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Studies on the Constituents of Leguminous Plants. VIII.[1] The Structure of a New Triterpenoid Saponin from the Fruits of *Gymnocladus chinensis* BAILLON

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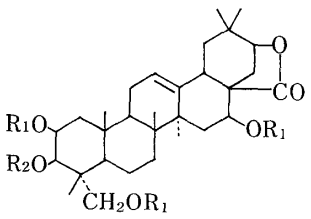
A new triterpene saponin, gymnocladus saponin D (**5**), having a glycosyl monoterpene carboxylate and a monoterpene carboxylate group, was isolated and its structure was characterized as the (6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoate of 2β,23-dihydroxy-3-*O*-α-L-rhamnopyranosyl-21-*O*-[(6*S*)-2-*trans*-2,6-dimethyl-6-α-L-arabinopyranosyloxy-2,7-octadienoyl]-acacic acid 28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→2)-[L-rhamnopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, on the basis of chemical and physicochemical evidence.

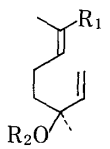
Keywords——*Gymnocladus chinensis*; Leguminosae; gymnocladus saponin D; 2β,23-dihydroxyacacic acid lactone; triterpenoid; saponin; monoterpene acid; monoterpene glycoside; ^{13}C-NMR

In the preceding paper,[1] we reported the structure elucidation of three triterpenoid saponins, gymnocladus saponins A (**2**), B (**3**) and C (**4**), isolated from the fruits of *Gymnocladus chinensis* BAILLON, as 3-*O*-glycosides of 2β, 23-dihydroxyacacic acid lactone (**1**), as shown in Chart 1. A new saponin, named gymnocladus saponin D (**5**), has now been isolated from the same source, and characterized as a 3,28-bisglycoside of 2β,23-dihydroxyacacic acid, bearing a monoterpene carboxylate and a glycosyl monoterpene carboxylate, on the basis of ^{13}C-nuclear magnetic resonance (NMR) spectrometry and some chemical degradations.

Gymnocladus saponin D (**5**) was isolated from the crude saponin fraction[1] by repeated chromatography on silica gel columns, gel filtration on Sephadex LH20 columns, and finally



1: $R_1=R_2=H$
2: $R_1=H$, $R_2=$–glc.$\overset{6\,1}{}$ara. (gymnocladus saponin A)
3: $R_1=H$, $R_2=$–glc.$\overset{2\,1}{}$glc. (gymnocladus saponin B)
4: $R_1=H$, $R_2=$–glc.$\overset{6\,1}{}$ara.$\overset{2\,1}{}$xyl. (gymnocladus saponin C)
6: $R_1=H$, $R_2=$L-rham.pyr.
7: $R_1=Me$, $R_2=$2,3,4-tri-*O*-Me–L-rham.pyr.
8: $R_1=Me$, $R_2=H$
9: $R_1=Me$, $R_2=Ac$



11: $R_1=COOH$, $R_2=H$
13: $R_1=CH_2OH$, $R_2=$2,3,4-tri-*O*-Me–L-ara.pyr.
16: $R_1=COOH$, $R_2=$L-ara.pyr.

Chart 1

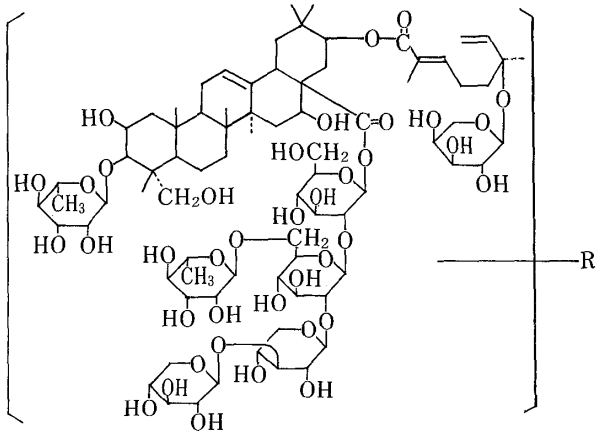
high performance liquid chromatography (HPLC), as a white powder, $C_{89}H_{142}O_{41}\cdot 6H_2O$, mp 196—200 °C, $[\alpha]_D^{25}$ −6.4°.

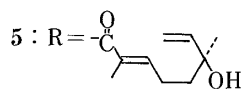
In the ^{13}C-NMR of **5**, three singlet signals of carbonyl carbons at δ 167.8, 168.1 and 174.5 were found instead of the signals due to a lactone group in **2**, **3** and **4**. On alkaline hydrolysis of **5** with 10% KOH, a prosapogenin (**6**) was obtained as a white powder. Acidic hydrolysis of **6** with 4 N H_2SO_4 in ethanol gave 2β,23-dihydroxyacacic acid lactone (**1**) and L-rhamnose. The permethylate (**7**) obtained from the prosapogenin (**6**) by Hakomori's method[2] was methanolyzed, and a sapogenin trimethyl ether (**8**) and methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside were obtained. The difference (−64°) of molecular rotation between the prosapogenin permethylate (**7**) and the sapogenin trimethylate (**8**) indicated the anomeric carbon of the rhamnosyl group to have α-configuration,[3] and therefore, the prosapogenin (**6**) is 3-*O*-α-L-rhamnopyranosyl-2β,23-dihydroxyacacic acid lactone.

In the infrared (IR) spectrum (1680 cm^{-1}) and the ultraviolet (UV) spectrum (λ_{max} 212 and 217 nm), the saponin D (**5**) showed the presence of conjugated acyl groups. On alkaline hydrolysis of **5** with 1% $NaHCO_3$ in ethanol, a partially deacylated compound (**10**) and a monoterpene carboxylic acid (**11**) were obtained. This acid (**11**) was identical with an authentic sample of (+)-2,6-dimethyl-6(*S*)-hydroxy-2-*trans*-2,7-octadienoic acid,[4] based on thin layer chromatographic (TLC), and IR and ^{1}H-NMR spectral comparisons. The ^{13}C-NMR spectrum (Table I) of **10** showed two carbonyl carbons at δ 167.8 and 174.5, and four olefinic carbons at δ 114.8, 128.7, 142.2 and 144.0, in addition to δ 123.8 (C-12) and 143.5 (C-13), and suggested the product (**10**) to have another very similar but less hydrolyzable acyl group.

On methylation by Hakomori's method, **5** and **10** gave the same permethylate (**12**), showing an absorption maximum at 212 nm in the UV spectrum, and signals due to a terminal olefin at δ 5.14 (dd, J=18, 2 Hz), 5.22 (dd, J=11, 2 Hz) and 5.85 (dd, J=11, 18 Hz), and seven anomeric protons of sugars at δ 4.29 (d, J=7 Hz), 4.68 (d, J=7 Hz), 4.71 (d, J=7 Hz), 4.81 (d, J=8 Hz), 4.86 (br s), 4.95 (br s) and 5.65 (d, J=7 Hz) in the ^{1}H-NMR spectrum. On methanolysis of **12**, six methylated monosaccharides were obtained and identified by TLC and gas liquid chromatographic (GLC) comparisons with authentic samples, as shown in Table II.

On reduction with lithium aluminium hydride ($LiAlH_4$), **12** gave a mixture of a monoterpene glycoside (**13**), a triterpene glycoside (**14**) and an oligosaccharide alcohol (**15**).





5 : R= (gymnocladus saponin D)

10 : R=H

Chart 2

TABLE I. Carbon-13 NMR Chemical Shifts (δ) in Pyridine-d_5[a]

Carbon	2	5	6[b]	10	16	11
Aglycone						
C-12	124.7	123.8	124.8	123.8		
C-13	140.2	143.2	140.2	143.5		
C-21	83.5		83.4			
C-28	181.2	174.5	181.1	174.5		
Monoterpene acid						
C-1′		168.1				168.4
C-2′		128.1				127.5
C-3′		143.5				143.5
C-7′		146.5				146.6
C-8′		111.6				111.7
C-9′		12.6				12.4
Glycosyl monoterpene acid						
C-1″		167.8		167.8	168.4	
C-2″		128.7		128.7	127.7	
C-3″		143.8		144.0	144.0	
C-7″		142.1		142.2	143.1	
C-8″		115.0		114.8	114.9	
C-9″		12.5		12.5	12.5	
Sugar		Anomeric[c]		Anomeric[c]		
		100.0	Rham.	99.9	Ara.	
		95.6	103.9	95.6	100.0	
		101.9	72.6	101.8	72.6	
		103.9	72.3	104.0	74.7	
		104.5	73.8	104.5	69.4	
		105.1	70.1	105.2	66.6	
		105.9	18.5	105.9	66.6	

a) Measured with TMS as an internal standard, at 22.5 MHz, on a JEOL FX90-Q NMR spectrometer. *b*) Other signals of the triterpene moiety were consistent with those of **2** within ±0.2 ppm. *c*) Other signals due to the sugars were overlapped.

TABLE II. Identification of Methylated Monosaccharides Obtained from the Permethylates **12**, **13**, **14**, **15**, **21**, **22** and **23**, by TLC and GLC[a]

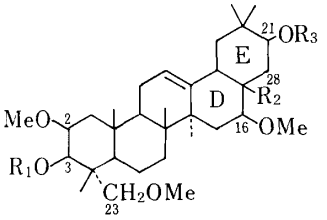
	12	13	14	15	21	22	23
Me 2,3,4-tri-*O*-Me-L-ara.pyr.	+	+	−	−	−	−	−
Me 2,3,4-tri-*O*-Me-L-rham.pyr.	+	−	+	+	+	+	+
Me 2,3,4-tri-*O*-Me-D-xyl.pyr.	+	−	−	+	−	−	−
Me 2,4-di-*O*-Me-D-xyl.pyr.	+	−	−	+	+	−	−
Me 3,4-di-*O*-Me-D-glc.pyr.	+	−	−	+	+	+	−
Me 3,4,6-tri-*O*-Me-D-glc.pyr.	+	−	−	−	−	−	−
Me 2,3,4-tri-*O*-Me-D-glc.pyr.	−	−	−	−	−	−	+
3,4,6-Tri-*O*-Me-D-glucitol[b]	−	−	+	+	+	+	−
1,3,4,5,6-Penta-*O*-Me-D-glucitol[b]	−	−	−	−	−	−	+

a) Conditions for GLC and TLC were the same as described in the previous paper.[6] *b*) Deduced from the TLC and GLC behavior, in comparison with the results for **12**.

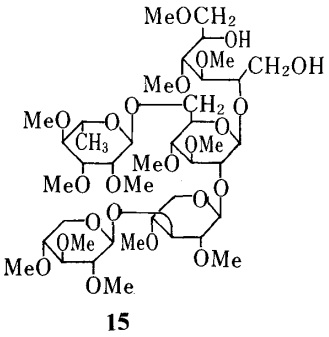
The monoterpene glycoside (**13**) was shown to be identical with (6*S*)-2-*trans*-6-α-L-arabinopyranosyloxy-2,6-dimethyl-2,7-octadienol (**13**) derived from the acid (**16**), which had been reported in the previous paper.[5] It is clear that the other acyl group in **10**, mentioned above, is this monoterpene glycoside (**16**).

The triterpene glycoside (**14**) was methanolyzed with 2 N hydrogen chloride (HCl) in

methanol to afford methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside and a triterpene alcohol (**17**). The ^{1}H-NMR spectrum of **17** showed the presence of three *O*-methyl groups at δ 3.28, 3.32 and 3.38 (3H, each s), and the mass spectrum (MS: Table III) exhibited prominent ion peaks at *m/z* 548 (M^+) and 280 (fragment-a; caused by retro-Diels–Alder cleavage). The tri-*O*-acetate (**18**), obtained on acetylation of **17** in the usual manner, exhibited mass fragment ion peaks at *m/z* 364, 332, 259 and 199 as shown in Table III, and suggested that two free hydroxy groups in **17** were present on the D/E ring part, and that the glycosyl monoterpene



14: R_1=2,3,4-tri-*O*-Me-L-rham.pyr., R_2=CH_2OH, R_3=H
17: R_1=R_3=H, R_2=CH_2OH
18: R_1=R_3=Ac, R_2=CH_2OAc
19: R_1=H, R_2=COOMe, R_3=Me
20: R_1=H, R_2=CH_2OH, R_3=Me
21: R_1=Ac, R_2=CH_2OAc, R_3=Me



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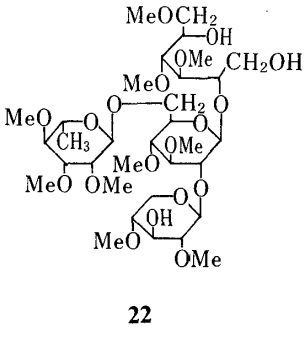

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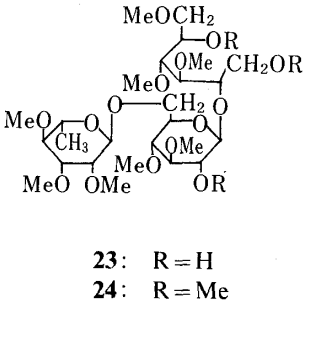
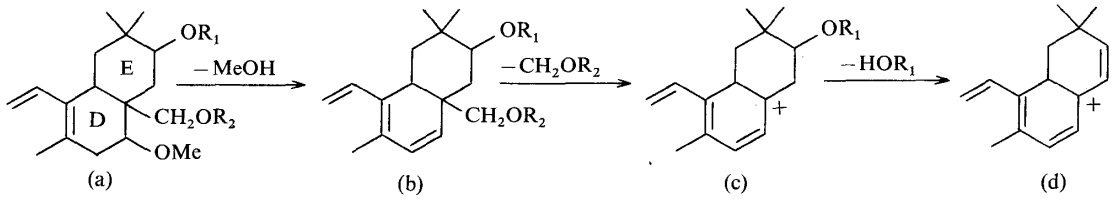


23: R=H
24: R=Me

Chart 3

TABLE III. MS Prominent Ion Peaks of **17**, **18**, **20** and **21** (*m/z*)

Fragment	**17**	**18**	**20**	**21**
M^+	548	674	562	646
$M-H_2O$	530		544	
M–MeOH				614
M–HOAc		614		586
–HOAc		554		
M–(MeOH, CH_2OH)			499	
–CH_2OH	499			
–MeOH	467	522	467	
(a)	280	364	294	336
(b)	248	332	262	304
(a)–HOAc				276
(a)–CH_2OAc				263
(c)	217	259	231	231
(d)	199	199	199	199



17: R_1=R_2=H
18: R_1=R_2=Ac
20: R_1=Me, R_2=H
21: R_1=Me, R_2=Ac

TABLE IV. Carbon-13 NMR Chemical Shifts (δ) of **17**, **18**, **19**, **20** and **21**[a]

Carbon	**19**[b]	**20** (Δ_δ 20—19)	**21** (Δ_δ 21—20)	**17** (Δ_δ 17—20)	**18** (Δ_δ 18—17)
C-2	82.2 d[c]	82.2 (±0)	79.0 (−3.2)	82.3 (+0.1)	79.0 (−3.3)
C-3	72.1 d	72.1 (±0)	75.1 (−3.0)	72.2 (+0.1)	75.1 (+2.9)
C-4	42.7 s	42.7 (±0)	42.2 (−0.5)	42.7 (±0)	41.9 (−0.8)
C-5	47.7 d	47.7 (±0)	47.3 (−0.4)	47.8 (+0.1)	47.2 (−0.6)
C-12	123.5 d	123.2 (−0.3)	124.0 (+0.8)	123.1 (−0.1)	124.2 (+1.1)
C-13	142.8 s	143.7 (+0.9)	142.6 (−1.1)	144.0 (+0.3)	142.2 (−1.8)
C-14	41.9 s	42.0 (+0.1)	41.9 (−0.1)	42.1 (+0.1)	42.3 (+0.2)
C-16	83.7 d	85.0 (+1.3)	84.3 (−0.7)	85.1 (+0.1)	84.1 (−1.0)
C-17	51.5 s	44.0 (−7.5)	41.5 (−2.5)	44.2 (+0.2)	41.5 (−2.7)
C-19	47.7 t	48.9 (+1.2)	48.5 (−0.4)	49.0 (+0.1)	47.9 (−1.1)
C-20	35.9 s	36.1 (+0.2)	36.0 (−0.1)	36.6 (+0.5)	35.0 (−1.6)
C-21	84.6 d	85.3 (+0.7)	84.8 (−0.5)	74.6 (−10.7)	77.6 (+3.0)
C-22	39.5 t	39.6 (+0.1)	40.0 (+0.4)	39.7 (+0.1)	36.1 (−3.6)
C-23	76.6 t	76.6 (±0)	76.0 (−0.6)	76.7 (+0.1)	75.9 (−0.8)
C-28	176.0 s	69.9	71.5 (+1.6)	70.1 (+0.2)	71.2 (+1.1)
C-29	29.5 q	29.8 (+0.3)	29.6 (−0.2)	30.1 (+0.3)	29.1 (−1.0)
C-30	18.6 q	18.6 (±0)	18.5 (−0.1)	18.2 (−0.4)	18.7 (−0.5)

a) Measured in pyridine-d_5 with TMS as an internal standard, at 20 MHz on a Varian FT-80A NMR spectrometer. *b*) The δ_C values for C-1, C6-11, C-15, C-18 and C-24—27 were consistent with those of **20**, **21**, **17** and **18** within ±0.3 ppm. *c*) Multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; observed in off-resonance experiments.

acid was present on the hydroxy group at either C-16 or C-21, as an ester.

The ^{1}H-NMR spectrum of **18** showed the signals of three *O*-acetyl groups at δ 2.02, 2.04 and 2.11 (3H, each s), and protons (CH–O–) at δ 3.74 (AB d, J=12 Hz), 4.99 (d, J=4 Hz) and 5.25 (dd, J=12, 5 Hz), in addition to the three *O*-methyl groups. In the ^{13}C-NMR spectra (Table IV), the signals attributable to C-3, C-21 and C-28, at 72.2, 74.6 and 70.1, respectively, in **17**, were found to be shifted 2.9, 3.0 and 1.1 ppm, respectively, to lower field in the acetate (**18**). Those of C-2, C-4, C-20, C-22 and C-17 (β-position of C-3, C-21 and C-28) were observed to be shifted to higher field. These acylation shifts observed at C-3, C-28 and in their vicinities, were smilar to the shifts found between 3,28-diol (**20**) and 3,28-diacetate (**21**) derived from saponin A (**2**)[1] (Table IV). These observations indicate the positions of the glycosyl monoterpene carboxylate group and the rhamnopyranosyl-oxy group in saponin D (**5**) to be C-21 and C-3, respectively.

The ^{1}H-NMR spectrum of the methylated oligosaccharide (**15**) showed four signals of anomeric protons at δ 4.67 (d, J=8 Hz), 4.70 (d, J=8 Hz), 4.82 (d, J=8 Hz) and 4.95 (d, J=2 Hz), and methanolysis of **15** with 2 N HCl in methanol gave four methylated monosaccharides, as shown in Table II. On partial methanolysis of **15** with 1 N HCl in methanol, an oligosaccharide (**22**) showing three signals of anomeric protons at δ 4.62 (d, J=8 Hz), 4.83 (d, J=8 Hz) and 4.96 (d, J=3 Hz) in the ^{1}H-NMR, and methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside were obtained. This oligosaccharide (**22**) gave methyl 2,4-di-*O*-methyl-D-xylopyranoside, methyl 3,4-di-*O*-methyl-D-glucopyranoside and methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside on methanolysis with 2 N HCl in methanol. On partial methanolysis of **22** with 1.5 N HCl in methanol, a trisaccharide (**23**) and methyl 2,4-di-*O*-methyl-D-xylopyranoside were obtained. The permethylate (**24**) obtained from **23** on permethylation by Hakomori's method, was methanolyzed to afford methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside and methyl 2,3,4-tri-*O*-methyl-D-glucopyranoside. Based on these results, the oligosaccharide moiety at C-28 in **10** was characterized as xylopyranosyl-(1→3)-xylopyranosyl-(1→2)-[rhamnopyranosyl-(1→6)]-glucopyranosyl-(1→2)-glucopyranosyl. From the coupling constants of the anomeric protons (each 8 Hz) in the ^{1}H-NMR spectra of

15 and **22**, the glycoside linkages of the two xylose and two glucose were deduced to have β-configuration.

The structure of gymnocladus saponin D was thus elucidated as shown in the formula (**5**). This compound is believed to be the first recorded saponin bearing a glycosyl monoterpene carboxylate as an acyl side-chain.

Studies on the configuration of the rhamnose in the oligosaccharide moiety, and on the position of the ester with the monoterpene carboxylic acid (**11**) are in progress.

Experimental

Melting points are uncorrected. Unless otherwise stated, ^{1}H-NMR spectra were measured on a Varian FT-80A NMR spectrometer in $CDCl_3$ at 80 MHz. MS were taken on a Hitachi M-80 mass spectrometer. UV spectra were measured on a Shimadzu UV240 spectrometer in 95% EtOH. Conditions of TLC and GLC for identification of methylated monosaccharides were as reported in the previous paper.[6]

Isolation of Gymnocladus Saponin D (5)——The crude saponin fraction[7] was repeatedly chromatographed on silica gel with $CHCl_3$: MeOH : H_2O = 65 : 35 : 10 (lower layer), and separated by gel-filtration on a Sephadex LH20 column in MeOH. The major fraction was purified by preparative HPLC (Waters μ-Bondapak C_{18}, MeOH : H_2O = 7 : 3), and **5** was obtained as a hygroscopic white powder, mp 196—200 °C, $[\alpha]_D^{25}$ −6.4° (c = 1.00, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3400—3500 (OH), 1730 (COOR), UV λ_{max}^{EtOH} nm (ε): 212 (10000), 217 (12000). ^{13}C-NMR: Table I. *Anal.* Calcd for $C_{89}H_{142}O_{41}\cdot 6H_2O$: C, 54.09; H, 7.86. Found: C, 53.84; H, 7.99. Yield: 0.0075%.

Hydrolysis of 5 with 10% KOH——A solution of **5** (300 mg) and 10% KOH (20 ml) in EtOH (20 ml) was refluxed for 2.5 h. The reaction mixture was neutralized with Dowex 50W × 8, and evaporated to half the initial volume *in vacuo*. The *n*-BuOH extract of the concentrated solution was purified on a silica gel column ($CHCl_3$: MeOH : H_2O = 8 : 3 : 1, lower layer) to give a prosapogenin (**6**, 80 mg) as a white powder (from MeOH : H_2O), mp 248—251 °C, $[\alpha]_D^{20}$ −3.9° (c = 0.95, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1760 (γ-lactone). ^{13}C-NMR: Table I. *Anal.* Calcd for $C_{36}H_{56}O_{10}\cdot H_2O$: C, 64.84; H, 8.77. Found: C, 64.72; H, 8.95.

This prosapogenin (**6**) was hydrolyzed with 4 N H_2SO_4 to afford 2β,23-dihydroxyacacic acid lactone (**1**) and L-rhamnose, and the products were identified by comparison with authentic samples.[1]

Permethylation of 6——According to Hakomori's method, NaH (1 g) was stirred with dimethyl sulfoxide (DMSO, 100 ml) at 80 °C for 0.5 h under an N_2 gas stream. To this reagent mixture (5 ml), a solution of **6** (50 mg) in DMSO (5 ml) was added, and the whole was stirred for 1 h at room temperature under an N_2 stream. After addition of CH_3I (5 ml), the reaction mixture was stirred for 3 h at room temperature, then poured into ice-water and extracted with Et_2O. The Et_2O layer was washed with H_2O, dried over $MgSO_4$ and evaporated to give a yellow syrup (60 mg). This syrup was purified by preparative TLC (silica gel, benzene : acetone = 5 : 1) to afford a permethylate (**7**, 25 mg). $[\alpha]_D^{25}$ −3.9° (c = 0.87, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (COOR). ^{1}H-NMR δ: 0.88 (3H, s, CH_3), 0.89 (6H, s, CH_3 × 2), 1.00, 1.02 (3H, each s, CH_3 × 2), 1.19 (6H, s, CH_3 × 2), 3.28, 3.31, 3.34, 3.48, 3.51, 3.54 (3H, each s, OCH_3 × 6), 4.18 (1H, d, J = 5 Hz, C-21-H), 4.85 (1H, br s, anomeric H), 5.43 (1H, t like, C-12-H).

Methanolysis of 7——A solution of **7** (10 mg) in methanolic 2 N HCl (5 ml) was refluxed for 3 h, then neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness, and the residue was purified by preparative TLC (silica gel, benzene : acetone = 3 : 2) to afford a sapogenin trimethyl ether (**8**), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760 (γ-lactone), ^{1}H-NMR δ: 0.85, 0.89, 0.96, 1.01, 1.03, 1.21 (3H, each s, CH_3 × 6), 3.36 (6H, s, OCH_3 × 2), 3.67 (3H, s, OCH_3), 4.20 (1H, d, J = 5 Hz, C-21-H), 5.45 (1H, br s, C-12-H), MS m/z: 544 (M^+), 276 (D/E ring residue), 244 (base, 276 − MeOH), 200 (244 − CO_2), and 2,3,4-tri-*O*-methyl-L-rhamnopyranoside, which was identified by TLC and GLC comparisons with an authentic sample.

Acetylation of 8——A solution of **8** (10 mg) in pyridine (1 ml) and Ac_2O (1 ml) was allowed to stand at room temperature for 18 h to give a monoacetate (**9**, 7 mg), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (γ-lactone), 1730 ($OCOCH_3$), ^{1}H-NMR δ: 0.95, 0.97, 1.00, 1.02, 1.19, 1.25 (3H, each s, CH_3 × 6), 2.12 (3H, s, $OCOCH_3$), 3.33, 3.34, 3.62 (3H, each s, OCH_3 × 3), 4.18 (1H, d, J = 5 Hz, C-21-H), 4.99 (1H, d, J = 4 Hz, C-3-H), 5.40 (1H, t like, C-12-H). MS m/z: 586 (M^+), 526 (M − HOAc), 276 (D/E ring residue), 244 (base, 276 − HOMe), 200 (244 − CO_2).

Hydrolysis of 5 with 1% $NaHCO_3$——A solution of saponin D (**5**, 340 mg) and 1% $NaHCO_3$ (20 ml) in EtOH (20 ml) was refluxed for 30 min. The reaction mixture was neutralized with Dowex 50W × 8, and concentrated to half the initial volume *in vacuo*. The residue was extracted successively with AcOEt, and *n*-BuOH saturated with water. The AcOEt extract was evaporated to dryness, followed by separation on a silica gel column, and gave (+)-2,6-dimethyl-6(*S*)-hydroxy-2-*trans*-2,7-octadienoic acid (**11**, 15 mg), TLC *Rf*: 0.85 ($CHCl_3$: MeOH : H_2O = 8 : 3 : 1), and an artefact derived from **11**[4] (20 mg), TLC *Rf*: 0.55. Both products were identified by direct comparison with authentic samples. The *n*-BuOH extract was evaporated and purified on a silica gel column ($CHCl_3$: MeOH : H_2O = 65 : 35 : 10) to give a partially deacylated compound (**10**, 140 mg) as a white hygroscopic powder from MeOH–H_2O, mp 211—215 °C, $[\alpha]_D^{25}$ −10.1° (c = 0.85, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3600, 3400 (OH), 1740 (COOR), UV λ_{max}^{EtOH} nm (ε):

212 (10000), ^{13}C-NMR: Table I. *Anal.* Calcd for $C_{79}H_{126}O_{39}\cdot 8H_2O$: C, 51.45; H, 7.76. Found: C, 51.13; H, 8.01.

Permethylation of 5 and 10——A solution of **5** (250 mg) in DMSO (20 ml) was permethylated according to Hakomori's method as described above to afford a permethylate (**12**, 120 mg) as an amorphous powder, $[\alpha]_D^{25}$ −15.6° (c=1.02, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (COOR), 1100 (C–O–C), UV λ_{max}^{EtOH} nm (ε): 212 (10000), ^{1}H-NMR (200 MHz) δ: 0.76, 0.84, 0.88, 1.01, 1.19, 1.25 (3H, each s, $CH_3\times 6$), 1.31, 1.33, (3H, each d, J=6 Hz, $CH_3\times 2$), 3.25—3.65 (66H, m, $OCH_3\times 22$), 4.29 (1H, d, J=7 Hz, anomeric H), 4.68 (1H, d, J=7 Hz, anomeric H), 4.71 (1H, d, J=7 Hz, anomeric H), 4.81 (1H, d, J=8 Hz, anomeric H), 4.86 (1H, br s, anomeric H), 4.95 (1H, br s, anomeric H), 5.14 (1H, dd, J=18, 2 Hz, $\overset{H}{R}\!>\!C\!=\!C\!<\!\overset{H}{H}$), 5.22 (1H, dd, J=11, 2 Hz, $\overset{H}{R}\!>\!C\!=\!C\!<\!\overset{H}{H}$), 5.38 (1H, t like, C–12–H), 5.65 (1H, d, J=7 Hz, anomeric H), 5.85 (1H, dd, J=18, 11 Hz, $\overset{H}{R}\!>\!C\!=\!C\!<\!\overset{H}{H}$). This permethylate (**12**) was also obtained from **10** in the same manner, and the two products were confirmed to be identical by TLC, and ^{1}H-NMR and IR spectral comparisons.

Methanolysis of 12——A solution of **12** (20 ml) in 2 N methanolic HCl (20 ml) was refluxed for 2.5 h, then neutralized with Ag_2CO_3, and filtered. The filtrate was evaporated and the residue wa examined by TLC and GLC. As shown in Table II, six different methylated monosaccharides were identified by comparison with authentic samples.

Reductive Cleavage of Permethylate (12) with $LiAlH_4$——$LiAlH_4$ (50 mg) was added to a solution of the permethylate (**12**, 90 mg) in anhydrous THF (20 ml), and the mixture was refluxed for 2.5 h. The excess $LiAlH_4$ was decomposed with wet Et_2O and the mixture was extracted with Et_2O and AcOEt successively. The Et_2O extract was chromatographed on silica gel (benzene : acetone = 5 : 4) to give (6*S*)-2-*trans*-6-(2,3,4-tri-*O*-methyl-α-L-arabinopyranosyloxy)-2,6-dimethyl-2,7-octadienol (**13**, 5 mg) as a colorless oil, $[\alpha]_D^{21}$ −9.7° (c=0.86, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1100 (C–O–C), ^{1}H-NMR δ: 0.98 (3H, s, CH_3), 1.28 (3H, d, J=5 Hz, CH_3), 3.43, 3.49, 3.58 (3H, each s, $OCH_3\times 3$), 5.13 (1H, dd, J=11, 2 Hz), 5.20 (1H, dd, J=18, 2 Hz), 5.85 (1H, dd, J=18, 11 Hz), 4.33 (1H, d, J=6 Hz, anomeric H), 5.40 (1H, m, olefinic H). This compound was identical with the corresponding monoterpene glycoside alcohol obtained from an authentic sample of **16**[5] in the same manner.

From the second fraction, a triterpenoid glycoside alcohol (**14**, 20 mg) was obtained as a colorless amorphous powder, $[\alpha]_D^{25}$ +3.9° (c=1.01, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1100 (C–O–C), ^{1}H-NMR (200 MHz) δ: 0.88, 0.89, 0.91, 0.96, 1.22 (3H, each s, $CH_3\times 5$), 1.28 (3H, d, J=6 Hz, CH_3), 1.30 (3H, s, CH_3), 3.28 (6H, s, $OCH_3\times 2$), 3.31, 3.48, 3.51, 3.53 (3H, each s, $OCH_3\times 4$), 4.02 (1H, dd, J=11, 6 Hz, C–18–H), 4.85 (1H, br s, anomeric H), 5.27 (1H, t like, C–12–H).

The AcOEt extract was purified by preparative TLC (benzene : acetone = 5 : 4) to give an oligosaccharide alcohol (**15**, 18 mg) as a colorless oil, $[\alpha]_D^{25}$ +84.3° (c=1.02, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1100 (C–O–C), ^{1}H-NMR (200 MHz) δ: 1.32 (3H, d, J=6 Hz, CH_3), 3.36, 3.42, 3.45, 3.46, 3.47, 3.50, 3.52, 3.54, 3.55, 3.67 (3H, each s, $OCH_3\times 10$), 3.61 (6H, s, $OCH_3\times 2$), 3.63 (3H, s, OCH_3), 4.67 (1H, d, J=8 Hz, anomeric H), 4.70 (1H, d, J=8 Hz, anomeric H), 4.82 (1H, d, J=8 Hz, anomeric H), 4.95 (1H, d, J=2 Hz, aomeric H).

Methanolysis of 14——A solution of **14** (20 mg) in 2 N methanolic HCl (10 ml) was refluxed for 3 h. The reaction mixture was treated in the usual manner, as mentioned above, to afford methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside, which was identified by comparison with an authentic sample, and a triterpene alcohol (**17**, 7 mg), $[\alpha]_D^{25}$ −17.3° (c=0.64, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1100 (C–O–C), ^{1}H-NMR : 0.82, 0.87, 0.91, 0.96, 1.16, 1.30 (each 3H, s, $CH_3\times 6$), 3.28, 3.32, 3.38 (3H, each s, $OCH_3\times 3$), 4.01 (1H, dd, J=11, 6 Hz, C–18–H), 5.28 (1H, t, J=3 Hz, C–12–H).

Acetylation of 17——A solution of **17** (15 mg) in pyridine (2 ml) and Ac_2O (2 ml) was allowed to stand at room temperature for 20 h to give a triacetate (**18**, 10 mg), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 ($OCOCH_3$), 1100 (C–O–C), ^{1}H-NMR δ: 0.87, 0.94, 0.95, 1.23 (3H, each s, $CH_3\times 4$), 1.25 (6H, s, $CH_3\times 2$), 2.02, 2.04, 2.11 (3H, each s, $OCOCH_3\times 3$), 3.26, 3.28, 3.37 (3H, each s, $OCH_3\times 3$), 3.74 (2H, AB d, J=12 Hz, C–28–H_2), 4.99 (1H, d, J=4 Hz, C–3–H), 5.30 (1H, br s, C–12–H), 5.25 (1H, dd, J=12, 5 Hz, C–21–H). MS: Table III. ^{13}C-NMR: Table IV.

2,16,21,23-Tetra-*O*-methyl-3,28-diol (20) and Its Diacetate (21)——2,16,21,23-Tetra-*O*-methyl-acacic acid methyl ester (**19**) obtained from gymnocladus saponin A (**2**) by permethylation followed by methanolysis,[1] was treated with $LiAlH_4$ in the usual manner to afford the 2,16,21,23-tetra-*O*-methyl-3,28-diol (**20**). Acetylation of **20** in the usual manner gave its diacetate (**21**), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 ($OCOCH_3$), 1100 (C–O–C), ^{1}H-NMR δ: 0.86, 0.93 (3H, each s, $CH_3\times 2$), 0.95 (6H, s, $CH_3\times 2$), 1.25, 1.30 (3H, each s, $CH_3\times 2$), 2.05, 2.11 (3H, each s, $OCOCH_3\times 2$), 3.26, 3.28, 3.31, 3.36 (3H, each s, $OCH_3\times 4$), 3.75 (2H, ABd, J=12 Hz, C–28–H_2), 4.99 (1H, d, J=4 Hz, C–3–H), 5.28 (1H, t, J=3 Hz, C–12–H). MS: Table III. ^{13}C-NMR: Table IV.

Partial Methanolysis of the Oligosaccharide Alcohol (15)——A solution of **15** (75 mg) in 1 N methanolic HCl (30 ml) was allowed to stand at room temperature for 24 h. After neutralization with Ag_2CO_3, the filtrate was evaporated and the residue was purified by preparative TLC (benzene : acetone = 3 : 4) to give **22** (30 mg) as a colorless syrup, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1100 (C–O–C), ^{1}H-NMR (200 MHz) δ: 1.32 (3H, d, J=6 Hz, CH_3), 3.37, 3.42, 3.46, 3.51, 3.52, 3.54, 3.57, 3.59, 3.60, 3.62 (3H, each s, $OCH_3\times 10$), 4.62 (1H, d, J=8 Hz, anomeric H), 4.83 (1H, d, J=8 Hz, anomeric H), 4.96 (1H, d, J=3 Hz, anomeric H), and methyl 2,3,4-tri-*O*-methyl-D-xylopyranoside. The latter was identified by TLC and GLC comparisons with an authentic sample.

Partial Methanolysis of 22——A solution of **22** (60 mg) in 1.5 N methanolic HCl (20 ml) was refluxed for 0.5 h, then neutralized and filtered. The filtrate was evaporated and the residue was purified by preparative TLC (benzene : acetone = 3 : 5) to give a colorless syrup (**23**, 21 mg) and methyl 2,4-di-*O*-methyl-D-xylopyranoside. The latter was identified by TLC and GLC comparisons with an authentic sample. Methylation of **23** by Hakomori's method afforded its permethylate (**24**, 8 mg) as a colorless syrup, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1100 (C–O–C), ^{1}H-NMR δ: 1.32 (3H, d, J = 6 Hz, CH_3), 3.35—3.65 (33H, m, $OCH_3 \times 11$), 4.72 (1H, d, J = 8 Hz, anomeric H), 4.95 (1H, d, J = 3 Hz, anomeric H).

Methanolysis of 15, 22, 23 and 24——A solution of **15**, **22**, **23** or **24** in 2 N methanolic HCl was refluxed for 2 h, and treated in the usual manner as described above. All of the methylated monosaccharides obtained from the permethylates, were identified by TLC and GLC comparisons with authentic samples.[6] The results are summarised in Table II.

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References and Notes

1) Part VII: T. Konoshima, T. Sawada and T. Kimura, *Chem. Pharm. Bull.*, **32**, 4833 (1984).
2) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
3) W. Klyne, *Biochem. J.*, **47**, xli (1950); H. Okabe, N. Koshito, K. Tanaka and T. Kawasaki, *Chem. Pharm. Bull.*, **19**, 2394 (1971). Methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside; $[M]_D = -99°$. Methyl 2,3,4-tri-*O*-methyl-β-L-rhamnopyranoside; $[M]_D = +230°$.
4) Y. Okada, K. Koyama, K. Takahashi, T. Okuyama and S. Shibata, *Planta Medica*, **40**, 185 (1980); T. Konoshima and T. Sawada, *Chem. Pharm. Bull.*, **30**, 2747 (1982).
5) T. Konoshima and T. Sawada, *Chem. Pharm. Bull.*, **32**, 2617 (1984).
6) T. Konoshima, H. Imai, K. Sato, M. Yonezawa and T. Sawada, *Chem. Pharm. Bull.*, **28**, 3473 (1980).
7) Details of the extraction and fractionation were reported in ref. 5.