Supporting Information to:

Three New Phenolic Glycosides and a New Triterpenoid from the Stems of *Scolopia chinensis*

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Materials and Methods

Optical rotations were recorded on a Perkin-Elmer 243B digital polarimeter. NMR spectra were recorded on Inova 500 MHz NMR spectrometers. HR-ESI-MS spectra were measured on a Bruker APEX IV FTMS mass spectrometer in positive-ion mode. GC were measured on an Agilent 6890N instrument [HP-5 capillary column (28 m × 0.32 mm i.d.); detection, FID; detector temperature, 260 °C; column temperature, 180 °C; carrier gas, N2; flow rate, 40 mL/min)]. All solvents used were of analytical grade (Beijing Chemical and Industry Factory). Silica gel (200–300 mesh; Qingdao Mar. Chem. Ind. Co. Ltd.), Sephadex LH-20 gel (Pharmacia), and C18 reverse-phased silica gel (150–200 mesh; Merck, performed by applying an N2 pressure of 0.12 Mpa) were used for column chromatography. Bis-(p-nitrophenyl) phosphate (Sigma N-3002), L-cysteine (Tianjin BODI Chemical Co. Ltd.), and EDTA (Sigma, purity is above 98 %) were used for the bioactivity assay.

The stems of S. chinensis were collected in December 2004 from Guangxi Province, P. R. China. The plant was identified by Chao-Liang Zhang, Medical Material Company of Guangxi Province. A voucher specimen (SC20041205) is deposited in the herbarium of the Peking University Modern Research Center for Traditional Chinese Medicine.

Extraction and isolation

The dried stems (57.8 kg) of S. chinensis were extracted 2 times with 80% EtOH (2 × 200 L). After evaporation of the solvent, the soluble fraction was suspended in H2O and extracted successively with petroleum ether (PE, 4 × 5 L), EtOAc (4 × 5 L), and n-BuOH (3 × 5 L).

The EtOAc extract (248 g) was subjected to column chromatography on silica gel (100–200 μm, 15 × 100 cm, 1.5 kg) and eluted with a gradient of PE–Me2CO (5:1, 2:1, 1:1, and 1:5; each 8 L) to give fractions 1–10. Fraction 2 (2 g) was subjected to silica gel column chromatography (200–300 μm, 5 × 40 cm) and eluted with PE–Me2CO (5:1, 2 L) to give 10 as colorless needles (22 mg).

Fraction 3 (40 g) was subjected to silica gel column chromatography (100–200 μm, 10 × 80
cm) and eluted with CHCl₃–MeOH (15:1, 10 L) to give 7 fractions (Frs. 3.1–3.7). Fraction 3.3 (3 g) was subjected to silica gel column chromatography (200–300 μm, 5 × 40 cm) and eluted with n-hexane–Me₂CO (5:1, 3 L) to obtain 12 (14 mg). Fraction 3.4 (3 g) was subjected to silica gel column chromatography (200–300 μm, 5 × 40 cm) with elution of n-hexane–Me₂CO (3:1, 3 L) to yield 11 (32 mg). Fraction 3.5 (4 g) was subjected to silica gel column chromatography (200–300 μm, 5 × 50 cm) and eluted with n-hexane–Me₂CO (4:1, 3 L) to get 13 (26 mg). Compounds 14 (6 mg), 15 (11 mg), and 4 (5 mg) were obtained from Fr. 3.7 (1.2 g) by column chromatography on silica gel (200–300 μm, 4 × 40 cm) and eluted with CHCl₃–Me₂CO (5:1, 1.5 L).

Fraction 4 (6 g) was subjected to silica gel (100–200 μm, 5 × 60 cm) column chromatography and eluted with a gradient of CHCl₃–MeOH (100:1, 20:1, 10:1, 3:1, and 1:1, each 500 mL) to give 7 fractions (Frs. 4.1–4.7). Fr. 4.5 (1.2 g) was subjected to silica gel column chromatography (200–300 μm, 3 × 40 cm) and eluted with CHCl₃–EtOAc (1:1, 1 L) to obtain 1 (149 mg). Fr. 4.6 (1.4 g) was subjected to LH-20 size exclusion chromatography with CHCl₃–MeOH (1:1) elution (1 mL/min) to give 7 (23 mg). Fr. 4.7 (1.9 g) was chromatographed by silica gel (200–300 μm, 4 × 40 cm) with n-hexane–EtOAc (1:1, 1.5 L) to provide 5 (13 mg).

Fraction 5 (17 g) was chromatographed by silica gel (100–200 μm, 6 × 60 cm) with a gradient of CHCl₃–MeOH (30:1–5:1, 5 L) to give seven fractions (Frs. 5.1–5.7). Fr. 5.3 (2.3 g) was subjected to silica gel column chromatography (200–300 μm, 4 × 40 cm) and eluted with CHCl₃–Me₂CO (25:1, 10:1, 3:1, and 1:1, each 500 mL) to give three fractions (Frs. 5.3.1–5.3.3). Fr. 5.3.2 was applied to semi-preparative HPLC with 40% MeOH/0.1% TFA-H₂O (2.5 mL/min) to yield 3 (11 mg, t_R = 86 min) and 8 (22 mg, t_R = 92 min). Fr. 5.4 (2.1 g) was subjected to silica gel column chromatography (200–300 μm, 4 × 40 cm) and eluted by CHCl₃–MeOH–HCOOH (10:1:0.02, 2 L) to give 8 (210 mg) and 9 (15 mg).

Fraction 6 (37.5 g) was chromatographed by silica gel (100–200 μm, 10 × 60 cm) and eluted
with CHCl₃–MeOH–H₂O (lower layer, 5:1:1, 10 L) to obtain four fractions (Frs. 6.1–6.4). Frs. 6.1 (10.1 g) and 6.2 (2.7 g) were recrystaled respectively in EtOAc to yield 19 (2.6 g) and 6 (199 mg). Fr. 6.3 (2.3 g) was subjected to silica gel column chromatography (200–300 μm, 3 × 40 cm) with n-hexane–ethyl formate (1:5, 1.2 L) to give 2 (16 mg). Fr. 6.4 (3.8 g) was subjected to LH-20 with MeOH (1 mL/min) to obtain 16 (11 mg).

The n-BuOH extract (1.5 kg) was subjected to column chromatography on HPD 100 (15 × 100 cm) and eluted successively with H₂O, 20% EtOH, 40% EtOH, and 95% EtOH. The 20% EtOH residue (93.1 g, dryness) was subjected to silica gel column chromatography (100–200 μm, 10 × 100 cm) with a gradient of CHCl₃–MeOH (8:1, 4:1 and 2:1, each 5 L) to obtain seven fractions (Fr. B1–B7). Fr. B2 (20 g) was subjected to Sephadex LH-20 column chromatography with 70% MeOH (2 mL/min) to give 17 (21 mg) and 18 (16 mg).

Bioactivity assay

The bioactivity assay was carried out with 33 mM Tris–HCl buffer, pH 8.8, with 0.00035 U/well final concentration using a microtiter plate assay and 0.33 mM bis-(p-nitrophenyl) phosphate as substrate. L-Cysteine and EDTA were used as positive controls (with inhibitory rates of 5.65% and 72.4% at 5 μM, respectively). After 30 min pre-incubation of the enzyme with the test samples (all with its purity >95%), enzyme activity was monitored spectrophotometrically at 37 °C on a microtiter plate reader (Bio-Rad model 680) by following the rate (change in OD/min) of release of p-nitrophenol from p-nitrophenyl phosphate at 405 nm. All assays were conducted in triplicate.
Fig. 1S Structures of compounds 5–19.
Fig. 2S ¹H-NMR spectrum of compound 1 (acetone-\(d_6\), 500 MHz).

Fig. 3S ¹³C-NMR spectrum of compound 1 (acetone-\(d_6\), 125 MHz).
Fig. 4S HR-ESI-MS spectrum of compound 1.

Fig. 5S $^1$H-NMR spectrum of compound 2 (acetone-$d_6$, 500 MHz).
Fig. 6S $^{13}$C-NMR spectrum of compound 3 (acetone-$d_6$, 500 MHz).

Fig. 7S HR-ESI-MS spectrum of compound 2.
Fig. 8S $^1$H-NMR spectrum of compound 3 (CD$_3$OD, 500 MHz).

Fig. 9S $^1$H-NMR spectrum of compound 3 (CD$_3$OD, 500 MHz).
**Fig. 10S** $^{13}$C-NMR spectrum of compound 3 (CD$_3$OD, 500 MHz).

**Fig. 11S** HR-ESI-MS spectrum of compound 3.
Fig. 12S $^1$H-NMR spectrum of compound 4 (acetone-$d_6$, 500 MHz).

Fig. 13S $^1$H-NMR spectrum of compound 4 (acetone-$d_6$, 500 MHz). [[Authors: This is the same]
Fig. 14S $^{13}$C-NMR spectrum of compound 4 (acetone-$d_6$, 125 MHz).
Fig. 15S HR-ESI-MS spectrum of compound 4.