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AURONE CONSTITUENTS FROM THE FLOWERS OF *ROSA RUGOSA* AND THEIR BIOLOGICAL ACTIVITIES

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Abstract – Three new aurones, rugaurones A–C (**1-3**), together with four known aurones (**4-7**) were isolated from the flowers of *Rosa rugosa*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D- NMR techniques. Compounds **1-7** were tested for their anti-HIV-1 activity and cytotoxicities. The results showed that compound **3** has significant potential anti-HIV-1 activity, compounds **2** have high cytotoxic abilities, and other compounds also have moderate biological activities.

INTRODUCTION

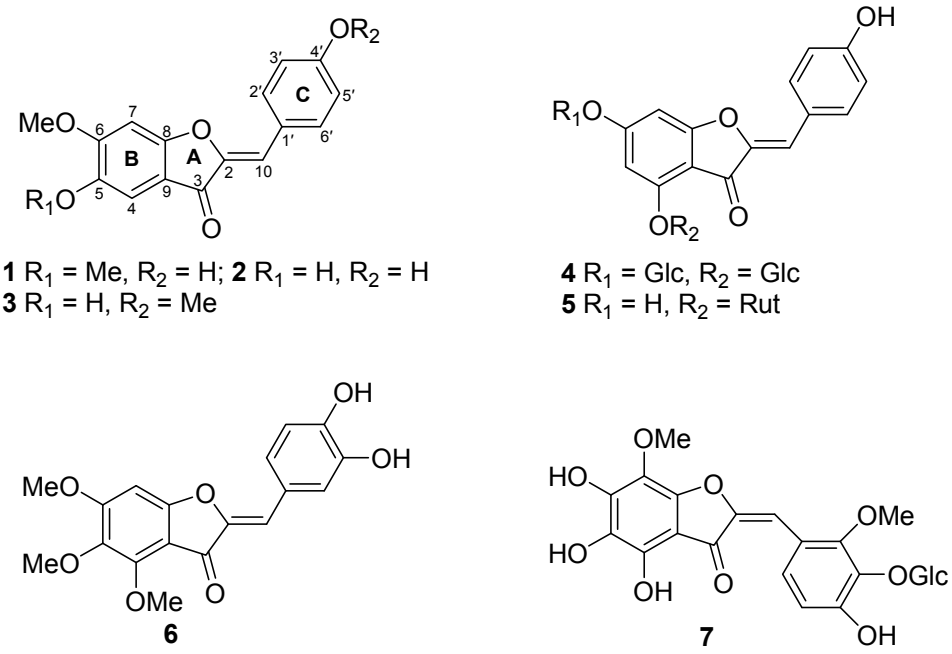
The species of *Rosa rugosa* are one of the most important ornamental plants for it large and attractive flowers. This species widely distributed in temperate regions of eastern Asia including Japan, Korea and China,[1] and had widely been cultivated in several areas of Yunnan Province because of high 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.[3,4] The previous phytochemical researches on *R. rugosa* has revealed that tannins, flavonoids, as well as terpenoids are major components isolated from this plant,[5-9] and recent studied reveal that *R. rugosa* also have anti-HIV and antitumor activity.[8,9]

With the aim of multipurpose utilization of *R. rugosa* and identify bioactive natural products from this plants, the phytochemical investigation on *R. rugosa* was carried out. As a result, three new aurones (**1-3**) together with four known aurones (**4-7**), were isolated from this plant. In addition, the anti-HIV-1 activity

and cytotoxicities of compounds **1-7** were evaluated. This article deals with the isolation, structural elucidation and biological activities of the compounds.

RESULTS AND DISCUSSION

A 70% aq. methanol extract prepared from the flowers of *R. rugosa* was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compounds **1-7**, including three new aurones, named rugaurones A–C (**1-3**), together with four known aurones, 4,6,4′-trihydroxy-aurone-4,6-di-*O*-*β*-D-glucopyranoside **(4)**,[10] sulfuretin **(5)**,[11] hamiltrone **(6)**,[12] (*E*)-3′-O-*β*-D-glucopyranosyl-4,5,6,4′-tetrahydroxy-7,2′-dimethoxyaurone (**7**).[13] The structures of the compounds **1-7** were as shown in Figure 1.



1 R_1 = Me, R_2 = H; **2** R_1 = H, R_2 = H
3 R_1 = H, R_2 = Me

4 R_1 = Glc, R_2 = Glc
5 R_1 = H, R_2 = Rut

6

7

Figure 1. The structures of aurones from the flowers of *R. rugosa*

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as $C_{17}H_{14}O_5$ by HR-ESI-MS m/z 321.0732 $[M+Na]^+$ (calcd 321.0739). The ^1H and ^{13}C NMR spectra of **1** (Table 1) along with analysis of the DEPT spectra displayed 17 carbon signals and 14 proton signals, respectively, corresponding to an aurone nucleus[12,13] (δ_C 147.0 s, 180.2 s, 113.0 d, 145.8 s, 154.0 s, 102.1 d, 158.3 s, 116.6 s, 110.5 d, 122.5 s, 134.5 d, 115.1 d, 156.7 s,

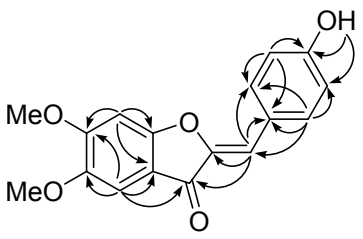


Figure 2 Selected HMBC (↷) correlations of **1**

115.1 d, 134.5 d), two methoxyl groups (δ_C 55.8 q, 56.0 q), and one phenolic hydroxyl proton (δ_H 10.88). Strong absorption bands accounting for hydroxyl (3421 cm^{-1}), carbonyl group (1667 cm^{-1}) and aromatic groups (1610, 1492, 1441 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 338, 256 and 210 nm, which confirmed the existence of the aromatic functions. The HMBC correlations (Figure 2) of H-10 (δ_H 6.79) with C-2 (δ_C 147.0), C-3 (δ_C 180.2), C-1′ (δ_C 122.5), C-2′ (δ_C 134.5), of H-2′ (δ_H 7.86) with C-10 (δ_C 110.5), and of H-4 (δ_C 7.29) with C-3 (δ_C 180.2), also supported the aurone nucleus.

Table 1. ^1H NMR and ^{13}C NMR data of compounds **1** - **3** in C_5ND_5(125 and 500 MHz)

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
2	147.0 s		147.7 s		147.0 s	
3	180.2 s		180.2 s		180.6 s	
4	113.0 d	7.29, s	114.9 d	7.30, s	115.0 d	7.31, s
5	145.8 s		140.0 s		141.0 s	
6	154.0 s		155.5 s		155.1 s	
7	102.1 d	6.69, s	102.8 d	6.62, s	102.3 d	6.62, s
8	158.3 s		158.4 s		158.2 s	
9	116.6 s		116.9 s		116.7 s	
10	110.5 d	6.79, s	110.7 d	6.80, s	110.2 d	6.82, s
1′	122.5 s		122.2 s		122.0 s	
2′	134.5 d	7.86, d, *J*=8.6	133.9 d	7.85, d, *J*=8.6	133.0 d	7.92, d, *J*=8.6
3′	115.1 d	7.02, d, *J*=8.6	115.7 d	7.02, d, *J*=8.6	113.5 d	7.05, d, *J*=8.6
4′	156.7 s		157.0 s		160.6 s	
5′	115.1 d	7.02, d, *J*=8.6	115.7 d	7.02, d, *J*=8.6	113.5 d	7.05, d, *J*=8.6
6′	134.5 d	7.86, d, *J*=8.6	133.9 d	7.85, d, *J*=8.6	133.0	7.92, d, *J*=8.6
OMe-5	55.8 q	3.76, s				
OMe-6	56.0 q	3.80, s	55.6 q	3.80, s	56.0 q	3.80, s
OMe-4′					55.7 q	3.82, s
OH-5				11.10, s		11.10, s
OH-4′		10.88, s		10.83, s		

The signals for four coupled aromatic protons at δ_H 7.02 (d, *J*=8.6 Hz, 2H), and 7.86 (d, *J*=8.6, 2H), suggested a 4′-monosubstituted for C ring. The proton signals for two singlets at δ_H 7.29 (s, 1H), and δ_H 6.69 (s, 1H) also revealed that the substituents for B-ring should be located at C-5 and C-6. The HMBC

correlations of the hydroxyl proton signal δ_H 10.88 with C-3′ (δ_C 115.1), C-4′ (δ_C 156.7), C-5′ (δ_C 115.1), suggested the attachment position of the phenolic hydroxyl group at C-4′. The HMBC correlation of two methoxy proton signals (δ_H 3.76, 3.80) with C-5 (δ_C 145.8) and C-6 (δ_C 154.0) suggested two methoxyl groups located at C-5 and C-6 respectively. Thus, the structure of **1** was established as 4′-hydroxy-5,6-dimethoxyl-aurone, and given the trivial name of rugaurone A.

Compounds **2** and **3** (rugaurones B and C) were also obtained as pale yellow gum. By comparison of their IR, UV, ^{1}H, and ^{13}C NMR spectra with those of **1**, compounds **2** and **3** were also assigned as 4′,5,6-substituted-aurone. The obvious chemical shift differences resulted from the substituents group variations in the aromatic rings. The NMR spectra of **2**, compared to those of **1**, displayed an additional phenolic hydroxyl proton (δ_H 11.10), and the disappearance of a methoxy group signals. This indicated that a methoxy group in **1** was replaced by a hydroxyl group in **2**, and the additional hydroxyl group located at C-5 was supported by the the HMBC cross-peak between this hydroxyl group (δ_H 11.10) with C-4 (δ_C 114.9), C-5 (δ_C 140.0), and C-6 (δ_C 155.5). The ^{1}H and ^{13}C NMR spectra of **3** displayed 17 carbon signals and 14 proton signals. The obvious differences are the downfield-shifted signals for C-4′ (δ_C 160.4) and the upfield-shifted signal for C-5 (δ_C 141.0) when compared to those of **1**. This suggested that the substituents group variations should be at C-5 and C-4′. The hydroxyl group located at C-5 was supported by the HMBC correlations of hydroxyl group (δ_H 11.10) with C-4 (δ_C 115.0), C-5 (δ_C 141.0), and C-6 (δ_C 155.1), and the methoxy groups located at C-4′ and C-6 were supported by the HMBC correlations of methoxy proton signals (δ_H 3.82, 3.80) with C-4′ (δ_C 160.6) and C-6 (δ_C 155.1), respectively. Thus, the structures of **2** and **3** were established as shown.

Since the configuration of the olefinic bond in aurones can be established on the basis of the chemical shift of olefic methine resonance as it absorbs at 119.9-121.5 ppm in *E*-aurones and at 105.9-112.8 ppm in *Z*-aurones.[14] The configuration of the olefinic bond in **1-3** was defined as *Z* by the chemical shift value of carbon atom C-10.

Table 2. Anti-HIV activity of compounds **1** - **7**

compounds	CC_{50} (μg/mL)	EC_{50} (μg/mL)	TI [a]
1	> 200	6.38	>31.34
2	184	3.25	56.70
3	> 200	1.28	>156.9
4	68.18	4.48	15.22
5	186.5	18.2	10.27
6	170.3	5.18	32.87
7	90.01	2.82	31.92

[a] TI (therapeutic index) = CC_{50}/ EC_{50}.

For anti-HIV-1 activity assay, the cytotoxicity against C8166 cells (CC_{50}) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}), using AZT as a positive control (EC_{50} = 0.045 μg/mL and CC_{50} > 200 μg/mL).[15] The results are shown in Table 2. The results reveal that compound **3** showed significant potential anti-HIV-1 activity with therapeutic index (TI) values above 156.9. Compounds **1**, **2**, **6**, and **7** also showed moderate anti-HIV-1 activitiy with TI values above 30.

The cytotoxicity tests for the isolates were performed using a previously reported procedure.[16] All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared with untreated cells. The cytotoxic abilities against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control) were shown in Table **3**. The results showed that compound **2** have high cytotoxic abilities with IC_{50} values close to these of the positive control. Other compounds also showed moderate cytotoxic abilities.

Table 3. Cytotoxicity of compounds **1-7**

Compound	Cell lines			
	HL-60	HepG2	KB	MDA-MB-231
1	5.46	6.12	8.24	7.61
2	2.41	2.37	3.10	3.93
3	6.15	9.49	6.23	8.26
4	11.67	12.20	6.56	21.37
5	9.92	11.11	16.56	12.65
6	14.87	6.63	10.35	6.12
7	11.67	9.94	8.46	9.13
Camptothecin	1.82	0.86	1.82	2.20

Data of IC_{50} values in μmol/L. For a compound to be deemed effective, an IC_{50} value < 100 μmol/L is required. Camptothecin was used as a positive control.
HL-60, human acute promyelocytic leukemia; Hep-G2, human hepatocellular carcinoma; KB, human oropharyngeal epidermoid carcinoma; MDA-MB-231, human breast cancer cells.

EXPERIMENTAL

General. Optical rotation was measured in Horiba SEPA-300 high sensitive polarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10～40 μm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

Plant material. 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 flowers of *R. rugosa* (2.5 kg) were extracted four times with 70% MeOH (4 × 2.0 L) at room temperature and filtered. The crude extract (92 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$-acetone 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 C (8:2, 11.5 g) by silica gel column chromatography, eluted with $CHCl_3$-MeOH (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures D1–D5. Fraction D1 (9:1, 1.57 g) was subjected to preparative HPLC (35% MeOH, flow rate 12 mL/min) to give **1** (15.2 mg), **2** (13.8 mg), **3** (8.26 mg), and **6** (13.2 mg). The further separation of fraction F (1:1, 23.6 g) by silica gel column chromatography, and preparative HPLC (15% MeOH, flow rate 12 mL/min) to give **4** (22.1 mg) **5** (28.4 mg), and **7** (16.3 mg).

Anti-HIV1 Assays. The cytotoxicity assay against C8166 cells (CC_{50}) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}).[15]

Cytotoxicity Assay. The cytotoxicity tests for the isolates were performed by against HL-60, Hep-G2, KB and MDA-MB-231 tumor cell lines by MTT-assay (with camptothecin as the positive control).[16]

Rugaurone A (1). Obtained as a pale yellow gum; UV (MeOH), λ_{max} (log ε) 338 (3.69), 256 (3.87), 210 (4.18) nm; IR (KBr) ν_{max} 3421, 2908, 1667, 1610, 1492, 1441, 1269, 1143, 1078, 851, 822, 775 cm^{-1}; ^1H NMR and ^{13}C NMR data (C_5ND_5, 500 MHz and 150 MHz, respectively), Table 1; ESIMS (positive ion mode) *m/z* 321 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 321.0732 $[M+Na]^+$ (calcd 321.0739 for $C_{17}H_{14}O_5Na$).

Rugaurone B (2). Obtained as a pale yellow gum; UV (MeOH), λ_{max} (log ε) 336 (3.62), 258 (3.81), 210 (4.22) nm; IR (KBr) ν_{max} 3423, 2905, 1665, 1612, 1490, 1444, 1267, 1143, 1075, 852, 825, 774 cm^{-1}; ^1H NMR and ^{13}C NMR data (C_5ND_5, 500 MHz and 150 MHz, respectively), Table 1; ESIMS (positive ion mode) *m/z* 307 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 307.0588 $[M+Na]^+$ (calcd 307.0582 for $C_{16}H_{12}O_5Na$).

Rugaurone C (3). Obtained as a pale yellow gum; UV (MeOH), λ_{max} (log ε) 340 (3.65), 256 (3.88), 210 (4.14) nm; IR (KBr) ν_{max} 3425, 2906, 1662, 1616, 1493, 1440, 1264, 1148, 1076, 850, 822, 776 cm^{-1}; ^1H NMR and ^{13}C NMR data (C_5ND_5, 500 MHz and 150 MHz, respectively), Table 1; ESIMS (positive ion mode) *m/z* 321 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 321.0746 $[M+Na]^+$ (calcd 321.0739 for $C_{17}H_{14}O_5Na$).

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