A New Natural Compound with Cytotoxic Activity from *Tripterygium hypoglaucum*

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Abstract: **Objective** To find antitumor constituents from *Tripterygium hypoglaucum*. **Methods** The chloroform extract of *T. hypoglaucum* was separated by silica gel column chromatography and preparative HPLC. The structures of compounds isolated were identified by spectral analysis and chemical evidence. **Results** Seven compounds were isolated and identified as rhein ethyl ester (**1**), chrysophenol (**2**), physcion (**3**), emodin (**4**), wilfordine (**5**), wilforgine (**6**), and wilforine (**7**). The cytotoxic activities of the compounds against cancer cell lines were assayed. **Conclusion** Compound **1** is a new natural compound with strong activities against human cancer cell lines (A2780 and OVCAR-3). Compounds **2**－**4** are isolated from this genus plants for the first time. The possible structure-activity relationship among compounds **1**－**4** shows that the methoxy group or oxyethyl moiety might be responsible for the cytotoxity.

Key words: antitumor; chrysophenol; emodin; rhein ethyl ester; *Tripterygium hypoglaucum*

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Introduction

Widely distributed in China, *Tripterygium hypoglaucum* (Levl.) Hutch. (Celastraceae)., has a long history of use in traditional Chinese medicine for treating swelling, fever, chills, sores, joint pain, and inflammation. Previous phytochemical studies on this plant revealed the presence of alkaloids (Duan, Kawazoe, and Takaishi, 1997; Duan *et al*, 2000), diterpenes (Zhang, Wang, and Wu, 1992; Milanova, Han, and Moore, 1995; Zhang *et al*, 2007; Wang *et al*, 2011), and triterpenes (Morota *et al*, 1995a; 1995b; 1995c; Peng and Yang, 2004). In order to find antitumor constituents from this plant, the further chemical research was performed. Seven compounds were isolated from its chloroform extract and the cytotoxic activities of all compounds were tested. Among them, anthraquinone compounds were isolated from the plants of *Tripterygium* Hook. f. for the first time and might be the main substance with antitumor effect in chloroform extract of *T. hypoglaucum*.

Materials and methods

Equipments

Melting points were measured on a Yanaco micro-hot-stage apparatus. Silica gel (200－300 mesh, Qingdao Haiyang Chemical Co., Ltd.), Sephadex LH-20 (Pharmacia), RP-HPLC (Beijing CXTH 3000 system), UV3000 Spectrophotometric Detector, and C_{18} column (Beijing CXTH, Daisogel-C_{18}, 250 mm × 20 mm, 10 μm) were used for isolation, purification, and analysis. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker—ARX—300 Spectrometer, using TMS as an internal standard. DMSO and MTT were purchased from Sigma Chemical Co., Ltd. (USA).

Extraction and isolation

The roots of *Tripterygium hypoglaucum* (Lev1.) Hutch. were collected from Yunnan Province, China, and identified by Prof. SUN Qi-shi in Shenyang Pharmaceutical University (Shenyang, China). A voucher specimen (20080910) has been deposited in Shenyang Pharmaceutical University. The dried roots of

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T. hypoglaucum (60 kg) were extracted with 60% ethanol for three times to afford ethanol extract, and then was suspended in water and extracted with petroleum ether, chloroform, and *n*-butanol, successively. By evaporating the solvent, the chloroform extract was obtained and the extraction rate was 0.09%. Then the chloroform extract was separated by silica gel into five fractions. Fr. A—E eluted with petroleum ether (60—90 ℃) and acetone. Fr. A was chromatographied over Sephadex LH-20 eluted with chloroform and methanol, and silica gel eluted with petroleum ether (60—90 ℃) and ethyl acetate to give compounds **1** (15 mg), **2** (16 mg), **3** (15 mg), and **4** (13 mg). Fr. D was separated by silica gel and preparative HPLC (ODS, 60% MeOH) to give compounds **5** (50 mg), **6** (70 mg), and **7** (66 mg) (Fig. 1).

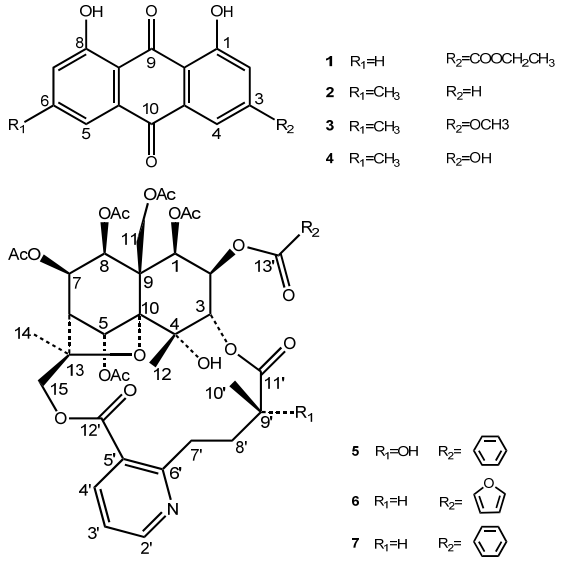

Fig. 1 Structures of compounds 1—7

Antitumor bioassay

Antitumor activity was evaluated by MTT assay (Tan *et al*, 1996). The ovarian cancer A2780 and OVCAR-3 cell lines (ATCC) were used as the target cells in the cytotoxic assay. Normal cell was IOSE144 cell.

For drug exposure experiments, after exposing the drug with cells for 48 h, MTT solution (100 μL) was added to each cell, and the tumor cells were incubated at 37 ℃ in a humidified atmosphere of 5% CO_2 air for 24 h. At the end of incubation, the growth medium was removed and replaced with DMSO (100 μL) at room temperature. The concentrations of samples were 100, 80, 60, 40, 20, and 10 μg/mL. After agitating on a vortex for 10 min, the absorbance was determined at 570 nm on a Bio-Rad microplate reader to calculate IC_{50}.

Results and discussion

Compound **1** was isolated as a yellow needle. Borntrager's reaction was positive indicating that compound **1** was an anthraquinone. The NMR spectral data of compound **1** closely resembled the known compound rhein (Danielsen, Aksnes, and Francis, 1992) except the signals of one O-CH_2CH_3 moiety in the NMR spectra. The O-CH_2CH_3 proton appeared as an AB spin system with a quartet at δ 4.44 (2H, q, J = 7.2 Hz) and a triplet at δ 1.42 (3H, t, J = 7.2 Hz), which corresponded to the carbons resonated at δ 62.1 and 14.2, respectively. This indicated that compound **1** was an ethyl ester of rhein. Two hydroxyl signals were observed at δ 12.02 (1-OH) and 11.97 (8-OH), indicating that the two hydrogen bonds were formed between 1-OH, 8-OH and keto carbonyl group. Thus the O-CH_2CH_3 moiety must not be connected with 1-OH or 8-OH, and the only possible connection was on the carbonyl group. So the structure of compound **1** was established as rhein ethyl ester.

Comparison of ^{1}H-NMR and ^{13}C-NMR data and physical data with those reported in the literature confirmed that compounds **2**—**7** were chrysophenol (**2**) (Danielsen, Aksnes, and Francis, 1992), physcion (**3**) (Isao *et al*, 1992), emodin (**4**) (Danielsen, Aksnes, and Francis, 1992), wilfordine (**5**) (Lin, Ying, and Liu, 1995), wilforgine (**6**), and wilforine (**7**) (Xu, Miao, and Huang, 1995). Among them, compounds **2**—**4** were isolated from this genus plants for the first time.

The cytotoxic activities of all compounds against human cancer cell lines *in vitro* were evaluated by MTT methods. Compounds **1** and **3** exhibited strong activities, while other compounds had no effect (Table 1). The results indicated that the methoxy group or oxyethyl moiety in anthraquinone might be the active group for the cytotoxic activity. In addition, compound **3** with methoxy group displayed cytotoxic activity against the human cancer cell lines as well as normal cells. Compared with compound **3**, compound **1** had no cytotoxic activity against the normal cells, which might be attributed to the existence of ester bond.

Rhein ethyl ester (**1**): yellow needle, mp 268—269 ℃; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 12.02 (1H, s, 1-OH), 11.97 (1H, s, 8-OH), 8.42 (1H, d, J = 1.5 Hz, H-4), 7.94 (1H, d, J = 1.5 Hz, H-2), 7.73 (1H, dd, J = 8.0, 7.4 Hz, H-6), 7.33 (1H, br d, J = 8.0 Hz, H-7), 7.87 (1H, br d, J =

Table 1 IC_{50} values of compounds isolated from *T. hypoglaucum* against ovarian cancer cell lines *in vitro*

Compounds	IC_{50} / (μg·mL^{-1})		
	A2780 cell	OVCAR-3 cell	IOSE144 cell
1	13.1	12.4	>150
2	>100	>100	>100
3	20.0	12.1	17.2
4	>300	>300	>300
5	>100	>100	>100
6	>100	>100	>100
7	>100	>100	>100
Ginsenoside Rg_3	42.4	18.2	>200

7.4 Hz, H-5), 4.44 (2H, q, J = 7.2 Hz, -COOC$\underline{H}_2$CH$_3$), 1.42 (3H, t, J = 7.2 Hz, -COOCH$_2$C$\underline{H}_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.4 (C-1), 125.3 (C-2), 138.2 (C-3), 120.4 (C-4), 120.2 (C-5), 137.7 (C-6), 124.9 (C-7), 162.8 (C-8), 192.8 (C-9), 181.0 (C-10), 133.8 (C-4α), 133.5 (C-10α), 118.2 (C-8α), 115.8 (C-9α), 164.4 (-$\underline{C}$OOCH$_2$CH$_3$), 62.1 (-COO$\underline{C}$H$_2$CH$_3$), 14.2 (-COOCH$_2\underline{C}$H$_3$).

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