HETEROCYCLES, Vol. 96, No. 1, 2018, pp. 137 - 143. © 2018 The Japan Institute of Heterocyclic Chemistry
Received, 26th October, 2017, Accepted, 24th November, 2017, Published online, 29th November, 2017
DOI: 10.3987/COM-17-13828

FLAVONES FROM THE FRUITS OF *VERNICIA FORDII* AND THEIR ANTI-TOBACCO MOSAIC VIRUS ACTIVITY

Min Zhou,[1,2] Rui-Qi Zhang,[2] Yan-Jun Chen,[1] Ling-Min Liao,[2] Yan-Qi Sun,[1] Zu-Hong Ma,[1] Qiao-Fen Yang,[1] Ping Li,[2] Wei-Guang Wang,[1,2]* and Qiu-Fen Hu[1,2]*

[1] School of Ethnic Medicine, Yunnan Minzu University, Kunming 650031, P.R. China. [2] Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University, Kunming 650031, P.R. China. E-mail: wwg@live.cn, huqiufena@aliyun.com

Abstract – Three new flavones (**1**–**3**), together with six known flavones (**4**–**9**), were isolated from the fruits of *Vernicia fordii* collected from Hunan province of China. Their structures were characterized by means of extensive spectroscopic analyses. Compounds **1**–**3** and **9** were evaluated for their anti-tobacco mosaic virus (anti-TMV) activity. The results showed that compound **9** exhibited high anti-TMV activity with inhibition rate of 32.6%. This inhibition rate is close to that of positive control (33.8%). Compounds **1**–**3** also showed potential anti-TMV activities with inhibition rates of 26.8%, 29.2%, and 25.7%, respectively.

Vernicia fordii (Euphorbiaceae), commonly known as tung oil tree, is originated from China and also cultivated in Korea.[1] The fruits and leaves have been used as folk medicine to treat inflammation-related diseases, such as sore throat, swollen glands in neck, and skin rash.[2,3] In previous phytochemical investigation, a series of chemical constituents, such as coumarins, diterpenoids, flavones, tannins and triterpenoids, had been reported from the fruits, leaves and roots of this plant.[4-7] Among them, flavones represent a class of polyphenols with a basic skeleton of 2-phenylchromen-4-one mainly found in fruits, vegetables, medical herbs, and microorganism.[8-10] Flavones have been widely used for the prevention and treatment for many common diseases, such as cardiac-cerebral vascular disease, viruses and bacterial infections, inflammation, cancer, osteoporosis and diarrhea, due to their potential ability to scavenge oxygen free radicals, to inhibit specific enzymes, and to regulate the release of some hormones.[8-10] In the course of our search for bioactive principles from Euphorbiaceae plants, three new flavones (**1**–**3**), together

with six known ones (**4**–**9**), were obtained from the acetone extracts of this plant. Herein, we mainly describe the isolation, characterization, and biological evaluation of these compounds.

A 95% aq. ethanol extract prepared from the fruits of *V. fordii* was subjected repeatedly to column chromatography and preparative HPLC to yield three new flavones, 7-hydroxymethyl-6,2'-dimethoxyflavone (**1**), 8-hydroxy-7-hydroxymethyl-6,2'-dimethoxyflavone (**2**), and 6,2'-dimethoxy-7-methylflavone (**3**), together with five known analogues (**4**–**9**). The structures of the isolated compounds were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of **1**–**3** were listed in Table 1. The known compounds, luteolin (**4**),[11] quercetin (**5**),[11] 5-hydroxy-8-hydroxymethyl-7,4'-dimethoxyflavone (**6**),[12] kaempferol (**7**),[11] 5,4'-dihydroxy-3,7-dimethoxy-6-methylflavone (**8**),[13] and 5-methyl-7-hydroxy-4'-methoxyflavone (**9**)[14] were established by comparison of their spectroscopic data with these reported in the literature.

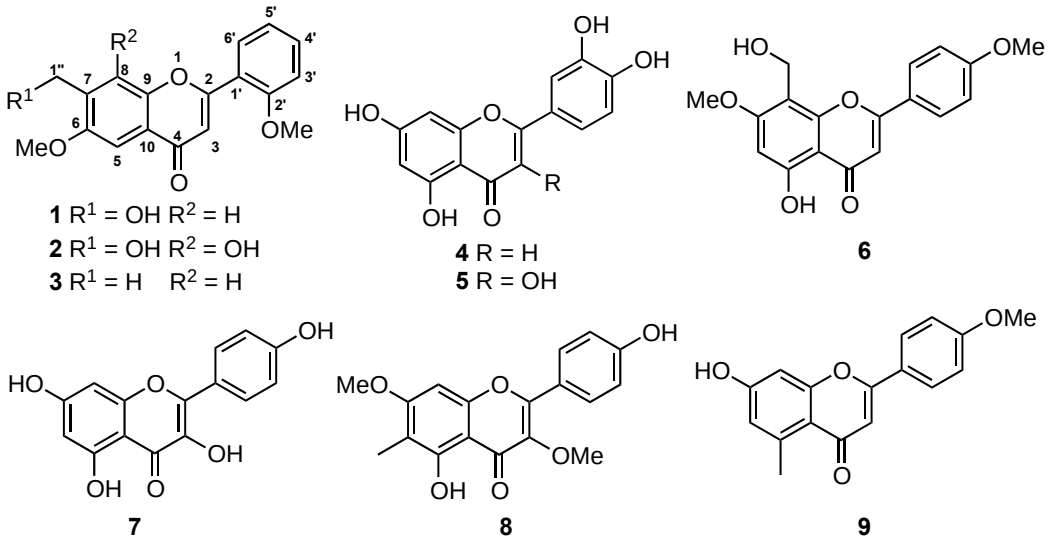

Figure 1. Flavones from the fruits of *V. fordii*

Compound **1** was obtained as an orange-yellow gum. It has the molecular formula $C_{18}H_{16}O_5$ from HRESIMS (*m/z*: 335.0898 $[M + Na]^+$, calcd $C_{18}H_{16}NaO_5$ for 335.0895). Its IR spectral data showed the presence of hydroxy (3426 cm^{-1}), carbonyl groups (1665 cm^{-1}) and phenyl groups (1614, 1552, and 1439 cm^{-1}). The UV absorptions at 364, 260, and 210 nm also showed a substituted aromatic ring. The ^{13}C and ^{1}H NMR spectra of **1** (Table 1) along with analysis of the DEPT spectra displayed 18 carbon signals and 16 proton signals, respectively, corresponding to a 1,2,4,5-tetrasubstituted aromatic ring (C-5~C-10; H-5 and H-8), a 1,2-disubstituted aromatic ring (C-1'~C-6'; H-3'~H-6'), an *α,β*-unsaturated ketone (C-2~C-4; H-3), one hydroxymethyl group (δ_C 63.9 t, δ_H 4.62 s), and two methoxy groups (δ_C 56.0 q, 56.3 q, δ_H 3.80 s, 3.82 s). The typical NMR signals of two aromatic rings and *α,β*-unsaturated ketone should be formed a flavone nucleus.[15] This was also supported by the HMBC correlations (Figure 2) from H-3 to C-2, C-4, C-10, C-1',

from H-5 to C-4, C-9, C-10, from H-8 to C-9, C-10, and from H-6' to C-2. Since the nucleus of compound was determined, the additional signals (a hydroxymethyl and two methoxy groups) were accounted for the remaining substituents.

Table 1. ^{1}H and ^{13}C NMR data for compounds **1**–**3** (δ in ppm, in C_5D_5N, 400 and 100 MHz)

No.	**1**		**2**		**3**	
	δ_C	δ_H (multi., *J*, Hz)	δ_C	δ_H (multi., *J*, Hz)	δ_C	δ_H (multi., *J*, Hz)
2	163.0 s		163.3 s		163.5 s	
3	108.5 d	6.71 s	108.3 d	6.73 s	108.3 d	6.69 s
4	177.0 s		176.7 s		176.7 s	
5	116.9 d	7.05 s	109.2 d	6.58 s	116.4 d	6.99 s
6	152.7 s		154.1 s		153.9 s	
7	133.9 s		126.9 s		131.2 s	
8	118.2 d	6.50 s	147.2 s		119.9 d	6.42 s
9	148.8 s		144.8 s		148.0 s	
10	122.9 s		125.9 s		122.1 s	
1'	118.6 s		118.1 s		118.0 s	
2'	157.4 s		156.9 s		157.1 s	
3'	116.0 d	6.92 (d) 7.6	115.9 d	6.91 (d) 7.6	115.9 d	6.91 (d) 7.6
4'	131.2 d	7.27 (t) 7.6	131.4 d	7.28 (t) 7.6	130.3 d	7.28 (t) 7.6
5'	120.8 d	6.87 (t) 7.6	120.8 d	6.86 (t) 7.6	121.1 d	6.85 (t) 7.6
6'	128.3 d	7.76 (d) 7.6	129.0 d	7.74 (d) 7.6	128.6 d	7.76 (d) 7.6
1"	63.9 t	4.62 s	55.3 t	4.50 s	16.9 q	2.37 s
6-OMe	56.0 q	3.80 s	56.0 q	3.80 s	56.0 q	3.79 s
2'-OMe	56.3 q	3.82 s	56.4 q	3.83 s	56.3 q	3.82 s
8-OH				11.11 s		
1"-OH		4.95 s		4.96 s		

The HMBC correlations of two methoxy protons (δ_H 3.80 s, 3.82 s) with C-6 (δ_C 152.7 s) and C-2' (δ_C 157.4 s) suggested the attachment position of the two methoxy groups at C-6 and C-2', respectively. The hydroxymethyl group located at C-7 was supported by the HMBC of correlations from H_2-1" (δ_H 4.62 s) to C-6 (δ_C 152.7 s), C-7 (δ_C 133.9 s), and C-8 (δ_C 118.2 d), and from H-8 (δ_H 6.50 s) to C-1" (δ_C 63.9 t). Furthermore, the typical protons signals (H-5, H-8, H-3'~H-6') also supported a 6,7-disubstituted for ring A and a 2'-monosubstituted for ring B. The structure of 7-hydroxymethyl-6,2'-dimethoxyflavone (**1**) was established as shown in Figure 1.

The ^{1}H and ^{13}C NMR spectra of 8-hydroxy-7-hydroxymethyl-6,2'-dimethoxyflavone (**2**) were similar to

those of **1**. The marked differences between them were due to the inexistence of an aromatic proton signal, and appearance of a phenolic hydroxy signal (δ_H 11.11 s) on ring A in compound **2**. This change indicated that an additional phenolic hydroxy group should be substituted on ring A in compound **2**. The HMBC correlations of phenolic hydroxy signal with C-7 (δ_C 126.9 s), C-8 (δ_C 147.2 s), and C-9 (δ_C 144.8 s) supported this phenolic hydroxy group located C-8. In addition, the other substituents positions also determined by the further analysis of its HMBC correlations. Thus, the structure of **2** was determined as shown.

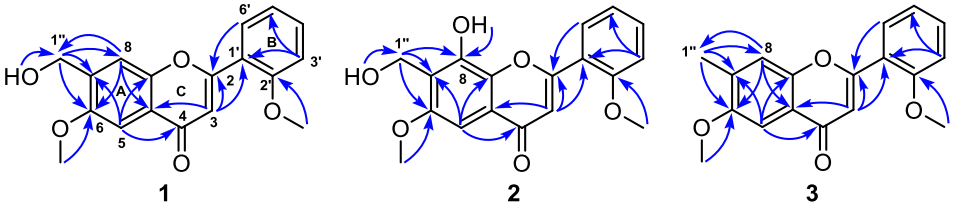

Figure 2. Selected HMBC (H → C) correlations of compounds **1**–**3**

Compound **3** was isolated as an orange-yellow gum and it gave a $[M + Na]^+$ peak at *m/z* 319.0938, consistent with a molecular formula of $C_{18}H_{16}O_4$, with 11 degrees of unsaturation. Its ^1H and ^{13}C NMR spectroscopic data were similar to those of **1**, which suggested that compound **3** was also structurally related to **1**. The marked differences between them were due to the inexistence of a hydroxymethyl group, and appearance of a methyl group (C-1", H_3-1") in compound **3**. These changes indicated that a hydroxymethyl group in **1** was replaced by a methyl group in compound **3**. This deduction was also supported by the HMBC correlations from H_3-1" (δ_H 2.37 s) to C-6 (δ_C 153.9 s), C-7 (δ_C 131.2 s), and C-8 (δ_C 119.9 d), and from H-8 (δ_H 6.42 s) to C-1" (δ_C 16.9 q). Moreover two methoxy groups located at C-6 and C-2' was supported by the HMBC correlations of two methoxy proton signals (δ_H 3.79 s, 3.82 s) with C-6 and C-2', respectively. Based on above, the structure of **3** was formulated as 6,2'-dimethoxy-7-methylflavone.

Since certain of the flavones from plants exhibit potential anti-TMV activity,[16-19] the selected compounds **1**–**4**, **8** and **9** were tested for their anti-TMV activity. The inhibitory activity of the related compounds against TMV replication were tested using the half-leaf method at the concentration of 20 μM.[20] Ningnanmycin (with inhibition rate of 33.2%), a commercial product for plant disease in China, was used as a positive control. The results showed that compound **9** exhibited high anti-TMV activity with inhibition rate of 32.6%. This inhibition rate is close to that of positive control (33.8%). Compounds **1**–**3** also showed potential anti-TMV activities with inhibition rates of 26.8%, 29.2%, and 25.7%, respectively. No significant activities of compounds **4** and **8** were detected at the concentration of 20 μM.

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 Bruker AV-400 spectrometer with TMS as the internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were performed on a VG Autospec-3000 spectrometer under 70 eV. Column chromatography was performed with silica gel (200-300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatography with a Welch Ultimate XB-Phenyl or Ultimate XB-C_{18} (10 μm, 4.6 mm × 25 cm). Preparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with a Shimadzu PRC-ODS (K) column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 8% H_2SO_4 in EtOH. All solvents including petroleum ether (60-90 ^oC) were distilled prior to use.

Plant Material

The fruits of *Vernicia fordii* were collected from Xiangtan, Hunan province of China, in October 2015 and verified by Prof. Ning Yuan. A voucher specimen (YNNI-15-10-12) has been deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission and Ministry of Education, Yunnan Minzu University.

Extraction and Isolation

The air-dried and powdered fruits of *V. fordii* (5.5 kg) were extracted with 95% (10 L) aqueous EtOH (three times, 72 h for each time) at room temperature. The combined solvent was evaporated in vacuo to yield a crude extract (280 g), which was suspended in water, and then extracted with CH_2Cl_2 and EtOAc, successively. The EtOAc layer provided 53.6 g of residue, which were purified by silica gel column eluted with CH_2Cl_2-acetone gradient system (1:0, 9:1, 8:2, 7:3, and 1:1) to yield five main fractions A-E. Fr. D (CH_2Cl_2/acetone 7:3, 6.84 g) eluted with PE/CH_2Cl_2 (5:1, 2:1, 1:1, 1:2, 1:5, and 0:1) and yielding subfractions D1-D6. Subfraction D4 (2.26 g, PE/CH_2Cl_2 1:2) was run on preparative HPLC (55% MeOH-H_2O, flow rate 20 mL/min) to yield compounds **1** (11.2 mg), **2** (10.3 mg), **3** (12.6 mg), **8** (8.2 mg), and **9** (11.9 mg). Fr. E (CH_2Cl_2/acetone 1:1, 8.22 g) eluted with PE/CH_2Cl_2 (5:1, 2:1, 1:1, 1:2, 1:5, and 0:1) and yielding subfractions E1-E6. Subfraction E5 (2.87 g, PE/CH_2Cl_2 1:5) was run on preparative HPLC (46% MeOH-H_2O, flow rate 20 mL/min) to yield compounds **4** (18.6 mg), **5** (22.4 mg), **6** (16.4 mg), and **7** (23.6 mg).

7-Hydroxymethyl-6,2'-dimethoxyflavone (**1**), $C_{18}H_{16}O_5$; obtained as orange-yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.65), 260 (3.86), 364 (3.62) nm; IR (KBr) ν_{max} 3426, 1665, 1614, 1552, 1439, 1402, 1370, 1143, 1067 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 335 $[M + Na]^+$; HRESIMS *m/z* 335.0898 $[M + Na]^+$ (calcd for $C_{18}H_{16}NaO_5$, 335.0895).

8-Hydroxy-7-hydroxymethyl-6,2'-dimethoxyflavone (**2**), $C_{18}H_{16}O_6$; obtained as orange-yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.60), 262 (3.82), 366 (3.57) nm; IR (KBr) ν_{max} 3441, 1662, 1612, 1559, 1420, 1396, 1357, 1148, 1060 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 351 $[M + Na]^+$; HRESIMS *m/z* 351.0836 $[M + Na]^+$ (calcd for $C_{18}H_{16}NaO_6$, 351.0845).

6,2'-Dimethoxy-7-methylflavone (**3**), $C_{18}H_{16}O_4$; obtained as orange-yellow gum; UV (MeOH) λ_{max} (log ε) 210 (4.62), 258 (3.80), 359 (3.58) nm; IR (KBr) ν_{max} 3087, 2926, 1668, 1611, 1564, 1445, 1418, 1357, 1156, 1072 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 319 $[M + Na]^+$; HRESIMS *m/z* 319.0938 $[M + Na]^+$ (calcd for $C_{18}H_{16}NaO_4$, 319.0946).

Anti-TMV Assays

The anti-TMV activities were tested using the half-leaf method,[18] and Ningnanmycin (2% water solution), a commercial product for plant disease in China, was used as a positive control.

ACKNOWLEDGMENTS

This project was supported by Project of Yunnan Provincial Education Department (No. 2015Z115) and China Tobacco Yunnan Industrial Co., Ltd (2016539200340107).

REFERENCES

1. A. Schönemann and H. G. M. Edwards, *Anal. Bioanal. Chem.*, 2011, **400**, 1173.
2. G. Pencreac'h, J. Graille, M. Pina, and R. Verger, *Anal. Biochem.*, 2002, **303**, 17.
3. Y. Ito, S. Yanase, H. Tokuda, M. Kishishita, H. Ohigashi, M. Hirota, and K. Koshimizu, *Cancer Lett.*, 1983, **18**, 87.
4. Y.-H. Pei, O.-K. Kwon, J.-S. Lee, H.-J. Cha, K.-S. Ahn, S.-R. Oh, H.-K. Lee, and Y.-W. Chin, *Chem. Pharm. Bull.*, 2013, **61**, 674.
5. Y.-F. Xie, Z.-M. Tao, H.-B. Wang, and G.-W. Qin, *Chin. J. Nat. Med.*, 2010, **8**, 264.
6. B. I. Fozdar, S. A. Khan, T. Shamsuddin, K. M. Shamsuddin, and J. P. Kintzinger, *Phytochemistry*, 1989, **28**, 2459.
7. T. Okuda, T. Yoshida, S. Koike, and N. Toh, *Phytochemistry*, 1975, **14**, 509.
8. G. L. Hostetler, R. A. Ralston, and S. J. Schwartz, *Adv. Nutr.*, 2017, **8**, 423.
9. E. Middleton, C. Kandaswami, and T. C. Theoharides, *Pharmacol. Rev.*, 2000, **52**, 673.

10. B. H. Havsteen, *Pharmacol. Ther.*, 2002, **96**, 67.
11. H. Wagner, V. M. Chari, and J. Sonnenbichler, *Tetrahedron Lett.*, 1976, **17**, 1799.
12. Y.-C. Yang, Y. Qin, X.-X. Wu, C.-F. Xia, Y.-L. Meng, B. Zhou, Y.-Q. Ye, X.-M. Gao, Y.-K. Li, and Q.-F. Hu, *Bull. Kor. Chem. Soc.*, 2015, **36**, 1161.
13. R. Rasamoelisendra, B. Voirin, J. Favre-Bonvin, M. Andriantsiferana, and Z. Rabesa, *Phytochemistry*, 1989, **28**, 1996.
14. E. Wenkert and H. E. Gottlieb, *Phytochemistry*, 1977, **16**, 1811.
15. B. Zhou, Y. Li, X. Wu, M. Li, Y. Ye, X. Gao, and Q. Hu, *Chem. Nat. Compd.*, 2015, **51**, 840.
16. W. Zhao, X. Zeng, T. Zhang, L. Wang, G. Yang, Y.-K. Chen, Q. Hu, and M. Miao, *Phytochem. Lett.*, 2013, **6**, 179.
17. J. Chen, H. Leng, Y. Duan, W. Zhao, G. Yang, Y. Guo, Y. Chen, and Q. Hu, *Phytochem. Lett.*, 2013, **6**, 144.
18. M. Zhou, K. Zhou, N.-J. Xiang, L. Yang, C.-M. Zhang, Y.-D. Wang, W. Dong, J. Lou, B.-K. Ji, X.-M. Gao, M.-M. Miao, and Q.-F. Hu, *J. Asian Nat. Prod. Res.*, 2015, **17**, 882.
19. 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.
20. M. Zhou, M.-M. Miao, G. Du, X.-N. Li, 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.