Secondary Metabolites from *Andrographis paniculata*

Wenkui Li,[a,b,c] Xudong Xu,[a,b,c] Hongjie Zhang,[a,b] Cuiying Ma,[a,b] Harry Fong,[a,b] Richard van Breemen,[a,b] and John Fitzloff*[a,b,c]

[a] *Department of Medicinal Chemistry and Pharmacognosy (m/c 781), University of Illinois at Chicago;* [b] *Program for Collaborative Research in the Pharmaceutical Sciences (PCRPS, m/c 877), University of Illinois at Chicago; and* [c] *Functional Food for Health (FFH) Core Analytical Laboratory, College of Pharmacy, University of Illinois at Chicago; 833 South Wood Street, Chicago, IL 60612, U.S.A.* Received September 4, 2006; accepted November 27, 2006

Two new flavonoid glycosides, 5-hydroxy-7,8-dimethoxy (2*R*)-flavanone-5-*O*-β-D-glucopyranoside (1) and 5-hydroxy-7,8,2′,5′-tetramethoxy-flavone-5-*O*-β-D-glucopyranoside (2), and a new diterpenoid, andrographic acid (3), along with andrographidine A (4) were isolated from *Andrographis paniculata*, and their structures were determined on the basis of physicochemical and spectroscopic analysis. Compound 3 was evaluated for cytotoxicity to KB cells along with andrographolide, isoandrographolide, neoandrographolide and 14-deoxy-11,12-didehydroandrographolide obtained from *A. paniculata* in the present study. Cytotoxicity was observed for andrographolide and isoandrographolide with ED_{50} values of 6.5 and 5.1 μg/ml, respectively.

Key words *Andrographis paniculata*; secondary metabolite; flavonoid; diterpenoid; LC-MS/MS; cytotoxicity

Andrographis paniculata Nees (Acanthaceae) is one of the most important medicinal plants and having been widely used in Chinese and Ayurvedic medicine for the treatment of gastric disorders, infectious diseases and common colds for many years. Pharmacological and clinical studies have demonstrated that *A. paniculata* possesses anti-inflammatory, antiallergenic, immuno-stimulatory, antiviral, antioxidant, hepatoprotective, cardiovascular activities, *etc.*, and these studies have been well-reviewed.[1,2] Diterpenoids and flavonoids are the main chemical constituents of *A. paniculata*, and these compounds are believed to be responsible for the biological activities of the plant.[1,2] More than 20 diterpenoids and over 10 flavonoids have been reported from this species in the past three decades.[1,2] Recently, several studies have been conducted to investigate the cytotoxic or antitumor activities of *A. paniculata* and/or its chemical constituents, especially andrographolide, which has been revealed to inhibit *in vitro* proliferation of different tumor cell lines.[3—5] In the present study, a sample of *A. paniculata* was investigated and found to contain three novel secondary metabolites. Bioassay revealed that both andrographolide and isoandrographolide possess cytotoxicity to KB cell line.

Results and Discussion

Compounds **1** and **4** were isolated as pale yellow powders with melting points of 123—125 and 168—169 °C, and $[\alpha]^{20}$ values of −70° (c=0.3 mg/ml, MeOH) and −103.6° (c=0.28 mg/ml, MeOH), respectively. In the high resolution electrospray ionization mass spectra obtained with a Micromass Q-TOF-2 mass spectrometer operated in positive ionization mode, **1** and **4** show protonated molecular ions at m/z 463.1642 and 463.1623 [$C_{23}H_{27}O_{10}$, theoretical 463.1604], respectively. The ^{1}H- and ^{13}C-NMR spectra (in DMSO-d_6) of **1** displayed the characteristic signals for a phenyl group [δ 7.33—7.43 (5H, m) and 127.2 (d), 129.3 (d) and 139.3 (s)], two methoxy groups [δ 3.57 (3H, s) and 3.77 (3H, s), and 61.8 (q), 55.8 (q)], a flavanone skeleton [δ 5.54 (1H, dd, J=11.9, 3.1 Hz), 2.70 (1H, dd, J=3.1, 16.3 Hz), 3.07 (1H, dd, J=11.9, 16.3 Hz), and 79.3 (d), 45.5 (t)], and a β-D-glucopyranosyl moiety, for which the signals due to the five *trans*-diaxial oxymethine protons (J=7.3 Hz) and one oxymethylene group were observed. The presence of the flavanone skeleton was further supported by the UV spectrum of **1** in methanol (235, 287, 332.5 nm).[6] In the ^{13}C-NMR spectrum of **1**, the characteristic chemical shift (δ 61.8) for a methoxy group with two substitutions at both neighboring carbons was observed.[7] The full assignment of the ^{1}H- and ^{13}C-NMR spectra of **1** employed a combination of 1D and 2D NMR techniques, including one-band proton–carbon chemical shift correlation (HMQC) and long-range heteronuclear multiple bond connectivity (HMBC). The starting points of the assignment were the unique ^{1}H resonance of the protons at C-6 position of the flavanone (δ 6.60, s), the anomeric position of the glucose (δ 4.75, d, J=7.3 Hz) and the methoxy groups (δ 3.77, 3.57, each s). The HMQC experiment provided the assignment for the signals attributed to C-6 (δ 96.5), OMe (δ 55.8), OMe (δ 61.8) and the anomeric carbon of the glucose (δ 103.1). In the contour of the HMBC experiments, correlation's were observed between H-6 (δ 6.60) and C-5 (δ 155.7), C-7 (δ 159.1), C-8 (δ 132.6), and C-10 (δ 107.5). The protons of the methoxy groups at δ 3.77 and 3.57 show correlation with the carbons at δ 159.1 (C-7) and 132.6 (C-8), respectively. The anomeric proton of the glucose (δ 4.75) gives correlation with C-5 (δ 155.7). The above information was combined showing the identity of **1** to be 5-hydroxy-7,8-dimethoxyflavanone-5-*O*-β-D-glucopyranoside (Fig. 1). The IR, UV, HR-MS (see above) and NMR (in MeOH-d_4) properties of compound **4** were highly similar to those of **1** (Table 1). As can be seen in Table 1, compound **4** also possesses the spectroscopic properties of 5-hydroxy-7,8-dimethoxyflavanone-5-*O*-β-D-glucopyranoside. In order to confirm the presence of these two compounds, in the raw material used, a LC-MS/MS experiment was conducted to compare the response ratios of three ion transitions by multiple reaction monitoring (MRM). Both compounds (in 50% methanol) were infused to a Micromass Quattro II mass spectrometer with CID (collision-induced-dissociation) in the negative ionization mode. The electrospray and CID parameters were optimized. The three most abundant fragment ions (m/z 461→284, m/z 461→299, and m/z 461→269) were selected for the multiple reaction monitoring (MRM) in LC-MS/MS. A representative LC-MS/MS chromatogram of the

* To whom correspondence should be addressed. e-mail: fitzloff@uic.edu

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of Compounds **1**, **2** and **4** (δ_{ppm})

Position	δ_H			δ_C		
	1 (DMSO-d_6)	**4** (in MeOH-d_4)	**2** (in MeOH-d_4)	**1** (DMSO-d_6)	**4** (in MeOH-d_4)	**2** (in MeOH-d_4)
2	5.54 (dd, J=11.9, 3.1 Hz)	5.51 (dd, J=12.2, 2.7 Hz)	—	79.3	79.3	161.0
3	3.07 (dd, J=11.9, 16.3 Hz)	3.10 (dd, J=12.2, 16.4 Hz)	7.07 (s)	45.5	45.5	113.4
	2.70 (dd, J=16.3, 3.1 Hz)	2.79 (dd, J=16.4, 2.7 Hz)				
4	—	—	—	192.1	192.1	180.9
5	—	—	—	155.7	155.7	155.1
6	6.60 (s)	6.72 (s)	7.16 (s)	96.5	96.5	102.1
7	—	—	—	159.1	159.1	158.7
8	—	—	—	132.6	132.6	133.6
9	—	—	—	155.7	155.7	153.2
10	—	—	—	107.5	107.5	111.8
1′	—	—	—	139.8	139.8	121.2
2′	7.43 (m)	7.50 (m)	—	127.2	127.2	155.1
3′	7.36 (m)	7.38 (m)	7.14 (d, br s, J=8.7 Hz)	129.3	128.5	119.6
4′	7.33 (m)	7.33 (m)	7.11 (dd, J=8.7, 2.7 Hz)	129.3	128.3	118.8
5′	7.36 (m)	7.38 (m)	—	129.3	128.5	154.2
6′	7.43 (m)	7.50 (m)	7.56 (d, J=2.7 Hz)	127.2	127.2	114.7
7-OMe	3.77 (s)	3.88 (s)	3.99 (s)	55.8	55.8	57.1
8-OMe	3.57 (s)	3.71 (s)	3.93 (s)	61.8	61.2	61.9
2′-OMe			3.93 (s)			56.7
5′-OMe			3.84 (s)			56.2
Glc-1	4.75 (d, J=7.3 Hz)	4.80 (d, J=7.3 Hz)	4.84 (d, J=7.8 Hz)	103.1	103.1	105.7
				74.2	74.2	74.8
				77.2	77.2	77.5
				70.9	70.9	71.7
				78.4	78.4	78.9
				61.8	61.8	62.9

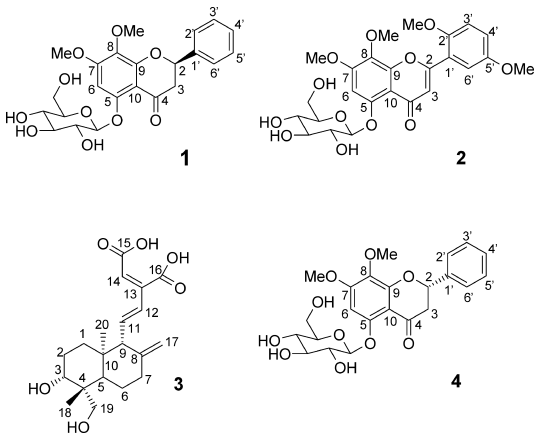

Fig. 1. Chemical Structures of Compounds **1**—**4**

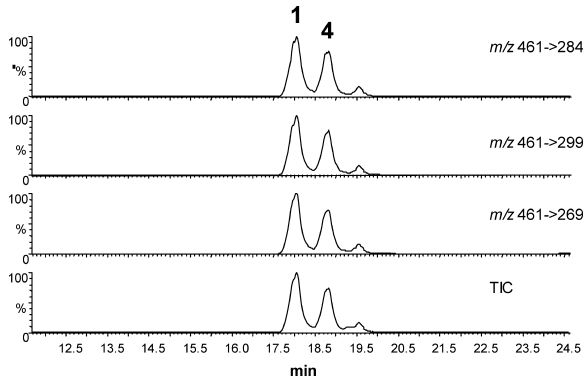

Fig. 2. Representative LC-MS/MS Chromatogram of Compounds **1** and **4** in the Methanolic Extract of Raw Material

methanolic extract of *A. paniculata* raw material is shown in Fig. 2. As can be seen in Fig. 2, both **1** and **4** were detected from the extract, with the relative signal abundance ratios of the three precursor/product ion pairs (*m*/*z* 461→284: *m*/*z* 461→299: *m*/*z* 461→269) estimated as 1 : 0.65 : 0.44 for **4**, which is identical to that of **1** (1 : 0.65 : 0.44). Although the NMR and MS/MS data of **1** and **4** were virtually identical, their retention times (t_R) on the analytical RP-HPLC column was significantly different with **1** and **4** observed at 18.1 and 19.04 min, respectively. This is primarily due to the difference in the stereochemistry at the C-2 position. Based on the melting points, **1** and **4** were tentatively identified to be 5-hydroxy-7,8-dimethoxyflavanone (2*R*)-5-*O*-β-D-glucopyranoside and andrographidine A,[7] respectively. It must be indicated that our assignments of ^{13}C-NMR spectral data of **4** are largely in agreement with that previously reported.[7] However, the previously reported ^{13}C-NMR signals for C-5 and C-7 need to be reversed.[7]

Compound **2**, a pale-yellow powder, mp 125—127 °C, gave a protonated molecular ion at *m*/*z* 521.1703 [$C_{25}H_{29}O_{12}$, theoretical 521.1659] in the high-resolution positive ion electrospray mass spectrum. The molecular formula, $C_{25}H_{28}O_{12}$, of compound **2** was supported by the analysis of its ^{1}H- and ^{13}C-NMR spectra, which showed the presence of four methoxy groups and a glucopyranosyl moiety with a 5-oxygenated flavone skeleton.[7] Of the four methoxy groups, one was substituted at both neighboring carbons (δ 61.9),[7] which showed correlation with the protons at δ 3.93 (3H, s) in the HMQC experiment. Other methoxy carbon signals appeared at δ 57.1, 56.7 and 56.2, respectively, in the ^{13}C-NMR spectrum, and they showed correlation with the protons at δ 3.99

(3H, s), 3.93 (3H, s) and 3.84 (3H, s), respectively, in the HMQC experiment. Two aromatic proton singlets were observed at δ 7.07 and 7.16, respectively, and they were correlated with the carbons at C-3 (δ 113.4) and C-6 (δ 102.1) in the HMQC spectrum, respectively. While a signal at δ 180.9 was assigned to the C-4 flavone ketone,[5] an ABX spin system of δ 7.14 (1H, d, br s, J=8.7 Hz), 7.11 (1H, d, d, J=8.7, 2.7 Hz) and 7.56 (1H, d, J=2.7 Hz) was attributed to H-3′, 4′ and 6′ in ring B of the flavone, and these protons correlated with the carbon signals at δ 119.6 (C-3′), 118.8 (C-4′) and 114.7 (C-6′), respectively, in the HMQC experiment. Signals observed for five *trans*-diaxial oxymethine protons (J= 7.8 Hz) and one oxymethylene group indicated the presence of a β-D-glucopyranosyl moiety for **2**. In the HMBC spectrum, the correlation between H-3′ (δ 7.14, d, br s, J=8.7 Hz) and the carbons at C-1′ (δ 121.2), H-4′ (δ 7.11 (1H, dd, J=8.7, 2.7 Hz)) with the carbons at C-2′ (δ 155.1), C-5′ (δ 154.2), H-6′ with C-4′ (δ 118.0), C-5′ (δ 154.2) and C-1′ (δ 121.2) were observed. The assignment of carbon signals at 57.1, 61.9, 56.7 and 56.2 to the carbons of methoxy groups at C-7, 8, 2′ and 5′, respectively, was based on the presence of the correlations between the proton signals of these methoxy groups with the carbons at C-7 (δ 158.7), 8 (δ 133.6), 2′ (δ 155.1) and 5′ (δ 154.2), respectively. The glucopyranosyl residue was located at the 5-*O*-position of **2** by HMBC correlation between C-5 (δ 155.1) and the anomeric proton (δ 4.84). Thus, compound **2** was identified to be 5-hydroxy-7,8,2′,5′-tetramethoxyflavone-5-*O*-β-D-glucopyranoside (Fig. 1).

Compound **3** was purified as an amorphous powder, mp >300 °C. Its high resolution negative ion elestrospray mass spectrum showed the presence of the deprotonated molecular ion $[M-H]^-$ at *m/z* 363.1813, corresponding to a molecular formula of $C_{20}H_{28}O_6$ (theoretical $M-H^-$, *m/z* 363.1808). In the IR spectrum, a broad absorption band of ν 3200—2500 cm^{-1} was observed for the compound, suggesting the presence of one or more carboxyl groups in the molecule, which was supported by the presence of the ^{13}C signals (Table 2) at δ 168.0 and 168.3 in the ^{13}C-NMR spectrum. The ^{13}C-NMR (Table 2) and DEPT spectra of **3** showed signals due to 20 carbons comprising two methyl, four methylene, two methine, three olefinic methine, an exocyclic methylene, one oxymethylene, one oxymethine, two quaternary carbons, two carboxyl carbons and two fully substituted olefinic carbons. These fragments further confirmed the molecular formula ($C_{20}H_{28}O_6$) of the compound. The ^1H- and ^{13}C-NMR (in DMSO-d_6) spectra of **3** suggested a diterpenoid compound with a structure similar to that of 14-deoxy-11,12-didehydroxy-andrographolide from *A. paniculata*,[8] except that there are two carboxyl carbons in **3**, instead of one carboxyl carbon (C-16) as in 14-deoxy-11,12-didehydroxyandrographolide.[8] In the ^1H-NMR spectrum of **3**, two methyl singlets were observed at δ 0.72 and 1.06, respectively, and they are all connected to quaternary carbons.[8] The characteristic exocyclic methylene protons for *Andrographis* diterpenoids were observed at δ 4.69 (br s) and 4.41 (br s), respectively, and they showed correlation's with the carbons at C-9 (δ 61.1) and C-7 (δ 37.1) in the HMBC experiment.[5] The COSY experiments clearly showed the relationships between the neighboring protons of H-9, 11 and 12. The H-9 signal was present at δ 2.30 (d, J=8.7 Hz), which correlated to the carbon signal at δ 61.1 and its β-position was deduced from comparison with similar diterpenoids reported from *Andrographis*.[5] The long-range correlations of H-14 (δ 6.08, br s) with a carboxyl carbon at δ 168.3 and H-12 (δ 6.25, d, J=15.5 Hz) with another carboxyl carbon at δ 168.0 were observed in the HMBC experiments. In the negative ESI mass spectrum of **3**, two major fragments were present at *m/z* 319 and 275, respectively, due to the consecutive neutral loss of carbon dioxide from the deprotonated molecule (figure not shown). The combined results indicated that the structure of andrographic acid is **3** (Fig. 1).

Table 2. ^1H- and ^{13}C-NMR Chemical Shifts of Compound **3** (δ_{ppm})

Position	δ_H	δ_C	HMBC
1	1.14 (m)	38.8 t	
2	1.93 (m)	28.5 t	
3	3.19 (m)	79.5 d	2, 4, 18, 19
4		43.1 s	
5	1.14 (m)	54.6 d	6, 7, 10, 20
6	1.33 (m), 1.66 (m)	24.0 t	
7	1.94 (m)	37.1 t	5, 8, 17
8		149.9 s	
9	2.30 (d, J=8.7 Hz)	61.1 d	4, 5, 8, 11, 12, 17, 19
10		39.2 s	
11	6.13 (dd, J=15.5, 9.6 Hz)	133.1 d	12, 13
12	6.25 (d, J=15.5 Hz)	133.3 d	11, 13, 14, COOH (δ 168.0)
13		144.7 s	
14	6.08 (br s)	128.4 d	13, COOH (δ 168.3)
15		168.3 s	
16		168.0 s	
17	4.69 (br s), 4.41 (br s)	108.9 t	7, 8, 9
18	1.06 (s)	23.8 q	3, 4, 5, 8, 9, 19, 23
19	3.83 (d, J=10.9 Hz), 3.24 (d, J=10.9 Hz)	63.5 t	3, 4, 18
20	0.72 (s)	16.3 q	5, 9, 10

The bioassay results showed that andrographolide and isoandrographolide possess cytotoxicity to the KB cell line with ED_{50} values of 6.5 and 5.1 μg/ml, respectively. In contrast, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide and andrographic acid did not show significant cytotoxicity with both ED_{50} values over 20 μg/ml.

Experimental

General Experimental Procedures Melting points were recorded with a Fisher–Johns melting apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were recorded at room temperature on an ATI Mattson Genesis FT-IR spectrophotometer. NMR spectral data were recorded on Bruker DPX-360 and DRX-500 MHz spectrometers with tetramethylsilane (TMS) as internal standard. High resolution electrospray exact mass measurements were conducted on a Micromass (Manchester, U.K.) QTOF-2 mass spectrometer. LC-MS/MS experiments were carried out using a Micromass (Manchester, U.K.) Quattro II triple quadrupole mass spectrometer equipped with a Waters (Milford, MA, U.S.A.) Alliance 2690 HPLC system and an electrospray ionization source.

Materials *A. paniculata* product was provided by Pharmavite (Mission Hills, CA, U.S.A.). A voucher specimen of the sample was deposited at the Functional Food for Health Core Analytical Laboratory, University of Illinois at Chicago.

Extraction and Isolation The *A. paniculata* samples (85 g) were extracted with methanol (3×250 ml). The resulting extracts were combined, evaporated under the reduced pressure at 40—45 °C, and the residue (25 g) was partitioned with hexane, chloroform and ethyl acetate, successively. The chloroform and ethyl acetate extracts, showing similar HPLC profiles under the HPLC condition as illustrated below, were combined (5.0 g) and run on an open silica gel column (4×100 cm) eluted with chloroform and methanol

in gradient to yielded 14 fractions (A—N). The fractions I—N (280 mg) were combined and run on a Supelco RP-18 column (2.1 cm×25 cm, 5 μm) eluted with 35% acetonitrile at 8 ml/min to give compound **1** (3.6 mg), **2** (2.5 mg), and **4** (11.0 mg), respectively. The water soluble fraction (10 g) was subjected to Diaion resin chromatography eluted with water and methanol, respectively. The obtained methanol fraction (0.5 g) was run on silica gel (2×100 cm) eluted with chloroform–methanol–water in gradient to give 28 subfractions. Subfraction 16 was subjected to Sephadex LH-20 chromatography eluted with methanol to yield three subfractions (I—III) and subfraction III was further subjected to preparative HPLC on Supelco RP-18 column (2.1 cm×25 cm, 5 μm) as above with the mobile phase of 15% acetonitrile in water to give compound **3** (3.6 mg).

5-Hydroxy-7,8-dimethoxyflavanone (2*R*)-5-*O*-β-D-glucopyranoside (**1**): Pale yellow powder; mp 123—125 °C; $[\alpha]^{20}$ −70° (c=0.3 mg/ml, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400, 1675, 1602, 1594, 1205, 1145, 1075; UV λ_{max} (MeOH): 210.5, 235.5, 240, 287, 333, 332.5. ^{1}H- and ^{13}C-NMR as shown in Table 1.

5-Hydroxy-7,8,2′,5′-tetramethoxyflavone-5-*O*-β-D-glucopyranoside (**2**): Pale-yellow powder; mp 125—127 °C; IR ν_{max} (KBr) cm^{-1}: 3420, 1635, 1580, 1470, 1340, 1035; UV λ_{max} (MeOH): 214.5, 264.5, 352. ^{1}H- and ^{13}C-NMR as shown in Table 1.

Andrographic Acid (**3**): Amorphous powder; mp >300 °C; IR ν_{max} (KBr) cm^{-1}: 3200—2500, 1735, 985. ^{1}H- and ^{13}C-NMR as shown in Table 2.

Andrographidine A (**4**): Pale yellow powder; mp 168—169 °C; $[\alpha]^{20}$ −103.6° (c=0.28 mg/ml, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400, 1672, 1602, 1595, 1205, 1145, 1075; UV λ_{max} (MeOH): 235.5, 286, 320.5. ^{1}H- and ^{13}C-NMR as shown in Table 1.

LC-MS/MS Analysis LC-MS/MS experiments were carried out using a Micromass (Manchester, U.K.) Quattro II triple quadrupole mass spectrometer equipped with a Waters (Milford, MA, U.S.A.) Alliance 2690 HPLC system and an electrospray ionization source. A Supelco Discovery C-18 column (4.6×250 mm, 5 μm) was used for HPLC separations with a mobile phase consisting of a 40-min linear gradient from 20—50% aqueous acetonitrile at a flow rate of 1 ml/min. The injection volume was 10 μl. The column effluent was split so that approximately 25% of the column effluent was introduced into the electrospray ionization source. The electrospray ionization source was operated at 140 °C in the negative ion mode. Nitrogen was used as both nebulizing and drying gas at a flow rate of 20 l/h and 450 l/h, respectively. Following the data obtained by infusing the reference standard solutions prepared with aqueous methanol (50%) at a flow rate of 5—20 μl/min, LC-MS/MS multiple reaction monitoring (MRM) was performed. After the deprotonated molecule ions of the compounds of interest were selected by using the first quadrupole (Q1), collision-induced dissociation (CID) was carried out with argon as the collision gas at 1.0×10^{-3} mbar in the collision cell (Q2), and then specific product ions were selected using the third quadrupole (Q3). During these multiple reaction monitoring (MRM) measurements, the dwell time was 0.2 s for each of the precursor/product ion pairs. Both the first (Q1) and last quadrupole (Q3) were operated at unit mass resolution.

Cytotoxicity Bioassay Human cancer cell line, the oral epidermoid carcinoma KB, was used to screen the cytotoxicity of the compounds of interest based on an established protocol.[9]

Acknowledgements This research was partially supported by the University of Illinois Functional Foods for Health Program.

References

1) Tang W., Eisenbrand G., "Chinese Drugs of Plant Origin, Chemistry, Pharmacology and Use in Traditional and Modern Medicine," Springer Verlag, Berlin, 1992, pp. 97—103.
2) Saxena S., Jain D. C., Bhakuni R. S., Sharma R. P., *Indian Drugs*, **35**, 458—467 (1998).
3) Kumar R. A., Sridevi K., Kumar N. V., Nanduri S., Rajagopol S., *J. Ethnopharmacol.*, **92**, 291—295 (2004).
4) Rajagopal S., Kumar R. A., Deevi D. S., Satyanarayana C., Rajagopolan R., *J. Exp. Ther. Oncol.*, **3**, 147—158 (2003).
5) Cheung H. Y., Cheung S. H., Li J., Cheung C. S., Lai W. P., Fong W. F., Leung F. M., *Planta Med.*, **71**, 106—111 (2005).
6) Mabry T. J., Markham K. R., Thomas M. B., "The Systematic Identification of Flavonoids," Springer, New York, 1970, pp. 261—273.
7) Kuroyanagi M., Sato M., Ueno A., Nishi K., *Chem. Pharm. Bull.*, **35**, 4429—4435 (1987).
8) Matsuda T., Kuroyanagi M., Sugiyama S., Umehara K., Ueno A., Nishi K., *Chem. Pharm. Bull.*, **42**, 1216—1225 (1994).
9) Likhitwitayawauid K., Angerhofer C. K., Cordell G. A., Pezzuto J. M., *J. Nat. Prod.*, **56**, 30—38 (1993).