Studies on the Constituents of *Aster scaber* THUNB. III.[1] Structures of Scaberosides B_7, B_8 and B_9, Minor Oleanolic Acid Glycosides Isolated from the Root

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Three new oleanolic acid 3,28-*O*-bisdesmosides, scaberosides B_7, B_8 and B_9, were isolated as minor saponins from the root of *Aster scaber* THUNB. (Compositae), and their structures were determined based on spectral and chemical evidence as follows. Scaberoside B_7 is 3-*O*-*β*-D-glucopyranosyluronic acid oleanolic acid 28-{*O*-*β*-D-apiofuranosyl-(1→3)-[*O*-*β*-D-xylopyranosyl-(1→4)]-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} ester, scaberoside B_8, 3-*O*-*β*-D-glucopyranosyl oleanolic acid 28-[*O*-*β*-D-xylopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, and scaberoside B_9, 3-*O*-*β*-D-glucopyranosyluronic acid oleanolic acid 28-{*O*-α-L-rhamnopyranosyl-(1→2)-[*O*-*β*-D-xylopyranosyl-(1→6)]-*β*-D-glucopyranosyl} ester. Scaberosides B_7 and B_9 were obtained as their methyl esters.

Keywords *Aster scaber*; Compositae; scaberoside; triterpene glycoside; oleanolic acid; 3,28-*O*-bisdesmoside

In the preceding papers of this series, we reported the structures of four echinocystic acid glycosides, scaberosides A_1—A_4,[1a] and six oleanolic acid glycosides, scaberosides B_1—B_6,[1b] isolated from the root of *Aster scaber* THUNB. (Compositae). Further investigation of the less polar glycoside fraction has resulted in the isolation of three additional triterpene glycosides, I, II and III. This paper deals with their structures.

Compound I was obtained as colorless needles. It showed an $[M+Na]^+$ ion at *m/z* 1211 in the positive ion fast atom bombardment mass spectrum (FAB-MS), while the negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 1187, indicating the molecular weight to be 1188. High-resolution FAB-MS gave the molecular formula $C_{58}H_{92}O_{25}$. The nuclear magnetic resonance (NMR) signals suggested that I is a triterpene acid glycoside, similar to scaberosides described in the preceding papers. On acid hydrolysis, I gave L-arabinose, L-rhamnose, D-xylose, D-apiose and D-glucuronic acid as the component sugars. The ^{13}C-NMR spectrum of I showed signals of six C–C bonded quaternary carbons (*δ* 30.9, 36.9, 39.5, 39.9, 42.1, 47.3), two ester carbonyl carbons (*δ* 170.7, 176.2), a pair of olefinic carbons (*δ* 123.0, 144.1) (Table I), and five sugar anomeric carbons (*δ* 93.0, 100.9, 105.2, 107.2, 111.7) (Table II). The ^{1}H-NMR spectrum showed signals of seven tertiary methyl groups (*δ* 0.84, 0.94, 0.97, 1.02, 1.06, 1.28, 1.30), one trisubstituted olefinic proton (*δ* 5.44, dd, *J*=3, 3 Hz) and five sugar anomeric protons (*δ* 4.96, d, *J*=8 Hz; 5.32, d, *J*=8 Hz; 5.59, d, *J*=2 Hz; 5.97, d, *J*=4 Hz; 6.53, d, *J*=2 Hz) (Table III). Overall features of the NMR spectra suggested I to be an oleanolic acid 3,28-*O*-bisdesmoside containing L-arabinose, L-rhamnose, D-xylose, D-apiose and D-glucuronic acid methyl ester. The ^{13}C-NMR spectrum of I was compared with that of scaberoside A_3 methyl ester (IV),[1a] an echinocystic acid 3,28-*O*-bisdesmoside having the same component sugars as I. The chemical shifts of the sugar carbons of I were quite similar to those of IV suggesting that I and IV have the same sugar moiety. When I was treated with CF_3COOH–MeOH, the apiosyl group was cleaved to give a desapiosyl compound, which was identical with scaberoside B_5 methyl ester (V).[1b] The nuclear Overhauser effect (NOE) difference spectrum of I measured with irradiation at the frequency of the anomeric proton of the apiofuranosyl group showed signals of C_2-H, C_3-H and C_4-H of the rhamnopyranosyl group. The most intense signal, that of C_3-H, indicated that the D-apiofuranosyl group is linked to the C_3-hydroxyl group of the L-rhamnopyranosyl group. The configuration of the apiofuranosyl group was determined to be *β* from the difference ($\Delta[M]_D$ −295.9°) between the molecular rotation ($[M]_D$ −647.5°) of I and that ($[M]_D$ −351.6°) of V.[2] The $^1C_4$ conformation of the ester-linked α-L-arabinopyranosyl group was determined by the J_{H_1,H_2} value (2 Hz), and the J_{C_1,H_1} value (174 Hz) is consistent with this conformation.[3] From the above-mentioned data, the structure of I was determined to be as shown in the chart, and I was named scaberoside B_7 methyl ester.

Compound II was obtained as an amorphous powder

TABLE I. ^{13}C-NMR Chemical Shifts (*δ*) of the Aglycone Moieties of I—V

C No.	I	II	III	IV[1a]	V[1b]
1	38.7	38.8	38.7	38.8	38.7
2	26.5	26.5	26.5	26.6	26.5
3	89.2	88.9	89.1	89.2	89.2
4	39.5	39.4	39.4	39.5	39.5
5	55.8	55.9	55.8	55.9	55.8
6	18.5	18.5	18.5	18.5	18.5
7	33.2	33.2	33.2	33.5	33.2
8	39.9	39.9	39.9	40.0	39.9
9	48.0	48.0	48.0	47.1	48.0
10	36.9	37.0	37.0	37.0	36.9
11	23.8	23.8	23.7	23.8	23.8
12	123.0	122.9	122.6	122.8	122.9
13	144.1	144.1	144.1	144.4	144.1
14	42.1	42.1	42.3	42.0	42.1
15	28.2	28.3	28.7	36.0	28.3
16	23.2	23.2	23.4	74.0	23.2
17	47.3	47.3	47.2	49.6	47.3
18	41.7	41.7	41.9	41.3	41.7
19	46.2	46.2	46.4	47.1	46.3
20	30.9	30.8	30.7	30.9	30.9
21	34.1	34.1	34.1	36.1	34.1
22	32.7	32.6	32.3	32.0	32.7
23	28.2	28.2	28.1	28.2	28.2
24	16.9	17.0	16.8	16.9	16.9
25	15.5	15.5	15.5	15.6	15.5
26	17.5	17.4	17.4	17.6	17.4
27	26.0	26.0	25.9	27.2	26.0
28	176.2	176.2	176.5	175.9	176.2
29	33.2	33.1	33.1	33.2	33.1
30	23.7	23.6	23.8	24.8	23.7

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Table II. ^{13}C-NMR Chemical Shifts (δ) of the Sugar Moieties of I—III

	I	II	III
3-*O*-Sugar	GlcUAMe	Glc	GlcUAMe
1	107.2 (159)	106.8 (157)	107.2 (159)
2	75.4	75.7	75.4
3	77.9	78.7	77.9
4	73.1	71.8	73.1
5	77.2	78.1	77.1
6	170.7	63.0	170.7
COOMe	52.0	—	51.9
28-*O*-Sugars	Ara	Ara	Glc
1	93.0 (174)	93.4 (171)	94.7 (165)
2	75.6	75.1	75.4
3	68.7	70.0	79.5
4	65.2	66.1	71.3
5	62.0	63.0	77.8
6	—	—	69.1
	Rha	Rha	Rha
1	100.9 (172)	101.0 (171)	101.3 (172)
2	71.5	71.8	72.2
3	82.4	72.6	72.5
4	78.1	84.3	73.8
5	68.7	68.5	69.7
6	18.6	18.3	18.7
	Xyl	Xyl	Xyl
1	105.2 (160)	107.1 (162)	105.4 (157)
2	75.5	76.0	74.6
3	78.2	78.5	77.5
4	71.2	70.9	71.0
5	67.2	67.4	66.9
	Api		
1	111.7 (174)		
2	77.5		
3	79.5		
4	74.5		
5	64.4		

Abbreviations: GlcUAMe, 6-*O*-methyl-β-D-glucuronopyranosyl; Glc, D-glucopyranosyl; Ara, L-arabinopyranosyl; Xyl, D-xylopyranosyl; Rha, L-rhamnopyranosyl; Api, D-apiofuranosyl. Figures in parentheses are $^1J_{C_1H_1}$ values in hertz (Hz).

Table III. ^{1}H-NMR Chemical Shifts (δ) of the Sugar Moieties of I—III

	I	II	III
3-*O*-Sugar	GlcUAMe	Glc	GlcUAMe
1	4.96 (d, 8)	4.91 (d, 8)	4.97 (d, 8)
2	4.05 (dd, 8, 9)	*ca.*4.02	4.07 (dd, 8, 9)
3	4.23 (dd, 9, 9)	*ca.*4.25	4.25 (dd, 9, 9)
4	4.45 (dd, 9, 9)	*ca.*4.25	4.46 (dd, 9, 9)
5	4.55 (d, 9)	3.57 (m)	4.57 (d, 9)
6	—	*ca.*4.40	—
		*ca.*4.57	
COOMe	3.73		3.73
28-*O*-Sugars	Ara	Ara	Glc
1	6.53 (d, 2)	6.46 (d, 3)	6.12 (d, 8)
2	*ca.*4.50	*ca.*4.57	4.39 (dd, 8, 8)
3	*ca.*4.55	*ca.*4.50	*ca.*4.25
4	*ca.*4.40	*ca.*4.40	*ca.*4.25
5	3.95 (dd, 4, 11)	3.93 (dd, 4, 11)	*ca.*4.06
	*ca.*4.53	*ca.*4.50	
6	—	—	4.28 (dd, 5, 11)
			4.63 (dd, 2, 11)
	Rha	Rha	Rha
1	5.59 (d, 2)	5.78 (br s)	6.52 (d, 2)
2	4.72 (dd, 2, 3)	*ca.*4.57	4.75 (dd, 2, 3)
3	4.42 (dd, 3, 9)	*ca.*4.57	4.52 (dd, 3, 9)
4	*ca.*4.50	4.35 (dd, 9, 9)	4.29 (dd, 9, 9)
5	4.34 (m)	*ca.*4.40	*ca.*4.53
6	1.76 (d, 6)	1.79 (d, 6)	1.76 (d, 6)
	Xyl	Xyl	Xyl
1	5.32 (d, 8)	5.10 (d, 7)	4.89 (d, 7)
2	3.94 (dd, 8, 8)	*ca.*4.02	3.95 (dd, 7, 8)
3	4.08 (dd, 8, 8)	4.05 (dd, 8, 8)	4.09 (dd, 8, 8)
4	4.12 (m)	4.14 (m)	4.13 (m)
5	3.45 (dd, 10, 11)	3.50 (dd, 10, 11)	3.64 (dd, 10, 11)
	*ca.*4.17	*ca.*4.25	*ca.*4.25
	Api		
1	5.97 (d, 4)		
2	4.74 (d, 4)		
3	—		
4	4.16 (d, 9)		
	4.56 (d, 9)		
5	*ca.*4.04 (2H)		

Figures in parentheses are coupling constants in hertz (Hz).

and high-resolution FAB-MS gave the molecular formula $C_{52}H_{84}O_{20}$. It gave L-arabinose, L-rhamnose, D-xylose and D-glucose on acid hydrolysis, and the NMR spectra indicated that II is also an oleanolic acid 3,28-*O*-bisdesmoside. Compound II showed NMR signals similar to those of V except that the signals of the glucuronopyranosyl group in V were replaced by those of the glucopyranosyl group in II. Compound II was obtained by $NaBH_4$ reduction of V. Thus, the structure of II was determined to be as shown in the chart, and II was named scaberoside B_8.

Compound III, $C_{54}H_{86}O_{22}$, was obtained as an amorphous powder and its component sugars were L-rhamnose, D-xylose, D-glucose and D-glucuronic acid. The NMR signals indicated that III is an oleanolic acid 3,28-*O*-bisdesmoside which has a β-D-glucuronic acid methyl ester linked to C_3-OH, and a trisaccharide composed of L-rhamnose, D-xylose and D-glucose linked to the carboxyl group of the aglycone. The ^{13}C-NMR spectrum (Table II) suggested the presence of a terminal rhamnopyranosyl group and a xylopyranosyl group, and as a result, it was presumed that the β-D-glucopyranosyl unit is linked to the C_{28}-carboxyl group of the aglycone. The fragment anions at *m/z* 939 ([M− H−rha]$^-$) and 953 ([M−H−xyl]$^-$) in the negative ion FAB-MS also supported the presumption that the ester-linked sugar moiety is a branched chain trisaccharide, rhamnopyranosyl-[xylopyranosyl]-glucose. The rotating frame nuclear Overhauser effect (ROE) difference spectrum obtained by irradiation at the frequency of the anomeric proton of the rhamnopyranosyl group (rha-1H, δ 6.52) showed NOE at the signals (δ 4.39, dd, J=8, 8 Hz) assigned to glc-2H, indicating that the rhamnopyranosyl group is linked to C_2-OH of the glucopyranosyl group. The position of linkage of the xylopyranosyl group was unambiguously determined to be C_6-OH by the chemical shift (δ 69.1) of C_6 of the glucopyranosyl group. The structure of III was, therefore, determined to be as shown in the chart, and III was named scaberoside B_9 methyl ester.

Experimental[4)]

Isolation of I—III Extraction and fractionation procedures were described in the first paper[1a)] of this series. Fraction B-9 (1.09 g), obtained from the less polar glycoside fraction (Fr. B, CH_2N_2 treated), was repeatedly chromatographed on a reversed-phase octadecyl silica (ODS) column (packed column RQ-2, Fuji Gel Co., Ltd.; eluant, 75% MeOH) and a silica gel column (EtOAc : 1-propanol : H_2O, 10 : 3 : 0.5 and $CHCl_3$: MeOH : H_2O, 15 : 4 : 0.5) to yield I (83 mg). Fraction B-8 (805 mg) was also chromatographed on a Fuji Gel ODS column (75% MeOH) and finally purified by preparative high performance liquid chromatography

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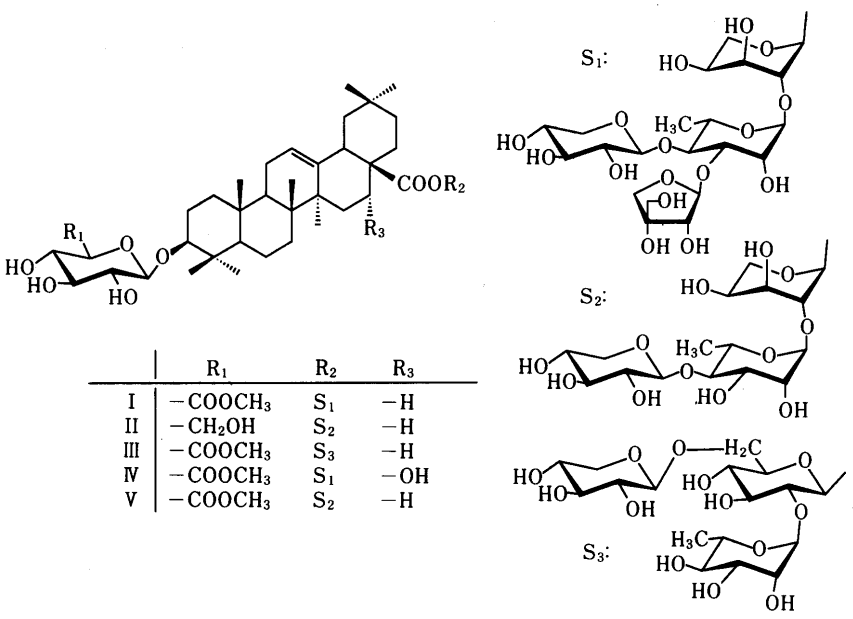

	R_1	R_2	R_3
I	$-COOCH_3$	S_1	$-H$
II	$-CH_2OH$	S_2	$-H$
III	$-COOCH_3$	S_3	$-H$
IV	$-COOCH_3$	S_1	$-OH$
V	$-COOCH_3$	S_2	$-H$

Chart 1

(HPLC) (column, Capcell Pak C18-AG120Å, 250 mm × 20 mm i.d., Shiseido Company Ltd.; eluant, 80% MeOH; recycled three times) to give II (15 mg) and III (10 mg).

Scaberoside B_7 Methyl Ester (I): Colorless needles from H_2O–MeOH, mp 230—233 °C, $[\alpha]_D^{20}$ −54.5° (c=0.9, MeOH). Positive ion high-resolution FAB-MS m/z: 1211.583 ($[M+Na]^+$). $C_{58}H_{92}NaO_{25}$ requires 1211.582. Negative ion FAB-MS m/z: 1187 ($[M-H]^-$). ^{1}H-NMR δ: aglycone moiety: 3.36 (dd, J=4, 12 Hz, C_3-H), 5.44 (dd, J=3, 3 Hz, C_{12}-H), 3.30 (dd, J=4, 14 Hz, C_{18}-H), 1.30 (C_{23}-H), 0.97 (C_{24}-H), 0.84 (C_{25}-H), 1.06 (C_{26}-H), 1.28 (C_{27}-H), 0.94 (C_{29}-H), 1.02 (C_{30}-H). Sugar moiety: shown in Table III. ^{13}C-NMR: shown in Tables I and II.

Scaberoside B_8 (II): A white powder. $[\alpha]_D^{29}$ −24.7° (c=0.7, MeOH). Positive ion high-resolution FAB-MS m/z: 1051.545 ($[M+Na]^+$). $C_{52}H_{84}NaO_{20}$ requires 1051.545. Negative ion FAB-MS m/z: 1027 ($[M-H]^-$). ^{1}H-NMR δ: aglycone moiety: 3.39 (dd, J=4, 12 Hz, C_3-H), 5.45 (dd, J=3, 3 Hz, C_{12}-H), 3.28 (dd, J=4, 14 Hz, C_{18}-H), 1.31 (C_{23}-H), 1.00 (C_{24}-H), 0.85 (C_{25}-H), 1.07 (C_{26}-H), 1.28 (C_{27}-H), 0.93 (C_{29}-H), 1.00 (C_{30}-H). Sugar moiety: shown in Table III. ^{13}C-NMR: shown in Tables I and II.

Scaberoside B_9 Methyl Ester (III): A white powder. $[\alpha]_D^{29}$ −31.6° (c=0.5, MeOH). Positive ion high-resolution FAB-MS m/z: 1109.551 ($[M+Na]^+$). $C_{54}H_{86}NaO_{22}$ requires 1109.550. Negative ion FAB-MS m/z: 1085 ($[M-H]^-$). ^{1}H-NMR δ: aglycone moiety: 3.34 (dd, J=4, 12 Hz, C_3-H), 5.42 (dd, J=3, 3 Hz, C_{12}-H), 3.17 (dd, J=4, 14 Hz, C_{18}-H), 1.24 (C_{23}-H), 0.94 (C_{24}-H), 0.89 (C_{25}-H), 1.08 (C_{26}-H), 1.27 (C_{27}-H), 0.89 (C_{29}-H), 0.95 (C_{30}-H). Sugar moiety: shown in Table III. ^{13}C-NMR δ: shown in Tables I and II.

Determination of Sugar Species and Their Absolute Configurations A glycoside (1—2 mg) was dissolved in 1 N HCl–MeOH (1 ml) and heated at 95 °C for 2 h. The acidic solution was neutralized with Ag_2CO_3 and the precipitates were centrifuged off. The supernatant was bubbled through with H_2S and concentrated. The residue was trimethylsilylated with trimethylsilylimidazole and checked by gas liquid chromatography (GC). The standard sugar samples and apiin were treated in the same way and retention times were compared with those of methanolysates of saponins. GC conditions were as follows: column, Shimadzu HiCap-CBP-1 (50 m × 0.2 mm i.d.); column oven temperature, 190 °C (for pentose and methylpentose) and 210 °C (for glucose and glucuronic acid); injection port temperature, 290 °C; carrier gas, He (linear velocity, 20 cm/s); split ratio, 1/110; make-up gas, He (50 ml/min). The sugar species identified for each saponin are given in the text.

Determination of the absolute configuration was performed according to the method reported by Hara *et al.*[5] Thus, a glycoside (1—2 mg) was hydrolyzed in a few drops of 10% H_2SO_4/1,4-dioxane mixture (1 : 1) at 100 °C for 2 h. The reaction solution was diluted with H_2O and then passed through an Amberlite MB-3 column. The eluate was concentrated to dryness 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 under an N_2 stream, and the residue was trimethylsilylated and checked by GC. The absolute configuration of glucuronic acid was determined as glucose in the same way after $NaBH_4$ reduction of the glycoside methyl ester. Standard sugar samples and the apiin hydrolysate (as D-apiose standard) were treated in the same way and the retention times were compared. The GC conditions were the same as those described above except for the column oven temperature (250 °C).

Partial Methanolysis of I Compound I (70 mg) was dissolved in 2 N CF_3COOH–MeOH solution (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 ($CHCl_3$: MeOH : H_2O, 15 : 4 : 0.5) and purified by HPLC (Capcell Pak C18, 80% MeOH) to give a thin-layer-chromatographically homogeneous desapiosyl compound (21 mg) as an amorphous powder. $[\alpha]_D^{29}$ −32.0° (c=1.0, MeOH). Positive ion FAB-MS m/z: 1079 ($[M+Na]^+$). Negative ion FAB-MS m/z: 1055 ($[M-H]^-$). The ^{1}H- and ^{13}C-NMR spectra were identical with those of V.

$NaBH_4$ Reduction of V Compound V (130 mg) and $NaBH_4$ (150 mg) were dissolved in MeOH and the solution was stirred for 24 h at room temperature. The reaction mixture was neutralized with CH_3COOH and evaporated to dryness. The residue was dissolved in 50% MeOH and passed through an Amberlite MB-3 column. The reaction product was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O, 15 : 4 : 0.5) and the reduction product (100 mg) was obtained as an amorphous powder. $[\alpha]_D^{29}$ −23.9° (c=2.6, MeOH). Positive ion FAB-MS m/z: 1051 ($[M+Na]^+$). Negative ion FAB-MS m/z: 1027 ($[M-H]^-$). The ^{1}H- and ^{13}C-NMR spectra were identical with those of II.

Acknowledgements The authors are grateful to Ms Y. Iwase, Miss J. Honda and Mr. H. Hanazono for measurements of NMR spectra and MS.

References and Notes

1) *a*) Part I: T. Nagao, R. Tanaka and H. Okabe, *Chem. Pharm. Bull.*, **39**, 1699 (1991); *b*) Part II: T. Nagao, R. Tanaka, H. Shimokawa and H. Okabe, *ibid.*, **39**, 1719 (1991).
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4) The instruments and materials used in this work were the same as those described in the preceding paper.[1a] All melting points are uncorrected. The ^{1}H- and ^{13}C-NMR spectra were measured at 400 and 100 MHz, respectively, in pyridine-d_5 solution, and the chemical shifts were expressed in the δ scale using tetramethylsilane as an internal standard.
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