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Studies on the Constituents of Leguminous Plants. VII.[1] The Structure of Triterpenoid Saponins from Fruits of *Gymnocladus chinensis* BAILLON

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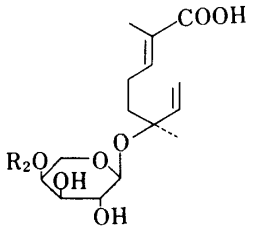
Three triterpenoid saponins, gymnocladus saponins A (**12**), B (**18**) and C (**20**), were isolated from the fruits of *Gymnocladus chinensis* BAILLON (Leguminosae). On the basis of chemical and physicochemical evidence, these saponins were characterized as 2β,23-dihydroxy-3-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]acacic acid 28,21-lactone (**12**), 2β,23-dihydroxy-3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]acacic acid 28,21-lactone (**18**) and 2β,23-dihydroxy-3-*O*-[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]acacic acid 28,21-lactone (**20**).

Keywords——triterpene saponin; gymnocladus saponin A; gymnocladus saponin B; gymnocladus saponin C; *Gymnocladus chinensis*; Leguminosae

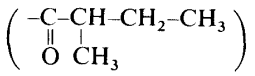
In the preceding paper,[1] we reported the isolation and structure elucidation of two monoterpene glycosides (**1** and **2**) obtained from the fruits of *Gymnocladus chinensis* BAILLON. In this paper, we report the structure elucidation of triterpenoid saponins obtained from the same materials.

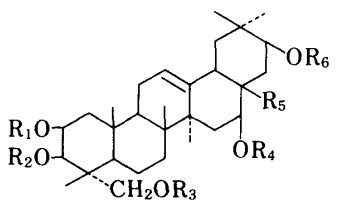
From the crude saponin fractions, three new saponins, gymnocladus saponins A, B and C, were isolated. On hydrolysis of each saponin with 4 N sulfuric acid (H_2SO_4), the same sapogenin (**3**) was obtained as colorless needles.



1: $R_1 = R_2 = H$

2: R_1 = glucose, R_2 = 2-methylbutyroyl





	R_1	R_2	R_3	R_4	R_5	R_6
3	–H	–H	–H	–H	–CO–	
4	–Ac	–Ac	–Ac	–Ac	–CO–	
5	–H	–H	–H	–H	–COOMe	–H
6	–Ac	–Ac	–Ac	–Ac	$-CH_2OAc$	–Ac
7	$-C(CH_3)_2-$		–Ac	–Ac	–CO–	
8	–H	$-C(CH_3)_2-$		–Ac	–CO–	
9	–H	–Ac	–Ac	–Ac	–CO–	
10	–H	–H	–Ac	–Ac	–CO–	
11	–H	–H	–H	–Ac	–CO–	
15	–Me	–H	–Me	–Me	–COOMe	–Me
16	–Me	–Ac	–Me	–Me	–COOMe	–Me

Chart 1

The sapogenin (**3**) showed the presence of a γ-lactone ring at 1760 cm^{-1} in the infrared (IR) spectrum, thirty carbons (–C– × 6, –CH × 3, $-CH_2$ × 7, $-CH_3$ × 6, –CH–O × 4, $-CH_2O$ × 1, –CH=C< × 1 and –C=O × 1) in the ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectrum (Table II), and six kinds of methyl protons and C_{18}–βH (at δ 2.75 (dd, J = 11 and 6 Hz)) in the proton magnetic resonance (^1H-NMR) spectrum (Table I). On acetylation of **3** with acetic anhydride (Ac_2O) in pyridine, a tetraacetate (**4**) was obtained, and on alkaline hydrolysis followed by methylation with diazomethane, a methyl ester (**5**) was obtained. On acetylation of **3** after reduction with lithium aluminum hydride ($LiAlH_4$), a hexaacetate (**6**) was obtained. Two kinds of acetonide acetates (**7**) and (**8**) were obtained from **3** by treatment with acetone and anhydrous cupric sulfate followed by acetylation. From these results, it was deduced that this sapogenin was an oleanene-type triterpene having four hydroxyl groups (as in more than one glycol system) and a lactone ring. In the mass spectra (MS) of **3** and **4**, prominent ion peaks at m/z 262 and 304, respectively, arising from the D and E ring due to retro Diels–Alder cleavage[2] were seen. Therefore, it was clear that one hydroxyl group was on the D or E ring and a glycol system was on the A or B ring.

The ^1H-NMR spectrum of the tetraacetate (**4**) showed the presence of four acetyl methyl group at δ 2.00 (3H), 2.01 (3H) and 2.06 (6H), and showed C_2–αH (δ 5.42 (br s)), C_3–αH (δ 4.91 (d, J = 3.9 Hz)) and C_{23}–H_2 (δ 3.72 and 3.85 (ABq, J = 12.0 Hz)). These chemical shifts and coupling constants are very similar to those of the tetraacetate of polygalacic acid.[3] The values and coupling constants of C_{16}–αH (δ 5.03 (dd, J = 11.6 and 5.1 Hz)) and C_{21}–αH (δ 4.23 (d, J = 5.1 Hz)) are also similar to those of the diacetate of acacic acid lactone.[4] These ^1H-NMR signals of **3** and **4** are also in accord with those of 2β,23-dihydroxy acacic acid lactone and its tetraacetate reported by Parkhurst *et al.*,[5] as shown in Table I.

Therefore, it was deduced that four hydroxyl groups of **3** were located at 2β, 3β, 16β and 23 (including stereochemistry). This structure was also supported by the following findings. On partial acetylation (Ac_2O and pyridine at −10 °C) of **3**, the 3β,16β,23-tri-*O*-acetyl derivative (**9**) was obtained. The 16β,23-diacetyl derivative (**10**) and 16β-monoacetyl derivative (**11**) were obtained, respectively, from **7** and **8**. Therefore the hydroxyl group at C_{16} was easily acetylated, but the hydroxyl group at C_2 was resistant to acetylation,[3] suggesting that the former is β-equatorial and the latter is β-axial. The MS of **3**—**11** exhibited the base ion peak at m/z 244 and a prominent ion peak at m/z 200. These ion peaks are formally derived from retro Diels–Alder cleavage of the C ring followed by dehydration or deacetylation, and then by elimination of a carbon dioxide unit. These fragmentations are typical of triterpenoids having the lactone bridge at C_{28} and C_{21}, for example, machaeric acid lactone,[6] acacic acid lactone[4] and platycogenic acid B dilactone.[7]

In conclusion, the sapogenin (**3**) is identical with the triterpene, 2β,23-dihydroxy acacic acid lactone, that was isolated from *G. dioica* by Parkhurst *et al.*[5,8]

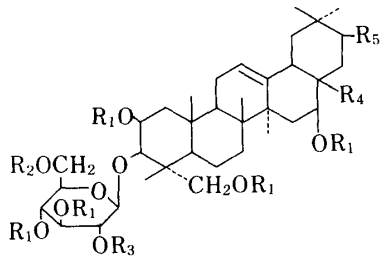
Gymnocladus saponin A (**12**) was obtained as a white powder, and hydrolyzed with 4 N H_2SO_4 to afford the sapogenin (**3**), prosapogenin (**13**), glucose and arabinose.[9] Compound **12** showed the presence of the γ-lactone ring at 1760 cm^{-1} in the IR spectrum and at δ 181.2 (lactone carbonyl) and 83.4 (γ-carbon of the lactone) in the ^{13}C-NMR spectrum. The prosapogenin (**13**) was permethylated by Hakomori's method[10] to afford the nona-*O*-methyl derivative (**14**), and its ^1H-NMR spectrum showed the presence of an anomeric proton at δ 4.19 (d, J = 6.5 Hz). On methanolysis with 2 N hydrochloric acid (HCl) in methanol, **14** afforded methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and the 2,16,21,23-tetra-*O*-methyl derivative (**15**). In the ^1H-NMR spectrum of **15**, four *O*-methyl protons at δ 3.31 (3H), 3.32 (3H), 3.34 (3H) and 3.35 (3H) and carbomethoxy protons at δ 3.62 (3H) were seen. On acetylation of **15**, the 3-*O*-acetyl-2,16,21,23,28-penta-*O*-methyl derivative (**16**) was obtained. In the ^1H-NMR spectrum of **16**, acetyl methyl protons at δ 2.11 (3H) and C_3–αH at 4.98 (d, J = 4.1 Hz) were seen. On the other hand, **12** was permethylated in the same manner as

TABLE I. ^{1}H-NMR Chemical Shift Values and Coupling Patterns of **3**, **4**, **6**, **9**, **10** and **11** in $CDCl_3$

	$-C\underline{H}_3$	$-OCOC\underline{H}_3$	$C_2-\alpha H$	$C_3-\alpha H$	$C_{12}-H$	$C_{16}-\alpha H$	$C_{21}-\alpha H$	$C_{23}-H_2$
3[a]	0.91 (3H) 1.00 (3H) 1.04 (3H) 1.11 (3H) 1.22 (3H) 1.29 (3H)		4.10 (br s)	3.61 (br s)	5.40 (t, J=3.5)	4.00 (dd, J=12.3, 4.5)	4.24 (d, J=5.6)	3.42, 3.71 (ABd, J=10.2)
4[a]	1.02 (6H) 1.04 (3H) 1.06 (3H) 1.22 (3H) 1.24 (3H)	2.00 (3H) 2.01 (3H) 2.06 (6H)	5.42 (br s)	4.91 (d, J=3.9)	5.41 (t, J=3.5)	5.03 (dd, J=11.6, 5.1)	4.23 (d, J=5.1)	3.72, 3.85 (ABd, J=12.0)
6	0.90 (3H) 0.94 (3H) 0.96 (3H) 1.03 (3H) 1.25 (6H)	2.00 (3H) 2.02 (3H) 2.05 (6H) 2.06 (3H) 2.21 (3H)	5.37[b] (br s)	4.92 (d, J=4.0)		5.01 (dd, J=11.5, 6.0)	5.37[b] (br s)	3.70, 3.86 (ABd, J=12.0)
9	1.03 (9H) 1.11 (3H) 1.24 (3H) 1.31 (3H)	1.98 (3H) 2.05 (3H) 2.12 (3H)	4.15 (br s)	4.90 (d, J=4.0)	5.47 (t like)	5.05 (dd, J=11.5, 4.5)	4.22 (d, J=4.5)	3.68, 3.78 (ABd, J=12.0)
10	1.00 (6H) 1.02 (6H) 1.21 (3H) 1.27 (3H)	1.99 (3H) 2.08 (3H)	4.17 (br s)	3.42 (d, J=3.8)	5.46 (t like)	5.02 (dd, J=11.0, 5.0)	4.21 (d, J=4.5)	3.80, 4.15 (ABd, J=11.4)
11	1.00 (6H) 1.03 (3H) 1.10 (3H) 1.21 (3H) 1.28 (3H)	1.99 (3H)	4.12 (br s)	3.57 (d, J=4.5)	5.46 (t like)	5.04 (dd, J=11.0, 5.2)	4.21 (d, J=4.6)	3.40, 3.70 (ABd, J=10.3)

a) These data were measured on a JEOL FX-200 spectrometer (200 MHz).
b) These signals were overlapping.

described for **13** to afford the permethylate (**17**), and its ^{1}H-NMR spectrum showed two anomeric proton signals at δ 4.17 (d, J=5.0 Hz) and 4.23 (d, J=7.1 Hz). On methanolysis of **17**, methyl 2,3,4-tri-*O*-methyl-L-arabinopyranoside, methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside and compound **15** were obtained; they were shown to be identical with authentic samples by thin layer chromatography (TLC) and gas liquid chromatography (GLC). The molecular rotation difference between **12** and **13** was $-33°$.[11] The ^{13}C chemical shifts of the sugar moiety also supported the proposed structure of **12** as shown in Table II. Therefore, the structure of gymnocladus saponin A (**12**) was characterized as 2β,23-dihydroxy-3-*O*-[α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]acacic acid 28,21-lactone.



13 : $R_1=R_2=R_3=H$, $R_4-CO-O-R_5$
14 : $R_1=R_2=R_3=Me$, $R_4=COOMe$, $R_5=OMe$

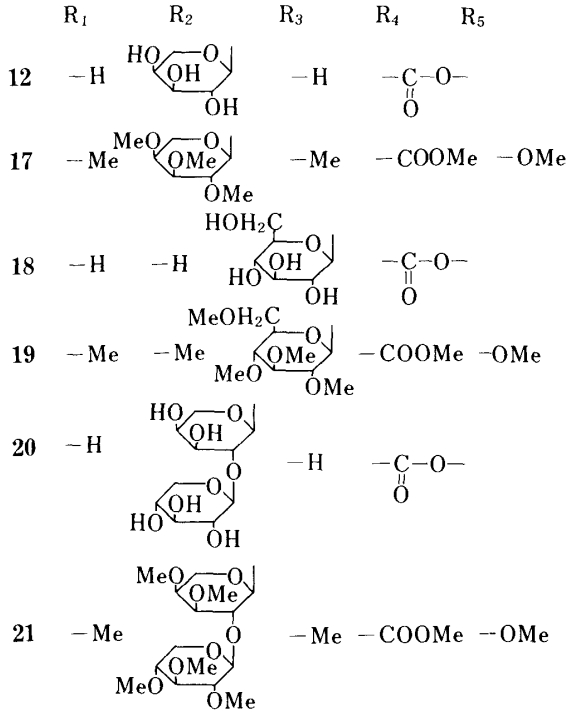


Chart 2

Gymnocladus saponin B (**18**) was obtained as a white powder, and hydrolyzed with 4 N H_2SO_4 to afford the sapogenin (**3**), prosapogenin (**13**) and glucose. Compound **18** showed the presence of a γ-lactone ring at 1760 cm^{-1} in the IR spectrum and δ 181.2 (lactone carbonyl) and 83.4 (γ-carbon of the lactone ring) in the ^{13}C-NMR spectrum, in the same way as **14**. On permethylation of **18** in the same manner as described for **13**, the permethylate (**19**) was obtained and its ^{1}H-NMR spectrum showed two anomeric proton signals at δ 4.67 (d, J= 7.5 Hz) and 4.22 (d, J=7.6 Hz). On methanolysis of **19**, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside and compound **15** were obtained; they were identified by TLC and GLC comparisons with authentic samples. As shown in Table II, the ^{13}C chemical shift values of the sugar moiety support the structure of **18** in comparison with those of **13**. Therefore, the structure of gymnocladus saponin B (**18**) was established as 2β,23-dihydroxy-3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]acacic acid 28,21-lactone.

Gymnocladus saponin C (**20**) was a major saponin of this plant and was obtained as a white powder. The IR spectrum and ^{13}C-NMR spectrum of **20** showed the presence of a γ-lactone ring at 1760 cm^{-1} and at δ 181.2 (lactone carbonyl) and 83. 4(γ-carbon of the lactone ring), respectively. On acid hydrolysis of **20** with 2 N H_2SO_4, the sapogenin (**3**), prosapogenin (**13**), glucose, arabinose and xylose were obtained. The structure of the sugar moiety was determined from the ^{13}C-NMR spectra obtained by the partially relaxed Fourier transfor-

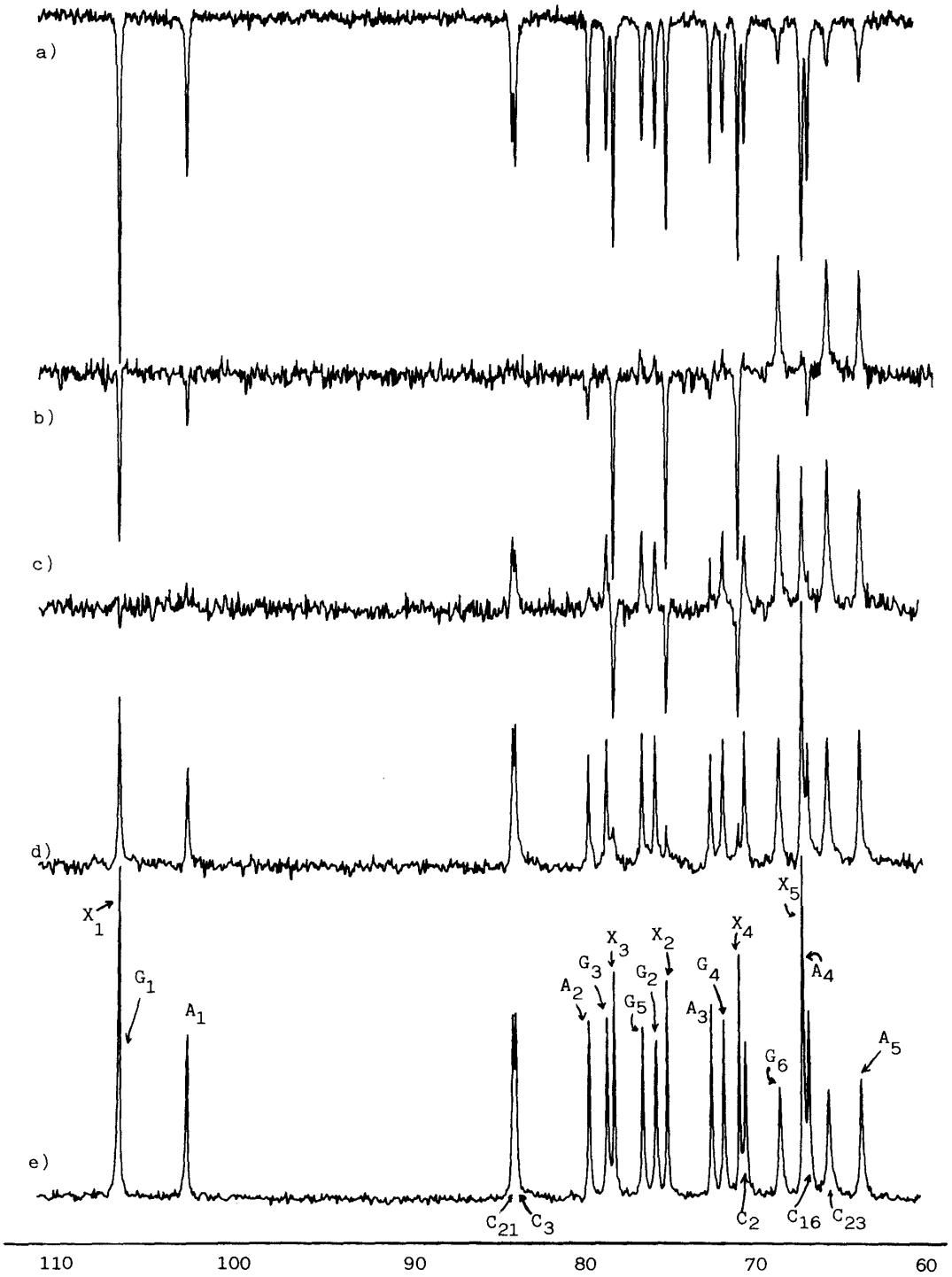


Fig. 1. PRFT-CMR Spectra of Gymnocladus Saponin C (**20**) in C_5D_5N on a JEOL FX-90Q Spectrometer at 22.50 MHz Using a 10 mm Tube

Pulse interval (s): a) 0.03, b) 0.11, c) 0.14, d) 0.24, e) 1.00. Number of cycles: 10000. X_1—X_5: xylose, A_1—A_5: arabinose, G_1—G_6: glucose.

mation (PRFT) method,[12] as shown in Fig. 1. The signals at δ 105.5, 74.8, 77.7, 70.7 and 67.1 due to the carbons having longer spin–lattice relaxation time (T_1) must be assigned to the terminal monosaccharide, so these signals were attributed to β-xylopyranoside. The signals at δ 105.4, 75.4, 78.1, 71.6, 76.1 and 68.4 due to the carbons having shorter T_1 can reasonably be assigned to 6-substituted β-glucopyranoside. Finally, the signals at δ 101.7, 79.2, 72.3, 67.1

TABLE II. ^{13}C-NMR Chemical Shifts of **3**, **12**, **13**, **18** and **20** in Pyridine-d_5[a]

		3[b]	**13**	**18**	**12**	**20**
Triterpene moiety						
	1	44.8 (t)[c]	43.8	43.8	43.8	43.8
	2	71.5 (d)	70.3	70.3	70.7	70.3
	3	72.9 (d)	82.9	82.8	83.2	83.2
	4	42.3 (s)	42.6	42.6	42.6	42.5
	20	34.1 (s)	34.0	34.1	34.1	34.0
	21	83.4 (d)	83.4	83.4	83.6	83.5
	23	67.5 (t)	65.3	65.7	65.6	65.7
	24	14.5 (q)	14.9	14.7	15.0	14.8
	28	181.2 (s)	181.2	181.2	181.1	181.2
Oligosaccharide moiety						
	1		glc. 105.3	glc. 103.0	glc. 105.0	glc. 105.4
	2		75.2	83.3	75.1	75.4
	3		78.3	76.6	78.3	78.1
	4		71.3	71.0	71.8	71.6
	5		78.0	78.0	76.3	76.1
	6		62.5	62.5	69.7	68.4
	1			glc. 105.7	ara. 104.6	ara. 101.7
	2			74.7	72.2	79.2
	3			78.3	73.9	72.3
	4			71.2	68.7	67.1[d]
	5			77.9	66.1	63.8
	6			62.4		
						xyl. 105.5
						74.8
						77.7
						70.7
						67.1[d]

a) The δ_C values for $C_{5-19,22,25-27,29-30}$ are 48.3 (d), 18.2 (t), 32.3 (t), 40.5 (s), 47.8 (d), 37.1 (s), 23.9 (t), 124.7 (d), 140.2 (s), 43.4 (s), 38.1 (t), 66.7 (d), 49.9 (s), 41.7 (d), 42.9 (t), 27.1 (t), 16.2 (q), 17.3 (q), 28.5 (q), 28.8 (q), 24.3 (q), respectively, within ± 0.2.
b) Signal assignments were referred to reported data for polygalacic acid.[15]
c) Abbreviations in parentheses denote signal patterns observed in the off-resonance experiments.
d) These signals were overlapping.

and 63.8 due to the carbons having medium T_1 can be assigned to the 2-substituted α-arabinopyranoside. Therefore, the oligosaccharide moiety of gymnocladus saponin C (**20**) was characterized as β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside. This was confirmed by examination of the ^{1}H-NMR spectrum of the permethylate (**21**) obtained from **20**, and methylated monosaccharide derived from **21** by methanolysis. The permethylate (**21**) showed the presence of three anomeric protons at δ 4.19 (d, J=6.7 Hz), 4.42 (d, J=6.7 Hz) and 4.53 (d, J=4.0 Hz). On methanolysis of **21**, three kinds of methylated monosaccharides (methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside, methyl 3,4-di-*O*-methyl-L-arabinopyranoside and methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside) were obtained and identified by TLC and GLC comparisons with authentic samples. On the other hand, gymnocladus saponin C (**20**) was partially hydrolyzed with 1 N H_2SO_4 to afford gymnocladus saponin A (**12**). Therefore, gymnocladus saponin C (**20**) was characterized as 2β,23-dihydroxy-3-*O*-[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-β-D-glucopyranosyl]acacic acid 28,21-lactone.

Studies on the structures of other glycosides obtained from more polar fractions are in progress.

Experimental

Melting points are uncorrected. Unless otherwise stated, ^{1}H-NMR spectra were measured on a Varian FT-80A instrument in $CDCl_3$ at 80 MHz. MS were measured on a Hitachi M-80 mass spectrometer. GLC was carried out on a Varian Aerograph 2100 gas chromatograph with 15% NEGS on Chromosorb W (0.3 cm × 200 cm).

Isolation of Gymnocladus Saponin A (12), B (18) and C (20)——Crude saponin fraction was repeatedly chromatographed on silica gel with $CHCl_3$–MeOH–H_2O = 8 : 3 : 1 (lower layer) as the developing solvent, and then gel-filtered on Sephadex LH20 with MeOH. The separated saponins were repeatedly precipitated from MeOH–Et_2O, yielding gymnocladus saponin A (**12**) as a hygroscopic white powder, mp 214—217 °C, $[\alpha]_D^{25}$ +0.6° (c = 0.72, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1760 (γ-lactone). ^{13}C-NMR data; see Table II. *Anal.* Calcd for $C_{41}H_{64}O_{15}\cdot 2H_2O$: C, 59.12; H, 8.23. Found: C, 59.38; H, 8.19.

Gymnocladus saponin B (**18**) was obtained as a hygroscopic white powder, mp 227—229 °C, $[\alpha]_D^{25}$ +6.7° (c = 0.89, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1760 (γ-lactone). ^{13}C-NMR data: see Table II. *Anal.* Calcd for $C_{42}H_{66}O_{16}\cdot 3/2H_2O$: C, 59.07; H, 8.14. Found: C, 58.87; H, 8.18.

Gymnocladus saponin C (**20**) was obtained as a hygroscopic white powder, mp 187—189 °C, $[\alpha]_D^{33}$ −10.0° (c = 0.98, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1760 (γ-lactone). ^{13}C-NMR data: see Table II. *Anal.* Calcd for $C_{46}H_{72}O_{19}\cdot 6H_2O$: C, 53.27; H, 8.16. Found: C, 53.33; H, 7.78.

Acid Hydrolysis of Saponins——A solution of gymnocladus saponin C (**20**, 85 mg) and 4 N H_2SO_4 (10 ml) in EtOH (10 ml) was refluxed for 2.5 h. The reaction mixture was neutralized with Amberlite IR 45, and the neutral solution was concentrated to half the initial volume. The residue was extracted with AcOEt, and the organic layer was washed with H_2O, then evaporated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O = 9 : 1 : 0.1 (lower layer), and repeatedly crystallized from MeOH–H_2O, to afford the sapogenin (**3**, 19 mg) as colorless needles, mp 265—267 °C, $[\alpha]_D^{33}$ +13.2° (c = 0.53, MeOH) (lit.,[3] mp 189—192 °C, $[\alpha]_D^{21}$ +16.2° (c = 1.64, $CHCl_3$)). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1760 (γ-lactone). ^{1}H-NMR (200 MHz) data: see Table I. (pyridine-d_5): 0.92, 0.95, 1.07 (3H, each s, C$\underline{H}_3$ × 3), 1.31 (6H, s, C$\underline{H}_3$ × 2), 1.55 (3H, s, C$\underline{H}_3$), 2.75 (1H, dd, J = 12 and 6 Hz, C_{18}–αH). MS m/z: 502.3278 (M^+, $C_{30}H_{46}O_6$ requires 502.3291), 262.1572 ($C_{16}H_{22}O_3$ requires 262.1568), 244.1416 ($C_{16}H_{20}O_2$ requires 244.1462), 200.1524 ($C_{15}H_{20}$ requires 200.1563). The same compound (**3**) was also obtained from gymnocladus saponin A (**12**) and gymnocladus saponin B (**18**) in the same manner.

Tetraacetate (4) of 3——A solution of **3** (32 mg) in pyridine (2 ml) and Ac_2O (2 ml) was allowed to stand for 18 h, giving the tetraacetate (**4**, 25 mg) as colorless needles, mp 159—162 °C from MeOH, $[\alpha]_D^{24}$ +13.9° (c = 0.74, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone), 1735 (CH_3COO–). ^{1}H-NMR data (200 MHz): see Table I. *Anal.* Calcd for $C_{38}H_{54}O_{10}\cdot 1/2H_2O$: C, 67.13; H, 8.16. Found: C, 67.34; H, 8.18.

Methyl Ester (5)——A solution of sapogenin (**3**, 67 mg) and 1% KOH (5 ml) in EtOH (20 ml) was refluxed for 1 h. The reaction mixture was neutralized with Dowex 50W × 8, and concentrated. The residue was extracted with AcOEt, and the extract was evaporated to dryness, then treated wih ethereal diazomethane in MeOH for 18 h at room temperature. The solution was concentrated to dryness, and the residue was chromatographed on silica gel with MeOH–$CHCl_3$ (5 : 95) to afford the methyl ester (**5**, 27 mg), ^{1}H-NMR (pyridine-d_5) δ: 0.98, 1.29, 1.33, 1.37, 1.66, 1.76 (3H, each s, CH_3 × 6), 3.67 (3H, s, OCH_3). MS m/z: 534 (M^+), 502 (M − 32), 244.

Hexaacetate (6)——A solution of **3** (0.2 g) in tetrahydrofuran (30 ml) was treated with $LiAlH_4$ (0.1 g) and refluxed for 3 h. The excess $LiAlH_4$ was decomposed with wet ether and water at 0 °C, and the reaction mixture was extracted with AcOEt. The organic layer was washed with water and evaporated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O = 8 : 3 : 1 (lower layer) to afford a hexaol as a white powder, mp > 300 °C, $[\alpha]_D^{24}$ +53.7° (c = 0.54, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 1.07, 1.33, 1.36, 1.39, 1.67, 1.83 (3H, each s, C$\underline{H}_3$ × 6), 4.26 (1H, d, J = 4.1 Hz). ^{13}C-NMR:[13] 144.5 (s), 122.8 (d), 74.8 (d), 73.7 (d), 73.2 (d), 71.5 (d), 70.1 (t), 67.9 (t), 30.1 (q), 27.3 (q), 18.2 (q), 17.4 (q), 17.1 (q) 14.5 (q). *Anal.* Calcd for $C_{30}H_{50}O_6\cdot 1/4H_2O$: C, 70.48; H, 9.96. Found: C, 70.44; H, 9.97. The hexaol (40 mg) was stirred with Ac_2O (3 ml) in pyridine (3 ml) for 10 h at 60—80 °C, to give a hexaacetate (**6**). ^{1}H-NMR data: see Table I. MS m/z: 753 (M^+), 698 (M^+ − HOAc), 638 (698 − HOAc), 578 (638 − HOAc), 625 (698 − CH_2OAc), 565 (638 − CH_2OAc), 365 (AB ring residue), 392 (DE ring residue), 305 (365 − HOAc), 332 (392 − HOAc), 272 (332–HOAc), 199 (base, 272 − CH_2OAc).

Two Acetonide Derivatives (7) and (8)——A mixture of **3** (100 mg) in acetone (10 ml) and anhydrous $CuSO_4$ (300 mg) was stirred for 2 h, then filtered. The filtrate was evaporated to dryness to afford a yellow oil (120 mg). This oil was examined by TLC ($CHCl_3$–MeOH–H_2O = 9 : 1 : 0.1, lower layer) and showed two major spots (Rf 0.57 and 0.75). This mixture was acetylated with pyridine and Ac_2O at room temperature in the usual manner. The product was chromatographed on aluminum oxide with benzene–$CHCl_3$ (7 : 3) to afford acetonide acetate (**7**, 54 mg) as a white powder and acetonide acetate (**8**, 36 mg) as colorless needles. **7**: mp 146—149 °C, $[\alpha]_D^{24}$ +15.3° (c = 0.59, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2850 (CH_3), 1770 (γ-lactone), 1730 ($ROCOCH_3$). ^{1}H-NMR δ: 0.94, 0.98, 1.01, 1.03, 1.15,

1.20, 1.32, 1.48 (3H, each s, $C\underline{H}_3 \times 8$), 1.98, 2.06 (3H, each s, $OCOC\underline{H}_3 \times 2$), 3.70, 3.09 (2H, ABq, $J=11.2$ Hz), 4.08 (1H, d, $J=6.7$ Hz), 4.21 (1H, d, $J=4.7$ Hz), 4.37 (1H, t, $J=6.7$ Hz), 5.02 (1H, dd, $J=10.7$, 4.8 Hz), 5.47 (1H, t like). **8**: mp 175—177 °C, $[\alpha]_D^{24}$ −8.0° ($c=1.19$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH, weak), 1770 (γ-lactone), 1740 ($ROCOCH_3$). ^{1}H-NMR δ: 0.99, 1.01, 1.03, 1.22, 1.28, 1.33, 1.43, 1.46 (3H, each s, $C\underline{H}_3 \times 8$), 1.98 (3H, s, $OCOC\underline{H}_3$), 3.46 (2H, br s), 4.01 (1H, br s), 4.21 (1H, d, $J=4.5$ Hz), 5.02 (1H, dd, $J=11.0$, 4.9 Hz), 5.46 (1H, t like).

Diacetate (10)——A solution of acetonide acetate (**7**, 40 mg) in MeOH (5 ml) and 2 N methanolic HCl (5 ml) was stirred for 1 h. The reaction mixture was neutralized with Ag_2CO_3, and concentrated to afford an oily product (36 mg). This oily product was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O = 9 : 1 : 0.1 (lower layer) to afford the diacetate (**10**, 24 mg) as a white powder, mp 220—223 °C, $[\alpha]_D^{20}$ −5.8° ($c=0.85$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH), 1770 (γ-lactone), 1730 ($ROCOCH_3$). ^{1}H-NMR data: see Table I. MS *m/z*: 586 (M^+), 568 ($M^+ - H_2O$), 526 ($M^+ - HOAc$), 304, 244 (base), 200.

Monoacetate (11)——A solution of acetonide acetate (**8**, 30 mg) in MeOH (5 ml) and methanolic HCl (5 ml) was stirred for 0.5 h. The reaction mixture was treated in the same manner as described for **7** to afford the 16β-monoacetate (**11**, 18 mg) as a hygroscopic white powder, mp 243—245 °C, $[\alpha]_D^{24}$ −2.0° ($c=0.55$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH), 1770 (γ-lactone), 1740 ($ROCOCH_3$). ^{1}H-NMR data: see Table I. MS *m/z*: 544 (M^+), 526 ($M^+ - H_2O$), 304, 244 (base), 200. *Anal.* Calcd for $C_{32}H_{48}O_7 \cdot 1/4H_2O$: C, 69.98; H, 8.90. Found: C, 70.10; H, 8.86.

Partial Acetylation of 3——A solution of **3** (40 mg) in pyridine (2 ml) and Ac_2O (2 ml) was allowed to stand for 3 h at −10 °C. The product was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O = 9 : 1 : 0.1 (lower layer), and crystallized from MeOH–H_2O to afford the triacetate (**9**, 28 mg) as a white powder, mp 226—228 °C, $[\alpha]_D^{26}$ +4.0° ($c=0.87$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH, weak), 1770 (γ-lactone), 1735 ($ROCOCH_3$). ^{1}H-NMR data: see Table I. MS *m/z*: 628 (M^+), 610 ($M^+ - H_2O$), 568 ($M^+ - HOAc$), 508 (568 − HOAc), 304, 244 (base), 200, 323 (AB ring residue), 305 (323 − H_2O), 263 (323 − HOAc). *Anal.* Calcd for $C_{36}H_{52}O_9 \cdot 1/2H_2O$: C, 67.79; H, 8.38. Found: C, 67.77; H, 8.34.

Prosapogenin (13)——A solution of **12** (100 mg) in EtOH (20 ml) and 2 N H_2SO_4 (20 ml) was refluxed for 1 h, and concentrated to half the initial volume. The solution was extracted with AcOEt and the extract was washed with H_2O saturated with NaCl. The organic layer was evaporated to dryness, and the residue was examined by TLC with $CHCl_3$–MeOH–H_2O = 8 : 3 : 1 (lower layer); it showed two major spots (*Rf*: 0.85 and 0.35). The residue was chromatographed on silica gel with the same solvent. The product corresponding to *Rf* 0.85 was identified as the sapogenin (**3**) from the TLC behavior and IR spectrum. On the other hand, the product corresponding to *Rf* 0.35 was crystallized from MeOH to give the prosapogenin (**13**, 37 mg) as a white powder, mp 231—234 °C, $[\alpha]_D^{23}$ +11.9° ($c=0.63$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1760 (γ-lactone). ^{1}H-NMR (pyridine-d_5) δ: 0.88, 0.91, 1.06, 1.31, 1.35, 1.52 (3H, each s, $C\underline{H}_3 \times 6$). MS (FD-MS);[14] 687 $[M+Na]^+$, 665 $[M+H]^+$, 647 $[(M+H)-H_2O]^+$, 503 $[(M+H)-162]^+$, 485 $[(M+H)-H_2O-162]^+$. *Anal.* Calcd for $C_{36}H_{56}O_{11} \cdot 3H_2O$: C, 60.14; H, 8.69. Found: C, 60.19; H, 8.56. ^{13}C-NMR data: see Table III. This prosapogenin was hydrolyzed with 4 N H_2SO_4 to afford the sapogenin (**3**) and glucose. Gymnocladus saponin B and gymnocladus saponin C were also hydrolyzed in the same manner to afford the same prosapogenin.

Permethylation of Prosapogenin (13)——According to Hakomori's method, NaH (2 g) was stirred with dimethylsulfoxide (DMSO, 50 ml) at 80 °C for 0.5 h under N_2 gas. To this reagent (6 ml), a solution of **13** (50 mg) in DMSO (5 ml) was added and the mixture was stirred for 1 h at room temperature under N_2 gas. CH_3I (10 ml) was then added and the whole was stirred for 3 h at room temperature. The reaction mixture was poured into ice-water and extracted with Et_2O. The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated to afford a syrup (65 mg). This syrup was purified by preparative TLC (silica gel, benzene–acetone = 5 : 2) to afford the permethylate (**14**, 28 mg), $[\alpha]_D^{25}$ +12.4 ($c=0.74$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (RCOOMe), 1100. ^{1}H-NMR δ: 0.69, 0.91 (3H, each s, $C\underline{H}_3 \times 2$), 0.93 (6H, s, $C\underline{H}_3 \times 2$), 1.19, 1.26 (3H, each s, $C\underline{H}_3 \times 2$), 3.30 (3H, s, OCH_3), 3.33 (6H, s, $OC\underline{H}_3 \times 2$), 3.34, 3.36, 3.51, 3.60, 3.61, 3.62 (3H, each s, $OC\underline{H}_3 \times 6$), 4.19 (1H, d, $J=6.5$ Hz, anomeric H), 5.34 (1H, t, $J=3.3$ Hz, C_{12}–H). *Anal.* Calcd for $C_{45}H_{76}O_{12}$: C, 66.80; H, 9.47. Found: C, 66.74; H, 9.73.

Methanolysis of 14——A solution of **14** (30 mg) in methanolic 2 N HCl (10 ml) was refluxed for 3 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness, and the residue was purified by preparative TLC (silica gel, solvent; benzene–acetone = 3 : 2) to afford compound **15**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550 (OH, weak), 1725 (RCOOMe), 1100. ^{1}H-NMR δ: 0.70, 0.82, 0.91, 0.94, 1.13, 1.27 (3H, each s, $C\underline{H}_3 \times 6$), 3.31, 3.32, 3.34, 3.35, 3.62 (3H, each s, $OC\underline{H}_3 \times 5$), 5.35 (1H, t, $J=3.5$ Hz, C_{12}–H). MS *m/z*: 590 (M^+), 558 ($M - CH_3OH$), 526 ($M - 2 \times CH_3OH$), 322 (DE ring residue), 290 (322 − CH_3OH), 258 (322 − $2 \times CH_3OH$), 199 (base, 258 − COOMe), and methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (identified by TLC and GLC comparisons with an authentic sample).

Acetylation of 15——A solution of **15** (40 mg) in pyridine (2 ml) and Ac_2O (2 ml) was allowed to stand for 18 h at room temperature. The reaction mixture was treated in the same manner as described above. The product was chromatographed on silica gel with acetone–benzene (1 : 99) to afford the 3-*O*-acetyl derivative (**16**, 28 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1725, 1250, 1100. ^{1}H-NMR δ: 0.71, 0.91 (3H, each s, $C\underline{H}_3 \times 2$), 0.95 (6H, s, $C\underline{H}_3 \times 2$), 1.20, 1.27 (3H, each s, $C\underline{H}_3 \times 2$), 2.11 (3H, s, $OCOC\underline{H}_3$), 3.25, 3.28, 3.34, 3.35 (3H, each s, $OC\underline{H}_3 \times 4$), 3.60 (3H, s, $COOC\underline{H}_3$), 4.98 (1H, d, $J=4.1$ Hz, C_3-αH), 5.35 (1H, t, $J=3.5$ Hz, C_{12}–H). MS *m/z*: 632 (M^+), 600 ($M - CH_3OH$), 568 ($M - 2 \times CH_3OH$), 322 (DE ring residue), 290 (322 − CH_3OH), 258 (322 − $2 \times CH_3OH$), 199 (base, 258 − COOMe).

Permethylation of 12, 18 and 20——A solution of gymnocladus saponin A (**12**, 100 mg) in DMSO (15 ml) was permethylated according to Hakomori's method as described for the prosapogenin (**13**) to afford the permethylate (**17**, 64 mg) as an amorphous product, $[\alpha]_D^{22}$ +20.0° (c=1.35, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (RCOOMe), 1100. ^{1}H-NMR δ: 0.70, 0.90 (3H, each s, $CH_3 \times 2$), 0.93 (6H, s, $CH_3 \times 2$), 1.19, 1.25 (3H, each s, $CH_3 \times 2$), 3.29, 3.33, 3.34, 3.35, 3.44, 3.47, 3.50, 3.55, 3.60 (3H, each s, $OCH_3 \times 9$), 3.62 (6H, s, $OCH_3 \times 2$), 4.17 (1H. d, J=5 Hz, anomeric H), 4.24 (1H, d, J=7.1 Hz, anomeric H), 5.54 (1H, t like, C_{12}–H). *Anal.* Calcd for $C_{52}H_{88}O_{16}$: C, 64.44; H, 9.15. Found: C, 64.38; H, 9.24. Gymnocladus saponin B (**18**, 100 mg) was permethylated in the same manner as described above to afford the permethylate (**19**, 57 mg) as an amorphous product, $[\alpha]_D^{22}$ −18.2° (c=0.99, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (RCOOMe), 1100. ^{1}H-NMR δ: 0.69, 0.88, 0.92, 0.93, 1.19, 1.25 (3H, each s, $CH_3 \times 6$), 3.31—3.64 (36H, m, $OCH_3 \times 12$), 4.22 (1H, d, J=7.6 Hz, anomeric H), 4.67 (1H, d, J=7.5 Hz, anomeric H), 5.35 (1H, t like, C_{12}–H). *Anal.* Calcd for $C_{54}H_{92}O_{17}$: C, 64.00; H, 9.15. Found: C, 63.72; H, 9.28. Gymnocladus saponin C (**20**, 100 mg) was permethylated in the same way as **18** to afford the permethylate (**21**, 48 mg) as an amorphous product, $[\alpha]_D^{22}$ −9.9° (c=0.86, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (RCOOMe), 1100. ^{1}H-NMR δ: 0.70, 0.91 (3H, each s, $CH_3 \times 2$), 0.93 (6H, s, $CH_3 \times 2$), 1.20, 1.25 (3H, each s, $CH_3 \times 2$), 3.30—3.62 (39H, m, $OCH_3 \times 13$), 4.19 (1H, d, J=6.7 Hz, anomeric H), 4.42 (1H, d, J=6.7 Hz, anomeric H), 4.53 (1H, d, J=4.0 Hz, anomeric H), 5.34 (1H, t like, C_{12}–H). *Anal.* Calcd for $C_{59}H_{100}O_{20}$: C, 62.74; H, 8.93. Found: C, 62.91; H, 9.20.

Methanolysis of Permethylates (17, 19 and 21)——Compound **17** was methanolyzed in the same way as described for **14** to give methyl 2,3,4-tri-*O*-methyl-L-arabinopyranoside and methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside; these products were identified by TLC and GLC comparisons with authentic samples. The methylated sapogenin (**15**) was obtained and identified by TLC and IR spectral analysis. Compound **19** was methanolyzed in the same way as described for **14** to give methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside and methylated sapogenin (**15**); these were identified by TLC, GLC and IR comparisons with authentic samples. Compound **21** was methanolyzed in the same way as described for **14** to give methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside, methyl 3,4-di-*O*-methyl-L-arabinopyranoside, methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside and methylated sapogenin (**15**), which were identified by TLC, GLC and IR comparisons with authentic samples.

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