ent-Kaurane Glycosides from *Tricalysia okelensis*

Wen-Hui Xu,[a,b] Melissa Ruth Jacob,[a] Ameeta Kishore Agarwal,[a] Alice Mae Clark,[a,c] Zong-Suo Liang,[b] and Xing-Cong Li*[,a,c]

[a] *National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi;* [c] *Department Pharmacognosy, School of Pharmacy, The University of Mississippi; University, MS 38677, U.S.A.: and* [b] *College of Life Sciences, Northwest A & F University; Yangling, Shaanxi 712100, People's Republic of China.* Received October 9, 2009; accepted November 15, 2009; published online November 19, 2009

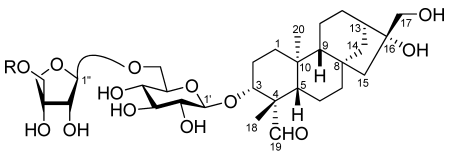
Tricalysiosides V and W, two new *ent*-kaurane glycosides with an acylated disaccharide moiety at the C-3 position, were isolated from the roots of *Tricalysia okelensis* and their structures established by spectroscopic and chemical methods as *ent*-kauran-3α,16α,17-triol-19-al 3-*O*-[5-*O*-vanilloyl-β-D-apiopyranosyl(1→6)]-β-D-glucopyranoside (1) and *ent*-kauran-3α,16α,17-triol-19-al 3-*O*-[5-*O*-*E*-sinapoyl-β-D-apiopyranosyl(1→6)]-β-D-glucopyranoside (2).

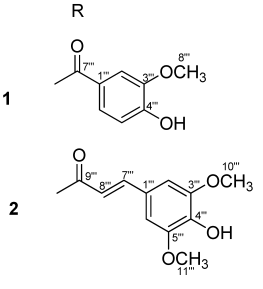
Key words *Tricalysia okelensis*; *ent*-kaurane glycoside; tricalysioside V; tricalysioside W

Tricalysia is a genus of the plant family Rubiaceae, with approximately 50 species distributed in subtropical and tropical regions in Asia and Africa, some of these used as folk medicine.[1,2] Extensive phytochemical studies on *Tricalysia dubia* led to the isolation of a number of structurally diverse diterpenoids (primarily with an *ent*-kaurane skeleton)[3—6] and their glycosides[2,7,8] and alkaloids,[9] while a bioassay-guided fractionation scheme identified the triterpenoids oleanolic acid and ursolic acid from *Tricalysia niamniamensis* that showed inhibitory activity against human ligase I.[10]

The organic extract of the roots of *Tricalysia okelensis* from the U.S. National Cancer Institute (NCI) Open Repository showed marginal antifungal activity but failed to produce enriched antifungal activity upon fractionation. A phytochemical investigation focusing on its diterpenoid constituents was thus conducted, leading to the identification of two new *ent*-kaurane glycosides, tricalysiosides V (**1**) and W (**2**). Herein we report the isolation and structure elucidation of the two compounds.

Column chromatography of the organic extract of *T. okelensis* on silica gel and reversed-phase silica gel, followed by HPLC purification afforded tricalysiosides V (**1**) and W (**2**) as an amorphous pale yellow powder. The positive-ion high-resolution electrospray ionization (ESI)-MS of compound **1** showed a pseudomolecular peak at *m/z* 803.3474 $[M+Na]^+$. In conjunction with the ^{13}C-NMR spectrum displaying 39 resonances, its molecular formula was determined as $C_{39}H_{56}O_{16}$. The ^{1}H- and ^{13}C-NMR spectroscopic data of **1** revealed the presence of a sugar moiety of two monosaccharides and one trisubstituted aroyl system with one methoxy group ($\delta_{H/C}$ 3.71/56.0), in addition to a diterpenoid aglycone moiety (Table 1). A distortionless enhancement by polarization transfer (DEPT) NMR experiment permitted differentiation of the 39 resonances into two singlet methyl, 13 methylene, 14 methine, nine quaternary, and one methoxy carbons. Upon acid hydrolysis, **1** afforded D-glucose and D-apiose. This was consistent with the anomeric protons of the β-D-glucopyranosyl and β-D-apiofuranosyl units at δ_H 4.96 (d, J=7.0 Hz) and 5.80 (br s) that correlated with anomeric carbons at δ_C 101.9, and 110.0, respectively, in the heteronuclear multiple quantum coherence (HMQC) spectrum. The presence of a vanilloyl moiety in **1** was evident from the eight resonances at δ_C 166.7 (s), 153.4 (s), 148.5 (s), 125.0 (d), 121.7 (s), 116.3 (d), 113.7 (d), and 56.0 (q, OMe),[11] which was further supported by the long range H–C correlations from the aromatic proton at δ_H 7.82 (1H, s, H-2‴) to the carbons at δ_C 166.7 (C-7‴), 153.4 (C-4‴), 148.5 (C-3‴), 125.0 (C-6‴), and 121.7 (C-1‴) and from the methoxy protons (δ_H 3.71) to C-3‴ in the heteronuclear multiple bonding connectivity (HMBC) spectrum. The IR spectrum of **1** also showed a strong aromatic ester absorption at 1677 cm^{-1}, in accordance with the UV maxima at 219 and 255 nm.[12] The vanilloyl moiety was proven to be attached to the C-5 hydroxy group of the apiosyl unit by the HMBC correlation between the methylene protons at δ_H 4.86 (2H) and the ester carbon at δ_C 166.7 (C-7‴) since these two protons also showed clear three-bond correlations with C-2″ and C-4″ within the apiosyl unit. It was noted that the acylation at this position resulted in the downfield shift of the two geminal protons of the apiosyl unit to different degrees, making them to converge at the same resonating position. The correlation between the anomeric proton of the apiose at δ_H 5.80 and the methylene carbon at δ_C 69.2 indicated that the apiosyl unit was linked to the C-6 hydroxy group of the glucosyl unit, while the correlation between the anomeric proton of the glucosyl unit at δ_H 4.96 and a carbon at δ_C 82.5 (CH) established the glucosyl unit to be attached to one hydroxy group of the diterpenoid moiety.





* To whom correspondence should be addressed. e-mail: xcli7@olemiss.edu

The proton and carbon resonances of the aglycone moiety of **1** included one typical aldehyde ($\delta_{H/C}$ 10.24/206.4), one methine ($\delta_{H/C}$ 3.88/82.5), one quaternary (δ_C 81.8), one methylene ($\delta_{H/C}$ 3.99, 4.06/66.6), and two tertiary methyl ($\delta_{H/C}$ 1.55/22.1 and 0.81/18.7) groups. Comparison of these and the remaining resonances with those of tricalysiosides H and I isolated from *T. dubia* [8] suggested that the aglycone possessed an *ent*-kaurane skeleton with 3α-OH, 16α-OH, 17-OH, and 19-CHO functionalities, which was confirmed by 2D NMR of HMBC and rotated frame Overhauser effect spectroscopy (ROESY) as follows. In the HMBC spectrum, the 19-CHO proton correlated with C-4 (δ_C 53.1) and Me-18 (δ_C 22.1), while the Me-18 protons (δ_H 1.55) showed cross peaks with C-4, C-5 (δ_C 57.6), C-19, and the carbon at δ_C 82.5 that is apparently C-3 of the aglycone. Thus, the sugar moiety was determined to be attached to the C-3 hydroxy group of the aglycone. The location of the oxygen-bearing C-16 quaternary carbon in the aglycone was evident from its HMBC correlation with H-15 at δ_H 1.74. The 16α,17-dihydroxy system in the aglycone was further confirmed by com-

Table 1. NMR Spectroscopic Data of **1** and **2** in Pyridine-d_5 (δ, ppm)[a]

Position	**1**				**2**	
	δ_H (J in Hz)	δ_C, mult.	HMBC	ROESY	δ_H (J in Hz)	δ_C, mult.
Aglycone						
1	0.91	37.9 (t)			0.92	37.9 (t)
2	2.17 α, d (12.5)	24.3 (t)		H: 19, 20	2.17 d (10.0)	24.3 (t)
	2.29 β, d (10.5)				2.29 d (10.0)	
3	3.88 dd (11.5, 4.0)	82.5 (d)	C: 5	H: 2β, 5, 1′	3.94	82.6 (d)
4		53.1 (s)				53.1 (s)
5	1.08 d (10.0)	57.6 (d)	C: 19	H: 18	1.10 d (10.0)	57.5 (d)
6	1.41	21.4 (t)	C: 8	H: 20	1.41	21.4 (t)
7	1.61 α	42.4 (t)	C: 6		1.60	42.4 (t)
	1.41 β		C: 8	H: 15β	1.41	
8		44.6 (s)				44.6 (s)
9	0.95 d (10.5)	55.3 (d)	C: 1, 8, 12, 14	H: 15β	0.96 d (10.0)	55.3 (d)
10		39.1 (s)				39.1 (s)
11	1.41	19.1 (t)	C: 8, 13		1.41	19.1 (t)
	1.55				1.57	
12	1.43	26.6 (t)			1.43	26.6 (t)
	1.81				1.81	
13	2.37	46.0 (t)		H: 14α	2.37 s	45.9 (t)
14	1.90 α	37.7 (t)		H: 13, 20	1.90	37.7 (t)
	1.78 β				1.78	
15	1.74 α	53.7 (t)	C: 7	H: 9	1.74	53.7 (t)
	1.61 β		C: 16		1.60	
16		81.8 (s)				81.8 (s)
17	3.99	66.6 (t)	C: 13		4.00	66.5 (t)
	4.06			H: 13	4.06	
18	1.55 s	22.1 (q)	C: 3, 4, 5, 19	H: 5	1.57 s	22.1 (q)
19	10.24 s	206.4 (d)	C: 3, 4, 18	H: 2α, 20	10.24 s	206.5 (d)
20	0.81 s	18.7 (q)	C: 1, 5, 9	H: 2α, 6, 14α	0.81 s	18.7 (q)
Glc-1′	4.96 d (7.0)	101.9 (d)	C: 3	H: 3, 6′	4.96 d (7.0)	102.0 (d)
2′	3.93	75.2 (d)	C: 1′		3.99	75.1 (d)
3′	4.22	78.7 (d)	C: 2′		4.22	78.6 (d)
4′	3.94	72.1 (d)	C: 2′, 3′, 5′		3.95	72.0 (d)
5′	4.09	77.5 (d)	C: 6′		4.08	77.4 (d)
6′	4.18	69.2 (t)		H: 1″	4.18	69.0 (t)
	4.70				4.70	
Api-1″	5.80 br s	111.0 (d)	C: 6′, 4″	H: 6′, 2″	5.80 br s	110.8 (d)
2″	4.68	78.4 (d)	C: 5″	H: 1″, 5″	4.62	78.3 (d)
3″		78.9 (s)				78.8 (s)
4″	4.41 d (9.0)	75.1 (t)	C: 1″, 2″, 4″		4.39 d (10.0)	75.1 (t)
	4.51 d (9.0)		C: 5″		4.48 d (10.0)	
5″	4.86	67.9 (t)	C: 2″, 4″, 7‴		4.81	67.7 (t)
Ar-1‴		121.7 (s)				125.3 (s)
2‴	7.82 s	113.7 (d)	C: 1‴, 3‴, 4‴, 6‴, 7‴	H: 5‴, 8‴	7.02 s	107.0 (d)
3‴		148.5 (s)				149.4 (s)
4‴		153.4 (s)				140.9 (s)
5‴	7.16 d (7.0)	116.3 (d)	C: 1‴, 3‴	H: 2‴		149.4 (s)
6‴	7.86 d (7.5)	125.0 (d)	C: 2‴, 4‴		7.02 s	107.0 (d)
7‴		166.7 (s)			7.94 d (16.0)	146.4 (d)
8‴	3.71 s	56.0 (q)	C: 3‴	H: 2‴	6.62 d (16.0)	115.3 (d)
9‴						167.5 (s)
10‴					3.85 s	56.6 (q)
11‴					3.85 s	56.6 (q)

a) Data recorded at 125 MHz for ^{13}C-NMR and 500 MHz for ^{1}H-NMR. Assignments were based on DEPT and 2D NMR spectra. Well-resolved couplings for ^{1}H-NMR data are expressed with coupling patterns and coupling constants in Hz in parentheses. Some geminal protons were denoted as a or b based on NOE evidence.

parison of the ^{13}C-NMR data with those of the *ent*-kaurane glycosides tricalysiolides A—C, E, and F[2] that possess such a system. A 16β,17-dihydroxy system in the *ent*-kaurane diterpenoids such as cussovantoside A[13] exhibits distinctly different chemical shifts for C-13, C-16, and C-17. The ROESY data of **1** also provided stereochemical information regarding the *ent*-kaurane skeleton: NOE correlations were observed between H-3β and H-5β, between Me-20α and 19-CHO, H-14α, H-2α, and H-6α, and between H-9β and H-15β. Other key NOE and HMBC correlations of **1** are shown in Table 1 and fully supports the structure *ent*-kauran-3α,16α,17-triol-19-al 3-*O*-[5-*O*-vanilloyl-β-D-apiopyranosyl (1→6)]-β-D-glucopyranoside.

Tricalysioside W (**2**) possesses a molecular formula of $C_{42}H_{60}O_{17}$ that was determined by the high-resolution ESI-MS (*m/z* 859.3777 Calcd for $[C_{42}H_{60}O_{17}+Na]^+$, 859.3723) and ^{13}C-NMR data. The NMR data of **2** due to the aglycone and sugar moieties were almost identical to those of **1** (Table 1). It appears that the only differences between **1** and **2** are the acyl moieties substituted at the C-5 hydroxy group of the apiosyl unit. Compared to **1**, the additional resonances in the ^{1}H-NMR spectrum at δ_H 7.94 and 6.62 (1H each, d, *J*= 16.0 Hz) that correlated with the carbon resonances at δ_C 146.4 (d) and 115.3 (d), respectively, in the HMQC spectrum indicated the presence of a typical *trans*-double bond system in the acyl moiety of **2**. In conjunction with other resonances at δ_H 7.02 (s, 2H) and 3.85 (s, 6H) and δ_C 167.5 (s), 149.4 (s, 2C), 146.4 (d), 140.9 (s), 125.3 (s), 115.3 (d), 107.0 (d), and 56.6 (q, OMe×2), the presence of a *trans*-sinapoyl moiety[14] in **2** was confirmed. Thus, the structure of **2** was established as *ent*-kauran-3α,16α,17-triol-19-al 3-*O*-[5-*O*-*E*-sinapoyl-β-D-apiopyranosyl(1→6)]-β-D-glucopyranoside. The assigment of its NMR data (Table 1) was facilitated by the HMQC and HMBC experiments and comparison with those of compound **1**.

ent-Kaurane diterpenoids are particularly rich in Labiatae plants,[15] with more than 500 compounds from the single *Isodon* genus.[16] However, there are only a few *ent*-kaurane 3-*O*-glycosides reported in the literature.[17—23] This is the first report of such compounds from the *Tricalysia* genus.

Experimental

General Experimental Procedures Optical rotations were measured with an Autopol IV polarimeter. UV was obtained from an HP 8453 diode array spectrophotometer. IR spectra were recorded using a Thermo Nicolet IR 300 FT/IR spectrometer. The 1D and 2D NMR (COSY, HMQC, HMBC and ROESY) spectra using standard pulse programs were recorded at room temperature in pyridine-d_5 on a Bruker Avance DRX 500 FT spectrometer operating at 500 (^{1}H) or 125 (^{13}C) MHz. The chemical shift values are relative to the NMR solvent residue ($\delta_{H/C}$ 8.73/149.9). ESI-MS data were obtained on an Agilent Series 1100 SL mass spectrometer. Column chromatography was performed using normal phase silica gel (J. T. Baker, 40 μm) and reversed-phase silica gel (RP-18, J. T. Baker, 40 μm). Semi-preparative HPLC was conducted on a C_{18} column (Gemini, 250×10 mm, 5 μm) with UV detection at 254 nm. Analytical HPLC was performed on a C_{18} column (Gemini, 4.6×150 mm, 5 μm) with UV detection at 250 nm. TLC was carried out on silica gel sheets (Alugram® Sil G/UV$_{254}$, Macherey-Nagel, Germany) and reversed-phase glass plates (RP-18 F$_{254S}$, Merck, Germany) with visualization by UV at 254 nm or spraying with 10% H_2SO_4 followed by heating.

Plant Material The roots of *Tricalysia okelensis* was collected in South Sandwick Islands Park Manovo-Gounda-St. Floris, Central Africa Republic (coordinates: 21°12′00″E, 08°29′00″N) on May 25, 1987, and identified by J. M. Fay. A voucher specimen is stored at National Smithsonian Institute in Washington D.C., U.S.A.

Extraction and Isolation The powdered, air-dried roots of *T. okelensis* was extracted with CH_2Cl_2–MeOH (1 : 1) under the standard extraction protocol at NCI. The crude extract (7.1 g) was subjected to silica gel chromatography (4.5×45 cm) using stepwise gradient elution $CHCl_3$/MeOH at 20 : 1 (1000 ml), 10 : 1 (900 ml), 8 : 1 (900 ml), 6 : 1 (700 ml), 4 : 1 (800 ml), 2 : 1 (600 ml), 1 : 1 (300 ml), and finally with MeOH (500 ml) to afford 11 pooled fractions (A—L) according to TLC. Fr. F (900 mg) was chromatographed on a C_{18} reversed-phase column (3.0×35 cm) using CH_3CN/H_2O at 40 : 60 (1800 ml), 50 : 50 (2000 ml), 60 : 40 (1000 ml), 70 : 30 (600 ml), and finally with MeOH (400 ml) to afford 8 pooled fractions according to TLC. Fr. 3 (102 mg) was further purified on a semi-preparative C_{18} reversed-phase HPLC column using 24% CH_3CN/H_2O (0.1% trifluoroacetic acid (TFA)), at a flow rate of 4 ml/min, to afford compound **2** (7.1 mg, t_R 12.1 min). Fr. G (750 mg) was similarly chromatographed on a C_{18} reversed-phase column (3.0×30 cm) using a stepwise gradient elution of MeOH/H_2O at 30 : 70 (1800 ml), 40 : 60 (2100 ml), and 50 : 50 (1000 ml) to give 7 pooled fractions. Fr. 5 (48 mg) was further purified on a semi-preparative C_{18} reversed-phase HPLC column using 22% CH_3CN/H_2O (0.1% TFA), at a flow rate of 4 ml/min, to afford compound **1** (17.6 mg, t_R 12.4 min).

Compound **1**: Amorphous pale yellow powder; $[\alpha]_D^{25}$ −42.4 (*c*=0.03, MeOH); UV (MeOH) λ_{max} (ε) 219 (741), 255 (524), 295 (229) nm; IR (neat) ν_{max} 3379, 2937, 1677, 1594, 1428, 1283, 1201, 1134, 799, 722 cm^{-1}; NMR data (pyridine-d_5), see Table 1; HR-ESI-MS *m/z* 803.3474 (Calcd for $[C_{39}H_{56}O_{16}+Na]^+$, 803.3461).

Compound **2**: Amorphous pale yellow powder; $[\alpha]_D^{25}$ −20.0 (*c*=0.04, MeOH); UV (MeOH) λ_{max} (ε) 240 (7943), 330 (5248), nm; IR (neat) ν_{max} 3346, 2934, 1673, 1514, 1458, 1280, 1123, 1040, 800, 722 cm^{-1}; NMR data (pyridine-d_5), see Table 1; HR-ESI-MS *m/z* 859.3777 (Calcd for $[C_{42}H_{60}O_{17}+Na]^+$, 859.3723).

Acid Hydrolysis of Compound 1 A solution of compound **1** (2.2 mg) in 1 M HCl/dioxane (1 : 1, 2 ml) was refluxed at 95 °C for 1.5 h. After cooling, the reaction mixture was diluted with H_2O (2 ml) and extracted with $CHCl_3$ (3 ml×3). The aqueous layer was neutralized by passing through an Amberlite MB-150 column eluting with H_2O. The eluent was concentrated to dryness to yield a sugar residue, which was analyzed by TLC (silica gel, $CHCl_3$–MeOH–AcOH–H_2O, 70 : 30 : 10 : 5) in comparison with standard samples. Glucose (*Rf*=0.29) and apiose (*Rf*=0.47) were detected. Determination of the absolute configuration of the sugars followed a recently reported procedure.[24] Briefly, the sugar residue (about 0.7 mg) and L-cysteine methyl ester (1 mg) was dissolved in pyridine (0.2 ml) and heated at 60 °C for 1 h, and then *o*-tolyl isothiocyanate (1 mg) was added to the mixture and heated at 60 °C for another 1 h. The reaction mixture (10 μl) was analyzed by analytical HPLC eluting with 20% aqueous CH_3CN containing 0.1% TFA at a flow rate of 1 ml/min over 35 min-run. D-Glucose (t_R=17.63 min) and D-apiose (t_R=27.02 min) were identified by comparing their retention times with those of the authentic samples, while L-glucose showed a different retention time at 16.21 min. L-Apiose was not included in the experiment due to unavailability.

Acknowledgments The authors thank the Natural Products Branch Repository Program at the National Cancer Institute for providing the plant extract, Dr. B. Avula for recording HR-ESI-MS spectra, and Mr. F. T. Wiggers for obtaining NMR spectra. This work was supported by the NIH, NIAID, Division of AIDS, Grant No. AI027094, the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009, and the China Scholarship Council.

References

1) Xiao C. H., Lu Y. R., "Chinese Medicinal Chemistry," Shanghai Science Technology Press, Shanghai, 1987, pp. 1—7.
2) He D. H., Otsuka H., Hirata E., Shinzato T., Bando M., Takeda Y., *J. Nat. Prod.*, **65**, 685—688 (2002).
3) Nishimura K., Hitotsuyanagi Y., Sugeta N., Sakakura K., Fujita K., Fukaya H., Aoyagi Y., Hasuda T., Kinoshita T., He D. H., *Tetrahedron*, **62**, 1512—1519 (2006).
4) Nishimura K., Hitotsuyanagi Y., Sugeta N., Sakakura K., Fujita K., Tachihara S., Fukaya H., Aoyagi Y., Hasuda T., Kinoshita T., Takeya K., *Tetrahedron*, **63**, 4558—4562 (2007).
5) He D. H., Matsunami K., Otsuka H., Shinzato T., Aramoto M., Bando M., Takeda Y., *J. Nat. Med.*, **61**, 46—50 (2007).
6) Tamaki N., Matsunami K., Otsuka H., Shinzato T., Aramoto M., Takeda Y., *J. Nat. Med.*, **62**, 314—320 (2008).
7) Otsuka H., Shitamoto J., He D. H., Matsunami K., Shinzato T., Aramoto M., Takeda Y., Kanchanapoom T., *Chem. Pharm. Bull.*, **55**,

1600—1605 (2007).
8) He D. H., Matsunami K., Otsuka H., Shinzato T., Aramoto M., Bando M., Takeda Y., *Phytochemistry*, **66**, 2857—2864 (2005).
9) Nishimura K., Hitotsuyanagi Y., Sugeta N., Fukaya H., Aoyagi Y., Hasuda T., Kinoshita T., Takeya K., *J. Nat. Prod.*, **70**, 758—762 (2007).
10) Tan G. T., Lee S., Lee I. S., Chen J. W., Leitner P., Besterman J. M., Kinghorn A. Douglas., Pezzuto J. M., *Biochem. J.*, **314**, 993—1000 (1996).
11) Harput U. S., Saracoglu I., Nagatsu A., Ogihara Y., *Chem. Pharm. Bull.*, **50**, 1106—1108 (2002).
12) El-Gindy A., Emara S., Hadad G. M., *J. Pharm. Biomed.*, **33**, 231—241 (2003).
13) Harinantenaina L., Kasai R., Yamasaki K., *Phytochemistry*, **61**, 367—372 (2002).
14) Kobayashi W., Miyase T., Suzuki S., Noguchi H., Chen X. M., *J. Nat. Prod.*, **63**, 1066—1069 (2000).
15) Zhao Y., Pu J. X., Huang S. X., Ding L. S., Wu Y. L., Li X., Yang L. B., Xiao W. L., Chen G. Q., Sun H. D., *J. Nat. Prod.*, **72**, 988—993 (2009).
16) Sun H. D., Huang S. X., Han Q. B., *Nat. Prod. Rep.*, **23**, 673—698 (2006).
17) Garcia P. A., Oliveira A. B., Batista R., *Molecules*, **12**, 455—483 (2007).
18) Li X., Zhang D. Z., Onda M., Konda Y., Iguchi M., Harigaya Y., *J. Nat. Prod.*, **53**, 657—661 (1990).
19) Castro V., Jakupovic J., Dominguez X. A., *Phytochemistry*, **28**, 2727—2729 (1989).
20) Jin W. S., Wang Z. M., Feng H., Liu F. S., *Zhongguo Zhongyao Zazhi*, **22**, 676—678 (1997).
21) Peng G. P., Lou F. Chang., *Yao Xue Xue Bao*, **37**, 950—954 (2002).
22) Wang Z. M., Feng H., Zhang Q., Liu F. S., Jin W. S., Mu M., Fan Q. H., Kong M., He Y. W., *Yao Xue Xue Bao*, **33**, 207—211 (1998).
23) Zhao Y., Yang L. B., Huang S. X., Xiao W. L., Pu J. X., Li L. M., Han Q. B., Sun H. D., *Chin. Chem. Lett.*, **19**, 1096—1098 (2008).
24) Tanaka T., Nakashima T., Ueda T., Tomii K., Kouno I., *Chem. Pharm. Bull.*, **55**, 899—901 (2007).