Lignan Constituents from *Chloranthus japonicus* Sieb.

Hai-xue Kuang, Yong-gang Xia, Bing-you Yang, Qiu-hong Wang, and Shao-wa Lü
Heilongjiang University of Chinese Medicine, No. 24, Heping Road, Harbin 150040, P. R. China

(Received October 29, 2008/Revised February 19, 2009/Accepted March 6, 2009)

A new coumarinolignan glucoside named yinxiancaoside C, along with five known benzofuran lignans, have been isolated from the whole plant of *Chloranthus japonicus* Sieb. The structures of compounds 1-6 were elucidated by chemical and spectroscopic methods including 1D-NMR, 2D-NMR, ESI-MS and HR-ESI-MS. Five known benzofuran lignans were firstly discovered in the Chloranthaceae. In addition, the cytotoxic activity of the isolated compounds against human hepatoma (Hepg-2), ovarian carcinoma (OV420), and breast cancer (MCF-7) cells was investigated by MTT method.

**Key words:** Chloranthaceae, *Chloranthus japonicus* Sieb., Coumarinolignan glucoside, Benzofuran lignans, Cytotoxicity

**INTRODUCTION**

The genus *Chloranthus* has been taxonomically placed in the Chloranthaceae, and comprises ca. 12 species in China. Some species in this genus have long been used as folk medicine for their anti-tumor, anti-fungal, and anti-inflammatory activities. The natural products described in the literatures as constituents of genus *Chloranthus* are volatile oil, simple coumariins, amide alkaloids and sesquiterpene lactones with unusual 3,5,6-ring system (Kawabata et al., 1984). It is worth mentioning that *Chloranthus* plants are chemotaxonomically characteristic due to the presence of typical sesquiterpene lactones having a lindenane skeleton named shizukanolides and chloranthalactones (Kawabata et al., 1979 and 1985; Uchida et al., 1980; Tahara et al., 1981). Their structures and biological activities have been of interest to natural product chemists. Recently, a series of dimeric lindenanes have been isolated from *Chloranthus* spp. (Kawabata et al., 1995 and 1998; Li et al., 2006).

*Chloranthus japonicus*, named yinxiancao in China, is a perennial herbaceous plant, which has comprehensively distributed in China. This herb has the long history to be used as Chinese folk medicine for the treatment of cough, bone fracture and rheumatalgia. In the course of searching for biologically active substances from *C. japonicus*, a new coumarinolignan glucoside, along with five known benzofuran lignans were isolated (Fig. 1). This paper deals with the experimental details of isolation and structural elucidation of these compounds and the cytotoxicity-assay results.

**MATERIALS AND METHODS**

**General**

The melting points were measured on Kofler micromelting point apparatus (uncorrected). The optical rotations were recorded on a Perkin-Elmer 341. The UV and NMR spectra were recorded on SHIMADZU UV-1601 and Bruker DPX 400 (400 MHz for $^1$H-NMR and 100 MHz for $^{13}$C-NMR), respectively. Chemical shifts are given as $\delta$ values with reference to tetramethylsilane (TMS) as an internal standard, and coupling constants are given in Hz. The HR-ESI-MS analyses were conducted on IonSpec Ultima 7.0T FTICR; ESI-MS were carried out on Finnigan MAT LCQ mass spectrometer. A Hypersil ODS II (6 µm, 4.6×250 mm, Yilite, Da Lian, China) was employed for analytical HPLC (Waters, 2695-2996). Preparative HPLC (Waters, Delta 600-2487) was performed on Pegasil ODS II (5 µm, 10×250 mm, Senshu Pak, Japan). Macroporous absorption...
resin (AB-8 Crosslinked Polystyrene, Nan Kai, Tian Jin, China) and silica gel (200-300 mesh, Yinhai, Qing Dao, China) were employed for column chromatography. ODS-A (120 A, 50 µm) was obtained from YMC Co..

Plant materials
The whole plants of _C. japonicus_ were collected from Suiling district, Heilongjiang Province of China in August 2005. The voucher specimen (20050026) was deposited at Heilongjiang University of Chinese Medicine, Harbin, China.

Test for cytotoxicity in vitro
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium hydrobromide (MTT) was used to determine the cytotoxicity of the compounds. The cytotoxic activity of each compound against three cultured human tumor cells was examined in vitro at Tumor Hospital of Harbin Medical University. The tumor cell lines were Hepg-2 (human hepatoma cells), OV420 (ovarian carcinoma cells), and MCF-7 (breast cancer cells). Details of the cytotoxicity were provided in our previously published paper (Kuang et al., 2008).

Extraction and isolation
The whole air-dried plants (17.5 kg) of _C. japonicus_ were ground to the particle size through standard mesh sieve No. 10 and extracted with 95% EtOH under reflux (10 L, 3×) for 2 h and the combined solution was filtered and evaporated in vacuum to a syrup, suspended in H₂O (2 L) and partitioned with petroleum ether (1 L, 3×). The water layer was evaporated. The residue (530 g) was suspended in H₂O (1 L), which was subjected to AB-8 Crosslinked Polystyrene, sequentially eluted with H₂O, 50% EtOH, and 95% EtOH, respectively. The 50% EtOH elution fraction was concentrated under vacuum to yield a syrup residue, and this crude residue (120 g) was then subjected to silica gel CC and eluted successively with CHCl₃/MeOH (15:1→1:1, v/v) gradient to give 8 fractions (Fr. 1→8). Fr. 2 (5 g) continues silica gel with CHCl₃/MeOH (1:0→5:1, v/v) and ODS column chromatography with MeOH/H₂O (3:7→1:0, v/v) to yield a yellow powder (800 mg), purified by preparative HPLC on a Hypersil-ODS II column (10 µm, 20×300 mm, flow rate 8 mL/min) with MeOH/H₂O (2:3) to afford compound 2 (500 mg, tᵣ = 38.6 min). Fr.5 (10 g) was subjected to silica gel with CHCl₃/MeOH (1:0→1:1, v/v) to afford a number of sub-fractions F5₁-F5₇. F5₃ (3 g) was passed over ODS column chromatography with MeOH/H₂O (3:7→1:0, v/v) and finally purified by preparative HPLC on a Hypersil-ODS II column (10 µm, 20×300 mm, flow rate 8 mL/min) with MeOH/H₂O (2:3) to afford compounds 3 (58 mg, tᵣ = 26.5 min), 5 (60 mg, tᵣ = 23.8 min) and 6 (36 mg, tᵣ = 28.6 min). F5₁ (1 g) was passed over Sephadex LH-20 open column with MeOH/H₂O (1:9→1:0, v/v) to yield two white powders B₁ and B₂ (80 and 123 mg), purified by semi-preparative HPLC on a Pegasus ODS II column (5 µm, 10×250 mm, flow rate 3 mL/min) with MeOH/H₂O (1:4) to afford compound 1 (43 mg, tᵣ = 13.5 min) from B₁ and compound 4 from B₂ (51 mg, tᵣ = 15.7 min), respectively.

Acid hydrolysis
Each compound 1, 3, 4, and 5 (10 mg) was refluxed with 4 N HCl for 2 h. The product was extracted with EtOAc. The aqueous layers of the acid hydrolysis of each compound were adjusted to pH 6 with NaHCO₃ and then concentrated. Glucose was

![Fig. 1. Structures of compounds 1-6](image-url)