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NOTE

Phenylethanoids from the Root of *Rosa rugosa* and their Biological Activities

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A new phenylethanoids, rugethanoid C (**1**), together with five known phenylethanoids (**2-6**), were isolated from the roots of *Rosa rugosa*. Their structures were determined by means of HRESIMS, extensive 1D and 2D NMR spectroscopic studies and chemical evidence. rugethanoid C (**1**) was tested for their anti HIV-1 activities and cytotoxicities. The results showed that rugethanoid C (**1**) has moderate potential cytotoxic ability and anti HIV-1 bioactivities.

Key Words: *Rosa rugosa*, Phenylethanoids, Anti HIV-1 activity, Cytotoxic ability.

Rosa rugosa is widely distributed from temperate regions of eastern Asia including Japan, Korea and China[1]. This species had become an important economic plant. It had widely been cultivated in several areas of Yunnan Province and had widely been used as ornamental flowers, food and incense materials[2]. Meanwhile, the roots of *R. rugosa* have been used in traditional Chinese medicine for treating stomach ache, diarrhoea and women's diseases[3,4]. Recent studies also revealed that *R. rugosa* also 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 the roots of *R. rugosa* was carried out. As a result, a new phenylethanoid, together with five known phenylethanoids, were isolated from the roots of this plant. In addition, the anti HIV-1 activities and cytotoxicities of compound **1** were evaluated.

The roots 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 roots of *R. rugosa* (2.5 kg) were extracted four times with 70 % methanol (4.0 L × 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 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 (35 % methanol, flow rate 12 mL/min) to give **1** (14.3 mg). Fraction D2 (8:2, 2.24 g) was subjected to preparative HPLC (30 % methanol, flow rate 12 mL/min) to give **4** (18.5 mg) and **5** (15.8 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 2 (26.6 mg), 3 (17.5 mg) and 6 (28.6 mg). The optical rotations, HPLC and CC were performed by reported method[13].

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})[14].

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)[15].

Rugethanoid C (1): Obtained as white powder; UV (MeOH) λ_{max} (log ε) 325 (2.59), 288 (4.18), 248 (3.38), 210 (4.70) nm; IR (KBr, ν_{max}, cm^{-1}): 3518, 3452, 2926, 2855, 1753, 1717, 1634, 1516, 14475, 1435, 1359, 1172, 1083, 974, 830; ^1H and ^{13}C NMR data (C_5D_5N, 500 and 125 MHz, respectively), Table-1; positive ESIMS m/z 319 $[M + Na]^+$; HRESIMS m/z 319.0787 $[M + Na]^+$ (calcd. (%) 319.0794 for $C_{14}H_{16}NaO_7$).

TABLE-1
^1H AND ^{13}C NMR DATA (IN C_5D_5N) OF COMPOUNDS **1**

	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	133.5 s	–
2	106.4 d	6.68, s
3	145.6 s	–
4	137.8 s	–
5	149.6 s	–
6	105.0 d	6.72, s
7	34.8 t	2.72, t, *J* = 7.1
8	66.2 t	4.36, t, *J* = 7.1
1'	173.6 s	–
2'	28.9 t	2.64, t, *J* = 6.4
3'	34.3 t	2.86, t, *J* = 6.4
4'	211.2 s	–
5'	68.0 t	4.51, s
-OCH_2O-	101.3 t	5.80, 5.93 s
Ar-OH	–	11.25 s

A 70 % aq. methanol extract prepared from the roots of *R. rugosa* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1-6**, including two a phenylethanoid, named rugethanoid C (**1**), together with five known phenylethanoids 2-(3-O-β-D-glucopyranosyl-4-hydroxyphenyl)-ethanol (**2**)[10], 2-(3-hydroxy-4-O-D-β- glucopyranosylphenyl)- ethanol (**3**)[10], 2-(3,4-dihydroxy-phenyl)-ethanol (**4**)[10], tyrosol (**5**)[11], salidroside (**6**)[12]. The structures of the compounds **1-6** were as shown in Fig. 1.

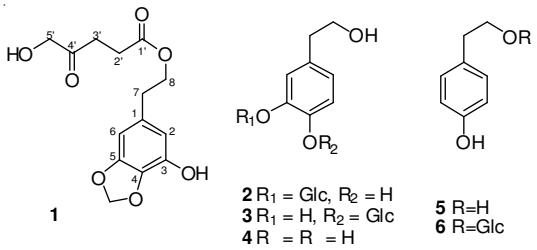

Fig. 1. Structure of compounds **1-6**

Compound **1** was obtained as white powder. Its molecular formula was determined as $C_{14}H_{16}O_7$ by HR-ESI-MS m/z 319.0787 $[M + Na]^+$ (calcd. (%) 319.0794). Its ^1H and ^{13}C NMR spectra (Table-1) showed signals to 16 hydrogens and 14 carbons, respectively, corresponding to one aromatic ring (δ_C 133.5, 106.4, 145.6, 137.8, 149.6, 105.0) with two aromatic protons (δ_H 6.72 s, 6.68 s), three methylene groups (δ_C 34.8, 28.9, 34.3), two oxidated methylene group (δ_C 66.2, 68.0), one methylenedioxyl group (δ_C 101.3; δ_H 5.80, 5.93 s), one ketone group (δ_C 211.2), an ester carbonyl group (δ_C 173.6) and a phenolic hydroxy group (δ_H 11.25). The ^1H-^1H COSY of H-7/H-8; together with HMBC correlations (Fig. 2) of H-6 (δ_H 6.72) with C-7 (δ_C 34.8), of H-8 (δ_H 4.36) with C-1 (δ_C 133.5), of the phenolic hydroxyl proton signal (δ_H 11.25) with C-2 (δ_C 106.4), C-3 (δ_C 145.6) and C-4 (δ_C 137.8), of methylenedioxyl proton signals (δ_H 5.80, 5.93 s) with C-4 (δ_C 137.8) and C-5 (dC 149.6) revealed that the exist of a 3-hydroxyl-4,5-methylenedioxylphenylethanoid structural unit. In addition, the ^1H-^1H COSY of H-2'/H-3' together with HMBC correlations of H-5' (δ_H 4.51) with C-4' (δ_C 211.2), C-3' (δ_C 34.3), of H-3' (δ_H 2.86) with C-1' (δ_C 173.6), C-2' (δ_C 28.9), C-4' (δ_C 211.2), C-5'(δ_C 68.0), of H-2' (δ_H 2.64) with C-1' (δ_C 173.6), C-3' (δ_C 34.3), C-4' (δ_C 211.2) also suggested that the exist of a 5-hydroxy-4-oxoamylacyl group (-OC(O)-CH_2CH_2C(O)CH_2OH). The HMBC of H-8 (δ_H 4.36) with C-1' (δ_C 173.6) (Fig. 2) 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 C.

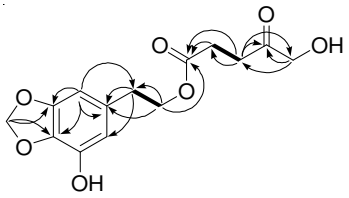

Fig. 2. Selected HMBC (↷) and ^1H-^1H COSY (—) correlations of **1**

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)[13]. Compound **1** shows anti HIV-1 activity with EC_{50} of 2.31 μg/ml, CC_{50} of above 200 μg/mL and TI (therapeutic index) valve of above 86.58.

The cytotoxicity tests for the isolates were performed using a previously reported procedure[14]. 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 was tested by MTT-assay (with doxorubicin as the positive control) and compound **1** showed cell line valves of 3.22, 2.67, 4.85, 6.55 for HL-60, Hep-G2, KB and MDA-MB-231 tumor cell, respectively.

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