: Colorless needles from aqueous MeOH. $[\alpha]_D^{28}$ +28.4° (c=1.0, MeOH). EI-MS m/z: 486 ($[M]^+$), 468, 278, 260, 219, 201. High-resolution EI-MS m/z: 486.371. $C_{31}H_{50}O_4$ requires m/z 486.370. ^{1}H-NMR: shown in Table II. ^{13}C-NMR: shown in Table I.

Permethylation of Methyl Glycosides, Methanolysis and Analysis of Methylated Monosaccharides Methylation of the methyl glycosides was performed according to Hakomori's method.[16] Details of methanolysis of permethylates and identification of the methylated monosaccharides were presented in the previous paper[15] from this laboratory. The results are described in the text.

Partial Hydrolysis of Aster Saponin Hd Methyl Ester (V) Compound V (60 mg) was dissolved in 2 N CF_3COOH–MeOH (2 ml) and the solution was warmed at 60 °C for 6 h. After evaporation of the solvent, the residue was chromatographed on silica gel (solvent: EtOAc–MeOH–H_2O, 8 : 1 : 0.5) to give a desapiosyl compound (35 mg), which was identified as IV by comparison of the NMR data.

Saponification of III Compound III (103 mg) was dissolved in 0.5 N NaOH–MeOH and stirred at room temperature for 30 min. The reaction mixture was diluted with H_2O and passed through a column of Amberlite IRC-84 to give an acidic saponin (99 mg). The ^{13}C-NMR chemical shifts were almost the same as those of foetidissimoside A reported by Dubois *et al.*[9]

Enzymatic Hydrolysis of IV Compound IV (500 mg) and cellulase (Sigma Type II) (4.6 g) were dissolved in 16% EtOH (300 ml) and the solution was shaken at 37—40 °C for 10 d. After evaporation of the solvent, the residue was extracted with MeOH. The MeOH extract was dissolved in 50% MeOH and passed through a column of styrene polymer Diaion CHP-20P. After washing of the column with 50% MeOH, the glycosides were eluted with MeOH. The MeOH eluate (353 mg) was treated with CH_2N_2 and chromatographed on silica gel (solvent: $CHCl_3$–MeOH, 15 : 2→15 : 4) to give II (41 mg), III (45 mg), VI (43 mg) and XIV (18 mg). Compound IV (30 mg) was recovered.

XIV: Amorphous white powder. $[\alpha]_D^{29}$ −31.9° (c=0.9, MeOH). FAB-MS m/z: 773 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; >C$\underline{H}_3$; 0.97, 1.02 (×2), 1.13, 1.16, 1.21, 1.79. >C$\underline{H}$–OH; 3.50 (dd, J=5, 10 Hz, C_3-H), >C=C$\underline{H}$–; 5.65 (br dd, J=3, 3 Hz, C_{12}-H). sugar moiety; anomeric H; 6.52 (d, J=3 Hz, Ara), 5.78 (d, J=2 Hz, Rha). ^{13}C-NMR δ: aglycone moiety; shown in Table I. sugar moiety; arabinosyl group; 93.5 (C_1), 75.3 (C_2), 70.4 (C_3), 66.0 (C_4), 62.9 (C_5). rhamnopyranosyl group; 101.4 (C_1), 72.3 (C_2), 72.5 (C_3), 73.8 (C_4), 70.0 (C_5), 18.5 (C_6).

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