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Anti-inflammatory furostanol saponins from the rhizomes of *Smilax china* L

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ABSTRACT

Seven new furostanol saponins (**1–7**), chongrenosides A-G, were isolated from the rhizomes of *Smilax china* L., together with nine known furostanol saponins (**8–16**). The structures of the new furostanol saponins (**1–7**) were elucidated by extensive spectroscopic data analyses (1D and 2D NMR, HRESIMS) and chemical evidence. Compounds **1–6** and **8–16** were evaluated for TNF-α mRNA expression inhibitory activity on LPS induced RAW264.7 cells. Of them, **1**, **4**, **6**, and **11** inhibited the TNF-α mRNA expression by 88%, 87%, 67%, and 93%, respectively, at the concentration of 10 μM.

1. Introduction

The genus *Smilax* (Liliaceae family) comprises about 300 species of climbing flowering shrub. They are widely distributed in the warm areas of East Asia and North America [1]. It is recorded that 19 *Smilax* species are used as folk medicine to treat inflammatory disorders in China [2]. Previous chemical investigations and biological studies on the genus *Smilax* revealed that steroidal saponins are the characteristic constituents with a wide spectrum of biological activities, such as antifungal, anti-inflammatory, and cytotoxic activities [2].

The rhizome of *S. china* L, known as “Jin Gang Teng” in Chinese Pharmacopeia, has been extensively used to treat chronic pelvic inflammation [3]. In a previous study, 20 phenolic compounds were identified from the rhizomes of *S. china* [4]. In a continuous effort to find active anti-inflammatory compounds, reinvestigation on the rhizomes of *S. china* led to the isolation of seven new furostanol saponins (**1–7**), and nine known furostanol saponins (**8–16**). Their structures were determined by extensive spectroscopic data analyses and chemical method. The anti-inflammatory activities of these isolates were also reported.

2. Experimental procedures

2.1. General

Optical rotations were measured with an MCP-500 polarimeter (Anton Parr). IR spectra were recorded in KBr pellets with an IR Affinity-1 spectrometer (Shimadzu). NMR spectra were acquired on an AV-400 spectrometer (Bruker) with TMS as the internal standard. ESI-MS-MS were made on AB Sciex API 4000 QTRAP. HRESIMS experiments were obtained on a Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer. HPLC analysis was carried out on a Shimadzu Essentia LC-16 system equipped with a DAD detector and an analytical Maisch Reprosil C18 column (5 μm, 4.6 × 150 mm, i.d). Column Chromatography (CC) were performed using polyamide (80–100 mesh, Basf), silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd). Sephadex LH-20 (25–100 μm, GE healthcare), MCI gel CHP20P (stationary phase: polystyrene, 75–150 μm, Mitsubishi), Toyopearl HW40F (stationary phase: polyacrylamide, Tosoh Co., Ltd), or ODS (stationary phase: C18, 40–63 μm, Merck).

2.2. Plant material

The rhizomes of *S. china* L. were collected from Xianning City, Hubei Province, China, and identified by co-author, En Fang, from Xianning Institute of Drug Supervision and Inspection. A voucher specimen (NO. SMU-NPC-201601) has been deposited in the Natural Product Chemistry Lab, School of Pharmaceutical Sciences, Southern Medical University.

2.3. Extraction and isolation

Air-dried and powdered rhizomes (2.5 kg) were refluxed with MeOH (3 × 5 L, each 2 h). The resulted solutions were combined and

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Table 1
^{1}H NMR (400 MHz) spectroscopic data (*J* in Hz) for the aglycone moieties of **1–7** (pyridine-d_5).

Positions	1	2	3	4	5	6	7
1ax	0.98 m	0.99 m	0.97 m	0.98 m	0.99 m	0.99 m	0.96 m
1 eq	1.75 m	1.76 m	1.71 m	1.74 m	1.76 m	1.75 m	1.76 m
2ax	1.87 m	1.88 m	1.85 m	1.87 m	1.86 m	1.87 m	1.84 m
2 eq	2.09 m	2.10 m	2.08 m	2.08 m	2.11 m	2.09 m	2.07 m
3	3.89 m	3.89 m	3.89 m	3.96 m	3.89 m	3.90 m	3.87 m
4ax	2.73 t (12.3)	2.74 m	2.73 m	2.72 t (11.8)	2.73 m	2.73 m	2.72 m
4 eq	2.79 dd (4.1, 12.3)	2.81 m	2.78 m	2.79 dd (4.1 11.8)	2.83 m	2.81 m	2.80 m
6	5.33 br.d (4.9)	5.33 br. d (4.7)	5.35 br.d (4.2)	5.31 br.d (4.8)	5.33 br.d (4.9)	5.35 br.s	5.33 br.d (4.8)
7ax	1.48 m	1.48 m	1.50 m	1.45 m	1.46 m	1.45 m	1.48 m
7 eq	1.88 m	1.89 m	1.88 m	1.86 m	1.88 m	1.86 m	1.86 m
8	1.48 m	1.49 m	1.56 m	1.55 m	1.50 m	1.49 m	1.47 m
9	0.93 m	0.94 m	0.96 m	0.88 m	0.92 m	0.91 m	0.89 m
11ax	1.46 m	1.48 m	1.38 m	1.43 m	1.50 m	1.43 m	1.37 m
11 eq	1.46 m	1.48 m	1.53 m	1.43 m	1.50 m	1.47 m	1.44 m
12ax	0.98 m	0.95 m	1.39 m	1.06 m	1.20 m	1.19 m	1.18 m
12 eq	1.68 m	1.68 m	2.69 m	1.69 m	1.78 m	1.76 m	1.72 m
14	0.81 m	0.83 m	0.94 m	0.98 m	0.90 m	0.90 m	0.87 m
15ax	2.37 dd (13.4, 7.2)	2.38 m	2.46 m	1.93 m	4.31 m	4.35 m	1.50 m
15 eq	1.18 m	1.19 m	1.24 m	1.45 m	–	–	2.11 m
16	5.40 m	5.40 m	5.36 m	4.87 m	4.87 m	4.89 m	4.84 m
17	1.22 m	1.23 m	1.63 m	1.94 m	2.53 d (10.5)	2.55 d (10.5)	2.55 d (10.7)
18	0.78 s	0.80 s	1.21 s	0.85 s	0.83 s	0.74 s	1.04 s
19	1.06 s	1.08 s	1.08 s	1.04 s	1.08 s	1.09 s	0.69 s
20a	1.35 m	1.37 m	4.48 m	2.41 m	–	–	–
20b	1.51 m	1.54 m	–	–	–	–	–
21	0.91 t (7.2)	0.91 t (7.4)	1.46 d (5.9)	1.29 d (7.1)	1.79 s	1.80 m	2.13 s
23a	2.47 m	2.48 m	2.45 m	2.11 m.	1.56 m	1.51 m	–
23b	2.47 m	2.48 m	2.45 m	2.47 m	2.16 m	2.12 m	–
24a	1.61 m	1.64 m	1.63 m	1.72 m	1.63 m	1.90 m	2.66 m
24b	2.02 m	2.01 m	2.02 m	1.94 m	2.31 m	2.09 m	3.07 m
25	1.94 m	1.95 m	2.00 m	1.98 m	2.33 m	2.24 m	2.69 m
26a	3.94 dd (9.5, 6.2)	3.98 m	3.98 m	3.97 m	4.13 m	4.17 m	4.01 m
26b	3.56 dd (9.5, 5.9)	3.50 dd (9.3, 6.7)	3.50 dd (9.5, 5.9)	3.63 m	3.55 m	3.59 m	3.58 m
27	0.96 d (6.2)	0.99 d (6.6)	0.97 d (6.3)	1.04 d (5.4)	1.13 d (6.3)	1.18 d (6.6)	1.04 d (6.0)
15a	–	–	–	–	3.73 m; 3.42 m	3.68 m; 3.39 m	–
15b	–	–	–	–	1.60 m	1.61 m	–
15c	–	–	–	–	1.45 m	1.48 m	–
15d	–	–	–	–	0.90 t (7.4)	0.90 t (7.4)	–

concentrated to give crude extract (490 g). The crude extracts were suspended in H_2O (2 L), and partitioned with EtOAc (6 × 3 L) and *n*-BuOH (6 × 3 L), successively. The *n*-BuOH soluble fraction (175 g) was subjected to CC over a polyamide column (9.0 × 25.0 cm) eluting with 20%, 40%, and 60% MeOH/H_2O (1L for each gradient), MCI CHP20P (MeOH/H_2O, 40%–80%, 200 mL for each gradient), and Toyopearl HW40F (MeOH/H_2O, 10%–60%, 200 mL for each gradient) to afford total steroidal saponin fraction (26.0 g). The total steroidal saponin fraction was chromatographed over silica gel column ($CHCl_3$:MeOH:H_2O, 8:2:0.5–6:4:1) to yield two fractions A-B. Fr. A (6.0 g) was repeatedly subjected to CC over silica gel ($CHCl_3$:MeOH:H_2O, 7:3:1–5:4:1) and ODS (MeOH/H_2O, 40%–80%) to yield compounds **2** (11 mg), **3** (27 mg), **5** (60 mg), **6** (23 mg), **7** (61 mg) and **14** (16 mg). Fr. B (4.4 g) was subjected to ODS CC (MeOH/H_2O, 40%-80%) to yield four sub-fractions (Fr. B1–B4). Fr. B1 (0.5 g) was purified over ODS (MeOH-H_2O, 40%-80%) to give compounds **8** (41 mg), **12** (21 mg), and **15** (70 mg). Fr. B2 (2.0 g) was applied to a silica gel CC eluted with $CHCl_3$-MeOH-H_2O (7:3:1–6:4:1), and ODS CC (MeOH/H_2O, 50%-100%) to yield compounds **9** (60 mg), **10** (23 mg), and **11** (24 mg). Fr. B3 (0.7 g) was chromatographed over a silica gel column (EtOAc:EtOH:H_2O, 8.8:1.2:1–8.3:1.7:1), and ODS (MeOH-H_2O, 60%–100%) to yield compound **16** (13 mg). Likewise, Fr. B4 (1.0 g) was purified over silica gel (EtOAc:EtOH:H_2O, 8.7:1.3:1–8.2:1.8:1), and ODS (MeOH/H_2O, 40%–60%) to yield compounds **1** (20 mg), **4** (13 mg), and **13** (23 mg).

2.3.1. *Chongrenoside A* (**1**)

White amorphous powder; [α] -34.5 (*c* 0.10, MeOH); IR(KBr)ν_{max}: 3363, 2933, 2960, 1750, 1500, 1040 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz), see Tables 1 and 2; ^{13}C NMR (pyridine-d_5, 100 MHz) see Tables 3 and 4; ESI-MS-MS: *m/z* 1047.6 $[M-H]^-$, 901.6 $[M-H-146]^-$, 755.0 $[M-H-146\times2]^-$; HRESIMS: *m/z* 1047.5375 $[M-H]^-$ (calcd for $C_{51}H_{83}O_{22}$, 1047.5371).

2.3.2. *Chongrenoside B* (**2**)

White amorphous powder; [α] −55.7 (*c* 0.21, MeOH); IR(KBr)ν_{max}: 3356, 2932, 2852, 1730, 1379, 1020 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz), see Tables 1 and 2; ^{13}C NMR (pyridine-d_5, 100 MHz) see Tables 3 and 4; ESI-MS-MS: *m/z* 1193.8 $[M-H]^-$, 1047.8 $[M-H-146]^-$, 901.4 $[M-H-146\times2]^-$, 755.1 $[M-H-146\times3]^-$; HRESIMS: *m/z* 1193.5936 $[M-H]^-$ (calcd for $C_{57}H_{93}O_{26}$, 1193.5949).

2.3.3. *Chongrenoside C* (**3**)

White amorphous powder; [α] −68.0 (*c* 0.17, MeOH); IR(KBr)ν_{max}: 3362, 2932, 1714, 1379, 1018 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz), see Tables 1 and 2; ^{13}C NMR (pyridine-d_5, 100 MHz) see Tables 3 and 4; HRESIMS: *m/z* 1209.5919 $[M-H]^-$ (calcd for $C_{57}H_{93}O_{27}$, 1209.5904).

2.3.4. *Chongrenoside D* (**4**)

White amorphous powder; [α] −67.9 (MeOH, c 0.1); IR(KBr)ν_{max}: 3682, 3363, 2960, 2933, 1130, 1039 cm^{-1}; (−)-ESIMS: *m/z* 1064