Studies on the Constituents of *Aster tataricus* L. f. III.[1] Structures of Aster Saponins E and F Isolated from the Root

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Two new oleanane-type triterpene glycosides, aster saponins E and F, were isolated from the less polar saponin fraction of the root of *Aster tataricus* L. f. (Compositae), and their structures were elucidated on the basis of spectral evidence and by correlation to the other aster saponins reported earlier. Aster saponin E is 3-*O*-[*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]-2β,3β,16α-trihydroxyolean-12-en-28-oic acid (asterogenic acid) 28-[*O*-β-D-xylopyranosyl-(1→3)-*O*-α-L-arabinopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl] ester. Aster saponin F is a 3β,16α-dihydroxyolean-12-en-28-oic acid (echinocystic acid) glycoside, the sugar moiety of which is the same as that of aster saponin E.

Keywords *Aster tataricus*; Asteris Radix; Compositae; triterpene glycoside; 3,28-*O*-bisdesmoside; aster saponin; asterogenic acid; 2β,3β,16α-trihydroxyolean-12-en-28-oic acid; echinocystic acid; 3β,16α-dihydroxyolean-12-en-28-oic acid

In the preceding paper[1] of this series, we reported the structures of four oleanane-type triterpene glycosides, aster saponins A (I), B (II), C (III) and D (IV), isolated from the root of *Aster tataricus* L. f. (Compositae). Further investigation of the less polar saponin fraction has resulted in the isolation of two additional new triterpene glycosides, aster saponins E (V) and F (VI). This paper deals with their structures.

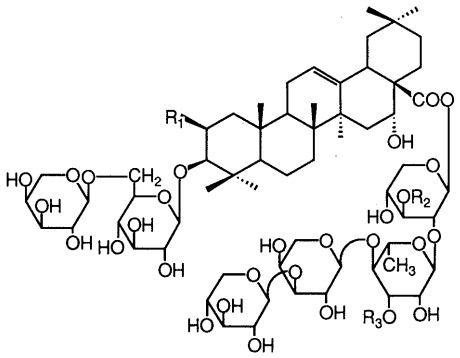
Aster saponin E (V) was obtained as an amorphous white powder. The fast atom bombardment mass spectrum (FAB-MS) showed an $[M+Na]^+$ ion at *m/z* 1347 and an $[M-H]^-$ ion at *m/z* 1323, indicating its molecular weight to be 1324. The results of the elemental analyses were consistent with the molecular formula $C_{62}H_{100}O_{30}\cdot 5/2H_2O$. Compound V gave D-glucose, L-rhamnose, L-arabinose and D-xylose on acid hydrolysis. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed the signals of seven tertiary methyl groups (δ 1.00, 1.10, 1.12, 1.30, 1.35, 1.44 and 1.81), one trisubstituted olefinic proton (δ 5.60) and six anomeric protons [δ 4.78 (d, *J*=7 Hz), 4.85 (d, *J*=8 Hz), 5.17 (2H, d, *J*=7 Hz), 6.18 (d, *J*=6 Hz) and 6.22 (br s)]. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table) revealed the presence of six saturated quaternary carbons (δ 30.8, 37.0, 38.6, 40.2, 42.3 and 49.5), a pair of olefinic carbons (δ 122.8 and 144.3), one ester carbonyl carbon (δ 176.0) and six anomeric carbons (δ 95.2, 101.3, 104.8, 105.6, 106.0 and 106.3). These spectral data suggested that V is an oleanane-type triterpene carboxylic acid glycoside having a sugar moiety linked to the carboxyl group with an ester linkage. When V was heated with LiI in a mixture of MeOH and 2,6-lutidine, the ester-linked sugar moiety was selectively cleaved and a prosapogenin and methyl oligoglycosides were obtained.[2] The methyl ester (VII) of the prosapogenin showed in the FAB-MS an $[M+Na]^+$ ion at *m/z* 819, and it gave L-arabinose and D-glucose on acid hydrolysis. The NMR spectra of VII were identical with those of 3-*O*-[*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]-asterogenic acid methyl ester which was obtained by the selective cleavage of the ester-linked sugar moiety of I.[1]

The methyl glycosides obtained by the selective cleavage of the ester-glycoside linkage of V gave L-rhamnose, L-arabinose and D-xylose on acid hydrolysis. The FAB-MS showed an $[M+Na]^+$ ion at *m/z* 597. The methyl glycoside fraction was subjected to high-performance liquid chromatography (HPLC) on a reversed-phase material and a β-anomer ($[\alpha]_D$ −71.5°, VIIIa) and an α-anomer ($[\alpha]_D$ −15.6°, VIIIb) were preparatively separated. By comparison of the NMR spectra, VIIIa and VIIIb were proved to be identical with methyl *O*-β-D-xylopyranosyl-(1→3)-*O*-α-L-arabinopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranoside and its α-anomer, respectively, which were obtained by the partial hydrolysis of the methyl glycosides of the ester-linked sugar of I.[1]

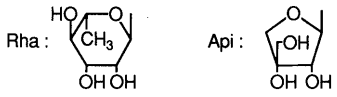
From the above-mentioned data, V was elucidated to be

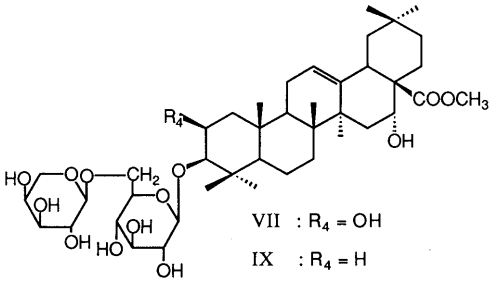
TABLE I. ^{13}C-NMR Chemical Shifts of the Aglycone Moieties of Aster Saponins and Related Compounds

Carbon No.	I	V	VII	II	VI	IX
1	44.0	44.0	43.9	38.9	38.8	38.8
2	69.5	69.5	69.6	26.7	26.7	26.7
3	90.0	90.0	89.9	89.1	89.0	89.0
4	38.6	38.6	38.6	39.5	39.5	39.5
5	55.9	56.0	55.9	56.0	55.9	55.8
6	18.5	18.6	18.4	18.6	18.6	18.5
7	33.5	33.5	33.4	33.5	33.4	33.3
8	40.2	40.2	40.0	40.1	40.0	39.8
9	47.5	47.4	47.4	47.1	47.1	47.0
10	37.0	37.0	37.0	37.0	37.0	37.0
11	23.9	23.9	23.8	23.8	23.8	23.7
12	122.8	122.8	122.8	122.7	122.6	122.7
13	144.3	144.3	144.3	144.3	144.3	144.4
14	42.3	42.3	42.0	42.1	42.1	41.9
15	36.0	36.0	35.9	36.1	36.2	36.0
16	74.1	74.1	74.4	74.1	74.0	74.3
17	49.5	49.5	49.1	49.5	49.5	49.0
18	41.6	41.5	41.3	41.5	41.5	41.2
19	47.4	47.4	47.0	47.4	47.4	47.0
20	30.8	30.8	30.8	30.8	30.8	30.8
21	36.0	36.0	35.9	36.0	36.0	35.9
22	31.9	32.0	32.5	31.9	32.0	32.5
23	29.8	29.9	30.0	28.2	28.2	28.2
24	18.8	18.8	18.8	17.1	17.0	17.0
25	16.7	16.7	16.6	15.7	15.7	15.6
26	17.5	17.5	17.2	17.6	17.5	17.2
27	27.1	27.1	27.2	27.1	27.0	27.1
28	176.0	176.0	177.7	176.0	175.9	177.7
29	33.1	33.1	33.2	33.2	33.1	33.2
30	24.7	24.6	24.6	24.7	24.6	24.6
OMe			51.7			51.7

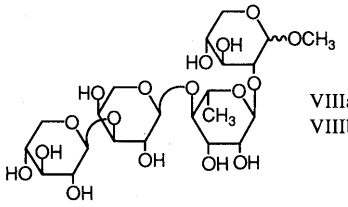


	R_1	R_2	R_3
I	OH	H	Api
II	H	H	Api
III	OH	Rha	Api
IV	H	Rha	Api
V	OH	H	H
VI	H	H	H





VII : R_4 = OH
IX : R_4 = H



VIIIa (β)
VIIIb (α)

desapiofuranosyl aster saponin A.

Aster saponin F (VI), $C_{62}H_{100}O_{29}$, was obtained as a white amorphous powder and FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1331 and an $[M-H]^-$ ion at *m/z* 1307, 16 mass units less than in the case of V. Compound VI gave D-glucose, L-rhamnose, L-arabinose and D-xylose on acid hydrolysis. The general features of the NMR spectra were quite similar to those of V, indicating that VI is a desoxy compound of V.

On selective cleavage of the ester-glycoside linkage, VI gave a prosapogenin and methyl glycosides (VIIIa and VIIIb). The methyl ester (IX) of the prosapogenin was concluded to be 3-*O*-[*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl]-echinocystic acid methyl ester from a comparison of the NMR spectra with those of the prosapogenin methyl ester derived from aster saponin B (II).[1]

Therefore, aster saponin F (VI) was concluded to be desapiofuranosyl aster saponin B.

The conformation and configuration of the sugar linkage of the ester-linked D-xylopyranosyl groups of V and VI were presumed to be $^1C_4$ and β, respectively, judging from the $J_{H1,H2}$ (6 Hz) and $J_{C1,H1}$ (169 Hz) values.[3] The ^{1}H-NMR signal pattern (δ 4.28, dd, J=6, 6 Hz) of C_2-H of the ester-linked xylopyranosyl group revealed by ^{1}H homodecoupling difference spectroscopy supported the above presumption.

Experimental[4]

Isolation of Aster Saponins E and F Extraction and fractionation procedures were described in the preceding paper.[1] The less polar fraction than that which contained I—IV was chromatographed on a reversed-phase octadecyl silica (ODS) column (packed column RQ-2, 360 mm × 24 mm i.d., Fuji Gel Co., Ltd.; eluant, 30% CH_3CN) and purified by HPLC (column, Capcell Pak C_{18}, 250 mm × 10 mm i.d., Shiseido Company Ltd.; eluants, 29—31% CH_3CN) to give aster saponins E (V, 379 mg) and F (VI, 81 mg).

Aster Saponin E (V): An amorphous white powder. $[\alpha]_D^{26}$ −33.7° (c=1.2, MeOH). *Anal.* Calcd for $C_{62}H_{100}O_{30}\cdot 5/2H_2O$: C, 54.34; H, 7.72. Found: C, 54.43; H, 7.76. FAB-MS *m/z*: 1347 ($[M+Na]^+$), 1323 ($[M-H]^-$). ^{1}H-NMR δ: aglycone moiety; ≥C$\underline{H}_3$; 1.00 (C_{29}-H), 1.10 (C_{30}-H), 1.12 (C_{26}-H), 1.30 (C_{23}-H), 1.35 (C_{24}-H), 1.44 (C_{25}-H), 1.81 (C_{27}-H). >C$\underline{H}$–O–; 3.36 (d, J=4 Hz, C_3-H), 4.65 (br s, C_2-H), 5.22 (br s, C_{16}-H). >C=C$\underline{H}$–; 5.60 (br dd, C_{12}-H). Sugar moiety; anomeric H; 4.78 (d, J=7 Hz), 4.85 (d, J=8 Hz), 5.17 (2H, d, J=7 Hz), 6.18 (d, J=6 Hz), 6.22 (br s). >CH–C$\underline{H}_3$; 1.73 (d, J=6 Hz). ^{13}C-NMR δ: aglycone moiety; shown in the Table. Sugar moiety; anomeric C (in the order of corresponding anomeric protons); 104.8, 105.6, 106.0, 106.3, 95.2, 101.3. C_6 of the rhamnopyranosyl group; 18.6.

Aster Saponin F (VI): An amorphous white powder. $[\alpha]_D^{28}$ −45.6° (c=0.9, MeOH), *Anal.* Calcd for $C_{62}H_{100}O_{29}$: C, 56.87; H, 7.57. Found: C, 56.96; H, 7.57. FAB-MS *m/z*: 1331 ($[M+Na]^+$), 1307 ($[M-H]^-$). ^{1}H-NMR δ: aglycone moiety; ≥C$\underline{H}_3$; 0.87 (C_{25}-H), 0.99 (C_{24}-H), 1.01 (C_{29}-H), 1.08 (C_{26}-H), 1.10 (C_{30}-H), 1.26 (C_{23}-H), 1.81 (C_{27}-H). >C$\underline{H}$–O–; 3.32 (dd, J=4, 12 Hz, C_3-H), 5.22 (br s, C_{16}-H). >C=C$\underline{H}$–; 5.60 (br dd). Sugar moiety; anomeric H; 4.82 (d, J=7 Hz), 4.95 (d, J=7 Hz), 5.15 (2H, d, J=7 Hz), 6.18 (d, J=6 Hz), 6.21 (br s), methyl group; 1.73 (d, J=6 Hz). ^{13}C-NMR δ: aglycone moiety; shown in the Table. Sugar moiety; anomeric C; 106.8, 105.2, 105.9, 106.2, 95.2, 101.3. C_6 of the rhamnopyranosyl group; 18.5.

Selective Cleavage of the Ester-Glycoside Linkages of V and VI Compound V (210 mg) was dissolved in 2,6-lutidine (4 ml) containing anhydrous MeOH (2 ml) and LiI (300 mg). The reaction mixture was heated at 180 °C for 6 h, then diluted with 50% MeOH and passed through a column of Amberlite MB-3 (20 ml). The eluate was concentrated *in vacuo* and treated with CH_2N_2. The product was chromatographed on styrene polymer (Diaion HP-20) using 50% MeOH and MeOH as eluants. The 50% MeOH eluate contained an anomeric mixture of methyl oligoglycosides (80 mg), and MeOH eluted a prosapogenin methyl ester (VII, 120 mg). The anomers of methyl oligoglycosides were separated preparatively by HPLC (Shiseido Capcell Pak C_{18}, 25% MeOH) to give a β-anomer (VIIIa, 22 mg) and an α-anomer (VIIIb, 35 mg).

VII: An amorphous white powder. $[\alpha]_D^{28}$ +8.3° (c=1.2, MeOH). FAB-MS *m/z*: 819 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; ≥C$\underline{H}_3$; 0.91 (C_{26}-H), 1.04 (C_{29}-H), 1.10 (C_{30}-H), 1.29 (C_{23}-H), 1.36 (C_{24}-H), 1.49 (C_{25}-H), 1.76 (C_{27}-H), 3.68 (COOC$\underline{H}_3$). >C$\underline{H}$–O–; 3.40 (d, J=4 Hz. C_3-H), 4.67 (br s, C_2-H), 5.01 (br s, C_{16}-H). >C=C$\underline{H}$–; 5.51 (br s). Sugar moiety; anomeric H; 4.77 (d, J=7 Hz, Ara), 4.86 (d, J=8 Hz, Glc). ^{13}C-NMR δ: aglycone moiety; shown in the Table. Sugar moiety; glucopyranosyl group; 105.8 (C_1), 75.2 (C_2), 78.6 (C_3), 72.1 (C_4), 76.5 (C_5), 69.7 (C_6), arabinopyranosyl group; 104.8 (C_1), 72.4 (C_2), 74.1 (C_3), 68.9 (C_4), 66.3 (C_5).

VIIIa: An amorphous white powder. $[\alpha]_D^{28}$ −71.5° (c=1.1, MeOH). FAB-MS *m/z*: 597 ($[M+Na]^+$). ^{1}H-NMR δ: anomeric H; 4.51 (d, J=7 Hz), 5.18 (d, J=7 Hz), 5.22 (d, J=8 Hz), 6.15 (br s). >CH–C$\underline{H}_3$; 1.73 (d, J=6 Hz). C$\underline{H}_3$O–; 3.53 (s). ^{13}C-NMR δ: anomeric C; 104.3, 106.4, 105.9, 102.3, C_6 of the rhamnopyranosyl group; 18.2. $\underline{C}H_3O$–; 56.3.

VIIIb: An amorphous white powder. $[\alpha]_D^{28}$ $-15.6°$ ($c=1.1$, MeOH). FAB-MS m/z: 597 ($[M+Na]^+$). ^{1}H-NMR δ: anomeric H; 5.16 (d, $J=7$ Hz), 5.20 (d, $J=4$ Hz), 5.21 (d, $J=7$ Hz), 5.69 (br s). $>$CH–CH$_3$; 1.67 (d, $J=6$ Hz). CH$_3$O–; 3.39 (s). ^{13}C-NMR δ: anomeric C; 106.3, 100.5, 105.9, 104.2. C_6 of the rhamnopyranosyl group; 18.5. CH$_3$O–; 54.9.

Compound VI (48 mg) gave prosapogenin methyl ester (IX, 20 mg) and methyl oligoglycosides (VIIIa: 6 mg, VIIIb: 7 mg).

IX: An amorphous white powder. $[\alpha]_D^{27}$ $-11.4°$ ($c=0.9$, MeOH). FAB-MS m/z: 803 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety; $\gtrless$CH$_3$; 0.88 (C_{26}-H), 0.92 (C_{25}-H), 1.01 (C_{24}-H), 1.05 (C_{29}-H), 1.11 (C_{30}-H), 1.27 (C_{23}-H), 1.77 (C_{27}-H), 3.68 (COOCH$_3$). $>$CH–O–; 3.35 (dd, $J=4$, 11 Hz, C_3-H), 5.02 (br s, C_{16}-H). $>$C=CH–; 5.52 (br s). Sugar moiety; anomeric H; 4.87 (d, $J=8$ Hz, Glc), 4.95 (d, $J=7$ Hz, Ara). ^{13}C-NMR δ: aglycone moiety; shown in the Table. Sugar moiety; glucopyranosyl group; 106.9 (C_1), 75.6 (C_2), 78.6 (C_3), 72.0 (C_4), 76.6 (C_5), 69.9 (C_6). Arabinopyranosyl group; 105.2 (C_1), 72.2 (C_2), 74.2 (C_3), 68.9 (C_4), 66.2 (C_5).

Determination of the Component Monosaccharides Identification of the sugars and determination of their absolute configurations were performed in the same manner as described in the preceding paper[1] and the results are described in the text.

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References and Notes

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4) The instruments and materials used in this work were the same as those described in the previous paper[5] from this laboratory. The ^{1}H-NMR and ^{13}C-NMR spectra were measured at 400 and 100 MHz, respectively, in pyridine-d_5 solution and chemical shifts were expressed in the δ scale using tetramethylsilane as an internal standard.
5) T. Nagao, H. Okabe, K. Mihashi and T. Yamauchi, *Chem. Pharm. Bull.*, **37**, 925 (1989).