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Anti-Inflammatory Secoiridoids from the Medicinal Herb *Gentianopsis barbata*

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Five new secoiridoids, gentianopsins A–E (**1**–**5**), along with two known analogues (**6** and **7**) were isolated from the whole plants of the medicinal herb *Gentianopsis barbata*. Their structures were elucidated by a comparison of extensive spectroscopic analysis (1D and 2D NMR, and HRMS) and quantum chemical calculations. Gentianopsins A (**1**) and B (**2**) represented two unusual skeletons of *trihomo*-secoiridoids. Anti-inflammatory activity of these isolates was evaluated via suppressing the secretion of cytokines TNF-α and IL-6 in LPS-induced macrophages RAW264.7. Significant inhibitory activity was observed for compounds **3** and **7** on IL-6 secretion with IC_{50} values of 10.22 and 13.30 μM, respectively.

Introduction

Secoiridoids, featured by a 7,8-*seco*-cyclopentano-pyranoid skeleton, are biogenetically formed by the oxidative cleavage and modification of the iridoids.[1] Naturally occurring secoiridoids only account for a small portion of cyclic ether terpenoids, and are mainly derived from the families Gentianaceae, Oleaceae, and Caprifoliaceae.[2] A variety of pharmacological effects including anti-diabetic, anti-inflammatory, immunosuppressive, and neuroprotective are reported for this class of monoterpene derivatives.[3,4]

The genus *Gentianopsis*, belonging to the Gentianaceae family, encompasses 24 species worldwide, among which five species are found in China.[5] *G. barbata*, widely distributed from the northeast to the southwest regions in China, is used in the traditional Mongolian medicine for treating liver and bile diseases, with efficacies of heat-clearing, detoxicating, anti-inflammation, and cholagogue.[6,7] However, to date there is only few study focusing on the phytochemical and pharmacological investigation of *G. barbata*, with several xanthones and two iridoid glucosides reported.[8–10]

In our efforts to discover bioactive molecules from traditional Chinese medicine, a comprehensive phytochemical investigation on the whole plants of *G. barbata* was undertaken, resulting in the isolation of five new secoiridoids, gentianopsins A–E (**1**–**5**), and two known analogues (**6** and **7**) (Figure 1). Gentianopsins A (**1**) and B (**2**) are the rare *trihomo*-secoiridoids that were seldom reported. Herein, the details of isolation, elucidation, and anti-inflammatory bioassay of these secoiridoids were described.

Results and Discussion

Gentianopsin A (**1**) was isolated as a colorless oil, with a molecular formula $C_{15}H_{19}NO_6$ as determined by its HRESIMS (*m/z* 308.1135 $[M-H]^-$, calcd 308.1134). The ^1H-NMR spectrum (Table 1) of **1** revealed signals of four olefinic protons [δ_H 7.43 (1H, s), 5.82 (1H, ddd, $J=17.3$, 10.5, 5.6 Hz), 5.18 (1H, ddd, $J=10.5$, 1.9, 1.0 Hz), 5.11 (1H, ddd, $J=17.3$, 1.9, 1.0 Hz)], and a methoxyl [δ_H 3.63 (3H, s)]. The ^{13}C NMR (DEPT) spectra (Table 1) displayed 13 carbon resonances, attributed to one methoxyl (δ_C 52.0), five methylenes (including an olefinic at δ_C 118.5), five methines (including two olefinic at δ_C 135.9 and 153.4, and an oxygenated at δ_C 83.9), and four quaternary carbons (including an olefinic at δ_C 111.6, and three ketos at δ_C 168.2, 172.9, and 178.1). Interpretation of the HSQC spectrum of **1** assigned all the proton signals to their respective carbons. The ^1H-^1H COSY spectrum of **1** (Figure 2) gave two proton spinning coupling systems, H-3/H_2-8/H-6/H-7/H-14/H_2-15 and H_2-10/H_2-11/H_2-12. The key HMBC correlations from H-3/H-6/H-14 to C-1, H_2-10 to C-1/C-3, and H_2-11 to C-13 (Figure 2), and the featured deshielded chemical shift of C-10 (δ_C 47.2), established the lactam core of the secoiridoid moiety with a butyric acid side chain. Besides, the HMBC correlations from H-3/H-6 to C-4, H_2-8 to C-5, H-6 to C-9, and the methoxyl to C-9, as well as the downfield chemical shifts of C-3 (δ_C 83.9) and C-4 (δ_C 153.4), deduced the fusion of a C_3-unit via C-5-C-6 and C-4-*O*-C-3 of the lactam, forming an additional six-membered ring. Thus, the planar structure of **1** was determined. Based on the small coupling constant between H-6 and H-7 ($J=1.7$ Hz) (Table 1), and the key NOESY correlations of H_2-15 with H-3/H_2-8 (Figure 2), the relative configuration of **1** was assigned. Finally, ECD calculations were performed, and the results (Figure 3) clarified

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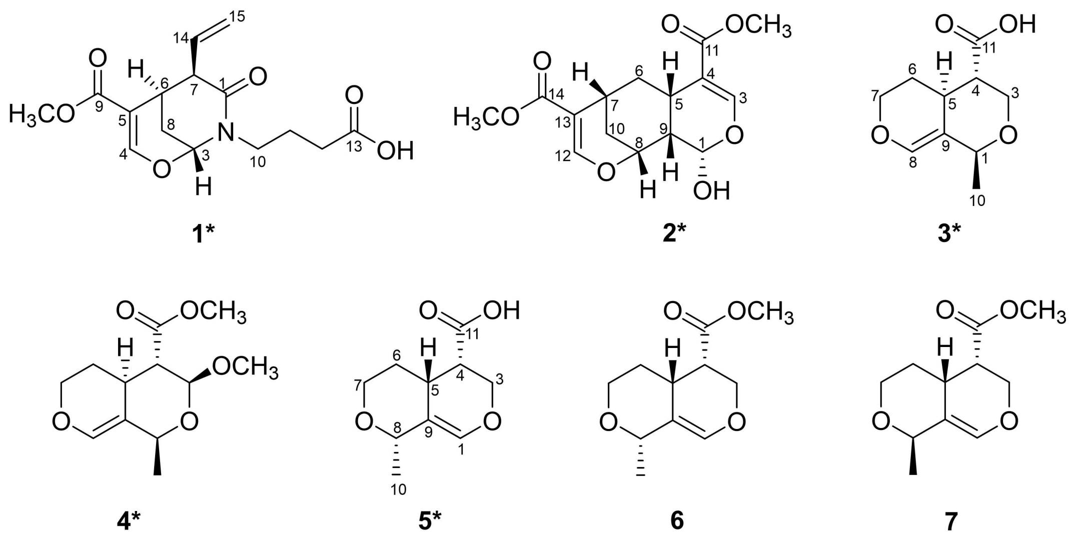

Figure 1. Chemical structures of compounds **1**–**7**.

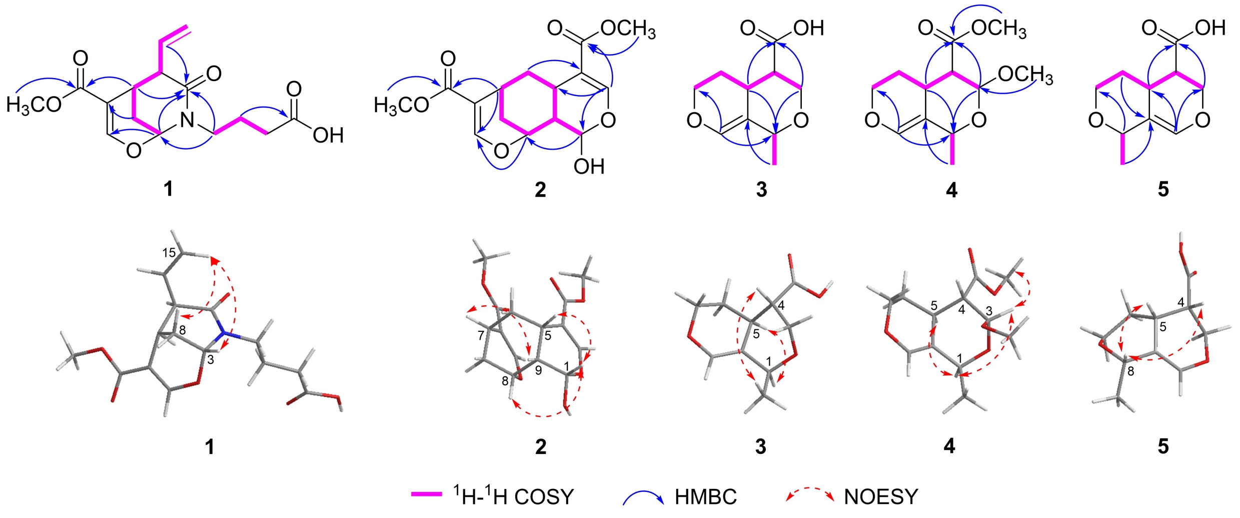

Figure 2. Key ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of compounds **1**–**5**.

the absolute configuration of **1** as shown in Figure 1. From a biogenetic consideration, the *trihomo*-secoiridoid skeleton of **1** could be formed by an enzymatic catalysis of a secoiridoid with a C_3-unit from malonic acid.[11,12]

Gentianopsin B (**2**) was also obtained as a colorless oil, having the molecular formula $C_{15}H_{18}O_7$ according to the HRESIMS (m/z 310.1057 $[M+H]^+$, calcd 310.1053). In the ^{1}H-NMR spectrum (Table 1), two olefinic protons [δ_H 7.16 (1H, d, $J=2.5$ Hz), 7.36 (1H, s)], two oxygenated methine protons [δ_H 4.69 (1H, m), 5.51 (1H, br s)], and two methoxyls [δ_H 3.60 (3H, s), 3.66 (3H, s)] were clearly shown. The ^{13}C NMR (DEPT) spectrum of **2** (Table 1) displayed 15 carbon resonances, corresponding to two methoxyls (δ_C 51.4, 51.5), two methylenes, seven methines (including two olefinic at δ_C 152.9 and 159.6, and two oxygenated at δ_C 74.5 and 95.7), and four quaternary carbons (including two olefinic at δ_C 110.2 and 109.7, and two ketos at δ_C 169.2). The key ^{1}H-^{1}H COSY relationship of H-1/H-9/H-8/H$_2$-10/H-7/H$_2$-6/H-5/H-9, and HMBC correlations from H-1 to C-3/C-5/C-8, H-3 to C-1/C-5/C-11, and H$_2$-6 to C-4 (Figure 2), established the dicyclic secoiridoid moiety. The additional C_3-unit was linked to the secoiridoid via C-13-C-7 and C-12-*O*-C-8, as evident by the HMBC correlations from H-7 to C-12/C-14, H$_2$-6/H$_2$-10 to C-13, and H-8 to C-12. The two methoxyls were located by their respective HMBC correlations to C-11 or C-14. The relative configuration of **2** was determined by the key NOESY correlations (H-1 with H-5/H-8, and H-7 with H-9) (Figure 2). Finally, the absolute configuration of **2** was assigned by calculated ECD method (Figure 3).

Gentianopsin C (**3**), colorless oil, possessed a molecular formula $C_{10}H_{14}O_4$ based on the HRESIMS (m/z 197.0821 $[M-H]^-$, calcd 197.0814). The ^{1}H-NMR spectrum of **3** (Table 1) clearly showed the presence of an olefinic proton [δ_H 6.43 (1H, br s)] and one methyl [δ_H 1.27 (3H, d, $J=6.3$ Hz)]. The ^{13}C NMR (DEPT) spectrum indicated 10 carbon resonances, classified as one methxyl (δ_C 17.2), three methylenes (including two oxygenated at δ_C 65.5 and 69.7), four methines (including an olefinic at δ_C

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Table 1. ^1H (600 MHz) and ^{13}C (150 MHz) NMR data of compounds **1**–**5** (δ in ppm, *J* in Hz).

No.	1		2		3		4		5	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	–	172.9 s	5.51 br s	95.7 d	3.95 m	74.1 d	4.06 qt (6.3, 1.5)	70.4 d	6.43 s	139.2 d
3a	5.52 m	83.9 d	7.16 d (2.5)	152.9 d	4.07 dd (11.3, 4.4)	69.7 t	4.46 d (8.5)	102.8 d	4.19 dd (10.5, 3.6)	66.8 t
3b	–	–	–	–	3.55 t (11.3)	–	–	–	3.71 t (10.5)	–
4	7.43 s	153.4 d	–	110.2 s	2.28 td (11.3, 4.4)	51.3 d	1.96 dd (11.5, 8.5)	55.0 d	2.49 td (10.5, 3.6)	48.0 d
5	–	111.6 s	3.02 m	24.2 d	2.56 m	34.4 d	2.49 m	33.1 d	2.58 m	35.2 d
6a	2.78 m	29.6 d	2.93 br d (14.3)	30.4 t	2.14 m	30.0 t	1.79 m	28.0 t	1.95 ddt (13.0, 4.0, 2.5)	34.0 t
6b	–	–	1.71 dd (14.3, 6.3, 3.4)		1.60 m	–	1.48 m	–	1.32 qd (13.0, 4.0)	–
7a	3.11 dt (5.6, 1.7)	52.8 d	2.72 m	25.4 d	4.04 m	65.5 t	3.92 dt (10.9, 3.8)	64.1 t	3.94 m	68.2 t
7b	–	–	–	–	3.77 dt (10.8, 3.8)	–	3.66 td (11.0, 2.2)	–	3.58 td (11.6, 2.0)	–
8a	1.82 m	23.6 t	4.69 m	74.5 d	6.43 br s	140.4 d	6.35 br s	139.6 d	3.96 m	73.7 d
8b	2.30 dt (13.8, 2.3)	–	–	–	–	–	–	–	–	–
9	–	168.2 s	2.21 br d (6.8)	44.8 d	–	115.1 s	–	112.4 s	–	117.0 s
10a	3.55 m	47.2 t	1.90 m	30.8 t	1.27 d (6.3)	17.2 q	1.23 d (6.3)	15.3 q	1.26 d (6.4)	17.4 q
10b	3.25 m	–	1.76 m	–	–	–	–	–	–	–
11	1.79 m	24.8 t	–	169.2 s	–	175.5 s	–	172.7 s	–	175.5 s
12	2.17 t (7.3)	33.2 t	7.36 s	159.6 d	–	–	–	–	–	–
13	–	178.1 s	–	109.7 s	–	–	–	–	–	–
14	5.82 ddd (17.3, 10.5, 5.6)	135.9 d	–	169.2 s	–	–	–	–	–	–
15a	5.18 dd (10.5, 1.9)	118.5 t	–	–	–	–	–	–	–	–
15b	5.11 dd (17.3, 1.9)		–	–	–	–	–	–	–	–
3-OMe	–	–	–	–	–	–	–	–	–	–
9-OMe	3.63 s	52.0 q	–	–	–	–	–	–	–	–
11-OMe	–	–	3.60 s	51.4 q	–	–	3.60 s	50.9 q	–	–
14-OMe	–	–	3.66 s	51.5 q	–	–	–	–	–	–

140.4, and an oxygenated at δ_C 74.1), and two quaternary carbons (including an olefinic at δ_C 115.1, and a keto at δ_C 175.5). The ^1H-^1H COSY relationships of H_2-3/H-4/H-5/H_2-6/H_2-7 and H-1/ H_3-10, and key HMBC correlations from H_2-3/H-5 to C-1/C-11, H-8 to C-1/C-7, and H_3-10 to C-9 (Figure 2) established the planar structure of **3**. Then, through analysis of the NOESY correlations (H-1 with H-5, and H-4 with H_3-10) (Figure 2), the large coupling constant between H-4 and H-5 ($J=11.3$ Hz) (Table 1), and the calculated ECD result (Figure 3), the structure of **3** was fully elucidated as shown in Figure 1.

Gentianopsin D (**4**) was also colorless oil with a molecular formula $C_{12}H_{18}O_5$ as determined from the HRESIMS (*m/z* 265.1049 $[M+Na]^+$, calcd 265.1052). Detailed comparison of the ^1H and ^{13}C NMR data of **4** and **3** (Table 1) suggested that they had the similar chemical structures. The major difference is that the C-3 methylene (δ_H 4.07, 3.55; δ_C 69.7) of **3** was replaced by a downfield methine (δ_H 4.46; δ_C 102.8) in **4**, and two methoxyls (δ_H 3.33, 3.60; δ_C 55.5, 50.9) were observed in **4**. From the ^1H-^1H COSY relationship of H-3/H-4/H-5/H_2-6/H_2-7, and the HMBC correlations from H-3 to C1/C11 and the two methoxyls to C-11/C-3 respectively, the planar structure of **4** was assigned. The NOESY correlations of H-1 with H-3/H-5, and H-3 with 11-OCH_3 (Figure 2), and the large coupling constants of H-3/H-4 ($J=8.5$ Hz) and H-4/H-5 ($J=11.5$ Hz) deduced the relative configuration of **4**. The absolute configuration of **4** was then confirmed by the ECD calculations (Figure 3).

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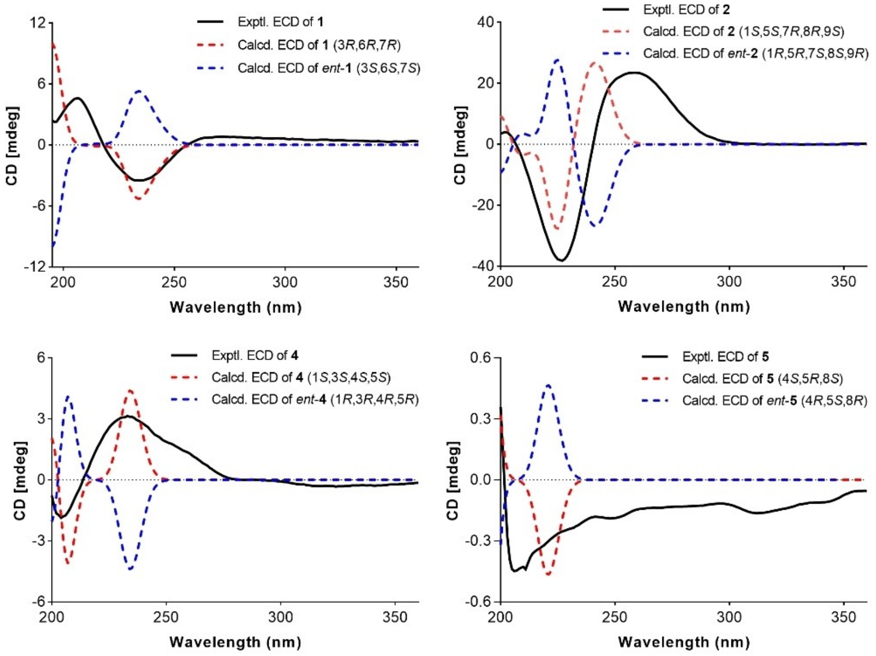

Figure 3. Experimental and calculated ECD spectra of compounds **1**–**5**.

Gentianopsin E (**5**) was obatined as a colorless oil with the molecular formula $C_{10}H_{14}O_4$ according to its HRESIMS (*m/z* 197.0824 [M−H]$^-$, calcd 197.0814). The ^{1}H and ^{13}C NMR data of **5** (Table 1) closely resembled those of hydrangine A (**6**), a known secoiridoid that was isolated from in this study.[13] The only obvious difference was that the methoxyl of **6** (δ_H 3.71, δ_C 51.5) disappeared in **5**, and the C-11 carbonyl shifted slightly downfield (**6**: δ_C 171.5; **5**: δ_C 175.5), indicating the presence of a carboxyl in **5**. Elucidation of the ^{1}H-^{1}H COSY relationship (H_2-3/H-4/H-5/H_2-6/H_2-7 and H-8/H_3-10) and the key HMBC correlations from H-1 to C-3/C-4/C-8, H_2-3/H-5 to C-11, H-8 to C-7, and H_3-10 to C-8 (Figure 2) confirmed the planar structure of **5**. Finally, the NOESY correlations of H-8 with H-4/H-5 (Figure 2) and the calculated ECD result (Figure 3) assigned the same absolute configuration of **5** as that of **6**.

The two known secoiridoids were identified as hydrangines A (**6**) and B (**7**) by comparison of their NMR data with those reported in the literatures.[13]

The anti-inflammatory activity of these secoiridoids was evaluated through suppressing the secretion of cytokines TNF-α and IL-6 in LPS-induced macrophages RAW264.7. As shown in Table 2, compounds **5**–**7** slightly inhibited the secretion of TNF-α with inhibitory rates of 11.50–27.68 % at 20 μM. The inhibitory rates of compounds **1**–**7** against IL-6 secretion ranged from 39.68 % to 62.05 % at 20 μM. The IC_{50} values of compounds **3** and **7** were measured as 10.22 and 13.30 μM, respectively.

Table 2. Anti-inflammatory activity of compounds **1**–**7**.

no.	TNF-α secretion	IL-6 secretion	
	Inhibitory rate (%, 20 μM)	Inhibitory rate (%, 20 μM)	IC_{50} (μM)
1	< 10	40.80 ± 2.95	–
2	< 10	39.68 ± 2.16	–
3	< 10	62.05 ± 3.08	10.22
4	< 10	45.46 ± 2.77	–
5	27.68 ± 1.66	44.32 ± 0.32	–
6	20.22 ± 2.25	42.61 ± 3.43	–
7	11.50 ± 1.31	56.77 ± 1.67	13.30
Dex[a]	92.13 ± 2.55	92.99 ± 3.41	–

[a] Dexamethasone (Dex) was used as a positive control.

Conclusions

In summary, phytochemical investigation towards the whole plants of the traditional Mongolian medicine *G. barbata* resulted in the isolation and identification of five new secoiridoids, gentianopsins A–E (**1**–**5**), and two known analogues (**6** and **7**). The *trihomo*-secoiridoid skeletons of gentianopsins A (**1**) and B (**2**) were rarely reported. Anti-inflammatory assay of these isoaltes revealed their different potencies in inhibiting cytokine secretion. These findings enriched the chemical diversity of secoiridoids, and contributed to a deeper understanding on the chemical composition and potential pharmacological application of *G. barbata*.

Experimental Section

General Experimental Procedures

Column chromatography (CC) was carried out on silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China),

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neutral Al_2O_3 (200–300 mesh, Shanghai Titan Scientific Co., Ltd., Shanghai, China), Sephadex LH-20 (20–100 μm, Amersham Pharmacia Biotech, Sweden), and MCI gel CHP-20 P (70–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan). Thin-layer chromatography (TLC) was performed on silica gel (GF_{254}, 10–40 μm, Qingdao Marine Chemical Factory). TLC spots were detected under UV light or by heating after spraying with 5% H_2SO_4 in EtOH (v/v). Semipreparative HPLC was performed on an Agilent 1260 series instrument with a Zorbax SB-C_{18} column (5 μm, 9.4×250 mm, 3 mL/min). NMR experiments were carried out using a Bruker AvanceNeo-600 with TMS as the internal standard. Mass spectra were obtained on a Waters Synapt XS or an Agilent G6230 spectrometer. ECD and UV spectra were measured on a Chirascan qCD spectrograph. Optical rotations were obtained on an Autopol-I spectropolarimeter.

Plant Material

The whole plants of *Gentianopsis barbata* (Froel.) Ma (Gentianaceae) were collected from Lijiang, Yunnan of China in October 2019, and identified by Wen-Qing He from Kunming Caizhi Biotechnology Co., Ltd. A voucher specimen (Gb-2019-10) was kept in the Innovative Institute of Chinese Medicine and Pharmacy, Chengdu University of Traditional Chinese Medicine.

Extraction and Isolation

Dried whole plants of *G. brabata* (5 kg) were extracted with methanol (30 L×3) at room temperature. The extracts were combined and evaporated to dryness under reduced pressure to yield an oily residue (510 g). The residue was subjected to silica gel CC eluted first with petroleum ether (PE) and then with a CH_2Cl_2/Me_2CO stepwise-gradient system (1:0, 9:1, 4:1, 1:1, 0:1, v/v) to give seven fractions (Fr. 1-Fr. 7). Fr. 2 (27 g) was applied to MCI gel CC eluted with a MeOH/H_2O stepwise-gradient system (from 5:5 to 10:0, v/v) to obtain four subfractions (Fr. 2-1-Fr. 2-4). Fr. 2-1 (4.5 g) was subjected to silica gel CC eluted with PE/Me_2CO stepwise-gradient system (8:1, v/v) to obtain three subfractions (Fr. 2-1a-Fr. 2-1c). Fr. 2-1c (300 mg) was separated by Sephadex LH-20 CC eluted with CH_2Cl_2/MeOH (1:1, v/v), and then applied to semi-preparative RP-HPLC using MeCN-H_2O (30:70, v/v) as the mobile phase to yield **7** (t_R 17.2 min, 1.2 mg), **6** (t_R 18.3 min, 1.2 mg), and **4** (t_R 27.1 min, 1.0 mg). Fr. 6 (35 g) was applied to MCI gel CC eluted with a MeOH/H_2O stepwise-gradient system (from 6:4 to 10:0, v/v) to obtain four subfractions (Fr. 6a-Fr. 6 d). Fr. 6a (3 g) was separated by Sephadex LH-20 CC eluted with CH_2Cl_2/MeOH (1:1, v/v) to get two subfractions (Fr. 6a-1-Fr. 6a-2). Fr. 6a-2 (1.2 g) was applied to Al_2O_3 CC using PE/EtOAc (2: 1, v/v) to obtain two subfractions (Fr. 6a-2a-Fr. 6a-2b). Fr. 6a-2b (300 mg) was subjected to semi-preparative RP-HPLC using MeCN-H_2O (30:70, v/v) as the mobile phase to yield **2** (t_R 5.5 min, 2.1 mg). Fr. 7 (80 g) was applied to MCI gel CC eluted with a MeOH/H_2O stepwise-gradient system (from 6:4 to 10:0, v/v) to obtain seven subfractions (Fr. 7a-Fr. 7 g). Fr. 7c (5 g) was performed Sephadex LH-20 CC eluted with CH_2Cl_2/MeOH (1:1, v/v) to get two subfractions (Fr. 7c-1-Fr. 7c-2). Fr. 7c-1 (1.4 g) was subjected to silica gel CC eluted with CH_2Cl_2/CH_3OH (35:1, v/v) to give four subfractions (Fr. 7c-1a-Fr. 7c-1 d). Fr. 7c-1c (340 mg) was applied to Al_2O_3 CC using PE/Me_2CO (4: 1, v/v), and then by semipreparative RP-HPLC using MeCN-H_2O (30:70, v/v) as the mobile phase to yield **1** (t_R 20.8 min, 1.6 mg). Fr. 7c-2 (1.2 g) was carried out on silica gel CC eluted with PE/Me_2CO (3:1, v/v) to give two subfractions (Fr. 7c-2a-Fr. 7c-2b). Fr. 7c-2a (600 mg) was applied to Al_2O_3 CC using CH_2Cl_2/CH_3OH (15:1, v/v), and then purified by semipreparative RP-HPLC using MeCN-H_2O (30:70, v/v) as the mobile phase to yield **5** (t_R 8.9 min, 8.6 mg) and **3** (t_R 11.0 min, 9.3 mg).

Gentianopsin A (**1**): colorless oil; [α]25 D −12.2 (c 0.1, MeOH); IR (KBr) ν_{max} 3430, 2952, 1707, 1661, 1438, 1384, 1289, 1090 cm^{-1}; HRESIMS m/z 308.1135 $[M-H]^-$ (calc. for $C_{15}H_{18}NO_6$, m/z 308.1134); ^1H and ^{13}C NMR data, see Table 1.

Gentianopsin B (**2**): colorless oil; [α]25 D −11.4 (c 0.1, MeOH); IR (KBr) ν_{max} 3438, 1696, 1633, 1437, 1383, 1297, 1105, 1063 cm^{-1}; HRESIMS m/z 310.1057 $[M+H]^+$ (calc. for $C_{15}H_{19}O_7$, m/z 310.1053); ^1H and ^{13}C NMR data, see Table 1.

Gentianopsin C (**3**): colorless oil; [α]25 D −9.2 (c 0.1, MeOH); IR (KBr) ν_{max} 3429, 2972, 1729, 1624, 1372, 1241, 1189, 1071 cm^{-1}; HRESIMS m/z 197.0821 $[M-H]^-$ (calc. for $C_{10}H_{13}O_4$, m/z 197.0814); ^1H and ^{13}C NMR data, see Table 1.

Gentianopsin D (**4**): colorless oil; [α]25 D −14.3 (c 0.1, MeOH); IR (KBr) ν_{max} 3428, 2963, 1729, 1622, 1384, 1240, 1179, 1064 cm^{-1}; HRESIMS m/z 265.1049 $[M+Na]^+$ (calc. for $C_{12}H_{18}O_5Na$, m/z 265.1052); ^1H and ^{13}C NMR data, see Table 1.

Gentianopsin E (**5**): colorless oil; [α]25 D −8.1 (c 0.1, MeOH); IR (KBr) ν_{max} 3434, 2923, 1728, 1631, 1463, 1383, 1261, 1081 cm^{-1}; HRESIMS m/z 197.0824 $[M-H]^-$ (calc. for $C_{10}H_{13}O_4$, m/z 197.0814); ^1H and ^{13}C NMR data, see Table 1.

ECD Calculations

Conformational analysis was performed by Spartan 14 software package using the MMFF minimization force field. The resulted conformers were further optimized with Gaussian 09 software by the density functional theory (DFT) calculations at the B3LYP/6-31+G(d,p) level in vacuum. The optimized conformations were further applied to ECD calculations using the TD-DFT method with basis set CAM–B3LYP/DGDZVP. ECD spectra of different conformers were simulated using a Gaussian function with a half-bandwidth ranging from 0.12 to 0.40 eV. The overall theoretical ECD spectra were then obtained according to the Boltzmann weighting and simulated experimental spectra by the SpecDis software. Details were provided in the supplemental material.

Anti-Inflammatory Assay

Anti-inflammatory assay was performed according to our previous reports.[14,15] In brief, RAW 264.7 cells were seeded in a 96-well plate at a density of 2×10^4 cells/well for 24 h maintained in Dulbecco's Modified Eagle's Medium (DMEM) media containing 10% fetal bovine serum (FBS), 1% penicillin and 1% streptomycin, and then treated with compounds at 20 μM. Dexamethasone (Dex, Solarbio) was assayed as a positive control. After 1 h, the cells were stimulated with LPS (1 μg/mL) (Beyotime Biotechnology) for 24 h. In addition, a total of 10 μL of CCK-8 (Bioground) was added for each well to check cell viability. After 30 min of incubation, the optical density at 450 nm was subsequently measured using a microplate reader (Molecular Devices). The levels of cytokines (TNF-α and IL-6) in cell supernatant were determined by Enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences Pharmingen) according to the manufacturer's instructions. Cmpounds with better inhibition rates at 20 μM were further selected to measure the IC_{50} values under different concentrations. Triplicate experiments were conducted.

Author Contributions

H. Liu and H.-Z. Cheng performed the isolation, anti-inflammatory assay, and wrote the manuscript. X.-Y. Qi and Y.-Z. Fan

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helped the isolation and structural elucidation. Z.-Z. Yuan and Y.-L. Xu helped the bioactivity assay. Yan Liu, Kai Guo, and Sheng-Hong Li designed the experiments and supervised this study.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Anti-inflammatory activity · Gentianopsins A–E · *Gentianopsis barbata* · Gentianaceae · Secoiridoids

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