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A New Flavone from Flower Buds of *Rosa rugosa*

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A new flavone, rugosaflavonoid H (**1**) were isolated from the flower buds of *Rosa rugosa*. Its structures were determined by spectroscopic methods including 1D and 2D-NMR technique. Compound **1** was also tested for its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) and it exhibited cytotoxicity against NB4 and MCF7 cells with IC_{50} values of 8.2 and 7.6 μM, respectively.

Keywords: Flavone, *Rosa rugosa*, Cytotoxicity.

INTRODUCTION

Rosa rugosa Thunb. (Rosaceae) is a common ornamental flower distributed in the temperate regions of eastern Asia and widely cultivated in the Yunnan Province[1,2]. The petals and buds of *R. rugosa* are often used as food, incense and as Chinese medicinal materials for the treatment of stomachache, diarrhea and gynecological problems[3]. Previous studies have shown the presence of tannins[4], terpenoids[5-7] and flavonoids[8,9] in this genus. Anti-inflammatory and cytotoxic activities have been observed with selected chemical ingredients isolated from *R. rugosa*[7,10]. Previous studies in our laboratories on this plant have led to the isolation of three new aurones that possess anti-HIV-1 and cytotoxic properties[9]. Continuing the effort to search for novel and bioactive metabolites from medicinal plants, we now report the isolation and characterization of a new flavone (**1**). In this paper, the structure elucidation of **1** and its biological evaluation are described.

EXPERIMENTAL

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectra. 1D- and 2D NMR spectroscopic data were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the TMS signal. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semi-preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C18 (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA), or MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Column fractions were monitored by TLC visualized by spraying with 5 % H_2SO_4 in EtOH and heating.

The air-dried flower buds of *R. rugosa* were purchased from Kunming Juhuacun Chinese Herb Medicine Market in September 2012. It was grown in Hanzhong Prefecture, Shaanxi Province and harvested in April 2012. The species was identified by Prof Chen Y.J.A voucher specimen (YNNI 12-9-40) was deposited in the herbarium of the Yunnan University of Nationalities.

Extraction and Isolation: The samples (2 kg) were crushed to 30 meshes and the powders were extracted with 95 % aqueous MeOH (4 × 3 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure and the crude extract (106 g) was applied to a silica gel (150-200 mesh) column eluted with $CHCl_3$-MeOH gradients (20:1, 9:1, 8:2, 7:3, 6:4, 5:5) to afford six fractions (A-F). Subsequent separation of fraction D (6.28 g) by silica gel column chromatography, eluted with petroleum ether-acetone (8:2-1:2), yielded subfractions D1-D5. Subfraction D3 (1.28 g) was purified by silica gel column chromatography using petroleum ether-ethyl acetate mixtures and semi-preparative HPLC (42 % MeOH-H_2O, flow rate 12 mL/min) to afford 1 (13.6 mg).

Rugosaflavonoid H (1): Pale yellow gum; $[\alpha]_D^{24.8}$-61.5 (c 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 356 (4.18), 316 (4.38),

262 (4.34), 210 (4.58) nm; IR (KBr, ν_{max}, cm^{-1}) 3462, 1685, 1654, 1612, 1543, 1505, 1460, 1348, 1216, 1157, 872, 765; ^1H and ^{13}C NMR data (in CD_3OD), (Table-1). Negative ESI-MS *m/z* 567 $[M-H]^-$; negative HRESI-MS *m/z* 581.1290 $[M-H]^-$ (Calcd. for $C_{29}H_{25}O_{13}$, 581.1295).

RESULTS AND DISCUSSION

The flower buds of *R. rugosa* were extracted with 95 % MeOH, followed by repeatedly column chromatography on silica gel, Sephadex LH-20 and RP-18 silica gel. Final purification by semi-preparative RP-HPLC afforded compound **1**. The structure of **1** is shown in Fig. 1 and its ^1H and ^{13}C NMR data are given in Table-1.

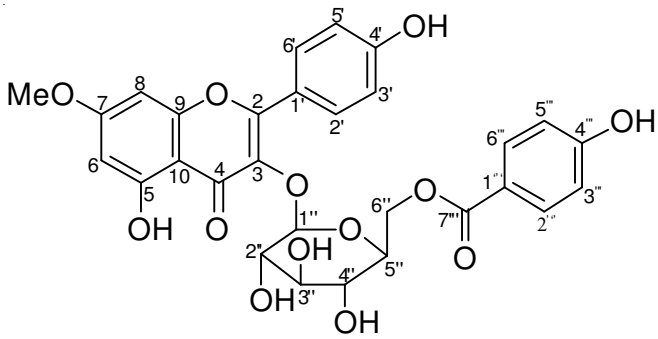

Fig. 1. Structure of compound **1**

TABLE-1
^1H AND ^{13}C NMR DATA OF COMPOUND **1**
(δIN PPM, IN CD_3OD, 500 AND 125 MHz)

Position	δ_C (m)	δ_H (m, *J*, Hz)	Position	δ_C (m)	δ_H (m, *J*, Hz)
2	158.0 s		1″	104.3 d	5.22 d (7.0)
3	135.3 s		2″	75.6 d	3.46 overlap
4	179.2 s		3″	78.2 d	3.45 overlap
5	162.5 s		4″	71.5 d	3. 31 m
6	98.6 d	6.22 s	5″	75.9 d	3.50 overlap
7	168.8 s		6″	64.6 t	4.15, 4.28 m
8	94.2 d	6.41 s	1‴	122.3 s	
9	159.0 s		2‴,6‴	132.5 d	7.90 d (8.9)
10	105.7 s		3‴,5‴	116.7 d	6.83 overlap
1′	122.3 s		4‴	161.5 s	
2′,6′	131.4 d	7.96 d (8.7)	7‴	168.6 s	
3′,5′	116.3 d	6.80 overlap	7-OMe	55.8 q	3.82 s
4′	161.2 s				

Compound **1** was obtained as a pale yellow gum. Its HRESIMS spectrum in the negative mode revealed a $[M-H]^-$ signal at *m/z* 581.1295, consistent with the molecular formula $C_{29}H_{26}O_{13}$ (seventeen degrees of unsaturation). Its IR spectrum exhibited the presence of hydroxy (3462 cm^{-1}), carbonyl (1685, 1654 cm^{-1}) and aromatic ring (1612, 1543 and 1505 cm^{-1}). Its ^{13}C and DEPT NMR spectra displayed signals for 29 carbons corresponding to a 5,7,4′-substituted flavonol [11][δ_C 158 s, 135.3 s, 179.2 s, 162.5 s, 98.6 d, 168.8 s, 94.2 d, 159 s, 105.7 s, 122.3 s, 131.4 d (2C), 116.3 d (2C), 161.2 s], a glucosyl moiet[12] (δ_C 104.3 d, 75.6 d, 78.2 d, 71.5 d, 75.9 d, 64.6 t), a 4-substituted benzoyloxy group[12] [δ_C 122.3 s, 132.5 d (2C), 116.7 d (2C), 161.5 s, 168.6 s] and a methoxy group (δ_C 55.8). The ^1H NMR signals (Table-1) also supported a 5,7,4′-substituted flavonol skeleton bearing a glucosyl, a 4-substituted benzoyloxy and a methoxy groups. The HMBC correlation (Fig. 2) of methoxy proton signal (δ_H 3.82) with C-7 (δ_C 168.8) indicated the methoxy group located at C-7. The long-range correlation observed between H-1″ (δ_H 5.22) and C-3 (δ_C 135.3) indicated the glucosyl group was located at C-3. The glucosyl group should be in a β-configuration due to the coupling constant value of H-1″ ($J = 7$ Hz)[13] and by comparing the ^1H and ^{13}C NMR data with those of glucose[11]. Furthermore, this was also confirmed by ROESY correlations of H-1″ with H-3″ and H-5″, H-2″ with H-4″. The attachment of the 4-substituted benzoyloxy group at C-6″ of glucose was supported by the HMBC correlations of H-6″ (δ_H 4.15, 4.28) with C-7‴ (δ_C 168.6). On the basis of the above evidence, the structure of **1** (rugosaflavonoid H) was established as shown in Fig. 1.

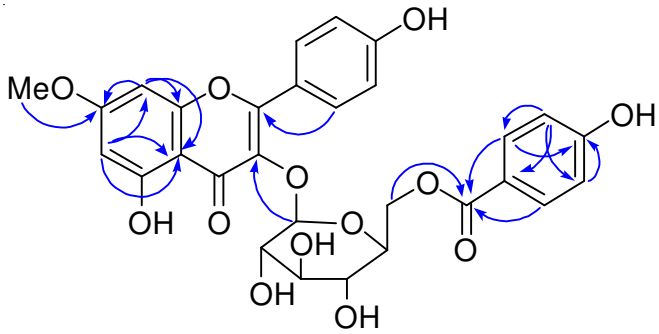

Fig. 2. Selected HMBC ( ) correlations of **1**

Many flavonoid derivatives are known to be cytotoxic[9,14-16] and antitumor activities have been reported for *R. rugosa*[7,9,10]. Compounds **1** was tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method, with paclitaxel as the positive control[17]. Compound **1** exhibited cytotoxicity against NB4 and MCF7 cells with IC_{50} values of 8.2 and 7.6 μM, respectively.

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