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Isolation of a new meroterpene and inhibitors of nitric oxide production from *Psoralea corylifolia* fruits guided by TLC bioautography

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ABSTRACT

A new meroterpene, psoracorylifol F (**1**), was isolated from *Psoralea corylifolia* fruits, along with two known meroterpenes (**2** and **3**), guided by TLC bioautography against $O_2^{\bullet -}$ radicals. The structure of **1** was elucidated by means of NMR, HRESIMS, and X-ray crystallographic analysis. All the three metroterpenes possessed potential inhibitory activity against LPS-induced NO production in RAW 264.7 cells with IC_{50} values ranging from 7.71 to 27.63 μM.

1. Introduction

Nitric oxide (NO) is a diatomic free radical synthesized from L-arginine by constitutive and inducible nitric oxide synthase (cNOS and iNOS) in various mammalian cells and tissues. iNOS is induced by either lipopolysaccharide (LPS) or cytokines such as interferon-γ (IFN-γ) in macrophages [1,2]. The NO produced in this way as well as superoxide ($O_2^{\bullet -}$) and their reaction product peroxynitrite ($ONOO^-$) are excessively produced during the host response against infections and contribute to inflammatory disorders by promoting oxidative stress and tissue injury [3]. Search and discovery of the effective inhibitors of NO production from natural products are in great demand. However, most of the current screening methods are not suitable for plant extracts due to the potential to produce false positive [4]. Therefore, a screening method that would demonstrate how many active compounds are present in a given extract is desirable. TLC bioautography method is such an approach and wildly applied as an effective separation technique during bio-guided fractionation because of its significant advantage of quick localization of the active components in a complicated plant extract.

In our preliminary screening of traditional Chinese medicines (TCM) for inhibitory effect on NO production, the 80% ethanolic extract of *Psoralea corylifolia* fruits was found to show the potential $O_2^{\bullet -}$ scavenging activity by TLC bioautography. *Psoralea corylifolia* Linn (Fabaceae) is an annual plant widely distributed in China and Southeast Asia. Its fruits, known as Psoraleae Fructus (*Buguzhi* in Chinese), are traditionally used for the treatment of spermatorrhea, pollakiuria, asthma, and nephritis [5]. Some compounds isolated from this herb have displayed a wide range of biological activities such as antioxidant [6,7], antibacterial [8], anti-inflammatory [9], and antidepressant-like activities [10] as well as inhibitory activities of baculovirus-expressed

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BACE-1 [11], DNA polymerase and topoisomerase II [12]. As part of our continuous efforts to screen antioxidant and anti-inflammatory agents from TCM [13,14], we have studied the fruits of *P. corylifolia* by TLC bioautography-directed fractionation and isolation. In this paper we described the isolation, structure elucidation, and preliminary biological evaluation of one new and two known meroterpenes (**1**–**3**).

2. Experimental

2.1. General

X-ray data were collected using a Bruker SMART APEX-II CCD diffractometer. UV spectra were recorded on a TU-1901 UV–VIS spectrometer (Purkinje General Instrument Co., Ltd, Beijing, China). IR spectra were recorded on a Bio-Rad FTS-185 spectrometer (Hercules, CA). The NMR spectra were measured on a Bruker Avance 500 NMR spectrometer (Bruker Daltonics, Bremen, Germany). HRESIMS was obtained on a Bruker Daltonics Apex Ultra Fourier Transform mass spectrometer (7.0 T, Bruker Inc., Bremen, Germany). Semi-preparative HPLC was performed on a Waters Prep LC 4000 system equipped with a Waters 2487 UV detector (254 nm) and a YMC-Pack ODS-A column (250×10 mm, 5 μm, 120 Å). Column chromatography was carried out on silica gel (200–300 mesh, Qingdao Marine Chemistry Company, Qingdao, China) and Sephadex LH-20 (Pharmacia, Sweden). Bovine milk xanthine oxidase (X4875-10UN), xanthine, Dulbecco's modified Eagle's medium (DMEM), Greiss reagent, nitroblue tetrazolium (NBT), lipopolysaccharide (*Escherichia coli* 026:B6), and cordycepin were all obtained from Sigma.

2.2. Plant material

The fruits of *P. corylifolia* were purchased from Kangqiao Decoction Pieces Factory (Shanghai, China), and authenticated by Dr. Lihong Wu of Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine where a voucher specimen (No. bgz-090309) is deposited.

2.3. Extraction and isolation

The dried fruits of *P. corylifolia* (8.0 kg) were powdered and extracted three times under reflux by 80% aqueous ethanol for 1 h. The extracts were combined, and ethanol was removed under the reduced pressure to afford 2.0 kg residue. Part of the residue (1.0 kg) was subjected to silica gel column chromatography (CC) eluted with petroleum ether–ethyl acetate mixtures of increasing polarity (30:1, 15:1, 9:1, 4:1, and 1:1) to obtain five fractions A–E according to TLC monitor. TLC bioautography showed that Fr. A and C contained active spots against $O_2^{\bullet -}$. Fr. A (98.0 g) was re-purified by silica gel CC with the eluting solvent of petroleum ether–ethyl acetate (30:1) to afford compound **3** (205 mg). Fr. C (12.7 g) was further separated by a silica gel column eluted with petroleum ether: ethyl acetate (35:1, 25:1, 15:1, 10:1, and 5:1) to afford five subfractions C_{1-5}. The subfraction C_3 (1.15 g) was observed with active spots against $O_2^{\bullet -}$ by TLC bioautography, which was then separated on a column of Sephadex LH-20 eluted with methanol–chloroform (1:1), followed by purification on a semi-preparative HPLC eluted with methanol–water (65:35) at a flow rate of 3 ml/min to give compounds **1** (15 mg) and **2** (30 mg).

Psoracorylifol F (**1**): colorless needles (MeOH); UV (MeOH) λ_{max} 226 and 278 nm; IR (KBr) ν_{max} 3361, 1645, 1614, 1557, and 1514 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 295.1668 $[M+Na]^+$ (cacld for $C_{18}H_{24}O_2Na$, 295.1669).

2.4. TLC bioautography analysis

The 80% ethanolic extract, Fr. A–E, and the pure isolates of *P. corylifolia* fruits were screened for their inhibitory activities against $O_2^{\bullet -}$ using a TLC bioautography method described by Ramallo et al. [15]. Briefly, an aliquot of 80% ethanolic extract methanol solution (10 mg/ml, 10 μl), Fr. A–E (10 mg/ml, 10 μl) methanol solution (2 mg/ml, 10 μl) or individual pure isolate methanol solutions (2 mg/ml, 10 μl) were directly deposited (as bands) onto TLC plates. The TLC plates were then developed in a presaturated solvent tank with petroleum ether (60–90 °C)–ethyl acetate–methanol (6:3:0.5) as developing reagents. The developing distance from application position was 8.0 cm. The developed TLC plate was removed from the solvents, and allowed to air-dry for 3 min, followed by being dipped with a 300 mU/ml XO in 100 mM phosphate buffer (pH 7.9). After being incubated for 20 min at 37 °C in an incubation chamber, the TLC plates were dipped with a mixture of 10 mM xanthine and 20 mM nitroblue tetrazolium (NBT) in 100 mM phosphate buffer (pH 7.9), followed by a second incubation for 20 min at 37 °C. Bands with the $O_2^{\bullet -}$ scavenging activity were observed as white yellow bands on a purple background.

2.5. X-ray crystallographic analysis of **1**

Suitable crystals for X-ray diffraction were obtained by recrystallization from a methanol solution of **1**. Intensity data

Table 1
NMR data of compound **1** in CD_3OD (500 MHz).

Position	Compound **1**	
	δ_H (*J* in Hz)	δ_C
1		134.9
2	6.90, d (8.5)	131.0
3	6.58, d (8.5)	116.1
4		156.8
5	6.58, d (8.5)	116.1
6	6.90, d (8.5)	131.0
7	2.54, t (11.0)	51.0
8	3.37, d (10.5)	80.6
9		43.3
10	1.44, m	37.6
	1.64, m	
11	1.48, m	28.9
	1.54, m	
12	2.24, m	53.4
13		149.1
14	4.36, s	112.3
	4.40, s	
15	1.41, s	20.2
16	5.87, dd (17.5, 11.0)	150.0
17	4.86, dd (11.0, 1.5)	111.9
	4.94, dd (18.0, 1.5)	
18	1.04, s	16.6