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# Three pairs of diastereoisomeric flavanone glycosides from *Viscum articulatum*

Haizhen Li [1], Zhun Hou [1], Chao Li, Yao Zhang, Tao Shen, Qingwen Hu, Dongmei Ren *

*Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44 Wenhuaxi Road, Jinan 250012, PR China*

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ABSTRACT

Phytochemical examination of the leaves and stems of *Viscum articulatum* resulted in the isolation of three pairs of new flavanone glycosides, 2*R*/2*S*-viscarticulide A–C (**1a/1b**–**3a/3b**), together with eight known compounds (**7**–**14**). Their structures were established by extensive spectroscopic data analyses. The diastereoisomers were separated by HPLC on a chiral phase and the absolute configuration at C-2 was determined by circular dichroism (CD) spectra. The protective effects of compounds **1**–**3** against $H_2O_2$-induced cytotoxicity with EA.hy926 cells were tested. The results showed that compounds **1**–**3** improved the survival of EA.hy926 cells after $H_2O_2$ exposure at the tested concentrations.

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## 1. Introduction

*Viscum articulatum* Burm, belonging to the family Loranthaceae, is distributed widely in south and southwest of China. The leaves and stems of the plant have been commonly used for the treatment of hemorrhage, pleurisy, gout, heart disease, arthritis, and hypertension in traditional Chinese medicine [1]. Previous phytochemical investigations have revealed that triterpenoids, organic acids and flavonoids are the major secondary metabolites of this plant [2–5]. Although there are a variety of biological activities reports have been published on some species of the genus *Viscum*, only a few of these reports is about *V. articulatum*. The extracts of this plant have been demonstrated to possess significant diuretic activity in rats [6], promising wound healing potential in rats [7], and antihypertensive effect in the NO deficient type of hypertension [1]. In our preliminary studies, the total flavonoids of *V. articulatum* showed cytoprotective activity against $H_2O_2$-induced oxidative stress in EA.hy926 cells. In the course of our ongoing survey for biologically active flavonoids, this phytochemical study on the leaves and stems of *V. articulatum* yielded three pairs of new flavanone glycosides (**1a/1b**–**3a/3b**), together with eight known compounds (**7**–**14**) (Fig. 1). In this paper, we describe the isolation, structure elucidation, stereoanalysis and bioassay of the novel flavanone glycosides.

## 2. Experimental

### 2.1. General experimental procedures

Optical rotation value was measured on an Anton Paar MCP 200 polarimeter. IR spectra were recorded on a Thermo Nicolet NEXUS 670 FTIR spectrometer with KBr disks. NMR spectra were measured on a Brucker AV-600 spectrometer with TMS as internal standard. High-resolution ESI-MS mass spectra were carried out on an LTQ-Orbitrap XL instrument. Semi-preparative HPLC was performed on an Agilent HP 1260 instrument, using an Agilent Eclipse XDB-$C_{18}$ (250 × 9.4 mm I.D., 5 μM) column. The chiral columns (250 × 4.6 mm I.D.) were amylose tris-(3, 5-dimethylphenylcarbamate) immobilized on 5 μm silica gel (Chiralpak IA) and amylose tris-(3, 5-dimethylphenylcarbamate) (Chiralpak AD-H) coated on 5 μm silica gel. The chiral columns were obtained from Daicel (Tokyo, Japan). CD spectra were measured on an applied

* Corresponding author. Tel.: +86 531 88382012; fax: +86 531 88382548.
*E-mail address:* rendom@sdu.edu.cn (D. Ren).
[1] These authors contributed equally to this work.

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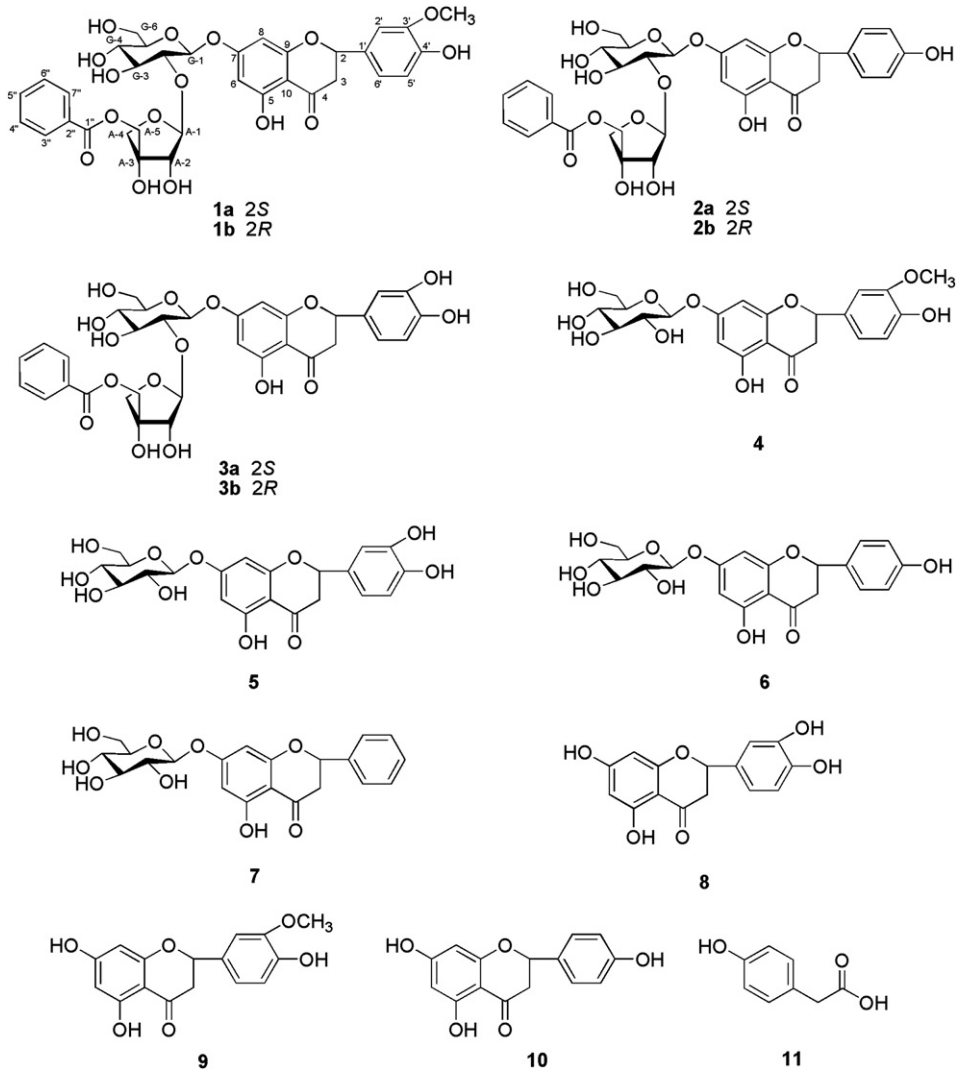


**Fig. 1.** Structure of compounds isolated from *V. articulatum*.

photophysics Chirascan spectrometer, using 1 mm cell. Silica gel (200–300 mesh, Haiyang Co., Qingdao, China), YMC-GEL ODS-A (50 μM, YMC Co., Ltd., Japan), Sephadex LH-20 gel (Amersham Biosciences) and Toyopearl HW-40F (Tosoh, Japan) were used for column chromatography. Precoated silica $GF_{254}$ plates (Haiyang Co., Qingdao, China) were used for TLC analysis.

### 2.2. Plant material

The whole plants of *V. articulatum* were collected from Xishuangbanna, Yunan Province and authenticated by one of the co-author, associate professor Tao Shen. A voucher specimen (No. 20101207-03) has been deposited in the School of Pharmaceutical Sciences, Shandong University.

### 2.3. Extraction and isolation

Air-dried and powdered whole plants of *V. articulatum* (10 kg) were extracted with EtOH–$H_2O$ (95:5, v/v, 20 L × 3, each 3 days) at room temperature. After removal of solvent under reduced pressure, the crude extract (944 g) was suspended in $H_2O$ and partitioned successively with petroleum ether, EtOAc and *n*-BuOH to give three different polar parts. The EtOAc fraction was evaporated in vacuo to give a residue (90 g), which was subjected to silica gel column chromatography (CC) using a gradient system of $CH_2Cl_2$–MeOH (100:0–0:100, v/v) as an eluent to afford five major fractions (EA1–EA5). Fraction EA2 (3.7 g) was further separated into three major subfractions (EA2A–EA2C) by chromatographing over silica gel column with a step gradient system of petroleum ether–EtOAc (30:1, 20:1, 10:1, 5:1, 1:1, v/v). Subfraction EA2B (200 mg) was subjected to Sephadex LH-20 CC eluted with $CH_2Cl_2$–MeOH (1:1) to yield compounds **11** (15 mg). Subfraction EA2C (1.2 g) was submitted to Sephadex LH-20 CC ($CH_2Cl_2$–MeOH, 1:1) followed by semi-preparative HPLC eluted with MeOH–$H_2O$ (65:35, v/v) to produce compounds **8** (10 mg), **9** (30 mg) and **10** (15 mg). Fraction EA4 (6.3 g) was subjected to CC over silica gel and eluted with a $CH_2Cl_2$–MeOH gradient system (50:1 → 1:1), producing three subfractions (EA4A–EA4D). Subfraction EA4A

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