Flavonoid and a Rare Benzophenone Glycoside from the Leaves of *Aquilaria sinensis*

Jin QI,[a] Jing-Jing LU,[a] Ji-Hua LIU,[a] and Bo-Yang YU*,[a,b]

[a] *Department of Complex Prescription of TCM, China Pharmaceutical University; and* [b] *Key Laboratory of Modern Chinese Medicines, China Pharmaceutical University, Ministry of Education; Nanjing 210038, P.R. China.*

Received July 20, 2008; accepted November 1, 2008; published online November 11, 2008

From the leaves of *Aquilaria sinensis* (LOUR.) GILG, a novel benzophenone glucoside, designated as aquilarinoside A (1), and a new flavonoid, as 7-*β*-D-glucoside of 5-*O*-methylapigenin (2), along with eight known compounds (3—10) were isolated. The structures of 1 and 2 were fully characterized by spectroscopic methods (1D, 2D NMR and MS) and the relative stereochemistry was assigned based on nuclear Overhauser effect spectroscopy (NOESY) correlations and analyses of coupling constants. The new compounds showed inhibition activity against polymorphonuclear neutrophils (PMNs) respiratory burst stimulated by PMA.

Key words *Aquilaria sinensis* (LOUR.) GILG; Thymelaeaceae; benzophenone glucoside; flavonoid; aquilarinoside A; 7-*β*-D-glucoside of 5-*O*-methylapigenin

The genus *Aquilaria* (Thymelaeaceae) is widely distributed in Asia. *Aquilaria sinensis* (LOUR.) GILG is of particular interest economically because it is the principal source of agarwood, one of the most highly valuable forest products currently traded internationally.[1)] The leaves of *Aquilaria sinensis* (LOUR.) GILG have been used traditionally in China for treatments of inflammation and anaphylaxis. Since little phytochemical study has yet been reported on the leaves of *Aquilaria sinensis*, an investigation of its ethanol extract was carried out. Herein we report the isolation, structure elucidation, and biological evaluation of a novel benzophenone glucoside and a new flavonoid (**1**, **2**). Some known compounds, including iriflophenone (**3**), mangiferin (**4**), 5-*O*-xylosylglucoside of 7-*O*-methylapigenin (**5**), 5-*O*-xylosylglucoside of 7,4′-di-*O*-methylapigenin (**6**), 5-*β*-D-glucoside of 7,3′-di-*O*-methylluteolin (**7**), luteolin (**8**), genkwanin (**9**) and hydroxygenkwanin (**10**) were also isolated from the plant. The structures of these compounds are given in Fig. 1.

Results and Discussion

Compound **1** was obtained as yellow amorphous powder, whose molecular formula of $C_{19}H_{18}O_9$ was determined on the basis of HR-ESI-MS (observed *m/z* 391.1023 $[M+H]^+$), ^{13}C-NMR spectrum and distortionless enhancement by polarization transfer (DEPT) spectrum, indicating 11 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3373 cm^{-1}), conjugated ketone (1640 cm^{-1}), and benzene ring (1608, 1509 cm^{-1}) functionalities. The UV spectrum showed absorption maxima at 229 and 309 nm. Analysis of the ^1H- and ^{13}C-NMR spectral data of **1** (Table 1) indicated that the molecule consisted of a benzophenone and a glucose moieties.[2)]

In the ^1H-NMR spectrum, two sets of doublets at δ_H 7.60 (2H, d, J=8.4 Hz) and 6.79 (2H, d, J=8.4 Hz), revealed the presence of a *para*-substituted symmetric phenolic ring derivative with two equivalent pairs of *ortho*-coupled protons, and the aromatic proton of the other phenolic ring displayed

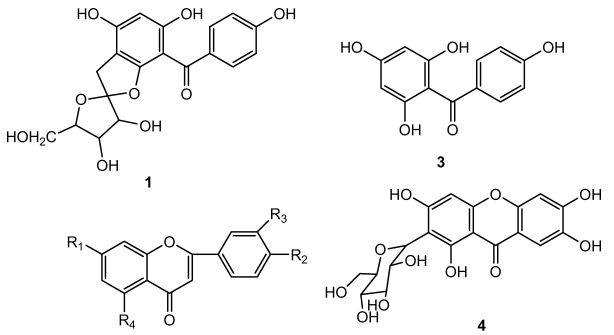

Fig. 1. The Structures of Compounds **1**—**10**

Table 1. ^1H- and ^{13}C-NMR Spectroscopic Data for (**1**) (DMSO-d_6, 500 MHz) (δ in ppm, J in Hz)

Atom	HSQC (δ_H)	δ_C	HMBC correlations
Aglycone			
1		102.0	
2		158.1	
3		102.7	
4		157.2	
5	5.96 (1H, s)	95.8	1, 4, 6
6		159.4	
7		193.0	
4-OH	10.02 (1H, s)		
6-OH	10.47 (1H, s)		1, 5
1′		129.9	
2′, 6′	7.60 (2H, d, J=8.4)	131.9	4′, 7
3′, 5′	6.79 (2H, d, J=8.4)	114.7	1′, 4′
4′		161.6	
4′-OH	10.20 (1H, s)		3′, 5′
1″	2.66 (1H, d, J=16.3)	32.2	2, 3, 2″
	3.28 (1H, d, J=16.3)		
2″		120.1	
3″	3.89 (1H, t, J=11.3)	80.9	2″, 4″
3″-OH	5.13 (1H, d, J=5.5)		4″
4″	3.62 (1H, m)	76.0	3″, 6″
4″-OH	5.69 (1H, d, J=5.6)		3″, 4″
5″	3.55 (1H, m)	82.1	4″
6″	3.55 (1H, m)	60.8	
	3.37 (1H, m)		
6″-OH	4.71 (1H, *t*, J=10.8)		

* To whom correspondence should be addressed. e-mail: boyangyu59@163.com

a singlet at δ_H 5.96 (1H, s). In addition, three singlets at δ_H 10.47 (1H, s), 10.20 (1H, s) and 10.02 (1H, s) were assigned to three phenolic-hydroxyl groups, respectively. These data indicated the aglycone moiety was analogous to iriflophenone except for the absence of one proton in ring A. In the ^{13}C-NMR spectrum (Table 1), 13 carbon signals from the aglycone moiety were similar to those of iriflophenone, but compared with those of iriflophenone the signals of C-2 (δ_C 158.1) and C-3 (δ_C 102.7) shifted upfield by $\Delta\,\delta_C$ 2.4 and 8.6 ppm, respectively. In addition, six carbon signals from a sugar moiety were evident. In the DEPT spectrum, six carbon signals from the sugar moiety resonated at δ_C 120.1 (C), 82.1 (CH), 80.9 (CH), 76.0 (CH), 60.8 (CH_2) and 32.2 (CH_2). All the information described above supported that compound **1** was a mono-glycoside derivative of iriflophenone.

Furthermore, in the heteronuclear multiple quantum correlation (HMQC) spectrum (Table 1), the signal at δ_C 32.2 was directly attached to the signals at δ_H 3.28 (d, J=18.7 Hz, H-1″a) and 2.66 (d, J=16.2 Hz, H-1″b). In the ^{1}H–^{1}H COSY spectrum, the signal at δ_H 3.62 (m, H-4″) correlated with signals at δ_H 3.89 (t, J=11.3 Hz, H-3″) and 3.55 (m, H-5″), while the signal at δ_H 3.37 (1H, m, H-6″b) correlated with signal at δ_H 3.55 (m, H-5″, 6″a). Consequently, this was taken into consideration of the HMQC and heteronuclear multiple bonding connectivity (HMBC) spectral analysis, the sugar was a ketohexose skeleton and the presence of fragment A was therefore confirmed (Fig. 2). Furthermore, the position of the glycosidic linkage was established on the basis of the following evidence. A downfield quaternary carbon (δ_C 120.1) of ketohexose C-2″ should be linked to the C-2 oxygen-atom due to the absence of a free 2-OH in the ^{1}H-NMR spectrum, and the signal of C-2 displayed an upfield shift ($\Delta\,\delta_C$ 2.4 ppm) compared with the corresponding signal of iriflophenone. The signal (δ_C 32.2) of ketohexose C-1″ was linked to C-3, which was confirmed by an upfield shift ($\Delta\,\delta_C$ 8.6 ppm) of C-3 and the HMBC (Fig. 2) in which the carbon signal at δ_C 102.7 (C-3) showed a key correlation with the protons at δ_H 3.28 (d, J=16.3 Hz, H-1″a) and 2.66 (d, J=16.3 Hz, H-1″b).[3] The NOESY spectrum (Fig. 2) showed cross peaks between protons at δ_H 3.67 (d, J=16.7 Hz, H-1″a) and 3.55 (m, H-6″), 3.37 (m, H-6″), at δ_H 3.62 (m, H-4″) and 3.55 (m, H-6″), 3.37 (m, H-6″), and proton at δ_H 3.89 (t, J=11.3 Hz, H-3″) and hydroxyl proton at δ_H 5.69 (d, J=5.6 Hz, 4″-OH), and proton at δ_H 3.62 (m, H-4″) and proton at δ 5.13 (d, J=5.5 Hz, 3-OH). These data indicated a relative configuration of the sugar moiety as α-fructofuranose.[4] Finally, the structure of aquilarinoside A was elucidated as **1**, a new benzophenone mono-glycoside.

Compound **2** was obtained as pale yellow powder, $[\alpha]_D^{25}$ −81.20° (c=0.05, MeOH) and showed a positive Mg–HCl color reaction. The molecular formula was assigned as $C_{22}H_{22}O_{10}$ based on the HR-ESI-MS (observed m/z 447.1285 $[M+H]^+$), ^{13}C-NMR spectrum and DEPT spectrum, indicating 12 degrees of unsaturation. The IR spectrum of **2** displayed absorptions for hydroxyl (3311 cm^{-1}), conjugated ketone (1643 cm^{-1}), and aromatic ring (1611, 1517, 1497 cm^{-1}) functionalities. The UV spectrum showed absorption maxima at 261 nm (band II) and 332 nm (band I) in MeOH, a characteristic of flavone derivatives.[5] Analysis of the ^{1}H- and ^{13}C-NMR spectral data of **2** (Table 2) indicated

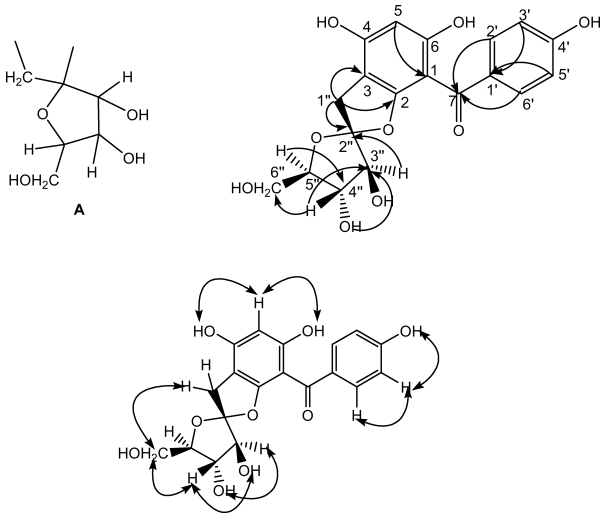

Fig. 2. Structure of Fragment A and the Key Correlation of HMBC (H→C) and ROESY for **1**

Table 2. ^{1}H- and ^{13}C-NMR Spectroscopic Data for (**2**) (DMSO-d_6, 500 MHz) (δ in ppm, J in Hz)

Atom	HSQC (δ_H)	δ_C	HMBC correlations
Aglycone			
2		161.5	
3	6.71 (1H, s)	105.9	2, 4, 1′
4		176.9	
5		163.6	
6	6.91 (1H, d, J=2.3)	103.6	5, 7, 8, 9
7		158.3	
8	7.06 (1H, d, J=2.3)	96.7	6, 7, 9
9		158.5	
10		109.4	
5-OCH_3	3.90 (3H, s)	56.1	5
1′		121.2	
2′, 6′	7.93 (2H, d, J=8.7)	128.2	3′, 5′, 4′
3′, 5′	6.93 (2H, d, J=8.7)	116.0	1′, 2′, 6′
4′		160.9	
4′-OH	10.26 (1H, s)		3′, 5′
Glucose			
1″	4.75 (1H, d, J=7.6)	104.4	7
2″	3.16 (1H, td, J=8.4, 3.4)	73.7	
3″	3.37 (1H, m)	75.9	
4″	3.32 (1H, dd, J=6.6, 1.4)	70.1	3″, 6″
5″	3.28 (1H, m)	77.5	4″
6″	3.76 (1H, dd, J=12.3, 5.6)	61.1	
	3.49 (1H, br d, J=12.3)		

that the molecule consisted of a flavanone, a glycose, and a methoxyl moiety.

The ^{1}H-NMR spectum suggested that **2** has an aglycon vertiaflavone (5-*O*-methylapigenin).[6] Signals at δ_H 7.93 (d, J=8.7 Hz, H-2′, 6′) and 6.93 (d, J=8.7 Hz, H-3′, 5′) were characteristic for a 4-disubstituted B ring of a vertiaflavone unit. Two doublets at δ_H 6.91 (d, J=2.3 Hz) and 7.06 (d, J=2.3 Hz), along with a singlet at δ_H 6.71 were due to the presence of protons at C-6, C-8 and C-3 in rings A and C of a vertiaflavone, respectively. Furthermore, two singlets at δ_H 3.90 (3H, s) and 10.26 (1H, s) were respectively assigned to protons of the methoxy group bonding to C-5 and the hydroxyl group bonding to C-4′ of a vertiaflavone. These were in sound agreement with the ^{13}C-NMR spectrum. The six carbon signals of the sugar moiety were at δ_C 104.4, 77.5,

Table 3. Inhibition of PMN-CL by Compounds **1**—**10** from the Leaves of *Aquilaria sinensis* (n=3)

	Samples									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
IC_{50} (μmol/l) Mean±S.D.	89.92±1.07	61.25±0.21	52.59±7.71	50.34±1.80	293.06±9.06	n.d.	n.d.	2.03±0.24	265.41±2.78	0.80±0.13

PMNs were stimulated by PMA. Chemiluminescence was measured with luminol.

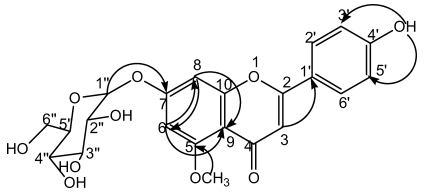

Fig. 3. Selected HMBC Correlations (H→C) for Compound **2**

75.9, 73.7, 70.1 and 61.1, suggesting that **2** was a vertiaflavone glycoside. The presence of the β-D-glucopyranosyl moiety was supported by the ^{13}C-NMR data and further confirmed by the acid hydrolysis of **2**, which resulted in a release of D-glucose by HPLC and GC in comparison with an authentic sugar sample. The configuration of the glucopyranosyl was assigned to be β-D based on the coupling constant of the anomeric proton H-1″ (δ_H 4.75, d, J=7.6 Hz). The glucosyl residue was located at the 7-*O*-position of the aglycon vertiaflavone by the appearance of HMBC cross peaks of the glucosyl anomeric proton H-1″ (δ_H 4.75, d, J=7.6 Hz) with the carbon signal at δ_C 158.3 (C-7). In addition, HMBC correlations (Fig. 3) of the methoxy group protons δ_H 3.90 (3H, s) with C-5 (δ_C 163.6) and of the hydroxyl group protons δ_H 10.26 with C-3′, 5′ (δ_C 116.0) further confirmed the above assignments. As a consequence, the structure of **2** was determined as 7-β-D-glucoside of 5-*O*-methylapigenin.

The structures of the known compounds were identified as iriflophenone (**3**),[7] mangiferin (**4**),[8] 5-*O*-xylosylglycoside of 7-*O*-methylapigenin (**5**),[9,10] 5-*O*-xylosylglucoside of 7,4′-di-*O*-methylapigenin (**6**),[11] 5-β-D-glucoside of 7,3′-di-*O*-methylluteolin (**7**),[12] luteolin (**8**),[13] genkwanin (**9**),[14] hydroxygenkwanin (**10**),[15] respectively, by comparison their NMR data with those reported in literatures.

Polymorphonuclear neutrophils (PMNs) are important cells involved in the bactericidal host defense system through the respiratory burst. PMNs respiratory burst plays a critical role in the immune-inflammatory processes. Inhibition of neutrophils respiratory burst has been one of the well-documented methods for the evaluation of anti-inflammatory activity for various synthetic compounds and natural products.[16—18] In the present study, we chose neutrophils respiratory burst assay as a model to evaluate the compounds isolated from the leaves of *Aquilaria sinensis* for their activity. The biological test results of the isolated compounds are shown in Table 3. The compounds **1**, **2**, **3**, **4**, **8** and **10** showed significant inhibitory activity against neutrophils respiratory burst stimulated by PMA with IC_{50} values ranging from 0.80 to 89.92 μmol/l, whereas compounds **5**, **9** only showed marginal inhibition activity.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO P-1020 polarimeter. NMR experiments were performed with a Brucker AV-500 spectrometer. ESI-MS spectra were recorded by an Agilent 1100 LC API MSD system. HR-ESI-MS was measured on a Bruker Daltonics, APEX III Instrument. Column chromatography was carried out with silica gel (200—300 mesh, Qingdao Marine Chemistry Company, People's Republic of China). Sephadex LH-20 was from Pharmacia (Sweden). The chemiluminescence values were recorded by BPCL-1-G-C Ultra-weak Luminescence Analyzer (Beijing Institutes for Biophysics, Chinese Academy of Science). Male SD rats weighing 200—250 g each were obtained from the Experimental Animal Center of Southeast University, Nanjing, China. Phorbol 12-myristate 13-acetate (PMA) and fetal calf serum (FCS) were obtained from Promega (U.S.A.) and Hyclone (U.S.A.), respectively.

Plant Material Leaves of *Aquilaria sinensis* (Lour.) Gilg were collected from Dianbai County, Guangdong province, China in October 2005 and was authenticated by Dr. Zenglai Xu (Nanjing Zhongshan Arboretum, Jiangsu province, China). The voucher specimen (BYY051020) was deposited at the Herbarium of China Pharmaceutical University.

Extraction and Isolation Dried leaves of *Aquilaria sinensis* (10 kg) were extracted with hot EtOH–H_2O (1 : 1) under refluxing (3 h×5), followed by removal of the solvent in vacuum, to yield a dried EtOH–H_2O extract (848 g). The EtOH extract (848 g) were suspended in H_2O and extracted successively with Petroleum Ether, EtOAc and *n*-BuOH. The *n*-BuOH fraction (200 g) was subjected to silica gel CC (1000 g) eluted successively with $CHCl_3$–CH_3OH (95 : 5), (9 : 1), (8.5 : 1.5), (8 : 2), (7 : 3), (6 : 4) and (1 : 1) to afford a total of eight fractions (Fr-1—Fr-8). Fr-3 (6 g) was further chromatographed over silica gel, eluting with $CHCl_3$–CH_3OH (95 : 5), and Sephadex LH-20, eluting with MeOH, to give **1** (10 mg). Fr-5 (20 g) was applied to a silica gel column eluting with $CHCl_3$–CH_3OH gradients, and then purified on Sephadex LH-20 eluting with $CHCl_3$–CH_3OH (1 : 1) to give compounds **7** (10 mg), **2** (20 mg) and **6** (38 mg). Same treatment for Fr-6 (21 g) as Fr-5 afforded pure **4** (100 mg) and **5** (40 mg). The EtOAc fraction (107 g) was subjected to silica gel CC (800 g) eluting successively with $CHCl_3$–CH_3OH (100 : 0), (98 : 2), (95 : 5), (9 : 1), (8 : 2), (7 : 3) and (1 : 1) to afford a total of eleven fractions (Fr-1—Fr-11). Fr-5 (15 g) was applied to a silica gel column eluted with Petroleum Ether–EtOAc gradients, and then purified by recrystallization to give compounds **8** (15 mg), **9** (2 g) and **10** (13 mg). Fr-7 (1.8 g) was further chromatographed over silica gel, eluting with $CHCl_3$–CH_3OH (95 : 5), and Sephadex LH-20, eluting with MeOH, to give **3** (18 mg).

Aquilarinoside A (**1**): Amorphous white powder, mp 140—142 °C. $[\alpha]_D^{25}$ −138.77° (c=0.05, MeOH). UV λ_{max} (MeOH) nm (log ε): 229 (sh), 309 (4.28). IR (KBr): 3373, 1640, 1608, 1509. NMR data are shown in Table 1; ESI-MS m/z: 391 $[M+H]^+$; HR-ESI-MS m/z: 391.1015 $[M+H]^+$ (Calcd for $C_{19}H_{19}O_9$: 391.1023).

7-β-D-Glucoside of 5-*O*-Methylapigenin (**2**): Pale yellow powder, mp 181—183 °C. $[\alpha]_D^{25}$ −81.20° (c=0.05, MeOH). UV λ_{max} (MeOH) nm (log ε): 261 (3.90), 332 (4.36). IR (KBr): 3311, 1643, 1611, 1517, 1497. NMR data are showed in Table 2; ESI-MS m/z: 447 $[M+H]^+$; HR-ESI-MS m/z: 447.1274 $[M+H]^+$ (Calcd for $C_{22}H_{23}O_{10}$: 447.1285).

Acidic Hydrolysis of Compound 2 A solution of compound **2** (2 mg), in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was refluxed for 4 h. After removing dioxane, the solution was diluted with H_2O and extracted with EtOAc (1 ml×3) to separate the aglycone. The water layer was neutralized by passing through an Amberlite IRA 400 column, eluting with water and concentrated in vacuum. Portion of the residue was examined by HPLC analysis comparing to standard sample [condition: column, Cosmosil carbohydrate analysis column (4.6×250 mm, 5 μm); solvent, CH_3CN–H_2O (85 : 15); flow rate, 1 ml/min; detector, Alltech ELSD 500 detector; drift tube temperature, 90 °C; retention time, D-glucose (15.7 min)]. The absolute configuration of the glucose was determined by GC after converted to its thiazolidine deriva-

tive.[19] A solution of water layer residue in pyridine (100 μl) was stirred with D-cysteine methyl ester for 1.5 h at 60 °C. Then, the hexamethyldisilazane (100 μl) and trimethylsilylchloride (100 μl) were added and the mixture was stirred for 30 min at 60 °C. The supernatant was analyzed by GC to lead to the identification of D-glucose by comparison of retention time with an authentic sample of this sugar [conditions retention time, D-glucose (10.4 min)].

Isolation of Neutrophils Whole blood was obtained from SD rats (Sprague-Dawley, 300—350 g) by eyeground puncture into heparinized tubes. Neutrophils were isolated and purified by dextran sedimentation, hypotonic lysis of erythrocytes, and centrifugation through Ficoll–Hypaque.[20] All the subsequent purification steps were performed at 4 °C using ice-cold buffers. Purified neutrophils were normally resuspended in Hanks' balanced salt solution (HBSS) containing 10 mM HEPES, pH 7.4, and 4 mM $NaHCO_3$, and kept in an ice-bath before use. The purified cells consisted of a >95% pure population of viable neutrophils as assessed by morphology and Trypan blue exclusion test.

Chemiluminescence Assay Details of chemiluminescence (CL) assay procedure were described in the literature.[21] Briefly, for the measurement of CL, 1.0 ml cells (10^7 or less neutrophils) were mixed in 5-ml quartz tubes with 10 μl testing solution and 200 μl 5 μM luminal. The tubes were placed in the BPCL-1-G-C Ultra-weak Luminescence Analyzer in dark and allowed to equilibrate for 10 min at 37 °C. Then, 20 μl phorbol 12-myristate 13-acetate (PMA) was added to activate the system and the light emission was recorded continuously. The IC_{50} values were obtained by linear regression analysis of the dose–response curves, which were plots of % inhibition *versus* concentration.[22] Testing compounds were prepared as 10 mM top stocks, dissolved in DMSO, and stored at 4 °C (Table 3).

Acknowledgements This work was supported by the grant No. 30572320 from the National Natural Science Foundation of China to Jihua Liu.

References

1) Soehartono T., Newton A. C., *Biol. Conserv.*, **96**, 83—94 (2000).
2) Tanaka T., Sueyasu T., Nonaka G. I., Nishioka I., *Chem. Pharm. Bull.*, **32**, 2676—2686 (1984).
3) Zhang P. C., Xu S. X., *Phytochemistry*, **57**, 1249—1253 (2001).
4) Agrawal P. K., *Phytochemistry*, **31**, 3307—3330 (1992).
5) Xu R. S., "The Chemistry of Natural Products," Science Press, Beijing, 1993, pp. 592—594.
6) Voigtländer H. W., Balsam G., *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **303**, 782—787 (1970).
7) Ito H., Nishitani E., Konoshima T., Takasaki M., Kozuka M., Yoshida T., *Phytochemistry*, **54**, 695—700 (2000).
8) Frahm A. W., Chaudhuri R. K., *Tetrahedron*, **35**, 2035—2038 (1979).
9) Ji C. R., Liu Y. Z., Feng W. S., Wang M. T., Zhao T. Z., *Chin. Tradit. Herb. Drugs*, **17**, 7—9 (1986).
10) Granados A. G., Saenz J. M., *Org. Magn. Reson.*, **13**, 462—463 (1980).
11) Zahir A., Jossang A., Bodo B., Provost J., Cosson J. P., Sevenet T., *J. Nat. Prod.*, **62**, 241—243 (1999).
12) Ulubelen A., Bucker R., Mabry T. J., *Phytochemistry*, **21**, 801—803 (1982).
13) Agrawal P. K., Thakur R. S., Bansal M. C., "Flavonoids," Vol. 39, Elsevier Science Publishers B.V., New York, 1989, pp. 95—182.
14) Chang P. T. O., Cordell G. A., Fong H. H. S., Farnsworth N. R., *Phytochemistry*, **16**, 1443—1445 (1977).
15) Ji C. R., Liu Y. Z., Wang M. T., Zhao T. Z., Zhang Z. W., *Acta Pharm. Sin.*, **18**, 61 (1983).
16) Jaisson S., Delevallee-Forte C., Toure F., Rieu P., Garnotel R., Gillery P., *FEBS Lett.*, **581**, 1509—1513 (2007).
17) Kuan Y. H., Lin R. H., Lin H. Y., Huang L. J., Tsai C. R., Tsao L. T., Lin C. N., Chuang L. C., Wang J. P., *Biochem. Pharmacol.*, **71**, 1735—1746 (2006).
18) Bengtsson T., Oreslius K., Wettero J., *Cell Biol. Int.*, **30**, 154—163 (2006).
19) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **34**, 1843—1845 (1986).
20) Wang J. P., Raung S. L., Kuo Y. H., Teng C. M., *Eur. J. Pharmacol.*, **288**, 341—348 (1995).
21) Pasmans F., Herdt P. D., Nerom A. V., Haesebrouck F., *Dev. Comp. Immunol.*, **25**, 159—168 (2001).
22) Chadfield M., Olsen J., *Vet. Immunol. Immunopathol.*, **80**, 289—308 (2001).