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STEROIDAL GLYCOSIDES FROM THE ROOT OF *CYNANCHUM VERSICOLOR*

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Key Word Index—*Cynanchum versicolor*; Asclepiadaceae; cynaversicoside C–E; glaucogenin D.

Abstract—Three new glycosides named cynaversicoside C, D and E with glaucogenin D as the aglycone were isolated from the Chinese crude drug 'Pai-Wei', *Cynanchum versicolor*. Their structures were determined on the bases of combined physio-chemical evidence as glaucogenin D:3-*O*-*β*-D-thevetopyranoside; glaucogenin D:3-*O*-*β*-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-*β*-D-thevetopyranoside; glaucogenin D:3-*O*-*β*-glucopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-*β*-D-thevetopyranoside.

INTRODUCTION

The Chinese drug 'Pai-Wei', which has been used as an antifebrile and diuretic, may be *Cynanchum versicolor* or *C. atratum* [1]. They are very similar in outward appearance, and the former has been studied by Zhang *et al.* [2, 3] who isolated some new glycosides. In this paper, we describe the isolation and identification of three new glycosides named cynaversicoside C, D and E from *C. versicolor*. These compounds contain an aglycone with 13,14; 14,15-diseco-pregnane-type skeleton which was found in 'Pai-Chien' (*Cynanchum glaucescens*) for the first time [4–6].

RESULT AND DISCUSSION

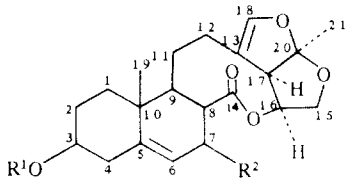
The new glycosides showed positive Liebermann–Burchard and Keller–Kiliani reactions suggesting a Δ^5-steroidal structure and a 2-deoxy sugar residue.

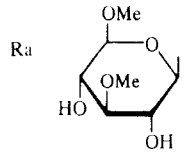
Cynaversicoside-C (**2**) had the molecular formula $C_{28}H_{40}O_{10}$ as determined by EIMS as well as by elemental analysis. It contained one more oxygen atom than glaucogenin C mono-D-thevetoside (**1**). ^{1}H NMR and ^{13}C NMR spectroscopy showed one anomeric proton signal at δ4.34 (*d*, $J=7.7$ Hz) and one anomeric carbon at 102.4, indicating that **2** is a mono-glycoside. The EI mass spectrum gave a molecular ion at *m/z* 536 and a fragment ion due to loss of the sugar moiety [M $-$sugar$-H_2O]^{+\cdot}$. Comparing the ^{1}H NMR spectrum of **2** with that of **1**, both were closely related except for the following: (i) the hydroxy methine proton appeared as a broad doublet at δ4.61 (*d*, $J=9.6$ Hz); (ii) the C-6 vinyl proton signal changed from a broad doublet ($J=6.0$ Hz) in the spectrum of **1** into a broad singlet in that of **2** at the same chemical shift (δ5.40). These facts suggested that there was a hydroxyl group attached at C-7, the small coupling constant between 7-H and 6-H (<1 Hz) and the coupling constant between 7-H and 8β-H(9.6 Hz) indicated that the hydroxyl group at C-7 was β-oriented and the aglycone was galucogenin D which was isolated from *C. paniculatum* by Sugama *et al.* [4]. The signals for the sugar moiety of **2** were identical with those of **1**, therefore, the sugar was assigned as D-thevetose. The ^{1}H NMR signals due to the anomeric proton at δ4.34 (1H, *d*, $J=7.7$ Hz) indicated the glycosidic linkage was β, thus the structure of **2** was deduced as glaucogenin D 3-*O*-*β*-D-thevetopyranoside.

Cynaversicoside-D (**3**) had the molecular formula $C_{42}H_{64}O_{16}$ as determined by EIMS and by elemental analysis. On mild acidic hydrolysis, **3** afforded **2**, diginose (**6**) and cymarose (**5**). The ^{1}H NMR signals due to anomeric protons at δ5.04 (1H, *d*, $J=9.2$ Hz), 5.12 (1H, *br d*, $J=9.6$ Hz) and 5.29(1H, *br s*) indicated the presence of one α and two β-glycosidic bonds. The terminal sugar was deduced to be D-cymarose and the middle one was L-diginose by comparing the ^{13}C NMR spectrum of **3** with that of methyl β-D-cymaropyranoside (**5b**) and methyl α-L-diginopyranoside (**6a**); the glycosidation chemical shift effects were observed at the C-4 carbons of D-thevetose and L-diginose. From these results, the structure of **3** was deduced as glaucogenin D 3-*O*-*β*-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-*β*-D-thevetopyranoside.

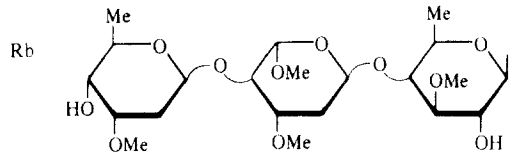
Cynaversicoside-E (**4**) had the molecular formula $C_{48}H_{74}O_{21}$ on the bases of EIMS and elemental analysis. The ^{1}H NMR spectrum of **4** showed four anomeric proton signals at δ4.91(1H, *d*, $J=7.6$ Hz), 5.04(1H, *d*, $J=9.2$ Hz), 5.14 (1H, *br d*, $J=9.6$ Hz), 5.26 (1H, *br s*) and four anomeric carbon signals at δ98.0, 99.3, 102.4 and 106.3. This indicated that there were four sugars in the sugar chain of **4** and one was α-linked, the others were β-linked. On mild acidic hydrolysis, **4** gave **2**, strophanthobiose (**7**) and diginose which were identified by TLC comparison with the authentic sample.

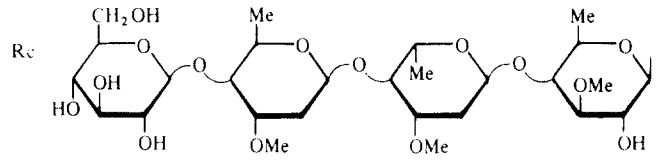
Enzymatic hydrolysis of **4** gave a deglaucosyl derivative which was identical to **3** by $[\alpha]_D$, IR and ^{1}H NMR spectral comparison. Glucose in the water layer was identified by TLC comparison with an authentic sample. Thus, the structure of **4** was established as glaucogenin D 3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-α-L-diginopyranosyl-(1→4)-*β*-D-thevetopyranoside.

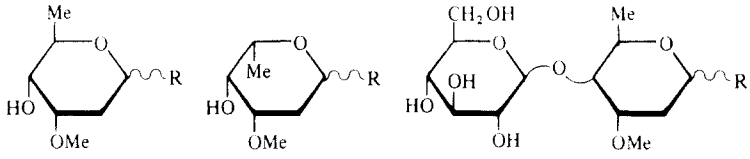




	R^1	R^2
1	R_a	H
2	R_a	OH
3	R_b	OH
4	R_c	OH







5 R = OH **6** R = OH **7** R = OH
5a R = α-OMe **6a** R = α-OMe
5b R = β-OMe **6b** R = β-OMe

EXPERIMENTAL

Mps: uncorr. ^{13}C NMR, ^{1}H NMR were run in $CDCl_3$ or C_5D_5N soln with TMS as int. standard. TLC was performed on Merck precoated kieselgel 60 F_{254} and RP-8 F254, RP-18 F_{254} CC was carried out on silica gel (200–300 mesh) and RP-8, RP-182 (Merck).

Plant material ('Pai-Wei') was obtained from Beijing market and was identified as the root of *Cynanchum versicolor* Bunge by Shie Tsung-Wan (Institute of Chinese Materia Medica, Academia of Chinese medicine, Beijing).

Cynaversicoside C (**2**). An amorphous powder, mp 124–128°; $[\alpha]_D$ +49.8(MeOH; *c* 0.562). Anal. calcd. for $C_{28}H_{40}O_{14}\cdot 3/2\ H_2O$: C, 59.08; H, 7.64. Found: C, 59.21; H, 7.61. IR ν_{max} cm^{-1}: 3450, 2950, 1730, 1650, 1450, 1380, 1300, 1170, 1080, 1040, 870. EIMS (*m/z*): 536 $[M]^+$, 358 $[M-\text{sugar chain}-H_2O]^+$. ^{1}H NMR ($CDCl_3$): δ0.98 (3H, *s*, 19-Me), 1.36 (3H, *d*, *J* = 6.4 Hz, 6′-Me), 1.54 (3H, *s*, 21-Me), 3.66 (3H, *s*, 3′-OMe), 3.84 (1H, *dd*, *J* = 9.3, 8.5 Hz, 15-CHβ), 4.18 (1H, *dd*, *J* = 9.3, 7.6 Hz, 15-CHα), 4.34 (1H, *d*, *J* = 8.0 Hz, 1′-CH), 4.61 (1H, *br d*, *J* = 9.3 Hz, 7-CH), 5.36 (1H, *dd*, *J* = 8.5, 7.6 Hz, 16-CH), 5.40 (1H, *br s*, 6-CH), 6.26 (1H, *br s*, 18-CH). ^{1}H NMR (C_5D_5N): δ0.82 (3H, *s*, 19-Me), 1.54 (3H, *s*, 21-Me), 1.60 (3H, *d*, *J* = 6.0 Hz, 6′-Me), 3.92 (3H, *s*, 3′-OMe), 4.14 (1H, *dd*, *J* = 10.0, 8.0 Hz, 15-CHβ), 4.44 (1H, *dd*, *J* = 10.0, 7.0 Hz, 15-CHα), 4.80 (1H, *d*, *J* = 7.6 Hz, 7-CH), 5.45 (1H, *dd*, *J* = 8.0, 7.0 Hz, 16-CH), 5.70 (1H, *br s*, 6-CH), 6.51 (1H, *br s*, 18-CH) ^{13}C NMR see Tables 1 and 2.

Table 1. ^{13}C NMR chemical shifts of aglycone moieties (ppm in C_5D_5N from int. TMS)

C	1	2	3	4
1	36.6	36.1	36.2	36.2
2	30.6	30.1	30.1	30.1
3	78.1	78.1	78.1	78.1
4	39.0	38.8	38.7	38.7
5	140.7	141.4	141.3	141.3
6	120.4	127.2	127.2	127.2
7	30.0	67.9	67.8	67.8
8	53.3	51.3	51.2	51.2
9	40.7	50.6	50.6	50.6
10	38.7	38.7	38.7	38.7
11	23.9	23.7	23.7	23.7
12	28.4	30.1	30.1	30.1
13	118.4	118.6	118.6	118.6
14	175.4	174.8	174.8	174.8
15	67.7	67.8	67.8	67.8
16	75.5	75.8	75.8	75.8
17	56.2	56.4	56.3	56.3
18	143.8	144.0	144.0	144.0
19	18.6	18.6	18.7	18.7
20	114.3	114.4	114.4	114.4
21	24.8	24.8	24.8	24.8

Table 2. ^{13}C NMR chemical shifts of sugar moieties (ppm from int. TMS in C_5D_5N)

C	1	2	3	4		
	D-the	D-the	D-the	D-the		
1′	102.4	102.5	102.4	102.4		
2′	75.0	75.0	74.8	74.8		
3′	88.0	88.0	85.5	85.5		
4′	75.9	75.9	80.1	80.1		
5′	72.6	72.7	72.0	72.0		
6′	17.9	17.7	16.7	17.6		
–OMe	60.8	60.8	60.2	60.2		
			L-dgn	L-dgn	**5a**	**5b**
1″			98.8	98.8	97.8	99.4
2″			32.2	32.2	31.9	35.1
3″			73.9	73.9	76.6	78.5
4″			74.8	74.8	73.3	74.0
5″			67.3	67.3	65.3	71.0
6″			17.6	17.6	18.5	18.9
–OMe			55.4	55.4	54.8	56.0
			D-cym	D-cym	56.7	57.8
1‴			99.3	99.3		
2‴			35.3	36.1		
3‴			79.0	78.1		
4‴			74.1	83.0		
5‴			71.1	69.5		
6‴			18.0	18.5		
–OMe			58.0	58.5		
				D-glc.	**6a**	**6b**
1⁗				106.4	99.2	101.8
2⁗				75.3	30.4	32.5
3⁗				78.3	75.9	79.0
4⁗				71.7	67.6	67.0
5⁗				78.3	66.8	71.5
6⁗				62.9	17.5	17.5
					54.5	55.3
					55.0	55.9

the = Thevetose; cym = cymarose; dgn = diginose; glc = glucose.

Cynaversicoside-D (**3**). An amorphous, mp 150–152°, $[\alpha]_D$ −8.7 (MeOH; *c* 0.517). Anal. Calcd. for $C_{42}H_{64}O_{16}\cdot 2H_2O$: C, 58.60; H, 7.90. Found: C, 58.43; H, 7.89. IR ν_{max} cm^{-1}: 3480, 2940, 1735, 1650, 1450, 1380, 1300, 1190, 1170, 1080, 1020, 810. EIMS (*m/z*): 536 [M − cym − dgn + H]$^+$, 358 [M − sugar chain − H_2O]$^+$. ^{1}H NMR (C_5D_5N): δ0.82 (3H, *s*, 19-Me), 1.38, 1.45 (each 3H, *d*, *J* = 6.0 Hz, 6″, 6‴-Me), 1.54 (3H, *s*, 21-Me), 1.60 (3H, *d*, *J* = 6.0 Hz, 6′-Me), 3.48, 3.55, 3.92 (each 3H, *s*, 3′, 3″, 3‴-OMe), 4.14 (1H, *dd*, *J* = 10.0, 8.0 Hz, 15-CHβ), 4.44 (1H, *dd*, *J* = 10.0, 7.0 Hz, 15-CHα), 4.80 (1H, *br d*, *J* = 7.6 Hz, 7-CH), 5.04 (1H, *d*, *J* = 9.2 Hz, 1′-CH), 5.12 (1H, *br d*, *J* = 9.6 Hz, 1‴-CH), 5.29 (1H, *br s*, 1″-CH), 5.45 (1H, *dd*, *J* = 8.0, 7.0 Hz, 16-CH), 5.70 (1H, *br*, *s*, 6-CH), 6.50 (1H, *br s*, 18-CH). ^{13}C NMR see Tables 1 and 2.

Acidic hydrolysis of **3**. To a soln of **3** (80 mg in 15 ml MeOH) was added 10 ml 0.05 M H_2SO_4 and the mixt. kept at 50° for 1 hr. The soln was diluted with 15 ml H_2O and concd to 25 ml. It was kept at 70° for 30 min, neutralized with aq. satd Ba $(OH)_2$ soln, and the ppt. filtered, the filtrate was concd to dryness under red. pres. and chromatographed on a column of silica gel (20 g) eluted with 2% MeOH–$CHCl_3$ to give 4 mg D-cymarose: $[\alpha]_D$ +50.3° (H_2O; *c* 0.53), 3 mg of L-diginose: $[\alpha]_D$ −60.5 (H_2O; *c* 0.54) and 15 mg of **2** which was identified with the authentic sample by TLC and ^{1}H NMR comparison. The R_f values of the sugars were: cymarose, 0.43, diginose, 0.46 and **2**, 0.32, respectively with the solvent system $CHCl_3$–MeOH (9:1); 0.37, 0.25 and 0.20, respectively, with solvent system Me_2CO–petrol (2:3).

Cynaversicoside E(**4**). An amorphous powder, mp 192–200°, $[\alpha]_D$ −16.7 (MeOH; *c* 0.57). Anal. calcd. for $C_{48}H_{74}O_{21}\cdot 3/2\ H_2O$: 56.86; H, 7.60. Found: C, 56.76; H, 7.58. IR ν_{max} cm^{-1}: 3450, 2940, 1730, 1640, 1450, 1380, 1300, 1190, 1160, 1080, 1020, 990, 920. EIMS (*m/z*): 536 [M − glc − dgn − cym + H]$^+$, 358 [536 − the − H_2O]$^+$. ^{1}H NMR (C_5D_5N): δ0.81 (3H, *s*, 19-Me), 1.38, 1.44, 1.64 (each 3H, *d*, *J* = 6.0 Hz, 6′, 6″, 6‴-Me), 1.54 (3H, *s*, 21-Me), 3.49, 3.52, 3.71 (each 3H, *s*, 3′, 3″, 3‴-OMe), 4.14 (1H, *dd*, *J* = 9.0, 8.0 Hz, 15-CHβ), 4.44 (1H, *dd*, *J* = 9.0, 7.0 Hz, 15-CHα), 4.80 (1H, *d*, *J* = 7.6 Hz, 7-CH), 4.91 (1H, *d*, *J* = 7.6 Hz, 1⁗-CH), 5.04 (1H, *d*, *J* = 9.2 Hz, 1′-CH), 5.14 (1H, *br d*, *J* = 9.6 Hz, 1‴-CH), 5.26 (1H, *br s*, 1″-CH), 5.45 (1H, *dd*, *J* = 8.0, 7.0 Hz, 16-CH), 5.70 (1H, *br s*, 6-CH), 6.50 (1H, *br s*, 18-CH). ^{13}C NMR see Tables 1 and 2.

Acidic hydrolysis of **4**. Compound **4** was treated with the same process as **3** and afforded **2**, diginose and strophanthobiose (**7**) which were identified by TLC comparison with the authentic sample, the R_f value of **7** was 0.35 with the solvent system $CHCl_3$–MeOH (4:1).

Enzymatic hydrolysis of **4**. A suspension of **4** (80 mg) and powdered snail digestive juice (80 mg) in 10 ml 0.3 M NaOAc buffer adjusted to pH 5.5 was allowed to stand at 37° for 7 days.

The TLC analysis with MeOH–$CHCl_3$ (1:9) system revealed the formation of **3**, the soln was concd and the residue subjected to silica gel CC eluted with 4% MeOH–$CHCl_3$ system to give **3** (25 mg) which was identified with authentic sample by TLC, [α], IR and ^1H NMR comparison.

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