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Notes

Phenylethanoids from the Flowers of *Rosa rugosa* and Their Biological Activities

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Rosa rugosa is widely distributed from temperate regions of eastern Asia including Japan, Korea and China.[1] As an important economic plant, this species had widely been cultivated in several areas of Yunnan Province, and had wildly been used as ornamental flowers, food, and incense materials.[2] Meanwhile, the petals and buds of *R. rugosa* have been used in traditional Chinese medicine for treating stomachache, diarrhoea and women's diseases.[3,4] Recent studies also revealed that *R. rugosa* have *anti*-HIV and antitumor activity.[5,6] 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]

Motivated by search for bioactive metabolites from this plant, the phytochemical investigation on *R. rugosa* was carried out. As a result, two new phenylethanoids, together with six known Phenylethanoids, were isolated from this plant. In addition, the *anti*-HIV-1 activities and cytotoxicities of compounds **1-8** 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 Si gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1-8**, including two new phenylethanoids, named rugethanoids A-B (**1-2**), together with six known phenylethanoids 2-(3-*O*-*β*-D-Glucopyranosyl-4-hydroxyphenyl)-ethanol (**3**),[10] 2-(3-hydroxy-4-*O*-D-*β*-glucopyranosylphenyl)-ethanol (**4**),[10] 2-(3,4-dihydroxy-phenyl)-ethanol (**5**),[10] acteoside **6**,[11] isoacteoside B **7**,[11] *β*-D-fructofuranosyl-*α*-D-(6-*O*-(*E*)-sinapoyl)glucopyranoside (**8**).[12] The structures of the compounds **1-8** were as shown in Figure 1.

Compound **1** was obtained as white powder. Its molecular formula was determined as $C_{14}H_{16}O_6$ by HR-ESI-MS *m/z* 303.0852 $[M+Na]^+$ (calcd 303.0845). Its ^1H and ^{13}C NMR spectra (Table 1) showed signals of 16 hydrogens and 14 carbons, respectively, corresponding to one aromatic ring (δ_C 129.3, 116.4, 147.0, 145.7, 117.1, 120.2) with three aromatic protons (δ_H 7.13, d, J = 1.8 Hz, 7.26, d, J = 7.8 Hz, 6.76, dd, J = 1.8, 7.8 Hz), three methylene groups (δ_C 34.5, 28.0, 33.1), two oxidated methylene groups (δ_C 65.6, 68.5), one methylenedioxyl group (δ_C 101.1, δ_H 5.82, 5.87 s), one ketone group (δ_C 210.1), and an ester carbonyl group (δ_C 172.5). The ^1H-^1H COSY of H-7/H-8; together with HMBC correlations (Figure 2) of H-6 (δ_H 6.76) with C-7 (δ_C 34.5), of H-8 (δ_H 4.32) with C-1 (δ_C 129.3), of methylenedioxyl

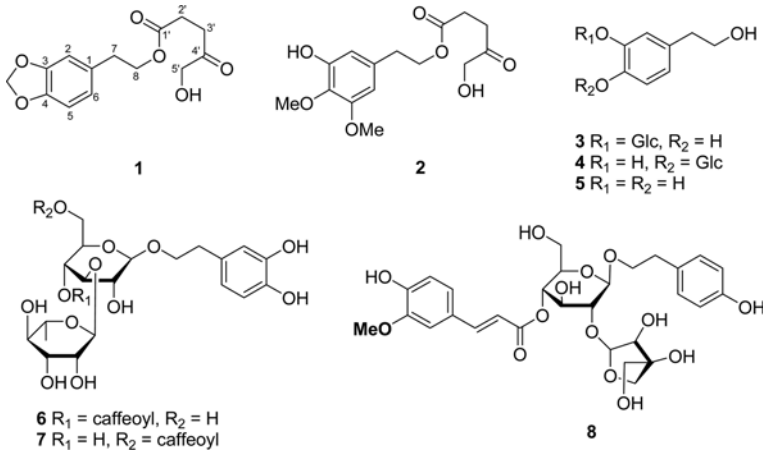

Figure 1. The structure of compounds **1-8**.

Table 1. ^{1}H NMR and ^{13}C NMR data (in C_5D_5N) of compounds **1**-**2**

No.	Compound **1**		Compound **2**	
	δ_C (mult.)	δ_H (mult, *J*, Hz)	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	129.3 s		133.8 s	
2	116.4 d	7.13, d, *J* = 1.8	103.9 d	6.82, s
3	147.0 s		152.8 s	
4	145.7 s		136.6 s	
5	117.1 d	7.26, d, *J* = 7.8	148.5 s	
6	120.2 d	6.76, dd, *J* = 1.8, 7.8	106.8 d	7.05, s
7	34.5 t	2.78, t, *J* = 7.1	34.6 t	2.79, t, *J* = 7.1
8	65.6 t	4.32, t, *J* = 7.1	65.8 t	4.32, t, *J* = 7.1
1'	172.5 s		172.2 s	
2'	28.0 t	2.69, t, *J* = 6.4	27.8 t	2.68, t, *J* = 6.4
3'	33.1 t	2.84, t, *J* = 6.4	33.0 t	2.82, t, *J* = 6.4
4'	210.1 s		210.3 s	
5'	68.5 t	4.49, s	68.5 t	4.49, s
OMe-4			61.0 q	3.80, s
OMe-5			55.8 q	3.78, s
-OCH_2O-	101.1 t	5.82, 5.87 s		
Ar-OH				11.11 brs

Figure 2. Selected HMBC (⌒) and ^{1}H-^{1}H COSY (—) correlations of compound **1**.

Table 2. *Anti*-HIV activity of compounds **1**-**8**

Compounds	CC_{50} (μg/mL)	EC_{50} (μg/mL)	TI [a]
1	116.5	6.71	17.4
2	20.4	2.95	6.91
3	52.5	3.25	16.1
4	243.8	36.8	6.62
5	169.5	16.5	10.2
6	154.8	13.8	11.2
7	72.7	5.29	13.7
8	> 200.0	3.79	> 52.8

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

Table 3. Cytotoxicity of compounds **1**-**8**

Compound	Cell lines			
	HL-60	HepG2	KB	MDA-MB-231
1	4.05	5.56	3.85	6.92
2	3.10	2.15	2.82	3.57
3	5.59	8.63	5.66	4.78
4	22.43	11.09	15.05	19.43
5	9.02	10.10	15.05	7.86
6	13.52	6.03	9.41	5.56
7	11.19	8.59	5.03	9.30
8	5.15	9.04	7.69	8.30
Camptothecin	1.85	0.92	1.86	2.23

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.

proton signals (δ_H 5.82, 5.87 s) with C-3 (δ_C 147.0) and C-4 (δ_C 145.7) revealed that the exist of a 3,4-methylenedioxylphenylethanoid structural unit.[13] In addition, the ^{1}H-^{1}H COSY of H-2'/H-3' together with HMBC correlations of H-5' (δ_H 4.49) with C-4' (δ_C 210.1), C-3' (δ_C 33.1), of H-3' (δ_H 2.84) with C-1' (δ_C 172.5), C-2' (δ_C 28.0), C-4' (δ_C 210.1), C-5'(δ_C 68.5), of H-2' (δ_H 2.69) with C-1' (δ_C 172.5), C-3' (δ_C 33.1), C-4' (δ_C 210.1) also suggested that the exist of a 5-hydroxy-4-oxoamylacyl group (-OC(O)-$CH_2CH_2C(O)CH_2OH$).[14] The HMBC of H-8 (δ_H 4.32) with C-1' (δ_C 172.5) indicated that the 5-hydroxy-4-oxoamylacyl group located at C-8. Thus, the structure of **1** was established and given the name as rugethanoid A.

Compounds **2** was obtained as white powder, and showed sodiated molecular ions at *m/z* 335.1100 $[M+Na]^+$ in the HRESIMS (calcd *m/z* 335.1107), corresponding to the molecular formula of $C_{15}H_{20}O_7$. The obvious difference between **1** and **2** are the substituents on the aromatic ring. In NMR spectra of **2**, the methylenedioxyl signals disappear, however, two methoxy groups signals (δ_C 61.0, 55.8) and a phenolic hydroxy group signal (δ_H 11.11) appear. This indicates that the substituents on the aromatic ring for compound **2** are two methoxy groups and one hydroxy group. Two methoxy groups located at C-4 and C-5 were assigned from the HMBC correlations of the methoxyl proton signals (δ_H 3.80, 3.78) with C-4 (δ_C 136.6) and C-5 (δ_C 148.5), respectively. The presence of a phenolic hydroxyl group at C-3 was supported by the HMBC correlations of the phenolic hydroxyl proton signal (δ_H 11.11) with C-2 (δ_C 103.9 d), C-3 (δ_C 152.8 s), and C-4 (δ_C 136.6 s), respectively. Thus, the structure of **2** was established, and it has been accorded the trivial name of rugethanoid B.

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} = 0.0045 μg/mL and CC_{50} > 200 μg/mL).[15] The results are shown in Table 2. The results reveal that compounds **8** showed therapeutic index (TI) values above 50. The other compounds also showed weak *anti*-HIV-1 activitiy.

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 compounds **2** have significant

potential cytotoxic abilities. It cell lines values are close to these of the positive control.

Experimental Section

General Procedures. 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 spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on DRX-500 spectrometers with TMS as internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. HRESIMS was performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a ZORBAX PrepHT GF (21.2 mm × 25 cm, 7.0 μm) column or a Venusil MP C18 (20 mm × 25 cm, 5.0 μm) column. Column chromatography was performed with Si gel (200-300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40-63 μm, Merck, Darmstadt, Germany) and MCI gel (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

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% methanol (4 × 20 L) at room temperature and filtered. The crude extract (92 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a chloroform-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 D (7:3, 19.6 g) by silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures D1-D5. Fraction D1 (9:1, 2.86 g) was subjected to preparative HPLC (38% methanol, flow rate 12 mL/min) to give **1** (12.6 mg), and **2** (18.6 mg). Fraction D2 (8:2, 2.24 g) was subjected to preparative HPLC (30% methanol, flow rate 12 mL/min) to give **5** (32.2 mg). The further separation of fraction F (1:1, 23.6 g) by silica gel column chromatography, and preparative HPLC (12% methanol, flow rate 12 mL/min) to give **3** (18.6 mg), **4** (25.4 mg), **6** (32.1 mg) **7** (16.7 mg), and **8** (22.1 mg).

***Anti*-HIV-1 Assay.** 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}).[13]

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 doxorubicin as the positive control).[14]

Rugethanoid A (1): Obtained as white powder; mp 151 ± 1 °C; UV (MeOH) λ_{max} (log ε) 325 (2.42), 288 (4.02), 248 (3.12), 210 (4.69) nm; IR (KBr) ν_{max} 3456, 2925, 2850, 1756, 1713, 1638, 1512, 1450, 1434, 1362, 1175, 1086, 971, 828 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; positive ESIMS *m/z* 303 $[M+Na]^+$; HRESIMS *m/z* 303.0852 $[M+Na]^+$ (calcd 303.0845 for $C_{14}H_{16}NaO_6$).

Rugethanoid B (2): Obtained as white powder; mp 182 ± 1 °C; UV (MeOH) λ_{max} (log ε) 350 (2.46), 279 (3.54), 210 (3.95) nm; IR (KBr) ν_{max} 3460, 2918, 2822, 1755, 1716, 1635, 1640, 1522, 1453, 1364, 1180, 1089, 973, 827 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table 1; positive ESIMS *m/z* 335 $[M+Na]^+$; HRESIMS *m/z* 335.1100 $[M+Na]^+$ (calcd 335.1107 for $C_{15}H_{20}NaO_7$).

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