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秦艽中的环烯醚萜甙成分

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摘要 秦艽(*Gentiana macrophylla* Pall.)为著名传统中药。从青海西宁产秦艽的甲醇提取物的水溶性部分分离到1个新裂环烯醚萜甙，命名为秦艽甙 (qinjiaoside) A，2个已知的环烯醚萜甙即龙胆苦甙 (gentiopicroside) 和哈巴甙 (harpagoside) 以及2个甾醇甙，胡萝卜甙和 β-谷甾醇-3-O-龙胆糖甙。这些化合物的结构通过红外光谱、紫外光谱、核磁共振波谱 (包括一维氢谱、碳谱、氢-氢相关谱、碳-氢相关谱、远程碳-氢相关谱) 和快原子轰击质谱分析得到推定。

关键词 秦艽，龙胆科，环烯醚萜甙，裂环烯醚萜甙，秦艽甙A，β-谷甾醇-3-O-龙胆糖甙

IRIDOID GLYCOSIDES FROM GENTIANA MACROPHYLLA

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Abstract From the roots of *Gentiana macrophylla*, a new secoiridoid glycoside, qinjiaoside A, has been isolated along with gentiopicroside and harpagoside as well as daucosterol and β-sitosterol-3-O-gentiobioside. Their structures were elucidated by means of spectral methods.

Key words *Gentiana macrophylla*, Gentianaceae, Iridoid glycosides, Secoiridoid glycosides, Qinjiaoside A, β-sitosterol-3-O-gentiobioside

INTRODUCTION

Gentiana macrophylla Pall.(Gentianaceae) as a traditional Chinese medicine (Chinese name: 'Qinjiao') [1] was early recorded in "Shennong Herbal", one of the earliest sources of folk knowledge in the use of herbs in Chinese history. It can be used for the treatment of rheumatism, headache, toothache, epidemic encephalomyelitis, etc. Earlier chemical investigation of this plant led to the isolation of three alkaloids, gentianine, gentianidine and gentianal [2]. Among which, gentianine was reported to have a depressive effect on the central nervous system, an anti-ulcerogenic action and an inhibitory action against the gastric secretion on experimental animals [3]. T. Hayashi et al. had first isolated gentiopicroside from this plant, and proposed that those alkaloids seem to be artefacts due to the presence of amine in the isolation process [4]. In order to evaluate the medical value of *G. macrophylla* and to search for biologically active compounds, we reinvestigated the chemical constituents of this plant. From the water soluble and liposoluble fractions of a methanol extract of the roots of *G. macrophylla*, fifteen

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compounds were obtained. We report herein the isolation and structure elucidation of a new secoiridoid glycoside, qinjiaoside A, along with gentiopicroside and harpagoside as well as daucosterol and β-sitosterol-3-O-gentiobioside from the water soluble fraction.

RESULTS AND DISCUSSION

The crude glycoside fraction was repeatedly chromatographed on silica gel and reversed phase silica gel to furnish compounds 1—5.

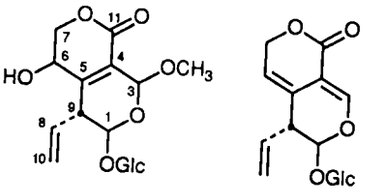
Compound **1** was obtained as a pale yellow powder. Its molecular formula $C_{17}H_{24}O_{11}$ was determined by the negative FAB mass spectrum which exhibited a quasi-molecular ion peak at m/z 403 $(M-H)^-$. It showed UV maxima at 205.5 and 214.5 nm and IR bands at 3400(OH), 2920(OCH_3), 1705, 1630 cm^{-1}, indicative of a skeleton of iridoid glycoside [5]. The ^1H NMR spectrum of **1** exhibited the signals at δ5.78(1H, ddd, J = 19.2, 10.7, 8.8Hz, H-8), 5.32(1H, dd, J = 19.8, 1.4Hz, H-10a) and 5.36(1H, dd, J = 11.6, 1.4Hz, H-10b), suggesting that 1 should be a secoiridoid glycoside. The ^{13}C NMR spectrum of **1** displayed signals at δ 125.26(>C=), 152.94(=C<) and 163.20(C=O), indicating the presence of a conjugated system of C=C-C=O. And absent was the vinylic H-3 at about δ 7.0 typical proton signal of most iridoids, and present were the signals at δ5.54(1H, d, J = 1.4Hz, H-3) and the methoxyl protons at δ 3.51, which indicated that the double bond was located between C-4 and C-5 positions and the methoxyl group might be attached to the C-3 position. In addition , three proton signals(ABX) at δ4.44(dd, J = 12.0, 1.7Hz), 4.41(dd, J = 12.0, 2.4Hz) and 4.15(1H, br s*) were observed, indicating the presence of an -O-CH_2-CH-O- unit and a hydroxyl group attached to C-6 position. In order to confirm the proposed structure, 2D NMR experiments were performed. The ^1H and ^{13}C NMR signals were fully assigned by a combination of ^1H-^1H and ^1H-^{13}C COSY spectra. In the COLOC spectrum $({}^nJ_{(CH)} = 4.0Hz)$ [6,7] of 1, the long range coupling cross peaks were observed between C-3 and methoxyl protons, methxyl carbon and H-3, C-7 and H-6, C-4 and H-6, and so on(see Table 1). This further confirmed that the methoxyl group was attached to C-3 and the hydroxyl group being attached to C-6. The remaining ambiguities for the configuration of the methoxyl and hydroxyl groups were unravelled as follows. The signals of H-7 at δ 4.44(dd, J = 12.0, 1.7Hz), 4.41(dd, J = 12.0, 2.4Hz) and H-6 at δ4.15(1H, br s*) showed that their coupling patterns were ae and ee. Thus, the H-6 should be α-orientated at C-6, and the hydroxyl group attached to C-6 should be β-configuration. For the configuration of methoxyl group, as the signal of H-3 at δ5.54(1H, d, J = 1.4Hz)displayed a small coupling constant which was deduced to be resulted from a W(or M) coupling with H-1, the H-3 should be α-configuration and the methoxyl group should be β-orientated at C-3. From the above evidence, the structure of **1** was established and was named qinjiaoside A.

Compounds **2** and **3** were identified as gentiopicroside and harpagoside, respectively, by comparison of ^1H, ^{13}C NMR spectra and TLC behaviour with those of authentic samples [8—11]. The former is a common iridoid glycoside in Gentianaceae plants [8,10—13] while the latter was reported to be present in the families of Labiatae, Pedaliaceae and Scrophulariaceae [11]. It is noted that harpagoside was isolated for

* This signal should be a double doublet. The reason for this signal to have become a br s(or m)is that the coupling constants(J = 1.7, 2.4Hz) are relatively small and to some extent further splitted by the proton of the hydroxyl group attached to C-6.

the first time from the family of Gentianaceae.

Compound **4** exhibited quasi-molecular ion peak at m/z 737[M−H]$^-$ in the negative FAB mass spectrum. On comparison of ^{13}C and ^{1}H NMR spectra with those of daucosterol(5), **4** showed an additional β-D-glucopyranosyl unit. It was deduced that this terminal glucose was attached to C-6 of the inner glucose, for the signal of C-6 was displaced downfield at δ69.54, characteristic of 6-O-glycosylated glucose. Thus, compound **4** was determined as β-sitosterol-3-O-gentiobioside.



1

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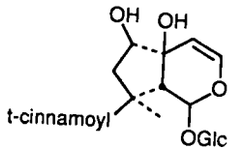

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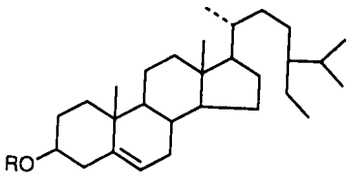

R

5 − Glc

4 − Glc−6−Glc

EXPERIMENTAL

^{1}H and ^{13}C NMR(DEPT) spectra were recorded at 400 MHz in CD_3OD unless otherwise noted using TMS as int. std. 2D NMR of ^{1}H—^{1}H and ^{1}H−^{13}C COSY and COLOC were performed with established usual pulse sequence.

Plant material. The whole plant of *Gentiana macrophylla* was collected in Xining, Qinghai province, and identified by Professor J.-S. Yang.

Extraction and isolation. The roots (2140 g)of dried plant were extracted with MeOH under reflux. After removal of solvent by evapn, the combined extracts(570 g) were suspended in H_2O, extracted successively with Et_2O and $CHCl_3$. The H_2O layer was concd and subjected to column chromatography on macroporous absorptive resin D-101 with aq. MeOH to give four frs. namely fr. 1(H_2O eluate; 16.0 g), fr. 2(30% MeOH eluate; 28.6 g), fr.3(60—80% MeOH eluate; 30.5 g) and fr.4(MeOH eluate; 16.0 g). Fr.2 was separated by repeated silica gel column chromatography with $CHCl_3$-MeOH (9 : 1) to give **1** (530 mg) and **2**(440 mg). Fr.3 was chromatographed on silica gel with $CHCl_3$-MeOH gradient (20 : 1 to 14 : 1) and then purified by RP-8 column chromatography with 60% MeOH to give **3**(12 mg). Fr.4 was repeatedly chromatographed on silica gel with $CHCl_3$-MeOH (20 : 1 to 15 : 1) to afford **4**(17 mg) and **5**(80 mg).

Qinjiaoside A (1). A pale yellow powder, $[\alpha]_D^{27}$−150.4 ° (MeOH; c=0.245); UVλ_{max}(EtOH)nm (lgε): 205.5(4.0), 214.5(4.0); IRν_{max}(KBr) cm^{-1}: 3400, 2920, 1705, 1630, 1400, 1070; FAB-MS(Neg.) m/z: 403[M−OH]$^-$, 387[M−OH]$^-$, 241[M−Glc−H]$^-$, ^{1}H and ^{13}C NMR (see Table 1).

Gentiopicroside (2). A pale yellow amorphous powder, ^{1}H NMR: δ5.66(1H, d, J=2.8Hz, H-1),

7.45(1H, s, H−3), 5.61(1H, m, H−6), 5.07, 4.97(2H, m, H−7), 5.74(1H, ddd, J = 17.5, 10.5, 7.0Hz, H−8), 3.15(1H, m, H−9), 5.23(2H, m, H−10), 4.64(1H, d, J = 8.0Hz, Glc H−1); ^{13}C NMR: aglycone moiety(C−1 to C−11) δ98.51, 150.61, 104.92, 127.00, 117.16, 70.87, 135.98, 46.58, 118.50, 166.27; Glc(C−1 to C−6)δ100.18, 74.52, 77.95, 71.50, 78.36, 62.74.

Harpagoside (3). A pale yellow amorphous powder, ^{1}H NMR: δ6.17(1H, s, H−1), 6.41(1H, d, J = 6.4Hz, H−3), 4.93(1H, dd, J = 6.4, 1.6Hz, H−4), 2.02(1H, dd, J = 15.2, 4.4Hz, H−7a), 2.27(1H, d, J = 15.2 Hz, H−7b), 2.93(1H, s, H−9), 1.55(3H, s, H−10), 4.62(1H, d, J = 8.0 Hz, Glc H−1), 7.61(2H, m, Cinn H−2, 6), 7.40(3H, m, Cinn H−3, 4, 5), 6.50(1H, d, J−16.0 Hz, Cinn α−H), 7.66(1H, d, J = 16.0 Hz, Cinn β−H); ^{13}C NMR: aglycone moiety(C−1 to C−10)δ94.40, 143.81, 106.36, 73.18, 77.22, 45.90, 88.38, 55.10, 22.64; Glc(C−1 to C−6)δ99.69, 74.11, 77.22, 71.40, 77.22, 62.75; Cinn(C−1 to C−9)δ135.24, 129.73, 128.96, 131.26, 128.96, 129.73, 145.77(α), 119.79(β), 168.28.

Table 1 NMR spectral data and COLOC correlations of compound 1 in CD_3OD(ppm)

C / H	^{1}H NMR (J, Hz)	^{13}C NMR	COLOC (^{n}J = 4.0 Hz)
1	5.43 d (4.3)	95.28	C−1 / H−3, H−1′
3	5.54 d (1.4)	95.44	C−3 / OCH_3
4		125.26	C−4 / H−6, H−3
5		152.94	
6	4.15 br s	61.76	C−6 / H−6, H−7
7	4.44 dd (12.0, 1.7)	73.34	C−7 / H−6, H−7
	4.41 dd (12.0, 2.4)		
8	5.78 ddd (19.2, 10.7, 8.8)	134.46	
9	3.26 dd (8.1, 4.4)	46.74	C−9 / H−10
10	5.32 dd (19.8, 1.4)	121.56	C−10 / H−10
	5.36 dd (11.6, 1.4)		
11		163.20	C−11 / H−7
OCH_3	3.51 s	56.63	OCH_3 / H−3
Glc−1′	4.69 d (8.0)	99.16	C−1′ / H−2′
2′	3.18 dd (8.0, 8.1)	74.65	C−2′ / H−3′
3′	3.41 dd (8.0, 7.7)	77.93	C−3′ / H−2′, H−3′
4′	3.31*	71.61	C−4′ / H−3′
5′	3.30*	78.40	C−5′ / H−5′
6′	3.87 dd (12.0, 1.8)	62.70	C−6′ / H−5′
	3.66 dd (11.9, 5.4)		

* Coupling patterns overlapped due to overcrowded.

β−sitosterol−3−O−gentiobioside (4). Colourless crystal, mp 253—259℃, IRν_{max}(KBr)cm^{-1}: 3420, 2925, 2910, 2840, 1600, 1462, 1440, 1312, 1403, 1371, 1310, 1275, 1260, 1215, 1178, 1155, 1100, 1075, 1035, 1020, 992; FAB−MS(Neg.)m / z: 737[M−H]$^-$; ^{1}H NMR(C_5D_5N): δ4.97(1H, d J = 7.7 Hz, Glc H−1), 5.15(1H, dJ = 7.6Hz, Glc′ H−1); ^{13}C NMR(C_5D_5N): sugar moiety, inner Glc(C−1 to C−6): δ102.97, 75.19, 78.51, 71.59, 77.23, 69.45; terminal Glc(C−1 to C−6): δ105.46, 75.26, 78.51, 71.71, 78.71, 62.80.

Daucosterol (5). Colourless crystal, mp 291—293℃; IR, ^{1}H, ^{13}C NMR spectra and TLC behaviour were identical with those of authentic sample.

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