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Studies on the Constituents of *Aster scaber* THUNB. V.[1] Structures of Six New Echinocystic Acid Glycosides Isolated from the Herb

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Six new echinocystic acid 3,28-*O*-bisdesmosides, scaberosides Hc_2, Hd, Hf, Hg, Hh and Hi, were isolated as their methyl esters from the ground part of *Aster scaber* THUNB. (Compositae), and their structures were determined based on spectral and chemical evidence as follows. Scaberoside Hc_2 is 3-*O*-[*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosiduronic acid] echinocystic acid 28-{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→3)]-*β*-D-xylopyranosyl}ester, scaberoside Hd, 3-*O*-[*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosiduronic acid] echinocystic acid 28-{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→3)-[*O*-*β*-D-xylopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→3)]-*β*-D-xylopyranosyl} ester, scaberoside Hf, 3-*O*-[*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranosiduronic acid] echinocystic acid 28-{*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→3)]-*β*-D-xylopyranosyl} ester, scaberoside Hg, 3-*O*-[*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranosiduronic acid] echinocystic acid 28-{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→3)]-*β*-D-xylopyranosyl} ester, scaberoside Hh, 3-*O*-{*O*-*β*-D-galactopyranosyl-(1→2)-[*O*-*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosiduronic acid} echinocystic acid 28-{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→3)]-*β*-D-xylopyranosyl} ester and scaberoside Hi, 3-*O*-[*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucopyranosiduronic acid] echinocystic acid 28-{*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-xylopyranosyl-(1→3)-[*O*-*β*-D-xylopyranosyl-(1→4)]-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*O*-*α*-L-rhamnopyranosyl-(1→3)]-*β*-D-xylopyranosyl} ester.

Keywords *Aster scaber*; triterpene glycoside; echinocystic acid; 3*β*,16*α*-dihydroxyolean-12-en-28-oic acid; scaberoside; Compositae

In the preceding paper[1] of this series, we reported the structures of four echinocystic acid glycosides, scaberosides Ha (I), Hb_1 (II), Hb_2 (III) and Hc_1 (IV), isolated from the ground part of *Aster scaber* THUNB. (Compositae). Further investigation of the polar glycoside fraction has resulted in the isolation of six additional triterpene glycosides. This paper deals with their structures.

The polar glycoside fraction obtained by silica gel chromatography was subjected to repeated chromatography on reversed-phase material (octadecyl silica (ODS)), and preparative HPLC. After methylation with CH_2N_2, methyl esters were again purified by silica gel chromatography and preparative HPLC to give compounds V, VI, VII, VIII, IX and X. All these new compounds showed very similar NMR spectra to those of the already reported scaberosides (I—IV). In particular, the signals of the aglycone moieties were almost superimposable. The NMR similarity indicated that the newly isolated polar glycosides are all echinocystic acid 3,28-*O*-bisdesmosides which differ from I—IV in the structures of their sugar moieties.

Compound V was obtained as a white powder, and the positive ion FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1505, while the negative ion FAB-MS showed an $[M-H]^-$ ion at *m/z* 1481, indicating the molecular weight to be 1482. The high-resolution (HR) FAB-MS gave the molecular formula $C_{69}H_{110}O_{34}$. On acid hydrolysis, V gave L-rhamnose, D-xylose and D-glucuronic acid as component sugars. The ^{1}H-NMR spectrum (Table III) showed seven sugar anomeric proton signals [δ 4.95 (d, $J=8$ Hz), 5.11 (d, $J=7$ Hz), 5.20 (d, $J=8$ Hz), 5.30 (d, $J=8$ Hz), 5.64 (d, $J=2$ Hz), 5.69 (d, $J=2$ Hz) and 6.48 (d, $J=4$ Hz)], and the ^{13}C-NMR spectrum (Tables I and II) also showed seven sugar anomeric carbon signals (δ 93.9, 100.9, 101.8, 105.9, 106.2, 106.3, 106.7). These NMR data suggested that V is an echinocystic acid heptaglycoside having an ester-linked sugar moiety. The similarity of the NMR spectra of V to those of scaberoside Hb_2 methyl ester (III) and the difference of molecular formula suggested that V is a xyloside of III.

The negative ion FAB-MS of V showed fragment ion peaks at *m/z* 793 and 661, which were assigned to a $[\text{prosapogenin}-H]^-$ ion and a $[793-\text{xylosyl group}]^-$ ion, respectively, thus suggesting that the prosapogenin is a xylosyl-glucosiduronic acid methyl ester of echinocystic acid. On selective cleavage of the ester-linked sugar moiety according to Ohtani's method[2] followed by treatment with CH_2N_2, V provided a prosapogenin methyl ester (XI) and an anomeric mixture of a methyl oligoglycoside (XII). The *β*-(XIIa) and *α*-anomer (XIIb) were separated. Their NMR spectra were superimposable on those of the corresponding anomers of the methyl oligoglycoside (the methyl glycoside of S_3 in Chart 1) obtained from III.

The prosapogenin methyl ester (XI) showed an $[M+Na]^+$ ion at *m/z* 831 in the positive ion FAB-MS, and it gave D-xylose and D-glucuronic acid on acid hydrolysis. The ^{13}C-NMR spectrum of XI was compared with that of 3-*O*-*β*-glucuronopyranosyl echinocystic acid dimethyl ester (XIII), the prosapogenin methyl ester derived from III. The chemical shift of C_3 (δ 86.2) of the glucuronopyranosyl group of XI was shifted downfield (Δ 8.3 ppm), indicating that XI is 3-*O*-[*O*-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucuronopyranosyl] echinocystic acid dimethyl ester. Thus, the structure of V was determined to be as shown in Chart 1, and V was named scaberoside Hc_2 methyl ester. The configuration and conformation of the ester-linked xylopyranosyl group of V were determined to be *β* and $^1C_4$ from

TABLE I. ^{13}C-NMR Chemical Shifts of Prosapogenin Moieties of Scaberosides[a] and Prosapogenin Methyl Esters

	I	V	VII	IX	XIII	XI	XV	XVII
1	38.8	38.7	38.8	38.7	38.7	38.6	38.7	38.7
2	26.6	26.5	26.6	26.5	26.6	26.5	26.6	26.5
3	89.1	89.4	89.2	89.7	89.1	89.3	89.2	89.7
4	39.5	39.5	39.5	39.6	39.5	39.5	39.5	39.5
5	55.9	55.8	55.9	55.9	55.8	55.8	55.8	55.8
6	18.4	18.5	18.5	18.5	18.4	18.4	18.4	18.4
7	33.5	33.4	33.5	33.4	33.3	33.3	33.3	33.3
8	40.0	40.0	40.0	39.9	39.8	39.8	39.8	39.8
9	47.1	47.1	47.1	47.0	47.0	47.0	47.0	47.0
10	36.9	36.9	36.9	36.9	37.0	36.9	36.9	36.9
11	23.8	23.8	23.8	23.7	23.7	23.7	23.7	23.7
12	122.7	122.8	122.8	122.8	122.6	122.6	122.6	122.6
13	144.3	144.3	144.3	144.3	144.4	144.4	144.5	144.4
14	42.0	42.0	42.0	42.0	41.9	41.9	41.9	41.8
15	36.1	36.1	36.1	36.1	35.9	35.9	35.9	35.9
16	73.9	73.9	73.9	73.9	74.3	74.3	74.3	74.3
17	49.4	49.4	49.4	49.3	49.0	49.0	49.0	49.0
18	41.4	41.4	41.4	41.4	41.2	41.2	41.2	41.2
19	47.1	47.1	47.1	47.0	46.9	46.9	46.9	46.9
20	30.8	30.8	30.8	30.8	30.8	30.8	30.8	30.8
21	35.9	35.9	35.9	35.9	35.8	35.9	35.9	35.9
22	32.1	32.2	32.2	32.2	32.4	32.4	32.4	32.4
23	28.1	28.0	28.1	28.0	28.1	28.0	28.1	28.0
24	16.9	16.9	16.7	16.7	16.9	16.9	16.7	16.7
25	15.6	15.5	15.6	15.5	15.5	15.5	15.5	15.5
26	17.4	17.5	17.5	17.5	17.2	17.2	17.2	17.1
27	27.0	27.0	27.0	27.0	27.1	27.1	27.1	27.1
28	175.7	175.8	175.7	175.7	177.7	177.7	177.7	177.7
29	33.1	33.2	33.1	33.1	33.1	33.1	33.1	33.1
30	24.6	24.7	24.6	24.6	24.6	24.6	24.6	24.5
–COOMe					51.7	51.7	51.7	51.7
GA-1	107.2	106.7	105.3	105.4	107.2	106.7	105.2	105.3
2	75.3	74.4	83.7	79.4	75.4	74.4	83.6	79.4
3	77.8	86.2	77.4	85.6	77.9	86.2	77.4	85.6
4	73.1	71.1	72.8	71.2	73.1	71.1	72.7	71.1
5	77.1	76.7	76.6	76.3	77.1	76.6	76.6	76.3
6	170.7	170.2	170.4	169.9	170.7	170.1	170.4	169.9
–COOMe	51.9	52.1	52.0	52.1	51.9	52.1	52.0	52.1
gal-1			107.1	104.5			107.0	104.5
2			74.6	73.7			74.5	73.7
3			74.8	75.1			74.8	75.2
4			69.5	69.7			69.5	69.7
5			76.8	76.5			76.8	76.6
6			61.3	61.5			61.3	61.5
xyl-1		106.2		104.9		106.1		104.9
2		75.2		75.1		75.2		75.1
3		78.0		78.4		78.0		78.4
4		70.8		70.7		70.8		70.7
5		67.3[b]		67.2		67.3		67.2

Abbreviations: GA, 6-*O*-methyl-*β*-D-glucuronopyranosyl; gal, *β*-D-galactopyranosyl; xyl, *β*-D-xylopyranosyl. *a*) Chemical shifts of the prosapogenin moieties of VI, VIII and X are almost the same as those of V, VII and VII, respectively. *b*) Assignment may be interchanged with Xc-5 of V in Table II.

TABLE II. ^{13}C-NMR Chemical Shifts of Sugar Moieties of Scaberosides

	V	VI[a]	VII	VIII	IX	X[a]
Xa-1	93.9	94.0	94.0	93.9	93.9	94.0
2	74.5	75.2	74.3	74.6	74.5	75.2
3	78.5		79.2	78.6	78.8	
4	68.1		68.3	68.1	68.1	
5	65.0		65.1	65.0	65.0	
Ra-1	100.9	100.8	100.8	100.9	100.9	100.6
2	72.5	71.3	72.5	72.5	72.5	71.2
3	71.6	82.1	71.6	71.6	71.6	82.1
4	83.5		83.8	83.5	83.5	
5	68.7		68.8	68.7	68.7	
6	18.4		18.4	18.4	18.4	
Rb-1	101.8	101.8	102.0	101.8	101.8	101.8
2	72.2	72.3	72.3	72.3	72.3	72.3
3	72.5	72.5	72.5	72.5	72.5	72.5
4	73.7		73.7	73.7	73.7	
5	70.5		70.6	70.6	70.5	
6	18.3		18.3	18.3	18.3	
Xb-1	106.3	105.1	106.9	106.3	106.3	105.1
2	74.7	75.6	75.8	74.8	74.8	75.5
3	87.0		78.4	86.9	86.9	
4	69.0		70.9	69.0	69.0	
5	66.8		67.4	66.8	66.7	
Xc-1	105.9			105.9	105.8	
2	75.2			75.2	75.1	
3	78.0			78.0	78.0	
4	70.8			70.8	70.8	
5	67.2[b]			67.2	67.2	
Xd-1		105.1				105.1
2		73.8				73.8
3		86.5				86.4
Xe-1		105.8				105.8

Abbreviations: X, *β*-D-xylopyranosyl; R, *α*-L-rhamnopyranosyl. *a*) Blank spaces indicate that signals were not assigned. *b*) Assignment may be interchanged with xyl-5 of V in Table I.

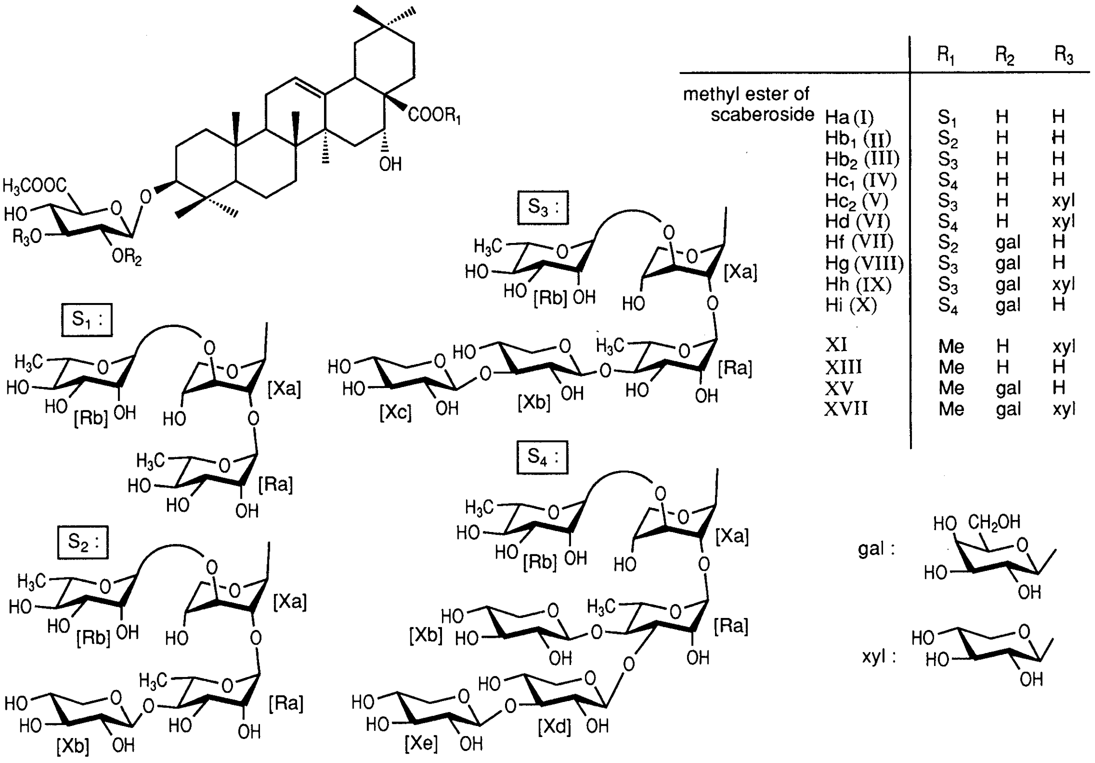
the values of $J_{H_1H_2}$ (4 Hz), $J_{H_2H_3}$ (4 Hz) and the chemical shift (δ 93.9) of the anomeric carbon.

Compound VI, $C_{74}H_{118}O_{38}$, was obtained as a white powder, and it gave L-rhamnose, D-xylose and D-glucuronic acid on acid hydrolysis. ^{1}H- and ^{13}C-NMR spectra suggested that VI is composed of the prosapogenin of V and the ester-linked sugar of scaberoside Hc$_1$ methyl ester (IV). Selective cleavage of the ester-linked sugar moiety followed by treatment with CH_2N_2, VI gave XI and an anomeric mixture of a methyl oligoglycoside (XIV). The *β*-anomer (XIVa) and the *α*-anomer (XIVb) of XIV were respectively identical with the *β*- and the *α*-anomer of the methyl oligoglycosides which were derived from IV. The structure of VI was, therefore, determined to be as shown in Chart 1, and VI was named scaberoside Hd methyl ester.

Compound VII, $C_{65}H_{104}O_{31}$, was obtained as a white powder, and the component sugars were identified as L-rhamnose, D-xylose, D-galactose and D-glucuronic acid. In the negative ion FAB-MS, VII showed an $[M-H]^-$ ion peak at *m*/*z* 1379, and fragment ion peaks at *m*/*z* 823 and 661 which were assigned to a $[\text{prosapogenin}-H]^-$ anion and an $[823-\text{galactosyl group}]^-$ anion, respectively. On selective cleavage of the ester-linked sugar moiety followed by treatment with CH_2N_2, VII gave a prosapogenin methyl ester (XV) and an anomeric mixture of a methyl oligoglycoside (XVI). Compound XV showed an $[M+Na]^+$ ion peak at *m*/*z* 861 in the positive ion FAB-MS, and it gave D-galactose and D-glucuronic acid on acid hydrolysis. By comparison of the ^{13}C-NMR data of XV with those of XIII, XV was presumed to be a galactopyranosyl XIII. The signal of C_2 (δ 83.6) of the glucuronopyranosyl group of XV appeared downfield (Δ 8.2 ppm) compared with that of XIII, and therefore, it is apparent that the galactopyranosyl group is linked to C_2-OH of the glucuronopyranosyl group. The *β*-anomer (XVIa) and the *α*-anomer (XVIb) of the methyl oligoglycoside XVI were identical with the respective anomers of the methyl oligoglycoside derived from scaberoside Hb$_1$ methyl ester (II). Therefore, the structure of VII was determined to be as shown in Chart

Table III. ^{1}H-NMR Chemical Shifts of Sugar Moieties of Scaberosides

	V	VI	VII	VIII	IX	X
3-*O*-Sugar moieties of						
GA-1	4.95 (d, $J=8$ Hz)	4.95 (d, $J=8$ Hz)	4.96 (d, $J=8$ Hz)	4.96 (d, $J=8$ Hz)	4.95 (d, $J=8$ Hz)	4.96 (d, $J=8$ Hz)
gal-1			5.18 (d, $J=8$ Hz)	5.18 (d, $J=8$ Hz)	5.52 (d, $J=8$ Hz)	5.19 (d, $J=8$ Hz)
xyl-1	5.30 (d, $J=8$ Hz)	5.29 (d, $J=8$ Hz)			5.29 (d, $J=8$ Hz)	
28-*O*-Sugar moieties of						
Xa-1	6.48 (d, $J=4$ Hz)	6.48 (d, $J=4$ Hz)	6.45 (d, $J=4$ Hz)	6.48 (d, $J=4$ Hz)	6.47 (d, $J=4$ Hz)	6.50 (d, $J=4$ Hz)
2	4.23 (dd, $J=4$, 4 Hz)	4.23 (dd, $J=4$, 4 Hz)	4.25 (dd, $J=4$, 4 Hz)	4.24 (dd, $J=4$, 4 Hz)	4.24 (dd, $J=4$, 4 Hz)	4.28 (dd, $J=4$, 4 Hz)
Ra-1	5.69 (d, $J=2$ Hz)	5.70 (s)	5.74 (d, $J=2$ Hz)	5.70 (s)	5.71 (s)	5.70 (s)
Rb-1	5.64 (d, $J=2$ Hz)	5.65 (s)	5.65 (d, $J=2$ Hz)	5.64 (s)	5.65 (s)	5.65 (s)
Xb-1	5.11 (d, $J=7$ Hz)	5.31 (d, $J=8$ Hz)	5.11 (d, $J=7$ Hz)	5.11 (d, $J=7$ Hz)	5.11 (d, $J=7$ Hz)	5.31 (d, $J=8$ Hz)
Xc-1	5.20 (d, $J=8$ Hz)			5.21 (d, $J=8$ Hz)	5.20 (d, $J=8$ Hz)	
Xd-1		5.17 (d, $J=7$ Hz)				5.17 (d, $J=7$ Hz)
Xe-1		5.08 (d, $J=7$ Hz)				5.08 (d, $J=8$ Hz)



		R₁	R₂	R₃
methyl ester of scaberoside	Ha (I)	S₁	H	H
	Hb₁ (II)	S₂	H	H
	Hb₂ (III)	S₃	H	H
	Hc₁ (IV)	S₄	H	H
	Hc₂ (V)	S₃	H	xyl
	Hd (VI)	S₄	H	xyl
	Hf (VII)	S₂	gal	H
	Hg (VIII)	S₃	gal	H
	Hh (IX)	S₃	gal	xyl
	Hi (X)	S₄	gal	H
	XI	Me	H	xyl
	XIII	Me	H	H
	XV	Me	gal	H
	XVII	Me	gal	xyl

Chart 1

1, and VII was named scaberoside Hf methyl ester.

Compound VIII, $C_{70}H_{112}O_{35}$, colorless needles, gave L-rhamnose, D-xylose, D-galactose and D-glucuronic acid as the component sugars. Compound VIII was presumed to be a galactopyranosyl III judging from its NMR data. On selective cleavage of the ester-linked sugar moiety followed by treatment with CH_2N_2, VIII provided a prosapogenin methyl ester XV and a methyl oligoglycoside XII. Thus, the structure of VIII was determined as shown in Chart 1, and VIII was named scaberoside Hg methyl ester.

Compound IX, $C_{75}H_{120}O_{39}$, a white powder, gave L-rhamnose, D-xylose, D-galactose and D-glucuronic acid on acid hydrolysis. In the negative ion FAB-MS, IX showed an $[M-H]^-$ ion peak at *m/z* 1643 and fragment ion peaks at *m/z* 955, 823, 793 and 661, which were assigned to anions of $[\text{prosapogenin}-H]^-$, $[955-\text{xylosyl group}]^-$, $[955-\text{galactosyl group}]^-$ and $[955-\text{xylosyl group}-\text{galactosyl group}]^-$, respectively. These fragment anions suggested that IX has a branched-chain sugar structure in the prosapogenin moiety. On selective cleavage of the ester-linked sugar moiety followed by treatment with CH_2N_2, IX gave a prosapogenin methyl ester XVII and a methyl oligoglycoside XII. Compound XVII showed an $[M+Na]^+$ ion at *m/z* 993 in the positive ion FAB-MS, and it gave D-xylose, D-galactose and D-glucuronic acid on acid hydrolysis. In order to determine the sugar linkage, the ^{1}H-NMR spectrum was carefully examined. All ^{1}H-NMR signals were assigned using the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) technique, and the rotating frame nuclear Overhauser effect (ROE) difference spectra were measured with irradiation at the frequencies of the anomeric protons of a xylopyranosyl group (xyl-1H, δ 5.29) and a galactopyranosyl group (gal-1H, δ 5.54). When the signal of xyl-1H was irradiated, nuclear Overhauser effect (NOE) was observed at the signal of C_3-H of a glucuronopyranosyl group [GA-3H, δ 4.28 (dd, $J=9$, 9 Hz)] (Fig. 1b). On the

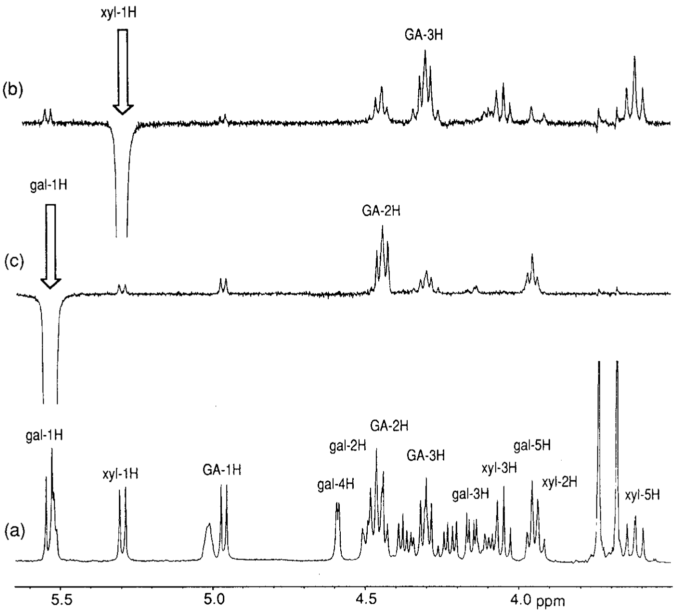


Fig. 1. ^{1}H-NMR Spectrum (a) and ROE Difference Spectra (b, c) of XVII

other hand, NOE was observed at the signals of GA-2H [δ 4.44 (dd, $J=8$, 9 Hz)] when gal-1H was irradiated (Fig. 1c). These results indicated that the galactopyranosyl group is linked to C_2-OH and a xylopyranosyl group is linked to C_3-OH of the glucuronopyranosyl group. The structures of XVII and IX were, therefore, determined to be as shown in the chart. Compound IX was named scaberoside Hh methyl ester.

Compound X, $C_{75}H_{120}O_{39}$, a white powder, gave L-rhamnose, D-xylose, D-galactose and D-glucuronic acid. On selective cleavage of the ester-linked sugar moiety followed by treatment with CH_2N_2, X provided a prosapogenin methyl ester XV and methyl oligoglycosides XIVa and XIVb. Thus, the structure of X was determined to be as shown in the chart, and X was named scaberoside Hi methyl ester.

Experimental[3)]

Isolation of V—X Extraction and primary fractionation of the crude glycoside fraction were reported in the preceding paper.[1)] Fractions 8—10, obtained by silica gel chromatography, were combined and subjected to repeated column chromatography on an ODS column (YMC-gel ODS-AQ-120-S50, YMC Co. Ltd.; eluant, 25—35% MeCN/0.5% AcOH) and preparative HPLC (Capcell pak C18-AG120 Å, 20 mm i.d. × 250 mm L., Shiseido Co. Ltd.; 45—50% acetone/0.5% AcOH). The fractions containing triterpene glycosides were treated with CH_2N_2, and further subjected to silica gel chromatography (EtOAc : MeOH : H_2O, 8 : 1 : 0.5—7 : 2 : 1) and preparative HPLC (Cosmosil $5C_{18}$-AR, 20 mm i.d. × 250 mm length, Nacalai Tesque; 48—55% acetone) to give V (117 mg), VI (204 mg), VII (72 mg), VIII (105 mg), IX (90 mg) and X (162 mg).

Scaberoside Hc$_2$ Methyl Ester (V): A white powder, $[\alpha]_D^{24}$ −67.4° ($c=1.0$, MeOH). Positive ion FAB-MS m/z: 1505 ($[M+Na]^+$), 711 ($[2rha+3xyl+Na]^+$). Positive ion high-resolution (HR) FAB-MS m/z: 1505.679. $C_{69}H_{110}NaO_{34}$ requires 1505.677. Negative ion FAB-MS m/z: 1481 ($[M-H]^-$), 793 ($[\text{prosapogenin}-H]^-$), 661 ($[793-\text{xyl}]^-$). ^{1}H-NMR δ: aglycone moiety: 3.34 (dd, $J=4$, 12 Hz, C_3-H), 5.60 (dd, $J=3$, 3 Hz, C_{12}-H), 5.18 (br s, C_{16}-H), 1.26 (C_{23}-H), 0.97 (C_{24}-H), 0.81 (C_{25}-H), 1.01 (C_{26}-H), 1.77 (C_{27}-H), 1.00 (C_{29}-H), 1.13 (C_{30}-H); sugar moiety: shown in Table III. ^{13}C-NMR: shown in Tables I and II.

Scaberoside Hd Methyl Ester (VI): A white powder, $[\alpha]_D^{26}$ −73.6° ($c=0.9$, MeOH). Positive ion FAB-MS m/z: 1637 ($[M+Na]^+$), 843 ($[2rha+4xyl+Na]^+$). Positive ion HR-FAB-MS m/z: 1637.719. $C_{74}H_{118}NaO_{38}$ requires 1637.719. Negative ion FAB-MS m/z: 1613 ($[M-H]^-$), 793 ($[\text{prosapogenin}-H]^-$), 661 ($[793-\text{xyl}]^-$). ^{1}H-NMR δ: aglycone moiety: 3.35 (dd, $J=4$, 12 Hz, C_3-H), 5.60 (dd, $J=3$, 3 Hz, C_{12}-H), 5.18 (br s, C_{16}-H), 1.27 (C_{23}-H), 0.98 (C_{24}-H), 0.84 (C_{25}-H), 1.04 (C_{26}-H), 1.77 (C_{27}-H), 0.99 (C_{29}-H), 1.15 (C_{30}-H); sugar moiety: shown in Table III. ^{13}C-NMR: shown in Tables I and II.

Scaberoside Hf Methyl Ester (VII): A white powder, $[\alpha]_D^{25}$ −67.7° ($c=1.1$, MeOH). Positive ion FAB-MS m/z: 1403 ($[M+Na]^+$), 579 ($[2rha+2xyl+Na]^+$). Postivive ion HR-FAB-MS m/z: 1403.645. $C_{65}H_{104}NaO_{31}$ requires 1403.645. Negative ion FAB-MS m/z: 1379 ($[M-H]^-$), 823 ($[\text{prosapogenin}-H]^-$), 661 ($[823-\text{gal}]^-$. ^{1}H-NMR δ: aglycone moiety: 3.28 (dd, $J=4$, 12 Hz, C_3-H), 5.60 (dd, $J=3$, 3 Hz, C_{12}-H), 5.20 (br s, C_{16}-H), 3.44 (dd, $J=4$, 14 Hz, C_{18}-H), 1.28 (C_{23}-H), 1.09 (C_{24}-H), 0.84 (C_{25}-H), 1.03 (C_{26}-H), 1.76 (C_{27}-H), 1.00 (C_{29}-H), 1.12 (C_{30}-H); sugar moiety; shown in Table III. ^{13}C-NMR: shown in Tables I and II.

Scaberoside Hg Methyl Ester (VIII): Colorless needles from H_2O–MeOH, mp 260—261 °C, $[\alpha]_D^{25}$ −67.3° ($c=1.0$, MeOH). Positive ion FAB-MS m/z: 1535 ($[M+Na]^+$), 711 ($[2rha+3xyl+Na]^+$). Positive ion HR-FAB-MS m/z: 1535.688. $C_{70}H_{112}NaO_{35}$ requires 1535.688. Negative ion FAB-MS m/z: 1511 ($[M-H]^-$), 823 ($[\text{prosapogenin}-H]^-$), 661 ($[823-\text{gal}]^-$). ^{1}H-NMR δ: aglycone moiety: 3.28 (dd, $J=4$, 12 Hz, C_3-H), 5.60 (dd, $J=3$, 3 Hz, C_{12}-H), 5.20 (br s, C_{16}-H), 1.28 (C_{23}-H), 1.09 (C_{24}-H), 0.83 (C_{25}-H), 1.02 (C_{26}-H), 1.76 (C_{27}-H), 0.99 (C_{29}-H), 1.13 (C_{30}-H); sugar moiety: shown in Table III. ^{13}C-NMR: shown in Tables I and II.

Scaberoside Hh Methyl Ester (IX): A white powder, $[\alpha]_D^{24}$ −56.9° ($c=0.9$, MeOH). Positive ion FAB-MS m/z: 1667 ($[M+Na]^+$), 711 ($[2rha+3xyl+Na]^+$). Positive ion HR-FAB-MS m/z: 1667.730. $C_{75}H_{120}NaO_{39}$ requires 1667.730. Negative ion FAB-MS m/z: 1643 ($[M-H]^-$), 955 ($[\text{prosapogenin}-H]^-$), 823 ($[955-\text{xyl}]^-$), 793 ($[955-\text{gal}]^-$), 661 ($[955-\text{xyl}-\text{gal}]^-$). ^{1}H-NMR δ: aglycone moiety; 3.27 (dd, $J=4$, 12 Hz, C_3-H), 5.60 (dd, $J=3$, 3 Hz, C_{12}-H), 5.18 (br s, C_{16}-H), 1.29 (C_{23}-H), 1.09 (C_{24}-H), 0.80 (C_{25}-H), 1.00 (C_{26}-H), 1.75 (C_{27}-H), 0.99 (C_{29}-H), 1.13 (C_{30}-H); sugar moiety; shown in Table III. ^{13}C-NMR: shown in Tables I and II.

Scaberoside Hi Methyl Ester (X): A white powder, $[\alpha]_D^{23}$ −64.4° ($c=0.8$, MeOH). Positive ion FAB-MS m/z: 1667 ($[M+Na]^+$), 843 ($[2rha+4xyl+Na]^+$). Positive ion HR-FAB-MS m/z: 1667.730. $C_{75}H_{120}NaO_{39}$ requires 1667.730. Negative ion FAB-MS m/z: 1643 ($[M-H]^-$), 823 ($[\text{prosapogenin}-H]^-$), 661 ($[823-\text{gal}]^-$). ^{1}H-NMR δ: aglycone moiety: 3.28 (dd, $J=4$, 12 Hz, C_3-H), 5.60 (dd, $J=3$, 3 Hz, C_{12}-H), 5.17 (br s, C_{16}-H), 1.29 (C_{23}-H), 1.10 (C_{24}-H), 0.85 (C_{25}-H), 1.04 (C_{26}-H), 1.75 (C_{27}-H), 0.99 (C_{29}-H), 1.14 (C_{30}-H); sugar moiety: shown in Table III. ^{13}C-NMR: shown in Tables I and II.

Determination of Sugar Species and Their Absolute Configurations A glycoside (*ca.* 3 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 concentrated and the residue was trimethylsilylated with trimethylsilylimidazole, and checked by GC. The GC conditions were as follows: column, Shimadzu HiCap-CBP-1 (50 m × 0.2 mm i.d.); column oven temperature, 200 °C; injection port temperature, 290 °C; carrier gas, He (linear velocity, 20 cm/s); split ratio, 1/110; make-up gas, He (50 ml/min). Determination of the absolute configuration was performed according to the method reported by Hara *et al.*[4)] A glycoside (5−10 mg) was hydrolyzed in 1 N HCl (1 ml) at 95 °C for 2 h. The acidic solution was neutralized in the same manner as described above. The hydrolysate was suspended in H_2O and extracted with $CHCl_3$ to remove the aglycone. The aqueous layer was concentrated 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 identification was performed by comparison of the retention time with that of an authentic sugar sample. The absolute configuration of glucuronic acid was determined in the same way after $NaBH_4$ reduction to 3-*O*-β-glucopyranoside. The GC conditions for the determination of the absolute configurations of the component monosaccharides were the same as those described above except for the column oven temperature (250 °C). The results are presented 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.*[2)] Compound V (65 mg) and anhydrous LiI (300 mg) were dissolved in a mixture of MeOH (1 ml) and 2,6-lutidine (2 ml). The reaction solution was heated at 180 °C for 3 h, then diluted with 50% MeOH and passed through an Amberlite MB-3 column. The eluate was concentrated to dryness, and the residue was dissolved in MeOH and treated with CH_2N_2. The reaction mixture was chromatographed on silica gel (EtOAc : MeOH : H_2O, 8 : 2 : 0.5) to give a prosapogenin methyl ester (XI, 25 mg) and an anomeric mixture of a methyl glycoside (XII, 23 mg).

Prosapogenin Methyl Ester (XI): A white powder, $[\alpha]_D^{26}$ $-9.6°$ ($c=0.9$, MeOH). Positive ion FAB-MS *m*/*z*: 831 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety: 3.38 (2H, C_3-H and C_{18}-H were overlapped), 5.53 (dd, $J=3$, 3 Hz, C_{12}-H), 5.02 (br s, C_{16}-H), 1.28 (C_{23}-H), 0.98 (C_{24}-H), 0.89 (C_{25}-H), 0.87 (C_{26}-H), 1.79 (C_{27}-H), 1.03 (C_{29}-H), 1.10 (C_{30}-H), 3.68 (-COOMe); sugar moiety: glucuronopyranosyl group, 4.97 (d, $J=8$ Hz, anomeric H), 4.08 (dd, $J=8$, 9 Hz, C_2-H), 4.27 (dd, $J=9$, 9 Hz, C_3-H), 4.36 (dd, $J=9$, 9 Hz, C_4-H), 4.55 (d, $J=9$ Hz, C_5-H), 3.74 (-COOMe); xylopyranosyl group: 5.29 (d, $J=8$ Hz, anomeric H), 3.99 (dd, $J=8$, 8 Hz, C_2-H), *ca.* 4.13 (2H, C_3- and C_4-H), *ca.* 3.68 (C_5-H), 4.31 (dd, $J=4$, 12 Hz, C_5-H). ^{13}C-NMR: shown in Table I.

XII: A white powder. Positive ion FAB-MS *m*/*z*: 743 ($[M+Na]^+$). Negative ion FAB-MS *m*/*z*: 719 ($[M-H]^-$), 587, 573, 455, 441, 309, 163. Compound XII was subjected to preparative HPLC to give a β-anomer (XIIa, 8 mg) and an α-anomer (XIIb, 9 mg) as white powders, and their ^{1}H- and ^{13}C-NMR spectra were superimposable on those of the anomers of methyl oligoglycosides which were derived from scaberoside Hb_2 methyl ester (III) in the same manner.

Compounds VI, VII, VIII, IX and X were treated in the same way as described for V to give a prosapogenin methyl ester, and anomers of a methyl oligoglycoside.

Compound VI (160 mg) gave XI (70 mg), XIVa (14 mg) and XIVb (20 mg).

XIVa, XIVb: A white powder. Positive ion FAB-MS *m*/*z*: 875 ($[M+Na]^+$). Negative ion FAB-MS *m*/*z*: 851 ($[M-H]^-$), 719, 705, 587, 573, 455, 441, 309, 163. The ^{1}H- and ^{13}C-NMR spectra of XIVa and XIVb were superimposable on those of the methyl oligoglycoside anomers derived from scaberoside Hc_1 methyl ester (IV).

Compound VII (65 mg) gave XV (25 mg), XVIa (8 mg) and XVIb (8 mg).

XV: A white powder, $[\alpha]_D^{24}$ $-9.3°$ ($c=1.0$, MeOH). Positive ion FAB-MS *m*/*z*: 861 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety: 3.31 (dd, $J=4$, 12 Hz, C_3-H), 5.53 (dd, $J=3$, 3 Hz, C_{12}-H), 5.01 (br s, C_{16}-H), 3.38 (dd, $J=4$, 14 Hz, C_{18}-H), 1.30 (C_{23}-H), 1.10 (C_{24}-H), 0.90 (C_{25}-H), 0.88 (C_{26}-H), 1.78 (C_{27}-H), 1.02 (C_{29}-H), 1.10 (C_{30}-H), 3.68 (-COOMe); sugar moiety: glucuronopyranosyl group: 4.98 (d, $J=8$ Hz, anomeric H), 4.22 (dd, $J=8$, 9 Hz, C_2-H), 4.29 (dd, $J=9$, 9 Hz, C_3-H), 4.40 (dd, $J=9$, 9 Hz, C_4-H), 4.49 (d, $J=9$ Hz, C_5-H), 3.72 (-COOMe); galactopyranosyl group: 5.20 (d, $J=8$ Hz, anomeric H), 4.54 (dd, $J=8$, 9 Hz, C_2-H), 4.20 (dd, $J=3$, 9 Hz, C_3-H), 4.66 (br d, $J=3$ Hz, C_4-H), 4.04 (br dd, $J=5$, 7 Hz, C_5-H), 4.38 (dd, $J=5$, 10 Hz, C_6-H), 4.57 (dd, $J=7$, 10 Hz, C_6-H). ^{13}C-NMR: shown in Table I.

XVIa, XVIb: A white powder. Positive ion FAB-MS *m*/*z*: 611 ($[M+Na]^+$). Negative ion FAB-MS *m*/*z*: 587 ($[M-H]^-$), 455, 441, 309, 163. The ^{1}H- and ^{13}C-NMR spectra of XVIa and XVIb were superimposable on those of the methyl oligoglycoside anomers derived from scaberoside Hb_1 methyl ester (II).

Compound VIII (85 mg) gave XV (30 mg), XIIa (10 mg) and XIIb (10 mg), IX (85 mg) gave XVII (30 mg), XIIa (12 mg) and XIIb (14 mg), and X (100 mg) gave XV (26 mg), XIVa (15 mg) and XIVb (18 mg).

XVII: A white powder, $[\alpha]_D^{24}$ $-3.1°$ ($c=1.5$, MeOH). Positive ion FAB-MS *m*/*z*: 993 ($[M+Na]^+$). ^{1}H-NMR δ: aglycone moiety: 3.30 (dd, $J=4$, 12 Hz, C_3-H), 5.52 (dd, $J=3$, 3 Hz, C_{12}-H), 5.02 (br s, C_{16}-H), 3.38 (dd, $J=4$, 14 Hz, C_{18}-H), 1.30 (C_{23}-H), 1.11 (C_{24}-H), 0.87 (C_{25}-H), 0.86 (C_{26}-H), 1.77 (C_{27}-H), 1.02 (C_{29}-H), 1.10 (C_{30}-H), 3.68 (-COOMe); sugar moiety: glucuronopyranosyl group: 4.97 (d, $J=8$ Hz, anomeric H), 4.44 (dd, $J=8$, 9 Hz, C_2-H), 4.28 (dd, $J=9$, 9 Hz, C_3-H), 4.32 (dd, $J=9$, 9 Hz, C_4-H), 4.45 (d, $J=9$ Hz, C_5-H), 3.74 (-COOMe); galactopyranosyl group: 5.54 (d, $J=8$ Hz, anomeric H), 4.48 (dd, $J=8$, 9 Hz, C_2-H), 4.15 (dd, $J=3$, 9 Hz, C_3-H), 4.59 (br d, $J=3$ Hz, C_4-H), 3.95 (br dd, $J=6$, 7 Hz, C_5-H), 4.37 (dd, $J=6$, 10 Hz, C_6-H), *ca.* 4.50 (C_6-H); xylopyranosyl group: 5.29 (d, $J=8$ Hz, anomeric H), 3.92 (dd, $J=8$, 9 Hz, C_2-H), 4.05 (dd, $J=9$, 9 Hz, C_3-H), 4.09 (m, C_4-H), 3.62 (dd, $J=10$, 11 Hz, C_5-H), 4.22 (dd, $J=5$, 11 Hz, C_5-H). ^{13}C-NMR: shown in Table I.

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References and Notes

1) T. Nagao, R. Tanaka, Y. Iwase, H. Okabe, *Chem. Pharm. Bull.*, **41**, 659 (1993).

2) K. Ohtani, K. Mizutani, R. Kasai, O. Tanaka, *Tetrahedron Lett.*, **25**, 4537 (1984).

3) The instruments used in this work were as follows: Yanaco micro melting point apparatus (melting point), JASCO DIP-360 digital polarimeter (specific rotation), JEOL JNM GX-400 spectrometer (^{1}H- and ^{13}C-NMR spectra), JEOL HX-110 spectrometer (MS), Shimadzu GC-8APF gas chromatograph (GC). Melting point is uncorrected. NMR spectra were measured in pyridine-d_5 solution and chemical shifts are expressed in the δ scale using tetramethylsilane as an internal standard.

4) S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.*, **35**, 501 (1987).