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青阳参的一个新 C_{21} 甾体苷

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摘要: 从萝藦科药用植物青阳参 (*Cynanchum otophyllum* Schneid) 的乙酸乙酯提取物的酸水解液中, 分离得到一个新的 C_{21} 甾体苷类化合物, 通过现代波谱技术, 确定其结构为去乙酰萝藦苷元 3-*O*-β-D-夹竹桃吡喃糖基-(1→4)-α-D-夹竹桃吡喃糖苷。

关键词: 青阳参; 萝藦科; C_{21} 甾体苷
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A New C_{21} Steroidal Glycoside from *Cynanchum otophyllum*

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Abstract: A new C_{21} steroidal glycoside was isolated from the acidic hydrolysis part of the ethyl acetate extract of *Cynanchum otophyllum* Schneid (Asclepiadaceae). Its structure was determined as deacetylmetaplexigenin 3-*O*-β-D-oleandropyranosyl-(1→4)-α-D-oleandropyranoside by spectral methods.

Key words: *Cynanchum otophyllum*; Asclepiadaceae; C_{21} steroidal glycoside

Cynanchum otophyllum Schneid, Qingyangshen, is a species of the genus *Cynanchum* L. (Asclepiadaceae), and a traditional Chinese medicine distributed extensively over southwest China. Pharmacodynamic and clinical experiments have established that the chloroform extract and the ethyl acetate extract of the rhizome are particularly effective against epilepsy and chronic hepatitis (Pei *et al*, 1981). Since 1984, Qingyangshen Tablets (the total glycosides of *C. otophyllum*) have been manufactured by Lijiang Pharmaceutical Co., Yunnan Baiyao Group, Lijiang, Yunnan, China. From the rhizome of *C. otophyllum*, Mu *et al* (1986) isolated nine constituents including two C_{21} steroidal glycosides. Consequently, Mu and co-workers developed *C. otophyllum* into three novel medicines (Patents of China: ZL 98 1 18938.5, ZL 98 1 18173.2, and ZL 96 1 11270.0). For maintaining the lead in the research into *C. otophyllum*, the authors carried out further investigations, which were very important. However, most compounds in the total glycosides were difficult to separate. To study these

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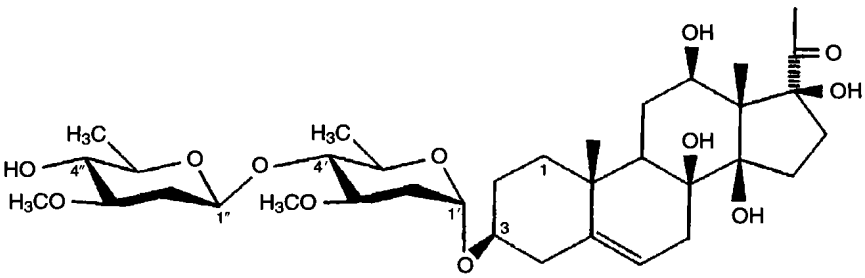
compounds, the authors used the acidic hydrolysis reaction universal in the research on glycosides to obtain secondary glycosides that are easy to separate. Moreover, some glycosides were easy to separate after other glycosides changed to secondary glycosides. From the acidic hydrolysis part of the ethyl acetate extract (the total glycosides) of the rhizome of *C. otophyllum*, the authors isolated four new carbohydrates (Zhao *et al*, 2004). Furthermore, this article reports a new C_{21} steroidal glycoside obtained from the same acidic hydrolysis part: deacetylmetaplexigenin 3-*O*-β-D-oleandropyranosyl-(1→4)-α-D-oleandropyranoside. Considering its structure, this compound might be a native glycoside or artificial product which was a fragment of corresponding native glycoside.



Results and Discussion

The novel glycoside was obtained as a white amorphous powder. The molecular formula was determined as $C_{35}H_{56}O_{12}$ by HRFAB-MS. The ^{13}C NMR and DEPT spectra showed one carbonyl, one pair of double bond, seven methyls, and numerous methylenes, methines, and quaternary carbons. These spectra were compared with the ^{13}C NMR and DEPT data (Zhang *et al*, 2000) of known C_{21} steroidal aglycones, and the aglycone was determined to be deacetylmetaplexigenin. The anomeric carbon at δ96.4 *d* corresponded to the proton at δ4.78 *m* in the HMQC, which had a long-range correlation with C—3 of the aglycone in the HMBC, and the signal for C—3 was at δ77.9, so this compound was a 3-*O*-glycoside of deacetylmetaplexigenin. The anomeric carbon resonances at δ96.4 *d* and 102.2 *d* revealed the presence of two sugar residues. In Table 1, the proton at δ4.78 *m* correlated with the signal at δ96.4 *d* in the HMQC, and had a correlation with H—2′ in the $^1H—^1H$ COSY. The assignment for C—2′ (δ37.4 *t*) was obtained from the correlation with H—2′ (δ1.40 *m*) in the HMQC. The proton at δ3.59 *m* (H—3′) correlated with the signal at δ77.9 *d* in the HMQC, and had a correlation with H—4′ in the $^1H—^1H$ COSY, from which C—4′ (δ83.5 *d*) was obtained. In this case, the carbons at δ96.4 *d*, 37.4 *t*, 77.9 *d*, 83.5 *d*, 69.0 *d*, and 18.7 *q*, were determined to be the carbons of the sugar by $^1H—^1H$ COSY and HMQC. The methoxy group (58.8 *q*) was located by the correlation of the resonance of δ3.07 *m*, with C—3′ in the HMBC spectrum. The ^{13}C NMR data of the carbons of the sugar were compared with the literature (Zhang *et al*, 2000) and the sugar was determined to be α-D-oleandropyranose. C—4′ was found to be at δ83.5 *d*, and in the HMBC, it showed a long-range correlation with the proton at δ4.26 *s*, which was correlated with the carbon at δ102.2 in the HMQC. Consequently, the O—4′ was linked with the sugar unit whose ano-

meric carbon (C-1″) was at δ102.2. On the basis of the correlations between the protons in the ^{1}H—^{1}H COSY and the long-range correlation of MeO- in the HMBC in Table 1, all of the ^{13}C NMR data of the second unit were determined. The data were compared with the literature (Zhang *et al*, 2000) and the second moiety was determined to be β-D-oleandropyranose. Since the resonance of C—4″ was at δ76.2, and there was no remaining sugar, so it was the terminal sugar moiety. Therefore, this glycoside was elucidated as deacetylmetaplexigenin 3-*O*-β-D-oleandropyranosyl-(1→4)-α-D-oleandropyranoside.

Table 1　NMR data for glycoside **1** in C_5D_5N

Carbon	^{13}C	^{1}H[a]	^{1}H-^{1}H COSY	HMBC	Carbon	^{13}C	^{1}H[a]	^{1}H-^{1}H COSY	HMBC
Deacetylmetaplexigenin					19	18.4 *q*	0.89 *m*; 3H	—	—
1	39.0 *t*	1.35 *m*; 1.40 *m*	—	—	20	209.6 *s*	—	—	—
2	30.0 *t*	1.59 *m*; 1.63 *m*	—	—	21	27.9 *q*	2.14 *m*; 3H	—	—
3	77.9 *d*	3.56 *m*	—	—	α-D-Ole				
4	39.4 *t*	1.93 *m*; 2.09 *m*	—	C-9; C-10	C-1′	96.4 *d*	4.78 *m*	H-2′	C-3
5	139.4 *s*	—	—	—	C-2′	37.4 *t*	1.40 *m*; 2H	H-1′	—
6	119.6 *d*	4.85 *m*	—	—	C-3′	77.9 *d*	3.59 *m*	H-4′	—
7	35.1 *t*	1.81 *m*; 2.03 *m*	—	—	C-4′	83.5 *d*	3.03 *m*	H-3′; H-5′	—
8	74.3 *s*	—	—	—	C-5′	69.0 *d*	3.74 *m*	H-4′; H-6′	—
9	45.0 *d*	1.11 *m*	H-11	—	C-6′	18.7 *q*	0.94 *m*; 3H	H-5′	C-5′
10	37.2 *s*	—	—	—	MeO-3′	58.8 *q*	3.07 *m*; 3H	—	C-3′
11	29.5 *t*	1.40 *m*; 1.93 *m*	H-9	C-9; C-12	β-D-Ole				
12	69.0 *d*	3.74 *m*	—	—	C-1″	102.2 *d*	4.26 *s*	H-2″	C-4′
13	60.4 *s*	—	—	—	C-2″	37.2 *t*	1.22 *m*; 2H	H-1″; H-3″	C-3″
14	89.3 *s*	—	—	—	C-3″	81.4 *d*	2.99 *m*	H-2″	—
15	34.3 *t*	1.60 *m*; 2H	H-16	C-16	C-4″	76.2 *d*	2.97 *m*	—	C-3″
16	32.8 *t*	2.85 *m*; 2H	H-15	C-15	C-5″	72.9 *d*	3.07 *m*	H-6″	—
17	92.6 *s*	—	—	—	C-6″	18.7 *q*	1.06 *m*; 3H	H-5″	C-4″; C-5″
18	9.5 *q*	1.49 *m*; 3H	—	C-12; C-13	MeO-3″	57.1 *q*	2.94 *m*; 3H	—	C-3″

[a] Coupling constants are in hertz.

Experimental

General experimental procedures　The mp was determined on a WC-1 micromelting point apparatus (Instrument Plant of Sichuan University, Sichuan, China) and was uncorrected. Optical rotation was measured on a Horiba Sepa-300 digital polarimeter. The IR spectrum was measured on a Perkin-Elmer 577 spectrophotometer. The UV spectrum was measured on a Shimadzu double-beam 210A spectrometer. FABMS were performed on a VG AutoSpec-3000 spectrometer. Bruker Am-400 and DRX-500 instruments were used to record ^{1}H NMR and 2D NMR (400 MHz), and ^{13}C NMR. C_5D_5N was the solvent and the internal standard at room temperature. Silica gel (200—300 mesh) for column chromatography and silica gel plate (GF-254) for thin-layer chromatography were the products of Qingdao Haiyang Chemical Group Co., Qingdao, China.

Plant material　The rhizome of *C. otophyllum* was bought from a drug market in Kunming. It was identified by Dr. Yue-Mao Shen and a voucher specimen (KUN No. 0776933) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

Extraction and isolation　The dried powder of the rhizome of *C. otophyllum* (40 kg) was refluxed with 95%

C_2H_5OH (120 L× 3). The extract was evaporated, was extracted with $CH_3CO_2C_2H_5$ (6 L), and was defatted with petroleum ether (1.4 L). The extract was the total glycosides (0.70 kg) (the above completed at the processing factory of the Institute). A part of the total glycosides (500 g) were dissolved in 2.25 L CH_3OH-0.025 mol/L H_2SO_4 (1∶2, v/v) in water-bath at 70 ℃. After two hours, $Ba(OH)_2$ solution was added until pH 7; $BaSO_4$ was filtered. The solution was dried up to give a crude aglycones (100 g).

The crude aglycones (100 g) were separated into twenty-one fractions (Fr. 1 to Fr. 21) through column chromatography over silica gel (300 g) by elution with $CHCl_3$ (1 874 ml), and then with a mixture of $CHCl_3$-CH_3OH (100∶1, v/v, 1 000 ml), with $CHCl_3$-CH_3OH (100∶3, 1 000 ml), and finally with $CHCl_3$-CH_3OH (100∶8.5, 1 582 ml). Fr. 9 (between 2 716 and 2 896 ml, 11.5 g) produced four fractions by silica gel column chromagraphy (77 g) eluted with $CHCl_3$-CH_3OH (100∶2, 2 000 ml; 100∶3, 1 000 ml; 100∶5, 500 ml), the second fraction in the four fractions yielded five fractions by silica gel column chromatography (55 g) eluted with petroleum ether-$(CH_3)_2CO$ (10∶5, 500 ml; 10∶6, 600 ml; 10∶7, 200 ml), the fourth fraction in the five fractions yielded two fractions by silica gel column chromatography (53 g) eluted with petroleum ether-$(CH_3)_2CO$ (10∶6, 700 ml), the first fraction in the two fractions produced three fractions by silica gel column chromatography (18 g) eluted with petroleum ether-$CH_3CO_2C_2H_5$ (35∶65, 200 ml; 2∶8, 100 ml; 1∶9, 100 ml), the third fraction in the three fractions produced three fractions (g, h, and i) by silica gel column chromatography (18 g) eluted with $CHCl_3$-$(CH_3)_2CO$ (75∶25, 390 ml), and the third fraction (i) was this new glycoside (between 245 and 350 ml, 16 mg).

Glycoside 1 White powder, mp 118—121 ℃; $[\alpha]_D^{20.0}$: +4.8° (EtOH, *c* 0.21); UV (EtOH): λ_{max} (log ε) 255.8 (3.03) nm; IR (KBr) ν_{max} 3443, 2933, 2364, 2339, 1699, 1635, 1457, 1367, 1099, 1062, 1002, 910, 538, 418 cm^{-1}; ^1H, ^{13}C and 2D NMR data, see Table 1; FAB-MS: *m*/*z* (rel. int.) = 667 [M-H]$^-$ (100), 513 (2), 434 (1.5), 379 (1.5), 339 (6.5), 80 (2); HRFAB-MS: *m*/*z* = 667.3677 [M-H]$^-$ (calcd. for $C_{35}H_{55}O_{12}$: 667.3694).

References:

Mu QZ, Lu JR, Zhou QL, 1986. Two new antiepilepsy compounds-otophyllosides A and B [J]. *Sci Sin, Ser B* (Engl Ed) (中国科学 B 辑 英文版), **29** (3): 295—301

Pei YQ (裴印权), Cao LG (曹龙光), Xie SJ (谢淑娟), *et al*, 1981. Central pharmacological action of *Cynanchum otophyllum* Schneid [J]. *Beijing Yixueyuan Xuebao* (北京医学院学报), **13** (3): 213—218

Zhang YH (张援虎), Wen YY (温远影), Kuang TY (匡廷云), 2000. The use of ^{13}C NMR in the structure analysis of C_{21} steroidal glycosides in the Asclepiadaceae [J]. *Tianran Chanwu Yanjiu Yu Kaifa* (天然产物研究与开发), **12** (3): 83—87

Zhao YB, Shen YM, He HP, *et al*, 2004. Carbohydrates from *Cynanchum otophyllum* [J]. *Carbohydr Res*, **339** (11): 1967—1972