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Studies on the Constituents of Asclepiadaceae Plants. LIV.[1] The Structures of Glaucoside-F and -G from the Chinese Drug "Pai-ch'ien," *Cynanchum glaucescens* Hand-Mazz

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The glycosides of the Chinese crude drug "Pai-ch'ien" have been further investigated. Two new glycosides named glaucoside-F (**8**) and -G (**9**) were isolated and their structures were characterized on the bases of spectroscopic evidence and analyses of their hydrolysates by thin-layer chromatography (TLC). They were found to possess α-L-cymaropyranose at the terminal of their sugar chains, like glaucoside-B (**4**), -C (**5**), -D (**6**), and -E (**7**).

Keywords——glaucoside-F, -G; ^{13}C-NMR; "Pai-ch'ien"; *Cynanchum glaucescens*; Asclepiadaceae

We have already reported in a previous paper[2] five glycosides named glaucoside-A (**3**), -B (**4**), -C (**5**), -D (**6**), and -E (**7**) isolated from Chinese crude drug "Pai-ch'ien"[3] 芫花叶白前, dried root of *Cynanchum glaucescens* Hand-Mazz (Asclepiadaceae), which has been used as an antitussive and expectorant in China. Compounds **4**, **5**, **6**, and **7** possess unique sugar chains, in that the terminal sugars of their sugar chains are α-linked L-cymaropyranose, while the other linkages are all β. This paper deals with the isolation and structural elucidation of two new

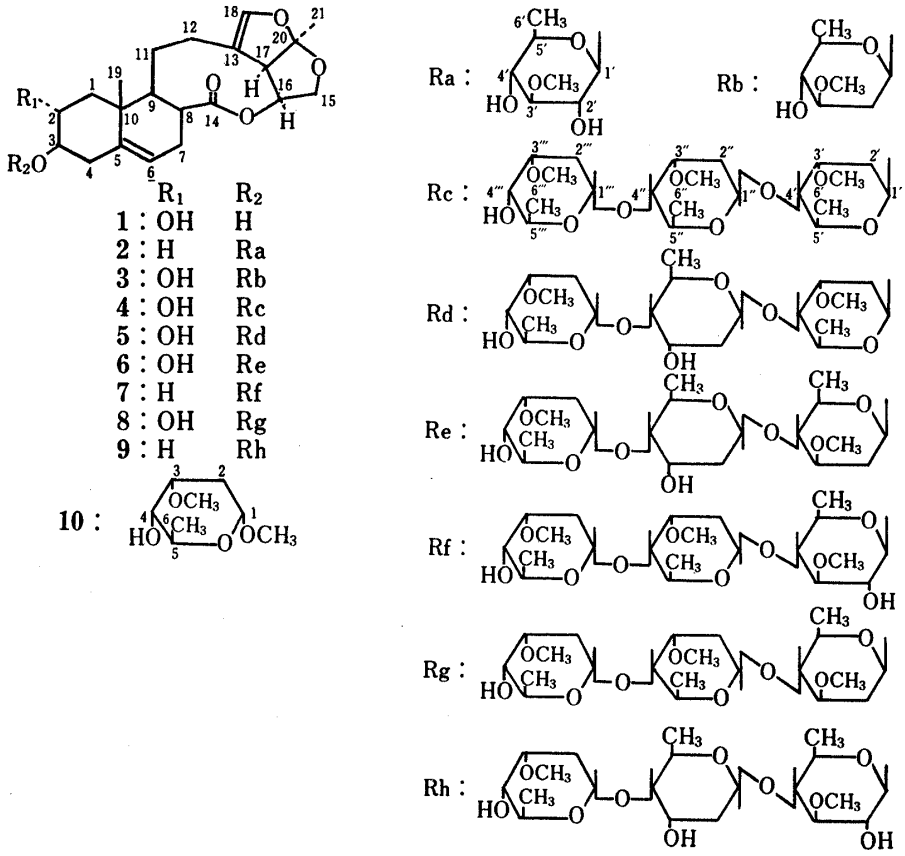

Chart 1

glycosides named glaucoside-F (**8**) and -G (**9**).

The less polar portion of the crude glycosides was subjected to repeated silica gel column chromatography with various solvent systems to yield **8** and **9** as amorphous white powders.

Glaucoside-F (**8**) has the molecular formula $C_{42}H_{64}O_{15}$, and gave glaucogenin-A (**1**), oleandrose, and cymarose on hydrolysis; these were identified by TLC comparison with authentic samples. The proton nuclear magnetic resonance spectrum (^{1}H-NMR) in deuterochloroform ($CDCl_3$) showed signals due to the sugars: three secondary methyls at δ 1.24, 1.27, and 1.31; three methoxyl methyls at δ 3.39, 3.42, and 3.48; and three anomeric protons at δ 4.49, 4.96 (each 1H, dd, J=10, 2 Hz), and 4.80 (1H, br d, J=4 Hz), indicating the presence of

Table I. ^{13}C-NMR Chemical Shifts for **1**, **2**, **8**, **9**, and **10** (ppm in C_5D_5N)

	1	**8**	**2**	**9**		**10**
C-1	45.5	44.5	36.6	36.4		
C-2	72.4	69.6(−2.8)	30.0	29.9		
C-3	76.7	85.0(+8.3)	78.1	78.0		
C-4	40.1	37.5(−2.6)	39.0	38.9		
C-5	140.9	139.4	140.7	140.3		
C-6	120.0	120.4	120.4	120.1		
C-7	30.1	29.9	30.0	29.0		
C-8	53.2	52.9	53.3	53.1		
C-9	40.4	40.1	40.7	40.6		
C-10	40.4	39.3	38.7	38.6		
C-11	23.9	23.8	23.9	23.9		
C-12	28.2	28.3	28.4	28.4		
C-13	118.5	118.1	118.4	118.2		
C-14	175.4	174.8	175.4	175.0		
C-15	67.8	67.5	67.7	67.7		
C-16	75.5	75.3	75.5	75.4		
C-17	56.2	56.0	56.2	56.0		
C-18	143.8	143.5	143.8	143.5		
C-19	19.2	18.9	18.6	18.6		
C-20	114.3	113.9	114.3	114.1		
C-21	24.8	24.7	24.8	24.8		
C-1′		98.6[a]	102.4	102.0		
C-2′		37.3	75.0	74.4		
C-3′		81.4	88.0	85.6(−2.4)		
C-4′		82.0	75.9	82.5(+6.6)		
C-5′		73.0	72.6	71.4(−1.2)		
C-6′		18.5	17.9	17.8		
-OMe		57.2	60.8	60.3		
C-1″		98.8[a]		98.6[b]		
C-2″		36.8		38.4		
C-3″		77.6		69.0		
C-4″		82.2		80.6		
C-5″		69.3		67.7		
C-6″		18.5		18.4[c]		
-OMe		58.2				
C-1‴		98.0		98.2[b]	C-1	97.6
C-2‴		31.9		32.1	C-2	31.9
C-3‴		76.1		76.3	C-3	76.5
C-4‴		73.1		72.6	C-4	73.2
C-5‴		66.0		66.9	C-5	65.2
C-6‴		18.5		18.5[c]	C-6	18.9
-OMe		56.3		56.6	-OMe	56.7
						54.7

a—*c*) Assignments may be interchanged.

two β-linkages and one α-linkage. The field desorption mass spectrum (FD-MS) of **8** displayed prominent fragment ion peaks at m/z: 664, 520, and 376 besides the molecular ion peak (m/z: 808); the former peaks may be attributed to successive loss of three sugars starting from the terminal as reported by Shulten and co-workers.[4] In the ^{13}C nuclear magnetic resonance spectrum (^{13}C-NMR) of **8** in pentadeuteropyridine (C_5D_5N) (Table I), glycosidation shifts[5] were observed at C-2 (−2.8 ppm), C-3 (+8.3), and C-4 (−2.6) in the aglycone moiety, as in the cases of **3**, **4**, **5**, and **6**, so that the position at which the sugar is linked should be the C-3 hydroxyl group of the aglycone. The presence of sugar carbon signals corresponding to those for methyl α-L-cymaropyranoside[2] (**10**) located the α-linked cymaropyranose at the terminal of the sugar chain in **8**, while the other sugars were assigned as β-linked cymaro- and oleandropyranose on the basis of glycosidation shifts at C-4. These spectroscopic data provided no further information on the sequence of sugars. Fortunately, however, acid hydrolysis of **8** under mild conditions gave glaucoside-A (**3**) in the partial hydrolysate; it was identified by comparison with an authentic sample. The occurrence of L-cymarose,[6] D-oleandrose,[7] and D-digitoxose[8] in the hydrolysate of this material had been confirmed;[2] therefore the structure of **8** was established as glaucogenin-A 3-*O*-α-L-cymaropyranosyl-(1→4)-β-L-cymaropyranosyl-(1→4)-β-D-oleandropyranoside. The lower hydrolyzability of the β-D-(1→3)-linkage compared with the others cannot be accounted for at present.

Glaucoside-G (**9**) has the molecular formula $C_{41}H_{62}O_{15}$, and gave glaucogenin-C mono-D-thevetoside (**2**), digitoxose, and cymarose on hydrolysis. The ^1H-NMR spectrum showed signals due to three sugars: three secondary methyls at δ 1.25, 1.26×2; two methoxyl methyls at δ 3.26 and 3.42; and three anomeric protons at δ 4.33 (1H, d, J=7.3 Hz), 4.91 (1H, br d, J=4 Hz), and 4.95 (1H, dd, J=9, 2 Hz), also suggesting the presence of two β-linkages and one α-linkage. The ^{13}C-NMR spectrum of **9** showed signal groups assignable to **2** glycosylated at the C-4 hydroxyl group of thevetose, β-linked digitoxopyranose glycosylated at the C-4 hydroxyl group, and α-linked cymaropyranose, so that the thevetose, digitoxose, and cymarose should be attached to the aglycone in that order. This was supported by the prominent fragment peaks at m/z: 650, 520, and 360 in the FD-MS of **9**. Thus, the structure glaucogenin-C 3-*O*-α-L-cymaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→4)-β-D-thevetopyranoside was assigned to glaucoside-G (**9**).

It should be noted that the terminal sugars of six trisaccharide glycosides so far obtained from this drug are all α-linked L-cymaropyranose, while the other linkages are all β. The more polar portion of the glycosides is currently under investigation.

Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temperature. Infrared (IR) spectra were recorded on a JASCO A-102 spectrometer. ^1H-NMR spectra were run on a JEOL FX-200 (200 MHz) in $CDCl_3$ solution and ^{13}C-NMR spectra on a JEOL FX-100 (25 MHz) in C_5D_5N solution with tetramethylsilane as a standard. Electron impact (EI)-MS were determined with a JEOL JMS-D-300 mass spectrometer and FD-MS with a JEOL JMS-01SG-2. TLC was performed on Merck precoated plates (Kieselgel 60 F_{254}), and silica gel column chromatography on Wakogel C-200 (200 mesh) or C-300 (300 mesh).

Isolation of 8 and 9——A part of the hexane–benzene (1: 1) and benzene soluble portion of the crude glycosides (40 g) reported in the previous paper[1] was applied to a column of silica gel (400 g of Wakogel C-200), and the material was eluted with solvents of increasing polarity from benzene–acetone (8: 1) to acetone. The fraction eluted with benzene–acetone (5: 1) contained **3** and **4**. Fraction 3 (8.0 g), which contained **5**, **6**, **7** and **8**, was obtained by further elution with the same solvent. Fraction 4 (9.3 g), eluted with benzene–acetone (4: 1), contained **6** and **9**. Fraction 3 (8.0 g) was rechromatographed with 1.5% methanol (MeOH) in chloroform ($CHCl_3$) to yield five fractions (fractions A to E). Fraction C (2.24 g) contained **5** and **7**, and fraction D (1.07 g) contained **6**. Fraction B (640 mg), containing **8**, was further rechromatographed with hexane–EtOAc (1: 2) to give a fraction (137 mg) containing mainly **8**, which was rechromatographed with 2% MeOH in benzene to furnish **8** (128 mg). Fraction 4 (9.30 g) was subjected to rechromatography with 3% MeOH in $CHCl_3$, hexane–EtOAc–MeOH (25: 25: 2), and 3% MeOH in benzene

in that order to give **9** (87 mg). Compounds **8** and **9** were obtained as amorphous white powders. The *Rf* values of **8** and **9** on TLC with 5% MeOH in $CHCl_3$ were 0.67 and 0.52, respectively.

Glaucoside-F (8)——An amorphous powder, mp 110—113°C, $[\alpha]_D$ −17.4° (c=1.21, $CHCl_3$). *Anal.* Calcd for $C_{42}H_{64}O_{15}\cdot H_2O$: C, 61.00; H, 8.05. Found: 61.00; H, 7.80. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3450, 1730, 1710, 1655, 1310, 1050, 1010, 880. FD-MS *m/z*: 808 (M^+, base peak), 664, (M^+−144), 520 (664—144), 376 (520—144). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 19-CH_3), 1.00 (1H, t, J=12 Hz, 1-CH_α), 1.24, 1.27, and 1.31 (each 3H, d, J=6 Hz, 5′-, 5″-, and 5‴-CH_3), 1.53 (3H, s, 21-CH_3), 1.74 (1H, dt, J=12, 5 Hz, 4-CH_α), 3.39, 3.42, and 3.48 (each 3H, s, 3′-, 3″-, and 3‴-OCH_3), 3.84 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.16 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.49 (1H, dd, J=10, 2 Hz, 1′-CH), 4.80 (1H, br d, J=4 Hz, 1‴-CH), 4.96 (1H, dd, J=10, 2 Hz, 1″-CH), 5.30 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.42 (1H, d, J=5 Hz, 6-CH), 6.27 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Table I.

Glaucoside-G (9)——An amorphous powder, mp 117—123°C, $[\alpha]_D$ −29.6° (c=0.81, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{62}O_{15}$: C, 61.95; H, 7.68. Found: C, 61.74; H, 7.92. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3300, 1730, 1710, 1655, 1310, 1100, 100, 930, 860. FD-MS *m/z*: 795 (M^++H, base peak), 794 (M^+), 650 (M^+−144), 520 (650—130), 360 (520—160). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, 19-CH_3), 1.25, 1.26 (3H and 6H, respectively, each d, J=6.4, 6.8 Hz, 6′-, 6″-, and 6‴-CH_3), 1.53 (3H, s, 21-CH_3), 3.26, 3.42 (each 3H, s, 3′-and 3‴-OCH_3), 3.84 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.16 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.33 (1H, d, J=7.3 Hz, 1′-CH), 4.91 (1H, br d, J=4 Hz, 1‴-CH), 4.95 (1H, dd, J=9, 2 Hz, 1″-CH), 5.35 (1H, ddd, J=10, 8, 7 Hz, 17-CH), 5.39 (1H, d, J=5 Hz, 6-CH), 6.25 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Table I.

Partial Acidic Hydrolysis of 8——A solution of 36 mg of **8** in 3 ml of MeOH was treated with 1 ml of 0.2 N H_2SO_4, and kept at around 60°C for 20 min. TLC analysis with $CHCl_3$–acetone (5: 1) revealed the formation of **3** (*Rf*, 0.37) and **1** (*Rf*, 0.22) as well as methyl glycosides. The solution was neutralized with saturated aqueous $Ba(OH)_2$ and the precipitates were filtered off. The filtrate was concentrated and the residue was subjected to silica gel column chromatography with $CHCl_3$–acetone (15: 1) to give **3** (5.6 mg) as an amorphous white powder, mp 85—90°C, $[\alpha]_D$ +8.93° (c=0.56, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3350, 1730, 1710, 1655, 1310, 1165, 1070, 950. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, s, 19-CH_3), 1.02 (1H, t, J=12 Hz, 1-CH_α), 1.36 (3H, d, J=6.4 Hz, 6′-CH_3), 1.54 (3H, s, 21-CH_3), 3.74 (1H, ddd, J=12, 10, 5 Hz, 2-CH_β), 3.85 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.16 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.55 (1H, dd, J=10, 2 Hz, 1′-CH), 5.35 (1H, ddd, J=10, 8, 7 Hz, 17-CH), 5.45 (1H, d, J=4.5 Hz, 6-CH). The $[\alpha]_D$, IR, MS, and ^{1}H-NMR data are identical with those of **3**. The identity of these two compounds was further confirmed by TLC with three solvent systems: *Rf* 0.56 (5% MeOH in $CHCl_3$); *Rf* 0.57 (benzene–acetone (5: 3)); *Rf* 0.68 (3% ethanol in methylene chloride).

Acidic Hydrolysis of 8 and 9——A solution of 3 mg of **8** in 2 ml of MeOH was treated with 2 ml of 0.1 N H_2SO_4, and kept at around 60°C for 30 min, then the solution was diluted with 2 ml of water and concentrated to 1/2 the initial volume. The solution was again kept at around 60°C for a further 30 min, then neutralized with saturated $Ba(OH)_2$, and the precipitates were filtered off. The filtrate was concentrated to give a yellow syrup, which was analyzed by TLC with three solvent systems: solvent A, $CHCl_3$–MeOH (9: 1); solvent B, methylene chloride–ethanol (9: 1); and solvent C, benzene–acetone (5: 3). The *Rf* values of **1**, **2**, cymarose, oleandrose, and digitoxose were 0.51, 0.55, 0.47, 0.43, and 0.21 with solvent A, 0.53, 0.56, 0.42, 0.33 and 0.21 with solvent B; and 0.43, 0.49, 0.43, 0.31, and 0.17 with solvent C, respectively. When **8** was hydrolyzed, **1**, oleandrose, and cymarose were identified by TLC comparisons with authentic samples (solvents A, B, and C). Similarly, 3 mg of **9** was hydrolyzed, and **2**, cymarose, and digitoxose were identified.

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