

## TWO NEW ANTIEPILEPSY COMPOUNDS ——OTOPHYLLOSIDES A AND B

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### ABSTRACT

Two new  $C_{21}$  steroidal constituents, named otophyllside A(IV) and otophyllside B(V), have been isolated from the roots of *Cynanchum otophyllum* Schneid, whose structures have been analysed in this paper by using X-ray crystallography and spectrometric data (UV, IR,  $^1H$ - and  $^{13}C$ -NMR, MS) and chemical reaction. They were found to be active against epilepsy, IV and V can protect rats from audiogenic seizures(AS) and its  $ED_{50}=10.20$  mg/kg.

We have been studying the chemical constituents, pharmacological action, clinical curative effect on epilepsy and domestication of the wild plant, *Cynanchum otophyllum* Schneid. Now, the experiment of cultivation of *Cynanchum otophyllum* Schneid has already begun and has come off all right in Yunnan Province. The result of pharmacological study shows that the crude glycoside has anticonvulsant action and therapeutic action on chronic epilepsy. Four experimental models of epilepsy, i.e. Kinging effect<sup>[1]</sup>, audiogenic seizures produced, seizures produced by electroshock and seizure produced by intracerebral injection of ferrous sulfate<sup>[2]</sup>, were examined. Furthermore, the clinical tests of the drug on 360 epilepsy cases were completed, and the effect ratio was 80%<sup>[3]</sup>.

It was reported in the preceding paper that there are three  $C_{21}$ -steroidal aglycones, caudatin (I), qingyangshengenin (II) and rostratamine (III) in this plant<sup>[4-6]</sup>. This paper describes the isolation of otophyllside A (IV) and otophyllside B(V) from this plant and their structures. These two compounds have an anticonvulsant action, they can protect rats from audiogenic seizures,  $ED_{50}=10.20$  mg/kg. So they are the active principles of this plant. The two new oligoglycosides were isolated by the procedure shown in Fig. 1.

The mass spectrum of IV indicated that molecular formula was  $C_{49}H_{72}O_{17}$  ( $M^+$  at  $m/e$  932), The ultraviolet spectrum of IV was the same with that of II. Its infrared spectrum showed the presence of methoxy group ( $2925, 1440\text{ cm}^{-1}$ ) and  $-C-O-C-O-$  ( $1195, 1160, 1080, 1050\text{ cm}^{-1}$ ). The nuclear magnetic resonance spectrum of IV indicated the presence of three methoxy groups at  $\delta$  3.39 (3H, s,  $OCH_3$ ), 3.45 (6H, s,  $2\times OCH_3$ ) and three methyl groups at  $\delta$  1.25 (6H, d,  $J=6\text{Hz}$ ,  $2CH_3$ ), 1.33 (3H, d,  $J=6\text{Hz}$ ,  $CH_3$ ), of which a methyl group and a methoxyl group could be assigned to  $C_3$  and  $C_6$  of a molecular desoxy sugar respectively. The fragment peaks  $m/e$  145( $C_7H_{13}O_3$ ), 144( $C_7H_{12}O_3$ ), 449( $C_7H_{13}O_3-C_7H_{12}O_3-C_7H_{12}O_4$ ) suggest that there are three desoxy sugar molecules. The mass spectrum cleavage of IV is shown in Fig. 2. The presence of three desoxy sugars is further proved by its CMR data, the sugar moiety of IV is almost



Table 1

<sup>13</sup>C-NMR Chemical Shifts of IV, V, II, I and Cynanchoside C<sub>2</sub>

	Aglycone Moiety					Sugar Moiety		
	IV	V	II <sub>a</sub>	I		IV	V	C <sub>2</sub>
C-1	38.99	38.94	38.54	38.98		Cymarose		
2	27.73	29.89	26.97	30.95	C-1	96.45	96.34	96.3
3	77.75	79.70	73.47	70.80	2	37.41	37.21	37.2
4	38.99	39.25	37.95	42.09	3	77.75	77.78	77.7
5	139.37	139.38	139.25	140.71	4	83.14	83.11	83.3
6	119.08	119.11	118.85	118.02	5	69.01	68.97	68.9
7	34.83	34.73	34.30	34.46	6	18.59	18.55	18.5
8	74.35	74.25	74.23	74.51	C-3-OMe	58.87	58.84	58.8
9	44.44	44.58	43.62	43.96		Cymarose		
10	37.40	37.21	37.00	37.17	C-1	100.44	100.45	100.3
11	24.64	24.98	24.22	24.61	2	37.40	37.38	37.2
12	73.35	72.55	73.16	71.93	3	77.75	77.78	77.6
13	58.86	58.84	58.23	58.03	4	83.14	83.39	83.1
14	89.53	89.39	88.12	88.70	5	69.01	68.97	68.9
15	33.83	33.79	33.42	33.35	6	18.59	18.55	18.5
16	32.01	32.86	31.96	32.36	C-3-OMe	58.87	58.84	58.8
17	92.46	92.36	91.53	91.98		Oleandrose		
18	10.78	10.62	9.61	10.07	C-1	102.14	102.10	102.0
19	18.41	18.55	18.41	18.77	2	37.23	37.21	37.0
20	208.50	209.27	209.55	209.66	3	81.38	81.35	81.3
21	29.79	27.47	27.50	27.49	4	76.22	76.18	76.1
C-1'	163.53	165.90	164.58	166.87	5	72.94	72.88	72.9
2'	127.50	114.19	127.53	113.27	6	18.18	18.16	18.6
3'	131.81	165.29	131.10	166.23	C-3-OMe	57.05	57.02	57.0
4'	121.74	38.46	121.71	38.39				
5'	151.07	20.86	154.56	21.10				
6'	122.49	20.86	121.71	21.10				
7'	131.81	16.45	131.10	16.87				

When IV was hydrolyzed with dilute sulfuric acid, cymarose, oleandrose and qingyangshengenin (II) were obtained. The latter which was acetylated with acetic anhydride-pyridine gave a diacetate (II<sub>a</sub>), its stereospecific structure was determined by X-ray diffraction and the bond length and bond angle corresponding to them are listed in Tables 2 and 3 respectively.

The mild acid hydrolysis of acetylate of IV with dilute sulfuric acid yields 4-O-acetyl-oleandrose, cymarose and monoacetate of II, being identical with authentic samples as determined by TLC and Gas-Liquid Chromatography (GLC), it shows that oleandrose is the terminal sugar.

Comparison of CMR spectra of IV with II shows that the chemical shift of C<sub>3</sub> of IV at  $\delta$  77.70 ppm moves 6.90 ppm downfield more than that of C<sub>3</sub> of II at  $\delta$  70.80 ppm, revealing that C<sub>2</sub>-OH is bonded with sugar moieties.

**Table 2**  
The Bond Length ( $\text{\AA}$ ) of II<sub>a</sub>

C1-C2	1.550(18)	C22-C23	1.471(17)
C1-C10	1.584(18)	C23-C24	1.391(21)
C2-C3	1.539(18)	C23-C28	1.396(20)
C3-C4	1.566(19)	C24-C25	1.396(19)
C4-C5	1.535(18)	C25-C26	1.335(21)
C5-C6	1.331(18)	C26-C27	1.412(21)
C6-C7	1.551(18)	C27-C28	1.418(20)
C7-C8	1.575(17)	C29-C30	1.477(19)
C8-C9	1.549(16)	C31-C32	1.494(24)
C9-C10	1.551(16)	O1-C31	1.818(21)
C9-C11	1.568(15)	O2-C3	1.456(15)
C10-C19	1.537(19)	O2-C31	1.325(20)
C11-C12	1.549(16)	O3-C8	1.414(15)
C12-C13	1.553(17)	O4-C14	1.449(15)
C13-C14	1.558(17)	O5-C17	1.456(16)
C13-C17	1.554(18)	O6-C20	1.207(14)
C13-C18	1.553(18)	O7-C12	1.461(13)
C14-C15	1.547(18)	O7-C22	1.325(16)
C15-C16	1.533(18)	O8-C22	1.229(16)
C16-C17	1.553(19)	O9-C26	1.386(16)
C17-C20	1.508(18)	O9-C19	1.380(18)
C20-C21	1.541(21)	O10-C29	1.215(19)

On the basis of the above results, we concluded that the structure of otophylloside A is kingyangshengenin 3-O- $\beta$ -D-oleandropyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranoside (IV). The comparison of the CMR spectrum of V with those of cynanchoside C<sub>2</sub><sup>[6]</sup> and I shows that the sugar moiety and the aglycone moiety of V and cynanchoside C<sub>2</sub> and I have almost the same chemical shifts (see Table 1). Its structure deduced from its UV, IR, PMR and Ms spectra is caudatin 3-O- $\beta$ -D-oleandropyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-cymaropyranoside B(V).

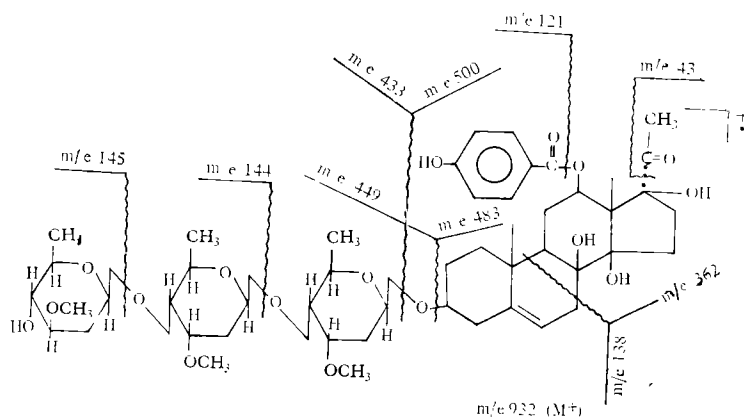


Fig. 3

Table 3

The Bond Angle of II<sub>a</sub> (Degree)

C1-C2-C3	109.5(1.0)	C13-C12-O7	105.6(0.8)
C1-C10-C5	109.9(1.0)	C13-C14-O4	109.8(0.9)
C1-C10-C9	106.0(1.0)	C13-C17-C16	104.1(1.0)
C1-C10-C19	109.1(1.0)	C13-C14-C15	103.7(1.0)
C2-C1-C10	112.5(1.0)	C13-C17-O5	108.3(1.0)
C2-C3-O2	108.9(1.0)	C13-C17-C20	115.3(1.1)
C2-C3-C4	107.0(1.0)	C14-C13-C17	102.0(0.9)
C3-C4-C5	108.4(1.0)	C14-C13-C18	113.6(1.0)
C3-O2-C31	117.6(1.1)	C14-C15-C16	106.8(1.0)
C4-C3-O2	106.3(1.0)	C15-C14-O4	115.6(1.0)
C4-C5-C6	120.2(1.2)	C15-C16-C17	105.6(1.1)
C4-C5-C10	116.3(1.1)	C16-C17-O5	103.6(1.0)
C5-C6-C7	126.9(1.2)	C16-C17-C20	118.6(1.1)
C5-C10-C9	109.3(0.9)	C17-C13-C18	112.0(1.0)
C5-C10-C19	108.2(1.0)	C17-C20-O6	121.1(1.4)
C3-C5-C10	123.4(1.1)	C17-C20-C21	118.0(1.2)
C6-C7-C8	110.5(1.0)	C20-C17-O5	105.9(1.0)
C7-C8-O3	110.6(1.0)	C21-C20-O6	120.5(1.3)
C7-C8-C9	109.6(0.9)	C22-C23-C24	118.1(1.2)
C7-C8-C14	108.0(0.9)	C22-C23-C28	120.8(1.3)
C7-C22-O8	122.1(1.1)	C23-C24-C25	118.5(1.3)
C8-C9-C10	114.9(0.9)	C23-C22-O7	114.6(1.1)
C8-C9-C11	109.1(0.9)	C23-C22-O8	123.2(1.2)
C8-C14-O4	102.2(0.9)	C23-C28-C27	120.5(1.3)
C8-C14-C13	116.2(1.0)	C24-C23-C28	120.6(1.2)
C8-C14-C15	115.6(1.0)	C24-C25-C26	120.9(1.4)
C9-C8-C7	108.7(0.9)	C25-C26-O9	122.4(1.3)
C9-C8-C14	110.9(0.9)	C25-C26-C27	112.9(1.2)
C9-C10-C19	114.4(1.0)	C26-O9-C29	117.6(1.1)
C9-C11-C12	108.2(0.9)	C27-C26-O9	114.4(1.2)
C10-C9-C11	112.4(0.9)	C28-C27-C26	116.2(1.2)
C11-C12-O7	107.6(0.9)	O1-C31-O2	121.2(1.5)
C11-C12-C13	111.0(1.6)	O9-C29-O10	119.6(1.3)
C12-C13-C14	106.2(0.9)	C30-C29-O9	114.1(1.2)
C12-C13-C17	110.5(1.0)	C30-C29-O10	126.3(1.4)
C12-C13-C18	112.0(1.0)	C32-C31-O1	123.8(1.6)
C12-O7-C22	117.7(0.9)	C32-C31-O2	114.7(1.4)

## EXPERIMENTAL

1. *Melting points.* Melting points were taken on a Kofler and were uncorrected. UV spectra were measured with a UV-210 type instrument. IR spectra were measured with an IR-450 infrared spectrophotometer. Mass spectra were measured with an MS/GC 4510-type machine. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were measured with WH-90 type spectrometer and tetramethylsilane (TMS) was used as an internal standard for spectra run in CDCl<sub>3</sub> and gas-liquid chromatographic analysis was carried out with a Shimadzu GC-1B. All samples were injected to produce their trimethylsilyl ether (TMS) deriva-

tives. L-Rhamnose TMS derivative was used as an internal standard. When a sugar showed more than one peak, the highest peak was compared with that of L-rhamnose. The analytical conditions are as follows: column 3% SE-30 (2 mm×1.75 m); column temperature 160°C; N<sub>2</sub> flow 20 ml/min. Reversed-phase chromatography was carried out on MCI gel (CHP 20 p) (Mitsubishi) column chromatography and silica gel column chromatography was performed on silica gel. TLC was carried out on HPTLC-Fertigplattchen (RP-18 F<sub>254s</sub>) (Merck) using the following solvent systems: (A) MeOH-H<sub>2</sub>O (70:30 v/v); (B) MeOH-H<sub>2</sub>O (80:20 v/v); (C) CHCl<sub>3</sub>-MeOH (95:5 v/v); (D) CHCl<sub>3</sub>-MeOH-25% NH<sub>4</sub>OH (95:4.5:0.5 v/v).

2. *Isolation of otophyllsides A and B.* The crude glycoside (100 g) was extracted with CCl<sub>4</sub>-AcOt (95:5; 85:15 v/v) in their order and the extract (14 g) of the former and the extract (28 g) of the latter were chromatographed on silica gel respectively, and they were eluted with CHCl<sub>3</sub>-MeOH respectively, the MeOH content of which was increased gradually from 1% to 10% (v/v). The CHCl<sub>3</sub>-MeOH (99:1) portion containing IV followed by CHP 20 p column chromatography with solvents (A) and (B) gave 500 mg glycoside A (IV) and 550 mg glycoside B (V). These two compounds gave positive Keller-Kiliani reactions, indicating that they contain 2-deoxy sugar.

3. *Identification of otophyllsides A.* Otophyllsides A (IV) is a colorless powder, m.p. 165–168°C,  $[\alpha]_D^{20} + 39.5$  ( $c = 2$ , MeOH), Anal. calcd. for C<sub>49</sub>H<sub>72</sub>O<sub>17</sub> · H<sub>2</sub>O (%): C, 61.89; H, 7.80. Found: C, 61.64; H, 8.44. UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 203(4.55), 260.5(4.49), 300(3.46). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3450 (OH), 1710 (C=O), 1606, 1510 (Aromatic ring). <sup>1</sup>H-NMR (in CDCl<sub>3</sub>)  $\delta$ : 1.12(3H, s, C<sub>19</sub>-CH<sub>3</sub>), 1.25(6H, d,  $J = 6$  Hz, 2 × sugar C<sub>6</sub>-CH<sub>3</sub>), 1.32(3H, d,  $J = 6$  Hz, sugar C<sub>6</sub>-CH<sub>3</sub>), 1.53 (3H, s, C<sub>18</sub>-CH<sub>3</sub>), 2.10(3H, s, C<sub>21</sub>-CH<sub>3</sub>), 3.39(3H, s, sugar C<sub>3</sub>-OCH<sub>3</sub>), 3.45(6H, s, 2 × sugar C<sub>3</sub>-OCH<sub>3</sub>), 4.51(1H, m, C<sub>3</sub>- $\alpha$ H), 4.84(1H, dd,  $J = 8$  and 4 Hz, C<sub>12</sub>- $\alpha$ H), 5.36 (1H, br, C<sub>6</sub>-H), 7.81 and 6.83(4H, dd,  $J = 9$  Hz, 1,4-disubstituted benzene).

4. *Mild acid hydrolysis of otophyllsides A (IV).* IV (5.4 mg) was hydrolyzed with 0.05 N HCl in 70% MeOH (15 ml) by reflux for 1 h. After cooling, the mixture was passed through Ionenaustauscher II (5g, Merck) column and eluted with methanol, the mixture was moved from the hydrolyzed product. Qingyangshengenin (II) ( $R_f$  0.19), cymarose ( $R_f$  0.45) and oleandrose ( $R_f$  0.27) were identified by TIC comparison with authentic samples. Moreover, cymarose and oleandrose were detected by GLC TMS-sugar relative retention time (min): 0.45 (cymarose), 0.27 (oleandrose). II acetylation with Ac<sub>2</sub>O-pyridine afforded a diacetate (II<sub>a</sub>). It is a colorless rectangular crystal, m.p. 240°C,  $[\alpha]_D^{20} + 21.4$  ( $c = 4$ , CHCl<sub>3</sub>), MS  $m/e$ : 541(M<sup>+</sup>-CH<sub>3</sub>CO), 542(M<sup>+</sup>-HAc), 463(M<sup>+</sup>-HO-C<sub>6</sub>H<sub>4</sub>CO), 446(M<sup>+</sup>-HO-C<sub>6</sub>H<sub>4</sub>COOH), 386(M<sup>+</sup>-HO-C<sub>6</sub>H<sub>4</sub>COOH-HAc), 121(HO-C<sub>6</sub>H<sub>4</sub>-CO), 43(CH<sub>3</sub>CO), Anal. Calc. for C<sub>32</sub>H<sub>40</sub>O<sub>10</sub> (%): C, 65.75; H, 6.85. Found: C, 66.09; H, 6.96. Its stereospecific structure was determined by X-ray diffraction and the molecular structure of II<sub>a</sub> is shown in Fig. 3.

5. *Acid hydrolysis of the acetate of otophyllsides A (IV).* Ac<sub>2</sub>O (2 ml) was added to a solution of IV (6.1 mg) in pyridine (2 ml), and the mixture was allowed to stand overnight at room temperature and warmed at 60°C for 1 h. The product was hydrolyzed under the above condition. The reaction mixture was also worked out as described above. The absence of oleandrose was confirmed by TLC comparison with authentic oleandrose.

drose. After dissolving in the mixture of Et<sub>2</sub>N-H<sub>2</sub>O-MeOH (2:5:10 v/v) and put for 24 h at room temperature, the reaction mixture was identified to be the same as the hydrolyzed product of otophyllside (IV) by TLC comparison with authentic samples.

6. *Identification of otophyllside B.* Otophyllside B (V) is a colorless powder, m.p. 143–146°C,  $[\alpha]_D^{20} + 12^\circ$  ( $c = 2$ , MeOH). Anal. calcd. for C<sub>49</sub>H<sub>79</sub>O<sub>16</sub> (%): C, 63.77; H, 8.69. Found C, 64.01; H, 8.69. UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 205(3.97), 221(4.13). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>-1</sup>: 3450(OH), 1710, 1640(C=CH-C=O). <sup>1</sup>H-NMR (in CDCl<sub>3</sub>)  $\delta$ : 1.06(6H, d,  $J = 6$  Hz, C'<sub>5</sub> and C'<sub>6</sub>-CH<sub>3</sub>), 1.12(3H, s, C<sub>19</sub>-CH<sub>3</sub>), 1.22(6H, d,  $J = 6$  Hz, 2  $\times$  sugar C<sub>6</sub>-CH<sub>3</sub>), 1.32(3H, d,  $J = 6$  Hz, sugar C<sub>6</sub>-CH<sub>3</sub>), 1.41(3H, s, C<sub>18</sub>-CH<sub>3</sub>), 2.12(3H, s, C<sub>7</sub>-CH<sub>3</sub>), 2.17(3H, s, C<sub>21</sub>-CH<sub>3</sub>), 3.45(6H, 2  $\times$  sugar C<sub>3</sub>-OCH<sub>3</sub>), 3.38(3H, s, sugar C<sub>3</sub>-OCH<sub>3</sub>), 4.54(1H, m, C<sub>3</sub>-2H), 4.80(1H, dd,  $J = 8$  and 4 Hz, C<sub>12</sub>-2H), 5.36(1H, br, C'<sub>6</sub>-H), 5.52(1H, s, C'<sub>2</sub>-H).

The action of (V) with Ac<sub>2</sub>O-pyridine by usual way gave monoacetate, the molecular formula of C<sub>51</sub>H<sub>83</sub>H<sub>17</sub>(M<sup>+</sup> at m/e 964) was given for monoacetate of (V). Anal. calcd for C<sub>51</sub>H<sub>83</sub>O<sub>17</sub> (%): C, 63.48; H, 8.29. Found: C, 63.32; H, 8.08. The mass spectrum suggests the presence of an ikemaoyl (3, 4-dimethyl-2-pentenoyl), and three desoxy sugars (m/e): 853(M<sup>+</sup>-C<sub>7</sub>H<sub>11</sub>O (ikemaoyl)), 473(M<sup>+</sup>-CH<sub>3</sub>CO-C<sub>7</sub>-H<sub>12</sub>O<sub>3</sub>-C<sub>7</sub>H<sub>12</sub>O<sub>3</sub>-C<sub>7</sub>H<sub>12</sub>O<sub>4</sub>), 362(473-C<sub>7</sub>H<sub>11</sub>O), 343(473-C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>), 344(362-H<sub>2</sub>O), 319(362-CH<sub>3</sub>CO), 301(319-H<sub>2</sub>O), 283(301-H<sub>2</sub>O), 265(283-H<sub>2</sub>O), 187(CH<sub>3</sub>CO-C<sub>7</sub>H<sub>13</sub>O<sub>3</sub>), 145(187-CH<sub>3</sub>CO), 111( $\begin{matrix} \text{CH}_3 \\ \diagup \\ \text{CH}-\text{C}=\text{CH}-\text{CO}^+ \\ \diagdown \\ \text{CH}_3 \end{matrix}$   $\begin{matrix} | \\ \text{CH}_3 \end{matrix}$ ), 113(C<sub>7</sub>H<sub>9</sub>O<sub>2</sub>), 95(113-H<sub>2</sub>O).

Glycoside B and its acetate were hydrolyzed and identified in the same way as otophyllside A (IV) and its acetate: caudatin (I), *Rf* 0.63; cymarose, *Rf* 0.45 and oleandrose, *Rf* 0.27.

The above experimental evidence leads us to assign the structure of qingyangshen-side B as (V).

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