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Flavonoids and Triterpenoids from the Roots of *Rosa laevigata*

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Abstract. Chemical investigation on the root of *R. laevigata* led the isolation and identification of two new flavonoids, (+)-catechin-8-acetic acid (**1**) and guibourtacacidine 4-methyl ether (**2**), one known flavonoid, guibourtacacidine (**3**), along with seven known triterpenoids, euscaphic acid (**4**), kajiichigoside F1 (**5**), nigaichigoside F2 (**6**), rubuside J (**7**), tomentic acid (**8**), rosamutin (**9**) and betulinic acid (**10**). Their structures were established on the basis of spectroscopic evidence and comparisons with literature data. Compounds **1**, **2** and **3** showed considerable radical scavenging activity in the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay.
Keywords: *Rosa laevigata*, flavonoids, triterpenoids, DPPH radical scavenging activity.

Resumen. La investigación química de las raíces de *R. laevigata* condujo al aislamiento de dos nuevos flavonoides, 8-(+)-catequin-ácido acético (**1**) y éter 4-metílico de guibourtacacidina (**2**); un flavonoide conocido, guibourtacacidina (**3**), y siete triterpenos conocidos, ácido euscáfico (**4**), kajichigósido F1 (**5**), nigaichigósido F2 (**6**), rubúsido J (**7**), ácido toméntico (**8**), rosamutina (**9**) y ácido betulínico (**10**). Las estructuras fueron establecidas de acuerdo a las evidencias espectroscópicas y comparaciones con los datos informados en la literatura. Los compuestos **1**, **2** y **3** mostraron actividad atrapadora de radicales en el ensayo del 1,1-difenil-2-picrilhidracilo (DFPH).
Palabras clave: *Rosa laevigata*, flavonoides, triterpenoides, actividad atrapadora de radicales, DFPH.

Introduction

Rosa laevigata Michx., is an evergreen climbing shrub belonging to family Rosaceae, commonly found in thickets at low altitudes, widely distributed in Southern China [1]. The roots, flowers, fruits and leave of *R. laevigata* have been recorded as traditional Chinese folk medicines since the Tang dynasty, and the roots were commonly used for the treatment of spermatorrhea, urinary incontinence, urinary frequency, uterine prolapse, menstrual irregularities, dysentery, rheumatism, scald, external injury, toothache and stomachache [2]. Previous phytochemical studies on different parts of *R. laevigata* have revealed the presence of pentacyclic triterpenoids [3-6], steroids [3, 7], flavonoids [3, 8, 9], tannins [3, 9], ligans [8], and polysaccharides [10] in this plant. Up to now, almost all the earlier studies on *R. laevigata* were focused on the fruits, and only five triterpenoids were obtained from the roots [5], here we described isolation and structural identification of three flavanoids and seven triterpenoids from the roots of this species.

Results and Discussion

The 80% EtOH extract of air-dried roots of *R. laevigata* was successively fractionated with petroleum ether, EtOAc and n-BuOH; and the petroleum ether and EtOAc were further seperated and purified over Sephadex LH-20, ODS, MCI and silica gel to give two new flavanols (**1**, **2**), one known flavanol (**3**) and seven known triterpenoids (**4**-**10**). The known ones were identified as guibourtacacidine (**3**) [11, 12], euscaphic acid (**4**)[7], kajiichigoside F1 (**5**)[7], nigaichigoside F2 (**6**)[13], rubuside J (**7**)[14], tomentic acid (**8**)[15, 16], rosamutin (**9**)[17] and betulinic acid (**10**)[18] by the spectroscopic evidences and comparing with literature data reported previously.

Compound **1** was obtained as a light yellow powder, its molecular formula was established as $C_{17}H_{16}O_8$ based on positive HR-ESI-MS data $[M+H]^+$ at 349.09161 (calcd. 349.09234), and ^{13}C NMR (including DEPT) data, The IR spectrum showed absorption bands due to hydroxyl groups, and aromatic rings at ν_{max} 3289, 1618 and 1455 cm^{-1}, respectively. The ^{13}C NMR (include DEPT) spectrum exhibited 17 carbon signals con-

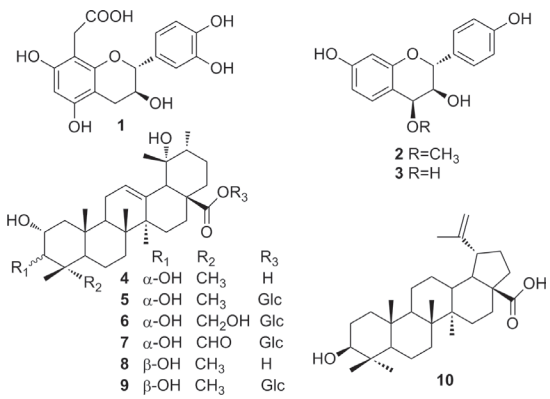

Fig. 1. Compounds **1**-**10** isolated from roots of *R. laevigata*.

tributed to two methylenes, six methines, and nine quaternary carbons, including one carboxylic carbon (δ_C 178.3, C-2''). The ^{13}C NMR and HSQC spectrum allowed the assignments of all the protons to their bonding carbons, the ^1H NMR spectrum revealed the presence of one 1, 3, 4-trisubstituted benzene ring characterized by signals at δ_H 6.72 (d, *J* = 1.86 Hz, H-2'), 6.64 (d, *J* = 8.10 Hz, H-5') and 6.61 (dd, *J* = 1.86, 8.10 Hz, H-6'); a pentasubstituted benzene ring [δ_H 5.91 (s, H-6)]; two methylenes proton at δ_H 2.71 (dd, *J* = 4.80, 16.08 Hz, H-4a), 2.45 (dd, *J* = 7.78, 16.08 Hz, H-4b), and 3.42 (d, *J* = 10.08 Hz, H-1''); two oxygen- bearing methine signals at δ_H 4.56 (d, *J* = 7.12 Hz, H-2) and 3.85 (m, H-3). A comparison of ^1H and ^{13}C NMR data of **1** with those of (+) -catechin [19], revealed that the two compounds were similar except an additional substituent group at C-8 position, this was further confirmed by the disappearance of proton signal at H-8, and the chemical shift down-fielded change of C-8 in ^{13}C NMR spectrum. In HMBC spectrum, the correlation of the methylenes proton signal (δ_H 3.42, H-1") with carboxylic carbon (δ_C 178.3, C-2'') revealed the existent moiety of -CH_2COOH, and correlations of H-1'' with C-7 (δ_C 156.1), C-8 (δ_C 102.3), C9 (δ_C 155.9) suggested the -CH_2COOH moiety was linked with C-8, the linkage was also reinforced by the downshift of C-8 from δ_C 94.8 to 102.3 in ^{13}C NMR. Therefore compound **1** was determined as (+)-catechin-8-acetic acid, whose structure was very similar to that of 8-carboxymethy- (+)-catechin, one (+)-catechin derivative synthesized by Van Der Merwe and Hundt [20].

Compound **2** was obtained as a light yellow powder, Its molecular formula was established as $C_{16}H_{16}O_5$ based on positive HR-ESIMS data $[M+H]^+$ at 289.10687, 289.10760 and ^{13}C NMR (including DEPT) data. The IR spectrum showed presence of OH (3394 cm^{-1}), and aromatic rings (1647, 1385 cm^{-1}), respectively. The ^{13}C NMR (include DEPT) spectrum exhibited 16 carbon signals assigned to one methyl, ten methines, and five quaternary carbons, the ^1H NMR spectrum revealed the presence of one 1,4-parasubstituted benzene ring characterized by signals at δ_H 7.26 (d, *J* = 8.56 Hz, H-2', 6') and 6.81 (d, *J* = 8.56 Hz, H-3', 5'); one 1,3,4-trisubstituted benzene ring [δ_H 7.10 (d, *J* = 8.28 Hz, H-5), 6.39 (dd, *J* = 8.28, 2.32 Hz, H-6), 6.28 (d, *J* = 2.32 Hz, H-8)]; three oxygen-bearing methine signals at δ_H 5.01(d, *J* = 9.76 Hz, H-2), 3.98 (dd, *J* = 9.76, 3.16 Hz, H-3) and 4.20 (d, *J* = 3.16 Hz, H-4), and one methoxyl [δ_H 3.47 (s, OMe)]. The ^1H and ^{13}C NMR spectrum analysis implied compound (**2**) was the characteristic flavan-3, 4-diol derivative, further comparison of the ^1H NMR data of compound (**2**) with those of guibourtacacidine (**3**) [11], one known flavan-3, 4-diol we have obtained in this experiment, the major differences between compound **2** and **3** were that the proton signal of H-4 up-fielded from δ_H 4.60 to 4.20, and the presence of an additional methoxyl signal at δ_H 3.47. The above evidences strongly suggested the methoxyl (δ_C 56.9) should be linked at C-4 position, and thus compound **2** was determined as guibourtacacidine 4-methyl ether, a new flavan-3, 4-diol derivative.

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay is widely used to evaluate antioxidant capacity [21]. The radical scavenging activities of the isolated compounds were determined by DPPH radical scavenging assay and the results are shown in table 2. Compounds **1-3**, flavonoids isolated from the roots of *R. laevigata* showed considerable radical scavenging capacity in DPPH assay compared with the positive control ascorbic acid (SC_{50} = 31.55 ± 0.58 μM); The flavonoids have been generally considered as important natural antioxidants [22], and were reported to play important roles in preventing the lipid peroxidation process [23] and diseases implicated with the cellular oxidative stress [24]. All the triterpenoids (**4-10**), obtained from *R. laevigata*, displayed very weak DPPH radical scavenging activity with the SC_{50} values more than 200 μM.

In conclusion, phytochemical and bioactive investigation in this study revealed that flavonoids and terpenoids were the two main types of principles in the roots of *R. laevigata*, and flavonoids were the important compounds responsible for the DPPH radical scavenging capacity. The above results will provide new insights to understand the chemicals and biological functions of roots of *R. laevigata,* and promote the reasonable usage of this medicinal herb.

Experimental Part

General experimental procedures

Optical rotation was measured on a Horiba SEPA-300 polarimeter. A UV-2401PC spectrometer was used to obtain the UV spectrum in methanol (MeOH). IR spectra were taken on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were measured on Bruker AM-400 or 600 spectrometers with TMS as an internal standard. ESI-MS and HR-ESI-MS were performed on an API-QSTAR-Pulsar-1 spectrometer. 1, 1-diphenyl-2-picryldydrazyl (DPPH) was purchased from Sigma Chemicals (St. Louis, MO). Column chromatography was carried out on Sephadex LH-20 gel (25-100 μm, Pharmacia Fine Chemical Co. Ltd.), MCI gel CHP-20P (75-150 μm, Mitsubishi Chemical Co.), Chromatorex ODS (30-50 μm, Fuji Silysia Chemical Co. Ltd.), and silica gel (SiO_2; 200-300 mesh, Qingdao Haiyang Chemical Co. Ltd., P. R. China). Thin layer chromatography (TLC) was carried out on silica gel G precoated plates (Qingdao Haiyang Chemical Co. Ltd.), and spots were detected by spraying with 5 % H_2SO_4 in EtOH followed by heating.

Plant material

The roots of *R. laevigata* were collected in August 2010 from Yuexi county, Anhui province, P. R. China, and authenticated by Prof. Kaijin Wang, from School of Life Sciences, Anhui University, where a voucher specimen (No. AHU20100806) is deposited.

Extraction and isolation

The air-dried roots of *R. laevigata* (10 kg) were cut into small pieces and extracted three times with 80% EtOH (40 L×3)

under reflux, and concentrated to give a dark-brown residue (1.6 kg). The residue was suspended in H_2O and followed by successive partition with petroleum ether, EtOAc and n-BuOH. The EtOAc extract (230 g) was fractionated by silica gel column eluted with gradient $CHCl_3$/MeOH mixture (40:1 to 1:5) to afford five fractions (E1 to E5). Fraction E5 (4.8 g) was applied to Sephadex LH-20 column eluted with MeOH to afford tree fractions (E5-1 to E5-5), E5-2 was repeatedly purified on column chromatography of ODS and MCI, eluted with MeOH/H_2O (0:100 to 100:0) to produce compound **1** (15.2 mg); E5-3 was subjected to MCI column eluted with MeOH/H_2O (0:100 to 100:0), then purified on ODS column, eluted with MeOH/H_2O (0:100 to 100:0) to afford compound **2** (7.5 mg) and **3** (28.0 mg). Fraction E4 (25.0 g) was subjected to Sephadex LH-20 column eluted with MeOH to afford four fractions (E4-1 to E4-5), fraction E4-3 (5.6 g) was apllied to Sephadex LH-20 column eluted with (MeOH/H_2O, 0:100 to 100:0), and followed by MCI column (MeOH/H_2O, 0:100 to 100:0) to produce **5** (20.0 mg), **9** (35.0 mg) and **6** (28.0 mg); fraction E4-2 (4.7 g) was applied to silica gel column eluted with a gradient $CHCl_3$/MeOH mixture (15:1 to 2:1), followed with silica gel column eluted with petroleum ether/ethyl acetate mixture (3:1) to afford compound **4** (32.0 mg) and **8** (18.5 mg), E4-4 (2.7 g) was separated by column chromatography on MCI, eluted with MeOH/H_2O (0:100 to 100:0), and then purified by silica gel column, eluted with gradient $CHCl_3$/MeOH mixture (10:1) to produced compound **7** (41.0 mg). The petroleum ether exract (43.0) was fractioned by on silica gel column eluted with a gradient petroleum ether/acetone mixture (20:1 to 1:1) to give five fractions (PE1-PE5), fraction PE-5 (2.5 g) was firstly crystallized in petroleum ether/ EtOAc (5:1), then re-crystallized in EtOAc to afford compound **10** (42.5 mg).

(+)-Catechin-8-acetic acid (1): light yellow amorphous powder, $[\alpha]_D^{21}$ = +18.75° (c 0.04, MeOH); IR (KBr) cm^{-1}: 3289, 2925, 1619, 1456, 1462, 1105, 805; UV (MeOH) λ_{max} nm (lgε): 300 (3.58); HR-ESI-MS (*m/z*): 349.09161 $[M+H]^+$ (calcd. 349.09234). ^1H and ^{13}C NMR data (CD_3OD) see Table 1.

Guibourtacacidine 4-methyl ether (2): light yellow amorphous powder, $[\alpha]_D^{21}$ = +21.67° (c 0.03, MeOH); IR (KBr) cm^{-1}: 3394, 2920, 1647, 1385, 1164, 1105, 606; UV (MeOH) λ_{max} nm (lgε): 205 (3.65), 230 (3.43), 280 (3.22); HR-ESI-MS (*m/z*): 289.10687 $[M+H]^+$ (calcd. 289.10760). ^1H and ^{13}C NMR data (CD_3OD) see Table 1.

DPPH radical scavenging assay

The DPPH assay was performed as previously described [25]. L-ascorbic acid was used as positive control, and reaction mixtures containing an ethanolic solution of 200 μM DPPH (100 μL) and twofold serial dilutions of sample (dissolved in 100 μL ethanol, with amounts of sample ranging from 2 to 1000 μg/mL) were placed in a 96 well microplate and incubated at 37 °C for 30 min. After incubation the absorbance was read at 517 nm by an Emax precision microplate reader and the mean

Table 1. The NMR Data for Compounds **1**and **2** (in CD_3OD, δ in ppm, J in Hz)[a].

Position	**1**		**2**	
	^1H	^{13}C	^1H	^{13}C
2	4.56 (1H, d, 7.12)	82.8 (d)	5.01 (1H, d, 9.76)	78.2 (d)
3	3.86 (1H, m)	68.9 (d)	3.98 (1H, dd, 9.76, 3.16)	77.6 (d)
4	2.71 (1H, dd, 4.80, 16.08) 2.45 (1H, dd, 7.78, 16.08)	28.4 (t)	4.20 (1H, d, 3.16)	71.7 (d)
5		156.1 (s)	7.10 (1H, d, 8.28)	132.7 (d)
6	5.91 (1H, s)	96.5 (d)	6.39 (1H, dd, 8.28, 2.32)	108.9 (d)
7		155.9 (s)		158.6 (s)
8		102.3 (s)	6.28 (1H, d, 2.32)	103.6 (d)
9		154.6 (s)		156.7 (s)
10		100.7 (s)		113.2 (s)
1’		132.6 (s)		131.4 (s)
2’	6.72 (1H, d, 1.86)	115.1 (d)	7.26 (1H, d, 8.56)	130.2 (d)
3’		146.1 (s)	6.81 (1H, d, 8.56)	116.0 (d)
4’		146.1 (s)		160.5 (s)
5’	6.64 ((1H, d, 8.10)	116.1 (d)	6.81 (1H, d, 8.56)	116.0 (d)
6’	6.61 (1H, dd, 1.86, 8.10)	119.8 (d)	7.26 (1H, d, 8.56)	130.2 (d)
1’’	3.42 (2H, d, 10.08)	30.3 (t)		
2’’(COO^-)		178.3 (s)		
OMe			3.47 (3H, s)	57.0 (t)

[a] ^1H-(600 MHz) and ^{13}C (150 MHz) for compound **1;** ^1H-(400 MHz) and ^{13}C (100 MHz) for compound **2**.

Table 2. DPPH radical scavenging activity of compounds isolated from the roots of *R. laevigata*.

Compound	SC_{50} (μM)	Compound	SC_{50}(μM)
(+)-catechin-8-acetic acid (**1**)	24.60 ± 0.45	nigaichigoside F2 (**6**)	> 200
guibourtacacidine 4-methyl ether (**2**)	37.15 ± 0.98	rubuside J (**7**)	> 200
guibourtacacidine (**3**)	33.85 ± 0.72	tomentic acid (**8**)	> 200
euscaphic acid (**4**)	> 200*	rosamutin (**9**)	> 200
kajiichigosie F1 (**5**)	> 200	betulinic acid (**10**)	> 200
L-ascorbic acid (positive control)	31.55 ± 0.58		

* The SC_{50} value > 200 means very weak DPPH radical scavenging activity.

of three readings was obtained. Scavenging activity was determined by following equation:

$$\% \text{ scavenging activity} = [\text{Absorbance}_{control} - \text{Absorbance}_{sample}]/\text{Absorbance}_{control} \times 100.$$

The SC_{50} values were obtained through extrapolation from linear regression analysis and denoted the concentrat ion of sample required to scavenge 50% of DPPH radicals. The antioxidant activities were evaluated by SC_{50} value.

Statistics

The data presented are means ± SD of three determinations.

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References

1. Editorial Committee of Flora of China. *Flora of China,* vol. 37, Science Press, Beijing, **1985**, 448-449.
2. Editorial Committee of China Herbal. *China Herbal,* vol. 10, Shanghai Science and Technology Press, Shanghai, China, **1999**, 223-227.
3. Fang, J.M.; Wang, K.C.; Cheng, Y.S. *J. Chin. Chem. Soc.* **1991**, *38*, 297-299.
4. Gao, P.Y.; Li, L.Z.; Peng, Y.; Piao, S.J.; Zeng, N.; Lin, H,W.; Song, S.J. *Biochem. Syst. Ecol*. **2010**, *38*, 457-459.
5. 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*, 2229-2237.
6. 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*, 732-738.
7. Fang, J.M.; Wang, K.C.; Cheng, Y.S. *Phytochemistry* **1991**, *30*, 3383-3387.
8. Li, X.; Cao, W.; Shen,Y.; Li, N.; Dong, X.P.; Wang, K. J.; Cheng, Y.X. *Food Chem.* **2012**, *130*, 575-580.
9. Yoshida, T.; Tanaka, K.; Chen, X.M.; Okuda, T. *Chem. Pharm. Bull.* **1989**, 37, 920-924.
10. Zhang, T.T.; Li, S.P.; Nie, L.W. *J. Biol*. **2002**, *19*, 27-29.
11. Carvalho, M. G; Nascimento, I. A.; Carvalho, A. G. *Quim. Nova* **2008**, *31*, 1349-1352.
12. Ali, M.; Bhutani, K. K.; Gupta, D. K. *Fitoterapia* **1997**, *68,* 82
13. Seto, T.; Tanaka, T.; Tanaka O.; Naruhashi, N. *Phytochemistry* **1984**, *23*, 2829-2834.
14. Li, W.; Fu, H. W.; Bai, H.; Sasaki, T.; Kato, H.; Koike, K. *J. Nat. Prod.* **2009**, *72,* 1755-1760.
15. Numata, A.; Yang, P.; Fujita, E. *Chem. Pharm. Bull.* **1989**, *37*, 648-651.
16. Yang, S. C.; Fang, J. M.; Cheng, Y. S. *J. Chin. Chem. Soc.* **1995**, *42*, 573-577.
17. Du, H. Q. *Acta Pharm. Sin.* **1983**, *18*, 314-316.
18. Ikuta, A.; Itokawa, H. *Phytochemistry* **1988**, *27*, 2813-2815.
19. Nonaka, G.; Ezaki, E.; Hayashi, K., Nishioka, I. *Phytochemistry* **1983**, 22, 1659-1661.
20. Van Der Merwe, P.J.; Hundt, K.K.L. *Xenobiotica* **1984**, *14*, 795-802.
21. Blois, M. S. *Nature*, **1958**,*181*, 1199-1200.
22. Pietta, P. G. *J.Nat.Prod.* **2000**, *63*, 1035-1042.
23. Mangiapane, H.; Thomson, J.; Salter, A.; Brown, S.; Bell, G.D.; White, D.A. *Biochem. Pharmacol.* **1992**, *43*, 445-450.
24. Halliwell, B. *Lancet*, **1994**, *334*, 721-724.
25. Wang, K.J.; Yang, C.R.; Zhang, Y.J. *Food Chem.* **2007**, *101*, 365-371.