[Chem. Pharm. Bull. 32(7)2617—2621(1984)]

Studies on the Constituents of Leguminous Plants. VI.[1] The Structure Elucidations of Monoterpene Glycosides from Fruits of *Gymnocladus chinensis* BAILLON

TAKAO KONOSHIMA* and TOKUNOSUKE SAWADA

Kyoto College of Pharmacy, Nakauchi-cho 5, Misasagi, Yamashina-ku, Kyoto 607, Japan

(Received November 7, 1983)

Two monoterpene glycosides **1** and **2** were isolated from the fruits of *Gymnocladus chinensis* BAILLON (Leguminosae). On the basis of chemical and physicochemical evidence, these glycosides were characterized as (6*S*)-2-*trans*-6-α-L-arabinopyranosyloxy-2,6-dimethyl-2,7-octadienoic acid and (6*S*)-2-*trans*-2,6-dimethyl-6-[3-*O*-(β-D-glucopyranosyl)-4-*O*-(2-methylbutyroyl)-α-L-arabinopyranosyloxy]-2,7-octadienoic acid.

Keywords——monoterpene glycoside; (6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid; acylated glycoside; *Gymnocladus chinensis*; Leguminosae; ^{13}C-NMR; acylation shift

Gymnocladus chinensis BAILLON (Leguminosae) is a plant widely distributed in south China, and the dried fruit of this plant is used as a crude drug 肥皂莢 (Hisohkyo in Japanese) in Chinese traditional medicine as an expectorant. This fruit contains a large amount of saponins, but their structures have not been reported. Recently, Parkhurst and his co-workers reported the structure elucidation of one of the sapogenins obtained from the seed pods of *G. dioica*.[2]

In this paper, we report the structure elucidations of two monoterpene glycosides **1** and **2**. The 70% methanolic extract of the dried fruits was extracted with ethyl acetate, then with *n*-butanol saturated with water. The ethyl acetate and *n*-butanol extracts were separated into seven fractions, I—VII, as described in the experimental sections. The monoterpene glycosides **1** and **2** were obtained from fractions I and II, respectively.

The glycoside **1**, $C_{15}H_{24}O_7$, mp 142—144 °C, $[\alpha]_D$ +4.3 °, contained an α,β-unsaturated carboxyl group, as judged from the infrared (IR) spectrum (1680 cm^{-1} and 1645 cm^{-1}) and the ultraviolet (UV) spectrum (216 nm, $\varepsilon = 13600$). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed the presence of the terminal olefinic protons as double-doublets of ABX type at δ 5.26 ($J = 10.5$ and 1.5 Hz), 5.40 ($J = 18$ and 1.5 Hz) and 6.24 ($J = 18$ and 10.5 Hz). Acid hydrolysis of **1** afforded L-arabinose and a monoterpene **3**. On acetylation followed by methylation with diazomethane, **1** afforded a triacetate methyl ester (**4**). Therefore, the location of the glycoside linkage is not at the carboxyl function, but at a hydroxyl group of the monoterpene. Methyl 2,3,4-tri-*O*-methyl-L-arabinopyranoside and the monoterpene methyl ester (**5**) were obtained by methanolysis of the permethylate (**6**) obtained from **1** by Hakomori's method.[3]

The monoterpene methyl ester (**5**) was identified as (6*S*)-2-*trans*-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid methyl ester, previously obtained from gleditsia saponin C (GS-C),[4,5] by thin layer chromatography (TLC), and IR, ^{1}H-NMR and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral analyses. The configuration of the arabinose was deduced to be α from the coupling constant ($J = 6$ Hz) of the anomeric proton signal at δ 4.33,[6] and from the molecular rotation difference[7] between the monoterpene **3** and **1** (−19 °).[8] Therefore, the

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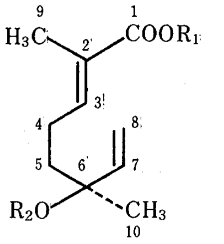
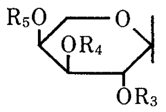
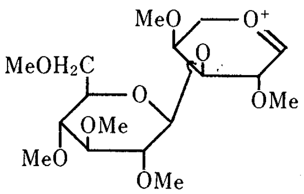
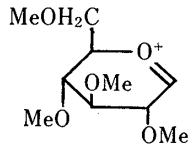


	R_1	R_2 (substituted arabinosyl derivative except **3** and **5**)
1:	H	$R_3=R_4=R_5=H$
2:	H	$R_3=H$, $R_4=\beta$-D-glucosyl, $R_5=-\overset{O}{\overset{\parallel}{C}}-\underset{CH_3}{CH}-CH_2-CH_3$
3:	H	H
4:	Me	$R_3=R_4=R_5=Ac$
5:	Me	H
6:	Me	$R_3=R_4=R_5=Me$
7:	H	$R_3=H$, $R_4=\beta$-D-glucosyl, $R_5=H$
8:	Me	$R_3=Me$, $R_4=2,3,4,6$-tetra-*O*-Me-β-D-glucosyl, $R_5=Me$

$R_2=$ 



9 *m/z* : 379



10 *m/z* : 219

Chart 1

glycoside **1** was characterized as (6*S*)-2-*trans*-6-α-L-arabinopyranosyloxy-2,6-dimethyl-2,7-octadienoic acid.

The glycoside **2**, $C_{26}H_{42}O_{13}$, $[\alpha]_D$ +38.8°, contained an α,β-unsaturated carboxyl group, as judged from the IR and UV spectra, which resembled those of **1** except for the absorbance at 1710 cm^{-1} due to the ester linkage. The ^{1}H-NMR spectrum of **2** showed the presence of a primary methyl group and a secondary methyl group at δ 0.82 (t, J=7 Hz) and 1.09 (d, J=7 Hz), respectively, and the ^{13}C-NMR spectrum of **2** showed the signals of five carbons at δ 176.7, 41.2 (d), 27.0 (t), 11.5 (q) and 16.5 (q). These signals could not be assigned to the monoterpene and/or sugar moieties. Thus, it was assumed that the glycoside **2** is acylated with an isoprene derivative. The deacyl glycoside (**7**) was obtained by hydrolysis with 1% KOH in ethanol, and a permethylate (**8**) was obtained from **7** by Hakomori's method. The mass spectrum (MS) of **8** exhibited prominent ion peaks at *m/z* 379 (**9**) and *m/z* 219 (**10**), and these peaks suggested the presence of a hexosyl-pentose moiety in the molecule of **8** as shown in Chart 1.[9] The permethylate (**8**) was methanolyzed with methanolic hydrochloride to afford methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside, methyl 2,4-di-*O*-methyl-L-arabinopyranoside and the monoterpene methyl ester (**5**). Consequently, it was concluded that the sugar moiety of **7** is a 3-*O*-D-glucopyranosyl-L-arabinopyranosyl group. This conclusion was supported by a comparison of the ^{13}C chemical shift values of **1** with those of **7**. On going from **1** to **7**, the carbon resonance due to C-3 of the arabinose was shifted downfield by +9.5 ppm and those due to C-2 and C-4 were shifted upfield by −1.0 ppm and −0.3 ppm, respectively.

The ^{13}C chemical shift values of the five carbons of the isoprene derivative described above were in accordance with those of methyl 2-methyl butyrate, and the ^{1}H chemical shift values and coupling patterns assigned to the primary methyl and the secondary methyl group were also reasonable.[10] Therefore, the acyl moiety in the molecule of **2** was a 2-methyl-butyroyl group. The location of the 2-methyl-butyroyl group was determined by comparison

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TABLE I. ^{13}C-NMR Chemical Shift Values (ppm) of Compounds **1**, **1′**, **2′** and **7′**[a,b]

No. of C	**1**	**1′**	$\Delta\delta$ (**1′**—**5**)	**7′**	$\Delta\delta$ (**7′**—**1′**)	**2′**	$\Delta\delta$ (**2′**—**7′**)	**5**
1	170.7	(168.4)	(±0)	168.4	(±0)	168.4	(±0)	168.4
2	129.1	(127.7)	(+0.2)	127.7	(±0)	127.7	(±0)	127.5
3	144.0	(144.0)	(+0.5)	144.1	(+0.1)	143.7	(−0.4)	143.5
4	23.6	(23.6)	(−0.4)	23.7	(+0.1)	23.6	(−0.1)	24.0
5	40.6	(40.4)	(−1.1)	40.4	(±0)	40.3	(−0.1)	41.5
6	79.5	(79.4)	(+7.3)	79.6	(+0.2)	79.8	(+0.2)	72.1
7	142.0	(143.1)	(−3.5)	143.1	(±0)	143.0	(−0.1)	146.6
8	114.9	(114.9)	(−3.2)	115.0	(+0.1)	115.2	(+0.2)	111.7
9	12.7	(12.5)	(+0.1)	12.7	(+0.2)	12.4	(−0.3)	12.4
10	23.7	(23.7)	(−4.8)	23.8	(+0.1)	23.7	(−0.1)	28.5
ara.								
1′	100.0	(100.0)		99.9	(−0.1)	99.7	(−0.2)	
2′	72.6	(72.6)		71.6	(−1.0)	71.7	(+0.1)	
3′	74.6	(74.7)		84.2	(+9.5)	81.4	(−2.8)	
4′	69.5	(69.4)		69.1	(−0.3)	71.6	(+2.5)	
5′	66.6	(66.6)		66.7	(+0.1)	64.1	(−2.6)	
glc.								
1″				106.3		106.1	(−0.2)	
2″				75.7		75.5	(−0.2)	
3″				78.6		78.4	(−0.2)	
4″				71.6		71.5	(−0.1)	
5″				78.4		78.2	(−0.2)	
6″				62.7		62.9	(+0.2)	
						176.7 (s)[e]		176.5[c,d]
						41.2 (d)		41.3
						27.0 (t)		27.2
						11.5 (q)		11.7
						16.5 (q)		16.8

a) δ ppm from internal TMS in C_5D_5N: Varian CFT 80 spectrometer at 20 MHz.
b) **1′**, **2′** and **7′** are the methyl esters of compounds **1**, **2** and **7**, obtained by methylation with diazomethane.
c) This column gives the values for methyl 2-methylbutyrate (commercial).
d) The ^{13}C chemical shift values of valeric acid and isovaleric acid (commercial) were, respectively, 176.0, 34.5, 27.7, 22.7, 14.0, and 175.8, 43.8, 25.8, 22.6 (two carbons).
e) Abbreviations in parentheses denote signal patterns observed in the off-resonance experiments.

of the ^{13}C chemical shift values of **7** with those of **2**. On going from **7** to **2**, the signal due to C-4 of the arabinose was shifted downfield by +2.5 ppm and those due to C-3 and C-5 were shifted upfield by −2.8 ppm and −2.6 ppm, respectively, while other carbon signals due to the glucose and C-1 of the arabinose remained unshifted. On the basis of the acylation shift rule,[11] it was concluded that the acyl moiety was located at C-4 of the arbainose. The configuration of the glucose was deduced to be β from the coupling constant (J=7 Hz) of the anomeric proton signal at δ 4.45. Therefore, the monoterpene glycoside (**2**) was characterized as (6*S*)-2-*trans*-2,6-dimethyl-6-[3-*O*-(β-D-glucopyranosyl)-4-*O*-(2-methylbutyroyl)-α-L-arabinopyranosyloxy]-2,7-octadienoic acid.

Studies on the structures of other glycosides obtained from *n*-butanol extract are in progress.

Experimental

Melting points are uncorrected. Neither compound could be obtained in crystalline form, but each showed a

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single spot on TLC. Unless otherwise stated, ^{1}H-NMR spectra were measured in $CDCl_3$ at 80 MHz. UV spectra were measured on a Shimadzu UV-240 spectrometer in 95% EtOH, and MS were measured on a Hitachi M-80 mass spectrometer.

Extraction and Isolation——The crushed dried fruits (1 kg) were extracted with 70% hot MeOH (6 l), and the extract was evaporated under reduced pressure. The residue was poured into water, and the solution was extracted with AcOEt. The aqueous layer was extracted with *n*-butanol saturated with H_2O, and each organic layer was concentrated to dryness under reduced pressure. The *n*-butanol extract was examined by TLC (silica gel, $CHCl_3$–MeOH–H_2O = 65 : 35 : 10 (lower layer)), and more than seven spots were detected. This extract was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O = 8 : 3 : 1 (lower layer) as the developing solvent to afford seven fractions (I—VII). The crude glycoside fractions (I and II) were repeatedly rechromatographed on silica gel using the same solvent and on Sephadex LH20 using MeOH. The monoterpene glycosides (**1**, 250 mg) and (**2**, 60 mg) were obtained, and the same compounds were also obtained from the AcOEt extract in the same manner. Isolation and structure elucidation of other glycosides from fractions III—VII are in progress.

Properties of the Glycosides 1 and 2——Glycoside **1** was obtained as colorless needles from MeOH, mp 142—144 °C, $[\alpha]_D^{27}$ +4.7° (*c* = 1.07, MeOH). UV λ_{max}^{EtOH} nm: 216 (ε = 13600). IR ν_{max}^{Nujol} cm^{-1}: 3400—3500 (OH), 1680 (COOH), 1645 (conjugated double bond). ^{1}H-NMR (C_5D_5N) δ: 1.56 (3H, s, C$\underline{H}_3$), 2.00 (3H, d, *J* = 1.5 Hz, C$\underline{H}_3$), 5.26 (1H, dd, *J* = 10.5, 1.5 Hz), 5.40 (1H, dd, *J* = 18, 1.5 Hz), 6.24 (1H, dd, *J* = 18, 10.5 Hz), 7.06 (1H, t, *J* = 8 Hz), ^{13}C-NMR data: see Table I, *Anal.* Calcd for $C_{15}H_{24}O_7 \cdot H_2O$: C, 53.88; H, 7.84. Found: C, 53.66; H, 7.90.

Glycoside **2** was obtained as a white powder from MeOH–Et_2O, mp 163—166 °C, $[\alpha]_D^{27}$ +38.8° (*c* = 1.25, MeOH). UV λ_{max}^{EtOH} nm: 216 (ε = 11000). IR ν_{max}^{Nujol} cm^{-1}: 3400—3500 (OH), 1710 (COOR), 1680 (COOH), 1645 (conjugated double bond). ^{1}H-NMR (C_5D_5N) δ: 0.82 (3H, t, *J* = 7 Hz, CH_2–C$\underline{H}_3$), 1.09 (3H, d, *J* = 7 Hz, CH–C$\underline{H}_3$), 1.51 (3H, s, C$\underline{H}_3$), 1.98 (3H, d, *J* = 1.5 Hz, C$\underline{H}_3$), *Anal.* Calcd for $C_{26}H_{42}O_{13} \cdot H_2O$: C, 53.78; H, 7.64. Found: C, 53.51; H, 7.82. ^{13}C-NMR data: see Table I.

Acid Hydrolysis of 1——A solution of the glycoside **1** in EtOH (30 ml) was treated with 4 N H_2SO_4 (2 ml), and the mixture was refluxed for 0.5 h. The reaction mixture was concentrated under reduced pressure, and extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$ and evaporated to dryness. The residue was examined by TLC ($CHCl_3$–MeOH–H_2O = 8 : 3 : 1 (lower layer)) and shown to be identical with an authentic sample obtained from gleditsia saponin C. The aqueous layer was neutralized with Amberlite IR 45 and concentrated. The residue was examined by partition paper chromatography (PPC) and TLC, and arabinose was identified.

Triacetate Methyl Ester of 1——The glycoside (**1**, 200 mg) in pyridine (4 ml) was treated with acetic anhydride (4 ml) at room temperature for 12 h, and the reaction mixture was poured into ice–water. The crude acetate was purified by chromatography on silica gel to afford the triacetate. Ethereal diazomethane was added to a solution of the triacetate in MeOH at room temperature, and the reaction mixture was treated in the usual way. The crude methyl ester was purified by chromatography on silica gel using $CHCl_3$ to afford the triacetate methyl ester (**4**, 120 mg) as a colorless oil, which showed a single spot on TLC (MeOH–$CHCl_3$ = 5 : 95, *Rf*: 0.80). Triacetate; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1685 (COOH), 1745 (OCOCH$_3$), 1640 (conjugated double bond). ^{1}H-NMR δ: 1.36 (3H, s, C$\underline{H}_3$), 1.81 (3H, d, *J* = 1.1 Hz, C$\underline{H}_3$), 2.01, 2.06, 2.14 (3H, each s, OCOC$\underline{H}_3$ × 3), 4.51 (1H, d, *J* = 6.5 Hz, anomeric H). Triacetate methyl ester (**4**); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1745 (OCOC$\underline{H}_3$), 1710 (COOC$\underline{H}_3$), 1645 (conjugated double bond). ^{1}H-NMR δ: 1.36 (3H, s, C$\underline{H}_3$), 1.81 (3H, d, *J* = 1.4 Hz, C$\underline{H}_3$), 2.01, 2.05, 2.14 (3H, each s, OCOC$\underline{H}_3$ × 3), 3.72 (3H, s, COOC$\underline{H}_3$), 4.52 (1H, d, *J* = 6.5 Hz, anomeric H).

Permethylation of 1——According to Hakomori's method, NaH (2 g) was stirred with dimethyl sulfoxide (DMSO, 50 ml) at 80—100 °C for 0.5 h under N_2 gas. A solution of **1** (250 mg) in DMSO (10 ml) was added to the above reagent (15 ml), and the reaction mixture was stirred for 1 h at room temperature. CH_3I (15 ml) was added and the whole was stirred for 3 h at room temperature under N_2 gas. The reaction mixture was then poured into ice–water, and the mixture was extracted with Et_2O. The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated to afford a yellow oil (230 mg). This oily product was purified by chromatography on silica gel (benzene–acetone = 7 : 3) to afford a permethylate (**6**, 180 mg) as a colorless syrup, $[\alpha]_D^{25}$ −20.5° (*c* = 0.43, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705 (COOCH$_3$), 1645 (conjugated double bond), 1100. ^{1}H-NMR δ: 1.38, 1.84 (3H, each s, C$\underline{H}_3$ × 2), 3.45, 3.50, 3.57, 3.72 (3H, each s, OCH$_3$ × 4), 4.33 (1H, d, *J* = 6 Hz, anomeric H).

Methanolysis of 6——A solution of **6** (10 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-L-arabinopyranoside by TLC and gas liquid chromatography (GLC) (column, 15% NEGS on Chromosorb W, 5 mm × 2 m; column temp., 210 °C; N_2 flow, 40 ml/min). The monoterpene methyl ester (**5**) was identical with an authentic sample obtained from gleditsia saponin C on the basis of TLC, and IR, ^{1}H-NMR, and ^{13}C-NMR spectral comparisons.

Alkaline Hydrolysis of 2——A mixture of **2** (30 mg) and 1% KOH (10 ml) in EtOH (10 ml) was refluxed for 0.5 h. The reaction mixture was neutralized with Dowex 50W × 8, and concentrated under reduced pressure. The residue was chromatographed on silica gel to afford a deacyl glycoside (**7**, 18 mg) as a colorless syrup, $[\alpha]_D^{18}$ −5.4° (*c* = 1.05, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3400—3500 (OH), 1680 (COOH), 1645 (conjugated double bond). *Anal.* Calcd for $C_{21}H_{34}O_{12} \cdot 2H_2O$: C, 49.02; H, 7.44. Found: C, 48.97; H, 7.62.

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Permethylation of 7——**7** (50 mg) was permethylated in the same way as **1** to afford a permethylate (**8**, 36 mg) as a colorless syrup, $[\alpha]_D^{25}$ −2.6° (c=1.70, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 ($COOCH_3$), 1645 (conjugated double bond), 1100. ^{1}H-NMR δ: 1.26 (3H, s, CH_3), 1.81 (3H, d, J=1.1 Hz, CH_3), 3.36, 3.45, 3.51, 3.56 (3H, each s, $OCH_3 \times 4$), 3.62 (6H, s, $OCH_3 \times 2$), 3.64 (3H, s, OCH_3), 4.25 (1H, d, J=6.5 Hz, anomeric H), 4.45 (1H, d, J=7 Hz, anomeric H). MS (EI; m/z): 576 (M^+, less than 1%), 379 (hexose-pentose permethylate, base peak), 219 (hexose permethylate).

Methanolysis of 8——A solution of **8** (15 mg) was methanolyzed in the same way as **6** and the products were identified as methyl 2,4-di-*O*-methyl-L-arabinopyranoside, methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside and the monoterpene methyl ester (**5**) by TLC and GLC.

Acknowledgements Our thanks are due to Emeritus Prof. S. Kitamura and Prof. G. Murata, Kyoto University, for supplying fruits of *Gymnocladus chinensis*. We are also grateful to Mr. Y. Fujiwara for the ^{1}H-NMR and ^{13}C-NMR measurements.

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