

Chemical Constituents of *Gentiana macrophylla* Pall. from Shaanxi

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A novel amide, qinjiaoamide and a novel iridoid, erythrocentaurin acid, together with seven known compounds [erythrocentaurin (**3**), roburic acid (**4**), oleanolic acid (**5**), gentiopicroside (**6**), swertiamarine (**7**), sweroside (**8**), and 6'-*O*- β -D-glucosylgentiopicroside (**9**)], were isolated from the root of *Gentiana macrophylla* Pall. from Shaanxi Province, China. The novel compounds were identified as *N*-pentacosyl-2-carboxy-benzoyl amide (**1**) and 5-carboxyl-3,4-dihydrogen-1H-2-benzopyran-1-one (**2**), on the basis of spectroscopic (UV, IR, 1D- and 2D-NMR) and mass spectrometric data. Among the known compounds, erythrocentaurin (**3**) was isolated from the genus *Gentiana* for the first time.

Keywords: Qinjiao, *Gentiana macrophylla* Pall., Gentianaceae, chemical constituents, qinjiaoamide, erythrocentaurin acid.

Roots of the large leaved *Gentiana macrophylla* Pall. (Gentianaceae) are an important traditional Chinese drug with a long history of application for "dispelling the wind, removing rheumatism, eliminating pyrexia, and relieving pain" [1]. It is also a genuine crude medicine in Shaanxi Province, China [2]. Recently, inhibitory effects of *G. macrophylla* extract on rheumatoid arthritis of rats were reported [3]. Previous studies of this species dealt with the roots of *G. macrophylla* from Gansu Province, China [4]. There have been no reports of the chemical constituents of this crude medicine from Shaanxi Province of China, and in this paper we report our phytochemical results from samples collected in this Province. Repeated chromatographic purification of an aqueous ethanolic extract of the roots of *G. macrophylla* afforded a novel amide (**1**), a novel iridoid (**2**), a known iridoid (erythrocentaurin; **3**), two triterpenes [roburic acid (**4**) and oleanolic acid (**5**)], together with four known secoiridoid glycosides [gentiopicroside (**6**), swertiamarine (**7**), sweroside (**8**), and 6'-*O*- β -D-glucosylgentiopicroside (**9**)].

Compound **1**, MP: 103-105°C (CHCl₃), exhibited a pseudo molecular ion peak at *m/z* 538 [M+Na]⁺ in the positive ESI-MS. The negative ESI-MS showed a base peak at *m/z* 514 [M-H]⁻. The molecular formula was established as C₃₃H₅₇O₃N, based on the high-resolution FAB mass spectrum, which showed a [M+H]⁺ ion at *m/z* 516.4393. On TLC, a spot of compound **1** turned light red after spraying with a modified Dragendorff reagent, fading in a short while. The IR spectrum showed absorptions corresponding to a secondary amine (3340 cm⁻¹), an aromatic ring (3088, 1608, 1586, 1531 cm⁻¹), 1,2-disubstituted benzoyl nucleus (753 cm⁻¹), and an alkyl chain (CH₂)_n with *n*>4 (2921, 2849, 1472, 1272 cm⁻¹). It can be inferred that there are more methylene than methyl groups in the alkyl chain since absorption at 1472 is stronger than that at 1377 cm⁻¹. The ¹H NMR spectrum displayed a carboxylic proton at δ 10.86, four aromatic protons (δ 8.09, 7.08, 7.56, and 8.75), an amide (δ 5.27) and a multiple peak (δ 1.40-1.24) integrating for 44 protons corresponding to the methylenes of the alkyl chain. In accordance with this, there are some strong peaks

(δ 29.64-29.14) in the ^{13}C NMR spectrum, in addition to a methyl carbon (δ 13.99) (Table 1). This evidence further indicated that the alkyl group is normal, having no branch. The HMBC spectrum (Figure 1) allowed us to conclude that compound **1** was *N*-pentacosyl-2-carboxy-benzoyl amide.

Table 1: ^1H and ^{13}C NMR (500 MHz, CDCl_3) spectral data of **1**.

Position	^{13}C	^1H (J, Hz)	
1	142.24		
2	113.71		
3	131.63	8.09 dd (8.0, 1.5)	1H
4	122.43	7.08 m	1H
5	135.51	7.56 m	1H
6	120.59	8.75 d (8.5)	1H
7	172.41		
8	171.01		
1'	38.68	2.43 t (7.0)	2H
2'	25.46	1.75 t (7.0)	2H
3'-22'	29.64-29.14	1.40-1.24 m	44H
23'	31.87		
24'	22.61		
25'	13.99		
-NH-		5.27 t	1H
-CO ₂ H		10.86 s	1H

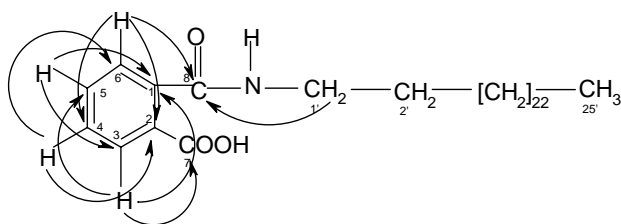


Figure 1: HMBC correlations for the aromatic fraction of compound **1**.

Compound **2**, obtained as colorless clear needles, MP: 251-253°C ($\text{C}_2\text{H}_5\text{OH}$), was assigned as $\text{C}_{10}\text{H}_8\text{O}_4$ on the basis of a quasimolecular ion at m/z 215 $[\text{M}+\text{Na}]^+$ in the positive ESI-MS, combined with the high-resolution EI mass spectral evidence. The observed molecular weight is 192.0429 (calculated for $\text{C}_{10}\text{H}_8\text{O}_4$: 192.0422). The IR spectrum revealed a hydroxyl group (3400 cm^{-1}), and two carbonyl groups [a lactone carbonyl (1732 cm^{-1}) and a carbonyl group conjugated with an aromatic ring (1696 cm^{-1})]. In the ^1H NMR spectrum three signals for aromatic protons appeared at δ 8.24 (1H, d, $J = 7.6\text{ Hz}$), 8.26 (1H, d, $J = 7.6\text{ Hz}$) and 7.53 (1H, t, $J = 8.0\text{ Hz}$), and a pair of methylene protons on the lactonic ring appearing at δ 3.53 and 4.53 (each 2H, t, $J = 6.0\text{ Hz}$). The above data suggested that the structure of compound **2** is identical to that of compound **3** (erythrocentaurin). The ^1H - ^1H COSY spectrum of **2** showed cross peaks

between δ 3.53 and 4.53; δ 7.53 and 8.24; and δ 7.53 and 8.26. The HMQC spectrum (Table 2) showed cross peaks between H-4 (δ 3.53) and C-4 (δ 27.32); H-3 (δ 4.53) and C-3 (δ 68.31); H-7 (δ 7.53) and C-7 (δ 128.19); H-6 (δ 8.26) and C-6 (δ 134.91); and H-8 (δ 8.24) and C-8 (δ 137.03). The structure of compound **2** was identified as 5-carboxyl-3,4-dihydrogen-1H-2-benzopyran-1-one, and named erythrocentaurin acid.

Table 2: ^1H and ^{13}C NMR (500 MHz, CDCl_3) spectral data of compounds **2** and **3**.

No.	^{13}C		^1H (J, Hz)		
	2	3	2	3	
1	166.96	164.09			
3	68.31	66.67	4.53 t (6.0)	4.52 t (6.0)	2H
4	27.32	24.56	3.53 t (6.0)	3.54 t (6.0)	2H
5	130.62	132.57			
6	134.91	138.35	8.26 d (7.6)	8.34 dd (7.5, 1.0)	1H
7	128.19	127.80	7.53 t (8.0)	7.59 t (8.0)	1H
8	137.03	135.58	8.24 d (7.6)	8.02 dd (7.5, 1.0)	1H
9	127.71	126.90			
10	143.19	141.07			
11	169.08	191.79		10.18 s	1H

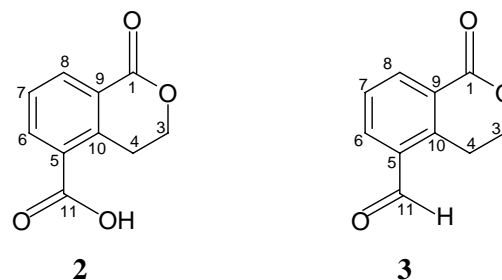


Figure 2: Structure of compounds **2** and **3** (erythrocentaurin)

Compounds **3**, **4**, **5** and **9** were identified as erythrocentaurin, roburic acid, oleanolic acid and 6'-O- β -D-glucosylgentiopicroside, respectively by comparison of the ^1H NMR and ^{13}C NMR spectral data with literature values [4-8]. Compounds **6**, **7** and **8** were identified as gentiopicroside, sweriamarine and sweroside by comparison with the spectral data and HPLC behavior of authentic samples [7,9]. These three secoiridoid glycosides are common constituents of Gentianaceae plants [10-12].

Experimental

General experimental procedures: The ^1H NMR and ^{13}C NMR spectra were recorded in either CD_3OD or CDCl_3 with a Bruker Avance DRX-500 spectrometer, using tetramethylsilane as internal standard. The UV spectra were recorded on a HITACHI U-2001 spectrophotometer. The IR spectra were obtained

using a UNICAM 1020C and Bruker EQUINOX 55 IR spectrometers. The ESI-mass spectra (positive and negative) were recorded with a BRUKER ESQUIRE-LC/MS mass spectrometer.

Plant material: Roots of *G. macrophylla* were purchased in 2000 in Long County, Shaanxi Province, P. R. China. The plant material was identified by Associate Professor Ya-Zou Wang and a voucher specimen was deposited in the Life Science College, Northwest University, China.

Extraction and isolation: The powdered, air-dried roots (1.2 kg) of *G. macrophylla* were extracted by refluxing three times with EtOH. Removal of solvent *in vacuo* gave a brown gum, which was subsequently suspended in water, and extracted successively with light petroleum and CHCl₃, and thus three parts were obtained. The CHCl₃ extract was concentrated and subjected to column chromatography (silica gel, 80-120 mesh), eluting with a gradient of CHCl₃-MeOH, and collecting every 100 mL, based on TLC monitoring. The fractions F8-F20 gave compound **3** (250 mg), and fractions F59-F68 gave compound **2** (110 mg). Separation of F21-F58 by repeated chromatography on a silica gel column gave compound **1** (80 mg), **4** (550 mg) and **5** (60 mg). The H₂O layer was subjected to column chromatography on macroporous absorption resin D101. Eluting successively with water and 30% EtOH, the fractions were collected based on TLC monitoring. Separation by silica gel column chromatography of the fraction eluted with 30% EtOH gave a substantial amount of compound **6** and a part containing mainly a mixture of three constituents. This mixture was concentrated, dissolved in water, and then filtered. The water solution was subjected to chromatography on a C₁₈ column, eluting with aq. MeOH, and monitored by HPLC. Three fractions were obtained (F1-F3). Purification of F1 and F2 by gel filtration over Sephadex LH-20 afforded compound **7** (85 mg) and compound **8** (35 mg). F3 was separated on a C₁₈ column and purified by Sephadex LH-20 to give compound **9** (20 mg).

Qinjiaoamide (1)

White, needle-shaped crystalline powder.

MP: 103-105°C (CHCl₃).

IR (KBr): 3340 (ν_{N-H}), 3088, 1608, 1586, 1531

(benzoyl ring), 2921, 2849 (ν_{C-H}), 1675 (ν_{O=C=}) cm⁻¹.

UV/Vis (MeOH) λ_{max} (log ε): 214 (4.28), 253 (4.098), 297 (3.556).

Positive ESI-MS: m/z 538 [M+Na]⁺.

Negative ESI-MS: m/z 514 [M-H]⁻.

HRMS-FAB: m/z [M + H]⁺ calcd for C₃₃H₅₇O₃N: 516.4416; found: 516.4393.

¹H NMR: Table 1.

¹³C NMR: Table 1.

Erythrocentaurin acid (2)

Colorless clear needles.

MP: 251-253°C (C₂H₅OH).

IR (KBr): 3400 (ν_{O-H}), 1732 (conjugated lactone), 1696 (conjugated carboxyl) cm⁻¹.

UV/Vis (MeOH) λ_{max} (log ε): 206 (4.619).

Positive ESI-MS: m/z 215 [M+Na]⁺.

Negative ESI-MS: m/z 191 [M-H]⁻.

HRMS-EI: m/z [M]⁺ calcd for C₁₀H₈O₄, 192.0422; found: 192.0429.

¹H NMR: Table 2.

¹³C NMR: Table 2.

Eerythrocentaurin (3)

Colorless needles, turn red when exposed to light.

MP: 145-147°C (CHCl₃).

IR (KBr): 3388 (ν_{O-H}), 1756 (conjugated lactone), 1698 (conjugated carboxy) cm⁻¹.

UV/Vis (MeOH) λ_{max} (log ε): 223.0 (4.485), 290.5 (3.31).

¹H NMR: Table 2.

¹³C NMR: Table 2.

Supplementary data: Physical and spectral data for roburic acid (**4**), oleanolic acid (**5**), gentiopicroside (**6**), swertiamarine (**7**), sweroside (**8**), and 6'-O-β-D-glucosylgentiopicroside (**9**).

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