Cytotoxic Diterpenes from the Root Tuber of *Curcuma wenyujin*

by **Wei Huang**, **Peng Zhang**, **Ye-Cheng Jin**, **Qiang Shi**, **Yi-Yu Cheng**, **Hai-Bin Qu***, and **Zhong-Jun Ma***

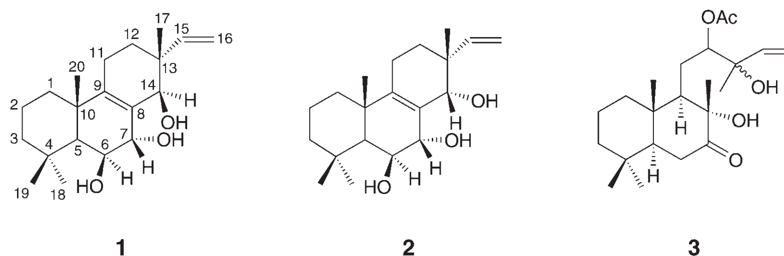
Institute of Pharmaceutical Informatics, School of Pharmaceutical Sciences, Zhejiang University Zijingang Campus, No. 388 Yuhangtang Rd., Hangzhou 310058, P. R. China
(phone: +86-571-88208427; fax: +86-571-88208428; e-mail: mazj@zju.edu.cn)

Bioassay-guided fractionation of ethanolic extract from the root tuber of *Curcuma wenyujin* afforded three new diterpenes, curcumrinols A–C (**1**–**3**), where **2** is the (14*S*)-epimer of **1**. The structures of **1**–**3** were established on the basis of spectroscopic analysis, mainly NMR and MS. **1**–**3** were tested *in vitro* for their cytotoxic activity against the HL-60 and K562 cancer cells. Among the compounds tested, **1** exhibited medium cytotoxicity against K-562 and HL60 human cancer cells with IC_{50} values of 11.2 and 3.2 μg/ml, respectively. However, **2** showed only weak activity against the above cancers cells, which suggested that C(14) may be an important position for cytotoxic activity.

Introduction. – *Curcuma wenyujin* Y. H. CHEN et C. LING is a member of the Zingiberaceae family (ginger family), which is distributed throughout tropical and subtropical regions of the world, and has been used for centuries as foods, spices, dyes, perfumes, as well as in traditional Chinese, Japanese, and Indian medicine [1–3]. The plant is distributed in South China and has been traditionally used for the treatment of jaundice, epilepsy, dysmenorrhea, thoracic-abdominal pain and hematuria [4][5]. Several species of *Curcuma* such as *C. domestica*, *C. zedoaria*, and *C. xanthorrhiza* have been extensively studied, and some kinds of compounds such as terpenoids [6][7], diarylheptanoids [8–13] were isolated from the *Curcuma* genus. There are some reports on the phytochemical constituents of *C. wenyujin*, some compounds such as germacrone and curdione were obtained from the plant volatile oil by HSCCC [5]. The components of the volatile oil were also investigated, and 72 compounds were identified by GC-MS [14][15]. However, there are no other reports on the chemical constituents of the plant. As a part of our phytochemical and pharmacological investigations of medicinal plants, a bioassay-guided isolation of chemical constituents of the root tuber of *Curcuma wenyujin* led to the isolation of two new pimarane diterpenes, curcumrinols A (**1**) and B (**2**), and one new labdane-type diterpene, curcumrinol C (**3**). The present report describes the isolation and structural elucidation as well as the determination of the cytotoxicity of these constituents.

Results and Discussion. – Curcumrinol A (**1**) was isolated as white needles, and its molecular formula was assigned to be $C_{20}H_{32}O_3$ by the peak in the HR-EI-MS at *m/z* 320.2334 (M^+, $C_{20}H_{32}O_3^+$; calc. 320.2351), indicating five degrees of unsaturation. LC-ESI-MS/MS analyses in the positive ion mode showed two main fragments at *m/z* 302 ($[M-H_2O]^+$) and *m/z* 171 ($[M-2\,H_2O-C_7H_{12}OH]^+$), suggesting that the molecule contains at least two OH groups. The ^{1}H-NMR spectrum displayed signals of four

© 2008 Verlag Helvetica Chimica Acta AG, Zürich



tertiary Me groups at δ 1.03 (*s*, 6 H), 1.24 (*s*, 3 H), and 1.37 (*s*, 3 H), of three oxygenated CH groups at δ 3.88 (*s*, 1 H), 3.95 (*s*, 1 H), and 4.28 (*s*, 1 H), and it also presented signals for one mono-substituted vinyl group at δ 5.17 (*dd*, $J = 1.2$, 18 Hz, 1 H), 5.22 (*dd*, $J = 1.2$, 10.9 Hz, 1 H), and 6.48 (*dd*, $J = 10.9$, 18.0 Hz, 1 H). The ^{13}C-NMR and HSQC spectra revealed the presence of 20 nonequivalent C-atoms including four Me groups at δ 22.1, 22.6, 24.3, and 33.3, three oxygenated C-atoms at δ 70.9, 76.8, and 78.1, five CH_2 groups, one $=CH_2$ group, three quaternary C-atoms, together with two C=C bonds at δ 115.2, 124.8, 143.0, and 147.1. The ^{1}H- and ^{13}C-NMR spectra were similar to those of the known sphaeropsidin D [16], except for the location of the OH groups. The positions of the OH groups were deduced to be at C(6), C(7), and C(14) from the HMBC correlations of δ 4.28 (H–C(6)) with δ 33.7 (C(4)), 48.9 (C(5)), 76.8 (C(7)), 124.8 (C(8)), and 22.1 (C(20)), of δ 3.95 (H–C(7)) with δ 48.9 (C(5)), 70.9 (C(6)), 124.8 (C(8)), 147.1 (C(9)), and 78.1 (C(14)), and of δ 3.88 (H–C(14)) with δ 76.8 (C(7)), 124.8 (C(8)), 147.1 (C(9)), 29.2 (C(12)), 39.9 (C(13)), 143.0 (C(15)), and 22.6 (C(17)). In addition, the ^{13}C-NMR signal for C(12) was shifted upfield from δ(C) 40.7 in sphaeropsidin D to δ(C) 29.2 in **1**, which corresponds to the absence of a OH group at C(11). Analysis of the heteronuclear 2D-NMR spectra resulted in an unambiguous assignment of all ^{1}H- and ^{13}C-NMR signals (*Table 1*). The relative configuration of **1** was assigned on the basis of a NOESY experiment, as depicted in the *Figure*. The compound exhibited NOEs between H-atoms δ 1.37 (Me(20)) and δ 2.08 (H_β–C(11)), δ 2.08 (H_β–C(11)) and δ 1.03 (Me(17)), δ 1.37 (Me(20)) and δ 1.24 (Me(19)), which suggested the orientation of Me(17) as β. A correlation between the H-atoms at δ 1.03 (Me(18)) and δ 4.28 (H–C(6)) was also detected in the NOESY spectrum. However, there were no correlations between H–C(5) at δ 1.53 and H–C(7) at δ 3.95, and between H–C(14) at δ 3.88 and H–C(7) at δ 3.95. Therefore, the orientations of the 7-OH and 14-OH groups are α and β, respectively. On the basis of these data, the structure was assigned as 6β,7α,14β-trihydroxyisopimara-8,15-diene, and named curcumrinol A.

Curcumrinol B (**2**) was isolated as white needles and was assigned the molecular formula $C_{20}H_{32}O_3$, as deduced from the HR-EI-MS data (*m*/*z* 320.2344 (M^+, $C_{20}H_{32}O_3^+$; calc. 320.2351)). Comparison of the spectroscopic data of **2** with those of **1** revealed many similarities, except for the moieties at C(7) and C(14). HMBC Correlations of H–C(7) with C(5), C(6), C(8), and C(9), and of H–C(14) with C(7), C(8), C(9), C(12), C(13), C(15), and C(17) were observed. Therefore, **2** was supposed to have a different configuration than **1**. This was confirmed by the NOESY data, which showed cross-peaks between δ 3.86 (H–C(14)) and δ 4.18 (H–C(7)). From this data, the

Table 1. *^{1}H- and ^{13}C-NMR Data of Curcumrinol A* (**1**) *and Curcumrinol B* (**2**) (in $CDCl_3$). δ in ppm, J in Hz.

Position	Curcumrinol A (**1**)			Curcumrinol B (**2**)		
	δ(C)[a]	δ(H)	Key HMBC[b]	δ(C)[a]	δ(H)	Key HMBC[b]
1	39.4 (*t*)	1.72 (br. *d*, J = 13.2, 2 H)	3	39.2 (*t*)	1.01–1.06 (*m*, H_β), 1.60–1.63 (*m*, H_α)	2, 3, 5, 9
2	19.2 (*t*)	1.53 (*s*, H_β), 1.72 (br. *d*, J = 13.2, H_α)	4, 5, 6, 10, 20	19.0 (*t*)	1.45–1.49 (*m*, H_β), 1.65–1.67 (*m*, H_α)	1
3	42.9 (*t*)	1.24–1.26 (*m*, H_β), 1.43 (br. *d*, J = 13.2, H_α)	1, 5	42.6 (*t*)	1.20–1.25 (*m*, H_β), 1.36–1.38 (*m*, H_α)	2, 4, 5, 18, 19
4	33.7 (*s*)			33.3 (*s*)		
5	48.9 (*d*)	1.53 (*s*, H_α)	4, 6, 20	48.7 (*d*)	1.32 (*s*, H_α)	3, 4, 6, 10, 18, 19, 20
6	70.9 (*d*)	4.28 (*s*, H_α)	4, 5, 7, 8, 20	70.6 (*d*)	4.26 (*s*, H_α)	4, 7, 8, 10
7	76.8 (*d*)	3.95 (*s*, H_β)	5, 6, 8, 9, 14	70.6 (*d*)	4.18 (*s*, H_β)	5, 6, 8, 9
8	124.8 (*s*)			125.5 (*s*)		
9	147.1 (*s*)			147.9 (*s*)		
10	38.4 (*s*)			37.9 (*s*)		
11	20.4 (*t*)	2.08 (*dt*, J = 18.0, 7.2, H_β), 2.27 (*dt*, J = 18.0, 6.0, H_α)	8, 9, 12, 13	21.6 (*t*)	2.00–2.03 (overlapped, 2 H)	8, 9, 12, 13
12	29.2 (*t*)	1.49 (*dt*, J = 18.0, 7.2, H_β), 1.81 (*dt*, J = 18.0, 6.0, H_α)	9, 11, 13, 14, 15, 17	28.6 (*t*)	1.34–1.38 (*m*, H_β), 1.56–1.59 (*m*, H_α)	2, 4, 9, 11, 13, 15, 19
13	39.9 (*s*)			38.8 (*s*)		
14	78.1 (*d*)	3.88 (*s*, H_α)	7, 8, 9, 12, 13, 15, 17	71.0 (*d*)	3.86 (*s*, H_β)	7, 8, 9, 12, 13, 15, 17
15	143.0 (*d*)	6.48 (*dd*, J = 18.0, 10.9, 1 H)	12, 13, 14, 17	143.4 (*d*)	5.72 (*dd*, J = 18, 11.2, 1 H)	12, 13, 14, 17
16	115.2 (*t*)	5.17 (*dd*, J = 18.0, 1.2, 1 H), 5.22 (*dd*, J = 10.9, 1.2, 1 H)	13, 15	113.0 (*t*)	4.97 (*d*, J = 11.2, 1 H), 5.05 (*d*, J = 18.0, 1 H)	13, 15
17	22.6 (*q*)	1.03 (*s*)	12, 13, 14, 15	24.1 (*q*)	1.04 (*s*)	12, 13, 14, 15
18	33.3 (*q*)	1.03 (*s*)	3, 4, 5, 18	33.6 (*q*)	0.98 (*s*)	3, 4, 5, 19
19	24.3 (*q*)	1.24 (*s*)	3, 4, 5, 19	23.8 (*q*)	1.16 (*s*)	3, 4, 5, 18
20	22.1 (*q*)	1.37 (*s*)	1, 5, 9	21.2 (*q*)	1.27 (*s*)	5, 9, 10

[a]) Chemical shifts and multiplicities, based on HSQC and HMBC correlation peaks. [b]) Numbers of the C-atoms correlating with the corresponding H-atom.

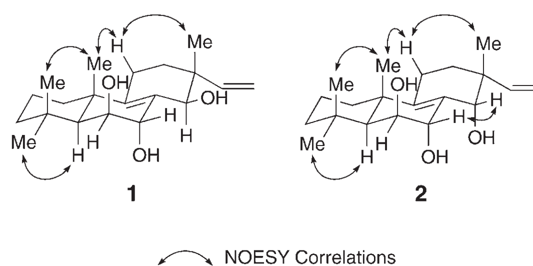


Figure. *Selected NOESY correlations of* **1** *and* **2**

orientation of the 14-OH group was determined to be α, and the structure was assigned as 6β,7α,14α-trihydroxyisopimara-8,15-diene, and named curcumrinol B.

Curcumrinol C (**3**) was obtained as white needles with the molecular formula $C_{22}H_{36}O_5$, as determined by the HR-EI-MS data (*m*/*z* 380.2581 (M^+, $C_{22}H_{36}O_5^+$; calc. 380.2563)). In the ^{1}H-NMR spectrum of **3**, six Me groups were present at δ 0.83 (*s*, 3 H), 0.86 (*s*, 3 H), 0.94 (*s*, 3 H), 1.22 (*s*, 3 H), 1.47 (*s*, 3 H), and 2.13 (*s*, 3 H). Additionally, a signal for one oxygenated CH group was observed at δ 5.33–5.37 (*m*, 1 H), and signals for one terminal C=C bond at δ 5.05 (*d*, J = 12.0 Hz, 1 H), 5.10 (*d*, J = 18.0 Hz, 1 H), and 6.06 (*dd*, J = 12.0, 18.0 Hz, 1 H) were present. 22 C-atom signals were observed in the ^{13}C-NMR spectrum, thereof one signal at δ 208.9 (C(7)), which suggested a ketone CO group; three signals for oxygenated C-atoms were detected at δ 70.4 (C(12)), 76.0 (C(13)), and 81.6 (C(8)), and for two C-atoms at δ 111.4 (C(15)) and 145.8 (C(14)) showed the presence of one C=C bond. Those data were more or less identical to those of the known compound ptychantin M [17]. However, the signal at δ 208.9 (C(7)) indicated, that the CH_2 group was replaced by a ketone CO group at C(7), which was confirmed by HMBC correlations (*Table 2*). In the NOESY spectrum, the relative configuration of Me(17) was determined to be β. Me(17) (δ 1.47) showed a correlation with Me(20) (δ 0.94). Therefore, the compound was assigned as 12-acetoxy-8α,13-dihydroxylabd-14-en-7-one, and named curcumrinol C.

Compounds **1**–**3** were tested for cytotoxicity against K-562 and HL-60 cells with the MTT[1]) method (*Table 3*), conducted in a manner reported previously [18][19]. Compounds **1** demonstrated medium inhibitory activity against K-562 (human chronic myelogenous leukemia) and HL-60 (human promyelocytic leukemia). However, **2**, the (14*S*)-epimer of **1**, showed only weak activity against the cancer cells, which suggested that position C(14) may be the important site for cytotoxicity of this kind of compounds.

The research was supported by the *Program for New Century Excellent Talents in University* (NCET-06-0515).

Experimental Part

General. Prep. HPLC: *Agilent 1100* system with a photodiode array detector; *C18* column (*ODS* 7 µm, 250 × 21.2 mm inner diameter, *ZORBAX*). Optical rotations: *Jasco P-1010* Polarimeter. IR: *Jasco FTIR-4100*. 1D- and 2D- (HSQC, HMBC, COSY, NOESY) NMR spectra: *Bruker 600* NMR

[1]) MTT = 3-(4,5-Dimethyl-1,3-thiazol-2-yl)-2,5-diphenyltetrazolium bromide.

Table 2. *^{1}H- and ^{13}C-NMR Data of Curcumrinol C* (**3**) (in $CDCl_3$). δ in ppm, J in Hz.

Position	δ(C)[a])	δ(H)	Key HMBC[b])
1	38.9 (*t*)	1.62 – 1.67 (overlapped, 2 H)	2, 10, 3, 5
2	18.1 (*t*)	1.49 – 1.52 (overlapped, 2 H)	4, 10
3	41.5 (*t*)	1.16 (*dd*, J = 12.8, 4.2, H_β), 1.45 (br. *s*, H_α)	1, 2, 4, 5, 18, 19
4	33.7 (*s*)		
5	56.4 (*d*)	1.40 (*dd*, J = 3.0, 14.0, H_α)	20, 4, 18, 19, 6, 9, 7
6	35.8 (*t*)	2.43 (*dd*, J = 14.0, 3.0, H_α), 2.57 (*t*, J = 14.0, H_β)	4, 10, 5, 7
7	208.9 (*s*)		
8	81.6 (*s*)		
9	50.4 (*d*)	1.96 – 1.98 (*m*)	20, 11, 10, 9, 12, 8, 17
10	36.3 (*s*)		
11	21.1 (*t*)	1.81 – 1.85 (*m*), 1.95 – 1.98 (*m*)	10, 12, 13, 8, 9
12	70.4 (*d*)	5.33 – 5.37 (*m*)	9, 12-OAc
13	76.0 (*s*)		
14	145.8 (*d*)	6.05 (*dd*, J = 18.0, 12.0)	12
15	111.4 (*t*)	5.05 (*d*, J = 12.0), 5.10 (*d*, J = 18.0)	16, 13, 14
16	26.7 (*q*)	1.22 (*s*)	12, 13, 1
17	22.2 (*q*)	1.47 (*s*)	9, 8, 7
18	32.6 (*q*)	0.83 (*s*)	19, 3, 4, 5
19	20.6 (*q*)	0.86 (*s*)	18, 3, 4, 5
20	15.0 (*q*)	0.94 (*s*)	10, 9, 5, 1
12-OAc	170.4 (*s*)		
	21.0 (*q*)	2.13 (*s*)	12, 12-OAc

[a]) Chemical shifts and multiplicities based on HSQC and HMBC correlation peaks. [b]) C-Atoms correlating with the corresponding H-atom.

Table 3. *Cytotoxic Activity of* **1** – **3** *against Cells HL-60 and K562*

IC_{50} [μg/ml]	**1**	**2**	**3**	Adriamycin[a])
K562	11.2	37.5	42.6	2
HL-60	3.2	18.9	36.5	2

[a]) Pos. control.

spectrometer (for compounds **2** and **3**), and *Bruker Biospin AMX-400* 400 MHz spectrometer (for compound **1**). EI-MS: *JEOL JMS SX-102A* mass spectrometer. ESI-MS: *LCQ DECA* system (*Thermofinnigan*) equipped with a hot ESI source (H-ESI, electrospray voltage: 3.0 kV, sheath gas: N_2, vaporizer temp.: 50°, capillary temp.: 250°, collision gas: Ar, collision pressure: 1.5 mTorr).

Plant Material. Dried, root tuber of *Curcuma wenyujin* Y. H. CHEN et C. LING collected in Pan'an, Zhejiang Province, P. R. China, in October 2006. The plant material was identified by authors. Voucher specimens (No. CW060901) are deposited at the college of Pharmaceutical Sciences, Zhejiang University, China.

Extraction and Isolation. The air-dried root tuber of *Curcuma wenyujin* Y. H. CHEN et C. LING (10 kg) were extracted with 95% EtOH (80 l × 4) to give a crude EtOH extract (247 g). This residue was dissolved in H_2O (500 ml), and then extracted successively with petroleum ether (PE) (60 – 90° 0.5 l × 3), CH_2Cl_2 (0.5 l × 3), and BuOH (0.5 l × 3). The CH_2Cl_2 (95.1 g) extract was chromatographed over a SiO_2 column with a PE/EtOAc gradient to afford ten fractions (*Fr. A – J*). *Fr. E* and *G* were divided into subfractions *E1 – E8* and *G1 – G6* by prep. HPLC, eluted with 70% and 40% $MeCN/H_2O$ resp. The *Subfr.*

E8 and *G6* were further separated by prep. HPLC using 45% and 60% MeCN/H_2O as the mobile phase (flow rate 10 ml/min) to yield compounds **3** (5.0 mg, t_R 43.7 min) and **1** (9.5 mg, t_R 25.3 min). *Fr. H* was chromatographed on reversed-phase column chromatography on *ODS C-18* (50 μm) eluted with a gradient solvent system of MeOH/H_2O (from 30% to 100%) to give six subfractions *H1 – H11*. *Subfr. H6* was further purified by prep. HPLC over a *ODS C-18* column with 48% MeCN/H_2O to afford compound **2** (10.4 mg, t_R 15.1 min).

Curcumrinol A (=*6β,7α,14β-Trihydroxyisopimara-8,15-diene*; **1**): White needles ($CHCl_3$). $[\alpha]_D^{25}$ = 37.4 (c = 0.1, $CHCl_3$). IR (KBr): 3423, 2924, 2864, 1640, 1385, 1367, 1040, 984, 910. ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): *Table 1*. ESI-MS: 321 ($[M+H]^+$). HR-EI-MS: 320.2334 (M^+, $C_{20}H_{32}O_3^+$; calc. 320.2351).

Curcumrinol B (=*6β,7α,14α-Trihydroxyisopimara-8,15-diene*; **2**): White needles ($CHCl_3$). $[\alpha]_D^{25}$ = 103.0 (c = 0.1, $CHCl_3$). IR (KBr): 3375, 2924, 2886, 1638, 1459, 1385, 1090, 1058, 947, 915. ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): *Table 1*. ESI-MS: 663 ($[2M+Na]^+$). HR-EI-MS: 320.2344 (M^+, $C_{20}H_{32}O_3^+$; calc. 320.2351).

Curcumrinol C (=*12-Acetoxy-8α,13-dihydroxylabd-14-en-7-one*; **3**): Whitle needles ($CHCl_3$). $[\alpha]_D^{25}$ = 74.3 (c = 0.1, $CHCl_3$). IR (KBr): 3389, 1678, 1647. ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): *Table 2*. ESI-MS: 319 ($[M-H]^-$). HR-EI-MS: 380.2581 (M^+, $C_{22}H_{36}O_5^+$; calc. 380.2563).

Cell Culture and Cytotoxic Bioassays. The human acute myeloid leukemia cell line HL-60 and human chronic myelogenous leukemia cell line K562 were maintained in *RPMI-1640* medium (*Gibco BRL*) with 3.7 g/l $NaHCO_3$, supplemented with 10% heat-inactivated FBS.

For the cytotoxicity assays with K562 and HL-60, adriamycin was chosen as a reference drug at a concentration of 4 μg/ml. All the above cell lines were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), seeded in 96-well tissue culture plates, and maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37° for 3 – 6 d before experimentation.

The cytotoxicity of all tested samples and compounds was assayed using the *in vitro* MTT reduction assay in 96-well microtiter plates. Altogether, the two cell lines HL-60 (human promyelocytic leukemia), K-562 (human chronic myelogenous leukemia) were employed. Briefly, cells in a logarithmic growth phase were harvested, diluted to 2×10^3 cells/ml with fresh medium and gently mixed. The test compound was dissolved in DMSO (concentration 50 mg/ml), and this soln. (0.88 μl) was added to the fresh medium (220 μl/well), and then the dilution (50 μl) and cell suspension (150 μl) were transferred into microtiter plates (concentration 50 μg/ml). If the compound was active at 50 μg/ml, a series of solns. was prepared by two-fold dilution, and exposed to cells as mentioned above, in order to obtain the IC_{50} values. Plates were incubated at 37° under 5% CO_2 atmosphere for 72 h. After the incubation period, 10% MTT was added, and the plates were incubated again at 37° for 4 h. The pure formazan product (produced by reduction of MTT by succinyl dehydrogenase in the mitochondria of living cells) was solubilized 150 μl DMSO for 10 min at r.t. The plate was read at 550 nm in a plate reader. Quadruplex wells were used for each drug concentration, and all of the reported experiments were performed at least four times. IC_{50} (drug concentration causing 50% growth inhibition growth) values were calculated with non-linear regression analysis. The percent inhibitions were obtained from the following equation:

$$\% \text{ Inhibition} = [(A_{\text{test}} - A_{\text{negative}})/(A_{\text{adriamycin}} - A_{\text{negative}})] \times 100.$$

REFERENCES

[1] K. Endo, E. Kanno, Y. Oshima, *Phytochemistry* **1990**, *29*, 797.
[2] C. K. Moon, K. S. Park, S. H. Lee, Y. P. Yoon, *Arch. Pharmacal Res.* **1985**, *8*, 42.
[3] T. N. B. Shankar, V. S. Murthy, *Indian J. Exp. Biol.* **1979**, *17*, 1363.
[4] C. Tohda, N. Nakayama, F. Hatanaka, K. Komatsu, *Evid.-Based Complement. Altern. Med.* **2006**, *3*, 255.
[5] J. Yan, G. Chen, S. Tong, Y. Feng, L. Sheng, J. Lou, *J. Chromatogr., A* **2005**, *1070*, 207.
[6] G. N. Roth, A. Chandra, M. G. Nair, *J. Nat. Prod.* **1998**, *61*, 542.

[7] C. H. Hong, Y. L. Kim, S. K. Lee, *Arch. Pharmacal Res.* **2001**, *24*, 424.
[8] V. Ravindranath, M. N. Satyanarayana, *Phytochemistry* **1980**, *19*, 2031.
[9] T. Masuda, A. Jitoe, J. Isobe, N. Nakatani, S. Yonemori, *Phytochemistry* **1993**, *32*, 1557.
[10] F. Kiuchi, Y. Goto, N. Sugimoto, N. Akao, K. Kondo, Y. Tsuda, *Chem. Pharm. Bull.* **1993**, *41*, 1640.
[11] S.-Y. Park, D. S. H. L. Kim, *J. Nat. Prod.* **2002**, *65*, 1227.
[12] S. Uehara, I. Yasuda, K. Akiyama, H. Morita, K. Takeya, H. Itokawa, *Chem. Pharm. Bull.* **1987**, *35*, 3298.
[13] W.-J. Syu, C.-C. Shen, M.-J. Don, J.-C. Ou, G.-H. Lee, C.-M. Sun, *J. Nat. Prod.* **1998**, *61*, 1531.
[14] J. Cao, M. Qi, L. Fang, S. Zhou, R. Fu, P. Zhang, *J. Pharm. Biomed. Anal.* **2006**, *40*, 552.
[15] J. Cao, M. Qi, Y. Zhang, S. Zhou, Q. Shao, R. Fu, *Anal. Chim. Acta* **2006**, *561*, 88.
[16] A. Evidente, L. Sparapano, G. Bruno, A. Motta, *Phytochemistry* **2002**, *59*, 817.
[17] C.-L. Wu, C.-J. Wang, M.-H. Yin, *J. Chin. Chem. Soc.* **2001**, *48*, 241.
[18] J. Carmichael, W. G. DeGraff, A. F. Gazdar, J. D. Minna, J. B. Mitchell, *Cancer Res.* **1987**, *47*, 936.
[19] P. R. Twentyman, M. A. Luscombe, *Br. J. Cancer* **1987**, *56*, 279.

Received November 26, 2007