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Legume Saponins of *Gleditsia japonica* Miquel. V.[1] ^{13}C Nuclear Magnetic Resonance Spectral Studies on the Structures of Gleditsia Saponins D_2, G and I

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Three triterpenoid saponins named gleditsia saponins D_2 (VII), G (IV) and I (VIII) were isolated from the legume of *Gleditsia japonica* cv.'Saponifera.' They were characterized as echinocystic acid 3,28-*O*-bisglycosides acylated with two monoterpene carboxylic acids IX and X. The locations of these monoterpenes were established by ^{13}C nuclear magnetic resonance spectroscopy.

Keywords——saponin; saponin acylated with monoterpene; gleditsia saponin D_2; gleditsia saponin G; gleditsia saponin I; echinocystic acid; *Gleditsia japonica*; Legumisosae; ^{13}C-NMR

In the preceding paper,[1] we reported the locations of two monoterpenes in the molecules of gleditsia saponins B (GS–B, I) and C (GS–C, II) which were isolated from *Gleditsia japonica* cv. 'Saponifera'; these saponins were characterized as formulae I and II on the basis of their ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectra. Further, the minor saponins, GS–E (III) and G (IV) had been isolated and the structures of the desmonoterpenyl compounds of these

Table I. ^{13}C Chemical Shifts (δ) of the Monoterpene Moieties of Saponins (IV, VIII and VII) (in C_5D_5N)

	GS–C (II)	GS–G (IV)	GS–I (VIII)	GS–D_2 (VII)	Me ester of monoterpene (IX)[a]
1	167.2	167.2	167.3	167.2	168.4
2	127.6	127.6	127.6	127.5	127.5
3	144.4	144.3	144.3	144.3	143.5
4	24.0	23.9	24.0	23.9	24.0
5	41.4	41.4	41.5	41.4	41.5
6	72.2[b]	72.2[b]	72.2[b]	72.1[b]	72.1
7	146.3	146.3	146.3	146.2	146.6
8	111.8[c]	111.8[c]	111.8[c]	111.8[c]	111.7
9	12.6	12.6	12.7	12.6	12.4
10	28.4	28.4	28.6	28.5	28.5
					51.5
1	167.0	167.1	167.1	167.0	(OCH_3)
2	132.8	132.8	132.8	132.7	
3	146.8	146.7	146.8	146.7	
4	24.1	24.1	24.2	24.1	
5	41.8	41.7	41.9	41.8	
6	72.2[b]	72.2[b]	72.2[b]	72.1[b]	
7	146.3	146.4	146.4	146.3	
8	111.8[c]	111.8[c]	111.8[c]	111.8[c]	
9	56.2	56.1	56.1	56.1	
10	28.3	28.3	28.5	28.4	
UV λ_{max}	215 nm (ε = 19000)	215 nm (ε = 24000)	215 nm (ε = 25000)	215 nm (ε = 21000)	

a) With regard to the carbon signals of the monoterpene (IX) in C_5D_5N, the ^{13}C-NMR spectrum of IX in $CDCl_3$ and that of GS-C in C_5D_5N were considered as reference spectra.[4]

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

TABLE II. ^{13}C Chemical Shifts (δ) of the Oligosaccharide Moieties of Saponins (IV, VIII and VII) and the Desmonoterpenyl Compounds of These Saponins (in C_5D_5N)

				(VI)		GS-G (IV)	$\Delta\delta$ (IV−VI)	XIII	GS-I (VIII)	$\Delta\delta$ (VIII−XIII)	GS-D_2 (VII)		
3-*O*-glycoside			1	106.7[b]		106.6	(−0.1)	106.5[b]	106.6	(+0.1)	106.7		
			2	75.7		75.6	(−0.1)	75.7	75.9	(+0.1)	75.4		
			3	78.6		78.6	(± 0)	78.0	78.2	(+0.2)	78.3		
			4	71.8		71.8	(± 0)	70.9	70.9	(± 0)	71.7		
			5	78.0		78.0	(± 0)	66.7	66.7	(± 0)	76.4		
			6	62.9		62.8	(−0.1)				69.7		
			1								105.1[c]		
			2								72.1		
			3								74.1		
			4								68.9		
			5								66.1		
		(XII)[a]	(XI)	$\Delta\delta$(XI−XII)		$\Delta\delta$(VI−XII)			$\Delta\delta$(XIII−VI)		$\Delta\delta$(VIII−IV)			$\Delta\delta$(VII−IV)	$\Delta\delta$(VII−VI)
28-*O*-glycoside	1	94.6	94.5	(−0.1)	94.5	(−0.1)	94.4	(−0.1)	94.4	(−0.1)	94.5	(+0.1)	(+0.1)	94.4	(± 0)	(−0.1)
glc	→2	79.4	79.3	(−0.1)	79.0	(−0.4)	78.9	(−0.1)	79.0	(± 0)	78.9	(± 0)	(−0.1)	78.9	(± 0)	(−0.1)
	3	77.6	77.4	(−0.2)	77.5	(−0.1)	77.3	(−0.2)	77.4	(−0.1)	77.4	(+0.1)	(± 0)	77.3	(± 0)	(−0.2)
	4	71.1	71.1	(± 0)	71.0	(−0.1)	70.9	(−0.1)	71.0	(± 0)	70.9	(± 0)	(−0.1)	70.9	(± 0)	(−0.1)
	5	76.1	76.2	(+0.1)	76.5	(+0.4)	76.7	(−0.2)	76.5	(± 0)	76.7	(± 0)	(+0.2)	76.6	(−0.1)	(+0.1)
	→6	66.5	66.5	(± 0)	66.6	(+0.1)	66.6	(± 0)	66.6	(± 0)	66.6	(± 0)	(± 0)	66.6	(± 0)	(± 0)
	—1	101.2	101.2	(± 0)	101.3	(+0.1)	101.2	(−0.1)	101.2	(−0.1)	101.4	(+0.2)	(+0.2)	101.3	(+0.1)	(± 0)
rham	2	71.9	71.8	(−0.1)	71.7	(−0.2)	71.6	(−0.1)	71.5	(−0.2)	71.6	(± 0)	(+0.1)	71.6	(± 0)	(−0.1)
	3	72.6	72.5	(−0.1)	72.4	(−0.2)	72.4	(± 0)	72.3	(−0.1)	72.4	(± 0)	(+0.1)	72.3	(−0.1)	(−0.1)
	→4	83.9	83.9	(± 0)	83.7	(−0.2)	83.9	(+0.2)	83.9	(+0.2)	83.9	(± 0)	(± 0)	83.9	(± 0)	(+0.2)
	5	68.4	68.4	(± 0)	68.3	(−0.1)	68.3	(± 0)	68.2	(−0.1)	68.4	(+0.1)	(+0.2)	68.3	(± 0)	(± 0)
	6	18.7	18.6	(−0.1)	18.6	(−0.1)	18.6	(± 0)	18.5	(−0.1)	18.7	(+0.1)	(+0.2)	18.5	(−0.1)	(−0.1)
	—1	106.9	106.9	(± 0)	106.2	(−0.7)	106.2	(+ 0)	106.2	(± 0)	106.3	(+0.1)	(+0.1)	106.2	(± 0)	(± 0)
xyl	2	76.0	76.0	(± 0)	74.9	(−1.1)	74.8	(−0.1)	74.8	(−0.1)	74.9	(+0.1)	(+0.1)	74.8	(± 0)	(−0.1)
	→3	78.6	78.5	(−0.1)	87.4	(+8.8)	87.4	(± 0)	87.4	(± 0)	87.4	(± 0)	(± 0)	87.4	(± 0)	(± 0)
	4	71.0	70.9	(−0.1)	69.0	(−2.0)	68.9	(−0.1)	68.9	(−0.1)	69.0	(+0.1)	(+0.1)	68.9	(± 0)	(−0.1)
	5	67.4	67.3	(−0.1)	67.2	(−0.2)	67.1	(−0.1)	67.2	(± 0)	67.2	(+0.1)	(± 0)	67.1	(± 0)	(−0.1)
	—1				105.9		105.7	(−0.2)	105.8	(−0.1)	105.9	(+0.2)	(+0.1)	105.8	(+0.1)	(−0.1)
xyl	2				75.0		74.9	(−0.1)	74.9	(−0.1)	75.0	(+0.1)	(+0.1)	74.9	(± 0)	(−0.1)
	3				78.0		77.8	(−0.2)	77.9	(−0.1)	78.0	(+0.2)	(+0.1)	77.9	(+0.1)	(−0.1)
	4				70.7		70.6	(−0.1)	70.6	(−0.1)	70.7	(+0.1)	(+0.1)	70.6	(± 0)	(−0.1)
	5				66.8		66.7	(−0.1)	66.7	(−0.1)	66.7	(± 0)	(± 0)	66.7	(± 0)	(−0.1)
	—1	101.8	98.1	(−3.7)	101.8	(± 0)	98.0	(−3.8)	101.7	(−0.1)	98.1	(+0.1)	(−3.6)	98.0	(± 0)	(−3.8)
rham	2	72.1	71.4	(−0.7)	72.0	(−0.1)	71.3	(−0.7)	71.9	(−0.1)	71.3	(± 0)	(−0.6)	71.3	(± 0)	(−0.7)
	3	72.6	73.3	(+0.7)	72.6	(± 0)	73.2	(+0.6)	72.6	(± 0)	73.3	(+0.1)	(+0.7)	73.2	(± 0)	(+0.6)
	4	73.9	70.8	(−3.1)	73.8	(−0.1)	71.0	(−2.8)	73.8	(± 0)	71.0	(± 0)	(−2.8)	71.0	(± 0)	(−2.8)
	5	69.7	69.6	(−0.1)	69.6	(−0.1)	69.5	(−0.1)	69.5	(−0.1)	69.6	(+0.1)	(+0.1)	69.5	(± 0)	(−0.1)
	6	18.7	18.6	(−0.1)	18.7	(± 0)	18.7	(± 0)	18.6	(−0.1)	18.8	(+0.1)	(+0.2)	18.6	(−0.1)	(−0.1)

a) These chemical shifts were assigned by the PRFT-CMR method in the preceding paper.[1]

b) With regard to the carbon signals of β-D-glucopyranoside and β-D-xylopyranoside in C_5D_5N, the literature data for Me-β-D-glucopyranoside (105.4, 74.8, 78.1, 71.4, 78.1 and 62.5) and Me-β-D-xylopyranoside (106.1, 74.6, 78.1, 70.9 and 66.9) were taken into consideration.[6]

c) With regard to the carbon signals of α-L-arabinopyranoside in C_5D_5N, the lit. data for Me-α-L-arabinopyranoside (105.8, 72.1, 74.2, 69.0 and 66.5) were taken into consideration.[9]

saponins had been characterized as formulae V and VI, respectively, in a previous paper.[2]

The present paper describes the isolation of gleditsia saponins D_2 (GS-D_2, VII) and I (GS-I, VIII), and ^{13}C-NMR spectral studies on the structures of IV, VII and VIII.

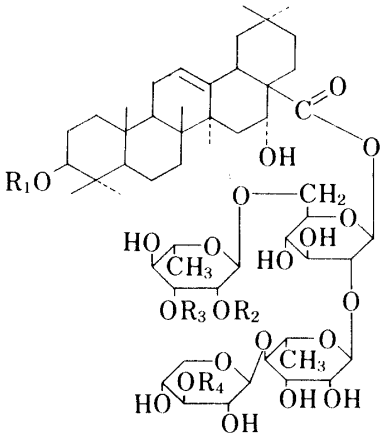
On the basis of ^{13}C-NMR spectra and ultraviolet (UV) spectra[3] of IV, VII and VIII, it was deduced that these saponins had the same monoterpenes (IX and X) as GS-C (II),[4] as shown in Table I. The prosapogenin (XI) was obtained from GS-G (IV) by enzymatic hydrolysis; the assignments of ^{13}C-NMR signals of the desmonoterpenyl prosapogenin (XII) obtained from XI had been established previously[1] by consideration of the PRFT-^{13}C-NMR spectra,[5] as shown in Table II.

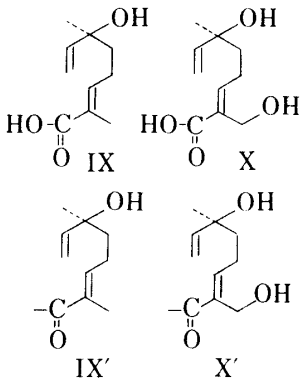
The ^{13}C-NMR spectrum of the desmonoterpenyl compound (VI), obtained from GS-G (IV), showed six kinds of signals due to anomeric carbons at δ 106.7, 94.5, 101.3, 106.2, 105.9 and 101.8. In this spectrum, six carbon signals (at δ 106.7, 75.7, 78.6, 71.8, 78.0 and 62.9) were reasonably assigned to carbons of glucose attached at the 3-*O*-hydroxy group of echinocystic acid by comparison with the chemical shift values of the 3-*O*-glucoside of methyl echinocystate[1] and with those of methyl β-D-glucopyranoside,[6] and five carbon signals (at δ 105.9, 75.0, 78.0, 70.7 and 66.8) were assigned to the terminal xylose by comparison with the chemical shift values of methyl β-D-xylopyranoside.[6] The three carbon signals at δ 74.9, 87.4 and 69.0 were reasonably assigned to C-2, C-3 and C-4 of the inner-located xylose by consideration of the chemical shift values of xylose carbons in the molecule of XII and glycosylation shifts.[7] (When XII is compared with VI, the signals of C-2, C-4 and C-3 are shifted to upper field by 1.1 ppm and 2.0 ppm and to lower field by 8.8 ppm, respectively.) Other carbon signals of VI were superimposable on those of XII. Therefore, all carbon signals due to the oligosaccharide moiety of VI can be assigned as shown in Table II.

The positions acylated with monoterpene carboxylic acids in GS-G (IV) were elucidated by comparison of the ^{13}C-NMR spectra of IV and XI with those of VI and XII, respectively. The carbon signals due to C-1 and C-4 of terminal rhamnose units in IV and XI were shifted to upper field by 3.8 and 2.8 ppm, and by 3.7 and 3.1 ppm, respectively, while other signals were superimposable on each other. On the basis of the acylation shifts,[8] it was concluded that the two monoterpenes in GS-G (IV) were located at C-2 and C-3 of the terminal rhamnose.

The desmonoterpenyl compound obtained from GS-D_2 (VII) by hydrolysis with 5% K_2CO_3 and its permethylate were identified as V and its permethylate, respectively, by comparison with samples obtained from GS-E.[2] In the molecule of the desmonoterpenyl compound (V), the structure of the oligosaccharide moiety attached at the C-3 hydroxy group of echinocystic acid was α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl, and the structure of the oligosaccharide moiety attached at the C-28 carboxyl group was the same as that of VI. On the basis of the chemical shift values of methyl β-D-glucopyranoside and methyl α-L-arabinopyranoside[9] and the glycosylation shifts, the ^{13}C-NMR signals due to the 3-*O*-glycoside of VII were assigned as shown in Table II. Comparison of the ^{13}C-NMR spectrum of GS-D_2 (VII) with that of VI revealed that the monoterpenes were located at C-2 and C-3 of the terminal rhamnose, because the signals due to C-1 and C-4 of this rhamnose were shifted to upper field by 3.8 ppm and 2.8 ppm, respectively. This result was the same as for GS-G (IV), and signals due to the 28-*O*-glycoside moiety of VII were superimposable on those of IV.

The desmonoterpenyl compound (XIII) was obtained from GS-I (VIII) on hydrolysis with 5% K_2CO_3, and its permethylate (XIV) was obtained on permethylation by Hakomori's method.[10] Compound XIV was reduced with $LiAlH_4$ to afford XV and the methylated oligosaccharide (XVI). The proton magnetic resonance (^{1}H-NMR) spectrum of XV revealed the presence of one anomeric proton at δ 4.24 (d, J=7 Hz) and four methoxyl groups at δ 3.27, 3.54, 3.60 and 3.62 (each s). On methanolysis of XV, methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside and 16-*O*-methyl primulagenin A were obtained and identified by comparison with authentic samples. The methylated oligosaccharide (XVI) was identical with the sample obtained from GS-G (IV) as judged by thin-layer chromatography (TLC), IR and ^{1}H-NMR





	R_1	R_2	R_3	R_4
GS-B (I)	-glc$^{6\,1}$ara$^{2\,1}$xyl	X′	X′	-xyl
GS-C (II)	-glc$^{6\,1}$ara$^{2\,1}$xyl	IX′	X′	-xyl
GS-G (IV)	-glc	IX′[a]	X′[a]	-xyl
VI	-glc	H	H	-xyl
XI	H	IX′[a]	X′[a]	H
XII	H	H	H	H
GS-D_2 (VII)	-glc$^{6\,1}$ara	IX′[a]	X′[a]	-xyl
V	-glc$^{6\,1}$ara	H	H	-xyl
GS-I (VIII)	-xyl	IX′[a]	X′[a]	-xyl
XIII	-xyl	H	H	-xyl

a) IX′ and X′ may be interchangeable, although the assignments given here are preferred on the basis of the results for GC-C.

Chart 1

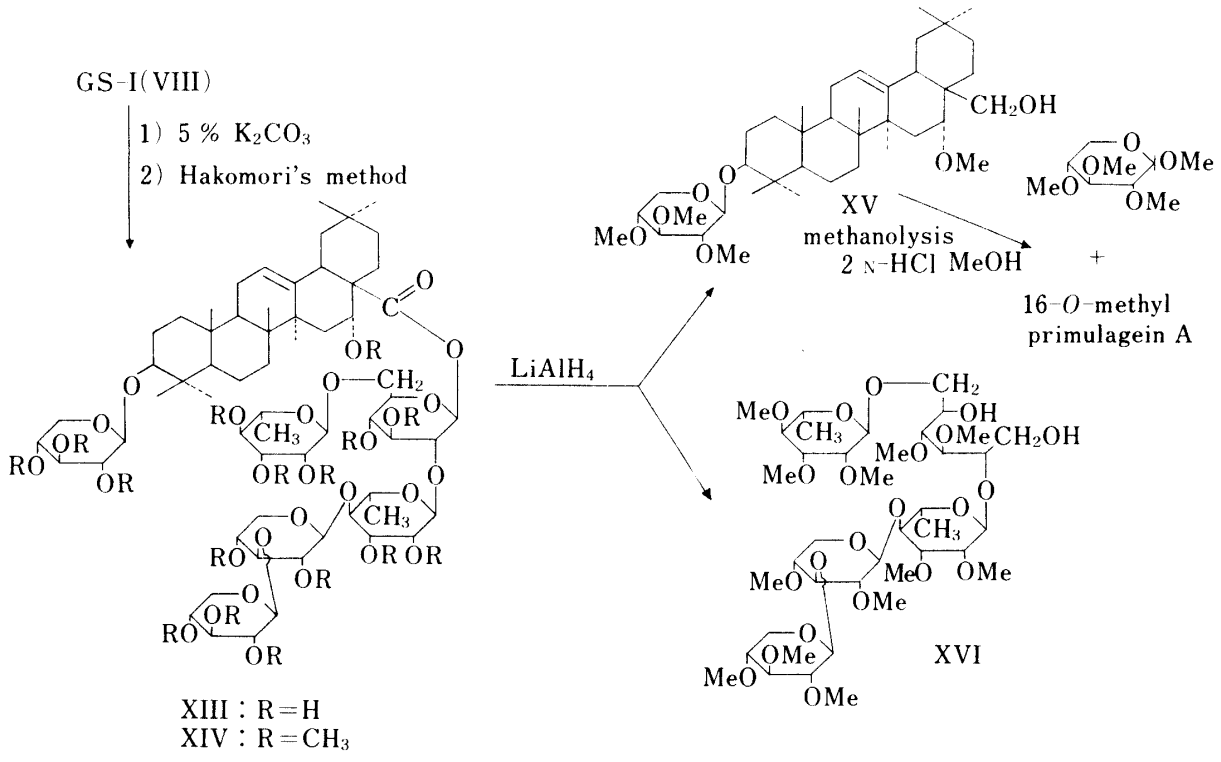


Chart 2

comparison.[2] Therefore, the desmonoterpenyl compound (XIII) obtained from GS-I (VIII) was characterized as echinocystic acid 3-*O*-β-D-xylopyranoside-28-*O*-[β-D-xylopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside. In the ^{13}C-NMR spectrum of XIII, five carbon signals (at δ 106.5, 75.7, 78.0, 70.9 and 66.7) were assigned to carbons of the xylose unit attached at the C-3 hydroxy group of echinocystic acid by comparison with the chemical shift values of methyl β-D-xylopyranoside, and other signals due to the 28-*O*-glycoside moiety were superimposable on those of VI. Comparison of the ^{13}C-NMR spectrum of GS-I (VIII) with that of its desmonoterpenyl compound (XIII) revealed that the monoterpenes were located at C-2 and C-3 of the terminal rhamnose, because the carbon signals due to C-1 and C-4 of this rhamnose were shifted to upper field by 3.6 ppm and 2.8 ppm, respectively.

Therefore, the structures of gleditsia saponins D_2, G and I were elucidated as VII, IV and VIII, respectively.

Experimental

Melting points are uncorrected. The ^{1}H-NMR spectra (100 MHz) were measured in $CDCl_3$, and ^{13}C-NMR spectra (50 MHz) were measured in C_5D_5N on a Varian XL 200 NMR spectrometer using 10 mm spinning tubes, with tetramethylsilane (TMS) as an internal standard. UV spectra were measured on Shimadzu UV-240 spectrometer in 95% EtOH. The properties and physical data of IV, V, VI, XI and XII were reported in the previous paper.[1,2]

Isolation and Properties of GS-D_2 (VII) and GS-I (VIII)——The crude saponin fraction was chromatographed repeatedly on a silica gel column (solvent; $CHCl_3$–MeOH–H_2O=8: 3: 1) until each fraction showed one spot on TLC. A fraction which consisted of a mixture of III and VII was purified by semi-preparative high performance liquid chromatography (HPLC) on a reversed phase C_{18} column (solvent, MeOH–H_2O=7: 3; flow rate, 2.0 ml/min), and gel filtration on Sephadex LH 20 (eluted with MeOH). The product was repeatedly precipitated from MeOH–Et_2O, yielding GS-D_2 (VII) as a white powder, mp 200—204°C, $[\alpha]_D^{23}$ −17.4° (c=0.85, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1725 (COOR). UV λ_{max}^{EtOH} (nm): 215 (ε=21000). *Anal.* Calcd for $C_{89}H_{140}O_{39}\cdot 5H_2O$: C, 55.85; H, 7.83. Found: C, 55.72; H, 7.51. ^{13}C-NMR data: see Tables I and II. GS-I (VIII) was also obtained by HPLC under the same conditions as described above to afford a white powder, mp 201—203°C, $[\alpha]_D^{25}$ −27.5° (c=0.77, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1725 (COOR). UV λ_{max}^{EtOH} (nm): 215 (ε=25000). *Anal.* Calcd for $C_{83}H_{130}O_{34}\cdot 3H_2O$: C, 57.76; H, 7.94. Found: C, 57.42; H, 8.20. ^{13}C-NMR data: see Tables I and II.

Hydrolysis of GS-D_2 (VII) with 5% K_2CO_3—A mixture of VII (20 mg) and 5% K_2CO_3 (10 ml) in EtOH (10 ml) was refluxed for 1 h. The reaction mixture was neutralized with Dowex 50W×8, and the neutral solution was concentrated under reduced pressure. The residue was extracted with ether, and the aqueous layer was extracted with *n*-BuOH saturated with water. The organic layer was evaporated to dryness under reduced pressure, and the residue was crystallized from MeOH–H_2O to afford the desmonoterpenyl compound V as a colorless powder, mp 265—269°C, $[\alpha]_D^{12}$ −37.8° (c=0.51, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1740 (COOR). ^{13}C-NMR data: see Table II. This product was identical with an authentic sample obtained from GS-E.[2]

Permethylation of V——According to Hakomori's method, NaH (1 g) was stirred with dimethyl sulfoxide (DMSO, 50 ml) at 90°C for 30 min under N_2 gas. A solution of V (100 mg) in DMSO (10 ml) was added to the above reagent (8 ml), and the whole was stirred for 1 h at room temperature. CH_3I (10 ml) was added and the whole was stirred for 3 h at room temperature. The reaction mixture was then poured into ice-water, and the mixture was extracted with Et_2O. The organic layer was washed with water, dried over $MgSO_4$ and concentrated to afford a syrup (130 mg). This syrup was purified by silica gel column chromatography (solvent; benzene–acetone=9: 1) to afford the permethylate (80 mg) as a white powder, $[\alpha]_D^{12}$ −35.3° (c=0.70, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (COOR). ^{1}H-NMR δ: 4.17 (1H, d, J=3 Hz, anomeric H), 4.29 (1H, d, J=7 Hz, anomeric H), 4.61 (1H, d, J=7 Hz, anomeric H), 4.70 (1H, d, J=7 Hz, anomeric H), 4.82 (1H, br s, anomeric H), 5.17 (1H, br s, anomeric H), 5.54 (1H, d, J=7 Hz, anomeric H). This permethylate was identical with an authentic sample obtained from GS-E on TLC, IR and ^{1}H-NMR comparison.

Hydrolysis of GS-I (VIII) with 5% K_2CO_3——GS-I (VIII, 10 mg) was hydrolyzed in the same way as GS-D_2 (V) to give the desmonoterpenyl compound (XIII, 7 mg) as colorless needles from MeOH–H_2O, mp 188—192°C, $[\alpha]_D^{12}$ −89.6° (c=0.72, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1740 (COOR). *Anal.* Calcd for $C_{63}H_{102}O_{29}\cdot H_2O$: C, 56.40; H, 7.81. Found: C, 56.22; H, 8.03. ^{13}C-NMR data: see Table II.

Permethylation of XIII——XIII (50 mg) was methylated in the same way as III to afford the permethylate (XIV, 35 mg) as a colorless syrup, $[\alpha]_D^{24}$ −53.7° (c=1.00, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (COOR). ^{1}H-NMR δ: 3.42—3.61 (48H, m, 16×OCH_3), 4.25 (1H, d, J=7 Hz, anomeric H), 4.63 (1H, d, J=7 Hz, anomeric

H), 4.69 (1H, d, $J=7$ Hz, anomeric H), 4.82 (1H, br s, anomeric H), 5.16 (1H, br s, anomeric H), 5.54 (1H, d, $J=7$ Hz, anomeric H). *Anal.* Calcd for $C_{92}H_{156}O_{37}$: C, 59.59; H, 8.48. Found: C, 59.40; H, 8.70.

Reduction of XIV with $LiAlH_4$——The permethylate (XIV, 20 mg) was dissolved in anhydrous THF (6 ml). $LiAlH_4$ (30 mg) was added, and the mixture was refluxed for 3 h. The excess $LiAlH_4$ was decomposed with wet Et_2O, and the mixture was extracted with Et_2O and AcOEt, in that order. Each organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. The ether extract was purified by preparative TLC (solvent; benzene–acetone=5: 2) to afford compound XV (8 mg). $[\alpha]_D^{10}$ $-12.5°$ ($c=0.20$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH, weak). ^{1}H-NMR δ: 0.85, 0.87, 0.88, 0.91, 0.94, 1.03 1.25 (3H, each s, $7\times CH_3$), 3.27, 3.54, 3.60, 3.62 (3H, each s, $4\times OCH_3$), 4.24 (1H, d, $J=7$ Hz, anomeric H). *Anal.* Calcd for $C_{39}H_{66}O_7$: C, 72.40; H, 10.28. Found: C, 72.27; H, 10.33. The AcOEt extract was purified by preparative TLC (solvent; benzene–acetone=3: 2) to afford methylated oligosaccharide (XV, 10 mg), IR $\nu_{max}^{CHCl_3}$ cm^{-1}:, 3450 (OH). ^{1}H-NMR δ: 1.24—1.36 ($3H\times 2$, $2\times CH_3$), 3.46—3.62 (36H, m, $12\times OCH_3$), 4.64 (1H, d, $J=7$ Hz anomeric H), 4.70 (1H, d, $J=7$ Hz, anomeric H), 4.87 (1H, br s, anomeric H), 5.05 (1H, br s, anomeric H). This product was found to be identical with a sample obtained by reduction of the permethylate of VI.[2]

Methanolysis of XV——A solution of XV (5 mg) in methanolic 2 N HCl was refluxed for 3 h and the reaction mixture was neutralized with Ag_2CO_3. The neutral solution was concentrated, and the residue was identified as methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside and 16-*O*-methyl primulagenin A by comparison with authentic samples.

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