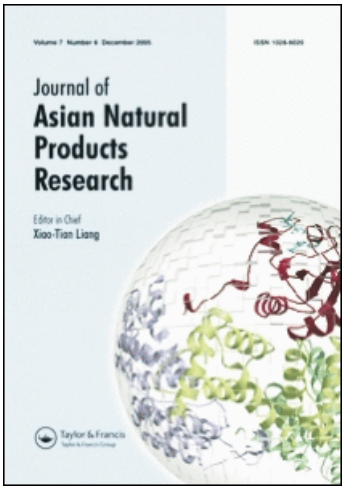

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new glycosides from *Breynia vitis-idaea*

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Online publication date: 15 June 2010

To cite this Article Meng, Da-Hai , Wu, Jian , Wang, Luo-Yi and Zhao, Wei-Min(2010) 'Two new glycosides from *Breynia vitis-idaea*', Journal of Asian Natural Products Research, 12: 6, 535 — 541
To link to this Article: DOI: 10.1080/10286021003745452
URL: http://dx.doi.org/10.1080/10286021003745452

Journal of Asian Natural Products Research
Vol. 12, No. 6, June 2010, 535–541

ORIGINAL ARTICLE

Two new glycosides from *Breynia vitis-idaea*

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(*Received 12 January 2010; final version received 3 March 2010*)

A new sulfur-containing spiroketal glycoside, breynin I (**1**), and a new terpenic glycoside, breyniaionoside E (**2**), together with 10 known compounds, were isolated from the aerial parts of *Breynia vitis-idaea* (Euphorbiaceae), a traditional Chinese medicine used for the treatment of chronic bronchitis and wounds. Their structures were elucidated on the basis of spectroscopic analysis and modified Mosher's method.

Keywords: *Breynia vitis-idaea*; Euphorbiaceae; sulfur-containing sesquiterpene glycoside; terpenic glycoside

1. Introduction

The genus *Breynia* (Euphorbiaceae) comprises 25 species of plants, among them, seven species are found in China. *B. vitis-idaea* C.E.C. Fisch. is distributed abundantly in South China and has been used as a traditional Chinese folk medicine for the treatment of chronic bronchitis and wounds. Previous phytochemical studies on the *Breynia* plants led to the identification of sulfur-containing spiroketal glycosides [1–4], alkaloids [5,6], terpenic, and phenolic glycosides [7], along with several other components [8,9]. Among those identified components, breynins A and B, the two sulfur-containing spiroketal glycosides exhibited significant oral hypocholesterolemic activity [1–4]. In our previous investigation, 10 sulfur-containing spiroketal glycosides were isolated from the aerial parts of *B. fruticosa* [10]. As part of our ongoing investigation on the discovery of new natural bioactive components from terrestrial plants, the aerial parts of *B. vitis-idaea* were studied systematically. This paper describes the isolation and structural elucidation of a new sulfur-containing spiroketal glycoside, breynin I (**1**), and a new terpenic glycoside, breyniaionoside E (**2**) (Figure 1) together with 10 known compounds. The absolute configuration of the aglycone of breyniaionoside E (**2**) was established by the modified Mosher's method.

2. Results and discussion

Compound **1** was isolated as an amorphous powder with the molecular formula of $C_{34}H_{46}O_{20}S$ deduced from its HR-ESI-MS and NMR spectral data. The ^{13}C NMR spectrum of **1** showed the presence of two sugar units (anomeric carbons at δ_C 104.2 and 107.1) and 22 carbons for the aglycone moiety, which were separated by the DEPT experiment into 1 methyl, 4 methylenes (one for oxygenated methylene), 11 methines (four oxygenated methines and four sp^2 methines), and 6

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286021003745452
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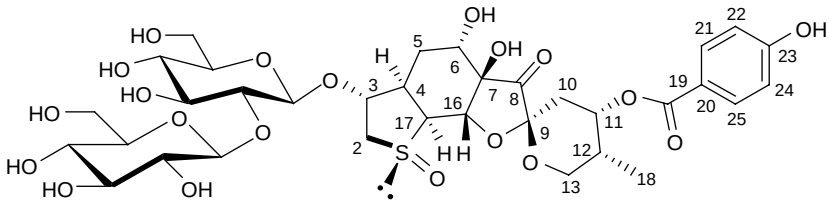

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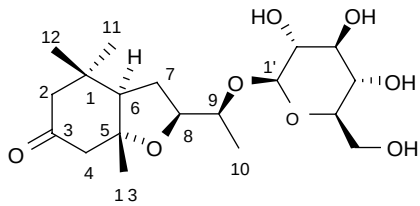

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Figure 1. Structures of compounds **1** and **2**.

quaternary carbons (one for ketal, one for keto, one for oxygenated quaternary carbon, one for carboxylate carbon, and two for sp^2 carbons). Analyses of its HSQC and $^1H-^1H$ COSY spectra led to the deduction of the fragments of C-2—C-3—C-4—(C-17—C-16)—C-5—C-6, C-10—C-11—C-12—(C-18)—C-13 and C-21—C—22. The planar structure of the aglycone of **1** was further established on the basis of its HMBC spectrum, in which $^1H-^{13}C$ long-range correlations were observed between H-2 and C-4/C-17; H-3 and C-17; H-5 and C-3; H-6 and C-4; H-10 and C-8/C-12; H-11 and C-13/C-19/C-18; H-13 and C-9/C-11/C-18; H-16 and C-6; H-17 and C-5/C-7; H-21/H-25 and C-19/C-23; and H-22/H-24 and C-20 (Figure 2). All of the above evidences indicated that compound **1** had the same aglycone as that of the known compound breynin B. As for its sugar moiety, two anomeric protons appeared at δ_H 4.12 (1H, d, $J = 7.4$ Hz) and 4.57 (1H, d, $J = 7.6$ Hz) in its ^1H NMR spectrum. Analyses of its $^1H-^1H$ COSY, HSQC, and TOCSY spectra enabled the assignments of all protons and carbons of the two sugar units (Table 1). From the characteristic NMR spectral data and NOE signals, the two sugar units were identified as β-glucopyranose [11]. The linkage sites of the two sugar units were derived from the HMBC experiment, in which $^{13}C-^1H$ long-range correlation signals were observed at H-$1_{glc\text{-}I}$/C-3 and H-$1_{glc\text{-}II}$/C-$2_{glc\text{-}I}$. Therefore, the structure of **1** was elucidated to be α-sulfinyl-breynogenin 3-*O*-β-glucopyranosyl-(1 → 2)-β-glucopyranoside. To the best of our knowledge, compound **1** is a new compound and has been assigned the trivial name breynin I.

Compound **2** was obtained as a colorless gum with the molecular formula of $C_{19}H_{32}O_8$, which was deduced from HR-ESI-MS and ^{13}C NMR spectral data. The ^1H NMR spectrum of **2** showed signals assignable to three singlet methyls (δ_H 0.90, 0.96, 1.23), one doublet methyl (δ_H 1.08, d, $J = 6.3$ Hz), and one anomeric proton signal (δ_H 4.38, 1H, d, $J = 8.2$ Hz). Enzymatic hydrolysis of **2** yielded glucose as its sugar component. The ^{13}C NMR spectrum of **2** indicated 19 carbon signals separated by the DEPT experiment into four methyls, four methylenes (including one oxygenated methylene), eight methines (including one anomeric carbon and

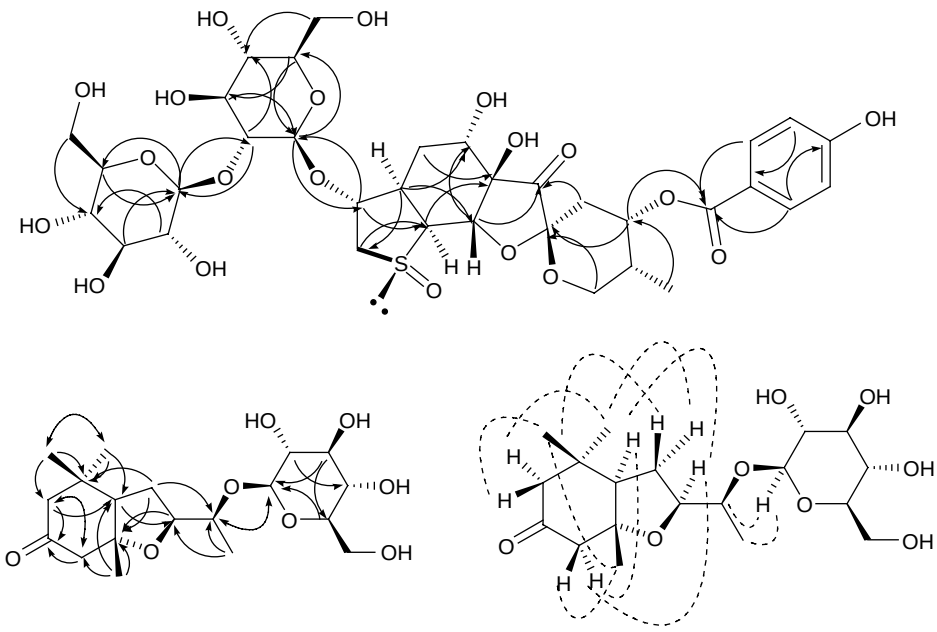

Figure 2. Key HMBC correlations ($^1H \rightarrow {}^{13}C$) of compounds **1** and **2** and main NOE correlations ($^1H-{}^1H$) of compound **2**.

six oxygenated methines), and three quaternary carbons (including one oxygenated quaternary carbon and one keto carbon). Apart from the sugar moiety, the structure of the aglycone of **2** with 13 carbons was deduced to be an ionone derivative similar to those of officinosides A and B, which were isolated from

Table 1. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** (CD_3OD, δ in ppm, J in Hz).

No.	^1H NMR	^{13}C NMR	No.	^1H NMR	^{13}C NMR
2	3.73 (m) (α); 3.34 (m) (β)	57.0 (t)	22	6.94 (d, 8.8)	117.1 (d)
3	4.56 (m)	88.8 (d)	23		163.9 (s)
4	3.13 (m)	39.1 (d)	24	6.94 (d, 8.8)	117.1 (d)
5	1.21 (m) (α); 1.70 (m) (β)	29.6 (t)	25	8.02 (d, 8.8)	133.8 (d)
6	3.92 (m)	70.8 (d)	*glc-I*		
7		75.6 (s)	1	4.57 (d, 7.6)	104.2 (d)
8		212.9 (s)	2	3.36 (m)	85.7 (d)
9		101.2 (s)	3	3.54 (m)	78.5 (d)
10	2.05 (m)	32.9 (t)	4	3.47 (m)	71.1 (d)
11	5.43 (m)	70.4 (d)	5	3.20 (m)	78.2 (d)
12	2.18 (m)	34.8 (d)	6	3.72 (m); 3.85 (dd, 12.0, 1.9)	62.9 (t)
13	3.66 (m) (a); 4.01 (m) (b)	64.6 (t)	*glc-II*		
16	4.90 (br s)	75.4 (d)	1	4.12 (d, 7.4)	107.1 (d)
17	3.99 (m)	72.1 (d)	2	3.18 (m)	76.8 (d)
18	0.92 (d, 6.9)	13.3 (q)	3	3.30 (m)	78.4 (d)
19		168.1 (s)	4	3.29 (m)	70.9 (d)
20		123.2 (s)	5	2.42 (dt, 9.2, 2.6)	77.8 (d)
21	8.02 (d, 8.8)	133.8 (d)	6	3.51 (m); 3.58 (m)	62.1 (t)

Calendula officinalis [12]. However, a noticeable difference was that the signals from two sp^2 carbons were absent and a carbonyl carbon was detected. A structural fragment —CH—CH_2—CH(O)—CH(O)—CH_3 could be deduced from its $^1H-^1H$ COSY and HSQC spectra. Further analysis of the HMBC spectrum enabled deduction of its planar structure (Figure 2). The $^{13}C-^1H$ long-range correlations at H-1′ (δ_H 4.38)/C-9 (δ_C 76.3) and H-9 (δ_H 3.73)/C-1′ (δ_C 103.4) in the HMBC spectrum, along with the NOE signal between H-9 and H-1′, located the sugar moiety at C-9 of the aglycone. The relative configuration of **2** was further established on the basis of the ROESY experiment (Figure 2). In order to clarify the absolute configuration of the aglycone, compound **2** was hydrolyzed by cellulase to afford **2a**. Mosher esters of **2a** were prepared by the usual method, and the absolute configuration of C-9 was established to be *S* (Figure 3). Thus, the structure of compound **2** was elucidated as (5*R*,6*R*,8*S*,9*S*)-5,8-epoxymegastigmane-9-ol-3-one 9-*O*-β-glucopyranoside (breyniaionoside E).

The 10 known compounds isolated from *B. vitis-idaea* were identified as benzyl 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside [13], (−)-5′-β-D-glucopyranosyl-oxyjasmonic acid [14], burselignan [15], aviculin [16], pinoresinol [17], (+)-syringaresinol [18], 9,9′-hydroxy-3,4-methylenedioxy-3′-methoxy[7-*O*-4′,8-5′]-neolignan [19], di-*O*-methylcrenatin [20], 2(*S*)-4′,5-dihydroxy-6,7-dimethoxyflavanon [21], and pentadecan-1-ol [22] by comparing their spectroscopic data with reported values.

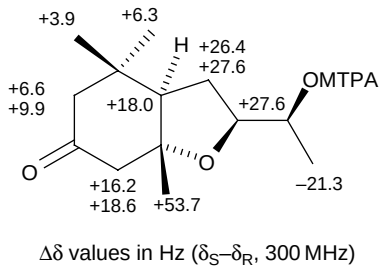

Figure 3. Values of $\Delta\delta$ ($\delta_S - \delta_R$) of the MTPA esters of **2a**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. EI-MS data were recorded on a MAT-95 mass spectrometer. ESI-MS were measured using a Finnigan LCQ-Deca instrument and HR-ESI-MS data were obtained on a Mariner mass spectrometer. The NMR experiments were run on a Bruker AM-400 spectrometer with TMS as an internal standard. Preparative HPLC was carried out using a Varian SD-1 instrument equipped with a Merck NW25 C_{18} column (20 mm × 250 mm, 10 μm; Merck Corporation, Darmstadt, Germany) and a ProStar 320 UV/Vis detector. Column chromatographic separations were carried out using Si gel H60 (300–400 mesh; Yantai Chemical Industrial Institute, Yantai, China), MCI gel CHP-20P (Mitsubishi Chemical, Tokyo, Japan), and HW-40 (TOSOH Corporation, Tokyo, Japan). HSGF254 Si gel TLC plates (Yantai Chemical Industrial Institute) and RP-18 WF_{254} TLC plates (Merck Corporation) were used for analytical TLC.

3.2 Plant material

The aerial parts of *B. vitis-idaea* were collected in the suburb of Nanning, Guangxi Province, China, in June 2005 and identified by Prof. Ding Fang of Guangxi Institute of Traditional Chinese Medicine. A voucher specimen (No. SIMM050612) has been deposited in the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3 Extraction and isolation

The aerial parts of *B. vitis-idaea* (1.0 kg) were powdered and extracted with 95%

EtOH four times at reflux. The extract was concentrated to dryness *in vacuo* and the aqueous residue was dissolved in methanol and subjected to chromatography over MCI with MeOH–H_2O (v/v, 0:1–1:0) as the eluant to afford Fr. 1–6. Fr. 1 was subjected to Si gel H60 chromatography with chloroform and methanol as the eluant (v/v, 10:1; 7:1; 4:1) to give Fr. 1a–1e. Repeated separation over preparative HPLC with gradient MeOH–H_2O as the eluant yielded burselignan (8 mg) and (−)-5′-β-D-glucopyranosyl-oxyjasmonic acid (14 mg) from Fr. 1a; compound **2** (12 mg) from Fr. 1b; di-*O*-methylcrenatin (11 mg) from Fr. 1c; and benzyl 6-*O*-β-D-apiofuranosyl-β-D-glucopyranoside (54 mg) from Fr. 1d. Compound **1** (13 mg) was purified from Fr. 1e by preparative HPLC eluted with gradient MeOH–H_2O, and Sephadex LH-20 column chromatography eluted with 95% ethanol, successively. Fr. 2 was subjected to preparative HPLC eluted with gradient MeOH–H_2O to yield aviculin (15 mg), 2(*S*)-4′,5-dihydroxy-6,7-dimethoxyflavanon (7 mg), and 9,9′-hydroxy-3,4-methylenedioxy-3′-methoxy[7-*O*-4′,8-5′]neolignan (24 mg). Fr. 4 was subjected to preparative HPLC eluted with gradient MeOH–H_2O and preparative TLC over SiO_2 to yield pinoresinol (21 mg) and (+)-syringaresinol (16 mg). Fr. 6 was separated over Si gel H60 column to give pentadecan-1-ol (100 mg).

3.3.1 Breynin I (*1*)

An amorphous powder; $[\alpha]_D^{22}$ + 12.7 (c = 0.30, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3358, 2924, 1782, 1697, 1608, 1516, 1277, 1167, 1074, 773; ^1H and ^{13}C NMR spectral data: see Table 1; ESI-MS (positive-ion mode) *m/z* 829.3 $[M + Na]^+$; HR-ESI-MS *m/z* 829.2211 $[M + Na]^+$ (calcd for $C_{34}H_{46}O_{20}SNa$, 829.2201).

3.3.2 Breyniaionoside E (*2*)

A colorless gum; $[\alpha]_D^{22}$ −10.0 (c = 0.25, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3390, 2968, 2931, 1709, 1647, 1379, 1267, 1026; ^1H and ^{13}C NMR spectral data: see Table 2; ESI-MS (positive-ion mode) *m/z* 411.2 $[M + Na]^+$; HR-ESI-MS *m/z* 411.1998 $[M + Na]^+$ (calcd for $C_{19}H_{32}O_8Na$, 411.1995).

3.4 Enzymatic hydrolysis of breyniaionoside E (2)

A solution of **2** (6 mg) in water was treated with cellulase (6 mg) and the whole mixture was kept at 37°C for 3 days. The reaction mixture was then extracted with $CHCl_3$. From the water layer, glucose was

Table 2. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound **2** (DMSO-d_6, δ in ppm, J in Hz).

No.	^1H NMR	^{13}C NMR	No.	^1H NMR	^{13}C NMR
1		34.3 (s)	11	0.96 (s)	29.5 (q)
2	2.55 (m) (α); 1.72 (d, 13.5) (β)	48.9 (t)	12	0.90 (s)	28.9 (q)
3		209.5 (s)	13	1.23 (s)	28.0 (q)
4	2.66 (m) (α); 2.00 (d, 13.6) (β)	49.7 (t)	1′	4.38 (d, 8.2)	103.4 (d)
5		84.1 (s)	2′	2.90 (m)	73.7 (d)
6	2.11 (m)	52.1 (d)	3′	3.07 (m)	76.8 (d)
7	2.12 (m) (α); 1.21 (m) (β)	29.6 (t)	4′	3.01 (t, 8.1)	70.1 (d)
8	3.81 (m)	78.2 (d)	5′	3.02 (m)	76.9 (d)
9	3.73 (m)	76.3 (d)	6′	3.40 (m); 3.65 (dd, 11.5, 5.4)	61.2 (t)
10	1.08 (d, 6.3)	18.7 (q)			

determined as the sugar moiety by co-TLC with an authentic sample (developed with EtOAc–MeOH–H_2O–HOAc, 13:3:3:4). The chloroform layer was purified by PTLC (developed with petroleum ether–acetone, 2:1) to give the aglycone **2a** (3 mg).

3.4.1 Compound *2a*

EI-MS: *m/z* 226 $[M]^+$; ^1H NMR (300 MHz, pyridine-d_5): δ_H 0.93, 0.94 (3H each, both s, H-11, 12), 1.38 (6H, m, H-10, 13), 2.01, 2.40 (1H each, both m, H-7), 2.20 (1H, m, H-6), 1.99, 2.58 (1H each, both d, $J = 13.9$ Hz, H-2), 2.42, 2.85 (1H each, both d, $J = 14.0$ Hz, H-4), 4.00 (2H, m, H-8, 9).

3.5 Preparation of (S)-MTPA ester (2aS) and (R)-MTPA ester (2aR) from 2a

Compound **2a** (3 mg) was divided into two halves and treated with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride and (*S*)-MTPA chloride (15 μl each), respectively. After adding 0.2 ml pyridine-d_5 and kept at room temperature for 6 h, ^1H NMR spectra of the two products (*S*)-MTPA ester (**2a***S*) and (*R*)-MTPA ester (**2a***R*) were measured, and $\Delta\delta$ ($\delta_S - \delta_R$) value was calculated.

3.5.1 Compound *2a*S

^1H NMR (300 MHz, pyridine-d_5): δ 0.875, 0.867 (3H each, both s, H-11, 12), 1.283 (3H, d, $J = 6.6$ Hz, H-10), 1.333 (3H, s, H-13), 1.842, 2.192 (1H each, both m, H-7), 2.033 (1H, m, H-6), 1.958, 2.533 (1H each, both d, $J = 13.9$ Hz, H-2), 2.400, 2.808 (1H each, both d, $J = 13.9$ Hz, H-4), 4.092 (1H, m, H-8), 5.325 (1H, m, H-9).

3.5.2 Compound *2a*R

^1H NMR (300 MHz, pyridine-d_5): δ 0.854 (6H, s, H-11, 12), 1.354 (3H, d, $J = 6.5$ Hz, H-10), 1.154 (3H, s, H-13), 1.754, 2.100 (1H each, both m, H-7), 1.973 (1H, m, H-6), 1.936, 2.500 (1H each, both d, $J = 13.9$ Hz, H-2), 2.318, 2.754 (1H each, both d, $J = 13.9$ Hz, H-4), 4.000 (1H, m, H-8), 5.287 (1H, m, H-9).

Acknowledgements

This work was financially supported by the National Science and Technology Major Project 'Key New Drug Creation and Manufacturing Program', China (No. 2009ZX09301-001) and Key Project of Chinese Academy of Sciences (No. KSCX2-YW-R-184). The authors wish to thank Professors Ying Zhou and Ding Fang of Guangxi Institute of Traditional Chinese Medicine for providing and identification of the plant material.

References

[1] H. Koshiyama, M. Hatori, H. Ohkuma, F. Sakai, H. Imanishi, M. Ohbayashi, and H. Kawaguchi, *Chem. Pharm. Bull.* **24**, 169 (1976).
[2] H. Ohkuma, T. Tsuno, M. Konishi, T. Naito, and H. Kawaguchi, *Chem. Pharm. Bull.* **39**, 942 (1991).
[3] A.B. Smith III, R.T. Gallagher, T.P. Keenan, G.T. Furst, and P.G. Dormer, *Tetrahedron Lett.* **32**, 6847 (1991).
[4] A.B. Smith III, T.P. Keenan, R.T. Gallagher, G.T. Furst, and P.G. Dormer, *J. Org. Chem.* **57**, 5115 (1992).
[5] N.H. Lajis, A. Retnam, H.A. Hassan, M.N. Khan, C.H. Chua, and M.V. Sargent, *Indian J. Chem.* **36B**, 206 (1997).
[6] N.H. Lajis, O.B. Guan, M.V. Sargent, B.W. Skelton, and A.H. White, *Aust. J. Chem.* **45**, 1893 (1992).
[7] H. Morikawa, R. Kasai, H. Otsuka, E. Hirata, T. Shinzato, M. Aramoto, and Y. Takeda, *Chem. Pharm. Bull.* **52**, 1086 (2004).
[8] G.M. Fu, Z.L. Xu, B.Y. Yu, and D.N. Zhu, *China J. Chin. Mater. Med.* **29**, 1052 (2004).
[9] G.M. Fu, Z.L. Xu, B.Y. Yu, and D.N. Zhu, *J. China Pharm. Univ.* **35**, 114 (2004).
[10] D.H. Meng, W.L. Chen, and W.M. Zhao, *J. Nat. Prod.* **70**, 824 (2007).
[11] P.K. Agrawal, *Phytochemistry* **31**, 3307 (1992).
[12] T. Marukami, A. Kishi, and M. Yoshikawa, *Chem. Pharm. Bull.* **49**, 974 (2001).
[13] L. Jiang, H. Kojima, K. Yamada, A. Kobayashi, and K. Kubota, *J. Agric. Food Chem.* **49**, 5888 (2001).

[14] T. Fujita, K. Terato, and M. Nakayama, *Biosci. Biotech. Biochem.* **60**, 732 (1996).
[15] A. Jutiviboonsuk, H. Zhang, G. Tan, C. Ma, V. Nguyen, M. Nguyen, N. Bunyapraphatsara, D. Soejarto, and H. Fong, *Phytochemistry* **66**, 2745 (2005).
[16] H. Kim, E. Woo, and H. Park, *J. Nat. Prod.* **57**, 581 (1994).
[17] H. Tatematsu, M. Kurokawa, M. Niwa, and Y. Hirata, *Chem. Pharm. Bull.* **32**, 1612 (1984).
[18] B. Baderschneider and P. Winterhalter, *J. Agric. Food Chem.* **49**, 2788 (2001).
[19] W.H. Lin, J.M. Fang, and Y.S. Cheng, *Phytochemistry* **50**, 653 (1999).
[20] X.C. Li, H.N. ElSohly, L.A. Walker, and A.M. Clark, *Planta Med.* **71**, 977 (2005).
[21] M. Uchida, P. Ruedi, and C.H. Eugster, *Helv. Chim. Acta* **63**, 225 (1980).
[22] Y.R. Jorapur and D.Y. Chi, *J. Org. Chem.* **70**, 10774 (2005).