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Phenolic Compounds from Tobacco Stem

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A new phenolic compound (tobphenol A) together with five known phenols was isolated from the tobacco stem. Their structures were determined by means of HRESIMS, extensive ^{1}D and ^{2}D NMR spectroscopic studies and chemical evidences. The anti HIV-1 activity of tobphenol A was also evaluated. It is shown that tobphenol A has moderate anti HIV-1 activity with a therapeutic index (TI) above 128.

Key Words: Tobacco stem, Phenolic compounds, Tobphenol A, Anti HIV-1 activity.

INTRODUCTION

Tobacco is one of the most important sources of income to farmers who live in over 100 countries and it has become one of the most commercially valued agricultural crops in the international markets[1,2]. In addition to its commercial value, tobacco leaf and stem also contain many useful chemical compounds, such as terpenes, alkaloids, lignans, polyphenols[3-9]. The utilizations of these active compounds in tobacco leaf and its stem were received more and more attentions[10,11].

In order to investigate the components of the tobacco stem and search for potential leads for drug development, in this work, the phytochemical investigation on tobacco stem was carried out. This study led to the isolation of a new phenolic compound (tobphenol A) (**1**), together with five known phenols, matairesinol (**2**)[12], (+)-1-hydroxy-1,3,5-bisabolatrien-10-one (**3**)[13], 2,4-dihydroxyphenylacetic acid (**4**)[14], caffeic acid (**5**)[15] and ferulic acid (**6**)[15]. The structures of compounds **1-6** (Fig. 1) were established by means of HRESIMS and extensive NMR spectra, respectively. The anti HIV-1 activity of tobphenol A was also evaluated.

EXPERIMENTAL

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 × 250 nm, 7.0 μm) column and DAD detector.

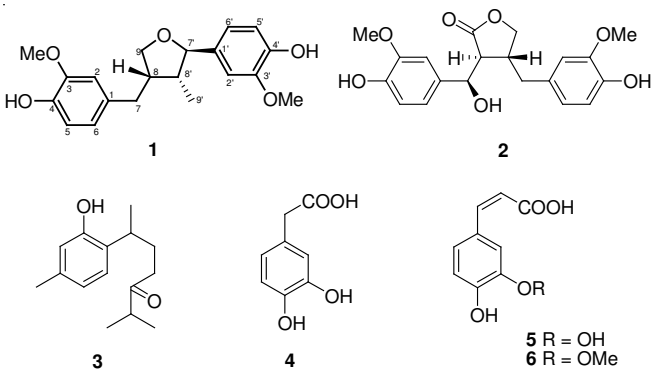

Fig. 1. Phenolic compounds from the tobacco stem

Plant material: The stem of *Nicotiana tabacum* L (tobacco stem) was collected from Yuxi County, Yunnan Province, P.R. China, in September 2008.

Extraction and isolation: The air-dried and powdered tobacco stem (2.5 kg) were extracted with 70 % aqueous ethanol (3.0 L × 3, 24 h each) at room temperature and the extract was concentrated under vacuum condition. The dried extract (68.5 g) was applied to Si gel (200-300 mesh) column chromatography eluting with a $CHCl_3$-Me_2CO gradient system (9:1, 8:2, 7:3, 6:4, 5:5, 2:1) to give six fractions A-F. The separation of fraction B ($CHCl_3$-Me_2CO 8:2, 21.2 g) by Si gel column chromatography eluted with $CHCl_3$-Me_2OH (9:1-1:2) yielded mixtures B1-B5. Fraction B2 (3.8 g) was subjected to preparative HPLC (45 % MeOH-H_2O, flow rate 12 mL/min) to give **1** (22.1 mg), **2** (44.8 mg) and **3** (116 mg). Fraction B4 (1.8 g) was subjected to preparative HPLC (28 % MeOH-H_2O, flow rate 12 mL/min) to give **4** (148 mg), **5** (56.7 mg) and **6** (182 mg).

Anti HIV-1 assay: The cytotoxicity assay against C8166 cells (CC50) was assessed using the MTT method and anti-HIV-1 activity was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC50)[16].

RESULTS AND DISCUSSION

Tobphenol A was obtained as white amorphous powder. The molecular formula of tobphenol A was determined as $C_{20}H_{24}O_5$ from its HRESIMS at m/z 367.1523 $[M + Na]^+$ (calcd. 367.1521). The ^1H and ^{13}C NMR data (Table-1) indicated the presence of aromatic rings. Strong absorption bands accounting for aromatic groups (1687, 1652, 1621, 1528, 1485, 1462) could also be observed in its IR spectrum. The UV spectrum of tobphenol A showed maximum absorption at 280 and 206 nm, which confirmed the existence of the aromatic functions. The ^1H, ^{13}C and DEPT NMR spectra exhibited 20 carbon atoms possessed two aromatic ring (Table-1) with six aromatic protons, two methoxyl groups on the aromatic rings, two methylene groups (include one oxygenated methylene group), three secondary methyl group (include one oxygenated secondary methyl group) and one methyl group. By comparison, the skeleton of tobphenol A was the same as that of known compound (kobusinol A)[17]. Detailed comparison of ^1D NMR spectra showed the only difference was a methoxy group in kobusinol A was substituted by a phenolic hydroxyl group in tobphenol A on the aromatic rings, which was supported by the disappearance of signal of a methoxy group in tobphenol A. According to the HMBC correlations from H-2 to C-7, H-7 to C-2 and C-1; H-2' to C-7', H-1' to C-7', H-7' to C-2' and C-1' (Fig. 2), two aromatic groups were attached to C-7 and C-7'. The ^1H-^1H COSY correlations of H-7/H-8, H-8/H-9, H-8/H-8', H-8'/H-9', H-8'/H-7', as well as the HMBC correlations from H-7 to C-8 and C-9, H-8 to C-9 and C-7', H-7' to C-8' and C-9', H-8' to C-8 and C-7, H-9' to C-8, C-9' and C-7' indicated tobphenol A is a tetrahydrofurofuran lignan and the signals methylene group were assigned to C-7, the signals oxygenated methylene group were assigned to C-9, two signals of secondary methyl groups were assigned to C-8 and C-8', the signals oxygenated secondary methyl group were assigned to C-7', the methyl groups (C-9') were attached to C-8', respectively. The methoxyls groups located at C-3, C-3' and the phenolic hydroxy groups located at C-4, C-4' can also be deduced from its HMBC spectrum. The proposed relative stereochemistry was further supported by the NOESY experiment (Fig. 3). Thus, the structure of tobphenol A was established as shown.

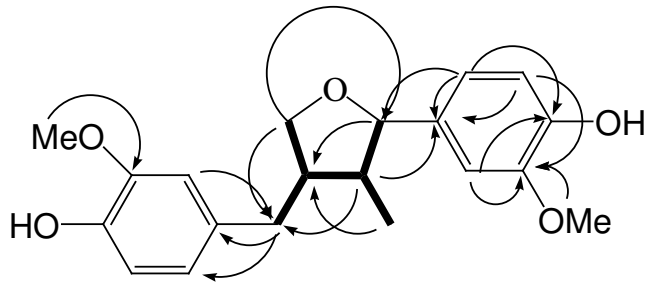

Fig. 2. ^1H, ^1H COSY (▬) and key HMBC (◠) correlations of tobphenol A

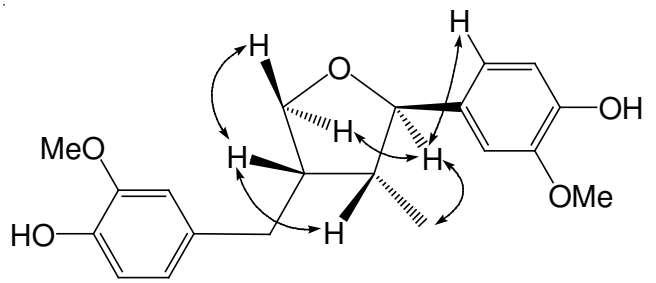

Fig. 3. Selected NOESY correlations for tobphenol A

The potencies of tobphenol A in preventing the cytopathic effects of HIV-1 in MT_4 cells, as well as compound-induced cytotoxicity in MT_4 cells in parallel with the antiviral activity were evaluated[16]. The results from the cell-based assays demonstrated potent anti HIV-1 activity with EC_{50} (median effect concentration) value of 2.18 μg/mL and a therapeutic index (TI) values is greater than 128. Tobphenol A shows moderate anti-HIV activity.

Tobphenol A: $C_{20}H_{24}O_5$, white amorphous solid; $[\alpha]^{24.2}_D$ + 3.62 (c 0.26, MeOH); UV (MeOH), λ_{max} (log ε) 280 (4.18), 206 (5.36) nm; IR (KBr, ν_{max}, cm^{-1}): 3462, 2958, 2874, 1687, 1652, 1621, 1528, 1485, 1462, 1398, 1354, 1245, 1208, 1121, 1098, 1055; ^1H and ^{13}C NMR data (C_5D_5N, 500 MHz), Table-1; HRESIMS (positive ion mode) m/z 367.1523 $[M + Na]^+$ (calcd. %) 367.1521 for $C_{26}H_{30}O_{10}Na$).

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TABLE-1
^1H NMR AND ^{13}C NMR DATA OF TOBPHENOL A IN C_5D_5N

No.	δ_C (mult.)	δ_H (mult, *J*, Hz)	No.	δ_C (mult.)	δ_H (mult, *J*, Hz)
1	132.1 s	–	1'	133.8 s	–
2	110.9 d	7.28 s	2'	113.2 d	6.96, s
3	150.4 s	–	3'	150.1 s	–
4	147.2 s	–	4'	146.6 s	–
5	116.3 d	7.26, d, *J* = 7.8 Hz	5'	116.6 d	7.19, d, *J* = 7.8 Hz
6	120.1 d	6.83, d, *J* = 7.8 Hz	6'	121.8 d	7.08, d, *J* = 7.8 Hz
7	38.5 t	2.58, dd, *J* = 8.7, 14.1 Hz	7'	89.2 d	4.44, d, *J* = 9.2 Hz
		2.90 dd, *J* = 5.4, 13.8 Hz	8'	49.2 d	1.89, m
8	49.5 d	2.31, m	9'	15.0 q	1.00, d, *J* = 6.5 Hz
9	73.4 t	3.98, dd, *J* = 8.2, 8.5 Hz	OMe-3	55.9 q	3.75, s
–	–	4.12, dd, *J* = 8.0, 8.2 Hz	OMe-3'	56.0 q	3.78, s

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