STEROIDS 72 (2007) 778–786

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/steroids

New pregnane glycosides from the roots of *Cynanchum otophyllum*

Xiao-Xia Ma[a,b], *Fang-Ting Jiang*[c], *Qing-Xiong Yang*[a], *Xiu-Hua Liu*[c], *Ying-Jun Zhang*[a,*], *Chong-Ren Yang*[a,**]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China*

[b] *Graduate School of Chinese Academy of Sciences, Beijing 100039, PR China*

[c] *College of Chemistry and Chemical Engineering, Henan University, Kaifeng 475001, PR China*

ARTICLE INFO

Article history:
Received 23 January 2007
Received in revised form 5 June 2007
Accepted 28 June 2007
Published on line 4 July 2007

Keywords:
Cynanchum otophyllum
Asclepiadaceae
Pregnane glycosides
Otophyllosides H–M

ABSTRACT

Six new pregnane glycosides with an acyl at C-12 and a straight sugar chain at C-3, namely otophyllosides H–M (**1–6**), were isolated from the roots of *Cynanchum otophyllum* (Asclepiadaceae) collected from Eryuan County in Yunnan province of China. Their structures were characterized to be qingyangshengenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**1**), qingyangshengenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**2**), qingyangshengenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**3**), qingyangshengenin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-thevetopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside (**4**), caudatin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranoside (**5**), caudatin 3-O-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside (**6**), respectively, on the basis of detailed spectroscopic analysis and chemical method.

1. Introduction

Cynanchum otophyllum Schneid (Chinese name Qingyangshen) is a folk medicinal plant endemic to Yunnan province of China, whose root was used for the treatment of epilepsy, rheumatic pain, kidney weakness, and muscle injures by the local people of its growing area. Based on the chemical and pharmacological experiments, it has been developed as an anti-epilepsy remedy and put into industrial production for many years in China [1–7]. The pregnane glycosides were determined as effective ingredients and otophyllosides A–G as main constituents were previously reported [2,8,9]. In a continuation of our phytochemical investigation of traditional Chinese medicinal plants to search for novel biologically active compounds, six new pregnane glycosides were isolated from the roots of this plant. Their structures were determined by detailed spectroscopic analysis, including 2D NMR techniques, and acidic hydrolysis. This paper describes the structure elucidation of these new compounds.

* *Corresponding author*. Tel.: +86 871 5223235; fax: +86 871 5150124.
** *Corresponding author*.
E-mail addresses: zhangyj@mail.kib.ac.cn (Y.-J. Zhang), cryang@mail.kib.ac.cn (C.-R. Yang).
0039-128X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2007.06.001

2. Experimental

2.1. General methods

Melting points were measured on an XRC-I micromelting point apparatus and were uncorrected. Optical rotations were obtained on a SEPA-300 automatic digital polarimeter. IR spectra were determined on a Bruker Tensor 27 spectrometer in KBr pellets. NMR spectra were performed in CD_3OD unless otherwise noted and recorded on Bruker DRX-400 and -500 instruments with TMS as internal standard. MS data were detected on a VG Auto Spec-3000 spectrometer. Silica gel HF_{254} prepared for TLC and silica gel (200–300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Company, Qingdao, China. Reversed phase silica gel Rp-18 and Rp-8 for CC were purchased from Merck & Co. Inc. L-Glucose, D-glucose, L-cysteine methyl ester hydrochloride, and 1-(trimethylsilyl)imidazole were purchased from Sigma (USA), Supelco (USA), Aldrich (USA), and Fluka (Switzerland), respectively.

2.2. Plant material

The roots of *C. otophyllum* Schneid were collected at Eryuan County, in the northwest of Yunnan province, China, and identified by Prof. C.R. Yang (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen is deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried roots of *C. otophyllum* (10 kg) were extracted with 90% EtOH at room temperature for three times (72, 72, and 48 h, 15 L × 3). After removal of the organic solvent *in vacuo*, the residue was suspended in water (2 L) and partitioned with $CHCl_3$ for three times (1.5 L × 3) to give a $CHCl_3$ extract (155 g), part of which (150 g) was subjected to a silica gel CC (11 cm × 120 cm) and eluted with $CHCl_3$–MeOH (10:1.5, 7.6 L) to give five fractions (Frs. 1–5). Fr. 3 (16.1 g) was repeatedly chromatographed over silica gel [$CHCl_3$–MeOH (10:0.8) and EtOAc–EtOH–H_2O (12:1:0.5)] and Rp-8 (MeOH–H_2O, 68% → 85%) to afford **1** (370 mg) and **4** (46 mg). Fr. 4 (6.4 g) was chromatographed on silica gel [$CHCl_3$–MeOH (9:1) and EtOAc–EtOH–H_2O (8:1:0.5)], Rp-8 and Rp-18 (MeOH–H_2O, 65% → 100%) columns to give **2** (87 mg), **3** (92 mg), and **5** (59 mg). Fr. 5 (20.0 g) was subjected repeatedly to silica gel ($CHCl_3$–MeOH, 9:1.5) and Rp-18 (MeOH–H_2O, 60% → 100%) CC to yield **6** (210 mg).

Another part of the $CHCl_3$ extract (5 g) was dissolved in MeOH (50 ml) and treated with 5% HCl (10 ml) at 90 °C for 30 min. After cooled down to room temperature and neutralized with NaOH (4 mol/L) to pH 6–7, the reaction mixture was evaporated under reduced pressure to remove of MeOH. The condensation was diluted with water and extracted by $CHCl_3$ for three times (50 ml × 3). The $CHCl_3$ phase (1.8 g) was chromatographed repeatedly on silica gel column to give **7** (76 mg) and **8** (93 mg).

Table 1 – ^{13}C NMR data for the aglycone moieties of 1–6[a] (δ in ppm, in CD_3OD, 100 MHz)

Position	1	2	3	4[a]	5	6[a]
1	39.8	39.8	39.8	39.2	39.8	39.3
2	30.1	30.1	30.1	29.9	30.2	29.9
3	79.3	79.3	79.3	77.6	79.3	77.7
4	39.8	39.8	39.8	39.1	39.8	39.0
5	140.3	140.3	140.3	139.4	140.3	139.4
6	119.4	119.6	119.6	119.2	119.6	119.2
7	35.2	35.2	35.2	34.8	35.2	34.8
8	75.0	75.0	75.0	74.3	75.0	74.4
9	45.1	45.1	45.1	44.5	45.2	44.6
10	38.1	38.1	38.1	37.4	38.1	37.5
11	25.5	25.5	25.5	25.2	25.4	25.1
12	73.7	73.5	74.0	73.4	73.3	72.6
13	59.1	59.1	59.1	58.4	58.7	57.5
14	90.0	90.0	90.0	89.6	89.9	89.5
15	34.3	34.3	34.3	33.9	34.2	33.9
16	33.4	33.4	33.5	33.2	33.2	33.0
17	93.1	93.0	93.1	92.5	93.0	92.4
18	10.6	10.6	10.6	10.9	10.4	10.7
19	18.6	18.6	18.5	18.2	18.5	18.2
20	211.9	211.8	212.0	209.9	211.5	209.5
21	27.7	27.7	27.8	27.9	27.5	27.6
1′	167.3	167.5	166.9	165.4	167.4	166.0
2′	119.6	118.3	121.5	122.0	114.3	114.2
3′	132.8	132.8	132.8	132.5	167.4	165.5
4′	117.6	118.1	116.6	116.2	39.3	38.2
5′	168.3	169.9	164.9	163.6	21.3	20.9
6′	117.6	118.1	116.6	116.2	21.2	21.0
7′	132.8	132.8	132.8	132.5	16.7	16.5

[a] The spectral data were obtained in C_5D_5N.

2.3.1. Otophylloside H (1)

White amorphous powder, mp 213–216 °C, $[\alpha]_D^{25}$ + 8.0° (*c* = 0.83, MeOH), IR (KBr) ν_{max} 3442, 2934, 1709, 1610, 1591, 1504, 1452, 1384, 1309, 1276, 1163, 1072, 911, 854, 773 cm^{-1}. FAB-MS (negative ion mode): *m/z* 1242 $[M]^-$, 1105 $[M-137]^-$, 943 $[M-137-162]^-$. HRFAB-MS (negative ion mode): *m/z* 1241.5628 $[M(C_{60}H_{90}O_{27})-H]^-$ (calcd. 1241.5591). ^1H and ^{13}C NMR: see Tables 1–3 .

2.3.2. Otophylloside I (2)

White amorphous powder, mp 201–203 °C, $[\alpha]_D^{27}$ + 1.5° (*c* = 0.55, MeOH), IR (KBr) ν_{max} 3447, 2934, 1708, 1609, 1590, 1504, 1452, 1383, 1369, 1309, 1276, 1163, 1093, 1004, 912, 854, 773 cm^{-1}. FAB-MS (negative ion mode): *m/z* 1080 $[M]^-$, 942 $[M-1-137]^-$, HRFAB-MS (negative ion mode): *m/z* 1079.5030 $[M(C_{54}H_{80}O_{22})-H]^-$ (calcd. 1079.5063). ^1H and ^{13}C NMR: see Tables 1–3.

2.3.3. Otophylloside J (3)

White amorphous powder, mp 179–182 °C, $[\alpha]_D^{27}$ + 12.1° (*c* = 0.46, MeOH), IR (KBr) ν_{max} 3449, 2934, 1711, 1610, 1594, 1515, 1452, 1382, 1276, 1165, 1094, 1005, 912, 866, 853, 772 cm^{-1}. ESI-MS (negative ion mode): *m/z* 1224 $[M]^-$, 1062 $[M-162]^-$. HRESI-MS (negative ion mode): *m/z* 1223.5840 $[M(C_{61}H_{92}O_{25})-H]^-$ (calcd. 1223.5849). ^1H and ^{13}C NMR: see Tables 1–3.

Table 2 – ^{13}C NMR data for the sugar moieties of 1–6[a] (δ in ppm, in CD_3OD, 100 MHz)

Position	1 β-D-digit	2 β-D-digit	3 β-D-digit	4[a] β-D-digit	5 β-D-cym	6[a] β-D-cym
1″	97.0	97.0	97.0	96.4	97.2	96.5
2″	38.8	38.8	38.8	38.9	36.7	37.3
3″	68.3	68.3	68.3	67.6	78.4	78.1
4″	83.7	83.6	83.6	83.2	83.9	83.4
5″	69.5	69.4	69.5	69.4	69.8	69.1
6″	18.5	18.5	18.5	18.5	18.6	18.7
OMe					58.4	58.7

Position	1 β-D-ole	2 β-D-cym	3 β-D-cym	4[a] β-D-cym	5 β-D-ole	6[a] β-D-cym
1‴	102.6	100.7	100.6	99.8	102.6	100.5
2‴	37.7	36.3	36.3	36.7	37.8	37.1
3‴	80.1	78.4	78.4	78.2	80.2	77.8
4‴	83.6	83.7	83.8	83.4	83.8	83.2
5‴	72.6	69.9	70.0	68.6	72.7	69.0
6‴	18.8	18.4	18.6	18.7	18.8	18.6
OMe	57.9	58.6	58.6	58.9	57.6	59.0

Position	1 β-D-cym	2 β-D-ole	3 β-D-ole	4[a] β-D-the	5 β-D-cym	6[a] β-D-ole
1⁗	100.6	102.6	102.7	106.0	101.2	102.0
2⁗	36.3	37.9	37.7	74.7	36.4	37.8
3⁗	78.4	80.1	80.0	85.9	78.5	78.8
4⁗	83.6	83.5	84.0	83.0	83.8	82.7
5⁗	70.0	72.7	72.4	72.0	70.0	71.8
6⁗	18.6	18.8	18.8	18.7	18.6	18.7
OMe	58.6	58.2	57.7	60.6	58.6	58.0

Position	1 β-D-glc	2 β-D-glc	3 β-D-cym	4[a] β-D-glc	5 β-D-glc	6[a] β-D-cym
1⁗′	104.0	104.1	99.6	104.9	104.1	98.4
2⁗′	75.3	75.5	36.5	75.9	75.3	36.8
3⁗′	76.7	78.2	78.8	78.7	76.7	78.2
4⁗′	81.0	71.7	83.8	72.0	81.1	83.2
5⁗′	76.4	78.0	70.4	78.0	76.5	69.7
6⁗′	62.1	63.0	18.6	63.1	62.2	18.7
OMe			58.5			58.9

Position	1 β-D-glc	3 β-D-glc	5 β-D-glc	6[a] β-D-glc
1⁗″	104.6	106.2	104.6	106.6
2⁗″	74.9	75.3	74.9	75.4
3⁗″	78.1	78.0	78.1	78.5
4⁗″	71.4	71.8	71.4	71.9
5⁗″	77.8	77.9	77.9	78.4
6⁗″	62.4	63.0	62.5	63.1

digit: digitoxopyranosyl; cym: cymaropyranosyl; ole: oleandropyranosyl; the: thevetopyranosyl; glc: glucopyranosyl.

[a] The spectral data were obtained in C_5D_5N.

2.3.4. *Otophylloside K* (**4**)

White amorphous powder, mp 169–172 °C, $[\alpha]_D^{28}$ + 4.0° (c = 0.21, MeOH), IR (KBr) ν_{max} 3442, 2934, 1710, 1610, 1516, 1453, 1383, 1369, 1277, 1165, 1076, 911, 865, 853, 772 cm^{-1}. FAB-MS (negative ion mode): *m/z* 1096 [M]$^-$, 933 [M − 1 − 162]$^-$. HRFAB-MS (negative ion mode): *m/z* 1095.5004 [M($C_{54}H_{80}O_{23}$)–H]$^-$ (calcd. 1095.5012). ^{1}H and ^{13}C NMR: see Tables 1–3.

2.3.5. *Otophylloside L* (**5**)

White amorphous powder, mp 191–193 °C, $[\alpha]_D^{29}$ + 1.3° (c = 0.74, MeOH), IR (KBr) ν_{max} 3443, 2968, 2934, 1713, 1642, 1452, 1383, 1369, 1317, 1224, 1164, 1060, 1004, 912, 864 cm^{-1}. FAB-MS (negative ion mode): *m/z* 1246 [M]$^-$, 1118 [M − 128]$^-$, 956 [M − 128 − 162]$^-$. HRFAB-MS (negative ion mode): *m/z* 1245.6261 [M($C_{61}H_{98}O_{26}$)–H]$^-$ (calcd. 1245.6268). ^{1}H and ^{13}C NMR: see Tables 1–3.

Table 3 – ^{1}H NMR spectral data of compounds 1–6[a] (δ in ppm, J in Hz, in CD_3OD, 500 MHz)

Position	1	2	3	4[a]	5	6[a]
3	3.54 m	3.57 m	3.57 m	3.86 m	3.57 m	3.83 m
6	5.38 d, 4.7	5.38 d, 4.8	5.38 d, 4.4	5.25 d, 3.2	5.34 d, 4.1	5.28 brs
12	4.72 dd, 11.6, 4.1	4.74 dd, 11.6, 4.1	4.78 dd, 11.6, 4.1	5.33 dd, 11.4, 4.1	4.48 dd, 11.6, 4.1	5.04[b]
18	1.63 s	1.66 s	1.67 s	2.09 s	1.50 s	1.97 s
19	1.14 s	1.18 s	1.18 s	1.29 s	1.14 s	1.31 s
21	2.08 s	2.11 s	2.10 s	2.41 s	2.17 s	2.50 s
2′					5.54 s	5.85 s
3′	7.73 d, 8.8	7.73 d, 8.8	7.81 d, 8.8	8.30 d, 8.6		
4′	6.71 d, 8.8	6.69 d, 8.8	6.82 d, 8.8	7.23 d, 8.6	2.40 m	2.44 m
5′					1.07 d, 6.8	0.94 d, 7.3
6′	6.71 d, 8.8	6.69 d, 8.8	6.82 d, 8.8	7.23 d, 8.6	1.07 d, 6.8	0.95 d, 7.4
7′	7.73 d, 8.8	7.73 d, 8.8	7.81 d, 8.8	8.30 d, 8.6	2.11 s	2.26 s
Position	**1** β-D-digit	**2** β-D-digit	**3** β-D-digit	**4**[a] β-D-digit	**5** β-D-cym	**6**[a] β-D-cym
1″	4.94 dd, 9.7, 1.7	4.98 dd, 9.7, 1.7	4.97 dd, 9.7,1.7	5.47 brd, 8.9	4.86 dd, 9.6, 1.6	5.26 brd, 9.4
2″	1.67 m	1.65 m	1.68 m	1.77 m	2.06 m	1.89 m
	1.93 m	1.93 m	1.96 m	2.03 m	2.17 m	2.30 m
3″	4.21 m	4.25 m	4.24 m	4.61 m	3.86 m	4.07 m
4″	3.27 m	3.20 m	3.19 m	3.47 m	3.26 m	3.66 dd, 8.7, 2.4
5″	3.80 m	3.79 m	3.82 m	4.20 m	3.82 m	4.21 m
6″	1.21 d, 5.8	1.23 d, 5.8	1.23 d, 6.3	1.49 d, 6.2	1.18 d, 6.3	1.38 d, 6.0
OMe					3.42 s	3.51 s
Position	**1** β-D-ole	**2** β-D-cym	**3** β-D-cym	**4**[a] β-D-cym	**5** β-D-ole	**6**[a] β-D-cym
1‴	4.60 dd, 9.6, 1.6	4.86 dd, 9.6, 1.6	4.86 dd, 9.6, 1.6	5.15 brd, 8.7	4.61 dd, 9.6, 1.6	5.10 brd, 9.5
2‴	1.41 m	1.59 m	1.63 m	1.75 m	1.94 m	1.77 m
	2.34 m	2.11 m	2.16 m	2.31 m	2.33 m	2.28 m
3‴	3.41 m	3.87 m	3.87 m	3.97 m	3.42 m	4.00 m
4‴	3.18 m	3.27 m	3.27 m	3.48 m	3.23 m	3.50 m
5‴	3.40 m	3.86 m	3.86 m	4.29 m	3.41 m	4.16 m
6‴	1.38 d, 6.2	1.24 d, 5.8	1.23 d, 6.2	1.42 d, 6.2	1.38 d, 6.1	1.37 d, 5.9
OMe	3.48 s	3.46 s	3.46 s	3.55 s	3.45 s	3.55 s
Position	**1** β-D-cym	**2** β-D-ole	**3** β-D-ole	**4**[a] β-D-the	**5** β-D-cym	**6**[a] β-D-ole
1⁗	4.82 dd, 9.6, 1.6	4.64 dd, 9.6, 1.6	4.60 dd, 9.6, 1.6	4.68 d, 7.7	4.78 dd, 9.6, 1.6	4.67 brd, 9.6
2⁗	1.64 m	1.44 m	1.44 m	3.88 m	1.58 m	1.81 m
	2.13 m	2.33 m	2.43 m		2.14 m	2.29 m
3⁗	3.84 m	3.43 m	3.38 m	3.70 t, 9.0	3.87 m	3.51 m
4⁗	3.24 m	3.41 m	3.11 m	3.85 m	3.20 m	3.46 m
5⁗	3.85 m	3.44 m	3.32 m	4.00 m	3.85 m	3.49 m
6⁗	1.20 d, 5.9	1.41 d, 6.1	1.30 d, 6.2	1.73 d, 6.1	1.21 d, 6.3	1.40 d, 5.8
OMe	3.46 s	3.50 s	3.45 s	3.93 s	3.42 s	3.50 s
Position	**1** β-D-glc	**2** β-D-glc	**3** β-D-cym	**4**[a] β-D-glc	**5** β-D-glc	**6**[a] β-D-cym
1⁗′	4.48 d, 7.9	4.48 d, 7.8	4.93 dd, 9.8, 1.8	5.13 d, 7.6	4.47 d, 8.0	5.24 brd, 8.2
2⁗′	3.23 m	3.22 m	1.60 m	4.03 m	3.22 m	2.32 m
			2.13 m			2.45 m
3⁗′	3.29 m	3.26 m	3.94 m	4.23 m	3.40 m	4.14 m
4⁗′	3.52 m	3.25 m	3.33 m	3.74 m	3.55 m	3.40 m
5⁗′	3.51 m	3.37 m	3.90 m	4.00 m	3.54 m	4.26 m
6⁗′	3.82 m[c]	3.67 dd, 5.8, 12.0	1.34 d, 6.2	4.35 dd, 5.4, 11.4	3.85 m[c]	1.61 d, 6.2
	3.92 dd, 2.2, 12.0	3.88 dd, 2.2, 12.0		4.53 dd, 2.2, 11.4	3.94 dd, 2.2, 12.0	
OMe			3.48 s			3.60 s

Table 3 (*Continued*)

Position	1	2	3	4[a]	5	6[a]
	β-D-glc		β-D-glc		β-D-glc	β-D-glc
1‴″	4.38 d, 7.8		4.36 d, 7.7		4.38 d, 7.8	4.92 d, 7.7
2‴″	3.21 m		3.18 m		3.19 m	3.98 m
3‴″	3.34 m		3.36 m		3.36 m	3.96 m
4‴″	3.28 m		3.26 m		3.31 m	4.17 m
5‴″	3.32 m		3.27 m		3.34 m	4.23 m
6‴″	3.64 dd, 5.8, 12.0		3.67 dd, 5.8, 12.0		3.69 dd, 5.8, 12.0	4.37 dd, 5.3, 11.5
	3.87 dd, 2.2, 12.0		3.91 m[c]		3.90 dd, 2.2, 12.0	4.57 brd, 9.6

digit: digitoxopyranosyl; cym: cymaropyranosyl; ole: oleandropyranosyl; the: thevetopyranosyl; glc: glucopyranosyl.
[a] The spectral data were obtained in C_5D_5N.
[b] Overlapped by H_2O signal.
[c] Overlapped by deoxy sugar signals.

2.3.6. Otophylloside M (6)

White amorphous powder, mp 162–164 °C, $[\alpha]_D^{29} + 2.4°$ (c = 0.91, MeOH), IR (KBr) ν_{max} 3456, 2970, 2934, 1713, 1643, 1452, 1382, 1369, 1317, 1280, 1224, 1165, 1088, 1004, 913, 865 cm^{-1}. FAB-MS (negative ion mode): *m/z* 1228 $[M]^-$, 1099 $[M-1-128]^-$. HRFAB-MS (negative ion mode): *m/z* 1227.6513 $[M(C_{62}H_{100}O_{24})–H]^-$ (calcd. 1227.6526). ^1H and ^{13}C NMR: see Tables 1–3.

2.3.7. Qingyangshengenin (7)

$C_{28}H_{36}O_8$, white amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz): δ 1.14 (3H, s, H-19), 1.56 (3H, s, H-18), 2.08 (3H, s, H-21), 3.53 (1H, m, H-3), 4.79 (1H, dd, *J* = 4.4, 11.4 Hz, H-12), 5.37 (1H, brs, H-6), 6.83 (2H, d, *J* = 8.7 Hz, H-4′,6′), 7.82 (2H, d, *J* = 8.7 Hz, H-3′,7′). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 38.5 (C-1), 30.4 (C-2), 71.4 (C-3), 41.5 (C-4), 140.0 (C-5), 117.7 (C-6), 34.0 (C-7), 73.9 (C-8), 43.4 (C-9), 36.7 (C-10), 24.1 (C-11), 72.5 (C-12), 58.0 (C-13), 88.2 (C-14), 33.0 (C-15), 31.9 (C-16), 91.5 (C-17), 9.5 (C-18), 18.2 (C-19), 210.2 (C-20), 27.2 (C-21), 165.4 (C-1′), 120.9 (C-2′), 131.6 (C-3′,7′), 115.1 (C-4′,6′), 161.5 (C-5′).

2.3.8. Caudatin (8)

$C_{28}H_{42}O_7$, white amorphous powder. ^1H NMR ($CDCl_3$, 500 MHz): δ 1.07 (6H, d, *J* = 6.8 Hz, H-5′, H-6′), 1.15 (3H, s, H-19), 1.47 (3H, s, H-18), 2.13 (3H, s, H-7′), 2.20 (3H, s, H-21), 3.52 (1H, m, H-3), 4.54 (1H, dd, *J* = 4.2, 11.5 Hz, H-12), 5.36 (1H, brs, H-6), 5.53 (1H, s, H-2′). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 38.5 (C-1), 30.3 (C-2), 71.3 (C-3), 41.5 (C-4), 140.1 (C-5), 117.6 (C-6), 33.9 (C-7), 73.9 (C-8), 43.5 (C-9), 36.7 (C-10), 24.1 (C-11), 71.4 (C-12), 57.4 (C-13), 88.2 (C-14), 32.8 (C-15), 31.7 (C-16), 91.5 (C-17), 9.4 (C-18), 18.2 (C-19), 209.6 (C-20), 27.0 (C-21), 166.7 (C-1′), 112.7 (C-2′), 166.0 (C-3′), 38.0 (C-4′), 20.7 (C-5′), 20.8 (C-6′), 16.4 (C-7′).

2.3.9. Acid hydrolysis of 1–6

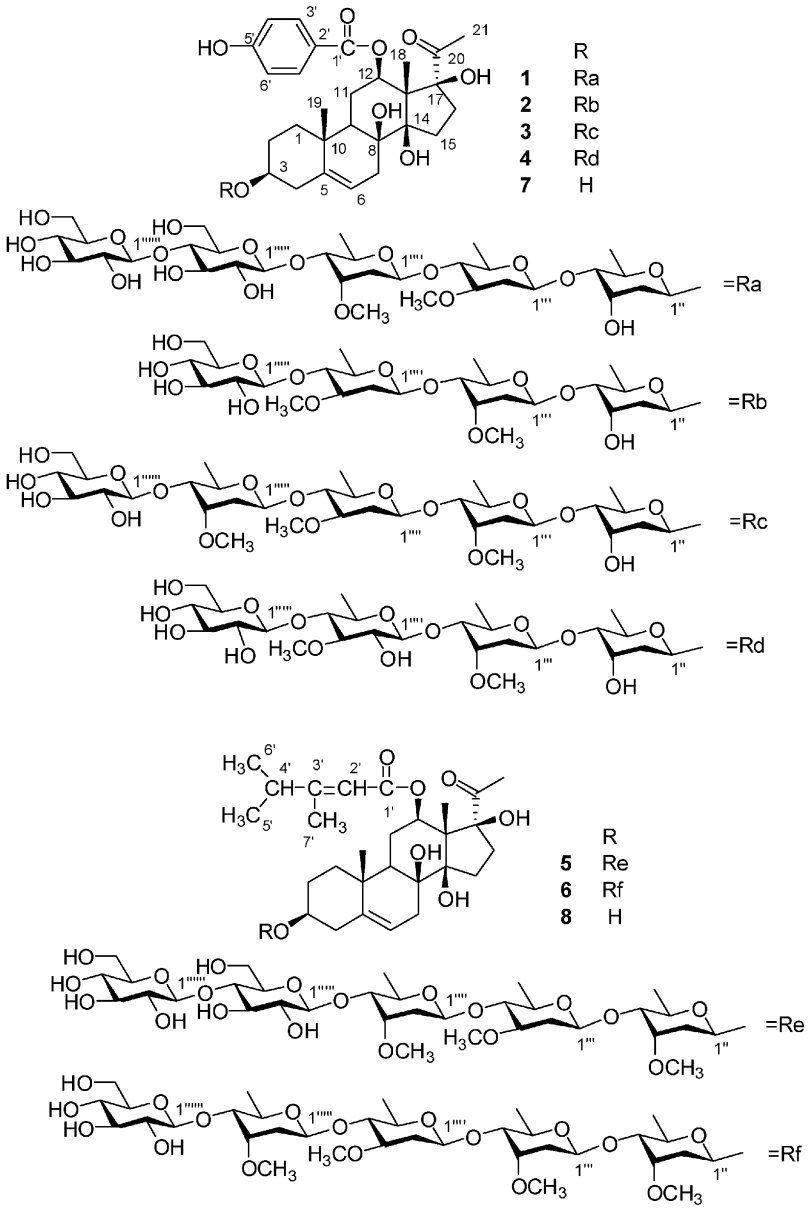
A solution of **1–6** (each 6 mg) in MeOH (5 ml) was treated separately with 5% HCl (5 ml) at 50 °C for 15 min. After added H_2O (5 ml), the reaction mixture was evaporated to 10 ml under vacuum for removing of MeOH, and then kept in 60 °C for another 15 min. The hydrolyzed mixture was neutralized to pH 7 with NaOH (4 mol/L) and condensed to dryness under reduced pressure. The residue was dissolved in MeOH, and compared by TLC analysis with authentic samples of qingyangshengenin (**7**) and caudatin (**8**) which thus revealed to be the aglycones of compounds **1–4** and **5–6**, respectively. The presence of the monosaccharides in the hydrolysates of each compound was confirmed by TLC comparison with authentic sugars, digitoxose was detected from **1–4**; oleandrose was detected from **1–3**, **5** and **6**; glucose and cymarose were detected from **1–6**. The R_f values of qingyangshengenin, caudatin, digitoxose, oleandrose, and cymarose were 0.35, 0.47, 0.16, 0.37, and 0.10 with solvent $CHCl_3$–MeOH (9:1); 0.10, 0.38, 0.09, 0.19, and 0.02 with solvent petroleum ether–Me_2CO (3:2); 0.23, 0.53, 0.19, 0.30, and 0.05 with solvent *n*-hexane–Me_2CO (1:1), respectively. The R_f values of glucose was 0.30 with solvent $CHCl_3$–MeOH–H_2O (7:3:0.5).

2.3.10. Determination of absolute configuration of glucose moieties

Each neutralized hydrolysates of **1–6** were condensed to dryness after TLC analyses and then suspended into water (2 ml) and extracted with $CHCl_3$ (2 ml × 3). The aqueous layer was concentrated to dryness and then dissolved in pyridine (2 ml) to give a monosaccharide mixture, which was added to L-cysteine methyl ester hydrochloride (1.5 mg) and kept at 60 °C for 1 h. After 1-(trimethylsilyl)imidazole was added to the reaction mixture slowly under ice bath condition, it was then kept at 60 °C for 30 min. The supernatant was subjected to GC analysis under the following conditions: Shimadzu GC-14C gas chromatograph equipped with an H_2 flame ionization detector. Column: 30QC2/AC-5 quartz capillary column (30 m × 0.32 mm). Column temperature: 180 °C/280 °C, programmed increase: 3 °C/min, carrier gas: N_2 (1.5 ml/min). Injector and detector temperature: 250 °C, injection volume: 4 μl, split ratio: 1/50. The absolute configurations of the glucoses were confirmed to be D-series by comparison of the retention times of glucose derivatives with those of standard samples: D-glucose (23.373 min) and L-glucose (23.832 min).

3. Results and discussion

Six new pregnane glycosides (**1–6**) were isolated from the $CHCl_3$ extracts of the roots of *C. otophyllum* through repeated column chromatography. All of them showed positive effects on Libermann–Buchard and Keller–Kiliani reactions, indicating the presence of a steroidal skeleton with 2-deoxysugar moiety. Spectroscopic analysis demonstrated that all the glycosides had a pregnane skeleton with an acyl group at C-12



Scheme 1 – Pregnane glycosides (1–6) isolated from *Cynanchum otophyllum*.

position and a straight sugar chain consisting of four or five sugar units connected to C-3 group position of the aglycone. Acidic hydrolysis of the $CHCl_3$ extract furnished two aglycone compounds which were identified to be qingyangshengenin (**7**) and caudatin (**8**) by comparison of their spectral data with those reported in the literatures [1,2,10–12] (Scheme 1).

The presence of the monosaccharides in the hydrolysates of each compound was confirmed by co-TLC comparison with authentic sugars. Further GC analysis of the corresponding trimethylsilylated L-cysteine adducts confirmed D-configuration of glucose. For the deoxysugars, since only D-form authentic samples could be obtained, their absolute configurations could not be assigned by GC analysis, but determined to be D-forms by comparison of their ^{13}C NMR spectroscopic data with those reported data. According to the literatures, the chemical shift of C-2 is <35.0 ppm in β-L-cymaropyranosyl unit and >36.0 ppm in β-D-cymaropyranosyl unit [13–15]. Therefore, all of the cymaropyranosyl units were determined to be D-configurations based on their ^{13}C NMR chemical shifts (>36.0 ppm). To the best of our knowledge, β-linked digitoxopyranosyl and oleandropyranosyl units were so far only found D-configurations from the Asclepiadaceae family, which were supposed for these sugars in this study as well, also due to their similar ^{13}C NMR chemical shifts with those reported in literatures [2,12,13,15–17].

3.1. *Otophylloside H (1)*

Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned to be $C_{60}H_{90}O_{27}$ on the basis of negative HRFAB-MS (*m/z* 1241.5628 $[M–H]^-$, calcd. 1241.5591) and ^{13}C NMR (DEPT) data (Tables 1 and 2). IR spec-

trum showed the absorption bands for hydroxyl (3442 cm^{-1}), carbonyl (1709 cm^{-1}) groups and benzene ring (1610 and 1591 cm^{-1}). The ^1H NMR spectrum of **1** revealed the presence of three singlet methyl groups [δ_H 1.14, 1.63, 2.08 (each 3H, s, Me-19, -18, -21)], one olefinic proton [δ_H 5.38 (d, J = 4.7 Hz, H-6)] and four aromatic protons on a *para*-substituted benzene ring [δ_H 6.71 (2H, d, J = 8.8 Hz, H-4′,6′) and 7.73 (2H, d, J = 8.8 Hz, H-3′,7′)] in its aglycone moiety. Its negative FAB-MS exhibited fragment ion peaks at m/z 1105 $[M-137]^-$ which can be ascribed to the loss of a hydroxybenzoyl ester group. The above observation suggested qingyangshengenin to be the aglycone of compound **1**. Proton signals were also assigned to three secondary methyl groups [δ_H 1.20 (d, J = 5.9 Hz), 1.21 (d, J = 5.8 Hz), and 1.38 (d, J = 6.2 Hz)], two methoxyl groups [δ_H 3.46 and 3.48] and five anomeric protons [δ_H 4.38 (d, J = 7.8 Hz), 4.48 (d, J = 7.9 Hz), 4.60 (dd, J = 9.6, 1.6 Hz), 4.82 (dd, J = 9.6, 1.6 Hz), and 4.94 (dd, J = 9.7, 1.7 Hz)] whose multiplicities suggested the presence of three 2,6-dideoxy-sugar in a pentasaccharides chain and the β-configuration of the five hexose units. Acid hydrolysis of **1** gave qingyangshengenin (**7**) as the aglycone, and glucose, digitoxose, oleandrose, as well as cymarose as sugar residues. The ^{13}C NMR shifts of each sugar unit were assigned unambiguously by HMQC, HMBC and HMQC-TOCSY analyses (Table 2). The existence of one D-digitoxopyranosyl, one D-oleandropyranosyl, one D-cymaropyranosyl and two D-glucopyranosyl units were confirmed by their comparison with the spectroscopic data in the literatures [2,12–17]. The glycosylation shifts effects of C-2 (−0.3 ppm), C-3 (+7.9 ppm), and C-4 (−1.7 ppm) showed the linkage position of the sugar moiety was at the C-3 hydroxyl group of the aglycone. The sequence of these five sugar units was demonstrated by HMBC spectrum, in which distinct correlations from δ_H 4.38 (H-1‴″″ of terminal β-D-glucopyranosyl) to δ_C 81.0 (C-4‴″″ of inner β-D-glucopyranosyl); from δ_H 4.48 (H-1‴″″ of inner β-D-glucopyranosyl) to δ_C 83.6 (C-4‴″ of β-D-cymaropyranosyl); from δ_H 4.82 (H-1‴″ of β-D-cymaropyranosyl) to δ_C 83.6 (C-4‴ of β-D-oleandropyranosyl); from δ_H 4.60 (H-1‴ of β-D-oleandropyranosyl) to δ_C 83.7 (C-4″ of β-D-digitoxopyranosyl); from δ_H 4.94 (H-1″ of β-D-digitoxopyranosyl) to δ_C 79.3 (C-3), were observed, respectively. Thus, the structure of otophylloside H (**1**) was established as qingyangshengenin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

3.2. *Otophylloside I (2)*

Based on the HRFAB-MS data (m/z 1079.5030 $[M-H]^-$, calcd. 1079.5063) and ^{13}C NMR (DEPT) spectrum, compound **2** was shown to have a molecular formula $C_{54}H_{80}O_{22}$. The acidic hydrolysis afforded **7** as the aglycone and the same sugar compositions as compound **1**. The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1** except that the signals due to one set of D-glucopyranosyl unit vanished in **2**. In the HMBC experiment, the sequence of the four sugar units was elucidated by significant correlations observed from δ_H 4.48 (H-1‴″″ of β-D-glucopyranosyl) to δ_C 83.5 (C-4‴″ of β-D-oleandropyranosyl); from δ_H 4.64 (H-1‴″ of β-D-oleandropyranosyl) to δ_C 83.7 (C-4‴ of β-D-cymaropyranosyl); from δ_H 4.86 (H-1‴ of β-D-cymaropyranosyl) to δ_C 83.6 (C-4″ of β-D-digitoxopyranosyl); from δ_H 4.98 (H-1″ of β-D-digitoxopyranosyl) to δ_C 79.3 (C-3). Therefore, compound **2** was deduced to be qingyangshengenin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside, and named otophylloside I.

3.3. *Otophylloside J (3)*

The molecular formula of **3** was determined as $C_{61}H_{92}O_{25}$ by its HRESI-MS (m/z 1223.5840 $[M-H]^-$, calcd. 1223.5849). The acidic hydrolysis afforded **7** as the aglycone and the same sugar compositions as compounds **1** and **2**. The ^1H NMR spectrum of **3** showed four secondary methyl signals [δ_H 1.23 (d, J = 6.3 Hz), 1.23 (d, J = 6.2 Hz), 1.30 (d, J = 6.2 Hz), and 1.34 (d, J = 6.2 Hz)] and three methoxyl methyl signals [δ_H 3.45, 3.46, and 3.48] together with five anomeric proton signals [δ_H 4.36 (d, J = 7.7 Hz), 4.60 (dd, J = 9.6, 1.6 Hz), 4.86 (dd, J = 9.6, 1.6 Hz), 4.93 (dd, J = 9.8, 1.8 Hz), and 4.97 (dd, J = 9.7, 1.7 Hz)]. These observations suggested that four 2,6-dideoxysugar were part of a pentahexose chain with β-linkages in **3**. The ^{13}C NMR signals of each sugar unit were assigned exactly by HMQC and HMBC analyses and indicated the existence of one β-D-digitoxopyranosyl, one β-D-oleandropyranosyl, one β-D-glucopyranosyl and two β-D-cymaropyranosyl units. According to the distinct long-range correlations from δ_H 4.36 (H-1‴″″ of β-D-glucopyranosyl) to δ_C 83.8 (C-4‴″″ of outer β-D-cymaropyranosyl); from δ_H 4.93 (H-1‴″″ of outer β-D-cymaropyranosyl) to δ_C 84.0 (C-4‴″ of β-D-oleandropyranosyl); from δ_H 4.60 (H-1‴″ of β-D-oleandropyranosyl) to δ_C 83.8 (C-4‴ of inner β-D-cymaropyranosyl); from δ_H 4.86 (H-1‴ of inner β-D-cymaropyranosyl) to δ_C 83.6 (C-4″ of β-D-digitoxopyranosyl); from δ_H 4.97 (H-1″ of β-D-digitoxopyranosyl) to δ_C 79.3 (C-3), the structure of otophylloside J (**3**) was confirmed to be qingyangshengenin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

3.4. *Otophylloside K (4)*

Compound **4** possessed the molecular formula $C_{54}H_{80}O_{23}$ on the basis of HRFAB-MS (m/z 1095.5004 $[M-H]^-$, calcd. 1095.5012). The combination of acidic hydrolysis, GC analysis and spectra studies indicated that **4** possessed qingyangshengenin (**7**) as aglycone, and D-digitoxose, D-cymarose, D-glucose and another deoxysugar as sugar residue. The undefined deoxysugar showed one secondary methyl [δ_H 1.73 (d, J = 6.1 Hz)], one methoxyl (δ_H 3.93), one methine [δ_H 3.70 (t, J = 9.0 Hz)], and an anomeric proton doublet [δ_H 4.68 (d, J = 7.7 Hz)] as well as seven carbons δ_C 106.0 (d), 74.7 (d), 85.9 (d), 83.0 (d), 72.0 (d), 18.7 (q), and 60.6 (q) in its NMR spectra. These NMR features were identical to those of β-thevetopyranosyl unit [13,16–17], and its configuration was supposed to be D-form because no L-thevetopyranose was found from the Asclepiadaceae family so far. The sequence of these four sugar units was determined by the important correlations from δ_H 5.13 (H-1‴″″ of β-D-glucopyranosyl) to δ_C 83.0 (C-4‴″ of β-D-thevetopyranosyl); from δ_H 4.68 (H-1‴″ of β-D-thevetopyranosyl) to δ_C 83.4 (C-4‴ of β-D-cymaropyranosyl); from δ_H 5.15 (H-1‴ of β-D-cymaropyranosyl) to δ_C 83.2

(C-4″ of β-D-digitoxopyranosyl); from δ_H 5.47 (H-1″ of β-D-digitoxopyranosyl) to δ_C 77.6 (C-3) observed in the HMBC spectrum. Hence, the structure of otophylloside K (**4**) was concluded to be qingyangshengenin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-thevetopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-digitoxopyranoside.

3.5. *Otophylloside L (5)*

The negative HRFAB-MS showed the quasi-molecular ion peak at *m/z* 1245.6261 ([M–H]$^-$, calcd. 1245.6268), in agreement with the molecular formula $C_{61}H_{98}O_{26}$, which was supported by the ^{13}C NMR and DEPT experiments. IR spectrum showed the absorption bands for hydroxyl (3443 cm^{-1}) and carbonyl (1713 cm^{-1}) groups as well as C=C band (1642 cm^{-1}). Acidic hydrolysis of **5** afforded a sugar mixture of cymarose, oleandrose and glucose, and the aglycone, which was identical to caudatin (**8**), by co-TLC comparison with authentic samples. Its negative FAB-MS exhibited fragment ion peaks at *m/z* 1118 [*M* – 128]$^-$ and 956 [*M* – 128 – 162]$^-$, revealing the existence of an ikemaoyl ester group in the aglycone and a terminal hexosyl unit in the sugar moiety. Inspection of the NMR spectral data of compound **5** showed that besides the signals arising from the aglycone (**8**), it contained five anomeric carbons at δ_C 97.2 (cym C-1), 101.2 (cym C-1), 102.6 (ole C-1), 104.1 (glu C-1), and 104.6 (glu C-1), corresponding to five anomeric proton signals at δ_H 4.86 (dd, *J* = 9.6, 1.6 Hz), 4.78 (dd, *J* = 9.6, 1.6 Hz), 4.61 (dd, *J* = 9.6, 1.6 Hz), 4.47 (d, *J* = 8.0 Hz), and 4.38 (d, *J* = 7.8 Hz), respectively. The signals of each sugar unit (Table 2) were assigned by HMQC and HMBC analyses, suggesting the existence of two D-cymaropyranosyl, one D-oleandropyranosyl, and two D-glucopyranosyl units compared with the spectroscopic data in the literatures [12,18–21]. Compared to the ^{13}C chemical shifts of **8**, glycosylation shifts were observed at the C-3 (+8.0 ppm), and C-4 (−1.7 ppm) positions, thus proving the attachment of the sugar chain at the C-3 hydroxyl group of the aglycone in **5**. The sugar sequence of **5** was demonstrated by HMBC correlations from δ_H 4.38 (H-1‴″ of terminal β-D-glucopyranosyl) to δ_C 81.1 (C-4‴″ of inner β-D-glucopyranosyl); from δ_H 4.47 (H-1‴″ of inner β-D-glucopyranosyl) to δ_C 83.8 (C-4‴″ of outer β-D-cymaropyranosyl); from δ_H 4.78 (H-1‴″ of outer β-D-cymaropyranosyl) to δ_C 83.8 (C-4‴ of β-D-oleandropyranosyl); from δ_H 4.61 (H-1‴ of β-D-oleandropyranosyl) to δ_C 83.9 (C-4″ of inner β-D-cymaropyranosyl); from δ_H 4.86 (H-1″ of inner β-D-cymaropyranosyl) to δ_C 79.3 (C-3). Consequently, the structure of **5** was established to be caudatin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranoside, and named otophylloside L.

3.6. *Otophylloside M (6)*

Compound **6** possessed a molecular formula $C_{62}H_{100}O_{24}$ based on its HRFAB-MS spectrum (*m/z* 1227.6513 [M–H]$^-$, calcd. 1227.6526). In the acid hydrolysis experiment, the same aglycone (**8**) and sugar components as those of **5** were obtained by TLC comparison with standard samples. Five anomeric proton signals at δ_H 4.67 (brd, *J* = 9.6 Hz), 4.92 (d, *J* = 7.7 Hz), 5.10 (brd, *J* = 9.5 Hz), 5.24 (brd, *J* = 8.2 Hz), and 5.26 (brd, *J* = 9.4 Hz), and five relevant anomeric carbon signals at δ_C 102.0 (ole C-1), 106.6 (glu C-1), 100.5 (cym C-1), 98.4 (cym C-1), and 96.5 (cym C-1) were observed in the NMR spectra, respectively. Based on the HMQC, HMBC and TOCSY spectra, the data of these five sugars were assigned to be three D-cymaropyranosyl, one D-oleandropyranosyl and one D-glucopyranosyl units comparing with the spectroscopic data in the literatures [18–21]. According to the glycosylation shifts, the sugar chain was determined to be attached to C-3 position of the aglycone. Following the same methodology described above, the sequence of the sugar moieties was assigned from HMQC and HMBC analysis using the well definite anomeric protons as starting signals, which was identical to that of caudatoside P, a glycoside of cynanchogenin isolated from *Cynanchum caudatum* [21]. Accordingly, the structure of otophylloside M (**6**) was elucidated to be caudatin-3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

Pregnane glycosides obtained from *C. otophllum* not only has prominent performance on epilepsy [2,7], but also can be used to treat Menier' syndrome and chronic hepatitis [8,9]. Our recent researches found that the total glycosides of *C. otophllum* showed potent influence on hypochondrium [22] and Alzheimer's disease (AD). All the isolated glycosides have a normal four-ring skeleton with an ester group at C-12 position and a single sugar chain mainly consisting of deoxy sugar units connected to C-3 group position of the aglycone. Initial studies on the structure–activity relationship revealed that the ester group and deoxy sugars would be important to pharmacological effects. Whether the various sugars constitute will affect the medical function remains to be investigated in future.

Acknowledgements

The authors are grateful to Professor Chen Jijun for supplying the deoxy sugar samples of D-digitoxose, D-cymarose, and D-oleandrose, and appreciate the measurements of all spectra performed by the colleagues of the Analytical Group in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany.

REFERENCES

[1] Mu QZ, Zhou QL. The constituents of qingyangshen (*Cynanchum otophyllum* scheind.). Acta Pharm Sinica 1983;18(5):356–62.
[2] Mu QZ, Lu JR, Zhou QL. Two new antiepilepsy compounds—otophyllosides A and B. Sci Sin Ser B (Engl Ed) 1986;29(3):295–301.
[3] Kuang PG, Wu YX, Meng FJ, Kuang PZ, Shao DS, Mu QZ. Treatment of grand mal seizures with "qingyangshen" (root of *Cynanchum otophyllum*) and observations on experimental animals. J Tradit Chin Med 1981;1(1):19–24.
[4] Hu YM, Zhang MQ, Kuang PG, Lang SY, Zhong YL, Wang CL, et al. Studies of qingyangshen (root of *Cynanchum otophyllum*). I. Effect of qingyangshen on copper and zinc metabolism. J Tradit Chin Med 1985;5(2):95–8.

[5] Kuang PG, Lang SY, Zhang FY, Yang ZL. Studies on qingyangshen (root of *Cynanchum otophyllum*). II. Effect of qingyangshen on metabolism of central monoamines. J Tradit Chin Med 1985;5(3):191–4.
[6] Kuang PG, Lang SY, Dai FL, Zhang DM, Zhang FY, Pu CQ, et al. Studies of qingyangshen (root of *Cynanchum otophyllum*). III. Effect of qingyangshen on acetylcholine concentration in mouse brain. J Tradit Chin Med 1985;5(4):267–70.
[7] Mu QZ, Shen YM, Zhou QL, inventors. An anti-epilepsy drug, CN 1060676C. Kunming Institute of Botany, Chinese Academy of Sciences; January 17, 2001.
[8] Mu QZ, Shen YM, Zhou QL, Hao XJ, inventors;. The pharmaceutical application of otophyllosides A and B, CN 1064235C. Kunming Institute of Botany, Chinese Academy of Sciences; April 11, 2001.
[9] Mu QZ, Shen YM, Zhou QL, Hao XJ, inventors. The preparation and application of otophyllosides C–G, CN 1064048C. Kunming Institute of Botany, Chinese Academy of Sciences; April 4, 2001.
[10] Zhang ZX, Zhou J. The chemical components of *Cynanchum wallichii*. Acta Bot Yunnan 1982;4(4):413–8.
[11] Yamagishi T, Mitsuhashi H. The structure of caudatin. Chem Pharm Bull 1972;20(3):625–6.
[12] Zhang ZX, Zhou J. The structure of wallicoside. Acta Chim Sin 1983;41(11):1059–64.
[13] Zhu NQ, Wang MF, Kikuzaki H, Nakatani N, Ho CT. Two C_{21}-steroidal glycosides isolated from *Cynanchum stauntoi*. Phytochemistry 1999;52(7):1351–5.
[14] Hamed AI, Sheded MG, Shaheen AESM, Hamada FA, Pizza C, Piacente S. Polyhydroxypregnane glycosides from *Oxystelma esculentum* var. *alpini*. Phytochemistry 2004;65(7): 975–80.
[15] Li XY, Sun HX, Ye YP, Chen FY, Pan YJ. C-21 steroidal glycosides from the roots of *Cynanchum chekiangense* and their immunosuppressive activities. Steroids 2006;71(1):61–6.
[16] Yoshikawa K, Matsuchika K, Takahashi K, Tanaka M, Arihara S, Chang HC, et al. Pregnane glycosides, gymnepregosides G–Q from the roots of *Gymnema alternifolium*. Chem Pharm Bull 1999;47(6):798–804.
[17] Yoshikawa K, Okada N, Kann Y, Arihara S. Steroidal glycosides from the fresh stem of *Stephanotis lutchuensis* var. *japonica* (Asclepiadaceae). Chemical structures of stephanosides A–J. Chem Pharm Bull 1996;44(10):1790–6.
[18] Warashina T, Noro T. Steroidal glycosides from the root of *Cynanchum caudatum* M. Chem Pharm Bull 1995;43(6):977–82.
[19] Warashina T, Noro T. Steroidal glycosides from roots of *Cynanchum caudatum* M, II. Chem Pharm Bull 1995;43(10):1734–7.
[20] Warashina T, Noro T. Steroidal glycosides from roots of *Cynanchum caudatum* M, III. Chem Pharm Bull 1996;44(2):358–63.
[21] Warashina T, Noro T. Steroidal glycosides from roots of *Cynanchum caudatum*. Phytochemistry 1997;44(5):917–23.
[22] Yang QX, Jin HK, Yang CR, inventors. Application of C_{21} steroid glycoside as antidepressants, CN 1634097A. Yunnan YunYao Laboratory Co. Ltd. July 6, 2005.