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FLAVONES FROM THE LEAVES OF YUNNAN LOCAL SUN CURED TOBACCO AND THEIR CYTOTOXICITY

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Abstract – Two new flavones, 4',7-dimethoxy-6-hydroxy-8-isobutyrylflavone (**1**), 4',7-dimethoxy-6-hydroxy-8-[(*E*)-3-hydroxy-2-methylprop-1-enyl]flavone (**2**), together with four known flavones (**3-6**) were isolated from the leaves of Yunnan local sun cured tobacco. Their structures were determined by means of HRESIMS and extensive 1D and 2D NMR spectroscopic studies. Compounds **1-6** were screened for the cytotoxic activity in a panel of human cancer cell lines, including NB4, A549, SHSY5Y, PC3, and MCF7. Compound **2** exhibited high cytotoxicity for A549 and SHSY5Y cells with IC_{50} values of 1.6 and 2.8 μM, respectively. The other compounds also exhibited cytotoxicity with IC_{50} values in the range of 4.3 – 9.5 μM, respectively.

Nicotiana tabacum L. is the most commonly grown of all plants in the *Nicotiana* genus, and its leaves are commercially grown in many countries to be processed into tobacco.[1,2] In addition, *N. tabacum* is also used as insecticide, anesthetic, diaphoretic, sedative, and emetic agents in Chinese folklore medicine because of it containing many useful chemical compounds.[1,3,4] In previous work, a number of bioactive compounds, such as terpenoids,[5-7] alkaloids,[8,9] lignans,[10,11] flavonoid,[12-14] phenylpropanoids,[15,16] chromanones,[17,18] biphenyls,[19] phenolic amides,[20] coumarins,[21,22] and the homologous, were isolated from this plant. Therefore, the multipurpose utilization of this plant is an interesting topical and attracts more and more attentions. Motivated by search for more bioactive metabolites from this plant, an investigation on the chemical constituents of the leaves of Yunnan local sun cured tobacco (a variant of *N. tabacum*) was carried out. As a result, two new (**1-2**) and four known flavones (**3-6**) were isolated from this plant. In addition, the cytotoxicity of compounds **1-6** was evaluated. This article deals with the isolation,

structural elucidation and biological activities of these flavones.

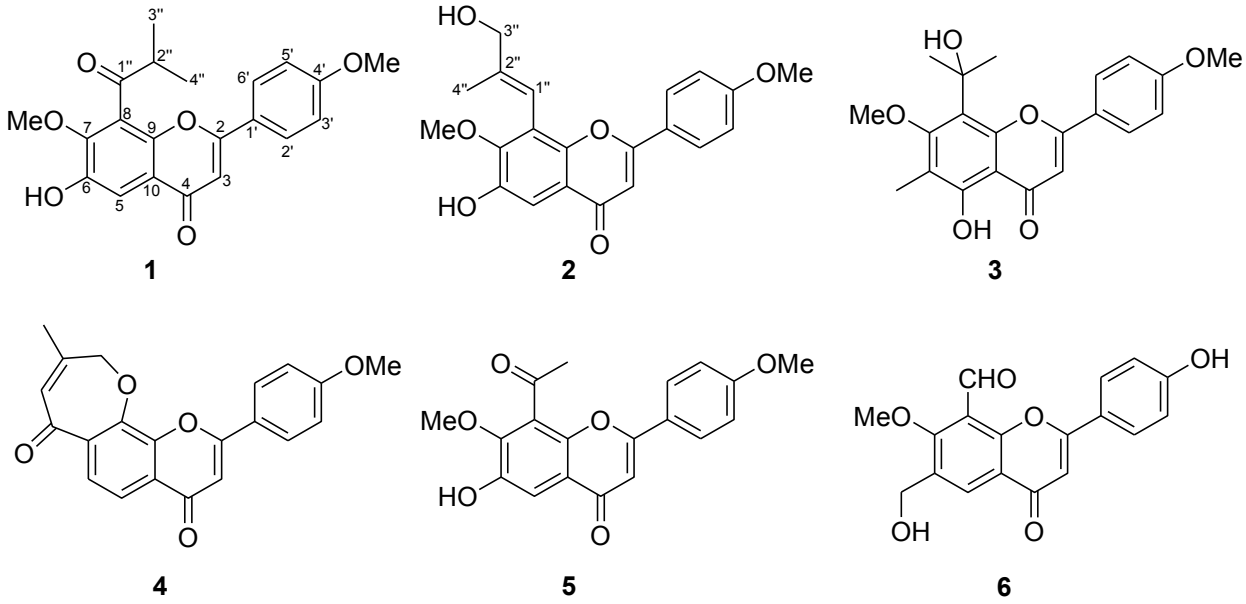

Figure 1. Flavones from the leaves of Yunnan local sun cured tobacco

A 70% aq. methanol extract prepared from the leaves of tobacco was subjected repeatedly to column chromatography on silica gel and preparative HPLC to afford two new flavones, 4',7-dimethoxy-6-hydroxy-8-isobutyrylflavone (**1**), 4',7-dimethoxy-6-hydroxy-8-[(*E*)-3-hydroxy-2-methylprop-1-enyl]-flavone (**2**), and four known flavones (**3**-**6**). The structures of the compounds **1**-**6** were shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1** and **2** were listed in Table 1. The known compounds, compared with literature data, were identified as 4',7-dimethoxy-5-hydroxy-8-(2-hydroxypropan-2-yl)flavone (**3**),[23] tobaflavone E (**4**),[2] paranicflavone C (**4**),[24] and tabaflavone B (**6**).[25]

Compound **1** was obtained as an orange gum. The molecular formula of $C_{21}H_{20}O_6$ was determined from the HRESIMS spectra showing the sodiated molecular ion at *m/z* 391.1166 $[M+Na]^+$ (calcd 391.1158), suggesting 12 degrees of unsaturation. The UV spectrum showed absorption maxima at 210, 254 and 362 nm, and the IR spectrum showed absorption bands at 3454, 1710, 1650, 1610, 1536, and 1455 cm^{-1}, indicating the presence of hydroxy group, carbonyl group, and aromatic ring. The ^{1}H and ^{13}C NMR spectrum of **1** (Table 1) along with analysis of the DEPT spectra displayed 21 carbon signals and 20 proton signals, respectively, corresponding to 4′,6,7,8-tetrasubstituted flavones nucleus (Table 1, C-2 ~ C10 and C-1′ ~ C-6′; H-3, H-5, H-2′,6′, and H-3′,5′),[24] one isobutyryl group (δ_C 209.1 s, 38.0 d, and 20.2 q; δ_H 4.42 m and 1.31 (d) 6.6),[26] two methoxy groups (δ_C 61.1 q and 55.9 q; δ_H 3.89 s and 3.83 s), and one phenolic hydroxy proton (δ_H 10.75 s). The HMBC correlations (Figure 2) of H-3 (δ_H 6.56 s) with C-10 (δ_C 119.8), C-2 (δ_C 164.9), C-4 (δ_C 176.2), and C-1′ (δ_C 122.7), of H-2′,6′ (δ_H 7.73) with C-2 (δ_C 164.9), of H-5 (δ_H 7.37) with C-4 (δ_C 176.2), C-6 (δ_C 143.2), C-7 (δ_C 159.0), C-9 (δ_C 148.0), and C-10 (δ_C 119.8),

also suggested that **1** should be a flavone. The HMBC correlations of two methoxy protons (δ_H 3.83 and 3.89) with C-4′ (δ_C 161.2) and C-7 (δ_C 159.0) concluded the linkage of the two methoxy groups at C-4′ and C-7, respectively. The location of the phenolic hydroxy group was assigned to C-6 positions on the basis of HMBC correlations of the phenolic hydroxy proton signal (δ_H 10.75) with C-5 (δ_C 121.0), C-6 (δ_C 143.2) and C-7 (δ_C 159.0). Finaly, the isobutyryl group located at C-8 was confirmed by the HMBC correlation of H-2″ (δ_H 4.42) with C-8 (δ_C 112.7). The typical proton signals of H-5 (δ_H 7.37), H-2′,6′ [δ_H 7.73 (d, J = 8.6)], and H-3′,5′ [(δ_H 6.71 (d, J = 8.6)] also supported this substituents pattern. Thus, the structure of **1** was established as 4',7-dimethoxy-6-hydroxy-8- isobutyrylflavone.

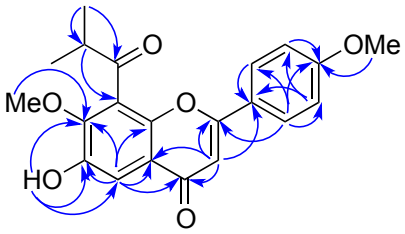

Figure. 2. Key HMBC (↷) correlations of **1**

Compound **2** was obtained as an orange gum. A molecular formula $C_{21}H_{20}O_6$ was assigned from HRESIMS (*m/z*: 391.1153 $[M+Na]^+$, calcd 391.1158). The ^1H and ^{13}C NMR data of **2** (Table 1) displayed 21 carbon and 20 proton signals, corresponding to a flavone nucleus[24] (Table 1, C-2 ~ C-10 and C-1′ ~ C-6′; H-3, H-5, H-2′,6′, and H-3′,5′), one (*E*)-3-hydroxy-2-methylprop-1-enyl moiety[27] (δ_C 125.8 d, 132.2 s, 58.9 t, 16.0 q; δ_H 6.61 (t, *J*=5.6), 4.41 and 4.44 (d) *J*=5.6, 1.85 s), two methoxy groups (δ_C 61.4 and 56.0, δ_H 3.90 and 3.86), and one phenolic hydroxy proton (δ_H 11.00). The ^1H and ^{13}C NMR spectral data of **2** were similar to those of **1**. The obvious chemical shift differences resulted from the substituent group variation on the benzene ring. The appearance of one (*E*)-3-hydroxy-2-methylprop-1-enyl moiety signals and the disappearance of one isobutyryl group signals [δ_C 209.1 s, 38.0 d, 20.2 q, and 20.2 q; δ_H 4.42 m, and 1.31 (d) 6.6] were observed in compound **2**. These changes indicated that the isobutyryl group in **1** was replaced by a (*E*)-3-hydroxy-2-methylprop-1-enyl moiety in **2**. The (*E*)-3-hydroxy-2-methylprop-1-enyl moiety located at C-8, the phenolic hydroxy group located at C-6,

Table 1. ^1H NMR and ^{13}C NMR Data (in C_5D_5N) of compounds **1** and **2**

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
2	164.9 s		163.1 s	
3	105.7 d	6.56 s	104.2 d	6.52 s
4	176.2 s		177.1 s	
5	121.0 d	7.37 s	117.0 d	6.97 s
6	143.2 s		142.1 s	
7	159.0 s		156.6 s	
8	112.7 s		116.1 s	
9	148.0 s		147.4 s	
10	119.8 s		117.6 s	
1′	122.7 s		122.6 s	
2′,6′	130.2 d	7.73 (d) 8.6	130.6 d	7.79 (d) 8.6
3′,5′	116.0 d	6.71 (d) 8.6	115.3 d	6.81 (d) 8.6
4′	161.2 s		161.2 s	
1″	209.1 s		125.8 d	6.61 (t) 5.6
2″	38.0 d	4.42 m	132.2 s	
3″	20.2 q	1.31 (d) 6.6	58.9 t	4.41, 4.44 (d) 5.6
4″	20.2 q	1.31 (d) 6.6	16.0 q	1.85 s
-OMe-7	61.1 q	3.89 s	61.4 q	3.90 s
-OMe-4′	55.9 q	3.83 s	56.0 q	3.86 s
Ar-OH		10.75 s		11.00 s

and two methoxy groups located at C-4′ and C-7, respectively, were confirmed by further analysis of its HMBC correlations. In addition, the ROESY correlation of H-1″ with H-3″ indicated an *E*-configuration for the C-1″, C-2" double bond. Thus, structure of 4',7-dimethoxy-6-hydroxy-8-[(*E*)-3-hydroxy-2-methylprop-1-enyl]flavone (**2**) was established.

The cytotoxicities of compounds **1-6** were tested using a previously reported procedure.[28,29] The cytotoxic abilities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by MTT-assay (with taxol as the positive control) were shown in Table 2. The results revealed that compound **2** exhibited high cytotoxicity for A549 and SHSY5Y cells with IC_{50} values of 1.6 and 2.8 μM. The other compounds also exhibited cytotoxicity with IC_{50} values in the range of 4.3 – 9.5 μM, respectively.

Table 2. Cytotoxic activities of compounds **1-6**

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	6.2	4.3	6.4	5.1	8.5
2	5.6	1.6	2.8	8.4	6.8
3	>10	8.2	8.8	>10	7.8
4	8.4	>10	6.9	7.2	>10
5	9.5	>10	6.8	>10	7.9
6	>10	8.2	>10	6.8	6.3
Taxol	0.03	0.02	0.1	0.1	0.05

NB4, human leukemia cell; A549, carcinomic human alveolar basal epithelial cell; SHSY5Y, human neuroblastoma cell; PC3, Human prostate cancer cell; MCF7, Human breast adenocarcinoma cell.

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu 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, and the chemical shifts (δ) were expressed in ppm. 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 Si gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, China). The fractions were monitored by TLC, and spots were visualized by heating Si gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant material. The sun cured tobacco leaves (Dali Heqing tobacco, a variety of *Nicotiana tabacum*) were collected in Dali Prefecture, Yunnan Province, People's Republic of China, in September 2013. The identification of the plant material was verified by Prof. H. W. Yang (School of Tobacco, Yunnan Agriculture University).

Extraction and Isolation. The air-dried and powdered tobacco leaves (4.8 kg) were extracted three times with 70% aqueous MeOH (3 × 6.0 L) at room temperature and filtered to yield a filtrate, which was

successively evaporated under reduced pressure to obtain a crude extract (325 g). This crude extract was applied to Si 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–F. The separation of fraction C (8:2, 20.5 g) was subjected to Si gel column chromatography eluting with $CHCl_3$-$(Me)_2CO$ and then run on preparative HPLC (55% MeOH-H_2O, flow rate 12 mL/min) to yield compounds **1** (13.6 mg), **2** (10.2 mg), and **4** (12.6 mg). Fraction C (14.6 g) was subjected to Si gel column chromatography eluting with $CHCl_3$-$(Me)_2CO$ and then run on preparative HPLC (46% MeOH-H_2O, flow rate 12 mL/min) to yield compounds **3** (12.2 mg), **5** (16.2 mg), and **6** (18.0 mg).

4',7-Dimethoxy-6-hydroxy-8-isobutyrylflavone (**1**): Obtained as orange gum; UV (CH_3OH), λ_{max} (log ε) 210 (4.18) 254 (3.69), 362 (3.58), nm; IR (KBr) ν_{max} 3454, 2927, 1710, 1650, 1610, 1536, 1455, 1376, 1236, 1176, 1068, 946, 818 cm^{-1}; ^{13}C NMR and ^{1}H NMR data (in C_5D_5N, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 391 $[M+Na]^+$; HRESIMS *m/z* 391.1166 $[M+Na]^+$ (calcd for $C_{21}H_{20}NaO_6$, 391.1158).

4',7-Dimethoxy-6-hydroxy-8-[(*E*)-3-hydroxy-2-methylprop-1-enyl]flavone (**2**): Obtained as orange gum; UV (CH_3OH), λ_{max} (log ε) 210 (4.22) 254 (3.69), 364 (3.58), nm; IR (KBr) ν_{max} 3438, 2932, 2875, 1652, 1605, 1574, 1527, 1462, 1358, 1241, 1156, 1038, 962, 826, 768 cm^{-1}; ^{13}C NMR and ^{1}H NMR data (in C_5D_5N, 500 and 125 MHz) see Table 1; positive ESIMS *m/z* 391 $[M+Na]^+$; HRESIMS *m/z* 391.1153 $[M+Na]^+$ (calcd for $C_{21}H_{20}NaO_6$, 391.1158).

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REFERENCES

1. The Editorial Committee of the Administration Bureau of Flora of China, 'Flora of China,' 67 vols. Beijing Science and Technology Press, Beijing, 2005.
2. M.-M. Miao, L. Li, Q.-P. Shen, C.-B. Liu, Y.-K. Li, T. Zhang, F.-M. Zhang, P. He, K.-M. Wang, R.-Z. Zhu, Y.-K. Chen, and G.-Y. Yang, *Fitoterapia*, 2015, **103**, 260.
3. A. Rodgman and T. A. Perfetti, 'The Chemical Components of Tobacco and Tobacco Smoke,' CRC Press, Taylor and Francis Group, Boca Raton, Florida, 2008.
4. A. P. Cavender and M. Alban, *J. Ethnobio. Ethnomed.*, 2009, **5**, 3.
5. X. Feng, Xin, J.-S. Wang, J. Luo, and L.-Y. Kong, *J. Asian Nat. Prod. Res.*, 2010, **12**, 252.

6. Y.-K. Chen, C.-Y. Meng, Z.-B. Su, L. Wang, G.-Y. Yang, and M.-M. Miao, *Asian J. Chem.*, 2014, **26**, 2246.

7. G. Y. Yang, W. Zhao, Y.-K. Chen, Z.-Y. Chen, Q.-F. Hu, and M.-M. Miao, *Asian J. Chem.*, 2013. **25**, 4932.

8. X. C. Wei, S. C. Sumithran, A. G. Deaciuc, H. R. Burton, L. P. Bush, L. P. Dwoskin, and P. A. Crooks, *Life Sci.*, 2005, **78**, 495.

9. T. Braumann, G. Nicolaus, W. Hahn, and H. Elmenhorst, *Phytochemistry*, 1990, **29**, 3693.

10. Y.-K. Chen, X.-S. Li, G.-Y. Yang, Z.-Y. Chen, Q.-F. Hu, and M.-M. Miao, *J. Asian Nat. Prod. Res*., 2012, **14**, 450.

11. X.-M. Gao, X.-S. Li, X.-Z. Yang, H.-X. Mu, Y.-K. Chen, G.-Y. Yang, and Q.-F. Hu, *Heterocycles*, 2012, **85**, 147.

12. Z.-Y. Chen, J.-L. Tan, G.-Y. Yang, M.-M. Miao, Z.-Y. Chen, and T.-F. Li, *Phytochem. Lett*., 2012, **5**, 233.

13. Y.-K. Li, Y.-L. Zhao, N.-J. Xiang, L. Yang, F. Wang, G.-Y. Yang, and Z.-Y. Wang, *Heterocycles*, 2014, **89**, 2771.

14. Y. Wang, C.-B. Liu, Q.-P. Shen, F.-M. Zhang, P. He, Z.-H. Liu, H.-B. Zhang, X.-D. Yang, M.-M. Miao, and G. Y. Yang, *Heterocycles*, 2015, **91**, 1198.

15. H.-Q. Leng, J.-X. Chen, Y. Hang, Y.-X. Duan, G.-Y. Yang, Y.-K. Chen, Y.-D. Guo, Q.-F. Hu, and M.-M. Miao, *Chem. Nat. Compd.*, 2014. **49**, 1028.

16. J.-L. Tan, Z.-Y. Chen, G.-Y. Yang, M.-M. Miao, Y.-K. Chen, and T.-F. Li. *Heterocycles*, 2011, **83**, 2381.

17. D.-R. Mou, W. Zhao, T. Zhang, L. Wan, G.-Y. Yang, Y.-K. Chen, Q.-F. Hu, and M.-M. Miao, *Heterocycles*, 2012, **85**, 2485.

18. G.-Y. Yang, W. Zhao, T. Zhang, Y.-X. Duan, Z.-H. Liu, M.-M. Miao, and Y.-K. Chen, *Heterocycles*, 2014, **89**, 183.

19. S.-Z. Shang, W.-X. Xu, P. Lei, W. Zhao, J.-G. Tang, M.-M. Miao, H.-D. Sun, J.-X. Pu, Y.-K. Chen, and G.-Y. Yang, *Fitoterapia*, 2014, **99**, 35.

20. S.-Z. Shang, Y.-X. Duan, X. Zhang, J.-X. Pu, H.-D. Sun, Z.-Y. Chen, M.-M. Miao, G.-Y. Yang, and Y.-K. Chen, *Phytochem. Lett.*, 2014, **7**, 413.

21. S.-Z. Shang, W.-X. Xu, L. Li, J.-G. Tang, W. Zhao, P. Lei, M.-M. Miao, H.-D. Sun, J.-X. Pu, Y.-K. Chen, and G.-Y. Yang, *Phytochem. Lett.*, 2015, **11**, 53.

22. C. Lei, W.-X. Xu, J.-Wu, S.-J. Wang, J.-Q. Sun, Z.-Y. Chen, and G.-Y. Yang, *Chem. Nat. Compd*., 2005, **51**, 43.

23. S. Nazreen, G. Kaur, M. M. Alam, S. Shafi, H. Hamid, and M. Ali, *Fitoterapia*, 2012, **83**, 1623.

24. W. Zhao, L. Li, P. Lei, L. Yang, S.-Z. Shang, J.-G. Tang, G.-Y. Yang, Y.-K. Chen, and M.-M. Miao, *Phytochem. Lett*., 2015, **12**, 125.

25. L. Yuan, W.-Z. Huang, C.-M. Zhang, N.-J. Xiang, C.-B. Liu, G.-Y. Yang, Y.-K. Chen, M.-M. Miao, and Y.-H. Ma, *Phytochem. Lett*., 2015, **12**, 75.

26. G. V. Ccana-Ccapatinta, E. D. Stolz, P. F. Costa, S. M. K. Rates, and G. L. Poser, *J. Nat. Prod*., 2015, **78**, 1112.

27. A. Andolfi, A. Boari, M. Evidente, A. Cimmino, M. Vurro, G. Ash, and A. Evidente, *J. Nat. Prod*., 2015, **78**, 623.

28. M. Zhou, K. Zhou, X.-M. Gao, Z.-Y. Jiang, J.-J. Lv, Z.-H. Liu, G.-Y. Yang, M.-M. Miao, C.-T. Che, and Q.-F. Hu, *Org. Lett*., 2015, **17**, 2638.

29. M. Zhou, M.-M. Miao, G. Du, S.-Z. Shang, W. Zhao, Z.-H. Liu, G.-Y. Yang, C.-T. Che, Q.-F. Hu, and X.-M. Gao. *Org. Lett*., 2014, **16**, 5016.