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Flavonol dimers from callus cultures of *Dysosma versipellis* and their *in vitro* neuraminidase inhibitory activities

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ABSTRACT

A chemical investigation of callus cultures of *Dysosma versipellis* led to the isolation of five new flavonol dimers, dysoverines A—E (**1**—**5**), together with 12 known compounds (**6**—**17**). The structures of new compounds were determined by the extensive spectroscopic data analyses. The biosynthetic pathway of the new compounds was proposed to involve *O*-methylation, prenylation, and Diels–Alder cycloaddition, which successively occurred in cultured plant cells. Compounds **1**—**17** exhibited *in vitro* neuraminidase inhibitory activities with the IC_{50} values of 31.0—93.9 μM.

1. Introduction

Dysosma versipellis (Hance) M. Cheng is a traditional Chinese herb in the southern regions of China belonging to the Berberidaceae family [1]. This herb is commonly utilized as a substitute resource for plants of the genus *Podophyllum*. Podophyllotoxin can be isolated from their rhizomes for the semi-synthesis of etoposide and teniposide which are clinically used in the treatment of lung cancer and leukemia [2–4]. Previous chemical investigations have described the isolation of a wide range of aryl tetralin lignans and flavonols from wild *D. versipellis* plant [1,5]. In view of the continuous destruction of wild populations of this endangered plant, the optimization of callus and suspension cultures and *in vitro* production of podophyllotoxin or related lignans have been reported [6–8]. However, systematic characterization of chemical components in cell cultures of this plant is limited. In this context, callus cultures derived from fresh leaves of *D. versipellis* have been established and iteratively sub-cultured for biomass accumulation (Fig. S1) and systematic chemical investigation in our group. Herein, we reports the isolation and structural elucidation of five new flavonoid dimers (**1**—**5**) together with 12 known compounds (**6**—**17**) (Fig. 1), their possible biosynthetic pathway and their neuraminidase inhibitory activities.

2. Experimental

2.1. General

Optical rotations were recorded on a Perkin-Elmer Model-343 digital polarimeter; CD spectra, on JASCO J-815 spectropolarimeter; UV spectra, on Shimadzu UV-160 spectrometer; IR spectra, on Nicolet 5700 FT-IR microscope spectrometer (FTIR Microscope Transmission). NMR spectra were performed on ARX-600 spectrometer (Bruker) using DMSO-d_6 as solvent and internal reference, chemical shifts (δ) are given in ppm, and coupling constants (J) are given in hertz (Hz). ESIMS data and HRESIMS data were measured on 6520 Accurate Mass Q-TOF LC/MS spectrometer (Agilent Technologies). Column chromatography (CC) was carried out with silica gel (200—300 mesh, Qingdao Marine Chemical Inc., Qingdao, PR China), and Sephadex LH-20 (GE Healthcare, Sweden). Semi-preparative HPLC was performed on Shimadzu HPLC instrument equipped with Shimadzu RID-10A detector and a C18 column (Microsorb 21.4 × 250 mm, 8 μm, VARIAN). TLC analysis was carried out on pre-coated silica gel GF254 plates (Qingdao Marine Chemical Industry, Qingdao, China) and visualized by spraying with 10% H_2SO_4 (in EtOH) followed by heating at 120 °C.

2.2. Plant material

The whole plant of *D. versipellis* (Hance) M. Cheng was collected from Longzhou, Guangxi in May 2009, and replanted in a flowerpot as

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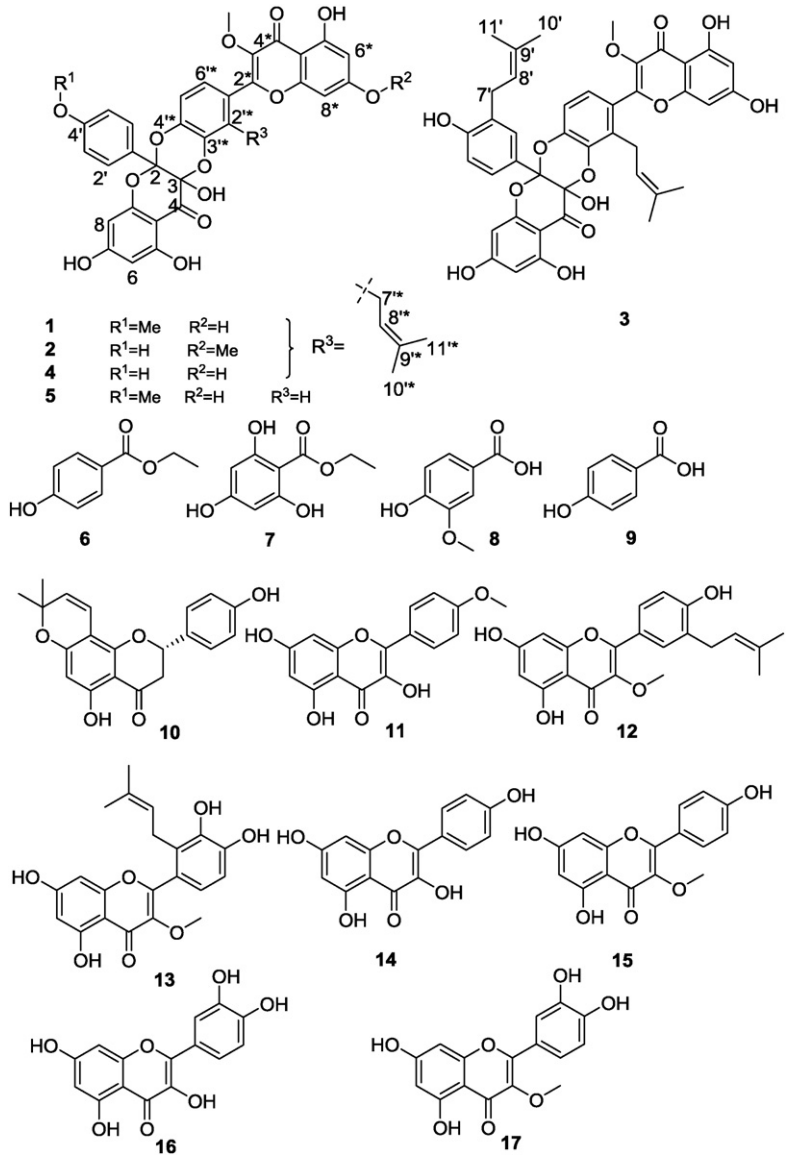

Fig. 1. Chemical structures of compounds **1**—**17**.

experimental sample. Plant material was identified by Prof. Jing-Quan Yuan (Guangxi Botanical Garden of Medicinal Plants). A voucher specimen (ID-26856) has been deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences, PR China.

2.3. Initiation of *D. versipellis* callus cultures

Callus cultures of *D. versipellis* have been initiated on Murashige and Skoog medium supplemented with 3% sucrose, 0.5 mg/L α-naphthaleneacetic acid (NAA), 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), and 0.7% agar. Subculture of initiated callus cultures were performed under conditions of 6,7-V medium supplemented with 3% sucrose, 0.2 mg/L NAA, 0.2 mg/L 6-benzylaminopurine (6-BA), 0.5 mg/L 2,4-D, and 0.7% agar at 25 °C under dark regimes. The callus cultures were subcultured every 15 days for biomass accumulation.

2.4. Extraction and isolation

The full-grown callus clumps were harvested at 1.7 kg of fresh weight. After evaporation of water at 55 °C, dry callus cultures (328 g) were extracted with 95% ethanol (1 L × 3, each 3 h). The ethanolic extract (84.6 g) was suspended in 1 L hot water, and extracted with $CHCl_3$ and EtOAc, successively. The EtOAc extract was concentrated to dryness to afford DE (34.3 g). The extract DE was separated on silica gel H (200 g) by medium pressure CC (6 × 80 cm) eluting with $CHCl_3$/EtOAC/MeOH (1:0:0 to 0:0:1, 20 mL/min) to afford 10 fractions (A–J). The fraction D (17.6 g) was chromatographed over medium pressure column (silica gel H, 6 × 80 cm, 500 g) by using a gradient of $CHCl_3$/MeOH (from 50:1 to 30:1, *v*/*v*; 1 L each) to yield eleven fractions (D1—D11). Fraction D5 (1.9 g) was subjected to Sephadex LH-20 CC (4 × 50 cm, 100 g) by eluting with MeOH to give compound **1** (1.1 g). Fraction D6 (2.5 g) was chromatographed over Sephadex LH-20 (4 × 50 cm, 100 g) by eluting with MeOH to give five fractions, D6a—D6e. Subfraction D6d (100 mg) was subjected to Sephadex LH-20 CC (2 × 40 cm, 20 g) and eluted with MeOH to afford compound **5** (14.2 mg). Purification of fraction D7 (652 mg) by Sephadex LH-20 CC (2 × 40 cm, 20 g) eluting with MeOH resulted in compounds **2** (4.9 mg) and **3** (8.1 mg). Fraction D9 (5.3 g) was purified by medium pressure CC (silica gel H, 6 × 80 cm, 500 g) by eluting with CH_2Cl_2/MeOH (30:1) to give four subfractions (D9a—D9d). Subfraction D9c (1.2 g) was subjected to Sephadex LH-20 CC (4 × 60 cm, 100 g) and eluted with MeOH to achieve compound **4** (1 g). Further information about the isolation of known compounds **6**—**17** is available in Supplementary data.