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Two new flavonoids from *Artemisa sacrorum* Ledeb and their antifungal activity

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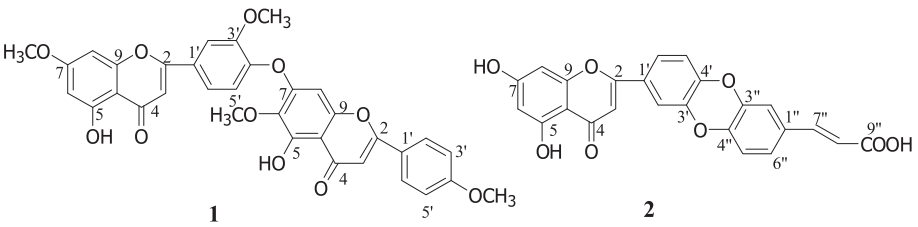
HIGHLIGHTS

- Two new flavonoids were isolated from *A. sacrorum.*
- This is the first report on the structure elucidation of two new compounds based on spectroscopic methods.
- The flavonoids exhibited appreciable inhibition of mycelial growth, with variable activities depending on Fod race.

GRAPHICAL ABSTRACT



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ABSTRACT

Two new flavonoids, named as sacriflavone A (**1**) and sacriflavone B (**2**), were isolated from the $CHCl_3$ extract of *Artemisa sacrorum* Ledeb (*A. sacrorum*). The structures of the isolated compounds have been elucidated unambiguously by UV, MS, and a series of 1D and 2D NMR analyses. The isolated compounds exhibited antifungal activity against different *Fusarium oxysporum* f. sp. dianthi pathotypes.

Introduction

Artemisa sacrorum, belonging to the family Composite, is found in various places in China [1]. Traditionally, it is used to treat ailments like swellings, stomach-ache, brain tingling, chicken pox and abscess [2]. Terpenoids [3–6], organic acids [7], coumarins [8,9], and flavonoids [10] have been isolated from this plant.

Flavonoids are a class of secondary metabolites generally located in plant leaves, flowers and stems [11]. These compounds are not only present in plants as constitutive agents but are also accumulated in plant tissues in response to microbial attack [12,13]. In a search for natural antifungal compounds from the plant, we have performed a phytochemical screening on the aerial parts of *A. sacrorum.* Previous study on *A. sacrorum,* led to he isolation of known coumarins and flavonoids. Now, from the $CHCl_3$ extract of *A. sacrorum* , we have isolated two new flavonoids, named as sacriflavone A (**1**) and sacriflavone B (**2**) (Fig. 1). The flavonoids have been subjected to antifungal tests on different Fod pathotypes to evaluate the possible involvement of *A. sacrorum* flavonoids in resistance tospectra pathogen attack.

Methods

General experimental procedures

The HR-ESI-MS spectra were measured on Bruker Daltonics Micro TOFQ (Bruker, Germany). NMR spectra were measured on a Bruker AV-500 spectrometer (Bruker, Germany) with tetramethylsilane (TMS) as the internal reference, and chemical shifts are

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expressed in δ (ppm). Semi-preparative HPLC (Shimadzu, Japan) was performed by using a Japanese liquid chromatograph equipped with a EZ0566 column. The UV spectra were recorded on a Shimadzu UV-2201 spectrometer (Shimadzu, Japan). The IR spectra were recorded in KBr discs on a Thermo Nicolet 200 double beam spectrophotometer (Shimadzu, Japan). Column chromatography was performed by using silica gel (200–300 mesh, Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Fractions were monitored by TLC (silica gel GF_{254}10–40 μm, Marine Chemical Factory, Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant material

The aerial parts of *A. sacrorum* were collected in Tongliao, Inner Mongolia of China, in June 2013, and identified by Prof. Buhebateer (Inner Mongolia University for Nationalities). A voucher (No. 20130728) has been deposited in the School of Traditional Mongolian Medicine of Inner Mongolia University for Nationalities.

Extraction

The air dried aerial parts of *A. sacrorum* (6.0 kg) were powdered and extracted twice under reflux 95% EtOH (50 L). Evaporation of the solvent under reduced pressure delivered the 95% EtOH extract. The extract was partitioned with petroleum ether (P.E.), $CHCl_3$, EtOAc and n-BuOH, sequentially. The $CHCl_3$ fraction (40.0 g) was isolated by column chromatography on silica gel (500 g) and gradiently eluted with $CHCl_3$–CH_3COCH_3 (60:1–10:1) to give 6 fractions (Fractions 1–6). Fraction 4 [530 mg, $CHCl_3$–CH_3COCH_3 (30:1) elute] was further chromatographed on Sephadex LH-20 column eluted with MeOH, and then separated by semipreparative HPLC (CH_3OH–H_2O, 45:55) yielding **1** (46 mg) and **2** (38 mg). The purity of compounds **1** and **2** was determined to be 95.6% and 99.3% by normalization of the peak areas detected by HPLC.

Biological assays

Fungitoxic activity of flavonoids (**1** and **2**) and commercial rutin (Sigma–Aldrich, MO), was evaluated by means of the poisoned medium technique [14]. Three different pathotypes of Fod, obtained by by Prof. Burie (Inner Mongolia University for Nationalities, China), were employed in this trial: Fod race 2 (isolate 2–75), Fod race 4 (isolate 1.06.04), and Fod race 8 (276). Weighted amounts of each tested compound were aseptically added, after dissolution in 200 mL of sterile H_2O and ultra-filtration (0.1 mm), to flasks containing 20 mL of sterilized potato dextrose agar (PDA), when still molten, to reach the final concentrations of 700, 350 and 35 mM. The medium was then poured into 35 mm i.d. Falcon culture dishes and allowed to solidify. A mycelial disc (2 mm i.d.) taken from 7-day-old culture was inoculated to each Petri dish. Plates containing non-poisoned medium served as control. Mycelial diameters, in control as well as in treatment sets, were recorded after incubation for 72 h at 26 °C. The data obtained on mycelial growth were pooled from four replicates and subjected to one-way ANOVA, after arcsin transformation. Treatments means were compared using critical difference at $P = 0.05$, similarity groups were determined using the Student–Newman–Keuls post hoc test.

Results and discussion

Structure elucidation

Compound **1** was obtained as a green solid. The positive reactions to the HCl–Mg tests suggested that the compound was a flavonoid. UV (MeOH) λ_{max} (nm) (log ε): 261 (3.78), 281 (4.01) and 346 (3.66); IR (KBr) ν_{max} (cm^{-1}): 1708, 1703, 1621, 1484, 1220 and 1103 cm^{-1}. The molecular formula was determined to be $C_{34}H_{26}O_{11}$ by HR-ESIMS at *m/z* 611.1548 $[M+H]^+$, which is consistent with biflavonoid. The ^1H and ^{13}C NMR spectra **1** (Table 1) suggested the presence of two hydrogen-bonded hydroxyl group (δ_H 12.79 and 13.08), a 1,3,4-trisubstituted B ring and a 1,4-disubstituted B′ ring, and a tetrasubstituted A ring and a pentasubstituted A′ ring. The A and B rings were called the partial structure I and the A′ and B′ rings as the partial structure II.

The ^1H NMR spectrum of **1** showed the signals of twelve aromatic protons at δ_H 7.85 (2H, d, $J = 8.0$ Hz), 7.03 (2H, d, $J = 8.0$ Hz), 7.50 (1H, dd, $J = 7.0$, 1.5 Hz), 7.06 (1H, d, $J = 7.0$ Hz), 7.33 (1H, d, $J = 1.5$ Hz), 6.39 (1H, d, $J = 1.5$ Hz) and 6.51 (1H, d, $J = 1.5$ Hz) indicated the presence of an A_2X_2 system for B′ ring (II), an AMX system for B ring (I) and an AB system for A ring (I), and two characteristic signals of H-3 at δ_H 6.59 (1H, s) and 6.58 (1H, s), which was confirmed according to correlations from δ_H 6.59 (H-3, I) to δ_C 182.4 (C-4, I), 164.1 (C-2, I), 105.5 (C-10, I) and 123.3 (C-1′, I), and δ_H 6.58 (H-3, II) to δ_C 182.9 (C-4, II), 164.2 (C-2, II), 105.7 (C-10, II) and 123.5 (C-1′, II) in Fig. 2. The remaining aromatic signal at δ_H 6.61 (1H, s) was assigned to H-8 (II) based on its correlations δ_C 130.4 (C-6, II), 105.7 (C-10, II). In addition, The ^1H NMR spectrum of **1** showed signals for four methoxy groups at 4.06 (3H, s), 4.02 (3H, s), 3.91 (3H, s) and 3.90 (3H, s). The HMBC correlation from δ_H 4.06 to C-6 (II), 4.02 to C-3′ (I), 3.91 to C-4′ (II) and 3.90 to C-7 (I) indicated that the methoxy groups were attached to C-6 (II), C-3′ (I), C-4′ (II) and C-7 (I).

Furthermore, the positive ion ESI-MS spectra of **1** showed a base peak at *m/z* 314, which suggested the possibility that linkages between the flavone units were through 5 (A)-O-5 (A′) and 4′ (B)-O-7 (A′). According to the characteristic resonances at δ_H 12.8 and 13.1 for HO-5 (A) and HO-5 (A′) in the ^1H NMR spectrum, the linkage between two flavone units could be 4′ (B)-O-7 (A′). The correlations observed in the HMBC spectrum (Fig. 2) confirmed further the structure of **1**, in which the correlations of H-6 (I) with C-8 (δc 92.7, I) and C-10 (δc 105.5, I), H-8 (I) with C-6 (δc 98.1, I) and C-10 (δc 105.5, I), H-2′ (I) with C-2 (δc 164.1, I), C-6′ (δc 120.8, I) and C-4′ (δc 149.3, I), H-5′ (I) with C-1′ (δc 123.3, I) and C-3′ (δc 146.8, I), H-6′ (I) with C-2 (δc 164.1, I), C-2′

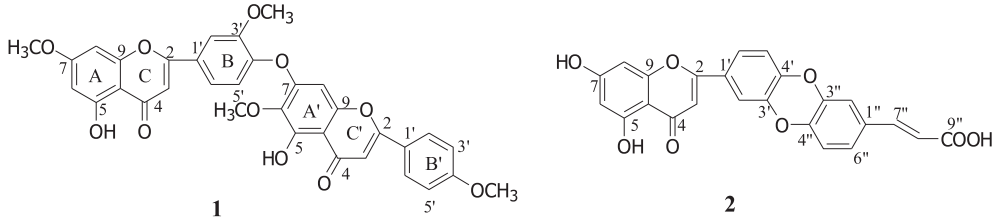

Fig. 1. Structures of compounds **1** and **2**.