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Studies on the Constituents of Asclepiadaceae Plants. LIV.¹⁾ The Structures of Glucoside-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 glucoside-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 glucoside-B (4), -C (5), -D (6), and -E (7).

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

We have already reported in a previous paper²⁾ five glycosides named glucoside-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

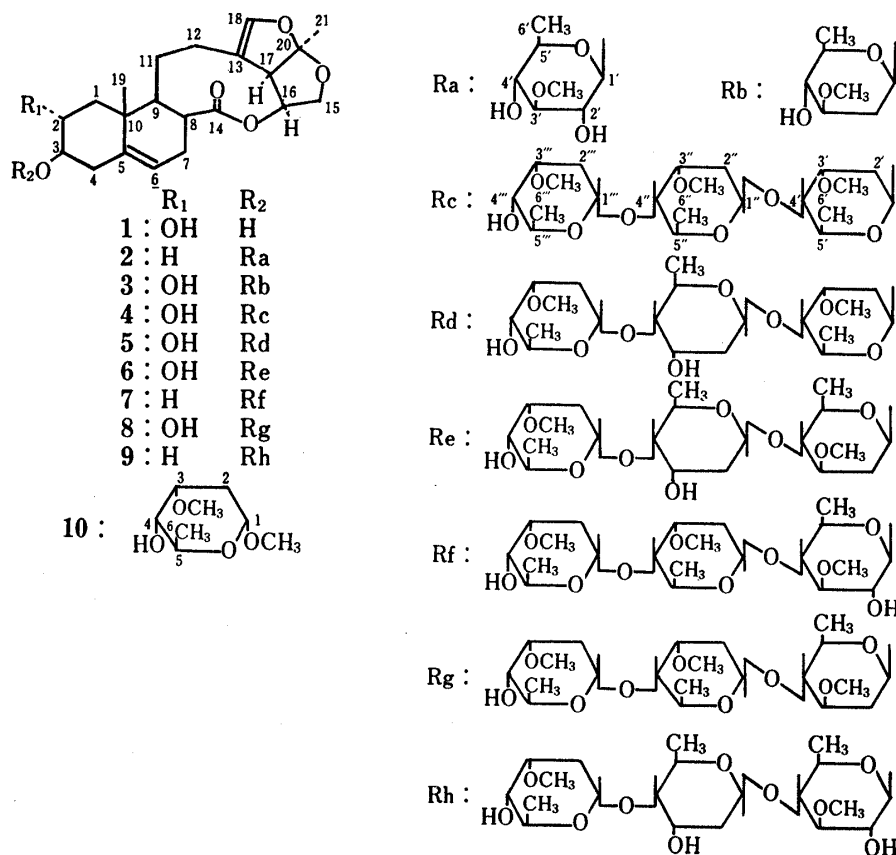


Chart 1

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 (1H -NMR) in deuteriochloroform ($CDCl_3$) 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_6D_6N)

	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	
-QMe		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)}	
-QMe		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
-QMe		56.3		56.6	-QMe 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 ($\text{C}_5\text{D}_5\text{N}$) (Table I), glycosidation shifts⁵⁾ 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-cymaropyranoside²⁾ (**10**) located the α -linked cymaropyranose at the terminal of the sugar chain in **8**, while the other sugars were assigned as β -linked cymar- 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-digitoxose⁸⁾ 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 $\rightarrow$ 4)- β -L-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-oleandropyranoside. The lower hydrolyzability of the β -D-(1 $\rightarrow$ 3)-linkage compared with the others cannot be accounted for at present.

Glaucoside-G (**9**) has the molecular formula $\text{C}_{41}\text{H}_{62}\text{O}_{15}$, 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 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 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 $\text{C}_5\text{D}_5\text{N}$ 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 F₂₅₄), 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 paper¹⁾ 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 *R_f* values of **8** and **9** on TLC with 5% MeOH in CHCl₃ were 0.67 and 0.52, respectively.

Glaucoside-F (8)—An amorphous powder, mp 110–113°C, $[\alpha]_D -17.4^\circ$ ($c=1.21$, CHCl₃). *Anal.* Calcd for C₄₂H₆₄O₁₅·H₂O: C, 61.00; H, 8.05. Found: C, 61.00; H, 7.80. IR $\nu_{\max}^{\text{CHCl}_3}$ 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). ¹H-NMR (CDCl₃) δ : 0.94 (3H, s, 19-CH₃), 1.00 (1H, t, $J=12$ Hz, 1-CH_a), 1.24, 1.27, and 1.31 (each 3H, d, $J=6$ Hz, 5'-, 5'', and 5'''-CH₃), 1.53 (3H, s, 21-CH₃), 1.74 (1H, dt, $J=12, 5$ Hz, 4-CH_a), 3.39, 3.42, and 3.48 (each 3H, s, 3'-, 3'', and 3'''-OCH₃), 3.84 (1H, dd, $J=10, 9$ Hz, 15-CH_β), 4.16 (1H, dd, $J=9, 7$ Hz, 15-CH_a), 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). ¹³C-NMR: see Table I.

Glaucoside-G (9)—An amorphous powder, mp 117–123°C, $[\alpha]_D -29.6^\circ$ ($c=0.81$, CHCl₃). *Anal.* Calcd for C₄₁H₆₂O₁₅: C, 61.95; H, 7.68. Found: C, 61.74; H, 7.92. IR $\nu_{\max}^{\text{CHCl}_3}$ 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). ¹H-NMR (CDCl₃) δ : 0.92 (3H, s, 19-CH₃), 1.25, 1.26 (3H and 6H, respectively, each d, $J=6.4, 6.8$ Hz, 6'-, 6'', and 6'''-CH₃), 1.53 (3H, s, 21-CH₃), 3.26, 3.42 (each 3H, s, 3'- and 3'''-OCH₃), 3.84 (1H, dd, $J=10, 9$ Hz, 15-CH_β), 4.16 (1H, dd, $J=9, 7$ Hz, 15-CH_a), 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). ¹³C-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 H₂SO₄, and kept at around 60°C for 20 min. TLC analysis with CHCl₃–acetone (5: 1) revealed the formation of **3** (*R_f*, 0.37) and **1** (*R_f*, 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 CHCl₃–acetone (15: 1) to give **3** (5.6 mg) as an amorphous white powder, mp 85–90°C, $[\alpha]_D +8.93^\circ$ ($c=0.56$, CHCl₃). IR $\nu_{\max}^{\text{CHCl}_3}$ cm⁻¹: 3600, 3350, 1730, 1710, 1655, 1310, 1165, 1070, 950. ¹H-NMR (CDCl₃) δ : 0.95 (3H, s, 19-CH₃), 1.02 (1H, t, $J=12$ Hz, 1-CH_a), 1.36 (3H, d, $J=6.4$ Hz, 6'-CH₃), 1.54 (3H, s, 21-CH₃), 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_a), 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 ¹H-NMR data are identical with those of **3**. The identity of these two compounds was further confirmed by TLC with three solvent systems: *R_f* 0.56 (5% MeOH in CHCl₃); *R_f* 0.57 (benzene–acetone (5: 3)); *R_f* 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 H₂SO₄, 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, CHCl₃–MeOH (9: 1); solvent B, methylene chloride–ethanol (9: 1); and solvent C, benzene–acetone (5: 3). The *R_f* 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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