NOTE

A New Phenyl Propanoid from the Roots of *Nicotiana tabacum* and Its Biological Activities

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A new phenylpropanoid, 3-hydroxy-1-(4,5-dimethoxyphenyl-2-O- β -D-glucopyranoside)-propan-1-one, was isolated from the roots of *Nicotiana tabacum*. Its structures were determined by means of HRESIMS, extensive 1D and 2D NMR spectroscopic studies and chemical evidence. Compound **1** was tested for its cytotoxicity against five human tumor cell lines and it show modest cytotoxic abilities against PC3 and MCF7 cell with IC₅₀ values of 5.6 and 2.8 μ M, respectively.

Key Words: 3-Hydroxy-1-(4,5-dimethoxyphenyl-2-O- β -D-glucopyranoside)-propan-1-one, *Nicotiana tabacum*, Cytotoxicity.

Nicotiana tabacum L. is one of the most commercially valued agricultural crops in the world^{1,2}. In addition to cigarette industry use, *N. tabacum* also contains many useful chemical compounds, such as sesquiterpenes^{3,4}, diterpenoids⁵⁻⁷, alkaloids^{8,9}, phenols¹⁰, etc. Motivated by search for bioactive metabolites from this plant, an investigation on the chemical constituents of the roots of *N. tabacum* was carried out. As a result, a new phenylpropanoid (Fig. 1) was isolated from this plant. In addition, the cytotoxicity of phenyl propanoid (**1**) was evaluated. This article deals with the isolation, structural elucidation and cytotoxicity of this new phenyl propanoid.

Fig. 1. Structure of compounds **1**

UV spectra were obtained using a 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. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. MS 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 $\times$ 25 cm, 7.0 μ m) column. Column chromatography was performed with Si gel (200.300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40.63 μ m, Merck, Darmstadt, Germany) and MCI gel (75.150 μ m, Mitsubishi Chemical Corporation, Tokyo, Japan). The fractions were monitored by TLC and spots were visualized by heating Si gel plates sprayed with 5 % H₂SO₄ in EtOH. Plant Material. The roots of the Chinese variety of *N. tabacum* (Honghua Dajinyuan) were collected in Dali Prefecture, Yunnan Province, P.R. China, in September 2012.

Extraction and isolation: The air-dried and powdered roots of *N. tabacum* (2.2 kg) were extracted four times with 70 % methanol (4 $\times$ 2 L) at room temperature and filtered. The crude extract (102 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 five fractions A to F. The further separation of fraction C (6:4, 35.2 g) by silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures C1-C5. Fraction C2 (8:2, 4.15 g) was subjected to preparative HPLC (30 % methanol, flow rate 12 mL/min) to give compound **1** (35.6 mg).

Compound **1** was obtained as pale yellow gum. Its molecular formula was determined as C₁₇H₂₄O₁₀ by HR-ESI-MS

m/z 387.1283 $[M-H]^-$ (calcd. 387.1291). Its ^1H and ^{13}C NMR spectra (Table-1) showed signals to 24 hydrogens and 17 carbons, respectively, corresponding to one aromatic ring (δ_C 102.8, 112.3, 112.8, 142.0, 154.2, 155.2) with two aromatic protons (δ_H 6.82 s, 7.36 s), one methylene group (δ_C 42.2), one oxidated methylene group (δ_C 58.9), one carbonyl group (δ_C 198.5), two methoxyl groups (δ_C 55.6, 56.2) and a glucosyl moiety (δ_C 104.3 d, 75.4 d, 78.3 d, 71.7 d, 78.0 d, and 62.8 t). Strong absorption bands accounting for hydroxyl (3458, 3376 cm^{-1}), carbonyl (1728, 1713 cm^{-1}) and aromatic group (1630, 1517, 1455, 1439 cm^{-1}) could also be observed in its IR spectrum. The UV spectrum of **1** showed absorption maxima at 285 nm confirmed the existence of the aromatic function. The ^1H - ^1H COSY of H-8/H-9; together with HMBC correlations (Fig. 2) of H-6 (δ_H 7.36) with C-7 (δ_C 198.5), of H-8 (δ_H 3.20) with C-1 (δ_C 112.3), of H-9 (δ_H 4.28) with C-7 (δ_C 198.5) and C-8 (δ_C 42.2) suggested that **1** is a 3-hydroxyl-1-phenyl-1-propanone (Ar-CO-CH₂-CH₂OH) and it possess two methoxyl groups and a glucosyl moiety on the aromatic ring. The HMBC correlations of two methoxyl proton signals (δ_H 3.82, 3.85) with C-4 (δ_C 154.2), C-5 (δ_C 142.0) indicated two methoxyl groups should be located at C-4 and C-5, respectively. The long-range correlations in the HMBC spectrum between H-1' (δ_H 4.72 d) and C-2 (δ_C 155.2 s) indicated the glucosyl was linked to C-2 and the coupling constant value of H-1' ($J = 7.35$ Hz) indicated that the glucosyl moiety was connected to the aglycone by a β -linkage^{11,12}. On the basis of the above evidence, the structure of **1** was established as 3-hydroxy-1-(4,5-dimethoxyphenyl-2-O- β -D-glucopyranoside)-propan-1-one.

TABLE-1

 ^1H AND ^{13}C NMR DATA (IN CD₃OD) OF COMPOUND **1**

No.	Compound 1	
	δ_C (mult.)	δ_H (mult, J, Hz)
1	112.3 s	—
2	155.2 s	—
3	102.8 d	6.82 s
4	154.2 s	—
5	142.0 s	—
6	112.8 d	7.36 s
7	198.5 s	—
8	42.2 t	3.20 t, $J = 6.1$
9	58.9 t	4.28 t, $J = 6.2$
OMe-4	55.6 q	3.82 s
OMe-5	56.2 q	3.85 s
1'	104.3 d	4.72, d, $J = 7.2$
2'	75.4 d	3.47 m
3'	78.3 d	3.46 m
4'	71.7 d	3.30 m
5'	78.0 d	3.30 m
—	—	3.68 m
6'	62.8 t	3.82 m

Fig. 2. Selected HMBC ($\curvearrowright$) correlations of **1**

Since certain of the phenyl propanoid derivatives exhibit potential cytotoxicity. 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 previously¹³. Taxol was used as the positive control. The results revealed the new compound showed low active (IC_{50} values $>10 \mu M$) for NB4, A549, and SHSY5Y tumor cell and showed modest cytotoxicity against PC3 and MCF7 cell with IC_{50} values of 5.6 and 2.8 μM , respectively.

3-Hydroxy-1-(4,5-dimethoxyphenyl-2-O- β -D-glucopyranoside)-propan-1-one: Obtained as pale yellow gum; UV (MeOH) λ_{max} (log ϵ) 320 (2.42), 285 (4.15), 250 (3.52), 210 (4.68) nm; IR (KBr, ν_{max} , cm^{-1}): 3458, 3376, 2922, 2854, 1728, 1713, 1630, 1517, 1455, 1439, 1363, 1282, 1165, 1137, 1080, 1048, 975, 860; ^1H and ^{13}C NMR data (C₅D₅N, 500 MHz), Table-1; positive ESIMS m/z 387 $[M-H]^-$; HRESIMS m/z 387.1291 $[M-H]^-$ (calcd. (%) 387.1291 for C₁₇H₂₃O₁₀).

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