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Chromones from the Flowers of *Rosa rugosa* and Their Cytotoxicity

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A new chromone, 2,2-dimethyl-4-oxo-8-(2-oxopropyl)-chroman-6-yl acetate (**1**), together with five known chromones (**2-6**), were isolated from the flowers of *Rosa rugosa*. The structures of **1-6** were elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Compounds **1** were evaluated for their cytotoxicity against five human tumor cell lines and it showed high cytotoxicity against A549 and MCF7 cell with IC_{50} values of 3.6 and 5.0 μM, respectively.

Key Words: Chromones, *Rosa rugosa*, Isolation, Structure elucidation, Cytotoxicity.

INTRODUCTION

The species of *Rosa rugosa* are one of the most important ornamental flowers widely distributed in temperate regions of eastern Asia including Japan, Korea and China[1]. This species had widely been cultivated in several areas of Yunnan Province because of its high commercial values[2]. Meanwhile, the petals and buds of *R. rugosa* have also been used as food, incense materials and in traditional Chinese medicine for treating stomachache, diarrhoea and women's diseases[3]. The recent study also report the previous phytochemical researches on *R. rugosa* has revealed that tannins[4], sesquiterpenes[5], terpenoids[6,7], as well as flavonoids[8,9] are major components isolated from this plant. The biological activities of antitumour had also been reported for recent study on this plant[7,9,10]. Motivated by a search for new bioactive metabolites from this plant, our group had reinvestigated the chemical constituents of the flowers of *R. rugosa*, which led to the isolation and characterization of a new chromone (**1**), along with five known chromones (**2-6**). The structures of the isolated compounds were established by means of spectroscopic methods including extensive 1D and 2D NMR techniques. This paper deals with the isolation, structural characterization and biological activities of these compounds.

EXPERIMENTAL

General experimental procedures: Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectrometry was obtained using a Shimadzu UV-2401A spectrophotometer. 1D and 2D NMR spectrometry were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) are expressed in ppm with reference to the solvent signals. 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 C_{18} (20 mm × 25 cm) columns. Column chromatography was performed using silica gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma-Aldrich, Inc, USA) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 5 % H_2SO_4 in EtOH.

The flowers of *R. rugosa* were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Prof. Chen Y.J. (Yunnan Nationalities University). A voucher specimen (YNNI 10-9-56) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered flower buds of *R. rugosa* (5.2 kg) were extracted four times with 95 % aqueous methanol (4 × 5 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure and the crude extract (465 g) was applied to silica gel (150-200 mesh) column chromatography, eluting with a $CHCl_3$-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give

six fractions A-F. The further separation of fraction B (9:1, 32.8 g) by silica gel column chromatography, eluted with petroleum ether-acetone (9:1-1:2), yielded mixtures B1-B6. Fraction B2 (8:2, 6.47 g) was subjected to silica gel column chromatography using petroleum ether-ethyl acetate and semi-preparative HPLC (52-55 % MeOH-H_2O or 40-45 % CH_3CN-H_2O, flow rate 12 mL/min) to give **1** (11.3 mg), **2** (25.4 mg) and **5** (20.1 mg). Fraction B3 (7:3, 6.22 g) was subjected to silica gel column chromatography using petroleum ether-ethyl acetate and semi-preparative HPLC (45-50 % MeOH-H_2O or 36-40 % CH_3CN-H_2O, flow rate 12 mL/min) to give **4** (18.4 mg) and **6** (13.6 mg). Further separation of fraction D (8:2, 31.8 g) by silica gel column chromatography, eluted with petroleum ether-acetone (8:2-1:2), yielded mixtures D1-D5. Fraction D3 (6:4, 8.22 g) was subjected to silica gel column chromatography using petroleum ether-ethyl acetate and semi-preparative HPLC (35-38 % MeOH-H_2O or 28-35 % CH_3CN-H_2O, flow rate 12 mL/min) to give **3** (21.4 mg).

2,2-Dimethyl-4-oxo-8-(2-oxopropyl)-chroman-6-yl acetate (1): Obtained as pale yellow oil; UV (MeOH) max (log ε) 210 (4.26), 262 (3.92), 355 (2.87) nm; IR (KBr, ν_{max}, cm^{-1}): 3435, 2914, 2876, 1716, 1680, 1668, 1612, 1550, 1439, 1356, 1132, 941, 853; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table-1; negative ESIMS m/z 313 $[M + Na]^+$; negative HRESIMS m/z 313.1044 $[M + Na]^+$ (calcd. (%) for $C_{16}H_{18}NaO_5$, 313.1052).

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

	δ_C (m)	δ_H (m, *J*, Hz)
2	79.7 s	–
3	50.7 t	2.67 s
4	192.2 s	–
5	112.0 d	7.42, d, *J* = 2.4
6	145.7 s	–
7	123.9 d	7.07, d, *J* = 2.4
8	130.8 s	–
9	154.2 s	–
10	120.1 s	–
11	49.6 t	4.04 s
12	206.3 s	–
13	30.2 q	2.22 s
14,15	25.2 q	1.49 s
-OAc	21.1 q, 169.6 s	2.03 s

RESULTS AND DISCUSSION

The flowers of *R. rugosa* were extracted with 95 % methanol. The extract was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and semi-preparative RP-HPLC separation to afford seven new chromone, 2,2-dimethyl-4-oxo-8-(2-oxopropyl)-chroman-6-yl acetate (**1**), together with five known chromones (**2-6**). The structures of the compounds **1-6** were as shown in Fig. 1 and the ^1H and ^{13}C NMR data of the compound **1** were listed in Table-1. The known compounds, compared with literature data, were identified as, peucenin-7-methyl ether (**2**)[11], mulberroside C (**3**)[12], greveichromenol (**4**)[13], 6-(3-hydroxy-4-methoxystyryl)-4-methoxy-2*H*-pyran-2-one (**5**)[14], pestaloficiol J (**6**)[15].

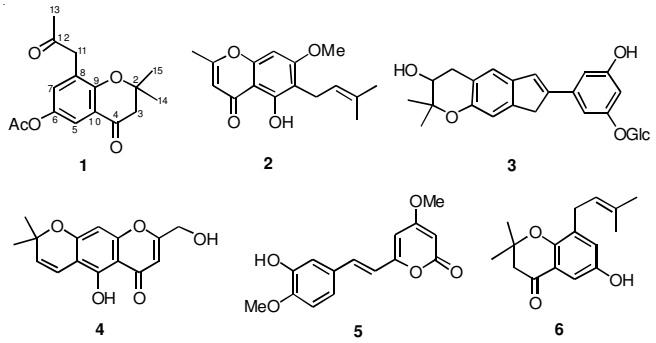

Fig. 1. Structure of chromones from the flowers of *Rosa rugosa*

Compound **1** was obtained as pale yellow oil. It gives a parent ion by HR-ESIMS at m/z 313.1044 $[M + Na]^+$ (calcd. (%) for 313.1052) corresponding to a molecular formula $C_{16}H_{18}O_5$, requiring eight degrees of unsaturation. The ^1H and ^{13}C NMR spectra of compound **1** along with analysis of the DEPT spectra (Table-1) displayed 16 carbon signals and 18 proton signals, respectively, corresponding to a chromanone nucleus[14] (δ_C 79.7 s, 50.7 t, 192.2 s, 112.0 d, 145.7 s, 123.9 d, 130.8 s, 154.2 s, 120.1 s, 25.2 q (2C)), an acetonyl group ($-CH_2C(O)CH_3$) (δ_C 49.6 t, 206.3 s, 30.2 q; δ_H 4.04 s, 2.21 s) and an acetoxy group (δ_C 21.2 q, 169.6 s; δ_H 2.03 s). Strong absorption bands accounting for hydroxy (3435 cm^{-1}), carbonyl group (1716, 1680, 1668 cm^{-1}) and aromatic groups (1612, 1550, 1439 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of compound **1** showed absorption maxima at 262 and 210 nm, which confirmed the existence of the aromatic functions. The HMBC correlations (Fig. 2) of H-11 (δ_H 4.04) with C-7 (δ_C 123.9), C-8 (δ_C 130.8) and C-9 (δ_C 154.2), of H-7 (δ_H 7.07) with C-11 (δ_C 49.6) indicated that the acetonyl group should be located at C-8 on the chromone ring (Fig. 2). The position of acetonyl group was determined, the acetoxy group should be located at C-6 to support the ^1H NMR signals of aromatic proton (δ_H 7.42, d, *J* = 2.4 and 7.07, d, *J* = 2.4). Thus, the structure of **1** was established as 2,2-dimethyl-4-oxo-8-(2-oxopropyl)-chroman- 6-yl acetate.

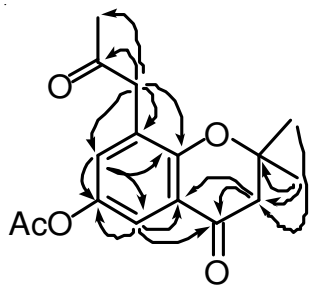

Fig. 2. Key HMBC correlations (↷) of **1**

Since certain chromone derivatives exhibit potential cytotoxicity[16,17], the compound **1** was tested for their cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method as reported previously[18]. The results showed that compound **1** showed high cytotoxicity against A549 and MCF7 cell with IC_{50} values of 3.6 and 5.0 μM, respectively.

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