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Studies on the Constituents of Asclepiadaceae Plants. LXI.[1] The Structure of Cynatratoside-F from the Chinese Drug "Pai-Wei," Dried Root of *Cynanchum atratum* BUNGE

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The chemical components of the Chinese crude drug "Pai-Wei," dried root of *Cynanchum atratum* BUNGE, have been further investigated. Glaucogenin-A (**1**), glaucoside-C (**2**), glaucoside-H (**3**) and a new glycoside named cynatratoside-F (**4**) were isolated. The structure of **4** was determined on the bases of spectroscopic evidence and analyses of its hydrolysate.

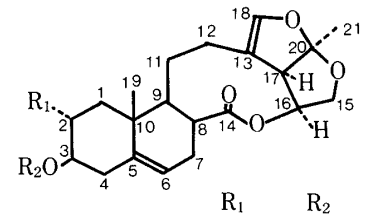
Keywords——*Cynanchum atratum*; glaucogenin-A; glaucoside-C; glaucoside-H; cynatratoside-F; D-cymarose; ^{13}C-NMR

We have already reported isolation[1] of glaucogenin-C (**5**) and five glycosides named cynatratosides-A (**6**), -B (**7**), -C (**8**), -D (**9**) and -E (**10**) from a Chinese crude drug "Pai-Wei," dried root of *Cynanchum atratum* BUNGE (Asclepiadaceae), which has been used as an antifebrile and diuretic in China. In the present paper, we wish to describe the isolation and structural elucidation of four other steroidal components **1**, **2**, **3** and **4** from the same source.

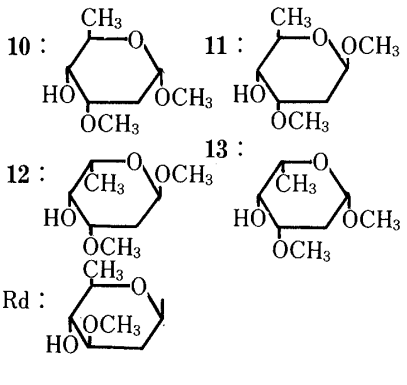
These four compounds gave a positive Liebermann–Burchard reaction and the compounds other than **1** showed a positive Keller–Kiliani reaction. These results suggested that there is a Δ^5 steroidal structure in each molecule, and that **2**, **3** and **4** contain a 2-deoxy sugar residues.[2]

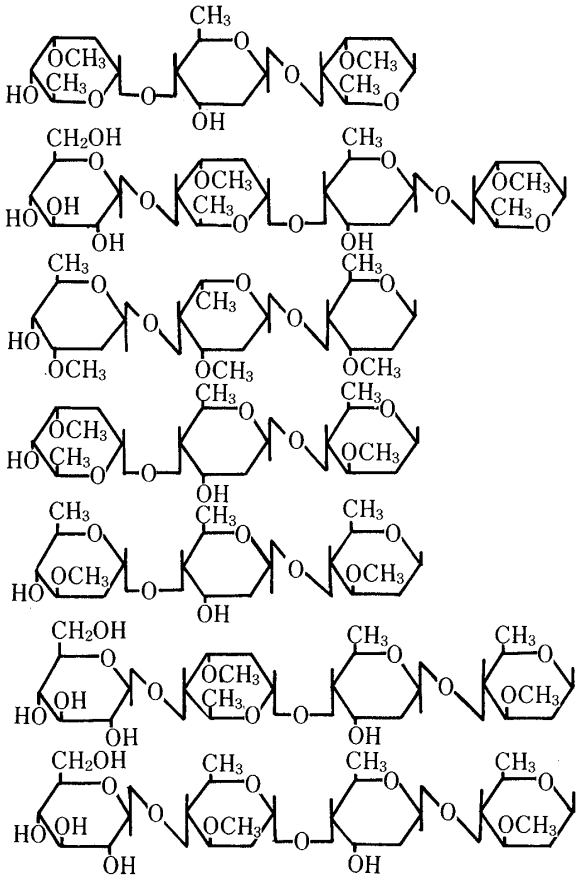
The proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) data, shown in Tables I and II, and the fragmentation patterns in the electron impact (EI) and field desorption (FD) mass spectra (MS) of **1**, **2** and **3** are identical with those of glaucogenin-A,[3] glaucoside-C[4] and glaucoside-H,[5] respectively, previously isolated from *C. glaucescens*. On a mild acidic hydrolysis, **2** gave **1**, digitoxose, and L-cymarose ($[\alpha]_D$ −49.2°). On enzymatic hydrolysis with β-glucosidase, **3** gave **2** and glucose. Therefore, **1**, **2** and **3** were identified as glaucogenin-A, glaucoside-C and glaucoside-H, respectively.

Cynatratoside-F (**4**) has a molecular formula $C_{42}H_{64}O_{15}$, on the bases of its elemental analysis and FD-MS. Mild acid hydrolysis of **4** afforded **1**, diginose and D-cymarose ($[\alpha]_D$ +52.4°). This is a good agreement with the fragmentation pattern, *m/z*: 808 (M^+), 664 ($M^+ - 144$), 520 (664 − 144), 376 (520 − 144) in the FD-MS. The ^{1}H-NMR signals due to the anomeric protons at δ 5.17 (1H, br d, J = 3.5 Hz), 4.88 (1H, dd, J = 10, 2 Hz), and 4.69 (1H, dd, J = 9, 2 Hz) indicated one α and two β glycosidic bond. Since glycosidation shifts[6] were observed at C-2 (−2.4 ppm), C-3 (+9.1 ppm) and C-4 (−2.5 ppm) in the ^{13}C-NMR spectrum of the aglycone moiety of **4**, the sugar moiety is linked to the C-3 hydroxyl group of the aglycone (**1**). The terminal sugar was deduced to be β-cymaropyranose and the other two sugars to be α-diginopyranose and β-cymaropyranose by comparison of the with ^{13}C-NMR chemical shifts with those of methyl α- and β-cymaropyranosides (**10**), and (**11**), and methyl α-



	R_1	R_2
1	OH	H
2	OH	Ra
3	OH	Rb
4	OH	Rc
5	H	H
6	H	Rd
7	H	Re
8	H	Rf
9	H	Rg
10	H	Rh





and β-diginopyranosides (**12**) and (**13**).[7] The sequence of the three sugars was assigned by the partially relaxed Fourier transform (PRFT) method[8] as follows. A set of signals with longer spin lattice relaxation time ($T_1 = 0.225$ s) than others among signals due to sugar carbons corresponded to those of the terminal β-cymaropyranose. The next two sugars are α-diginopyranose and another β-cymaropyranose linked to the aglycone based on the order of the T_1 values (0.175 s and shorter than 0.15 s, respectively). Since the diginose in this drug is in the L series as determined from the specific rotation, the structure of **4** was deduced to be glaucogenin-C 3-*O*-β-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-β-D-cymaropyranoside.

This sugar sequence is the same as that of wilfoside C3N from *Cynanchum wilfordi*,[7] though the aglycones are different. Some other wilfosides are unique in containing both D and L cymaroses in the same glycosidic sequence. Cynatratoside-F (**4**) is different from other cynatratosides with respect to the optical rotation of the cymarose; the former contains D cymarose and the latter compounds contain L-cymarose. It remains to be determined whether this crude drug, "Pai-Wei," consists of only one species of plant (*Cynanchum atratum*) or not. Such research is under way.

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. ^{1}H-NMR spectra were run on a JEOL FX-200 (200 MHz) instrument in $CDCl_3$ solution and ^{13}C-NMR spectra on a FX-200 (50 MHz) in C_5H_5N solution with tetramethylsilane as a standard. EI-MS were determined with a JEOL JMS-D-300 mass spectrometer and FD-MS with a JEOL JMS-OISG-2 machine. Thin layer chromatography (TLC) was performed on Merck precoated plates, Kieselgel $60F_{254}$ or RP-18 F_{254}. Column chromatography was carried out on Wakogel C-200 (200 mesh) and reversed phase gel (Fuji, ODS-Q3 column).

TABLE I. ^{13}C-NMR Chemical Shifts of the Aglycone Moieties

	1	2	3	4
C-1	45.6	44.8	44.7	44.8
C-2	72.4	70.0	70.0	70.0
C-3	76.7	85.4	85.4	85.4
C-4	40.1	37.6	37.6	37.6
C-5	140.9	139.8	139.7	139.8
C-6	120.1	120.8	120.7	120.7
C-7	30.1	30.1	30.0	30.1
C-8	53.2	53.1	53.0	53.1
C-9	40.5	40.3	40.2	40.3
C-10	40.4	39.5	39.5	39.5
C-11	23.9	23.9	23.8	23.9
C-12	28.5	28.5	28.5	28.5
C-13	118.5	118.5	118.5	118.5
C-14	175.5	175.4	175.3	175.3
C-15	67.8	67.8	67.8	67.8
C-16	75.6	75.6	75.5	75.6
C-17	56.2	56.2	56.2	56.2
C-18	143.8	143.8	143.8	143.8
C-19	19.2	19.0	18.9	19.0
C-20	114.4	114.4	114.3	114.3
C-21	24.8	24.8	24.8	24.8

Isolation of 1, 2, 3, and 4——The crude aglycone (18 g) reported in the previous paper[1] was subjected to silica gel column chromatography with a mixed solvent of increasing polarity from 3% MeOH–$CHCl_3$ to 40% MeOH–$CHCl_3$ to give four fractions. Fraction 2 (3 g) was rechromatographed with 4% MeOH–$CHCl_3$ and hexane–EtOAc (1 : 1) to give **1** (110 mg) as colorless needles from acetone. The benzene-soluble portion of the reported crude glycoside[1] was applied to a column of silica gel, and eluted with 2% MeOH–$CHCl_3$ to give five fractions. Fraction 3 (20 g) contained two glycosides **7** and **8**, which were separated by column chromatography. The residual mixture was separated repeatedly on a reversed-phase column and silica gel column with MeOH–H_2O (7 : 3) and 1% MeOH–$CHCl_3$, respectively, to give pure **2** and **4**. The benzene insoluble portion of the crude glycoside was applied to a silica gel column, and eluted with the solvents of increasing polarity from $CHCl_3$ to $CHCl_3$–MeOH (3 : 2) to afford six fractions. After isolation of **9** and **10** from fraction 5 (20 g) which contained mainly polar glycoside, the residue was repeatedly separated on a reversed phase column to give **3**.

Glaucogenin-A (1)——Colorless needles, mp 227—230 °C, $[\alpha]_D$ +80.5° (c=1.00, MeOH). *Anal.* Calcd for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50. Found: C, 66.72; H, 7.84. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 3400, 1700, 1620, 1350, 1280, 1110. EI-MS m/z: 376 (M^+), 358 ($M^+ - H_2O$), 330, 312, 137 (base peak), 43. ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, s, 19 CH_3), 1.06 (1H, t, J=12 Hz, 1-CH), 1.54 (3H, s, 21-H), 3.36 (1H, ddd, J=12, 9, 6 Hz, 3-CH), 3.46 (1H, dd, J=8, 2 Hz, 17-CH), 3.70 (1H, ddd, J=12, 9, 4 Hz, 2-CH), 3.86 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.17 (1H, dd, J=9, 7 Hz, 15-CH_α), 5.35 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.45 (1H, d, J=4.5 Hz, 6-CH), 6.27 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR data: see Table I.

Glaucoside-C (2)——An amorphous powder, mp 130—136 °C, $[\alpha]_D$ −16.0° (c=1.00, $CHCl_3$). *Anal.* Calcd for $C_{41}H_{62}O_{15}$: C, 61.69; H, 7.81. Found: C, 61.67; H, 7.96. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 3400, 1700, 1360, 1305, 1200, 1160, 1080, 1050. FD-MS m/z: 794 (M^+, base), 650 ($M^+ - 144$), 520 (650 − 130), 376 (520 − 144). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 19-CH_3), 1.08 (1H, t, J=12 Hz, 1-CH), 1.22 and 1.25 (each 3H, d, J=6.4 Hz), 1.53 (3H, s, 21-CH_3), 3.42 and 3.46 (each 3H, s, 3′- and 3‴-OCH_3), 4.15 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.79 and 4.88 (each 1H, dd, J=10, 2 Hz, 1′- and 1″-CH, 4.95 (1H, br d, J=3 Hz, 1‴-CH), 5.28 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.41 (1H, d, J=4.5 Hz), 6.27 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Tables I and II.

Acidic Hydrolysis of 2——A solution of 73 mg of **2** in 10 ml of MeOH was treated with 10 ml of 0.1 N H_2SO_4 and the mixture kept at 50 °C for 50 min. After the solution had been diluted with water (10 ml) and concentrated to 20 ml, it was again kept at 70 °C for 30 min, then neutralized with saturated $Ba(OH)_2$ aqueous solution, and the precipitate was filtered off. The filtrate was concentrated to dryness and the residue was chromatographed on a column of silica gel (16 g of Wakogel C-200) with 1% MeOH–$CHCl_3$ to obtain 5 mg of cymarose, which showed $[\alpha]_D$ −49.2° (c=0.5, H_2O) indicating that it belonged to the L series. Glaucogenin-A and digitoxose were identified by TLC in comparison with an authentic sample.

Glaucoside-H (3)——An amorphous powder, mp 150—155 °C, $[\alpha]_D$ −29.6° (c=1.00, $CHCl_3$), *Anal.* Calcd for

TABLE II. ^{13}C-NMR Chemical Shifts of the Sugar Carbons of **2**, **3**, **4**, **12**, and **13**

	2	**3**	**4**	**12**	**13**
	L-cym	L-cym	D-cym		
C-1′	97.9	97.8	97.5		
C-2′	37.1	37.0	35.5		
C-3′	77.9	77.8	77.5		
C-4′	82.9	82.9	82.0		
C-5′	69.4	69.4	69.6		
C-6′	18.2	18.2	18.8		
OMe	59.0	58.9	57.4		
	D-dig	D-dig	L-dgn		
C-1″	100.4	100.4	101.0	99.2	101.8
C-2″	38.4	38.5	32.4	30.4	32.5
C-3″	68.9	68.8	73.8	75.9	79.0
C-4″	80.8	80.9	74.6	67.6	67.0
C-5″	67.7	67.6	67.7	66.8	71.5
C-6″	18.4	18.5	17.9	17.5	17.5
OMe			55.3	54.5	55.3
				55.0	55.9
	L-cym	L-cym	D-cym		
C-1‴	98.4	98.3	99.5		
C-2‴	32.3	31.7	35.3		
C-3‴	76.6	73.5	78.9		
C-4‴	72.7	77.8	74.2		
C-5‴	67.2	66.2	71.1		
C-6‴	18.5	18.9	18.3		
OMe	56.8	57.1	58.0		
		D-glu			
C-1⁗		102.4			
C-2⁗		75.3			
C-3⁗		78.1			
C-4⁗		71.7			
C-5⁗		78.4			
C-6⁗		62.9			

cym, cymarose; dig, digitoxose; dgn, diginose; glu, glucose. Measured in pyridine-d_5.

$C_{47}H_{72}O_{20}\cdot 3/2H_2O$: C, 57.37; H, 7.63. Found: C, 57.14; H, 7.60. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500—3300, 1710, 1640, 1440, 1360, 1300, 1150, 1120, 1080, 1040, 980. FD-MS *m/z*: 979 $(M+Na)^+$, 957 $(M+H)^+$, 776, 650, 520. ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 19-CH_3), 1.08 (1H, t, J=12 Hz, 1-CH), 1.24 (9H, br d, J=6.4 Hz, sugar C-6 $CH_3\times 3$), 1.53 (3H, s, 21-CH_3), 3.42, 3.46 (each 3H, s, 3′- and 3‴-OCH_3), 4.74, 4.85 (each 1H, dd, J=10, 2 Hz, 1′- and 1″-CH), 4.88 (1H, br d, J=3 Hz, 1‴-CH), 5.33 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.43 (1H, d, J=4.5 Hz, 6-CH), 6.27 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Tables I and II.

Enzymatic Hydrolysis of 3 with Snail Enzyme (β-Glucosidase)——A suspension of **3** (80 mg) in 40 ml of 0.3 M NaOAc buffer adjusted to pH 5.5 was treated with a suspension of powdered snail digestive glands (80 mg) in 4 ml of the same buffer, and the mixture was allowed to stand at 37 °C for 90 h. TLC analysis with MeOH–$CHCl_3$ (1:9) revealed the formation of **1** (*Rf* 0.78). The solution was concentrated and the residue was subjected to silica gel column chromatography with 30% acetone in hexane to give **14** (28 mg). Further elution with MeOH–$CHCl_3$ (1:1) gave a fraction in which glucose was detected by TLC with $CHCl_3$–MeOH–H_2O (4:3:1, lower phase *Rf* 0.45).

Compound 14——mp 131—135 °C, $[\alpha]_D$ −15.3° (c=0.50, $CHCl_3$), *Anal.* Calcd for $C_{41}H_{62}O_{15}$: C, 61.95; H, 7.86. Found: C, 61.67, H, 7.86. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550—3300, 1720, 1695, 1630, 650 (M^+-144), 520 (650−130), 376 (520−144). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 19-CH_3), 1.07 (1H, t, J=12 Hz, 1-CH_α), 1.22 (3H, d, J=5.4 Hz, one 6-CH_3 of deoxysugars), 1.26 (6H, d, J=5.9 Hz, two 6-CH_3 of deoxysugars), 1.53 (3H, s, 21-CH_3), 3.42 and 3.46 (each 3H, s, 3′ and 3″-OCH_3), 3.84 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.15 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.97, 4.85 (each 1H, dd, J=10, 2 Hz, 1′- and 1″-CH), 4.91 (1H, br d, J=4 Hz, 1‴-CH), 5.32 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.41 (1H, d, J=4.5 Hz, 6-CH), 6.27 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR δ: 18.2, 18.4, 18.5, 19.0, 23.9, 24.8, 28.5, 30.1, 32.3, 37.0,

37.6, 38.4, 39.5, 40.3, 44.8, 53.1, 56.2, 56.8, 59.0, 67.2, 67.7, 67.8, 67.9, 69.4, 70.0, 72.7, 75.6, 76.6, 77.9, 80.8, 82.9, 85.4, 97.9, 98.4, 100.4, 114.4, 118.5, 120.8, 139.8, 143.8, 175.3. The specific rotation, elemental analysis, IR, FD-MS, ^{1}H-NMR and ^{13}C-NMR data were identical with those of glaucoside-C.[4)]

Cynatratoside-F (4)——An amorphous powder, mp 106—113 °C, $[\alpha]_D$ −32.8° (c=1.00, $CHCl_3$), *Anal.* Calcd for $C_{42}H_{54}O_{15}$: C, 62.38; H, 7.92. Found: C, 62.20; H, 8.12. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500—3300, 1700, 1620, 1420, 1350, 1280, 1130, 1060, 1020, 980, 960, 940, 900, 880, 830. FD-MS m/z: 808 (M^+), 664 (M^+-144), 520 (664−144), 376 (520−144). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 19-CH_3), 1.01 (1H, t, J=12 Hz, 1-CH_α), 1.24, 1.27, 1.30 (each 3H, d, J=6.4 Hz, 6′-, 6″-, and 6‴-CH_3), 1.53 (3H, s, 21-CH_3), 3.41, 3.43, 3.44 (each 3H, s, 3′-, 3″-, and 3‴-OCH_3), 4.15 (1H, dd, J=9, 7 Hz, 15-CH_α), 4.69, 4.77 (each 1H, dd, J=10, 2 Hz, 1′- and 1″-CH), 4.99 (1H, d, J=3 Hz, 1‴-CH), 5.33 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.42 (1H, d, J=4.5 Hz, 6-CH), 6.27 (1H, d, J=2 Hz, 18-CH). ^{13}C-NMR: see Tables I and II.

Acidic Hydrolysis of 4——**4** (74 mg) was hydrolyzed in the same way as described for **2**. The aglycone (**15**) (17 mg) and cymarose (5.3 mg) were obtained. This cymarose showed $[\alpha]_D$ +52.4° (c=1.00, H_2O). Diginose was identified by TLC in comparison with an authentic sample.

Compound 15——Colorless needles, mp 225—230 °C, $[\alpha]_D$ +81.2° (c=1.00, MeOH), *Anal.* Calcd for $C_{21}H_{28}O_6$: C, 67.00; H, 7.50. Found: C, 67.29; H, 7.67. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600—3300, 1700, 1620, 1350, 1280, 1110. EI-MS m/z: 376 (M^+), 358, 312, 137 (base peak), 43. ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, s, 19-CH_3), 1.04 (1H, t, J=12 Hz, 1-CH), 1.54 (3H, s, 21-CH_3), 3.36 (1H, ddd, J=12, 9, 6 Hz, 3-CH), 3.46 (1H, dd, J=8, 2 Hz, 17-CH), 3.70 (1H, ddd, J=12, 9, 4 Hz, 2-CH), 3.86 (1H, dd, J=10, 9 Hz, 15-CH_β), 4.17 (1H, dd, J=9, 7 Hz, 15-CH_α), 5.34 (1H, ddd, J=10, 8, 7 Hz, 16-CH), 5.44 (1H, d, J=4.5 Hz, 6-CH), 6.27 (1H, d, J=2 Hz, 18-CH). The specific rotation, elemental analysis, IR, EI-MS, and ^{1}H-NMR data were identical with those of glaucogenin-A (**1**).

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