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A New Isoflavonoid from the Rhizomes of *Cyperus rotundus*

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A new isoflavonoid, 5,7,4'-trihydroxy-2'-methoxy-3'-prenylisoflavone (**1**), along with eleven phenolic compounds (**2-12**), licoricone (**2**), 6-O-*p*-hydroxybenzoyl-6-*epi*-aucubin (**3**), 6-O-*p*-hydroxybenzoyl-6-*epi*-monomelittoside (**4**), 7-O-*p*-hydroxybenzoyl-8-*epi*-loganic acid (**5**), verproside (**6**), syringopicroside B (**7**), syringopicroside C (**8**), oleuropeinic acid (**9**), oleuroside (**10**), 10-hydroxyoleuropein (**11**), senburisideI (**12**), were obtained from the rhizomes of *Cyperus rotundus*. Their chemical structures were elucidated on the basis of UV, IR, MS, NMR spectroscopic analyses. Compound **12** showed considerable macrophages respiratory burst (MRB) inhibitory activity in the test with IC_{50} value of approximately 27 μM.

Keywords: *Cyperus rotundus*, Isoflavonoid, Phenolic compouds, Macrophages respiratory burst.

INTRODUCTION

The rhizome of *Cyperus rotundus* is an important traditional Chinese medicine, which is widely used in folk medicine as an antiinflammatory, antidepressant, antipyretic, analgesic and antiemetic remedy for dysentery and women's diseases[1,2]. Alkaloids, flavonoids, glycosides and furochromones and several novel sesquiterpenoids have been reported from *Cyperus rotundus*[3-6].

Our studies indicated that the 95% aqueous ethanol extract of rhizomes of *Cyperus rotundus* showed significant inhibition activity against macrophages respiratory burst[7]. More recently, using a bioactivity-guided approach, we have reported several phenolic compounds, flavonoids and iridoid glycosides that are responsible for the observed pharmacological activity in the macrophages respiratory burst inhibitory activity test[7-9]. Further investigation on the 95 % aqueous ethanol extract of the same plant has led to the isolation of 12 compounds. In this paper, we describe the isolation and structure determination of the new compound, together with the macrophages respiratory burst inhibitory activity test performed for all isolated compounds (**1-12**) (Fig. 1).

EXPERIMENTAL

UV spectra were recorded on a Hewlett-Packard HP-845 UV-visible spectrophotometer. Specific rotation measurements were recorded on a Perkin-Elmer 242 MC polarimeter. IR spectra were recorded on a Nicolet 470 spectrometer and MS on a Varian MAT-212 mass spectrometer and a Shimadzu GC-MS model QP2010 Plus spectrophotometer, respectively. NMR spectra were recorded on a Bruker DRX-300 (300 MHz for ^{1}H NMR) using standard Bruker pulse programs. Chemical shifts are given as d values with reference to tetramethylsilane (TMS) as internal standard. The chemiluminescence value was recorded by BPCL-1-G-C Ultra-weak Luminescence Analyzer (Beijing Institutes for Biophysics, Chinese Academy of Science). RPMI-1640, Phorbol 12-myristate 13-acetate (PMA) and fetal calf serum (FCS) were obtained from GIBCO (USA), respectively. Column chromatography separations were carried out on silica gel (200-300 mesh, Qingdao Haiyang Chemical Co. Ltd, Qingdao, P.R. China), ODS (50 mesh, AA12S50, YMC) and Diaion HP-20 (Pharmacia, Peapack, New Jersey, U.S.A). All other chemicals used were of biochemical reagent grade.

The rhizomes of *Cyperus rotundus* L. were collected in Zhanjiang, Guangdong Province of China in September 2009 and a voucher specimen (No.20090903) was kept at the Chemitry Science and Technology School, Zhanjiang Normal University.

Extraction and isolation: The dry rhizomes of *Cyperus rotundus* (10 kg) were extracted three times under reflux with 95 % aqueous EtOH (150 L × 2 h). After removing the solvent under reduced pressure, the residue was suspended in water

and then sequentially extracted with petroleum ether, CH_2Cl_2, EtOAc and *n*-BuOH. The EtOAc extract (88 g) was subjected to silica gel column chromatography (CC) using $CHCl_3$-MeOH mixtures (1:0 to 0:1) and divided into eight main fractions by TLC detection. Fraction 5 was separated by CC over silica gel using $CHCl_3$-MeOH (6:1) and Sephadex LH-20 CC using $CHCl_3$-MeOH (1:1) to afford **1** (19 mg). Fraction 7 was chromatographed on silica gel eluting with $CHCl_3$-MeOH-H_2O (9:1:0.1 to 7:3:0.3) and ODS silica gel with MeOH-H_2O (1:1 to 1:0) to furnish **2** (27 mg). The *n*-BuOH extract (152 g) was submitted through a column chromatography (CC) of high porous absorption resin (Diaion HP-20), eluting with H_2O and CH_3OH. The methanol fraction (98 g) was repeatedly CC over normal and reverse phase silica gel to afford four fractions (Frs.1-4). Fr.1 was subjected to ODS CC eluting with CH_3OH-H_2O (0:1- 1:0) and silica gel with $CHCl_3$-MeOH-H_2O (9:1:0.1) to give compounds **3** (21 mg), **4** (18 mg) and **5** (26 mg). Fr. 3 was subjected to ODS CC eluting with CH_3OH-H_2O (0:1- 1:0 and silica gel with $CHCl_3$-MeOH-H_2O (8.5:1.5:0.15-8:2:0.2) to give compounds **6** (19 mg), **7** (22 mg), **8** (20 mg), **9** (17 mg), **10** (22 mg) and **11** (28 mg). Fr. 4 was subjected to ODS CC eluting with CH_3OH-H_2O (7:3) and silica gel with $CHCl_3$-MeOH-H_2O (6.5:2.5:0.1-6.5:3.5:0.1) to give compound **12** (25 mg).

Macrophages respiratory burst inhibitory activity: The murine macrophage-like cell line RAW 264.7 was routinely cultivated at 37 °C, 5 % CO_2 in RPMI-1640 supplemented with 10 % FCS (Hyclone, America), 100 µg/mL streptomycin, 118 µg/mL ampicillin and 2 mg/mL sodium bicarbonate. After 72 h, the macrophage cells formed a confluent monolayer. The monolayer cells were digested with trypsin. After washed with PBS without Ca^{2+} and Mg^{2+}, the deposited cells were suspended with RPMI-1640 without FCS (approximately 2×10^6 cell/mL) in the vitreous culture flask[10,11].

The details of chemiluminescence assay procedure were according to the method described in the literature[12]. Tested compounds were prepared as 10 mM top stocks, dissolved in DMSO and stored at 4 °C. Phorbol 12-myristate 13-acetate (PMA) was applied as triggering agent. Data were collected at a frequency of 6 s/min and chemiluminescence was recorded for up to 0.5 h. The IC_{50} values were obtained by linear regression analysis of the dose response curves, which were ploted % inhibition *versus* concentration[13].

RESULTS AND DISCUSSION

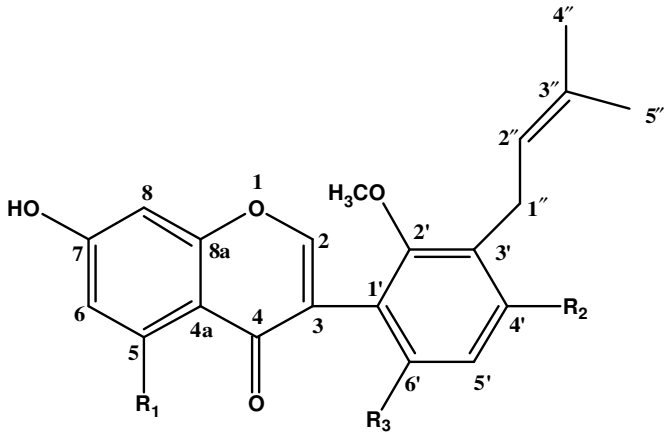
The phytochemical study of 95 % aqueous ethanol extract obtained from the rhizomes of *Cyperus rotundus* afforded 12 compounds. The novel compound, 5,7,4'-trihydroxy-2'-methoxy-3'-prenylisoflavone (**1**) was determined by the 1D and 2D NMR elucidations and mass spectral analysis. Eleven known phenolic compounds, including licoricone (**2**)[14], 6-O-*p*-hydroxybenzoyl-6-*epi*-aucubin (**3**)[15], 6-O-*p*-hydroxybenzoyl-6-*epi*-monomelittoside (**4**)[16], 7-O-*p*-hydroxybenzoyl-8-*epi*-loganic acid (**5**)[17], verproside (**6**)[18], syringopicroside B (**7**)[19], syringopicroside C (**8**)[19], oleuropeinic acid (**9**)[20], oleuroside (**10**)[21], 10-hydroxyoleuropein (**11**)[22] and senburisidel (**12**)[15] were characterized by comparison of their physical and spectroscopic data with those reported in the literature.

Compound **1** was isolated as a pale yellow amorphous powder with the m.f. determined as $C_{21}H_{20}O_6$ deduced from the observed $[M + H]^+$ peak at *m/z* 369.1336 (calcd. 369.1348) in the HREIMS. The IR spectrum showed absorption bands for hydroxyl (3617 cm^{-1}), carbonyl (1664 cm^{-1}) and alkene (1604 cm^{-1}) functional groups. Compound **1** was recognized as isoflavonoid by UV absorption maxima at 262 and 317 (sh) nm as well as by the characteristic proton singlet at δ 7.90 (H-2) in ^{1}H NMR spectrum[23]. In the ^{1}H NMR spectrum, two meta-coupled aromatic protons at δ 6.34 (1H, d, J = 2.4 Hz) and 6.25 (1H, d, J = 2.4 Hz) were attributed to the protons at H-6 and H-8 in ring A. On the other hand, two AB-type aromatic protons at δ 6.54 (1H, d, J = 8.4 Hz) and 6.97 (1H, d, J = 8.4 Hz) were attributed to the protons at H-5' and H-6' in ring B Table-1. The ^{1}H NMR spectra also exhibited signals of one aromatic methoxyl group [δ 3.71 (3H, s), δ 61.2], one chelated hydroxyl group (δ 12.47) and a set of signals that were assigned to one prenyl group [two methyl singlets at δ 1.68 and 1.78 (each 3H, s), one methylene doublet at δ 3.49 (2H, d, J = 6.6 Hz) and one triplet at δ 5.25 (1H, t, J = 6.6 Hz)][24]. According to the 2D NMR spectrum, the structure units of **1** (Fig. 1) were established. The HMBC specra (Fig. 2) showed long-range correlations of H-2 (δ 7.90) with C-3 (δ 123.8), C-4 (δ 182.0), C-8a (δ 157.9) and C-1' (δ 114.0), confirming its isoflavone structure. In the HMBC spectrum, cross-peaks between H-1″ (δ 3.49), H-4″ (δ 1.68) or H-5″ (δ 1.78) and C-2″ (δ 122.5), C-3″ (δ 132.2) and in the COSY spectrum coss-peaks between H-1″ and H-2″ revealed the

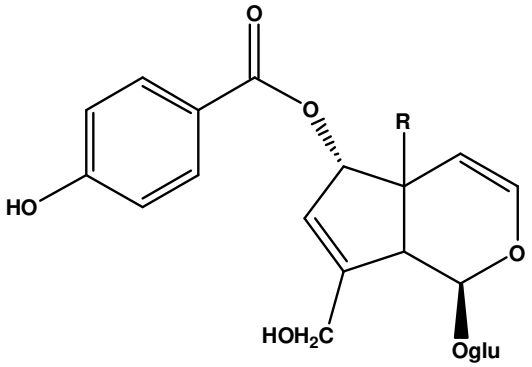
TABLE-1
^{1}H NMR AND ^{13}C NMR SPECTROSCOPIC DATA FOR COMPOUND **1**

Carbon No.	δ	δ	Carbon No.	δ	δ
2	7.90 (s)	155.8 (d)	3′	–	119.0 (s)
3	–	123.8 (s)	4′,	–	156.6 (s)
4	–	182.0 (s)	5′	6.54 (d, 8.4)	104.4 (d)
4a	–	105.6 (s)	6′	6.97 (d, 8.4)	127.7 (d)
5	–	162.6 (s)	1″	3.49 (d, 6.6)	22.7 (t)
6	6.25 (d, 2.4)	100.5 (d)	2″	5.25 (t, 6.6)	122.5 (d)
7	–	163.7 (s)	3″	–	132.2 (s)
8	6.34 (d 2.4)	94.6 (d)	4″	1.68 (s)	25.8 (q)
8a	–	157.9 (s)	5″	1.78 (s)	17.8 (q)
1′	–	114.0 (s)	2′-OMe	3.71 (s)	61.2 (q)
2′	–	156.2 (s)	5-OH	12.47 (s)	–

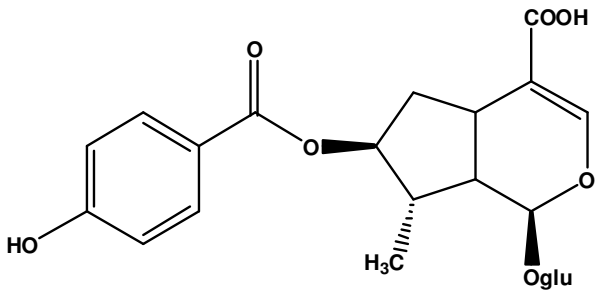
Recorded in $CDCl_3$ at 300 MHz (^{1}H NMR) or 75 MHz (^{13}C NMR). Chemical shifts and coupling constants are given in ppm and Hz, respectively



1 R_1 = OH, R_2 = OH, R_3 = H
2 R_1 = H, R_2 = OCH_3, R_3 = OH



3 R = H **4** R = β-OH



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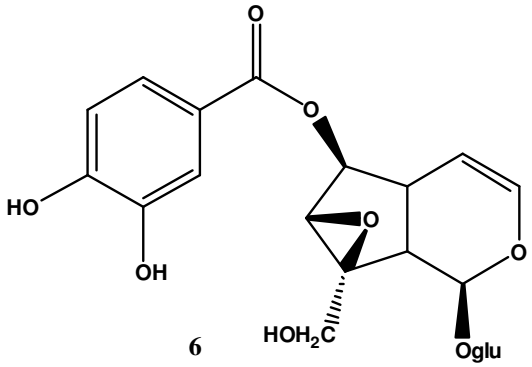

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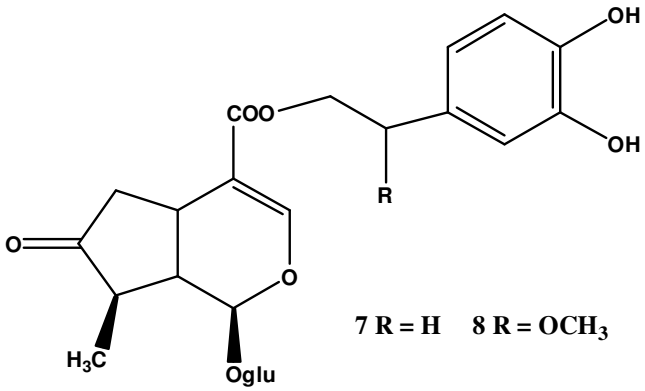
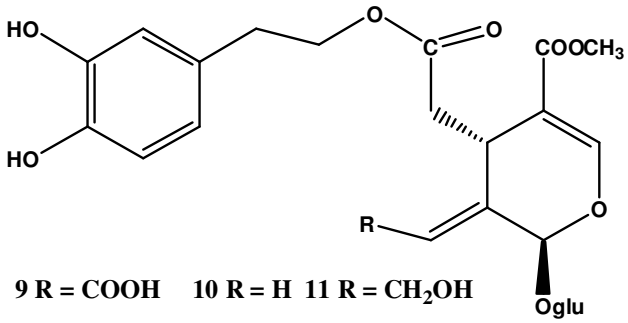


7 R = H **8** R = OCH_3



9 R = COOH **10** R = H **11** R = CH_2OH

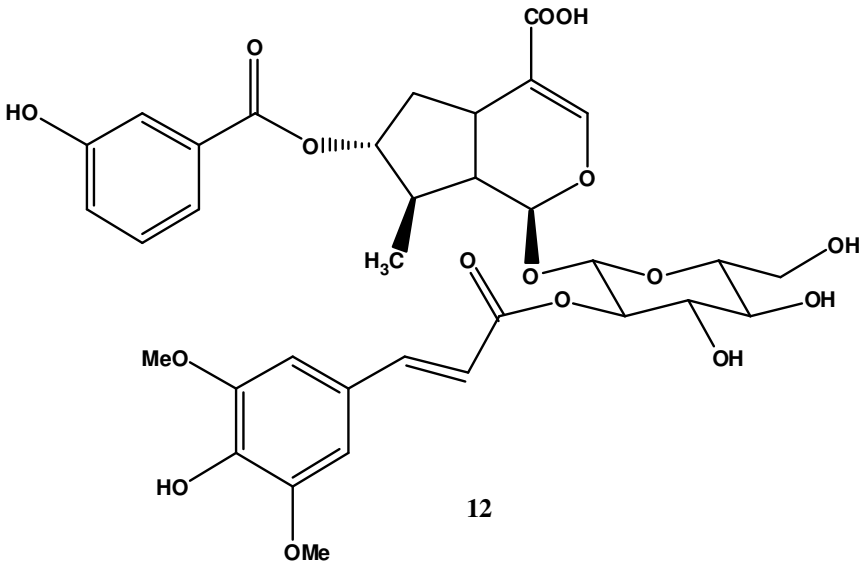


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Fig. 1. Chemical structures of compounds **1-12** isolated from the rhizomes of *Cyperus rotundus*

TABLE-2
MRB INHIBITORY ACTIVITY OF ALL THE ISOLATED COMPOUNDS FROM THE RHIZOMES OF *Cyperus rotundus*

Compound No.	IC_{50} (mean ± SD, μmol/L)	Compound No.	IC_{50} (mean ± SD, μmol/L)
1	903.27 ± 12.33	8	475.31 ± 3.72
2	864.08 ± 31.05	9	674.09 ± 31.91
3	277.16 ± 1.91	10	798.53 ± 14.47
4	218.55 ± 37.43	11	693.14 ± 17.35
5	391.04 ± 15.02	12	27.06 ± 1.33
6	413.28 ± 9.77	Rutin[a]	15.07 ± 2.51
7	316.91 ± 11.38	Dexamethasone[a]	355.14 ± 45.76

[a]control test reagents

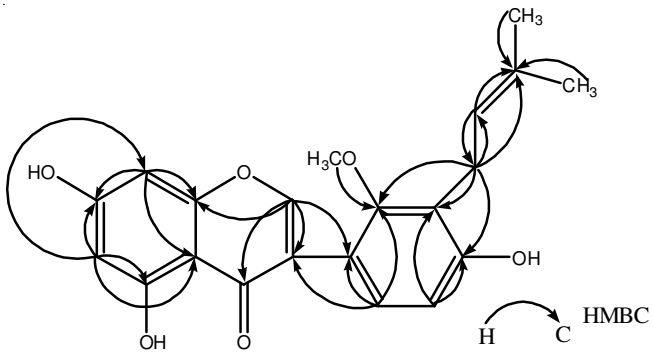

Fig. 2. Structure of **1** and its important H-C correlations in the HMBC spectrum

presence of the prenyl group. The C-1″ (δ 22.7) of the prenyl group was proven to be connected to C-3' (δ 119.0) by cross-peaks in the HMBC spectra from H-1″ to C-2' (δ 156.2), C-3' (δ 119.0) and C-4' (δ 156.6). In addition, the long range correlations from methoxyl protons (δ 3.71) to C-2' indicated that one methoxyl group was attached at C-2'. Full assignments of the ^{1}H and ^{13}C NMR signals were accomplished using 2D NMR experiments. Thus, compound **1** was identified as 5,7,4'-trihydroxy-2'-methoxy-3'-prenylisoflavone.

Macrophages respiratory burst (MRB) plays an important role in specific and nonspecific immune-inflammatory processes, which links to many inflammatory mediators such as cytokins, chemokins, nitric oxide synthase, phospholipases and free radical generation. And inhibition of MRB has been one of the well-documented methods for the evaluation of anti-inflammatory activity for various synthetic compounds and natural products. Many scientists have turned their interest to explore potential MRB inhibitors from traditional herbal plants. All the isolated compounds (**1-12**) were tested for their inhibitory activity against MRB with chemiluminescence detection. As shown in Table-2, compound **12** showed considerable activity with IC_{50} value of 27.06 ± 1.33 μM. While the other compounds showed inhibitory effect with higher IC_{50}.

Conclusion

A new compound, 5,7,4'-trihydroxy-2'-methoxy-3'-prenylisoflavone (**1**) was isolated from the rhizomes of *Cyperus rotundus*, along with eleven known components. The inhibitory activity of **1-12** on MRB was assayed. Compounds **3-12** were iridoid glycosides and compound **12** was found to inhibit sensitive MRB with the IC_{50} value of 27.06 ± 1.33 μM, while the others showed inhibitory effect with higher IC_{50}. Thus, this research suggested that the iridoid glycosides from the 95 % aqueous ethanol extract may be primary macrophages respiratory burst inhibitors.

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REFERENCES

1. O.A. Lawal and A.O. Oyedeji, *Molecules*, **14**, 2909 (2009).
2. Z.L. Zhou and Y.H. Liu, *Chin. J. Exp. Tradit. Med. Formulae*, **7**, 191 (2012).
3. N.A. Raut and N.J. Gaikwad, *Fitoterapia*, **77**, 585 (2006).
4. H.M. Sayed, M.H. Mohamed, S.F. Farag, G.A. Mohamed, O.R.M. Omobuwajo and P. Proksch, *Nat. Prod. Res.*, **22**, 1487 (2008).
5. S.J. Jeong, T. Miyamoto, M. Inagaki, Y.C. Kim and R. Higuchi, *J. Nat. Prod.*, **63**, 673 (2000).
6. M. Morimoto and K. Komai, *Weed Biol. Manage.*, **5**, 203 (2005).
7. Z.L. Zhou, W.Q. Yin, H.L. Zhang, Z.C. Feng and J.M. Xia, *Nat. Prod. Res.*, **27**, 1732 (2012).
8. Z.L. Zhou and C.Y. Fu, *Chem. Nat. Compd.*, **48**, 963 (2013).
9. Z.L. Zhou and W.Q. Yin, *Molecules*, **17**, 12636 (2012).
10. J. Qi, J.J. Chen, Z.H. Cheng, J.H. Zhou, B.Y. Yu and S.X. Qiu, *Phytochemistry*, **67**, 1372 (2006).
11. K.J. Cho, C.H. Yun, D.Y. Yoon, Y.S. Cho, G. Rimbach, L. Packer and A.S. Chung, *Toxicol. Appl. Pharmacol.*, **168**, 64 (2000).
12. F. Pasmans, P.D. Herdt, A.V. Nerom and F. Haesebrouck, *Dev. Comp. Immunol.*, **25**, 159 (2001).
13. M. Chadfield and J. Olsen, *Vet. Immunol. Immunopathol.*, **80**, 289 (2001).
14. W.W. Tao, J.A. Duan, N.Y. Yang, Y.P. Tang, M.Z. Liu and Y.F. Qian, *Fitoterapia*, **83**, 422 (2012).
15. C.A. Boros and F.R. Stermitz, *J. Nat. Prod.*, **53**, 1055 (1990).
16. A. Bianco, P. Passacantilli, G. Polidori, M. Nicolerti and R. Alves de Lima, *Gazz. Chim. Ital.*, **113**, 465 (1983).
17. M.F. Lahloub, M. Goda, M.S. Affifi and O. Sticher, *Planta Med.*, **55**, 627 (1989).
18. F.Ü. Afifi-Yazar and O. Sticher, *Helv. Chim. Acta*, **63**, 1905 (1980).
19. C. Scheele, E. Wollenweber and F.J. Arriaga-Giner, *J. Nat. Prod.*, **50**, 181 (1987).
20. C.A. Boros and F.R. Stermitz, *J. Nat. Prod.*, **54**, 1173 (1991).
21. H. Kuwajima, T. Uemura, K. Takaishi, K. Inoue and H. Inouyet, *Phytochemistry*, **27**, 1757 (1988).
22. K. Inoue, T. Nishioka, T. Tanahashi and H. Inouye, *Phytochemistry*, **21**, 2305 (1982).
23. Z.J. Huang, J.X. Yang, Z.G. She and Y.C. Lin, *Nat. Prod. Res.*, **26**, 11 (2012).
24. M.Q. Fu, S.X. Feng, N.L. Zhang, X.J. Zhou, R.M. Huang, H.J. Huang, Z.F. Xu, X. Li and S.X. Qiu, *Helv. Chim. Acta*, **95**, 598 (2012).