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Three new flavonoids from the leaves of oriental tobacco and their cytotoxicity

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ABSTRACT

Three new flavonoids, 6,7-dimethoxy-4′-hydroxy-8-formylflavon (**1**), 8-formyl-4′,6,7-trimethoxyflavon (**2**), 4′,7-dihydroxy-8-formyl-6-methoxyflavon (**3**), together with fifteen known flavonoids (**4**–**18**) were isolated from the leaves of oriental tobacco (a variety of *Nicotiana tabacum* L). Their structures were determined by means of HRESIMS, extensive 1D and 2D NMR spectroscopic studies and chemical evidences. The cytotoxicity against five human tumor (NB4, A549, SHSY5Y, PC3, and MCF7) cell lines of compounds **1**–**3** were also evaluated. The results showed that compounds **1** and **3** showed high cytotoxicity against PC3 and A549 cell lines with IC_{50} values of 2.6 and 1.6 μM, respectively.

1. Introduction

Nicotiana tabacum L, a perennial herbaceous plant, is one of the most commercially valued agricultural crops in the world (The Editorial Committee of the Administration Bureau of Flora of China, 2005; Hu and Mao, 2006). The leaves of *N. tabacum* are the most important row material for tobacco industry. In addition to being used in cigarette industry, *N. tabacum* is also used as insecticides, anesthetics, diaphoretics, sedatives, and emetic agents in Chinese folklore medicines because of it containing many useful chemical compounds (Rodgman and Perfetti, 2008).

Previous investigation of this species led to the discovery of a number of compounds, such as sesquiterpenes (Feng et al., 2010, 2009), diterpenoids (Shinozaki et al., 1996; Petterson et al., 1993), alkaloids (Wei et al., 2005; Braumann et al., 1990), lignans (Chen et al., 2012a; Gao et al., 2012), flavonoid (Chen et al., 2012b), phenylpropanoids (Tan et al., 2011). With the aim of continuing efforts to utilize *N. tabacum* and identify bioactive natural products, the phytochemical investigation of the leaves of oriental tobacco (a variant of *N. tabacum*) was carried out. As a result, three new flavonoids (**1**–**3**), together with fifteen known compounds (**4**–**18**) were isolated from this plant. In addition, the cytotoxicity of the compounds **1**–**3** was also evaluated. This paper deals with the isolation, structural elucidation and biological activities.

2. Results and discussion

A 70% aq. methanol extract prepared from the leaves of oriental tobacco was subjected repeatedly to column chromatography on silica gel, sephadex LH-20, RP-18 and preparative HPLC to afford three new flavonoids, 6,7-dimethoxy-4′-hydroxy-8-formylflavon (**1**), 8-formyl-4′,6,7-trimethoxyflavon (**2**), 4′,7-dihydroxy-8-formyl-6-methoxyflavon (**3**), together with fifteen known flavonoids (**4**–**18**). The structures of the compounds **1**–**18** were as shown in Fig. 1, and the ^{1}H and ^{13}C NMR data of **1**–**3** were listed in Table 1. The known compounds, compared with literature, were identified as kaempferol (**4**) (Yu and Yang, 1999), kaempferol-3-*O*-*β*-D-glycosides (**5**) (Yu and Yang, 1999), kaempferol-3-*O*-*β*-D-galactopranoside (**6**) (Yu and Yang, 1999), kaempferol-3-*O*-*β*-D-rutinsides (**7**) (Yu and Yang, 1999), quercetin (**8**) (Yu and Yang, 1999), quercetin-3-*O*-*β*-D-glycosides (**9**) (Yu and Yang, 1999), quercetin-3-*O*-*β*-D-galactopranoside (**10**) (Yu and Yang, 1999), rutin (**11**) (Yu and Yang, 1999), diosmetin-7-rutinoside (**12**) (Teslov and Zapesochnaya, 1978), taxifolin (**13**) (Yu and Yang, 1999), taxifolin 4′-methyl ether (**14**) (Ling et al., 2007), (−)-epicatechin (**15**) (Yu and Yang, 1999), (2*R*,3*R*)-3,5-dihydroxy-7-methoxyflavanone (**16**) (Rossi et al., 1997), candenatenin E (**17**) (Sarot et al., 2009), morachalcone A (**18**) (Delle Monache et al., 1995).

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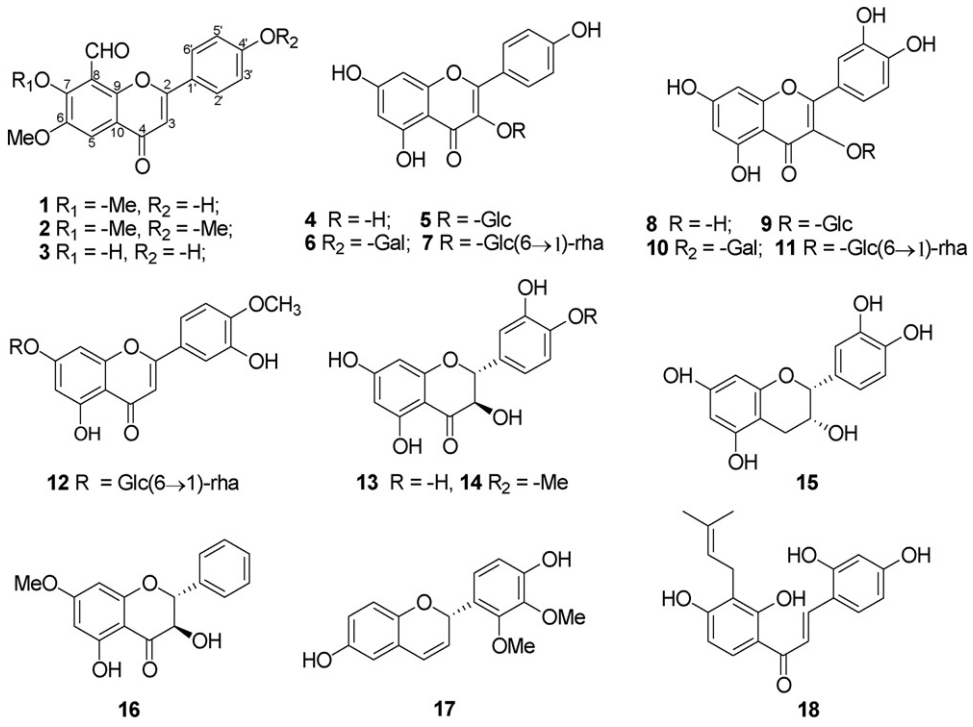

Fig. 1. The structures of flavonoids from oriental tobacco.

Compound **1** was obtained as an orange-yellow gum. It gives the molecular formula $C_{18}H_{14}O_6$ by HRESIMS (*m*/*z*: 349.0681 $[M+Na]^+$, calcd 349.0688). The ^1H and ^{13}C NMR spectrum of **1** (Table 1) along with analysis of the DEPT spectra displayed 18 carbon signals and 14 proton signals, respectively, corresponding to a flavon nucleus (Yu and Yang, 1999) (δ_C 164.3 s, 105.5 d, 178.4 s, 122.3 d, 146.9 s, 158.8 s, 115.0 s, 151.0 s, 119.8 s, 122.8 s, 132.0 d (2C), 116.5 d (2C), 160.8 s), one aldehyde group (δ_C 192.0; δ_H 10.19), two methoxy groups (δ_C 56.9, 61.1; δ_H 3.77, 3.80), and one low-field hydroxy proton (δ_H 10.83). The typical protons signals at (δ_H 7.97 d, J = 8.6, 2H) and (δ_H 6.78 d, J = 8.6, 2H) observed in the ^1H NMR spectrum revealed that the aromatic ring C of **1** is a 1,4-disubstituted phenolic moiety (Yu and Yang, 1999), and the HMBC of correlations (Fig. 2) of the hydroxy proton signals, δ_H 10.83 with C-3′,5′ (δ_C 116.5) and C-4′ (δ_C 160.8) also suggested the above. The aldehyde and two methoxy groups located at C-8, C-6, and C-7 were supported by the HMBC correlations of aldehyde proton signal at δ_H 10.19 with carbons C-7 (δ_C 158.8), C-8 (δ_C 115.0), C-9 (δ_C 151.0), and the methoxy proton signals at δ_H 3.77 and δ_H 3.80 with the carbons C-6 (δ_C 146.9) and C-7 (δ_C 158.8), respectively. Thus, the structure of **1** was established as 6,7-dimethoxy-4′-hydroxy-8-formylflavon.

Compound **2** was obtained as orange-yellow gum, and should be established a molecular ions at *m*/*z* 363.0840 $[M+Na]^+$ in the HRESIMS (calcd *m*/*z* 363.0845), corresponding to the molecular formula of $C_{19}H_{16}O_6$. The ^1H and ^{13}C NMR spectra of **2** were very similar to these of **1**. The only difference resulted from the appearance of an additional methoxy group in **2**, which indicated that the hydroxy group in **1** was substituted by a methoxy group in **2**. Further analysis of the HMBC spectrum of the methoxy proton

Table 1
^1H NMR and ^{13}C NMR Data (in C_5D_5N) of compounds **1**–**3**.

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (mult, *J*, Hz)	δ_C (m)	δ_H (mult, *J*, Hz)
2	164.3 s		164.7 s		164.5 s	
3	105.5 d	6.62 s	105.7 d	6.62 s	104.8 d	6.63 s
4	178.4 s		179.0 s		178.2 s	
5	122.3 d	7.39 s	122.1 s	7.39 s	121.3 s	7.43 s
6	146.9 s		147.0 s		146.0 s	
7	158.8 s		158.6 s		156.1 s	
8	115.0 s		114.9 s		114.0 s	
9	151.0 s		151.7 s		151.4 s	
10	119.8 s		119.9 s		119.0 s	
1′	122.8 s		122.5 s		122.8 s	
2′, 6′	132.0 d	7.97 d, *J* = 8.6	131.1 d	8.01 d, *J* = 8.6	131.6 d	8.00 d, *J* = 8.6
3′, 5′	116.5 d	6.78 d, *J* = 8.6	115.4 d	6.81 d, *J* = 8.6	116.7 d	6.89 d, *J* = 8.6
4′	160.8 s		163.0 s		160.0 s	
–CHO	192.0 s	10.19 s	192.9 s	10.20 s	192.2 s	10.20 s
–OMe-6	55.9 q	3.77 s	56.0 q	3.77 s	55.9 q	3.80 s
–OMe-7	61.1 q	3.80 s	61.2 q	3.82 s		
–OMe-4′			55.8 q	3.84 s		
Ar-OH-4′		10.83 brs				10.80 s
Ar-OH-7′						11.25 s