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Legume Saponins of *Gleditsia japonica* Miquel. IV.[1] ^{13}C-Nuclear Magnetic Resonance Spectral Studies for Structure Elucidation of Gleditsia Saponins B and C

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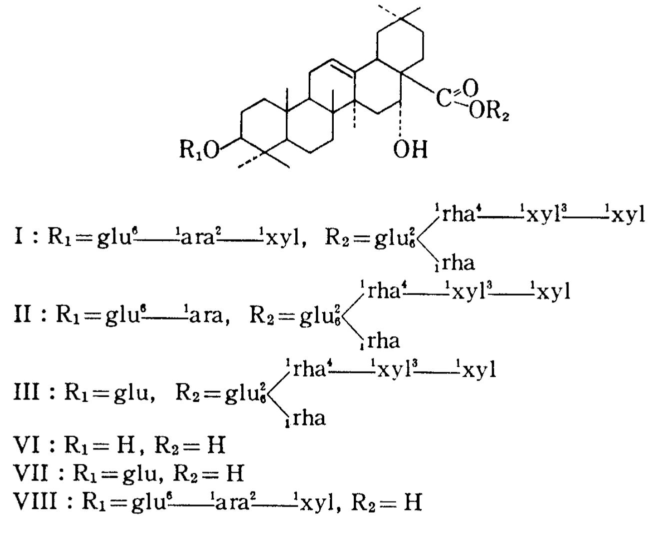
Two triterpenoid saponins, gleditsia saponins B (GS-B, XIX) and C (GS-C, XX) which were isolated from *Gleditsia japonica* cv. 'Saponifera', were characterized as 3,28-*O*-bisglycosides of echinocystic acid acylated with monoterpene carboxylic acids. On the basis of ^{13}C-nuclear magnetic resonance spectra and the results of chemical and enzymatic hydrolysis, it was established that the positions of acylation with the mono-terpenes (IV) and (V) were C-2 and C-3, respectively, of the terminal rhamnose of the 28-*O*-glycoside moiety in the molecules of GS-C and GS-B.

Keywords——saponin; saponin acylated with monoterpene; gleditsia saponin B; gleditsia saponin C; echinocystic acid; *Gleditsia japonica*; Leguminosae; ^{13}C-NMR; PRFT method; T_1

In preceding papers,[1,2] it was reported that the structure of the desmonoterpenyl compound of gleditsia saponins C (GS–C, XX) and B (GS–B, XIX), which were isolated from the legume of *Gleditsia japonica* cv. 'Saponifera', was characterized as echinocystic acid 3,28-*O*-bisglycoside, I, and the desmonoterpenyl compounds of GS–E and GS–G were characterized as II and III, respectively. Shibata *et al.* reported that[3] the structures of the two monoterpenes attached to the molecule of GS–C were IV and V. Recently, Shibata *et al.* and we indicated[4] that the one of these monoterpenes was attached at C-2 of the terminal rhamnose on the

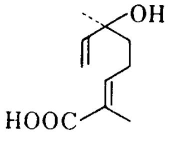


I : $R_1=\text{glu}^{6}\text{—}{}^{1}\text{ara}^{2}\text{—}{}^{1}\text{xyl},\ R_2=\text{glu}^{2}_{6}\left\langle\begin{matrix}{}^{1}\text{rha}^{4}\text{—}{}^{1}\text{xyl}^{3}\text{—}{}^{1}\text{xyl}\\ {}_{1}\text{rha}\end{matrix}\right.$

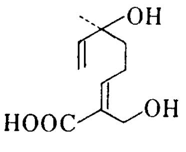
II : $R_1=\text{glu}^{6}\text{—}{}^{1}\text{ara},\ R_2=\text{glu}^{2}_{6}\left\langle\begin{matrix}{}^{1}\text{rha}^{4}\text{—}{}^{1}\text{xyl}^{3}\text{—}{}^{1}\text{xyl}\\ {}_{1}\text{rha}\end{matrix}\right.$

III : $R_1=\text{glu},\ R_2=\text{glu}^{2}_{6}\left\langle\begin{matrix}{}^{1}\text{rha}^{4}\text{—}{}^{1}\text{xyl}^{3}\text{—}{}^{1}\text{xyl}\\ {}_{1}\text{rha}\end{matrix}\right.$

VI : $R_1=H,\ R_2=H$
VII : $R_1=\text{glu},\ R_2=H$
VIII : $R_1=\text{glu}^{6}\text{—}{}^{1}\text{ara}^{2}\text{—}{}^{1}\text{xyl},\ R_2=H$



IV



V

Chart 1

basis of the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum, in which the signal of its anomeric carbon was shifted upfield.

In this paper, the determination of the positions of two monoterpenes in the molecules of GS–C and GS–B, is reported. The ^{13}C-NMR spectrum of GS–C was too complicated to be assigned directly. Thus, signal assignments of the ^{13}C-NMR spectra of echinocystic acid 3-*O*-glycoside, 28-*O*-glycoside and desmonoterpenyl 3,28-*O*-bisglycoside were carried out based on the partially relaxed Fourier transform (PRFT) method.5) The position of monoterpene was deduced from a consideration of the acylation shift6) and from the structures of prosapogenins obtained by enzymatic hydrolysis.

Many investigations of the ^{13}C-NMR spectra of triterpenes and their glycosides have been reported, but echinocystic acid and its glycosides have not yet been analyzed in detail. The full assignments of ^{13}C-NMR spectra of the methyl ester of echinocystic acid (VI) and the triterpenoid moieties of its 3-*O*-glycosides, VII and VIII, were accomplished (Table I) by consideration of the off-resonance spectra and by comparison with the literature data for oleanolic acid,7) hederagenin8) and other triterpenoids which have α-OH at C-16, platicodigenin,9) quillaic acid,8) 23-hydroxyprimulagenin A10) and saikogenin G.10) The assignment of the ^{13}C-NMR spectrum of the methyl ester of VII was accomplished by consideration of the literature data for oleanolic acid 3-*O*-glucoside7) and the chemical shift values of VI. The structure of VIII and the assignment of the ^{13}C-NMR signals of its oligosaccharide moieties has been reported.11) These assignments were supported by the PRFT–^{13}C-NMR signals of the oligosaccharide carbons of the methyl ester of VIII, as shown in Fig. 1. The spin-lattice relaxation time (T_1)12) of the terminal xylose should be longest, and T_1 of glucose attached to the hydroxyl

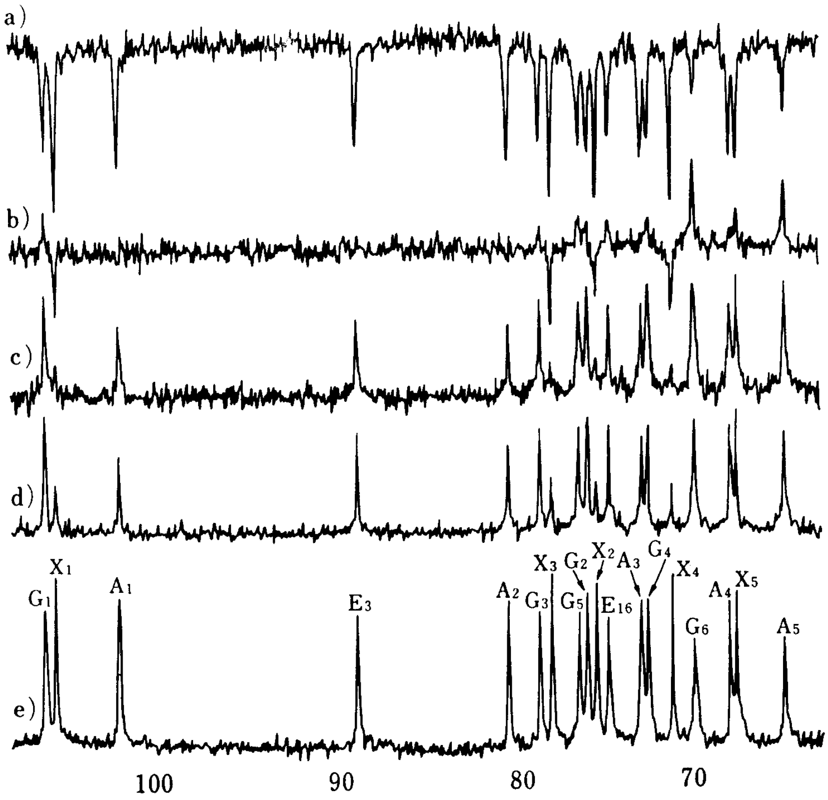


Fig. 1. PRFT–^{13}C-NMR Spectra of the Methyl Ester of the Prosapogenin (VIII) in C_5D_5N at 60°C (JEOL FX FX-90Q Spectrometer at 22.50 MHz using a 10 mm Tube)

Pulse interval (s): a) 0.03, b) 0.13, c) 0.23, d) 0.30, e) 1.00. Number of repetitions: 10000 times. G_1—G_6, glucose; X_1—X_5, xylose; A_1—A_5, arabinose; E, echinocystic acid.

TABLE I. ^{13}C Chemical Shifts (δ) of Methyl Esters of VI, VII and VIII in Pyridine-d_5

	Methyl ester of VI	Methyl ester of VII	$\Delta\delta$ (VII—VI)	Methyl ester of VIII	$\Delta\delta$ (VIII—VI)
1	39.0(t)[a]	38.9(t)[a]	(− 0.1)	38.8(t)[a]	(− 0.2)
2	28.1(t)	26.5(t)	(− 1.6)	26.7(t)	(− 1.4)
3	78.1(d)	88.9(d)	(+10.8)	88.6(d)	(+10.5)
4	39.4(s)	39.5(s)	(− 0.1)	39.5(s)	(− 0.1)
5	55.9(d)	56.0(d)	(+ 0.1)	55.9(d)	(± 0)
6	18.8(t)	18.5(t)	(− 0.3)	18.5(t)	(− 0.3)
7	33.4(t)	33.4(t)	(± 0)	33.4(t)	(± 0)
8	39.8(s)	39.9(s)	(+ 0.1)	39.8(s)	(± 0)
9	47.2(d)	47.1(d)	(− 0.1)	47.0(d)	(− 0.2)
10	37.4(s)	37.1(s)	(− 0.3)	37.0(s)	(− 0.4)
11	23.8(t)	23.7(t)	(− 0.1)	23.8(t)	(± 0)
12	122.7(d)	122.6(d)	(− 0.1)	122.6(d)	(− 0.1)
13	144.4(s)	144.5(s)	(+ 0.1)	144.4(s)	(± 0)
14	41.9(s)	41.9(s)	(± 0)	41.8(s)	(− 0.1)
15	35.9(t)[b]	35.9(t)[b]	(± 0)	35.9(t)[b]	(± 0)
16	74.4(d)	74.4(d)	(± 0)	74.3(d)	(− 0.1)
17	49.0(s)	49.1(s)	(+ 0.1)	48.9(s)	(− 0.1)
18	41.3(d)	41.3(d)	(± 0)	41.1(d)	(− 0.2)
19	47.0(t)	47.0(t)	(± 0)	46.8(t)	(− 0.2)
20	30.9(s)	30.8(s)	(− 0.1)	30.8(s)	(− 0.1)
21	35.9(t)[b]	35.9(t)[b]	(± 0)	35.9(t)[b]	(± 0)
22	32.5(t)	32.4(t)	(− 0.1)	32.5(t)	(± 0)
23	28.8(q)	28.3(q)	(− 0.5)	28.2(q)	(− 0.6)
24	16.6(q)	17.0(q)	(+ 0.4)	17.0(q)	(+ 0.4)
25	15.7(q)	15.6(q)	(− 0.1)	15.6(q)	(− 0.1)
26	17.2(q)	17.2(q)	(± 0)	17.2(q)	(± 0)
27	27.1(q)	27.2(q)	(+ 0.1)	27.2(q)	(+ 0.1)
28	177.7(s)	177.7(s)	(± 0)	177.7(s)	(± 0)
29	33.2(q)	33.2(q)	(± 0)	33.2(q)	(± 0)
30	24.6(q)	24.7(q)	(+ 0.1)	24.6(q)	(± 0)
O-CH_3	51.8(q)	51.7(q)		51.8(q)	
Glucose 1[c]		106.6		106.7	
2		75.7		75.6	
3		78.6		78.3	
4		71.9		72.1	
5		77.9		76.0	
6		63.1		69.5	
Arabinose 1[d]				102.3	
2				80.3	
3				72.5	
4				67.4	
5				64.2	
Xylose 1[e]				106.2	
2				75.3	
3				77.7	
4				70.7	
5				67.2	

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a) Abbreviations given in parentheses denote signal patterns observed in the off-resonance experiments.

b) These signals were overlapping.

c) With regard to carbon signals of β-glucopyranoside in pyridine-d_5 the literature data for Me oleanolate-3-*O*-β-glucopyranoside[7] were referred to (106.3, 75.6, 78.5, 72.1, 77.6 and 63.3).

d) The values for α-arabinopyranoside were compared with those of Me-α-arabinopyranoside, mp 131.5—132.5°C, $[\alpha]_D+4.7°$, measured in pyridine-d_5. (105.8, 72.1, 74.2, 69.0 and 66.5).[13]

e) With regard to carbon signals of β-xylopyranoside in pyridine-d_5, the literature data for chikusetsusaponin L_5 were referred to[5b] (106.7, 75.2, 78.4, 70.8 and 67.2).

group of C-3 of echinocystic acid should be shortest. As shown in Fig. 2, the average NT_1 values (N=number of proton(s) attached to specific carbons) for skeletal carbons of individual saccharide units were 0.34 (for xylose), 0.22 (for arabinose) and 0.18 (for glucose) s. Therefore, the signals at δ 106.2, 75.3, 77.7 and 70.7 can be assigned to the secondary carbons of xylose, the signals at δ 106.7, 75.6, 78.3, 72.1 and 76.0 can be assigned to the carbons of the glucose, and the signals at δ 102.3, 80.3, 72.5 and 67.4 can be assigned to the carbons of the arabinose, as shown in Table I.

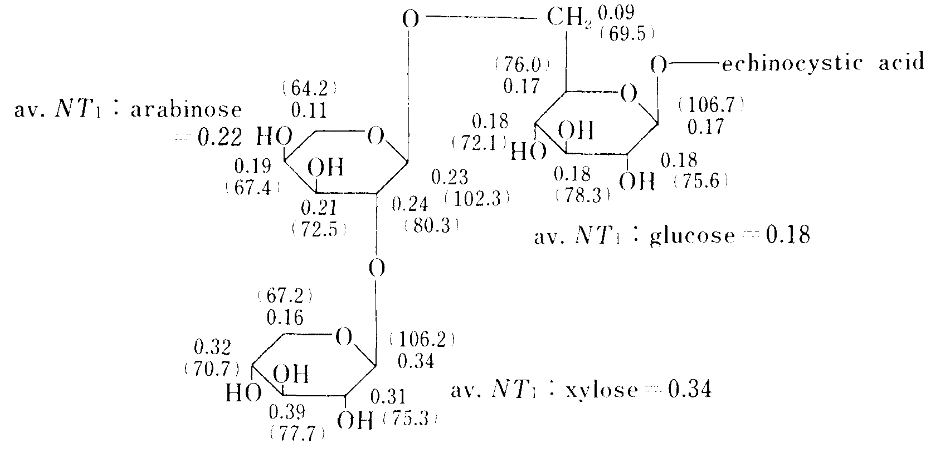


Fig. 2. T_1 and NT_1 Values (s) for the Methyl Ester of the Prosapogenin (VIII)

Chemical shift values assigned from PRFT spectra are shown in parentheses.

Then, we attempted to hydrolyze GS-G (XXI), one of the minor saponins of *G. japonica*, with an enzyme to obtain the 28-*O*-glycoside of echinocystic acid. The prosapogenin (IX) was obtained from XXI by hydrolysis with cellulase.[14] The ^{13}C-NMR spectrum of IX showed the presence of two carbonyl carbons at δ 167.3 and 167.0, eight olefinic carbons at δ 146.7, 146.4, 146.3, 144.3, 132.8, 127.6 and 111.8 (two carbons), and four anomeric carbons at δ 94.5, 98.1, 101.2 and 106.9. The ultra violet (UV) spectrum of IX showed an absorption maximum at 216 nm. Therefore, it was deduced that IX has two monoterpenes in its molecule (Table II). Hydrolysis of IX with 5% K_2CO_3 in ethanol afforded a desmonoterpenyl compound (X), and hydrolysis of IX with 20% KOH in ethanol afforded echinocystic acid, not 3-*O*-glucosyl echinocystic acid. The desmonoterpenyl compound (X) was permethylated by Hakomori's method[15] to afford the permethylate (XI). In the proton magnetic resonance (PMR) spectrum of XI, signals of four anomeric protons were seen at δ 4.61 (d, J=7 Hz), 4.82 (br s), 5.22 (br s) and 5.50 (d, J=7 Hz). On reduction of XI with $LiAlH_4$, the methylated oligosaccharide (XII) and 3,16-di-*O*-methyl primulagenin A were obtained. The PMR spectrum of XII showed three anomeric protons at δ 4.63 (d, J=7 Hz), 4.87 (br s) and 5.03 (br s). On methanolysis of XII, methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside, methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside, methyl 2,3-di-*O*-methyl-L-rhamnopyranoside and 3,4-di-*O*-methyl-D-glucitol were obtained and identified by comparison with authentic samples [thin layer chromatography (TLC) and gas liqued chromatography (GLC)]. Accordingly, it was concluded that the structure of the oligosaccharide moiety of the prosapogenin (IX) was [β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranosyl.

Then, the assignment of the ^{13}C-NMR spectrum of X was accomplished by comparison with the chemical shift values of deapioplatycodin D (XIII)[9] and those of the methyl glycoside, and by examination of the PRFT-^{13}C-NMR signals of the oligosaccharide moiety of X. T_1 of the terminal rhamnose and xylose should be longer than those of inner-located rhamnose and glucose. In the PRFT-^{13}C-NMR spectra, the signal recovery of the terminal rhamnose and xylose was indeed slower than that of inner-located rhamnose and glucose (Fig. 3).

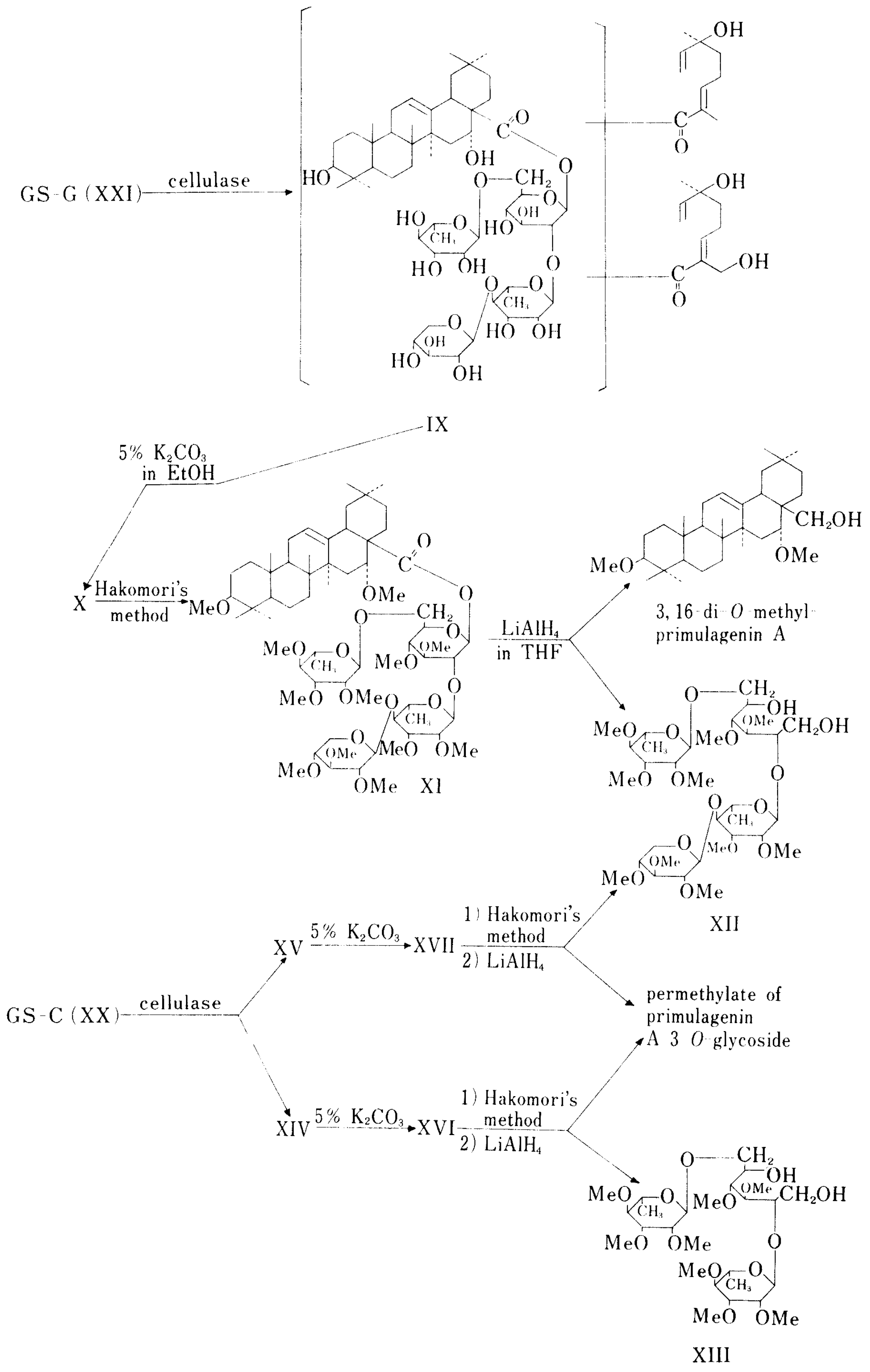

GS-G (XXI)
cellulase
IX
5% K_2CO_3 in EtOH
X
Hakomori's method
XI
$LiAlH_4$ in THF
3, 16-di-O-methyl-primulagenin A
XII
GS-C (XX)
cellulase
XV
5% K_2CO_3
XVII
1) Hakomori's method
2) $LiAlH_4$
permethylate of primulagenin A 3-O-glycoside
XIV
5% K_2CO_3
XVI
1) Hakomori's method
2) $LiAlH_4$
XIII

Chart 2

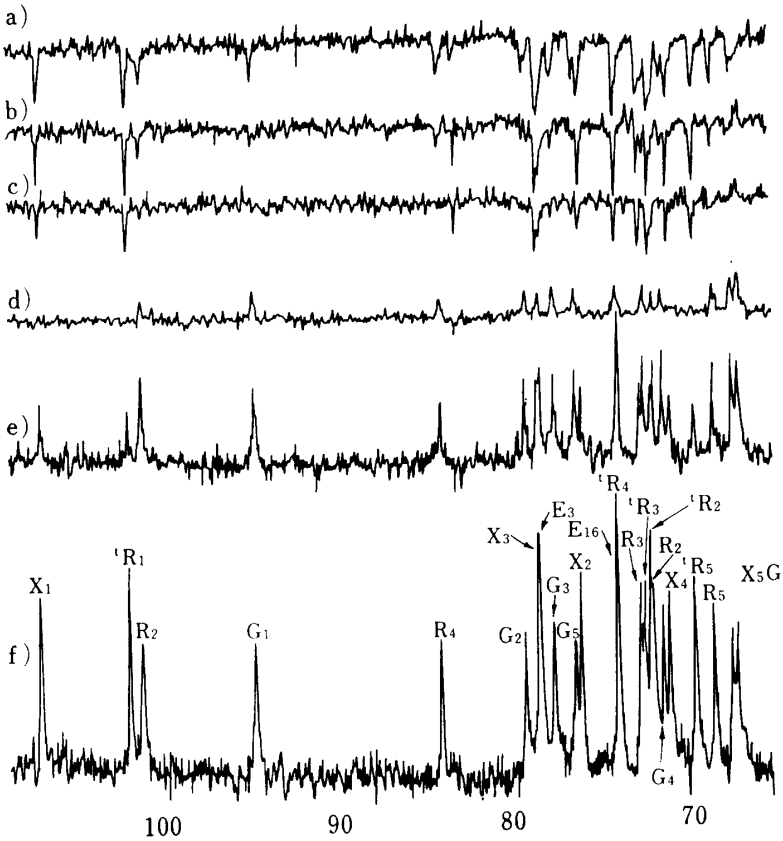


Fig. 3. PRFT-CMR Spectra of Prosapogeinn (X) in C_5D_5N at 60°C (Conditions as in Fig. 1)

Pulse interval (s): a) 0.03, b) 0.07, c) 0.09, d) 0.18, e) 0.30, f) 1.00. Number of repetitions: 10000 times. G_1—G_6, glucose; X_1—X_5, xylose; R_1—R_5, inner-located rhamnose; $'R_1$—$'R_5$, terminal rhamnose; E, echinocystic acid.

Therefore, the signals at δ 101.8, 72.1, 72.6, 73.9 and 69.7 can be assigned to carbons of the terminal rhamnose, signals at δ 106.9, 76.0, 78.6 and 71.0 can be assigned to those of xylose, signals at δ 94.6, 79.4, 77.6, 71.1 and 76.1 can be assigned to those of glucose, and signals at δ 101.2, 71.9, 72.6, 83.9 and 68.4 can be assigned to those of the inner-located rhamnose, as shown in Table III.

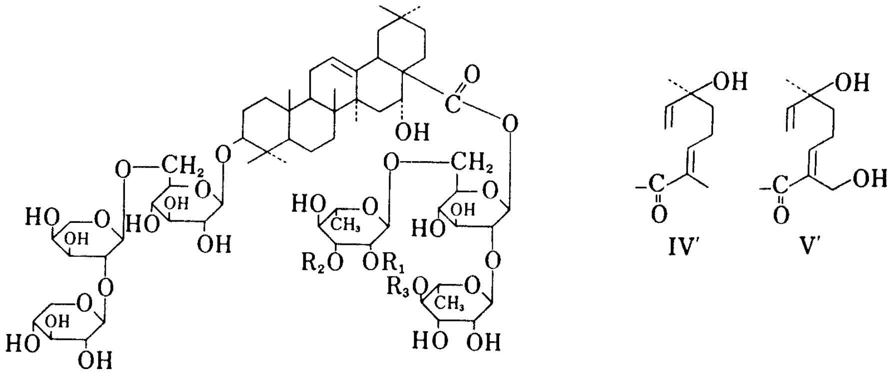
The ^{13}C-NMR spectrum of GS-C was very complicated. Therefore, in order to simplify the spectrum, GS-C was hydrolyzed with an enzyme, without loss of the monoterpenes. GS-C was hydrolyzed with cellulase to afford the prosapogenins (XIV) and (XV). The UV spectra of these prosapogenins each showed an absorption maximum at 216 nm, indicating the presence of conjugated carbonyl functions.[16] In the ^{13}C-NMR spectra, both XIV and XV showed three kinds of signals of carbonyl carbons at δ 175.9 (C-28 of echinocystic acid), 167.2 and 167.0, XIV showed six kinds of signals of anomeric carbons, and XV showed seven kinds of anomeric carbons. Therefore, it was deduced that both prosapogenins (XIV) and (XV) obtained by hydrolysis with cellulase retained the two monoterpene moieties (Table II).

Both XIV and XV were hydrolyzed with 10% KOH in ethanol to afford compound (VIII). Therefore, on hydrolysis of GS-C with cellulase, the oligosaccharide moieties attached to the C-28 carboxyl group of echinocystic acid were partially removed. XIV and XV were hydrolyzed with 5% K_2CO_3 to afford the desmonoterpenyl prosapogenins (XVI) and (XVII), respectively. The structures of XVI and XVII were supported by the PMR spectra of their permethylates, and by the spectra of the compounds obtained by reductive cleavage of these permethylates.[18] In the desmonoterpenyl prosapogenin (XVI), the structure of the oligosac-

TABLE II. ^{13}C Chemical Shifts (δ) of Monoterpene Moieties of Prosapogenins XIV, XV, XVIII, IX, XXII and Monoterpene (IV)[17)]

	XIV	XV	XVIII	IX	XXII	IV	(in $CDCl_3$)
1	167.2	167.2	167.7	167.3	167.2	168.4	(173.4)
2	127.5	127.6	127.8	127.6	132.6	127.5	(127.3)
3	144.3	144.3	143.8	144.3	147.5	143.5	(144.6)
4	23.9	24.0	23.9	24.0	23.9	24.0	(23.7)
5	41.4	41.4	41.3	41.5	41.6	41.5	(40.5)
6	72.2[a)]	72.2[a)]	72.1	72.2[a)]	72.3[a)]	72.1	(73.3)
7	146.2	146.2	146.3	146.3	146.2	146.6	(144.3)
8	111.8[b)]	111.8[b)]	111.7	111.8[b)]	111.9	111.7	(112.4)
9	12.6	12.6	12.5	12.7	56.1	12.4	(12.0)
10	28.5	28.5	28.4	28.6	28.4	28.5	(27.9)
1	167.0	167.0		167.0	166.9		
2	132.7	132.8		132.9	132.5		
3	146.8	146.8		146.7	147.2		
4	24.1	24.1		24.2	24.1		
5	41.8	41.8		41.9	41.8		
6	72.2[a)]	72.2[a)]		72.2[a)]	72.3[a)]		
7	146.3	146.3		146.4	146.1		
8	111.8[a)]	111.8[a)]		111.8[a)]	111.8[a)]		
9	56.2	56.2		56.2	56.1		
10	28.3	28.4		28.5	28.3		
UV: max	216 nm	216 nm	217 nm	216 nm	213 nm		

a, *b*) These pairs of signals were overlapping.



	R_1	R_2	R_3
GS-C (XX)	IV′	V′	—xyl^{3}——^{1}xyl
GS–B (XIX)	V′	V′	—xyl^{3}——^{1}xyl
I	H	H	—xyl^{3}——^{1}xyl
XIV	IV′	V′	H
XV	IV′	V′	—xyl
XVI	H	H	H
XVII	H	H	—xyl
XVIII	IV′	H	—xyl
XXII	V′	V′	—xyl

Chart 3

charide moiety attached at the C-28 carboxyl group of echinocystic acid was characterized as [α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranosyl. In the desmonoterpenyl prosapogenin (XVII), the structure of the oligosaccharide moiety attached at the C-28 carboxyl group was characterized as [β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl-(1→2)][α-L-rhamnopyranosyl (1→6)]-β-D-glucopyranosyl. Therefore, in the molecule of XVII, the structure of the oligosaccharide attached at the C-3 hydroxyl group of echinocystic acid is the same as in the prosapogenin (VIII), and the structure of the oligosaccharide attached at the C-28 carboxyl group of echinocystic acid was the same as in X.

As shown in Table III, the ^{13}C chemical shifts of the 3-*O*-glycoside moiety and 28-*O*-glycoside moiety of XVII were, respectively, superimposable on those of the methyl ester of VIII (3-*O*-glycoside) and X (28-*O*-glycoside). The PRFT–^{13}C-NMR spectra of XVII are shown in Fig. 4. In the molecule of XVII, there are three terminal sugars, two xyloses and a rhamnose, and signal recovery of these sugars should be slower than that of the other four sugars, glucoses, arabinose and the inner-located rhamnose. Therefore, signals at δ 106.7 and 106.0 can be assigned to anomeric carbons of terminal xyloses, the signal at δ 101.7 can be assigned to the anomeric carbon of the terminal rhamnose, and signals at δ 106.6, 102.1, 101.1 and 94.5 can be assigned to anomeric carbons of glucose (attached at C-3), arabinose, inner-located rhamnose and glucose (attached at C-28), respectively.

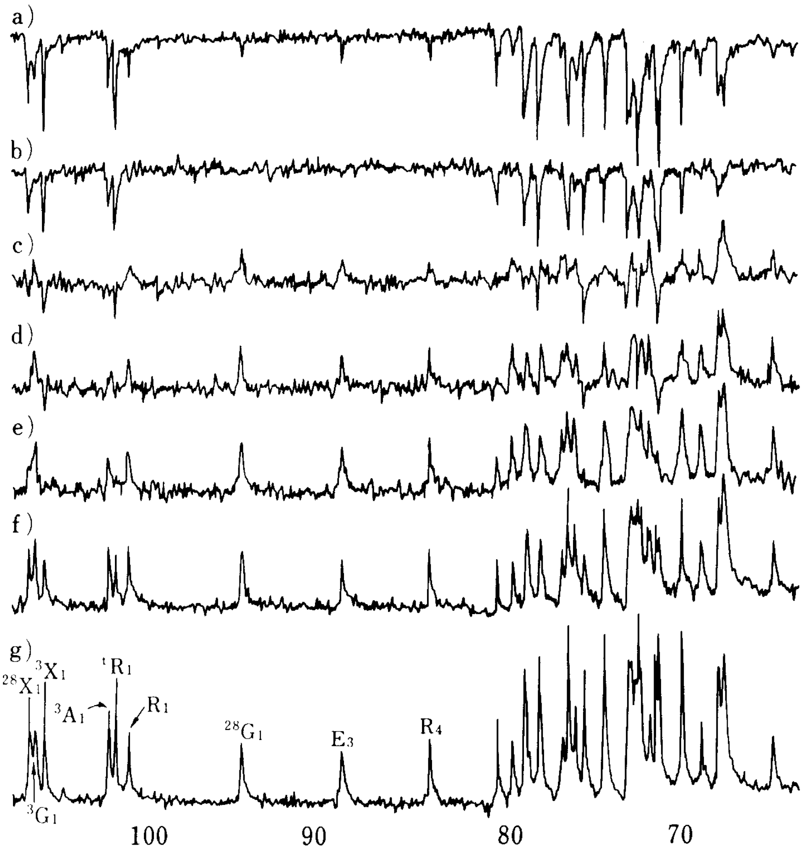


Fig. 4. PRFT-CMR Spectra of Prosapogenin (XVII) in C_5D_5N at 60°C (Condition as in Fig. 1)

Pulse interval (s): a) 0.03, b) 0.05, c) 0.09, d) 0.11, e) 0.15, f) 0.23, g) 1.00. Number of repetitions: 10000 times. ^{3}G, ^{3}X, ^{3}A, sugars at C-3 of echinocystic acid; ^{28}G, ^{28}X, sugars at C-28 of echinocystic acid; ^{t}R, terminal rhamnose; R, inner-located rhamnose.

The prosapogenin (XV) was hydrolyzed with 0.1% K_2CO_3 in ethanol to afford XVIII and desmonoterpenyl prosapogenin (XVII). XVIII showed an absorption maximum at 217 nm in its UV spectrum, and in the ^{13}C-NMR spectrum, XVIII showed the presence of two carbonyl carbons at δ 175.9 (C-28 of echinocystic acid) and 167.7, six kinds of signals of olefinic carbons at δ 146.3, 144.4 (C-13 of echinocystic acid), 143.8, 127.8, 122.8 (C-12 of echinocystic acid) and 111.7, and an allylic methyl carbon at δ 12.5. Therefore, in the prosapogenin (XVIII), the monoterpene (IV) remained, while the monoterpene (V) had been removed. It has been reported that the acyl group on C-2 or C-3 of rhamnosides readily undergoes acyl migration.[19] However, it was deduced that monoterpene migration had not occurred under the conditions

TABLE III. ^{13}C Chemical Shifts (δ) of Oligosaccharide Moities of Prosapogenins and Desmonoterpenyl Prosapogenins in C_5D_5N

			VIII	XVII	Δδ (XVII—VIII)	XVI	Δδ (XVI—VIII)	XVIII	Δδ (XVIII—XVII)	XV	Δδ (XV—XVII)	Δδ (XV—XVIII)	XIV	Δδ (XIV—XVI)	XXII	Δδ (XXII—XV)	Δδ (XXII—XVII)
3-*O*-Glycoside	glu	1	106.7	106.6	(−0.1)	106.7	(± 0)	106.6	(+0.1)	106.6	(+0.1)	(±0)	106.7	(±0)	106.6	(±0)	(+0.1)
		2	75.6	75.4	(−0.2)	75.6	(± 0)	75.4	(±0)	75.5	(+0.1)	(+0.1)	75.5	(−0.1)	75.5	(±0)	(+0.1)
		3	78.3	78.2	(−0.1)	78.3	(± 0)	78.1	(−0.1)	78.2	(±0)	(+0.1)	78.2	(−0.1)	78.2	(±0)	(±0)
		4	72.1	72.1	(±0)	72.1	(± 0)	72.0	(−0.1)	72.0	(−0.1)	(±0)	72.1	(±0)	72.0	(±0)	(−0.1)
		5	76.0	76.0	(±0)	76.0	(± 0)	76.0	(±0)	76.0	(±0)	(±0)	76.0	(±0)	76.0	(±0)	(±0)
		6	69.5	69.4	(−0.1)	69.6	(+ 0.1)	69.4	(±0)	69.4	(±0)	(±0)	69.5	(−0.1)	69.4	(±0)	(±0)
	ars	1	102.3	102.1	(−0.2)	102.2	(− 0.1)	102.1	(±0)	102.1	(±0)	(±0)	102.2	(±0)	102.0	(−0.1)	(−0.1)
		2	80.3	80.2	(−0.1)	80.4	(+ 0.1)	80.2	(±0)	80.2	(±0)	(±0)	80.3	(−0.1)	80.3	(+0.1)	(+0.1)
		3	72.5	72.4	(−0.1)	72.5	(± 0)	72.3	(−0.1)	72.4	(±0)	(+0.1)	72.5	(±0)	72.4	(±0)	(±0)
		4	67.4	67.3	(−0.1)	67.4	(± 0)	67.3	(±0)	67.3	(±0)	(±0)	67.4	(±0)	67.3	(±0)	(±0)
		5	64.2	64.1	(−0.1)	64.2	(± 0)	64.1	(±0)	64.1	(±0)	(±0)	64.2	(±0)	64.1	(±0)	(±0)
	xyl	1	106.2	106.0	(−0.2)	106.2	(± 0)	106.0	(+0.1)	106.0	(+0.1)	(±0)	106.1	(−0.1)	106.0	(±0)	(+0.1)
		2	75.3	75.2	(−0.1)	75.3	(± 0)	75.0	(±0)	75.2	(±0)	(±0)	75.3	(±0)	75.2	(±0)	(±0)
		3	77.7	77.7	(±0)	77.8	(+ 0.1)	77.7	(±0)	77.7	(±0)	(±0)	77.7	(−0.1)	77.7	(±0)	(±0)
		4	70.7	70.7	(±0)	70.8	(+ 0.1)	70.6	(−0.1)	70.7	(±0)	(+0.1)	70.7	(−0.1)	70.7	(±0)	(±0)
		5	67.2	67.1	(−0.1)	67.2	(± 0)	67.1	(±0)	67.1	(±0)	(±0)	67.2	(±0)	67.1	(±0)	(±0)
			(X)		Δδ(XVII—X)		Δδ(XVI—X)										
28-*O*-Glycoside	glu	1	94.6	94.5	(−0.1)	94.7	(+ 0.1)	94.4	(−0.3)	94.4	(−0.3)	(±0)	94.6	(−0.1)	94.4	(±0)	(−0.3)
		2	79.4	79.2	(−0.2)	79.4	(± 0)	79.1	(−0.1)	79.2	(±0)	(+0.1)	79.3	(−0.1)	79.1	(−0.1)	(−0.1)
		3	77.6	77.5	(−0.1)	77.5	(− 0.1)	77.3	(−0.2)	77.4	(−0.1)	(+0.1)	77.3	(−0.2)	77.5	(+0.1)	(±0)
		4	71.1	71.0	(−0.1)	71.1	(± 0)	70.9	(−0.1)	70.9	(−0.1)	(±0)	71.0	(−0.1)	70.9	(±0)	(−0.1)
		5	76.1	76.1	(±0)	75.9	(− 0.2)	76.1	(±0)	76.2	(+0.1)	(+0.1)	76.0	(+0.1)	76.3	(+0.1)	(+0.2)
		6	66.5	66.5	(±0)	66.5	(± 0)	66.4	(−0.1)	66.5	(±0)	(+0.1)	66.6	(+0.1)	66.6	(±0)	(+0.1)
	rha[a]	1	101.2	101.1	(−0.1)	101.4	(+ 0.2)	101.1	(±0)	101.2	(+0.1)	(+0.1)	101.4	(±0)	101.2	(±0)	(+0.1)
		2	71.9	71.7	(−0.2)	72.4	(− 0.5)	71.7	(±0)	71.7	(±0)	(±0)	72.4	(±0)	71.8	(+0.1)	(+0.1)
		3	72.6	72.4	(−0.2)	72.5	(− 0.1)	72.3	(−0.1)	72.4	(±0)	(+0.1)	72.5	(±0)	72.4	(±0)	(±0)
		4	83.9	83.7	(−0.2)	73.9	(−10.0)	83.7	(±0)	83.7	(±0)	(±0)	73.8	(−0.1)	83.8	(+0.1)	(+0.1)
		5	68.4	68.3	(−0.1)	69.9	(+ 1.6)	68.3	(±0)	68.3	(±0)	(±0)	69.6	(−0.3)	68.4	(+0.1)	(+0.1)
		6	18.7	18.6	(−0.1)	18.7	(± 0)	18.6	(±0)	18.6	(±0)	(±0)	18.7	(±0)	18.7	(+0.1)	(+0.1)
	xyl[a]	1	106.9	106.7	(−0.2)			106.7	(−0.1)	106.7	(−0.1)	(±0)			106.7	(±0)	(−0.1)
		2	76.0	75.9	(−0.1)			75.9	(±0)	75.9	(±0)	(±0)			75.9	(±0)	(±0)
		3	78.6	78.4	(−0.2)			78.3	(−0.1)	78.4	(±0)	(+0.1)			78.4	(±0)	(±0)
		4	71.0	70.9	(−0.1)			70.7	(−0.2)	70.9	(±0)	(+0.2)			70.9	(±0)	(±0)
		5	67.4	67.2	(−0.2)			67.2	(±0)	67.2	(±0)	(±0)			67.3	(+0.1)	(+0.1)
	rha	1	101.8	101.7	(−0.2)	101.8	(± 0)	98.4	(−3.3)	98.0	(−3.7)	(−0.4)	98.1	(−3.7)	98.1	(+0.1)	(−3.6)
		2	72.1	71.9	(−0.2)	72.0	(− 0.1)	74.1	(+2.2)	71.3	(−0.6)	(−2.8)	71.3	(−0.7)	71.3	(±0)	(−0.6)
		3	72.6	72.4	(−0.2)	72.6	(± 0)	70.3	(−2.1)	73.3	(+0.9)	(+3.0)	73.3	(+0.7)	73.3	(±0)	(+0.9)
		4	73.9	73.8	(−0.1)	73.9	(± 0)	73.7	(−0.1)	71.0	(−2.8)	(−2.7)	70.9	(−3.0)	71.0	(±0)	(−2.8)
		5	69.7	69.6	(−0.1)	69.6	(− 0.1)	69.6	(±0)	69.5	(±0)	(−0.1)	69.6	(±0)	69.6	(+0.1)	(±0)
		6	18.7	18.6	(−0.1)	18.7	(± 0)	18.6	(±0)	18.6	(±0)	(±0)	18.7	(±0)	18.6	(±0)	(±0)

[a] With regard to carbon signals, the literature data for deapioplatycodin D were referred to (H. Ishii *et al.*, *Chem. Lett.*, **1978**, 719).

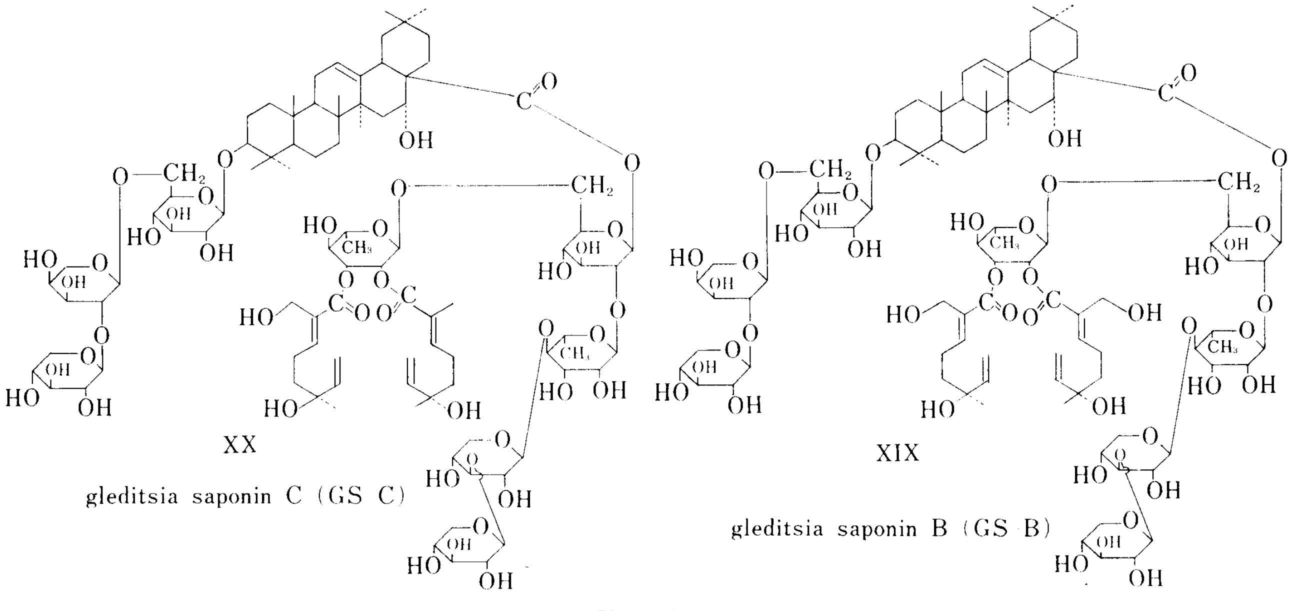
of this partial hydrolysis, because on treatment of XVIII with 0.1% K_2CO_3 at room temperature, the desmonoterpenyl prosapogenin (XVII) and XVIII were obtained but the migrated compound and/or an equilibrium mixture were not obtained.

Chemical shift values of oligosaccharide moieties attached at C-3 of echinocystic acid for XIV, XV and XVIII were almost superimposable on those of VIII, XVII and XVI, as shown in Table III. Therefore, the positions of monoterpenes in the molecules of XIV, XV and XVIII must be at sugars, glucose and/or two rhamnoses, attached at the C-28 carboxyl group of echinocystic acid, *i.e.* C-3, C-4 of glucosyl, C-2, C-3 of inner-located rhamnosyl and C-2, C-3, C-4 of the terminal rhamnosyl. If the C-2 and/or C-3 position of the inner-located rhamnose was acylated with monoterpene, the signal of the anomeric carbon and/or C-4 of the inner-located rhamnose should be shifted upfield by comparison of XV and XVIII with XVII. However, these signals were not shifted upfield, and were distinguishable from others in the spectra of XV, XVII XVIII, shown in Table III. Therefore, the positions of monoterpenes should be at C-3, C-4 of glucose or C-2, C-3, C-4 of the terminal rhamnose.

In the ^{13}C-NMR spectrum of XVIII, the signal of one anomeric carbon of rhamnose, which was assigned to the terminal rhamnose by examination of the PRFT-^{13}C-NMR spectra of X and XVII, was shifted upfield by 3.3 ppm (101.7 to 98.4 ppm), the signal of C-3 of the same rhamnose was shifted upfield by 2.1 ppm (72.4 to 70.3 ppm), and the signal of C-2 was shifted to lower field by 2.2 ppm (71.9 to 74.1 ppm), while other signals were almost superimposable on the signals of XVII within ±0.2 ppm. Further, acyl migration had not occurred in the molecule of XVIII as described above. Therefore, it was concluded that the monoterpene (IV) was attached at C-2 of the terminal rhamnose on the basis of the acylation shift.6)

Then, the position of the other monoterpene (V) was deduced as follows. The differences of chemical shift values between XV and XVIII [$\Delta\delta$(XV—XVIII)] are shown in Table III. The signals of C-2 and C-4 of the terminal rhamnose in XV were shifted upfield by 2.8 and 2.7 ppm, respectively, and the signal of C-3 was shifted to lower field by 3.0 ppm. Considering the differences of chemical shift values between XV and XVII [$\Delta\delta$(XV—XVII)] and between XIV and XVI [$\Delta\delta$(XIV—XVI)], the signal of anomeric carbon of the terminal rhamnose and the signal of C-4 of the same rhamnose, respectively, were shifted upfield from 2.8 to 3.7 ppm.

Therefore, it was deduced that, in the molecules of XIV, XV and further GS-C (XX), the monoterpene (IV) is attached at C-2 of the terminal rhamnose and the monoterpene (V) is attached at C-3 of the same rhamnose, as shown in Chart 4.



XX

gleditsia saponin C (GS C)

XIX

gleditsia saponin B (GS B)

Chart 4

Gleditsia saponin B (GS–B, XIX) afforded the same desmonoterpenyl compound (I) as GS–C on hydrolysis with 5% K_2CO_3,[2] and afforded prosapogenin (XXII) on hydrolysis with cellulase. The UV spectra of XIX and XXII showed an absorption maximum at 213 nm, and the prosapogenin (XXII) was hydrolyzed with 5% K_2CO_3 in ethanol to afford the same desmonoterpenyl prosapogenin (XVII) as XV. Therefore, it was deduced that the structure of GS–B (XIX) was similar to that of GS–C.

In the ^{13}C-NMR spectrum, XXII showed three kinds of signals of carbonyl carbons at δ 175.9 (C-28 of echinocystic acid), 167.1 and 166.9, ten kinds of signals of olefinic carbons, and two kinds of signals of allylic alcohol carbons at δ 56.1 and 56.0. On the other hand, the allylic methyl carbon signal which should appear at about δ 12.5 was not observed, as shown in Table III. Therefore, it was deduced that the two monoterpenes attached to the molecule of GS–B (XIX) were both monoterpene (V).

In the ^{13}C-NMR spectrum of XXII, signals of oligosaccharide moieties and the triterpene moiety were almost superimposable on those of the prosapogenin (XV), and in particular, the signal of the anomeric carbon and the signal of C-4 of the terminal rhamnose were shifted upfield by 3.6 ppm and by 2.8 ppm as in XV, so the positions of acylation with monoterpenes were deduced to be the same C-2 and C-3 of the terminal rhamnose as in GS–C.

Therefore, the structures of gleditsia saponins C and B were established as formulae XX and XIX.

Experimental

Mps are uncorrected. The PMR spectra (100 MHz) were measured in $CDCl_3$, and the ^{13}C-NMR spectra (50 MHz) were measured on a Varian XL 200 NMR spectrometer using 10 mm spinning tubes in C_5D_5N with TMS as an internal standard. UV spectra were measured on a Shimazu UV-240 spectrometer in 95% EtOH. The properties and physical data of VI, VII and VIII were reported in a previous paper.[11] Conditions of TLC and GLC for identification of methylated monosaccharides were the same as in previous papers.[1,2]

Hydrolysis of GS-G with Cellulase——A solution of GS-G (55 mg) and cellulase[14] (50 mg) in H_2O (30 ml) was incubated for 96 h at 37 °C. MeOH (10 ml) was added to this reaction mixture and the whole was heated for 5 min at 100°C. The precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The residue was extracted with *n*-BuOH saturated with water, and the organic layer was concentrated to dryness under reduced pressure. The residue was examined by TLC (solvent: $CHCl_3$–MeOH–H_2O = 13: 7: 2), and showed two major spots (*Rf* 0.75 and 0.58). The mixture was fractionated by chromatography on silica gel, and the fraction corresponding to *Rf* 0.75 was purified by HPLC (column: reversed phase C_{18}, solvent: MeOH–H_2O = 75: 25). The prosapogenin (IX) (15 mg) was obtained as a white powder by crystallization from MeOH–H_2O, mp 224—226°C, $[\alpha]_D^{20}$ $-2.19°$ (c = 0.91, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1730 (COOR). UV λ_{max}^{EtOH} (nm): 216 (ε = 27000). ^{13}C-NMR data: see Table II and III. *Anal.* Calcd for $C_{73}H_{114}O_{26}\cdot 2H_2O$: C, 60.73; H, 8.24. Found: C, 60.81; H, 8.23. GS-G was recovered from the fraction corresponding to *Rf* 0.58.

Hydrolysis of IX with 5% K_2CO_3——A solution of IX (100 mg) and 5% K_2CO_3 (10 ml) in EtOH (10 ml) was refluxed for 40 min. The reaction mixture was neutralized with Dowex 50W × 8 and concentrated to about half the original volume. The residue was extracted with *n*-BuOH, and the organic layer was concentrated to dryness. The residue was purified by chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O = 13: 7: 2), and recrystallized from MeOH–H_2O to afford 55 mg of X as colorless needles, mp 257—260°C, $[\alpha]_D^{17}$ $-11.7°$ (c = 1.00, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1730 (COOR). ^{13}C-NMR data: see Table III. *Anal.* Calcd for $C_{53}H_{86}O_{21}\cdot H_2O$: C, 59.09; H, 8.24. Found: C, 58.90; H, 8.44.

Hydrolysis of IX with 20% KOH——A solution of prosapogenin (IX) (10 mg) and 20% KOH (10 ml) in EtOH (10 ml) was refluxed for 2 h. The reaction mixture was neutralized with Dowex 50 W × 8, and concentrated to half the original volume under reduced pressure. The residue was extracted with AcOEt, and the extract was evaporated to dryness. The residue was crystallized from MeOH–H_2O to afford colorless needles (mp 306—309°C). This compound was identified as echinocystic acid by comparison with an authentic sample.

Permethylation of X and Reductive Cleavage of the Permethylate——The desmonoterpenyl compound (X) (50 mg) in dimethylsulfoxide (DMSO) (10 ml) was methylated by Hakomori's method[15] (NaI–DMSO–CH_3I) to afford a yellow oily product. This oily product was purified by chromatography on silica gel (solvent: C_6H_6–Me_2CO = 9: 1) to afford 30 mg of permethylate as a colorless syrup, $[\alpha]_D^{26}$ $-48.8°$ (c = 1.12, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740 (COOR). PMR δ: 3.44—3.61 (36H, m, $12 \times OCH_3$), 4.61 (1H, d, J = 7 Hz, anomeric H), 4.82 (1H, br s, anomeric H), 5.22 (1H, br s, anomeric H), 5.50 (1H, d, $J=7$ Hz, anomeric H). This permethylate (17 mg) was reduced with $LiAlH_4$ (20 mg) in tetrahydrofuran (THF) (6 ml) in the usual manner, and the reaction mixture was extracted with Et_2O and AcOEt, in that order. 3,16-Di-*O*-methylprimulagenin A (5 mg) was obtained from the Et_2O extract. PMR δ: 0.76 (3H, s, CH_3), 0.88—0.96 (15H, m, $5\times CH_3$), 1.96 (3H, s, CH_3), 3.28, 3.35 (each 3H, s, $2\times OCH_3$), 5.25 (1H, t, $J=3$ Hz). MS (m/z): 486 (M^+), 264. The methylated oligosaccharide (XII) (8 mg) was obtained from the AcOEt extract as a colorless syrup, $[\alpha]_D^{19}$ $-70.8°$ ($c=0.87$, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), PMR δ: 1.30, 1.31 (each 3H, d, $J=6$ Hz, $2\times CH_3$ of rhamnoses), 3.45—3.60 (30H, m, $10\times OCH_3$), 4.63 (1H, d, $J=7$ Hz, anomeric H), 4.87 (1H, br s, anomeric H), 5.03 (1H, br s, anomeric H).

Hydrolysis of GS-C with Cellulase——A solution of GS-C (500 mg) and cellulase (300 mg) in H_2O (300 ml) was incubated for 72 h at 37°C. MeOH (50 ml) was added to this reactin mixture and the whole was heated for 10 min at 80°C. This solution was treated in the same manner as described for GS-G. The *n*-BuOH extract was examined by TLC (solvent: $CHCl_3$–MeOH–H_2O = 13 : 7 : 2), and showed three major spots (*Rf* 0.35, 0.22 and 0.20). The mixture was fractionated by chromatography on silica gel, and purified by HPLC (column, reversed phase C_{18}; solvent, MeOH–H_2O = 7 : 3). The product corresponding to *Rf* 0.35 was precipitated from MeOH–Et_2O to afford 80 mg of the prosapogenin (XIV) as a hygroscopic white powder, mp 278—280°C, $[\alpha]_D^{37}$ $-14.6°$ ($c=1.12$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1730 (COOR). UV λ_{max}^{EtOH} (nm): 216 ($\varepsilon=31000$). ^{13}C-NMR data: see Table II and III. *Anal.* Calcd for $C_{84}H_{136}O_{36}\cdot 2H_2O$: C, 58.59; H, 7.96. Found: C, 58.71; H, 8.11. The product corresponding to *Rf* 0.22 was precipitated from MeOH–Et_2O to afford 250 mg of the prosapogenin (XV) as a hygroscopic white powder, mp 198—201°C, $[\alpha]_D^{25}$ $-13.4°$ ($c=1.10$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1730 (COOR). UV λ_{max}^{EtOH} (nm): 216 ($\varepsilon=26000$). ^{13}C-NMR data: see Table II and III. *Anal.* Calcd for $C_{89}H_{140}O_{39}\cdot 4H_2O$: C, 56.08; H, 7.83. Found: C, 56.38; H, 7.89. The product corresponding to *Rf* 0.20 was GS-C (100 mg).

Hydrolysis of XIV with 5% K_2CO_3——The prosapogenin (XIV, 100 mg) was hydrolyzed with 5% K_2CO_3 (10 ml) in EtOH (10 ml) in the same manner as described for IX. The *n*-BuOH extract was purified by chromatography on silica gel, and the product was crystallized from MeOH–H_2O to afford 53 mg of XVI as colorless needles, mp 295—298°C, $[\alpha]_D^{23}$ $-37.1°$ ($c=0.65$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1720 (COOR). ^{13}C-NMR data: see Table III.

Hydrolysis of XV with 5% K_2CO_3——The prosapogenin (XV) was hydrolyzed with 5% K_2CO_3 in EtOH in the same way as described for IX. The desmonoterpenyl compound (XVII) was obtained from the *n*-BuOH extract as colorless needles, mp 235—238°C, $[\alpha]_D^{25}$ $-37.3°$ ($c=0.70$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1730 (COOR). ^{13}C-NMR data: see Table III. *Anal.* Calcd for $C_{69}H_{112}O_{34}\cdot 2H_2O$: C, 54.46; H, 7.68. Found: C, 54.14; H, 7.72.

Hydrolysis of XIV and XV with 10% KOH——A solution of prosapogenin (XIV) or (XV) (10 mg) and 10% KOH (10 ml) in EtOH (10 ml) was refluxed for 1.5 h. The reaction mixture was neutralized with Dowex 50W×8, and concentrated to half the original volume under reduced pressure. The residue was extracted with AcOEt, and the extract was evaporated to dryness. The residue was crystallized from MeOH–H_2O to afford VIII (10 mg) as a white powder, mp 219—222°C. This compound was identified by comparison with an authentic sample.[11] (IR spectroscopy, TLC and mixed melting point determination).

Permethylation of XVI and Reductive Cleavage of the Permethylate with $LiAlH_4$——The desmonoterpenyl compound (XVI) (200 mg) in dimethyl sulfoxide (DMSO) (5 ml) was methylated by Hakomori's method to afford 90 mg of permethylate as a colorless syrup, $[\alpha]_D^{24}$ $-36.8°$ ($c=0.87$, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 (COOR), 1100 (C–O–C). PMR δ: 4.25 (1H, d, $J=7$ Hz, anomeric H), 4.46 (1H, d, $J=7$ Hz, anomeric H), 4.59 (1H, d, $J=5$ Hz, anomeric H), 4.82 (1H, br s, anomeric H), 5.33 (1H, br s, anomeric H), 5.44 (1H, d, $J=7$ Hz, anomeric H). This permethylate (70 mg) was reduced with $LiAlH_4$ (30 mg) in THF (5 ml) in the usual manner, and the reaction mixture was extracted with Et_2O and AcOEt, in that order. The compound obtained from the Et_2O extract was identical with the sample obtained from the permethylate of desmonoterpenyl GS-C[2] as judged by TLC, IR and PMR comparison. PMR δ: 4.25 (1H, d, $J=7$ Hz, anomeric H), 4.46 (1H, d, $J=7$ Hz, anomeric H), 4.59 (1H, d, $J=4$ Hz, anomeric H). The methylated oligosaccharide was obtained from the AcOEt extract as a colorless syrup, $[\alpha]_D^{25}$ $-53.7°$ ($c=1.50$, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400—3500 (OH), 1100 (C–O–C). PMR δ: 1.30 (3H, d, $J=6$ Hz, CH_3 of rhamnose), 1.32 (3H, d, $J=6$ Hz, CH_3 of rhamnose), 4.88 (1H, br s, anomeric H), 5.04 (1H, br s, anomeric H).

Permethylation of XVII and Reductive Cleavage of the Permethylate with $LiAlH_4$——The desmonoterpenyl compound (XVII) (200 mg) was methylated by the same method as described for X to afford 70 mg of permethylate as a colorless syrup, $[\alpha]_D^{19}$ $-47.1°$ ($c=1.01$, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745 (COOR), 1100 (C–O–C). PMR δ: 4.26 (1H, d, $J=7$ Hz, anomeric H), 4.46 (1H, d, $J=7$ Hz, anomeric H), 4.59 (1H, d, $J=4$ Hz, anomeric H), 4.62 (1H, d, $J=7$ Hz, anomeric H), 4.82 (1H, br s, anomeric H), 5.21 (1H, br s, anomeric H), 5.49 (1H, d, $J=7$ Hz, anomeric H). This permethylate was reduced with $LiAlH_4$ in the same manner as described for X. The compound obtained from the Et_2O extract was identical with the sample obtained from the permethylate of desmonoterpenyl GS-C as judged by TLC, IR and PMR comparison. The methylated oligosaccharide was obtained from the AcOEt extract as a colorless syrup $[\alpha]_D^{19}$ $-70.8°$ ($c=0.87$, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400—3500 (OH), 1100 (C–O–C). PMR δ: 1.30 (3H, d, $J=6$ Hz, CH_3 of rhamnose), 1.31 (3H, d, $J=6$ Hz, CH_3 of rhamnose), 4.63 (1H, d, $J=7$ Hz, anomeric H), 4.87 (1H, br s, anomeric H), 5.03 (1H, br s, anomeric H).

Hydrolysis of XV with 0.1% K_2CO_3——A solution of the prosapogenin (XV) (500 mg) and 0.1% K_2CO_3 (50 ml) in EtOH (50 ml) was stirred for 1.5 h at room temperature, then the reaction mixture was neutralized with Dowex 50W × 8 under ice cooling. The neutral solution was concentrated to about half the original volume under reduced pressure, and extracted with *n*-BuOH saturated with water. The organic layer was evaporated to dryness under reduced pressure. The residue was examined by TLC (solvent: $CHCl_3$-MeOH-H_2O = 13: 7: 2), and showed three major spots (*Rf* 0.23, 0.18 and 0.10). The mixture was fractionated by chromatography on silica gel, and the product corresponding to *Rf* 0.23 (220 mg) was found to be identical with prosapogenin (XV). The product corresponding to *Rf* 0.10 (80 mg) was identical with the desmonoterpenyl compound (XVII). The product corresponding to *Rf* 0.18 was purified by HPLC (column: reversed phase C_{18}, solvent: MeOH-H_2O = 68: 32, flow rate 1.5 ml/min), and crystallized from MeOH-H_2O to afford 100 mg of XVIII as hygroscopic colorless needles mp 216—218°C, $[\alpha]_D^{28}$ −34.7° (c = 0.64, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1730 (COOR). UV λ_{max}^{EtOH} (nm): 217 (ε = 11000). ^{13}C-NMR data: see Table II and III. *Anal.* Calcd for $C_{79}H_{126}O_{36} \cdot 5H_2O$: C, 54.47; H, 7.87. Found: C, 54.92; H, 7.72. On hydrolysis with 5% K_2CO_3 in EtOH, XVIII afforded the desmonoterpenyl compound (XVII), and the monoterpene (IV) which was identical with the sample obtained from GS-C (TLC, IR, PMR and ^{13}C-NMR of its methyl ester). XVIII was treated with 0.1% K_2CO_3 in ethanol for 3 h at room temperature, and the mixture was neutralized with Dowex 50W × 8 under ice cooling. The neutral solution was evaporated to dryness under reduced pressure. The residue was examined by TLC (solvent; the same as described above) and showed two spots (*Rf* 0.18 and 0.10). In the ^{13}C-NMR spectrum of the crude fraction corresponding to *Rf* 0.18, signals of anomeric carbons appeared at the same chemical shifts as in the case of XVIII. The product corresponding to *Rf* 0.10 was identical with an authentic sample of XVII. During measurement of the ^{13}C-NMR in pyridine, the ^{13}C chemical shifts of XVIII were not altered.

Hydrolysis of GS-B (XIX) with Cellulase——A solution of GS-B (XIX, 50 mg) and cellulase (30 mg) in H_2O (100 ml) was incubated for 72 h at 37°C. The solution was treated as described above to afford the prosapogenin (XXII) (12 mg) as a hygroscopic white powder, mp 204—206°C, $[\alpha]_D^{15}$ −22.2° (c = 0.81, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1740 (COOR). UV λ_{max}^{EtOH} (nm): 213 (ε = 27000). CMR data: see Tables II and III. *Anal.* Calcd for $C_{89}H_{140}O_{40} \cdot 4H_2O$: C, 55.61; H, 7.76. Found: C, 55.82; H, 7.69. The prosapogenin (XXII) was hydrolyzed with 5% K_2CO_3 in EtOH as described above to afford the desmonoterpenyl compound (XVII) which was identical with an authentic sample (TLC, IR, ^{13}C-NMR and mixed melting point).

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and olefinic carbon signals were at 167.4, 167.3, 146.6, 146.5, 144.2, 132.9, 127.9 and 111.8 measured in C_5D_5N).

18) These permethylates and reductive products afforded methylated monosaccharides upon methanolysis and these were identified by GLC and TLC comparisons with authentic samples. Conditions of GLC and TLC were the same as in our preceding paper.[2]

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