Triterpenoid Saponins from the Seeds of *Pharbitis nil*

Da Young JUNG,^a Hyekyung HA,^a Ho Young LEE,^a Chungsook KIM,^a Je-Hyun LEE,^b KiHwan BAE,^c Ju Sun KIM,^d and Sam Sik KANG^{*,d}

^aDepartment of Drug Research & Development, Korea Institute of Oriental Medicine, Daejeon 305–811, Korea; ^bCollege of Oriental Medicine, Dongguk University, Gyeongju 780–714, Korea; ^cCollege of Pharmacy, Chungnam National University, Daejeon 305–764, Korea; and ^dNatural Products Research Institute and College of Pharmacy, Seoul National University, Seoul 151–747, Korea. Received August 17, 2007; accepted October 19, 2007

From the seeds of *Pharbitis nil* (Convolvulaceae), two new oleanene-type triterpene glycosides, pharbitosides A (1) and B (2), together with β -sitosterol, β -sitosterol glucoside (daucosterol), caffeic acid, and methyl caffeate were isolated. The structure of pharbitoside A (1) was elucidated to be queretaroic acid 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside (1). Pharbitoside B (2) is a 21 α -hydroxyoleanolic acid saponin carrying the same sugar moiety as that of pharbitoside A (1).

Key words *Pharbitis nil*; Convolvulaceae; triterpenoid saponin; pharbitoside

Pharbitis nil CHOISY [= *Ipomoea nil* (L.) ROTH] is found throughout Southeast Asia. The seeds and the resin extracted from the plant are medicinal. The main use is as a purgative.¹⁾ The Japanese morning glory has been domesticated as an ornamental plant in Japan. There have been several reports on the isolation of resin glycosides,^{2–4)} a diterpenoid, pharbitic acid,⁵⁾ flavonoids, caffeic acid, and chlorogenic acid derivatives,⁶⁾ and anthocyanins^{7–10)} from the seeds and flowers of this plant but there has been no report on the chemical investigation of the saponins in the seeds. In continuation of our studies on phytochemical studies on naturally occurring bioactive glycosides,^{11–14)} we became interested in the saponin content of the seeds of the plant. This paper describes the structural determination of the two new saponins isolated from the MeOH extract of the seeds.

The powdered seeds of *P. nil* were sonicated with MeOH and partitioned as described in the Experimental. β -Sitosterol and its glucoside and caffeic acid and its methyl ester were isolated by successive chromatography using silica gel and reverse-phase C₁₈ from the hexane and EtOAc fractions, respectively. The BuOH-soluble fraction after repeated chromatographic purification over MCI gel and reverse-phase C₁₈ afforded two compounds designated pharbitosides A (1) and B (2).

Pharbitoside A (1) was obtained as colorless crystalline needles. High-resolution (HR) FAB-MS gave the composition C₄₈H₇₈O₁₈. An inspection of the ¹H- and ¹³C-NMR spectra of the compound readily indicated the presence of three monosaccharide units through easily identifiable signals for anomeric protons and carbons. Acid hydrolysis of 1 afforded glucose (Glc) and rhamnose (Rha) as the sugar components identified on TLC analysis by comparison with authentic samples. The absolute configurations of sugars were determined to be the L-form for Rha and D-form for Glc, respectively, which were identified by GLC analysis of the thiazolidine derivatives.¹⁵⁾ The negative-ion mode FAB mass spectrum of 1 exhibited an [M–H][–] ion at *m/z* 941, which is consistent with a trisaccharide glycoside carrying two Glc, one Rha, and an aglycon with a molecular mass of 472. The fragments at *m/z* 795 [(M–H)–146][–], 633 [(M–H)–146–162][–], and 471 [(M–H)–146–162–162][–] showed the presence of a linear sugar chain, and the sugar sequence appeared

to be that of Glc–Glc–Rha.¹⁶⁾ The spectroscopic data of the aglycon moiety of 1 were closely related to those of oleanolic acid¹⁷⁾; however, the molecular formula of the aglycon moiety was higher by one oxygen atom than that of oleanolic acid, implying the presence of one more hydroxyl group in addition to the C-3 hydroxyl group. The oxygenated methylene protons at δ 3.93 (d, *J* = 10.5 Hz) and 4.01 (d, *J* = 10.5 Hz) correlated with δ 65.6 in the heteronuclear multiple-quantum correlation (HMQC) spectrum and showed heteronuclear multiple-bond correlation (HMBC) correlations with C-19 (δ 42.1), C-20 (δ 35.9), and C-29 (δ 28.5), indicating the presence of a hydroxymethyl group at C-20. These results indicate that either the C-29 or the C-30 methyl group was hydroxylated. C-29 hydroxylation gives mesembryanthemoidigenic acid,¹⁸⁾ while C-30 hydroxylation gives queretaroic acid.¹⁹⁾ Comparison of the carbon chemical shift for the hydroxymethyl signal (δ 65.6) with both model compounds (δ 73.9 of C-29 for mesembryanthemoidigenic acid vs. 65.5 of C-30 for queretaroic acid) supported the identification as queretaroic acid. The glucosyl unit was shown to be directly attached at C-3 of the aglycon by an HMBC correlation between the signal of the anomeric proton of the glucosyl unit at δ 4.96 and C-3 of the aglycon at δ 89.6. The anomeric proton of the terminal rhamnosyl moiety at δ 6.43 showed a ³*J*_{C,H} correlation with C-2 of the inner glucosyl residue at δ

* To whom correspondence should be addressed. e-mail: sskang@snu.ac.kr

78.5, the anomeric proton of which at δ 5.86 in turn showed a long-range correlation with C-2 of the glucosyl moiety at δ 79.0. The ^1H -NMR signal multiplet patterns and coupling constants (Table 1) allowed the identification of an α -L-rhamnopyranosyl unit and two β -D-glucopyranosyl units in **1**. In light of the above observations, the structure of the pharbitoside A (**1**) was determined to be queretaroic acid 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside.

Pharbitoside B (**2**) was shown to have the same molecular formula $\text{C}_{48}\text{H}_{78}\text{O}_{18}$ as that of **1** on the basis of the (–)-HR-FAB-MS. Acid hydrolysis of **2** gave sugars identified as L-Rha and D-Glc as described in **1**.¹⁵ The sequence of the sugars and binding site at the aglycon of **2** were determined based on two-dimensional (2D) NMR experiments. These results indicate that **2** has the same sequence of sugar linkages as **1**. In the ^{13}C -NMR spectrum, the signal due to the C-30 hydroxymethyl carbon at δ 65.6 in **1** was displaced by a methyl carbon at δ 24.9 in **2**. All other signals were almost superimposable between **1** and **2**, with the exception of signals for C-16, 21, and C-22 of the aglycon, as shown in Tables 1 and 2. The hydroxymethine proton at δ 3.82 (t, $J=3.0$ Hz, H-21) showed spin couplings with the methylene protons at δ 2.38 (dd, $J=3.0$, 14.5 Hz, H-22a) and δ 2.40 (dd, $J=3.0$, 14.5 Hz, H-22b) in the ^1H - ^1H correlation spectroscopy (COSY) spectrum and HMBC correlation with C-

17 (δ 46.6), indicating the presence of a hydroxyl group at C-21. The configuration of the C-21 hydroxyl group was evident from the chemical shift and the small J value of H-21 (1H, t, $J=3.0$ Hz), characteristic of an equatorial proton. Comparison of the ^{13}C -NMR data of the aglycon moiety of **2** with oleanolic acid¹⁷ showed that the signals for C-19 and 29- CH_3 of the aglycon were significantly shifted upfield by -5.1 ppm, respectively, due to a γ -gauche effect,²⁰ which was consistent with the presence of the C-21 axial (α) hydroxyl group. Therefore, the structure of the aglycon was determined to be 21 α -hydroxyoleanolic acid.²¹ Thus the structure of the pharbitoside B (**2**) was determined to be 21 α -hydroxyoleanolic acid 3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside. In addition, β -sitosterol glucoside (daucosterol), and β -sitosterol²² from the hexane fraction and caffeic acid^{23,24} and its methyl ester²⁵ from the EtOAc fraction were isolated and identified by comparison of their physical and spectral data with those previously reported.

The presence of saponins from a plant belonging to the family Convolvulaceae is an unusual finding, since alkaloids, resin glycosides, and flavonoids are the typical secondary metabolites biosynthesized by the genera of this family.²⁶ Moreover, pharbitosides A (**1**) and B (**2**) have unusual aglycones, queretaroic acid and 21 α -hydroxyoleanolic acid, respectively. To date, only one saponin containing 21 α -hydroxy-

Table 1. NMR Spectroscopic Data (500 MHz, Pyridine- d_5) for Pharbitosides A (**1**) and B (**2**) (Aglycon Moieties)

Position	Pharbitoside A (1)			Pharbitoside B (2)		
	δ_{C} mult.	δ_{H} (J in Hz)	HMBC	δ_{C} mult.	δ_{H} (J in Hz)	HMBC
1	38.7, CH_2	0.91, brt (14.5); 1.38		38.5, CH_2	0.94, brt (14.5); 1.46	
2	26.4, CH_2	1.74–1.80, m; 2.15		26.2, CH_2	1.79; 2.18	
3	89.6, CH	3.33, dd (4.5, 12.0)	4, 23, 24, 1'	89.4, CH	3.35, dd (4.5, 12.0)	4, 23, 24, 1'
4	39.6, qC	—		39.4, qC	—	
5	55.9, CH	0.82		55.7, CH	0.82	24, 25
6	18.5, CH_2	1.38; 1.51, m		18.3, CH_2	1.50	
7	33.2, CH_2	1.30–1.37		33.1, CH_2	—	
8	39.8, qC	—		39.4, qC	—	
9	48.0, CH	1.66, brt (9.0)	1, 26	47.8, CH	1.70, t (9.0)	8, 10, 11, 14, 25, 26
10	37.0, qC	—		36.8, qC	—	
11	23.8, CH_2	1.51; 1.86		23.6, CH_2	1.96	
12	122.6, CH	5.48, brs		122.2, CH	5.58, t (4.0)	9, 14
13	144.9, qC	—		144.9, qC	—	
14	42.2, qC	—		42.3, qC	—	
15	28.4, CH_2	1.20; 2.23, brt (12.0)		28.6, CH_2	2.13, ddd (4.0, 14.5, 14.5)	
16	24.1, CH_2	2.05; 2.18	28	27.2, CH_2	3.13, ddd (4.0, 14.5, 14.5); 2.36	
17	46.6, qC	—		46.6, qC	—	
18	41.6, CH	3.45, br dd (4.0, 12.0)	12, 13, 17, 19, 28	41.9, CH	3.52, br dd (3.5, 14.5)	13, 17
19	42.1, CH_2	1.82		41.5, CH_2	2.62, t (14.0); 1.30, dd (3.0, 14.0)	18, 20, 29, 30
20	35.9, qC	—		35.7, qC	—	
21	29.7, CH_2	1.87		73.5, CH	3.82, t (3.0)	17
22	33.0, CH_2	1.88; 2.22	28	40.2, CH_2	2.38, dd (3.0, 14.5); 2.40, dd (3.0, 14.5)	
23	28.5, CH_3	1.38, s	3, 4, 5, 24	28.2, CH_3	1.38, s	3, 4, 5, 24
24	16.9, CH_3	1.09, s	3, 4, 5, 23	16.8, CH_3	1.11, s	3, 4, 5, 23
25	15.4, CH_3	0.78, s	5, 9, 10	15.3, CH_3	0.83, s	1, 5, 9, 10
26	17.4, CH_3	0.98, s	7, 8, 9, 14	17.3, CH_3	1.03, s	8, 9, 14
27	26.2, CH_3	1.35, s	8, 13, 15	25.7, CH_3	1.42, s	13, 14, 15
28	180.3, qC	—		180.0, qC	—	
29	28.5, CH_3	1.24, s	19, 20, 21, 30	28.3, CH_3	1.28, s	19, 20, 21, 30
30	65.6, CH_2	3.93, d (10.5); 4.01, d (10.5)	19, 20, 29	24.9, CH_3	1.16, s	19, 20, 21, 29

Table 2. NMR Spectroscopic Data (500 MHz, Pyridine-*d*₅) for Pharbitosides A (1) and B (2) (Sugar Moieties)

Position	Pharbitoside A (1)			Pharbitoside B (2)		
	δ_C mult.	δ_H (J in Hz)	HMBC	δ_C mult.	δ_H (J in Hz)	HMBC
1'	105.1, CH	4.96, d (7.5)	3	105.5, CH	4.98, d (7.5)	3
2'	79.0, CH	4.43, t (8.0)	1', 3', 1''	79.5, CH	4.46, dd (7.5, 9.0)	3', 1''
3'	79.5, CH	4.56, t (9.0)	2'	79.9, CH	4.57, t (9.5)	2', 4', 5'
4'	72.9, CH	4.09, t (9.0)	3', 6'	72.4, CH	4.11, t (9.5)	5', 6'
5'	77.9, CH	3.94, m		78.4, CH	3.95, m	4'
6'	62.8, CH ₂	4.37, dd (5.5, 12.0)		63.3, CH ₂	4.39, dd (5.0, 11.5)	
		4.52, dd (2.0, 12.0)			4.54, dd (2.0, 11.5)	
1''	102.0, CH	5.86, d (7.5)		102.5, CH	5.87, d (7.5)	2''
2''	78.5, CH	4.33, t (9.0)	3'', 1'''	79.0, CH	4.34, t (8.0)	3'', 1'''
3''	79.5, CH	4.25, t (9.0)	2''	80.0, CH	4.26, t (9.5)	2''
4''	72.4, CH	4.10, t (9.0)	3'', 6''	73.3, CH	4.10, t (9.5)	3'', 6''
5''	77.6, CH	3.85, m		78.0, CH	3.86, m	
6''	63.5, CH ₂	4.48, dd (3.0, 11.5)		63.9, CH ₂	4.49, dd (3.0, 11.5)	
		4.31, dd (6.0, 11.5)			4.32, dd (6.0, 11.5)	
1'''	102.1, CH	6.43, br s	2'', 3'', 5'''	102.5, CH	6.44, s	2'', 2''', 5'''
2'''	72.0, CH	4.77, dd (1.5, 3.5)	3''', 4'''	72.9, CH	4.78, dd (1.5, 3.5)	4'''
3'''	72.7, CH	4.71, dd (3.5, 9.5)	2''', 4'''	73.2, CH	4.71, dd (3.5, 9.5)	4'''
4'''	74.4, CH	4.35, t (9.5)	2''', 5''', 6'''	74.8, CH	4.38, t (9.5)	2''', 5''', 6'''
5'''	69.6, CH	5.06, m	4''', 6'''	70.1, CH	5.06, m	4'''
6'''	19.1, CH ₃	1.82, d (6.5)	4'', 5'''	18.8, CH ₃	1.82, d (6.5)	4'', 5'''

oleanolic acid as an aglycon was isolated from the roots of *Clematis chinensis* (Ranunculaceae).²¹⁾

Experimental

The optical rotations were determined on a JASCO P-1020 polarimeter. The IR spectra were recorded on a JASCO FT/IR-5300 spectrometer. FAB mass spectra were obtained in a glycerol matrix in negative-ion mode on a VG-VSEQ spectrometer. The NMR spectra were measured in pyridine-*d*₅ on a Varian Gemini 2000 instrument (300 MHz) or a Bruker AMX-500 instrument (500 MHz), and the chemical shifts were referenced to TMS. GC analysis was performed with a Hewlett Packard 5890 Series II gas chromatograph equipped with an H₂ flame ionization detector. The column was an HP-5 capillary column (30 m×0.32 mm×0.25 μm); column temperature, 200 °C; injector and detector temperature, 290 °C; and He flow rate, 30 ml/min. TLC was performed on silica gel 60 F₂₅₄ (Merck) and cellulose plates (art no. 5716, Merck).

Plant Material The seeds of *P. nil* were collected in September 2003 at Shinchun, Songpa-ku, Seoul, Korea, and authenticated by one of authors (J.-H. Lee). A voucher specimen (KIOM 03-3-20) was deposited in the Department of Herbal Pharmaceutical Medicine, Korea Institute of Oriental Medicine.

Extraction and Isolation The powdered seeds of *P. nil* (1.67 kg) were sonicated three times with 70% MeOH at room temperature. The MeOH extract (280 g) was evaporated under reduced pressure to dryness to give the precipitate, which was recrystallized from CHCl₃-MeOH to give β-sitosterol glucoside (30 mg). The MeOH extract was partitioned in succession between H₂O and hexane, EtOAc, and then *n*-BuOH, affording 7, 8, and 87 g of the respective extracts. The hexane-soluble fraction was subjected to column chromatography on silica gel eluted with CH₂Cl₂-MeOH (gradient) to give 15 subfractions. Subfraction 4 was recrystallized from CHCl₃-MeOH to give β-sitosterol (13 mg). The EtOAc-soluble fraction was chromatographed on silica gel with CH₂Cl₂-MeOH (gradient) as an eluent to yield 17 subfractions. Subfraction 4 was recrystallized from MeOH to give methyl caffeate (184 mg). Subfraction 9 was repeatedly chromatographed on LiChroprep RP-18 with 80% aqueous MeOH as eluent to yield caffeic acid (34 mg). The *n*-BuOH-soluble fraction was passed through a porous polymer MCI gel (CHP 20P, 75–150 μ) column. After washing the column with water, the adsorbed materials were eluted successively with 70%, 80%, and 90% aqueous MeOH and MeOH to give 15 subfractions. Subfraction 4 was subjected to column chromatography on RP-18 eluted with 70% aqueous MeOH to give compounds 1 (28 mg) and 2 (21 mg). Spectra measured for the isolated compounds of known structures were in good agreement with the reported data for β-sitosterol, β-sitosterol glucoside (daucosterol),²²⁾ caffeic acid,^{23,24)} and methyl caffeate.²⁵⁾

Pharbitoside A (1): $[\alpha]_D^{21} -0.4^\circ$ (*c*=0.14, MeOH). (–)-HR-FAB-MS *m/z*: 941.5125. Calcd for C₄₈H₇₇O₁₈: 941.5110. FAB-MS *m/z*: 941 [M–H][–], 795 [(M–H)–146][–], 633 [(M–H)–146–162][–], 471 [(M–H)–146–162–162][–]. NMR data (pyridine-*d*₅, 500 MHz): Tables 1 and 2.

Pharbitoside B (2): $[\alpha]_D^{21} -12.0^\circ$ (*c*=0.21, MeOH). (–)-HR-FAB-MS *m/z*: 941.5133. Calcd for C₄₈H₇₇O₁₈: 941.5110. FAB-MS *m/z*: 941 [M–H][–], 795 [(M–H)–146][–], 633 [(M–H)–146–162][–], 471 [(M–H)–146–162–162][–]. NMR data (pyridine-*d*₅, 500 MHz): Tables 1 and 2.

Acid Hydrolysis of 1 and 2 Saponins 1 and 2 (2 mg each) were refluxed separately with 5% HCl in 60% aqueous dioxane (10 ml) for 2 h. The reaction solution was evaporated under reduced pressure, and the hydrolysate was extracted with ether. The aqueous layer was neutralized with Ag₂CO₃, filtered, and the filtrate was concentrated under reduced pressure. The residue was compared with standard sugars using cellulose TLC [pyridine–EtOAc–HOAc–H₂O (36:36:7:21)], which showed the sugars to be Rha and Glc in both cases. The spots on TLC were visualized by spraying with aniline phthalate reagent followed by heating.

Determination of the Absolute Configuration of Sugars of 1 and 2

The dried sugar mixture was dissolved in pyridine (0.1 ml), and then the solution was added to a pyridine solution (0.1 ml) of L-cysteine methyl ester hydrochloride (2 mg) and warmed at 60 °C for 1 h. The solvent was evaporated under a N₂ stream and dried *in vacuo*. The residue was trimethylsilylated with TMS-HT (0.1 ml) at 60 °C for 30 min. After the addition of hexane and water, the hexane layer was removed and checked with GC. The retention times (*t*_R) of the peaks were 23.73 min (L-Rha) and 37.87 min (D-Glc). The *t*_R of the peaks of the authentic samples were 37.87 min (D-Glc), 39.90 and 40.65 min (L-Glc), and 23.73 min (L-Rha), respectively.

Acknowledgments This work was supported by a grant (PF0321102-00) from the Plant Diversity Research Center of the 21st century Frontier Research Program funded by the Ministry of Science and Technology of Korea. Our thanks are due to staff members of the National Center for Inter-University Research Facilities (NCIRF) of our University for NMR (500 MHz) and FAB-MS measurements.

References

- 1) Bensky D., Gamble A., "Chinese Herbal Medicine: Materia Medica," revised ed., Eastland Press, Seattle, 1993, p. 121.
- 2) Kawasaki T., Okabe H., Nakatsuka I., *Chem. Pharm. Bull.*, **19**, 1144–1149 (1971).
- 3) Okabe H., Koshito N., Tanaka K., Kawasaki T., *Chem. Pharm. Bull.*, **19**, 2394–2403 (1971).
- 4) Ono M., Noda N., Kawasaki T., Miyahara I., *Chem. Pharm. Bull.*, **38**, 1892–1897 (1990).
- 5) Yokota T., Yamazaki S., Takahashi N., Iitaka Y., *Tetrahedron Lett.*, **15**,

- 2957—2960 (1974).
- 6) Saito N., Cheng J., Ichimura M., Yokoi M., Abe Y., Honda T., *Phytochemistry*, **35**, 687—691 (1994).
 - 7) Saito N., Lu T. S., Yokoi M., Shigihara A., Honda T., *Phytochemistry*, **33**, 245—247 (1993).
 - 8) Saito N., Lu T. S., Akaizawa M., Yokoi M., Shigihara A., Honda T., *Phytochemistry*, **35**, 407—411 (1994).
 - 9) Saito N., Tatsuzawa F., Kasahara K., Yokoi M., Iida S., Shigihara A., Honda T., *Phytochemistry*, **41**, 1607—1611 (1996).
 - 10) Saito N., Toki K., Morita Y., Hoshino A., Iida S., Shigihara A., Honda T., *Phytochemistry*, **66**, 1852—1860 (2005).
 - 11) Zhao H. L., Sim J.-S., Shim S. H., Ha Y. W., Kang S. S., Kim Y. S., *Int. J. Obesity*, **29**, 983—990 (2005).
 - 12) Kim J. S., Shim S. H., Chae S.-W., Han S. J., Kang S. S., Son K. H., Chang H. W., Kim H. P., Bae K.-H., *Chem. Pharm. Bull.*, **53**, 696—700 (2005).
 - 13) Lee E. B., Kim O. J., Kang S. S., Jeong C.-S., *Biol. Pharm. Bull.*, **28**, 523—526 (2005).
 - 14) Ahn K. S., Noh E. J., Zhao H. L., Jung S. H., Kang S. S., Kim Y. S., *Life Sci.*, **76**, 2315—2328 (2005).
 - 15) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **35**, 501—506 (1987).
 - 16) Kang S. S., Kim J. S., Kim Y. H., Choi J. S., *J. Nat. Prod.*, **60**, 1060—1062 (1997).
 - 17) Seebacher W., Simic N., Weis R., Saf R., Kunert O., *Magn. Reson. Chem.*, **41**, 636—638 (2003).
 - 18) Shao C.-J., Kasai R., Xu J.-D., Tanaka O., *Chem. Pharm. Bull.*, **37**, 42—45 (1989).
 - 19) Ye Y., Kinoshita K., Koyama K., Takahashi K., Kondo N., Yuasa H., *J. Nat. Prod.*, **61**, 456—460 (1998).
 - 20) Eggert H., VanAntwerp C. L., Bhacca N. S., Djerassi C., *J. Org. Chem.*, **41**, 71—78 (1976).
 - 21) Mimaki Y., Yokosuka A., Hamanaka M., Sakuma C., Yamori T., Sashida Y., *J. Nat. Prod.*, **67**, 1151—1156 (2004).
 - 22) Xu Y. N., Kim J. S., Kang S. S., Son K. H., Kim H. P., Chang H. W., Bae K.-H., *Kor. J. Pharmacogn.*, **33**, 267—271 (2002).
 - 23) Islam M. S., Yoshimoto M., Yahara S., Okuno S., Ishiguro K., Yamakawa O., *J. Agric. Food Chem.*, **50**, 3718—3722 (2002).
 - 24) Choi C.-W., Jung H. A., Kang S. S., Choi J. S., *Arch. Pharm. Res.*, **30**, 1—7 (2007).
 - 25) Pieters L., Van Dyck S., Gao M., Bai R., Hamel E., Vlietinck A., Lemièrre G., *J. Med. Chem.*, **42**, 5475—5481 (1999).
 - 26) Species Index, “Dictionary of Natural Products,” Vol. 14, Chapman & Hall, London, 1994.