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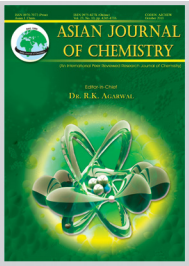

Phenolic Compounds from *Cestrum aurantiacum*

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A new phenolic compound (**1**), together with eight known compounds (**2-9**) were isolated from the leaves and stems of *Cestrum aurantiacum*. The structure was elucidated on the basis of extensive NMR and mass spectral means. The anti-HIV-1 and antioxidant activity of (**1**) was evaluated and showed weak anti-HIV-1 activity with Therapeutic Index 24.1 and antioxidant activity with an IC_{50} value of 3.68 μg/mL.

Key Words: Phenolic compounds, Leaves and stems of *Cestrum aurantiacum*, Anti-HIV-1 activity, Antioxidant activity.

INTRODUCTION

The *Cestrum aurantiacum* belong to the genus Solanaceae family. It is an evergreen, half-climbing shrub originated in South America. The *Cestrum aurantiacum* had introduced into China more than on century and it had widely distributed in south China (Guangdong, Fujian, Guangxi, Yunnan) now[1,2].

Previous phytochemical research on the genus of *Cestrum* has revealed that steroidal saponins[3-5], flavonols[6], terpenoids[7], lignans[8,9], as well as phenols[10] are major principles isolated from the plant of this genus. With the aim of continuing efforts to identify bioactive natural products from the plants, a chemical investigation on the leaves and stems of *Cestrum aurantiacum* indigenous to the Dali Prefecture of Yunnan Province of China was carried out. A new phenolic compound (**1**), together with eight known one (**2-9**) were separated from this plant. In addition, the anti-HIV-1 active and antioxidant activity of (**1**) were evaluated. The structure elucidation and biological activities of the isolated compounds are also reported.

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 spectroscopy with KBr pellets. ^{1}D and ^{2}D NMR spectra were recorded on DRX-500 spectrometer 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 μm) column or a Venusil MP C_{18} (20 mm × 25 cm, 5 μm) column. Column chromatography was performed with silica 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 silica gel plates sprayed with 5 % H_2SO_4 in EtOH.

The leaves and stems of *Cestrum aurantiacum* was collected in Dali Prefecture, Yunnan Province, P.R. China, in February 2009 and was identified by Prof. N. Yuan. A voucher specimen (No. YNNi 09-2-08) was deposited in our laboratory.

Extraction and isolation: The air-dried and powdered leaves and stems of *Cestrum aurantiacum* (2.5 kg) were extracted four times with 70 % aqueous Me_2CO (4 × 3.5 L) at room temperature and filtered to yield a filtrate, which was successively evaporated under reduced pressure and partitioned with EtOAc (3 × 4 L). The EtOAc partition (126 g) was applied to silica gel (200-300 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-E. The separation of fraction B (9:1, 18.9 g) by silica gel column chromatography eluted with $CHCl_3$-$(Me)_2CO$ (9:1-1:2) yielded mixtures B1-B6. Fraction B2 (8:2, 3.25 g) was subjected to silica gel column chromatography using petroleum ether-acetone and preparative HPLC

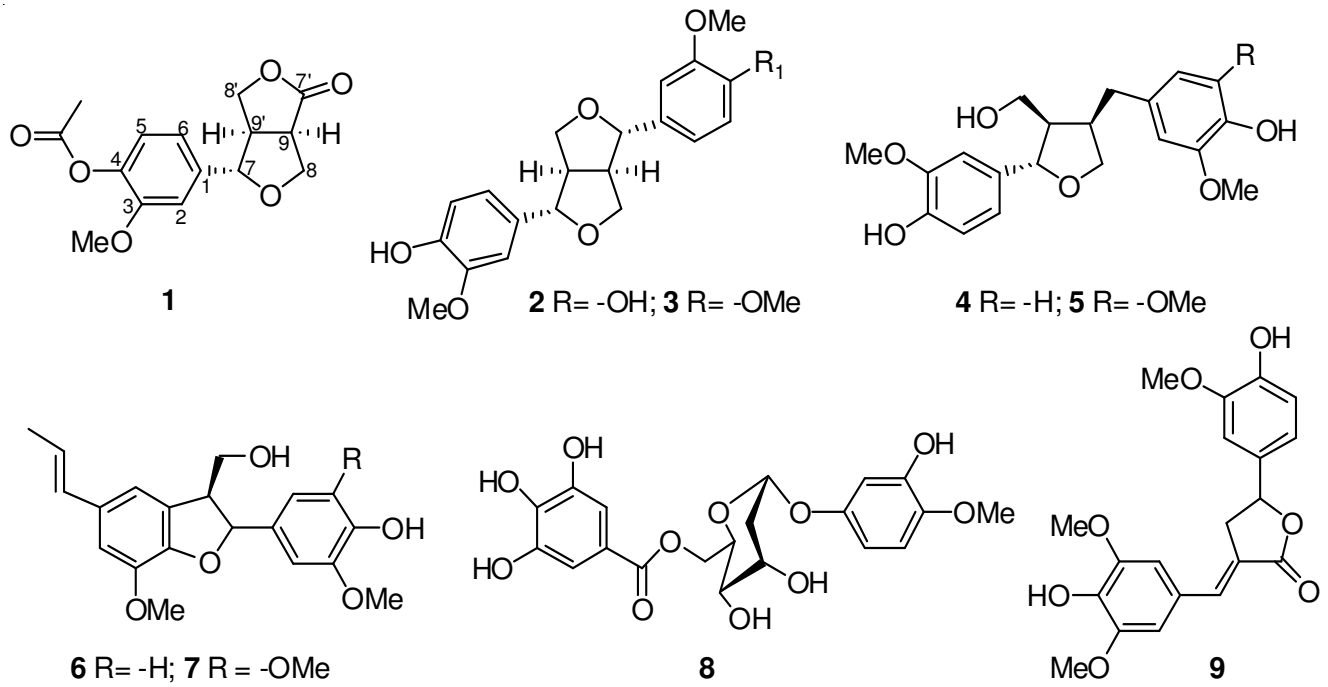

Fig. 1. Structure of phenolic compounds from the *Cestrum aurantiacum*

(68 % MeOH-H_2O, flow rate 12 mL /min) to give compounds **1** (12.6 mg), **3** (28.4 mg), **5** (18.5 mg), **7** (14.8 mg) and **9** (42.6 mg). Fraction B3 (7:3, 1.86 g) was subjected to silica gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (60 % MeOH-H_2O, flow rate 12 mL /min) to yield compounds **2** (8.62 mg), **4** (16.2 mg) and **6** (19.5 mg). Fraction B5 (1:1, 1.25 g) was subjected to silica gel column chromatography eluting with petroleum ether-acetone and then run on preparative HPLC (30 % MeOH-H_2O, flow rate 12 mL /min) to give compounds **8** (13.5 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})[11].

Antioxidant activity assay: Antioxidant activity was determined by the detection of the oxidative products with the 2',7'-dichlorofluorescin diacetate (DCFH) method as reported previously[12].

Aurantphenol A (1), $C_{15}H_{16}O_6$, white powder; $[\alpha]_D$ + 11.3 (c 0.025, CH_3OH); UV (CH_3OH), λ_{max} (log ε) 210 (4.92), 258 (3.26), 280 (2.89), 325 (1.87) nm; IR (KBr, ν_{max}, cm^{-1}) : 3025, 2940, 2850, 1712, 1655, 1582, 1528, 1478, 1380, 1032, 870, 789; ^{13}C NMR and ^1H NMR data (C_5D_5N, 500 MHz) (Table-1); positive ESIMS m/z 315 $[M+Na]^+$; HRESIMS m/z 315.0852 $[M+Na]^+$ (calcd. for $C_{15}H_{16}O_6Na$, 315.0845).

RESULTS AND DISCUSSION

A 70 % aq. acetone extract prepared from the leaves and stems of *Cestrum aurantiacum* was partitioned between EtOAc and H_2O. The EtOAc layer was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compounds **1-9** (Fig. 1), including one new phenolic compound, named aurantphenol A (**1**), together with eight known compounds, (+)-pinoresinol (**2**)[13], (+)-mediaresinol (**3**)[14], (+)-lariciresinol (**4**)[15], (+)- justiciresinol (**5**)[16], dehydrodiconiferyl alcohol (**6**)[17], (-)-simulanol, (**7**)[18], 4-hydroxy-3-methoxy-phenyl-1-*O*-(6'-*O*-galloyl)-β-D-glucopyranoside (**8**)[19] and oxyneolignan C (**9**)[8].

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

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	135.0 s	
2	110.8 d	7.18, d, *J* = 1.8
3	151.1 s	
4	142.5 s	
5	118.8 d	7.22, d, *J* = 8.2
6	120.9 d	7.02, dd, *J* = 1.8, 8.2
7	85.7 d	4.80, d, *J* = 6.6
8	70.2 t	4.30-4.45 overlap
9	46.7 d	3.59 m
7'	178.7 s	
8'	70.4 t	4.30-4.45 overlap
9'	48.6 d	3.19, m
3-OMe	55.9 q	3.75 s
4-OAc	21.2, 169.4	1.99 s

Compound (1), obtained as white amorphous powder, was assigned the molecular formula $C_{15}H_{16}O_6$ by HRESIMS m/z 315.0852 $[M+Na]^+$ (calcd. 315.0845). Its ^1H and ^{13}C NMR spectra showed signals to 16 hydrogens and 15 carbons, respectively, corresponding to one aromatic rings (3-methoxy-4-acetoxybenzyl), one carbonyl carbon (δ_C 178.7), two oxidated methylene groups, (δ_C 70.2, 70.4), one oxidated methine carbon (δ_C 86.7), two methine carbon (δ_C 46.7, 48.6), which were in accordance with the molecular formula, $C_{15}H_{16}O_6$. Strong absorption bands accounting for carbonyl group (1712) and aromatic groups (1655, 1582, 1528, 1478 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 258, 210, 280 nm, which confirmed the existence of the aromatic function. The ^1H-^1H COSY of H-7/H-9'/H-9/H-8, H-9'/H-8' (Fig. 2)

suggested that C-7 coupled to C-9', C-9' coupled to C-9, C-9 coupled to C-8 and C-9' coupled to C-8'. The carbonyl carbon was assigned to C-7' by the HMBC correlations of H-8' (δ_H 4.32), H-9 (δ_H 3.60), H-9' (δ_H 3.20) and H-8 (δ_H 4.43) with C-7'. The HMBC correlations of H-8' (δ_H 4.32) with C-7' (δ_C 178.7) and H-7 (δ_H 4.79) with C-8 (δ_C 70.2) indicated that C-8' coupled to C-7' and C-7 coupled to C-8 through a oxygen atom. The 3-methoxy-4-acetoxyphenyl linked to C-7 was deducted by the HMBC correlations of H-2 (δ_H 7.18), H-6 (δ_H 7.02) to C-7. Thus, the planar structure of **1** was established. The relative configurations at C-7, C-9' and C-9 of **1** could be established on the basis of ROESY correlations[13,20]. The proposed relative stereochemistry was further supported by the NOESY experiment (Fig. 3). Thus, the structure of aurantphenol A was established as shown.

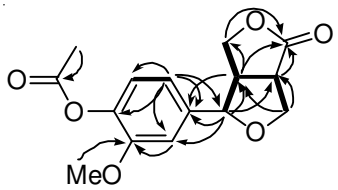

Fig. 2. Selected HMBC() and 1H-1H COSY (↷) of (▬)**1**

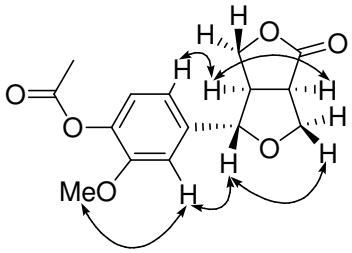

Fig. 3. Key ROESY(◄►) correlations of **1**

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})[11] aurantphenol A shows anti-HIV-1 activity with EC_{50} 3.86 μg/mL, CC_{50} 93.2 μg/mL and TI (Therapeutic Index) 24.1. This compound shows weak anti-HIV-1 activity.

The antioxidant activity of **1** was determined by the detection of the oxidative products with the 2',7'-dichlorofluorescin diacetate (DCFH) method reported previously[12]. It shows antioxidant activity with an IC_{50} value of 3.68 μg/mL. This compound shows high antioxidant activity.

ACKNOWLEDGEMENTS

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