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CHROMONE DERIVATIVES FROM THE LEAVES OF *NICOTIANA TABACUM* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITIES

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Abstract – Two new chromone derivatives, 5-hydroxy-9-methyl-1-(2-oxopropyl)benzo[*β*]oxepin-7(2*H*)-one (**1**), 5-hydroxy-2-methoxy-9-methyl-1-(2-oxopropyl)benzo[*β*]oxepin-7(2*H*)-one (**2**), together with five known chromone derivatives (**3**-**7**), were isolated from the leaves of *Nicotiana tabacum*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR techniques. Compounds **1**-**7** were tested for their anti-tobacco mosaic virus (anti-TMV) activities. The results showed that compound **2** exhibited high anti-TMV activity with inhibition rate of 31.5%. Its inhibition rate is close to that of positive control. The other compounds also showed potential anti-TMV activities with inhibition rates in the range of 11.2 ~ 23.5%.

Nicotiana tabacum, tobacco, is a stout herbaceous plant in the Solanaceae (nightshade family) that originated in the tropical Americas (South America, Mexico, and the West Indies) and now cultivated worldwide as the primary commercial source of tobacco, which is smoked or chewed as a drug for its mild stimulant effects.[1,2] In addition, *N. tabacum* is also used as insecticides, anesthetics, diaphoretics, sedatives, and emetic agents in Chinese folklore medicines because of its containing many useful chemical compounds.[1,3] In previous investigation of this species led to the discovery of a number of new compounds by our groups, and those compounds were found to be shown various bioactivities, such as anti-HIV-1, anti-TMV, and cytotoxicity.[4-9] With the aim of continuing efforts to utilize *N. tabacum* and

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identify bioactive natural products, the phytochemical investigation of the leaves of Honghua Dajinyuan (a variety of *N. tabacum*) was carried out, and led to the isolation of two new chromone derivatives (**1** and **2**), together with five known chromone derivatives (**3**-**7**). This paper reports the isolation, structural elucidation, and anti-TMV activity of these compounds.

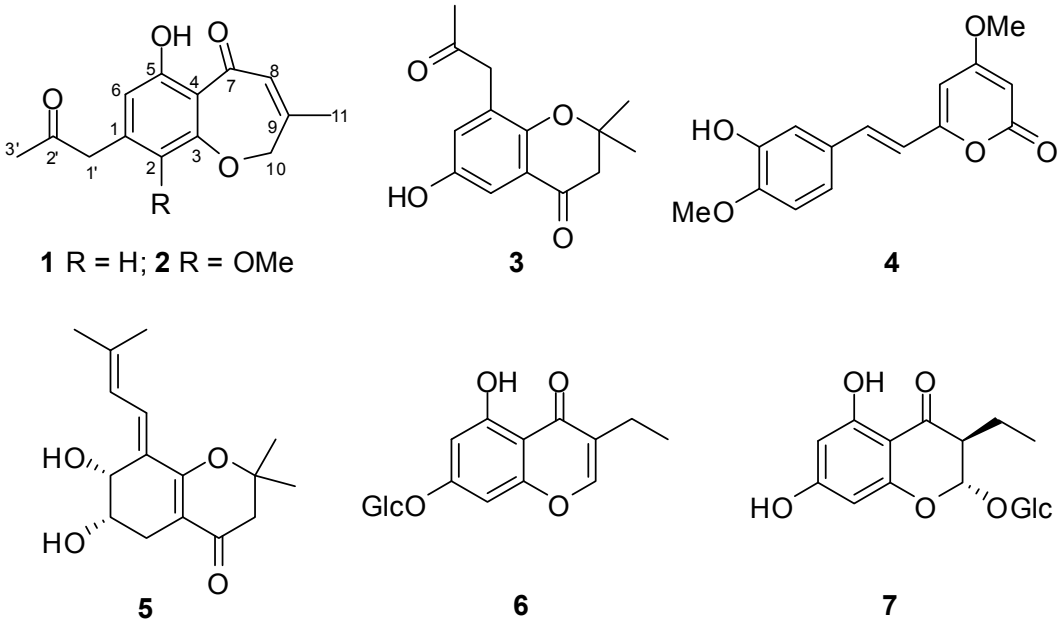

Figure 1. The chromone derivatives form *N. tabacum*

The 90% aqueous ethanol extract prepared from the leaves of *N. tabacum* was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford two new chromone derivatives, 5-hydroxy-9-methyl-1-(2-oxopropyl)benzo[*β*]oxepin-7(2*H*)-one (**1**), 5-hydroxy-2-methoxy-9-methyl-1-(2-oxopropyl)benzo[*β*]oxepin-7(2*H*)-one (**2**), together with five known chromone derivatives (**3**-**6**). The structures of compounds **1**-**7** were shown in Figure 1. The ^{1}H and ^{13}C NMR data of the compounds **1** and **2** were listed in Table 1. The known compounds, compared with literature data, were identified as: tabchromone A (**3**),[9] 6-(3-hydroxy-4-methoxystyryl)-4-methoxy-2*H*-pyran-2-one (**4**),[10] pestaloficiol G (**5**),[11] takanechromone B (**6**),[12] takanechromanone B (**7**).[12]

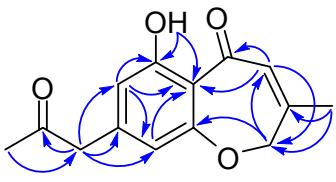

Figure 2. Key HMBC ( ) correlations of **1**

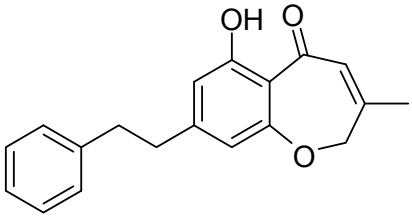

Figure 3. The structure in reference 13

Compound **1** was obtained as pale yellow oil. The electrospray ionization mass (ESI) spectrum of **1** gave a molecular ion peak at *m/z* 245 as the base peak, and high-resolution analysis showed a molecular formula $C_{14}H_{14}O_4$, confirming 8 degrees of unsaturation. Fourier transform (FT)-IR showed hydroxyl group at 3436, carbonyl group at 1705 and 1642, and aromatic group at 1604, 1570, and 1465 cm^{-1}. UV spectrum of **1** also exhibited absorption bands for aromatic ring at 292 and 253 nm. The ^{13}C-NMR spectrum of **1** (Table 1) contained 14 carbons, including eight olefinic (δ_C 144.3 s, 111.1 d, 160.8 s, 113.5 s, 164.6 s, 114.8 d, 128.0 d, and 154.1 s) carbons, two carbonyl (δ_C 193.5 s and 205.9 s) carbons, two methyl (δ_C 22.1 q and 30.5 q) carbons, and two methylene (δ_C 72.0 t and 50.2 t) carbons. The ^{1}H-NMR spectrum of **1** also showed signals of three aromatic ring protons (δ_H 6.50 (d) 1.8, 6.62 (d) 1.8, and 6.29 s), two singlet methyl protons (δ_H 2.01 s and 2.44 s), two singlet methylene protons (δ_H 4.11 s and 4.42 s), and a phenolic hydroxyl proton (δ_H 9.88 s). These signals suggested compound **1** containing a 1,5-substituted, 9-methylbenzo[*β*]oxepin-7(2*H*)-one moiety (Figure 3),[13] an 2-oxopropyl group,[9] and a phenolic hydroxy group. The heteronuclear multiple bond correlation (HMBC) (Figure 2) of H-8 (δ_H 6.29) with C-4 (δ_C 113.5), C-7 (δ_C 193.5), C-10 (δ_C 72.0), and C-11 (δ_C 22.1), of H-10 (δ_H 4.42) with C-3 (δ_C 160.8) and C-8 (δ_C 128.0), of H-11 (δ_H 2.01) with C-8 (δ_C 128.0), C-9 (δ_C 154.1), and C-10 (δ_C 72.0), also support the existence of the 9-methylbenzo[*β*]oxepin-7(2*H*)-one moiety. The long-range correlations between H-1′ (δ_H 4.11) and C-1 (δ_C 144.3), C-2 (δ_C 111.1), and C-6 (δ_C 114.8), demonstrates the 2-oxopropyl moiety located at C-1. Moreover, the HMBC correlations between Ar-OH (δ_H 9.88) and the signal of C-4 (113.5), C-5 (164.6), and C-6 (114.8) confirmed the hydroxy groups at C-5. Thus, the structure of **1** was established as 5-hydroxy-9-methyl-1-(2-oxopropyl)propyl)benzo[*β*]oxepin-7(2*H*)-one.

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds **1** and **2** in $CDCl_3$

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
1	144.3 s		135.1 s	
2	111.1 d	6.50 (d) 1.8	144.6 s	
3	160.8 s		153.9 s	
4	113.5 s		114.2 s	
5	164.6 s		156.0 s	
6	114.8 d	6.62 (d) 1.8	110.0 d	6.44 s
7	193.5 s		193.0 s	
8	128.0 d	6.29 s	127.4 d	6.34 s
9	154.1 s		154.4 s	
10	72.0 t	4.42 s	72.4 t	4.43 s
11	22.1 q	2.01 s	22.4 d	2.01 s
1'	50.2 t	4.11 s	49.8 t	4.08 s
2'	205.9 s		206.1 s	
3'	30.5 q	2.44 s	30.9 q	2.45 s
2-OMe			60.9 q	3.80 s
5-OH		9.88 s		9.67 s

Compound **2** was also obtained as pale yellow oil, and showed quasi molecular ion at *m/z* 299.0890

$[M+Na]^+$ in the HRESIMS (calcd *m/z* 299.0895), corresponding to the molecular formula of $C_{15}H_{16}O_5$. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**. The obvious chemical shift differences resulted from the disappearance of an olefinic proton signals, and appearance of a methoxy group (δ_C 60.9 q; δ_H 3.80 s) in compound **2.** This indicated that an additional methoxy group should be substituted on aromatic ring of **2**. The HMBC correlations of the methoxy proton signal (δ_C 3.80) with C-2 (δ_C 144.6) indicated that the methoxy group should be located at C-2. Thus, the structure of **2** was established as 5-hydroxy-2-methoxy-9-methyl-1-(2-oxopropyl)benzo[*β*]oxepin-7(2*H*)-one.

Since certain of the chromone derivatives exhibit potential anti-TMV activities,[14] compounds **1-7** were tested for their anti-TMV activity. The inhibitory activities of compounds **1-7** against TMV replication were tested using the half-leaf method.[14] Ningnanmycin (20 μM, in DMSO), a commercial product for plant disease in China, was used as a positive control. The antiviral inhibition rates of compounds **1-7** at the concentration of 20 μM were listed Table 2. The results showed that compound **2** exhibited anti-TMV activity with inhibition rate of 31.5%. Its inhibition rate is close to that of positive control. The other compounds also showed potential anti-TMV activities with inhibition rates in the range of 11.2 ~ 23.5%.

Table 2. TMV Infection Inhibition Activities of Compounds **1-7**

Compounds	Inhibition rates (%)	Compounds	Inhibition rates (%)
1	22.5 ± 2.6	**5**	15.4 ± 2.5
2	31.5± 2.8	**6**	18.1± 2.4
3	11.2±2.5	**7**	14.5±1.8
4	23.5 ± 2.6	ningnamycin	29.6 ± 3.0

All results are expressed as mean ± SD; n = 3 for all groups.

EXPERIMENTAL

General. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer. 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. ^1H, ^{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 mm × 250 mm, 7.0 μm) column and DAD detector.

Plant material. The leaves of Honghua Dajinyuan (a variety of *N. tabacum*) were collected in Yuxi Prefecture, Yunnan Province, People's Republic of China, in September 2012.

Extraction and Isolation. The air-dried and powdered leaves of *N. tabacum* (2.5 kg) were extracted four times with 90% aq. EtOH (4 Å~5.0 L) at room temperature and filtered. The crude extract (298 g) was applied to silica gel (200–300 mesh) column chromatography, eluting with a $CHCl_3$-MeOH (9:1, 8:2,

7:3, 6:4, 1:1), yielded mixtures A-E. The further separation of fraction A (9:1, 12.8 g) by silica gel column chromatography, eluted with $CHCl_3$-Me_2CO gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A1–A6. Subfraction A2 (9:1, 1.26 g) was subjected to preparative HPLC (58% MeOH, flow rate 12 mL/min) to give **1** (6.27 mg), **2** (12.2 mg), **3** (12.2 mg), **4** (8.26 mg), and **5** (5.27 mg). The further separation of fraction B (8:2, 12.8 g) by silica gel column chromatography and preparative HPLC (35% MeOH, flow rate 12 mL/min) to give **6** (18.2 mg) and **7** (12.2 mg).

5-Hydroxy-9-methyl-1-(2-oxopropyl)benzo[*β*]oxepin-7(2*H*)-one (**1**): pale yellow oil; UV (MeOH) λ_{max} (log ε) 210 (4.26), 253 (3.68), 292 (3.47) nm; IR (KBr) ν_{max} 3436, 2918, 2860, 1705, 1642, 1604, 1570, 1465, 1385, 1205, 1142, 970, 863 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1. Negative ESIMS *m/z* 245 $[M-H]^-$; negative HRESIMS *m/z* 245.0819 $[M-H]^-$ (calcd for $C_{14}H_{13}O_4$, 245.0814).

5-Hydroxy-2-methoxy-9-methyl-1-(2-oxopropyl)benzo[*β*]oxepin-7(2*H*)-one (**2**): pale yellow oil; UV (MeOH) λ_{max} (log ε) 210 (4.28), 250 (3.65), 295 (3.52) nm; IR (KBr) ν_{max} 3448, 2926, 2862, 1708, 1645, 1602, 1566, 1457, 1375, 1218, 1145, 965, 850 cm^{-1}; ^1H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1. Positive ESIMS *m/z* 299 $[M+Na]^+$; Positive HRESIMS *m/z* 299.0890 $[M+Na]^+$ (calcd for $C_{15}H_{16}NaO_5$, 299.0895).

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