

坚龙胆中的一个新裂环烯醚萜甙*

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摘要: 从坚龙胆 (*Gentiana rigescens*) 的根中分离得到 1 个新的裂环烯醚萜甙, 命名为坚龙胆甙 A (1), 以及 8 个已知化合物: 龙胆苦甙 (2), 6'-*O*- β -D-吡喃葡萄糖基-龙胆苦甙 (3), 马钱子酸 (4), 6'-*O*- β -D-吡喃葡萄糖基-马钱子酸 (5), 獐牙菜甙 (6), 2'-(邻, 间-二羟基苯甲酰基)-獐牙菜甙 (7), 獐牙菜苦甙 (8), 四乙酰开联番木鳖甙 (9)。它们的化学结构通过现代波谱解析得以鉴定。化合物 3, 5 和 9 为首次从坚龙胆中分离得到。

关键词: 坚龙胆; 裂环烯醚萜甙; 坚龙胆甙 A

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A New Secoiridoidal Glucoside from *Gentiana rigescens* (Gentianaceae)

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Abstract: A new acylated secoiridoidal glucoside, named gentiorigenoside A (1), was isolated from the root of *Gentiana rigescens* (Gentianaceae), together with eight known compounds, gentiopicroside (2), 6'-*O*- β -D-glucopyranosyl gentiopicroside (3), loganic acid (4), 6'-*O*- β -D-glucopyranosyl loganic acid (5), sweroside (6), 2'-(*o*, *m*-dihydroxybenzyl)-sweroside (7), swertiamarin (8) and secologanoside (9). Their structures were elucidated on the basis of detailed spectroscopic analysis. Iridoidal glucosides 3, 5 and 9 were isolated for the first time from the title plant.

Key words: *Gentiana rigescens*; Iridoidal glucosides; Gentiorigenoside A

“Long-Dan” is a well-known traditional Chinese medicinal (TCM) herb commonly used for treatment of inflammation, hepatitis, rheumatism, cholecystitis and tuberculosis. In the Chinese Pharmacopoeia, the roots of four plants from the genus *Gentiana* (Gentianaceae), *G. scabra*, *G. manshurica*, *G. triflora*, and *G. rigescens*, are used as the raw materials of “Long-Dan” (Editorial Board of Chinese Pharmacopoeia, 2005). Among them, the former three species

are mainly distributed in the northeast of China, while the later one is mainly growing in the southwest of China, particularly in the mountainous areas of Yunnan province and called as “Jian-Long-Dan”.

Like many of the *Gentiana* plants, previous study showed that *G. rigescens* contains rich iridoidal glucosides with gentiopicroside (2) as major constituents (Sun and Xia, 1984; Zhang and Tian, 1991). Our further detailed chemical investigation on this plant led

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to the isolation of a new acetylated secoiridoidal glycoside, named gentiorigenoside A (**1**), together with eight known iridoidal glucosides (**2–9**) from the root. Their structures were identified by detailed extensive NMR spectroscopic experiments, including ^1H - ^1H COSY, HMBC and HMQC. This paper describes the isolation and structure determination of these compounds. Furthermore, a possible biogenetic pathway of these compounds was also proposed.

Results and Discussion

The methanolic extract of the roots of *G. rigescens* was suspended into water and partitioned successively with EtOAc and *n*-butanol. Repeatedly chromatography of the EtOAc, *n*-butanol and H_2O fractions on Diaion HP20SS, MCI-gel CHP20P, Chromatorex ODS and silica gel column let to the isolation of a new compound **1**, together with 8 known constituents (**2–9**). The known compounds were identified as gentiopicroside (**2**) (Liu and Li, 1994), 6'-*O*- β -D-glucopyranosyl gentiopicroside (**3**) (Mpondo and Chulia, 1988), loganic acid (**4**) (Calis *et al.*, 1984), 6'-*O*- β -D-glucopyranosyl loganic acid (**5**) (Tomita and Mouri, 1996), sweroside (**6**) (Luo and Nie, 1992), 2'-(*o*, *m*-dihydroxybenzoyl)-sweroside (**7**) (Tan *et al.*, 1996), swertiamarin (**8**) (Luo and Nie, 1992) and secologanoside (**9**) (Calis and Sticher, 1984), respectively, by direct comparison of their spectral data (including IR, MS, and ^1H and ^{13}C NMR) with those of the literature values. Of them, compound **2** was the major constituent in the root, and **3**, **5** and **9** were isolated for the first time from the title plant.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned as $\text{C}_{22}\text{H}_{30}\text{O}_{12}$ on the basis of the HR-ESI-MS (m/z 485.1354 [M-H] $^-$, Calcd. 485.1346) and ^{13}C NMR data. The ^1H and ^{13}C NMR data of **1** were closely related to those of **2** (Liu and Li, 1994), except for the obvious downfield shift of C-4 (δ 121.2), C-5 (δ 136.3), and C-11 (δ 172.8), together with the appearance of two acetyl groups [δ_{H} 2.05, 2.07 (each 3H, s); δ_{C}

20.8, 20.7 and 172.8 ($\times 2$)] and one ethoxyl [δ_{H} 4.10 (2H, q, $J = 7.0$ Hz) and 0.88 (3H, t, $J = 7.0$ Hz); δ_{C} 61.2 and 14.4] group. These observations suggested that the six-membered lactone ring in **2** was opened, acetylated and esterified in **1**, which was further confirmed by the HMBC experiment.

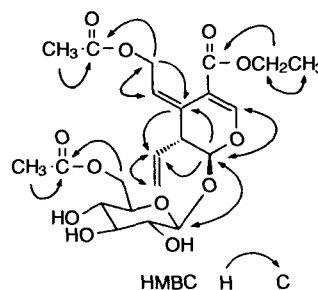


Fig. 1 Important HMBC correlations of **1**

In the HMBC spectra of **1** (Fig. 1), the methylene (δ 4.10) was correlated with the carbonyl carbon at δ 172.8 (C-11), indicating that C-11 was esterified with an ethyl group. The downfield chemical shift of C/H-7 (δ_{C} 70.9, δ_{H} 5.04 and 5.00) and C/H-6' (δ_{C} 64.5, δ_{H} 4.09 and 4.40), together with the HMBC correlations of H-7 and H-6' with two acetyl carbonyl carbon at δ 172.8 could assign the two acetyl groups located on C-7 position of the aglycone and C-6' position of the glucopyranosyl unit, respectively. In addition, other key HMBC correlations (Fig. 1) confirmed the structural of **1**. Thus, the structure of gentiorigenoside A was determined as shown in **1**, which should be a natural product since ethanol was avoided during all the extraction and isolation procedure.

The isolated compounds could be divided into three groups, iridoidal glucosides (**4**, **5**), secoiridoidal glucosides (**1**, **9**) and secoiridoidal glucosides with lactone rings (**2–3**, **6–8**). A possible biogenetic pathway of these compounds was proposed as shown in Fig. 2. It suggested that compound **4** could be a precursor of all the other iridoidal glucosides biosynthesized in *G. rigescens* and compound **2**, the major constituents in the root, is an important intermediate for the formation of **3**, **6**, **7** and **8**.

Experimental

General Experimental Procedures Optical rotations were measured on a SEPA-3000 automatic digital polarimeter. IR spectra were conducted on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were measured in CD_3OD and recorded on a Bruker AV-400 (for ^1H NMR and ^{13}C NMR) and DRX-500 (for 2D NMR) instrument with TMS as internal standard. ESI-MS (negative ion mode) and HR-ESI-MS (negative ion mode) spectra were recorded on VG AutoSpe 3000 and API Qstar Pulsar LC/TOF

Spectrometers, respectively. Column chromatography (CC) was performed on Diaion HP20SS (Mitsubishi Chemical Co.), MCI-gel CHP20P (75–150 μm , Mitsubishi Chemical Co.), Chromatorex ODS (100–200 mesh, Fuji Silysia Chemical Co. Ltd.), and silica gel (100–200 mesh, Qingdao Haiyang Chemical Co.). Thin layer chromatography (TLC) was carried on silica gel G pre-coated plates (Qingdao Haiyang Chemical Co.) with $\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (7:3:0.5). Spots were detected by spraying with 10% sulfuric acid reagents followed by heating.

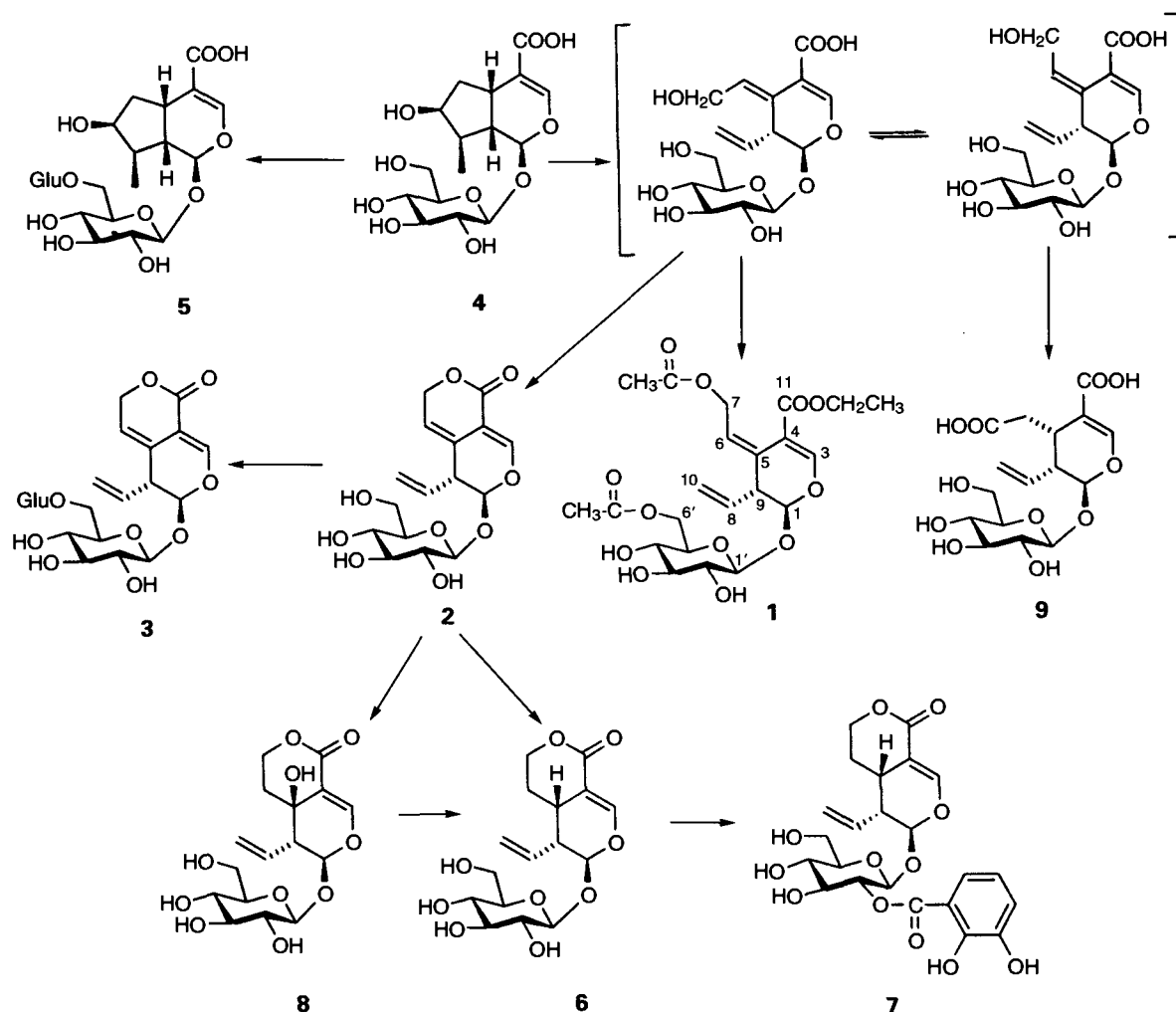


Fig. 2 A possible biogenetic pathway of the isolated iridoidal glucosides 1–9 in *G. rigescens*

Plant material The roots of *Gentiana rigescens* Fr. ex Hemsl. were collected from Chuxiong, Yunnan, China, and identified by Prof. Chong-Ren Yang. The voucher specimen (yang0204100) was deposited in Herbarium of Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS).

Extraction and Isolation The powdered air-dried roots of

G. rigescens (9.2 kg) were extracted with MeOH at room temperature. After concentrated to almost dryness under reduced pressure, the MeOH extract (1290 g) was suspended into H_2O and partitioned with EtOAc and *n*-BuOH, successively. TLC analysis showed that gentiopicoside (2) was the major component which was existed in all of the three fractions [$\text{CHCl}_3\text{:MeOH:H}_2\text{O}$ (7:3

:0.5), R_f 0.6]. The further isolation was then focused firstly on this major component and its related compounds.

The H_2O -layer was concentrated to a small volume (400 ml) and applied to a Diaion HP20SS column eluting with H_2O -MeOH (1:0-0:1) to give five fractions (A_1 - A_5). Fractions A_1 (11.5 g) and A_2 (51 g) was separately subjected to Chromatorex ODS and silica gel CC to give **3** (49 mg), **4** (8.367 g) and **5** (21 mg) from A_1 , and **2** (38.927 g), **4** (10.768 g), **8** (10 mg) and **9** (56 mg) from A_2 , respectively. Fraction A_4 (36 g) was chromatographed over MCI-gel CHP20P and silica gel columns to afford **2** (28.303 g).

The n -BuOH fraction (430 g) was chromatographed on a Diaion HP20SS column eluting with H_2O -MeOH (1:0-0:1) to give seven fractions (B_1 - B_7). Fraction B_2 (160 g) was subjected to MCI-gel CHP20P, Chromatorex ODS and silica gel CC to give **2** (85.146 g) and **4** (43.254 g). Fraction B_6 (4.6 g) was chromatographed over Chromatorex ODS, silica gel and MCI gel CHP-20P to give **1** (5 mg), **6** (57 mg), **7** (2.355 g).

Repeated CC of EtOAc fraction (10 g) on silica gel and Chromatorex ODS to afford **2** (5.983 g), β -sitosterol (186 mg) and daucosterol (78 mg).

Gentiorigenoside A (**1**): White amorphous powder, $[\alpha]_D^{26} = -89.4^\circ$ ($c = 0.17$ MeOH); UV λ_{max}^{MeOH} (log ϵ): 203.8 nm; IR (KBr): 3441, 2924, 2854, 1712, 1612, 1270, 1077 cm^{-1} . HR-ESI-MS: m/z 485.1354 [M ($C_{22}H_{30}O_{12}$)- H] $^-$, Calcd. 485.1346. ESI-MS: m/z 485 (M^-), 457, 419, 256; 1H -NMR (CD_3OD): δ 7.43 (1H, s, H-3), 5.73 (1H, ddd, $J = 9.5, 17.0, 4.3$ Hz, H-8), 5.60 (1H, m, H-6), 5.48 (1H, d, $J = 3.2$ Hz, H-1), 5.21 (1H, dd, $J = 9.5, 1.2$ Hz, H-10a), 5.18 (1H, dd, $J = 17.0, 1.2$ Hz, H-10b), 5.04 (1H, br d, $J = 16.7$ Hz, H-7b), 5.00 (1H, br d, $J = 16.7$ Hz, H-7a), 4.64 (1H, d, $J = 7.9$ Hz, H-1'), 4.40 (1H, dd, $J = 1.9, 11.9$ Hz, H-6b'), 4.10 (2H, q, $J = 7.0$ Hz, CH_3-CH_2-O), 4.09 (1H, dd, $J = 7.1, 14.2$ Hz, H-6a'), 3.55 (1H, m, H-5'), 3.35 (1H, dd, $J = 4.3, 3.2$ Hz, H-9), 3.30-3.35 (2H, m, H-3', 4'), 3.15 (1H, m, H-2'), 2.05, 2.07 (each 3H, s, $CH_3-C=O$), 0.88 (3H, t, $J = 7.0$ Hz, CH_3-CH_2-O). ^{13}C -NMR (CD_3OD): δ 172.8 ($\times 3$, C-11,

$2 \times CH_3-C=O$), 150.5 (C-3), 136.3 (C-5), 134.9 (C-8), 121.2 (C-4), 118.6 (C-10), 117.3 (C-6), 100.4 (C-1'), 98.8 (C-1), 77.8 (C-3'), 75.7 (C-5'), 74.5 (C-2'), 71.3 (C-4'), 70.9 (C-7), 64.5 (C-6'), 61.2 (CH_3-CH_2-O), 46.7 (C-9), 20.8, 20.7 ($CH_3-C=O$), 14.4 (CH_3-CH_2-O).

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