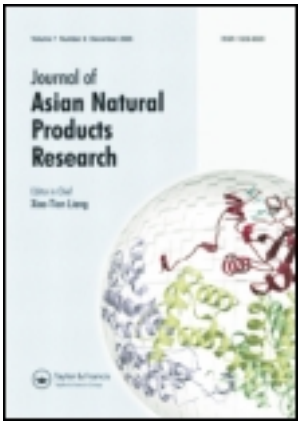

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Steroidal glycosides from *Cynanchum amplexicaule*

Gang Chen [a b], Huan Chen [a b], Wen Li [a b] & Yue-Hu Pei [a b]

[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, 110016, China

[b] Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang, 110016, China

Available online: 13 Jul 2011

To cite this article: Gang Chen, Huan Chen, Wen Li & Yue-Hu Pei (2011): Steroidal glycosides from *Cynanchum amplexicaule*, Journal of Asian Natural Products Research, 13:8, 756-760

To link to this article: http://dx.doi.org/10.1080/10286020.2011.588601

Journal of Asian Natural Products Research
Vol. 13, No. 8, August 2011, 756–760

Steroidal glycosides from *Cynanchum amplexicaule*

Gang Chen[ab], Huan Chen[ab], Wen Li[ab] and Yue-Hu Pei[ab]*

[a]*School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China;* [b]*Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China*

(*Received 9 December 2010; final version received 12 May 2011*)

Two new C_{21} steroidal glycosides with a new aglycone of amplexicogenin B were isolated from *Cynanchum amplexicaule* Sieb. et Zucc. Their structures were elucidated as amplexicogenin B-3-*O*-β-D-cymaropyranoside (**1**), amplexicogenin B-3-*O*-β-D-cymaropyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside (**2**) by means of spectral and chemical analysis.

Keywords: *Cynanchum amplexicaule*; C_{21} steroidal glycosides; 2,6-dideoxy-pyranoses

1. Introduction

Cynanchum amplexicaule (Sieb. et Zucc.), a Chinese folk medicine belonging to the family Asclepiadaceae, is widely distributed in China and used for the treatment of rheumatoid arthritis, hectic fever, and abscesses according to the famous Chinese medical book *China Herbal*. Previous studies indicated that this plant contained steroids with seco-ring at 13,14: 14,15 as main component [1]. In order to study this special type of constituents in *C. amplexicaule* (Sieb. et Zucc.), systematic constituent research was performed and two new with three known steroidal glycosides were isolated. In this paper, we report the isolation and structure elucidation of two new steroidal glycosides, having a new steroid amplexicogenin B as aglycone (Figure 1).

2. Results and discussion

Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ +10.6 ($c = 0.39$, MeOH). The molecular formula was determined to be $C_{28}H_{42}O_{11}$ by HR-FAB-MS at *m/z* 577.2616 $[M + Na]^+$. The ^{13}C NMR spectrum of **1** (Table 1) showed one anomeric carbon signal at δ 98.9. The carbon signals assignable to the aglycone moiety were similar to those of glaucogenin C [2], except that 5,6-double bond disappeared and four hydroxyl groups located in rings A and B.

In the HMBC experiment, correlations between H-1 at δ 2.31, 1.74 and C-19 at δ 16.5, C-10 at δ 47.3, C-3 at δ 80.0; H-2 at δ 2.42 and C-3 at δ 80.0, C-1 at δ 39.4; H-4 at δ 4.71 and C-2 at δ 38.4, C-10 at δ 47.3, C-5 at δ 89.3; H-6 at δ 4.41 and C-8 at δ 39.0, C-10 at δ 47.3, C-5 at δ 89.3 as well as H-19 at δ 1.37 and C-1 at δ 39.4, C-10 at δ 47.3, C-9 at δ 52.4, C-5 at δ 89.3 were observed (see Figure 2), which determined the locations of four hydroxyls were at C-3, C-4, C-5, and C-6. The stereo-configurations of rings A and B were determined by both the NOE spectrum and the coupling constants of H-3, 4, 6, and 8 (see Figure 3). In the NOE spectrum of compound **1**,

*Corresponding author. Email: peiyueh@vip.163.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.588601
http://www.informaworld.com

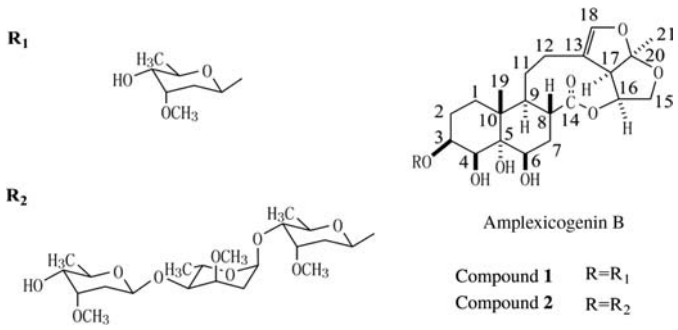

Figure 1. Structures of compound **1** and **2**.

correlations between H-19 at δ 1.37 and two axial proton signals at δ 2.42 (H-2), 3.12 (H-8) determined the *trans*-linkage between rings A/B, which suggested that both 19-Me and 5-OH are axial. The stereoconformations of H-3, 4, 6 were determined by their coupling constant values of 10.5 Hz for H-3, 4.8 Hz for H-4, and brs peak for H-6, which suggested H-3 was axial and H-4,6 were equatorial. Thus, the new aglycone moiety was determined as 3β, 4β, 5α, 6β-tetrahydroxyl-15, 20α: 18, 20β-diepoxy-13,14: 14,15-disecopregna-13(18)-eno-14-oic acid-16β-lactone and named amplexicogenin B (see Figure 3).

Besides the signals assignable to the aglycone, the signals assignable to a deoxypyranose were also observed. An acid hydrolysis experiment proved the existence of cymaropyranose and the absolute configuration was determined to be D by comparing its optical rotation value with that of authentic sample. Its anomeric proton at δ 5.00 (1H, d, $J =$ 9.6 Hz) indicated β-configuration for the anomeric proton. Based on the HMBC correlation between H-1′ at δ 5.00 and C-3 at δ 80.0, compound **1** was finally determined as amplexicogenin B-3-*O*-β-D-cymaropyranoside.

Compound **2** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ +8.5 ($c = 0.20$, MeOH). The molecular formula was determined to be $C_{42}H_{66}O_{17}$ by HR-FAB-MS at *m/z* 865.4189 $[M + Na]^+$. In the ^{13}C NMR spectrum of **2** (Table 1), the carbon signals assignable to the aglycone moiety were same as those of amplexicogenin B, which indicated that compounds **2** and **1** had the same aglycone.

The ^{13}C NMR spectrum of **2** showed three anomeric carbon signals at δ 98.8, 99.5, and 100.7. In the acid hydrolysis experiment, only cymarose was observed. Comparing the NMR spectral data with those of chekiangensoside B [3] and cynanversicoside A [4], the middle cymarose was determined as α-L-cymaropyranosyl, which was confirmed by its anomeric proton at δ 5.00 (br. d, 3.6) and other two cymarose were determined as β-D-cymaropyranosyl, which were confirmed by their anomeric protons at δ 4.97 (d, 10.6) and 5.10 (d, 9.0), respectively.

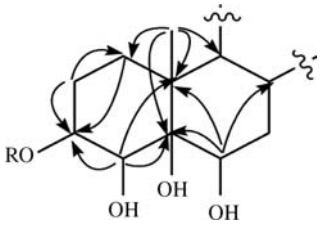

Figure 2. HMBC correlations in ring A and B for compound **1**.

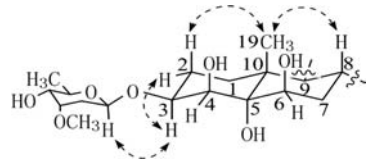

Figure 3. NOE correlations of compound **1**.

Table 1. The ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (600 MHz for ^{1}H and 150 MHz for ^{13}C, C_5D_5N).

1			**2**			**2**		
No.	δ_H (J Hz)	δ_C	No.	δ_H (J Hz)	δ_C	No.	δ_H (J Hz)	δ_C
1	2.31[a], 1.74[a]	39.4	1	2.26[a], 1.73[a]	39.4	α-L-Cym		
2	2.42 (dd, 13.5, 3.0) 2.21	38.4	2	2.37 (dd, 13.5, 3.0) 2.18	38.4	1″	5.00 (br. d, 3.6)	99.5
3	4.38 (dd, 11.5, 3.5)	80.0	3	4.29 (dd, 11.5, 3.5)	79.9	2″	2.18, 2.03	28.5
4	4.71 (t, 4.8)	80.2	4	4.64 (t, 5.4)	80.2	3″	3.99 (q like, 3.0)	75.8
5		89.3	5		89.3	4″	3.70[a]	77.3
6	4.41 (br. s)	69.7	6	4.37 (br. s)	69.7	5″	4.60 (m)	63.1
7	2.61[a], 2.21[a]	34.3	7	2.61[a], 2.18[a]	34.3	6″	1.33 (d, 6.0)	17.0
8	3.12 (t, 12.0)	39.0	8	3.08 (t, 12.0)	39.0	3″-OMe	3.34 (s)	56.3
9	1.88 (t, 10.2)	52.4	9	1.84 (t, 10.2)	52.4	β-D-Cym		
10		47.3	10		47.3		5.10 (d, 9.0)	100.7
11	2.63[a], 1.46[a]	24.8	11	2.63[a], 1.46[a]	24.8	2‴	2.42, 1.83	35.6
12	1.95[a], 1.51[a]	31.1	12	1.93[a], 1.47[a]	31.1	3‴	3.72[a]	78.9
13		118.7	13		118.7	4‴	3.52[a]	74.2
14		175.8	14		175.8	5‴	4.10[a]	71.0
15	4.22 (t, 7.8) 3.95 (t, 7.8)	67.9	15	4.20 (t, 7.8) 3.93 (t, 7.8)	67.9	6‴	1.48 (d, 6.0)	19.0
16	5.43 (q, 7.8)	75.2	16	5.40 (q, 7.8)	75.2	3‴-OMe	3.43 (s)	58.1
17	3.53[a]	56.3	17	3.47 (d, 7.8)	56.3			
18	6.47 (s)	143.8	18	6.45 (s)	143.8			
19	1.37 (s)	16.5	19	1.34 (s)	16.5			
20		114.7	20		114.7			
21	1.46 (s)	24.8	21	1.49 (s)	24.8			
β-D-Cym			β-D-Cym					
1′	5.00 (d, 9.6)	98.9	1′	4.97 (d, 10.6)	98.8			
2′	2.33, 1.78	35.5	2′	2.26[a], 1.73[a]	36.5			
3′	3.73 (brs)	78.8	3′	3.84 (q like, 3.0)	77.6			
4′	3.53[a]	74.2	4′	3.40 (dd, 9.6, 3.0)	82.2			
5′	4.04[a]	70.9	5′	4.08[a]	69.3			
6′	1.49 (d, 6.0)	19.1	6′	1.27 (d, 6.0)	18.6			
3′-OMe	3.41 (s)	58.0	3′-OMe	3.54 (s)	58.1			

Note: [a]Overlapped with other signals.

In the HMBC spectrum, the correlations between H-1′ at δ 4.97 and C-3 at δ 79.9, H-1″ δ 5.00, and C-4′ at δ 82.2 as well as H-1‴ at δ 5.10 and C-4″ at δ 77.3 were observed. Hence, compound **2** was elucidated as amplexicogenin B-3-*O*-β-d-cymaropyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained using a P-E 241 MC. IR spectra were recorded on a Bruker IFS-55 infrared spectrophotometer with KBr disks. The NMR spectral data were recorded on Bruker AV-600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) with TMS as an internal standard. HR-FAB-MS data were measured on Micromass Autospec-Ultima-TOF spectrometer. Silica gel GF254 for TLC and silica gel (200–300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company, Qingdao, China. Sweden. HPLC was carried on Shimadzu LC-8A (Kyoto, Japan) and the detector was Shimadzu SPD-10A. An analytical reversed-phase C18 column (Diamonsil C18 ∅ 4.6 mm × 250 mm, Zuanshi Company, Shanghai, China) and a preparative reversed-phase C18 column (Inertsil Prep-ODS ∅ 10 mm × 250 mm, Zuanshi Company) were employed.

3.2 Plant material

The roots of *C. amplexicaule* were collected in August 2005 at Xinxiang City, Henan Province, China. A voucher specimen was identified by Professor Qi-Shi Sun and has been deposited in the School of Traditional Chinese Materia Medica of Shenyang Pharmaceutical University (No. 6039).

3.3 Extraction and isolation

Roots of *C. amplexicaule* were extracted three times by means of reflux with hot 95% EtOH for 2 h each, and the combined solution was concentrated *in vacuo* to give a syrup (1100 g), followed by suspension in water. The suspension was then extracted with petroleum ether, chloroform, and *n*-butanol, successively. The chloroform extract (150 g) was further fractionated by silica gel column chromatography eluted with petroleum ether/acetone (100:1 → 1:1, v/v) to obtain night fractions. Fraction seven (500 mg) was further separated by HPLC eluted with MeOH 70% (v/v) to give compound **2** (15 mg). Fraction eight (2.2 g) was further separated by Sephadex LH-20 to give five sub-fractions. Sub-fraction five (300 mg) was subjected to HPLC eluted by MeOH 55% (v/v) to give compound **1** (12 mg).

3.3.1 Amplexicogenin B-3-O-β-D-cymaropyranoside (*1*)

A white amorphous powder; $[\alpha]_D^{20}$ +10.6 ($c = 0.39$, MeOH); IR (KBr) ν_{max}: 3443, 2935, 2890, 1738, 1383, 1309, 1164, 1106, 1061, 987 cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data (see Table 1); HR-FAB-MS *m/z* 577.2616 $[M + Na]^+$ (calcd for $C_{28}H_{42}O_{11}Na$, 577.2619).

3.3.2 Amplexicogenin B-3-O-β-D-cymaropyranosyl-(1 → 4)-α-L-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside (*2*)

A white amorphous powder; $[\alpha]_D^{20}$ +8.5 ($c = 0.20$, MeOH); IR (KBr) ν_{max}: 3445, 2935, 1733, 1652, 1450, 1383, 1308, 1164, 1104, 1062, 988 cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data (see Table 1); HR-FAB-MS *m/z* 865.4189 $[M + Na]^+$ (calcd for $C_{42}H_{66}O_{17}Na$, 865.4192).

3.4 Acid hydrolysis of compounds 1 and 2

A solution of **1** and **2** (each 10 mg) in MeOH (5 ml) was treated separately with 0.1 N H_2SO_4 (5 ml) at 50°C for 15 min.

After H_2O (5 ml) was added, the mixture was evaporated to 10 ml under reduced pressure to remove MeOH and then kept in 60°C for another 30 min. The hydrolyzed mixture was neutralized to pH 7 with $Ba(OH)_2$ and condensed to dryness under reduced pressure. The monosaccharide was isolated and determined to be cymarose for compounds **1** and **2** by means of preparative TLC developed by $CHCl_3$–MeOH–H_2O (20:3:1, *Rf* 0.55) and comparing with authentic sample. The absolute configuration was considered to be D-form for compound **1** by its optical value: cymarose $[\alpha]_D^{20} + 49.7$ ($c = 0.02$, H_2O).

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