http://dx.doi.org/10.5012/bkcs.2013.34.4.1263

Chalcones from the Flowers of *Rosa Rugosa* and Their *Anti*-tobacco Mosaic Virus Activities

Gang Du,[†,‡] Jing-Mei Han,[‡] Wei-Song Kong,[‡] Wei Zhao,[‡] Hai-Ying Yang,[†] Guang-Yu Yang,[†] Xue-Mei Gao,[†,*] and Qiu-Fen Hu[†,*]

[†]*Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities.* [*]*E-mail: huqiufena@yahoo.com.cn (Q. Hu); gao_xuemei@hotmail.com (X. Gao)*
[‡]*Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, Kunming 650106, P.R. China*
Received December 24, 2012, Accepted January 17, 2013

Key Words : *Rosa rugosa*, Chalcones, *Anti*-tobacco mosaic virus activity

The genus of *Rosa* is one of the most important ornamental plants in the world. Some species of *Rosa*, such as *R. rugosa*, *R. damascene*, and *R. centifolia*, are renowned for their beautiful flowers and fine fragrance, and had widely been cultivated in several areas of Yunnan Province because of high commercial values.[1,2] Meanwhile, their petals and buds also have been used as food or medicine for treating stomachache, diarrhoea and women's diseases.[3,4] The previous phytochemical researches have revealed that tannins,[5,6] flavonoids,[7-9] terpenoids,[10,11] as well as phenylethanoids[12] are major components isolated from this plant.

In our previous studies, some aurones[9] and phenylethanoids[12] were isolated from the flower of *R. rugosa*, and some of them showed *anti*-HIV-1 activities and cytotoxicities. Continuing the efforts to discover bioactive metabolites from local plants, we now investigated the chemical constituents of the flowers of *R. rugosa* growing in the Yuxi Prefecture, leading to the isolation of two new (**1** and **2**) and six known (**3-8**) chalcones. Since many phenolic compounds are known to exhibit *anti*-TMV activities.[12-16] Compounds **1-8** were tested for their *anti*-tobacco mosaic virus (*anti*-TMV) activities, and compounds **1** and **4** exhibited *in vitro anti*-TMV activities.

Results and Discussion

A 70% aq. methanol extract prepared from the flowers of *R. rugosa* was subjected repeatedly to column chromatography on Si gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford two new chalcones, rugchalcones A and B (**1** and **2**), and six known chalcones (**3-8**). The structures of the compounds **1-8** were as shown in Figure 1, and the ^{1}H and ^{13}C NMR data of the compounds **1** and **2** were listed in Table 1. The known compounds were identied as hamilcone (**3**),[17] 4,2'-dihydroxy-3',4',6'-trimethoxy-chalcone (**4**),[18] broussochalcone A (**5**),[19] broussochalcone B (**6**),[19] morachalcone A (**7**),[20] and crotmadine (**8**)[21] by comparison of experimental and reported spectroscopic data.

Compound **1** was obtained as yellow gum. The HRESIMS showed a quasi-molecular ion peak at *m/z* 321.0733 $[M+Na]^+$, corresponding to a molecular formula of $C_{17}H_{14}O_5$. The ^{1}H and ^{13}C NMR spectrum of **1** (Table 1) displayed 17 carbon and 14 proton signals, respectively, corresponding to one aromatic ring [C-4 (δ_C 105.2 d), C-5 (δ_C 145.8 s), C-6 (δ_C 148.7 s), C-7 (δ_C 101.0 d), C-8 (δ_C 156.3 s), C-9 (δ_C 126.0 s)] with two aromatic proton [H-4 (δ_H 7.15 s) and H-7 (δ_H 6.82 s)], one aromatic ring [C-1' (δ_C 123.5 s), C-2',6' (δ_C 130.9 d), C-3',5' (δ_C 117.2), C-4' (δ_C 160.0 s)] with four aromatic proton [H-2',6' (δ_H 7.74, d, J = 8.6) and H-3',5' (δ_H 7.03, d, J = 8.6)], a carbonyl (C-10, δ_C 186.5 s), a pair of double bond [C-2 (δ_C 152.0 s) and C-3 (δ_C 118.9 d); H-3 (δ_H 7.58 s)], two methoxy groups (δ_C 55.9 and 56.0; δ_H 3.89 and 3.82), and a phenolic hydroxy group (δ_H 9.27). Strong absorption bands accounting for hydroxy (3354 cm^{-1}), carbonyl (1679), and aromatic groups (1615, 1542, 1482 cm^{-1}) could be observed in its IR spectrum. The UV absorptions at 370, 309, 250 and 230 nm also suggested the presence of a conjugated aromatic ring system. The NMR data (Table 1), together with the HMBC correlations (Fig. 2) of H-3 (δ_H 7.58) with C-2 (δ_C 152.0)/C-4 (δ_C 105.2)/C-8 (δ_C 156.3)/C-9 (δ_C 126.0)/C-10 (δ_C 186.5), of H-4 (δ_H 7.15) with

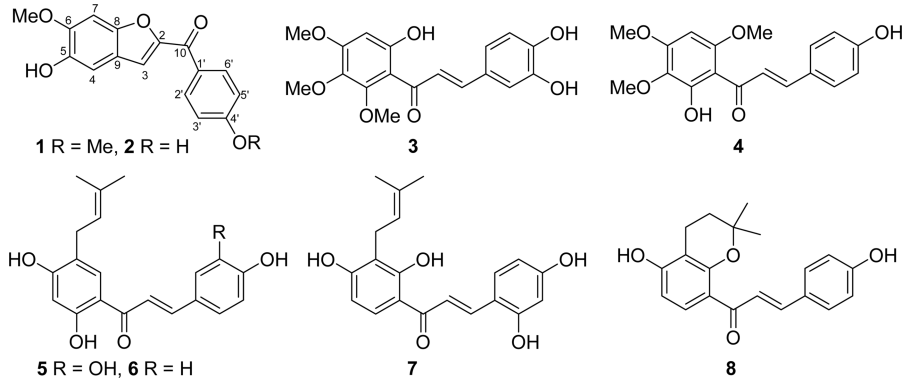

Figure 1. The structure of Chalcones from the Flowers of *R. Rugosa*.

Table 1. ^{1}H and ^{13}C NMR data of compounds **1** and **2** (Data obtained in $CDCl_3$, 500 and 125 MHz)

No.	Compound **1**		Compound **2**	
	δ_C (m)	δ_H (m, *J*, Hz)	δ_C (m)	δ_H (m, *J*, Hz)
2	152.0 s		151.2 s	
3	118.9 d	7.58, s	118.5 d	7.59, s
4	105.2 d	7.15, s	105.1 d	7.15, s
5	145.8 s		145.5 s	
6	148.7 s		148.5 s	
7	101.0 d	6.82, s	100.7 d	6.85, s
8	156.3 s		156.3 s	
9	126.0 s		126.0 s	
10	186.5 s		186.1 s	
1'	123.5 s		123.9 s	
2',6'	130.9 d	7.74, d, *J* = 8.6	131.0 d	7.76, d, *J* = 8.6
3',5'	117.2 d	7.03, d, *J* = 8.6	116.5 d	6.99, d, *J* = 8.6
4'	160.0 s		157.0 s	
OMe-6	55.9 q	3.89, s	56.0 q	3.87, s
OMe-4'	56.0 q	3.82, s		
Ar-OH-5		9.27, brs		9.16, brs
Ar-OH-4'				8.85, s

C-3 (δ_C 118.9), and of C-2',6' (δ_C 130.9) with C-10 (δ_C 186.5), suggested that **1** should be a chalcone derivative fused with a furan ring at C-2 and C-8.[22] The signals for four coupled aromatic protons at δ_H 7.03 (d, *J* = 8.6 Hz, 2H) and 7.74 (d, *J* = 8.6, 2H), suggested a 4-monosubstituted for C ring,[9] and the proton signals for two singlets at δ_H 7.15 (s, 1H) and δ_H 6.82 (s, 1H) also revealed that the substituents for B-ring should be located at C-5 and C-6.[9] The HMBC correlations between the methoxy proton signals (δ_H 3.89 and 3.82) and C-6 (δ_C 148.7)/C-4' (δ_C 160.0) suggested the positions of two methoxy groups at C-6 and C-4'. On the other hand, the phenolic hydroxy group at C-5 was supported by the HMBC correlations observed between the hydroxy proton (δ_H 9.27) and C-4 (δ_C 105.2), C-5 (δ_C 145.8), and C-6 (δ_C 148.7), respectively. Two methoxy groups at C-6 and C-4' were also supported by the cross-peak between one methoxy proton signal (δ_H 3.89) and H-7 (δ_H 6.82), and the other methoxy proton signal (δ_H 3.82) and H-3',5' (δ_H 7.03) in the NOESY experiment. On the basis of the above observations, the structure of **1** was elucidated as (5-hydroxy-6-methoxybenzofuran-2-yl)-(4-methoxyphenyl)-methanone.

Compound (**2**) was also obtained as yellow gum. It was assigned the molecular formula of $C_{16}H_{12}O_5$ by HRESIMS at *m/z* 307.0587 $[M+Na]^+$. IR absorption bands for hydroxy (3354 cm^{-1}), carbonyl (1679), and aromatic groups (1615, 1542, 1482 cm^{-1}), and UV absorptions (370, 309, 250 and 230 nm) for conjugated system were observed on its IR and UV spectra. The ^{1}H and ^{13}C NMR spectra of **2** (Table 1) were similar to those of **1**; the major difference being the replacement of a methoxy signal in **1** by a phenolic proton (δ_H 8.85) in **2**, and the up-shift of NMR data for C-4' from (δ_C 160.0) to (δ_C 157.0). This indicated that the methoxy group at C-4 in **1** should be substituted by a hydroxy group in **2**. The HMBC correlations of the hydroxy proton (δ_H 8.85) with C-4' (δ_C 157.0) and C-3'/5' (δ_C 116.5) indicated

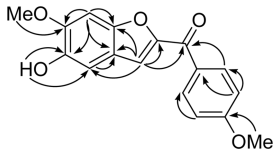

Figure 2. Selected HMBC (↷) correlations of compound **1**.

Table 2. *Anti*-TMV Activity of **1-8** on *Nicotiana glutinosa* leaf

Compound	% Inhibition at 20 μM	IC_{50} (μM)
1	22.2 ± 3.1	62.5
2	13.8 ± 2.2	122.6
3	6.16 ± 3.0	ND
4	25.8 ± 3.2	52.1
5	9.21 ± 2.2	ND
6	8.26 ± 2.3	ND
7	15.2 ± 2.0	98.3
8	6.24 ± 1.8	ND
Ningnanmycin	28.9 ± 2.3	49.7

All results are expressed as mean ± SD; n = 3. ND: Not determined

that a hydroxy group was located at C-4'. The other hydroxy group at C-5 and a methoxy group at C-6 were also supported by its HMBC correlation. Compound **2** is therefore the 4-hydroxyl derivative of **1**.

Compounds **1-8** were tested for *anti*-TMV activities using the half-leaf method.[23,24] Ningnanmycin, a biochemical pesticide against virus diseases on tobacco, was used as the positive control. The results are listed in Table 2. Compounds **1** and **4** (20 μM) showed moderate *anti*-TMV activity with inhibition rates of 22.2 and 25.8%, respectively, which were close to that of ningnanmycin (28.9%). For the compounds showing inhibitory rates above 10%, the IC_{50} values (Table 2) were determined. Compounds **1** and **4** exhibited the highest activities with IC_{50} values of 62.5 and 52.1 μM, respectively.

Experimental

General Procedures. 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 separation 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 flowers of *R. rugosa* were collected in Yuxi Prefecture, Yunnan Province, People's Republic of China, in September 2011. The identification of the plant material was verified by Prof. Chen Y. J (Yunnan Nationalities University). A voucher specimen (YNNI 11-9-32) has been deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered flowers of *R. rugosa* (4.5 kg) were extracted four times with 70% methanol (4 × 5.0 L) at room temperature and filtered. The crude extract (185 g) was applied to silica gel (200-300

mesh) column chromatography, eluting with a chloroform-acetone gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A–F. The further separation of fraction C (8:2, 22.3 g) by silica gel column chromatography, eluted with chloroform-methanol (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures C1–C5 Fraction C1 (9:1, 2.36 g) was subjected to preparative HPLC (48% methanol, flow rate 12 mL/min) to give **1** (13.6 mg) with retention time 35.8 min, **4** (15.8 mg) with retention time 32.4 min, **6** (18.2 mg) with retention time 27.6 min, and **8** (22.4 mg) with retention time 29.5 min. On the other hand, the separation of fraction C2 (7:3, 4.28 g) by silica gel column chromatography and preparative HPLC (44% methanol, flow rate 12 mL/min), to afford **2** (22.8 mg) with retention time 36.2 min, **3** (21.2 mg) with retention time 33.6 min, **5** (22.4 mg) with retention time 29.7 min, and **7** (16.3 mg) with retention time 27.4 min.

***Anti*-TMV Assays.** TMV (U1 strain) was obtained from the Key Laboratory of Tobacco Chemistry, Yunnan Academy of Tobacco Science. The virus was multiplied in *Nicotiana tabacum* cv. K326 and purified as previously described.[25] The concentration of TMV was adjusted to 20 mg/mL as determined by UV absorption. [virus concentration = (A_{260} × dilution ratio)/$E_{1\ cm}^{0.1\%.260\ nm}$]. The purified virus was kept at –20 °C and diluted to 32 μg/mL with 0.01 M PBS before use.

Nicotiana glutinosa plants were cultivated in an insect-free greenhouse. The experiments were conducted when the plants grew to 5-6-leaf stage. The tested compounds were dissolved in DMSO and diluted with distilled H_2O to the required concentrations. A solution of equal concentration of DMSO was used as negative control; and Ningnanmycin was used as positive control.

For the Half-Leaf Method,[23] the virus was mixed with the solution of test compound for 30 min before inoculated on the left side of a leaf of *N. glutinosa*, whereas the right side of the leaf was inoculated with a mixture of DMSO and virus as a control. The local lesion numbers were recorded 3-4 days after inoculation. Three leaf blades were used for each compound. The inhibition rates were calculated according to the formula: Inhibition Rate (%) = [(C-T)/C] × 100%, where C is the average number of local lesions in the control and T is the average number of local lesions in the treated leaves.

Rugchalcone A (1). Obtained as a yellow gum; UV (MeOH), λ_{max} (log ε) 370 (3.92), 309 (3.68), 250 (3.56), 230 (4.18) nm; IR (KBr) ν_{max} 3354, 2962, 2907, 1679, 1615, 1542, 1482, 1262, 1148, 1056, 876, 753 cm^{-1}; ^1H NMR and ^{13}C NMR data ($CDC1_3$, 500 MHz and 125 MHz, respectively), Table 1; ESIMS (positive ion mode) *m/z* 309 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 321.0733 $[M+Na]^+$ (calcd 321.0739 for $C_{17}H_{14}NaO_5$).

Rugchalcone B (2). Obtained as a yellow gum; UV (MeOH), λ_{max} (log ε) 368 (3.97), 311 (3.74), 253 (3.59), 230 (4.22) nm; IR (KBr) ν_{max} 3360, 2965, 2902, 1681, 1619, 1540, 1487, 1264, 1146, 1050, 872, 758 cm^{-1}; ^1H NMR and ^{13}C NMR data ($CDC1_3$, 500 MHz and 125 MHz, respectively), Table 1; ESIMS (positive ion mode) *m/z* 307 $[M+Na]^+$; HRESIMS (positive ion mode) *m/z* 307.0587 $[M+Na]^+$ (calcd 307.0582 for $C_{16}H_{12}NaO_5$).

Acknowledgments. This project was supported financially by the Excellent Scientific and Technological Team of Yunnan High School (2010CI08), the Yunnan University of Nationalities Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008), and Open Research Fund Program of Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University of Nationalities) (2010XY08). And the publication cost of this article was supported by the Korean Chemical Society. And the publication of this paper was supported by the Korean Chemical Society.

References

1. Zhang, X. H.; Xie, Z. Z. *Chin. J. Shandong. Forest. Sci. Tech.* **2012**, *42*, 5.
2. Lu, J. L. *China. Flowers. Horticul.* **2012**, *11*, 26.
3. Shibata, K. *A Cyclopedia of Useful Plant and Plant Products*; Enlarged and Revised Edition, The Hokuryukan: Tokyo, 1957; p 612.
4. Jiang, Y. *Flora of China*; Chinese Science Press: Beijing, 1977; Vol 63, p 289.
5. Ochir, S.; Park, B.; Nishizawa, M.; Kanazawa, T.; Funaki, M.; Yamagishi, T. *J. Nat. Med.* **2010**, *64*, 383.
6. Park, K. H.; Sung, K.; Choi, S. E.; Kwon, J. H.; Myung, H.; Lee, M. W. *Chem. Pharm. Bull*. **2010**, *58*, 1227.
7. Porter, E. A.; van den Bos, A. A.; Kite, G. C.; Veitch, N. C.; Simmonds, M. S. *Phytochemistry* **2012**, *81*, 90.
8. Kwon, E.; Lee, K. D. Y.; Lee, H.; Kim, D. O.; Baek, N.; Kim, Y. E.; Kim, H. Y. *J. Agric. Food. Chem*. **2010**, *58*, 882.
9. Gao, X. M.; Yang, L. Y.; Shu, L. D.; Shen, Y. Q.; Zhang, Y. J.; Hu, Q. F. *Heterocycles* **2012**, *85*, 1925.
10. Yuan, J. Q.; Yang, X. Z.; Miao, J. H.; Tang, C. P.; Ke, C. Q.; Zhang, J. B.; Ma, X. J.; Ye, Y. *Molecules* **2008**, *13*, 2229.
11. Zeng, N.; Shen, Y.; Li, L. Z.; Jiao, W. H.; Gao, P. Y.; Song, S. J.; Chen, W. S.; Lin, H. W. *J. Nat. Prod*. **2011**, *74*, 732.
12. Gao, X. M.; Shu, L. D.; Yang, L. Y.; Shen, Y. Q.; Zhang, Y. J.; Hu, Q. F. *Bull. Korean Chem. Soc*. **2013**, *34*, in press.
13. Gao, X. M.; Liying Yang, L. Y.; Shen, Y. Q.; Shu, L. D.; Li, X. M.; Hu, Q. F. *Bull. Korean Chem. Soc*. **2012**, *33*, 2447.
14. Geng, Z. L.; Shang, S. H.; Chen, X. J.; Cao, Y. *Chin. Tob. Sci*. **2011**, *32*, 84.
15. Gao, X. M.; Li, X. S.; Yang, X. Z.; Mu, H. X.; Chen, Y. K.; Yang, G. Y.; Hu, Q. F. *Heterocycles* **2012**, *85*, 147.
16. Hu, Q. F.; Zhou, B.; Gao, X. M.; Yang, L. Y.; Shu, L. D.; Shen, Y. Q.; Li, G. P.; Che, C. T.; Yang, G. Y. *J. Nat. Prod*. **2012**, *75*, 1909.
17. Huang, L.; Wall, M. E.; Wani, M. C.; Navarro, H.; Santisuk, T.; Reutrakul, V.; Seo, E. K.; Farnsworth, N. R.; Kinghorn, A. D. *J. Nat. Prod*. **1998**, *61*, 446.
18. Panichpol, K.; Waterman, P. G. *Phytochemistry* **1978**, *17*, 1363.
19. Matsumoto, J.; Fujimoto, T.; Takino, C.; Saitoh, M.; Hano, Y.; Fukai, T.; Nomura, T. *Chem. Pharm. Bull*. **1985**, *33*, 3250.
20. delle Monache, G.; Derosa, M. C.; Scurria, R.; Vitali, A.; Cuteri, A.; Monacelli, B.; Pasqua, G.; Botta, B. *Phytochemistry* **1995**, *39*, 575.
21. Bhakuni, D. S.; Chaturvedi, R. *J. Nat. Prod*. **1984**, *47*, 585.
22. Yang, Y.; Zhang, T.; Xiao, L.; Yang, L. X.; Chen, R. Y. *Fitoterapia* **2010**, *81*, 614.
23. Yan, X. H.; Chen, J.; Di, Y. T.; Fang, X.; Dong, J. H.; Sang, P.; Wang, Y. H.; He, H. P.; Zhang, Z. K.; Hao, X. J. *J. Agric. Food Chem*. **2010**, *58*, 1572.
24. Song, B. A.; Zhang, H. P.; Wang, H.; Yang, S.; Jin, L. H.; Hu, D. Y.; Pang, L. L.; Xue, W. *J. Agric. Food Chem*. **2005**, *53*, 7886.
25. Gooding, G. V. J.; Hebert, T. T. *Phytopathology* **1967**, *57*, 1285.