Antioxidative Flavonoids from *Cleistocalyx operculatus* Buds

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Four new flavonoids, 3′-formyl-4′,6′,4-trihydroxy-2′-methoxy-5′-methylchalcone (1), 3′-formyl-6′,4-dihydroxy-2′-methoxy-5′-methylchalcone 4′-*O*-*β*-D-glucopyranoside (2), (2*S*)-8-formyl-6-methylnaringenin (3), and (2*S*)-8-formyl-6-methylnaringenin 7-*O*-*β*-D-glucopyranoside (4) were isolated from the buds of *Cleistocalyx operculatus* (Myrtaceae). The structures of the new metabolites (1—4) were determined on the basic of spectroscopic analyses including 2 dimensional NMR. Compounds 1 and 3 exhibited 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity with IC_{50} values of 22.8 and 27.1 μM, respectively.

Key words *Cleistocalyx operculatus*; Myrtaceae; anti oxidant; flavonoid

Cleistocalyx operculatus (ROXB.) MERR. *et* PERRY (Myrtaceae) is a large green tree that grows in rural areas of North Vietnam where it is commonly called Voi. The flower buds (name as Nu Voi) and leaves (La Voi) have been used to make a beverage since ancient times.[1)] It is also a well-known medicinal plant whose buds are commonly used as an ingredient for tonic drinks in Southern China.[2)] The water extract of *C. operculatus* buds has been shown to increase the contractility and decrease the frequency of contraction in an isolated rat heart perfusion system.[3)] It showed strong protective effects on lipid peroxidation in rat liver microsomes, and the H_2O_2-induced trauma of rat pheochromocytima (PC12) cells.[4)] *C. operculatus* extract showed inhibitory activity against the α-glucosidase, rat-intestinal maltase, and sucrase activities. It is also considered a promising material for preventing and treating diabetes.[5)] Previous phytochemical attention characterized the oleanane-type triterpenes,[6,7)] and flavonoids.[2)] Analysis of its leaf oil by gas chromatography (GC) and chromatography/mass spectrometry (GC/MS) has also been reported.[8)] Chalcone compounds from this plant possess antioxidant and anticancer activities.[9—11)] In this report, we describe the isolation and structural determination of four new flavonoids (**1**—**4**), and evaluate their DPPH radical scavenging activity.

Results and Discussion

The MeOH extract of the buds of *C. operculatus* was partitioned into hexane-, EtOAc-, and BuOH-soluble fractions. Chromatographic purification of the EtOAc-soluble fraction led to the isolation of four compounds (**1**—**4**) (Fig. 1).

Compound **1** was isolated as a yellow amorphous powder. In the positive ion mode, electrospray ionization mass spectrometry (ESI-MS), a quasimolecular ion peak at *m/z* 329 $[M+H]^+$ was observed, and high-resolution ESI-MS (HR-ESI-MS) analysis indicated an ion peak at 329.1813 which corresponded to the molecular formula, $C_{18}H_{16}O_6$. The infrared (IR) absorptions at 3452, 2873 and 2758 cm^{-1} showed the presence of hydroxy and aldehyde groups. The ^1H nuclear magnetic resonance (NMR) spectrum of **1** indicated the presence of methoxy group at δ 3.82 (3H, s), aldehyde group at δ 10.41 (1H, s), and methyl group at δ 2.04 (3H, s). Proton signals were also observed at δ 7.54 (2H, dd, J=8.5, 2.0 Hz, H-2, 6) and δ 6.94 (2H, dd, J=8.5, 1.8 Hz, H-3, 5) and assigned to 4-substituted phenyl in the B ring of chalcone.[12)] An AB spin system with J=15.8 Hz at δ 7.73 and 7.78 indicated the presence of protons of an α,β-unsaturated ketone moiety.[2)] The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectrum of **1** showed 18 signals, including three oxygenated aromatic carbons at δ 165.7 (C-2′), 169.8 (C-6′) and 160.7 (C-4), one carbonyl carbon at δ 193.8 (C=O), one aldehyde carbon at δ 191.7 (3′-CHO), one methyl carbon at δ 10.6 (5′-CH_3), and four aromatic carbons at δ 130.2 (C-2, 6) and 118.3 (C-3, 5). The ^1H- and ^{13}C-NMR spectra (Table 1) were very similar to those of 3′-formyl-4′,6′-dihydroxy-2′-methoxy-5′-methylchalcone,[2,10)] except for the hydroxy group at the position C-4 of the B ring. The full NMR assignments and connectivities of **1** were determined by heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple-bond correlation (HMBC) spectroscopic data analyses. The HMBC spectrum confirmed the correlations between aldehyde proton (δ 10.41, s) and C-3′ (δ 108.7), between methoxy protons (δ 3.82, s) and C-2′ (δ 165.7), and between methyl protons (δ 2.04, s) and C-5′ (δ 110.2) (Fig. 2). Based on the above analyses, the structure of compound **1** was elucidated as 3′-formyl-4′,6′,4-trihydroxy-2′-methoxy-5′-methylchalcone.

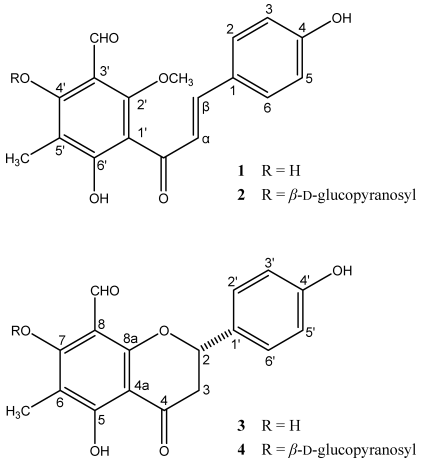

Fig. 1. Chemical Structures of Isolated Compounds

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Table 1. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) Spectral Data of Compounds **1** and **2** in MeOH-d_4 (δ ppm)

Position	**1**		**2**	
	^{1}H (mult., J in Hz)	^{13}C	^{1}H (mult., J in Hz)	^{13}C
1		128.3		127.5
2	7.54 (1H, dd, 8.5, 2.0)	130.2	7.57 (1H, dd, 8.5, 2.2)	130.3
3	6.94 (1H, dd, 8.5, 1.8)	118.3	6.95 (1H, dd, 8.5, 2.0)	117.9
4		160.7		158.4
5	6.94 (1H, dd, 8.5, 1.8)	118.3	6.95 (1H, dd, 8.5, 2.0)	117.9
6	7.54 (1H, dd, 8.5, 2.0)	130.2	7.57 (1H, dd, 8.5, 2.2)	130.3
α	7.73 (1H, d, 15.8)	125.8	7.72 (1H, d, 15.8)	125.9
β	7.78 (1H, d, 15.8)	145.3	7.79 (1H, d, 15.8)	145.6
1′		108.5		108.8
2′		165.7		166.2
3′		108.7		107.4
4′		166.9		166.8
5′		110.2		110.6
6′		169.8		169.9
C=O		193.8		193.5
2′-OCH$_3$	3.82 (3H, s)	68.5	3.85 (3H, s)	67.7
3′-CHO	10.41 (1H, s)	191.7	10.58 (1H, s)	190.8
5′-CH$_3$	2.04 (3H, s)	10.6	2.05 (3H, s)	10.5
Glc				
1″			5.08 (1H, d, 7.8)	101.7
2″			3.55 (1H, m)	74.5
3″			3.47 (1H, m)	78.6
4″			3.38 (1H, m)	70.8
5″			3.52 (1H, m)	78.9
6″			3.92 (1H, dd, 12.2, 2.0) 3.57 (1H, m)	62.9

Table 2. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) Spectral Data of Compounds **3** and 4 in MeOH-d_4 (δ ppm)

Position	**3**		**4**	
	^{1}H (mult., J in Hz)	^{13}C	^{1}H (mult., J in Hz)	^{13}C
2	5.41 (1H, dd, 12.5, 2.4)	79.8	5.45 (1H, dd, 12.4, 2.2)	80.3
3	2.91 (1H, dd, 16.5, 12.5) 2.58 (1H, dd, 16.5, 2.4)	44.7	2.87 (1H, dd, 16.5, 12.4) 2.58 (1H, dd, 16.5, 2.2)	45.6
4		188.3		185.2
4a		108.1		109.6
5		167.4		167.2
6		110.5		111.4
7		166.5		165.2
8		110.8		110.5
8a		160.4		159.7
1′		128.1		128.0
2′	7.50 (1H, dd, 8.5, 2.2)	127.0	7.55 (1H, dd, 8.5, 2.0)	126.8
3′	6.88 (1H, dd, 8.5, 2.0)	114.8	6.89 (1H, dd, 8.5, 1.8)	115.2
4′		159.2		158.4
5′	6.88 (1H, dd, 8.5, 2.0)	114.8	6.89 (1H, dd, 8.5, 1.8)	115.2
6′	7.50 (1H, dd, 8.5, 2.2)	127.0	7.55 (1H, dd, 8.5, 2.0)	126.8
8-CHO	10.28 (1H, s)	192.0	10.25 (1H, s)	192.2
6-CH$_3$	2.05 (3H, s)	10.1	2.12 (3H, s)	10.4
Glc				
1″			4.96 (1H, d, 7.5)	101.6
2″			3.35 (1H, m)	72.7
3″			3.38 (1H, m)	78.7
4″			3.27 (1H, m)	70.2
5″			3.51 (1H, m)	77.4
6″			3.74 (1H, dd, 12.2, 2.0) 3.50 (1H, m)	62.5

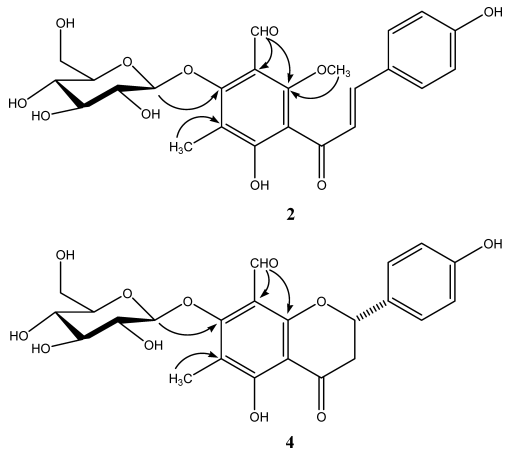

Fig. 2. Selected HMBC Correlations of **2** and **4**

Compound **2** was isolated as a yellow amorphous powder with a specific rotation $[\alpha]_D^{22}$ of −125.5 (c=0.2, MeOH). The HR-ESI-MS of **2** showed an $[M+H]^+$ peak at m/z 491.1585, which established a molecular formula of $C_{24}H_{26}O_{11}$, indicating that it was a glycosylated derivative of **1**. Its ^{1}H-NMR spectrum showed seven new characteristic signals typical of a sugar moiety, including a peak at δ 5.08 for an anomeric proton (H-1″). The ^{13}C-NMR spectrum also exhibited six carbon signals between δ 62.9 and 101.7, in addition to the signals assignable to **1**. The sugar was assigned as glucopyranose on the basis of NMR data and the *Rf* value compared with authentic glucose after enzymatic (naringinase) hydrolysis of **2**. The absolute configuration was determined to be D-glucose by GC. The $J_{H,H}$ value (7.8 Hz) of the anomeric proton (H-1″) indicated that glucose was linked *via* a β-linkage. In addition, the aromatic signal was shifted downfield at δ 166.8 (C-4′), which suggested that the OH group at C-4′ of **1** was glucosylated in **2**. This finding was confirmed by the HMBC correlation between H-1″ and C-4′ (Fig. 2). The NMR data were unambiguously assigned for both the aglycone and the sugar moiety by HMQC experiments. The structure of **2** was therefore established as 3′-formyl-6′,4-dihydroxy-2′-methoxy-5′-methylchalcone 4′-*O*-β-D-glucopyranoside.

Compound **3** was obtained as a yellow amorphous powder. Its molecular formula was found to be $C_{17}H_{14}O_6$ by the HR-ESI-MS (m/z 315.2788 $[M+H]^+$). The UV spectrum exhibited an absorption maxima at 270 and 335 nm, which suggested a flavanone nature for **3**.[13] Compound **3** displayed aromatic signals downfield at δ 7.50 (2H, dd, J=8.5, 2.2 Hz, H-2′, 6′), 6.88 (2H, dd, J=8.5, 2.0 Hz, H-3′, 5′), δ 127.0 (C-2′, 6′), 114.8 (C-3′, 5′) and 159.2 (C-4′) in the ^{1}H- and ^{13}C-NMR spectra, suggesting the hydroxygenated position to be at C-4′. Remarkable differences were observed between the ^{1}H- and ^{13}C-NMR spectra of compound **3** (Table 2) and those of compounds **1** and **2** (Table 1). The three one-proton coupled double doublets at δ 5.41 (1H, dd, J=12.5, 2.4 Hz, H-2), δ 2.91 (1H, dd, J=16.5, 12.5 Hz, H-3ax), and δ 2.58 (1H, dd, J=16.5, 2.4 Hz, H-3eq) suggested that the C ring was saturated. This splitting pattern was due to the coupling between the H-2 axial proton and the H-3 geminal protons. The absolute configuration at the C-2 stereocenter was established to be *S* on the basic of the high-amplitude, negative Cotton effect in the 270—300 nm region and the weak positive Cotton effect in the 325—350 nm region,[13] which indicated that stereospecificity was achieved in the process of C-ring cyclization.[14] The structure of **3** was also established by

Table 3. DPPH Radical Scavenging Activity of Compounds **1**—**4**

Sample	IC_{50} (μM)[a)]
1	22.8
2	117.2
3	27.1
4	105.8
α-Tocopherol[b)]	20.1

a) IC_{50} values were calculated from regression lines using five different concentrations in triplicate. *b*) Positive control.

comparing the 1D and 2D NMR spectra, including HMBC (Fig. 2), with those of (2*S*)-8-formyl-5-hydroxy-7-methoxy-6-methylflavanone isolated from the same plant.[2)] Thus, the structure of **3** was determined as (2*S*)-8-formyl-6-methylnaringenin.

Compound **4** was obtained as a white amorphous powder. The HR-ESI-MS spectrum showed the $[M+H]^+$ peak at *m*/*z* 477.2594, which established a molecular formula of $C_{23}H_{24}O_{11}$, indicating that it was a glycosylated derivative of **3**. In addition to all the signals assignable to **3**, the ^{1}H- and ^{13}C-NMR spectra of **4** showed seven and six characteristic signals of a sugar moiety in the region ranging from δ 3.27 to 4.96 and from δ 62.5 to 101.6, respectively. The sugar was assigned as glucopyranose on the basis of NMR data and the comparison of the *Rf* value with authentic glucose after enzymatic (naringinase) hydrolysis of **4**. The absolute configuration was determined to be D-glucose by GC. The $J_{H,H}$ value (7.5 Hz) of the anomeric proton (H-1″) indicated that glucose was linked *via* a β-linkage. The location of glucose was confirmed by HMBC correlation between H-1″ and C-7 (Fig. 2). On the basis of these data, the structure of **4** was assigned as (2*S*)-8-formyl-6-methylnaringenin 7-*O*-β-D-glucopyranoside.

Compounds **1**—**4** were tested for their *in vitro* antioxidant activity using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay (Table 3). Compounds **1** and **3** exhibited DPPH radical scavenging activity with inhibitory concentration (IC_{50}) values of 22.8 and 27.1 μM, respectively, while compounds **2** and **4** exhibited weak activity with IC_{50} values of 117.2 and 105.8 μM, respectively.

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO DIP 1000 digital polarimeter. UV spectra were recorded on a JASCO V-530 spectrophotometer, and CD spectra were recorded on a JASCO J-715 CD/ORD spectropolarimeter. IR spectra were obtained on a JASCO FT/IR 300-E spectrometer. NMR experiments were conducted on a Varian Unity INOVA 400 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, and tetramethylsilane was used as the internal standard. ESI-MS and HR-ESI-MS analyses were performed on a Micromass QTQF2 mass spectrometer. EI-MS spectra were obtained on a JEOL JMS-SX102A spectrometer. TLC was carried out on Merck silica gel F_{254}-precoated glass plates and RP-18 F_{254S} plates. HPLC was performed on a Waters 600E multisolvent delivery system connected to a UV detector using Supelco Supelcosil LC-SI (5 μm, 10×250 mm) and Isco Allsphere ODS-2 (10 μm, 10×250 mm) semipreparative columns.

Plant Material The buds of *Cleistocalyx operculatus* were purchased in Dong Xuan herbarium market, Hanoi, Vietnam, in July 2007 and identified by Professor Pham Thanh Ky, Department of Pharmacognosy, Hanoi College of Pharmacy. A voucher specimen (HN-0160) was deposited in the herbarium of the Hanoi College of Pharmacy.

Extraction and Isolation The buds (0.8 kg) were extracted with 2 l of MeOH, three times. The MeOH extract was combined and concentrated to yield a residue which was suspended in water and then successively partitioned with hexane, EtOAc, and BuOH. The EtOAc-soluble fraction (15.3 g) was separated by silica gel column chromatography using a gradient of hexane–EtOAc (from 30 : 1 to 5 : 1), then EtOAc–MeOH (from 20 : 1 to 1 : 1), to yield five fractions (E1—E5) according to their TLC profiles. Fraction E4 (0.8 g) was chromatographed over silica gel column using a gradient of EtOAc–MeOH (from 10 : 1 to 5 : 1), to yield five subfractions E4.1—E4.5. The E4.3 fraction was further purified by semi preparative HPLC [RS Tech Optima Pak C_{18} column (10×250 mm, 10 μm particle size); mobile phase MeOH–H_2O (85 : 15); flow rate 2 ml/min; UV detection at 254 nm] to obtain compound **1** (8.3 mg; t_R=26.4 min), and **3** (5.6 mg, t_R=31.4 min). The E5 fraction was separated by reversed-phase C_{18} (RP-18) column chromatography using a stepwise gradient of MeOH–H_2O (from 50 : 50 to 100 : 0 for each step), to afford ten subfractions (E5.1—E5.10). Fraction E5.3 was purified by semi preparative HPLC using the same condition above except that the gradient of mobile phase (50 to 100% MeOH in water over 50 min) to afford compound **2** (14.1 mg, t_R=18.2 min). Fraction E5.5 was purified by semi preparative HPLC using the same condition with that employed for compound **2** to afford compound **4** (12.7 mg, t_R=22.8 min).

3′-Formyl-4′,6′,4-trihydroxy-2′-methoxy-5′-methylchalcone (**1**): Yellow amorphous powder; UV λ_{max} (MeOH) nm (ε): 280 (4.12), 321 (3.87); IR (KBr) cm^{-1}: 3452, 2873, 2758, 1620, 1547, 1465, 780, 705; ESI-MS *m*/*z*: 329 $[M+H]^+$; HR-ESI-MS *m*/*z*: 329.1813 $[M+H]^+$ (Calcd for $C_{18}H_{16}O_6$: 329.1820); for ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

3′-Formyl-6′,4-dihydroxy-2′-methoxy-5′-methylchalcone 4′-*O*-β-D-Glucopyranoside (**2**): Yellow amorphous powder; $[\alpha]_D^{22}$ −125.5 (*c*=0.2, MeOH); UV λ_{max} (MeOH) nm (ε): 285 (4.05), 325 (3.80); IR (KBr) cm^{-1}: 3400, 2958, 2760, 1625, 1606, 1518, 1416, 1280, 1175, 1076 cm^{-1}; ESI-MS *m*/*z*: 491 $[M+H]^+$; HR-ESI-MS *m*/*z*: 491.1585 $[M+H]^+$ (Calcd for $C_{24}H_{26}O_{11}$: 491.1586); for ^{1}H- and ^{13}C-NMR spectral data, see Table 1.

(2*S*)-8-Formyl-6-methylnaringenin (**3**): Yellow amorphous powder; $[\alpha]_D^{22}$ +58.8 (*c*=0.2, MeOH); UV λ_{max} (MeOH) nm (ε): 232 (4.15), 270 (4.37), 385 (3.90); IR (KBr) cm^{-1}: 3425, 2870, 2770, 1686, 1630, 1527, 1385, 1278, 1090; ESI-MS *m*/*z*: 315 $[M+H]^+$; HR-ESI-MS *m*/*z*: 315.2788 $[M+H]^+$ (Calcd for $C_{17}H_{14}O_6$: 315.2790); for ^{1}H- and ^{13}C-NMR spectral data, see Table 2.

(2*S*)-8-Formyl-6-methylnaringenin 7-*O*-β-D-Glucopyranoside (**4**): Yellow amorphous powder; $[\alpha]_D^{22}$ −120.7 (*c*=0.2, MeOH); UV λ_{max} (MeOH) nm (ε): 230 (4.08), 272 (4.35), 335 (3.85); IR (KBr) cm^{-1}: 3430, 2870, 2770, 1685, 1640, 1529, 1380, 1276, 1086; ESI-MS *m*/*z* 477 $[M+H]^+$; HR-ESI-MS *m*/*z* 477.2590 $[M+H]^+$ (Calcd for $C_{23}H_{24}O_{11}$: 477.2594); for ^{1}H- and ^{13}C-NMR spectral data, see Table 2.

Enzymatic Hydrolysis of 2 and 4 Naringinase (100 mg, from *Penicillium decumbens*) was added to a suspension of **2** and **4** (5 mg) in 50 mM acetate buffer (pH 5.5), and the mixture was stirred at 37 °C for 5 h. The reaction mixture was extracted with EtOAc (10 ml×3), and the organic layer was evaporated to dryness. The residue was chromatographed on a preparative-TLC with $CHCl_3$–MeOH (9 : 1) to give **1** (1.5 mg, *Rf*, 0.62) and **3** (1.8 mg, *Rf* 0.55). The water layer was checked by silica gel TLC (EtOAc–MeOH–H_2O–AcOH, 65 : 20 : 15 : 15). The spot on the TLC plate was visualized by an anisaldehyde–H_2SO_4 reagent. The configuration of glucose was determined by a GC method,[15)] and the sugar derivative thus obtained showed a retention time of 21.30 min, identical with that of authentic D-glucose.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity The DPPH radical-scavenging activity was measured using a method described previously.[16)] Briefly, 10 μl of each sample dissolved in DMSO was prepared in 96-well plates, and then 190 μl of 200 μM ethanolic DPPH solution was added. The mixture was incubated at room temperature for 30 min, and the absorbance of the reaction mixture was measured at 517 nm.

Acknowledgements The authors acknowledge the financial support provided by Hanoi University of Pharmacy, Hanoi, Vietnam and Gyeongbuk Regional Innovation Agency (2007), Korea.

References

1) Loi D. T., "Vietnamese Medicinal Plants and Ingredients," Medical Publishing House, Hanoi, 2001, pp. 423—424.
2) Ye C. L., Lu Y. H., Wei D. Z., *Phytochemistry*, **65**, 445—447 (2004).
3) Woo A. Y., Waye M. M., Kwan H. S., Chan M. C., Chau C. F., Cheng C. H., *Vasc. Pharmacol.*, **38**, 163—168 (2002).
4) Lu Y. H., Du C. B., Wu Z. B., Ye C. L., Liu J. W., Wei D. Z., *Zhongguo Zhong Yao Za Zhi*, **28**, 964—966 (2003).
5) Mai T. T., Chuyen N. V., *Biosci. Biotechnol. Biochem.*, **71**, 69—76 (2007).
6) Nomura M., Yamakawa K., Hirata Y., Niwa M., *Shoyakugaku Zasshi*,

47, 408—410 (1993).
7) Zhang F. X., Liu M. F., Lu R. R., *Zhiwu Xuebao*, **32**, 469—472 (1990).
8) Dung N. X., Luu H. V., Khoi T. T., Leclercq P. A., *J. Essent. Oil Res.*, **6**, 661—662 (1994).
9) Ye C. L., Liu J. W., Wei D. Z., Lu Y. H., Qian F., *Cancer Chemother. Pharmacol.*, **56**, 70—74 (2005).
10) Ye C. L., Liu Y., Wei D. Z., *J. Pharm. Pharmacol.*, **59**, 553—559 (2007).
11) Ye C. L., Qian F., Wei D. Z., Lu Y. H., Liu J. W., *Leuk. Res.*, **29**, 887—892 (2005).
12) Norbedo C., Ferraro G., Coussio J. D., *J. Nat. Prod.*, **45**, 635—636 (1982).
13) Gaffield W., *Tetrahedron*, **26**, 4093—4108 (1970).
14) Karikome H., Mimaki Y., Sashida Y., *Phytochemistry*, **30**, 315—319 (1991).
15) Zhao J., Nakamura N., Hattori M., Kuboyama T., Tohda C., Komatsu K., *Chem. Pharm. Bull.*, **50**, 760—765 (2002).
16) Min B. S., Na M. K., Oh S. R., Ahn K. S., Jeong G. S., Li G., Lee S. K., Joung H., Lee H. K., *J. Nat. Prod.*, **67**, 1980—1984 (2004).