Two New Diterpenoids and Other Constituents from *Isodon japonicus*

by **Rui-Jie Hou**[a]), **Fu-Lin Yan***[a]), **Hai-Yang Zhang**[b]), **Yi-Xiao Bai**[a]), **Ming-Ming Ding**[a])

[a]) Pharmacy College, Xinxiang Medical University, Xinxiang 453003, P. R. China (phone: +86-13782513326; fax: +86-373-3029882; e-mail: yanfulin03@xxmu.edu.cn)
[b]) First Clinical College of Xinxiang Medical University, Weihui 453100, P. R. China

Two new *ent*-kaurene diterpenoids, 15α,20-dihydroxy-6,7-seco-entkaur-16-ene-7,1α(6,11α)-diolide (**1**), 6β-butyroxy-3β-hydroxy-6,7-seco-6,20-epoxy-7,1α-olide-entkaur-16-en-15-one (**2**), together with 25 known compounds, **3**–**27**, were isolated from the leaves of *Isodon japonicus*. Their structures were established by spectroscopic methods, including 2D-NMR techniques.

Introduction. – *Isodon japonicus* (BURM.f.) H.HARA is a perennial plant which is widely distributed in China, Korea, and Japan. It is being used as a folk medicine for the treatment of gastrointestinal disorders, tumors, and inflammatory diseases [1][2]. Phytochemical studies on this plant have led to the isolation of more than 40 *ent*-kaurane diterpenoids [3][4], some of which possess various biological activities, such as cytotoxic and antibacterial activities [5][6]. In search for new diterpenoids, we reinvestigated this species, which was collected in Shanxi Province of China, and we isolated two new compounds, 15α,20-dihydroxy-6,7-seco-entkaur-16-ene-7,1α(6,11α)-diolide (**1**), and 6β-butyroxy-3β-hydroxy-6,7-seco-6,20-epoxy-7,1α-olide-entkaur-16-en-15-one (**2**), together with 25 known diterpenoids (see *Fig. 1*).

Results and Discussion. – Compound **1** was obtained as colorless crystals. The HR-ESI-MS spectrum exhibited a molecular-ion peak at *m*/*z* 363.1805 ($[M+H]^+$; calc. 363.1808) indicating a molecular formula of $C_{20}H_{26}O_6$, with eight degrees of unsaturation. In the IR spectrum, the absorption bands at 3525, 3441, 1759, and 1701 cm^{-1} suggested the presence of OH and lactone CO groups. In addition, the ^{13}C-NMR and DEPT spectra (*Table*) displayed 20 C-atom resonances due to two lactone CO groups, four quaternary C-atoms (thereof one olefinic), six CH groups, thereof three O-bearing, six CH_2 groups (including one olefinic, one O-bearing), and two Me groups, which suggested that **1** was a typical kaurane diterpenoid, often found in *Isodon* plants. The above data of compound **1** showed close resemblance to the ones of rubescrystal A [7]. The notable difference is that the CHO group at C(10) of rubescrystal A (δ(C) 199.5, *d*) was reduced to a CH_2OH group in compound **1** (δ(C) 60.3, *t*), which caused a considerable upfield shift of C(10) to δ(C) 43.0. This was supported by the HMBCs between the signals of CH_2(20) (4.45 and 4.20, *AB*) and those of C(1) (δ(C) 78.8, *d*), C(5) (δ(C) 50.8, *d*), C(9) (δ(C) 39.3, *d*). In the NOESY, the signal of H–C(15) (δ(H) 5.59, *s*) showed correlations with those of H–C(14) (δ(H) 1.81, *dd*) and H–C(13) (δ(H) 2.61–2.69, *m*), but no correlation with the one of H–C(9)

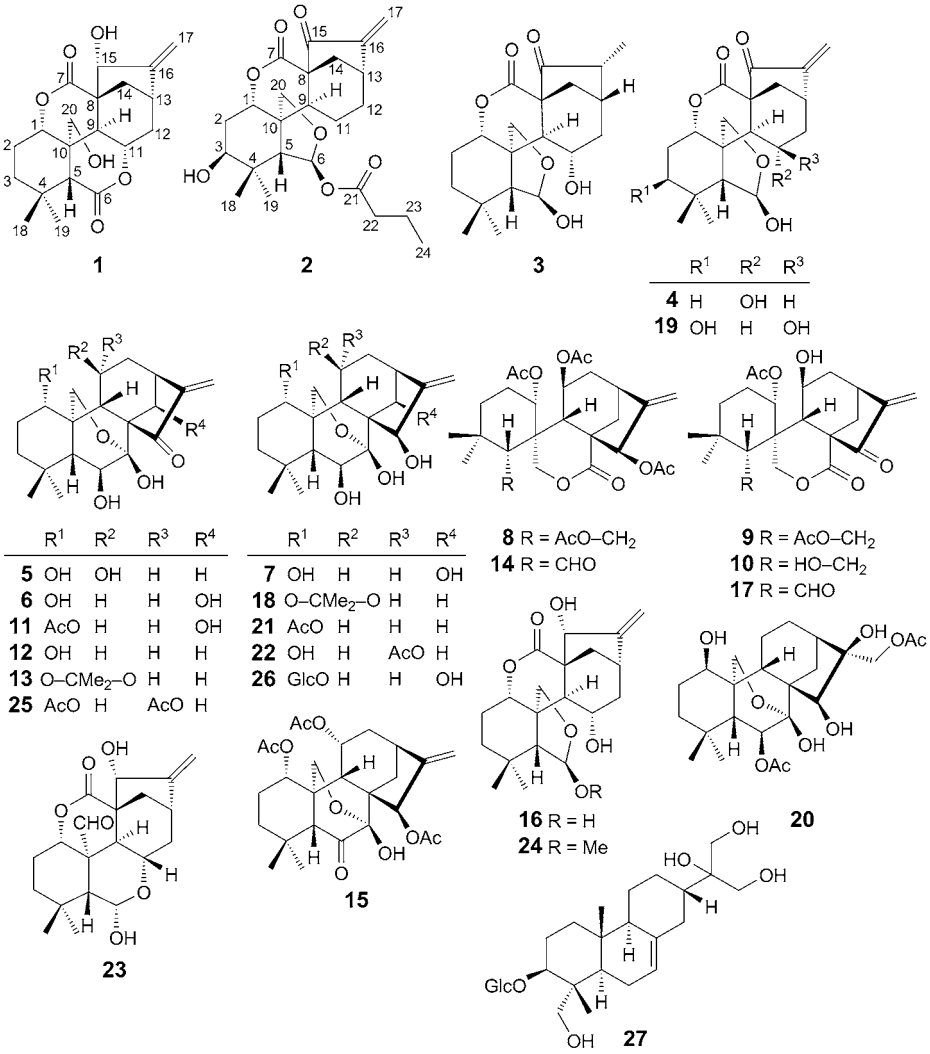


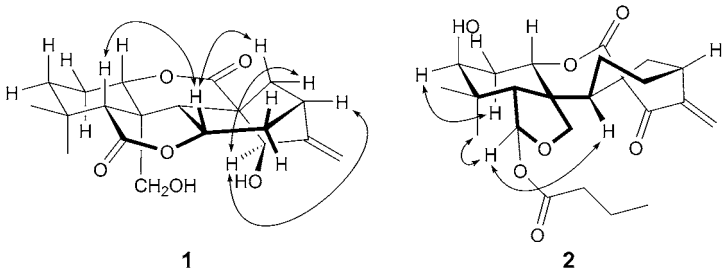
Fig. 1. *The structures of compounds* **1**–**27**

(δ(H) 3.43, *d*), which suggested that H–C(15) has β-orientation (*Fig. 2*). The signal for H–C(11) (δ(H) 4.89, *dt*) showed correlations with the signals of H–C(5) (δ(H) 2.52, *s*) and H–C(14) (δ(H) 2.20, *dd*), which indicated that H–C(11) likewise is in β-orientation. Therefore, compound **1** was unambiguously determined as 15α,20-dihydroxy-6,7-seco-entkaur-16-ene-7,1α(6,11α)-diolide. Due to biogenetic reasons, the absolute configuration of compound **1** is believed to be as shown in the formula collection.

Compound **2** was isolated as colorless needles, and the molecular formula $C_{24}H_{32}O_7$ was determined on the basis of HR-ESI-MS at *m*/*z* 433.2257 ($[M+H]^+$). The IR

Table. *^1H-* (400 MHz) *and ^{13}C-NMR* (100 MHz) *Data of Compounds* **1** *and* **2** *in (D_5)Pyridine* (δ in ppm)

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	4.64 (*dd*, *J* = 12.0, 4.0)	78.8 (*d*)	5.34 (*dd*, *J* = 12.0, 4.0)	73.3 (*d*)
CH_2(2)	1.89–1.92, 2.10–2.17 (2*m*)	25.0 (*t*)	2.18 (overlapped), 2.27–2.30 (*m*)	30.3 (*t*)
CH_2(3) or H–C(3)	1.40–1.48, 1.51–1.58 (2*m*)	40.4 (*t*)	3.75 (br. *s*)	74.3 (*d*)
C(4)		32.7 (*s*)		35.9 (*s*)
H–C(5)	2.52 (*s*)	50.8 (*d*)	2.62 (*s*)	49.2 (*d*)
C(6) or H–C(6)		171.4 (*s*)	6.58 (*s*)	102.1 (*d*)
C(7)		174.8 (*s*)		171.7 (*s*)
C(8)		50.9 (*s*)		56.4 (*s*)
H–C(9)	3.43 (*d*, *J* = 12.0)	39.3 (*d*)	2.64 (*dd*, *J* = 8.0, 4.0)	45.9 (*d*)
C(10)		43.0 (*s*)		49.8 (*s*)
H–C(11) or CH_2(11)	4.89 (*dt*, *J* = 11.6, 9.2)	70.0 (*d*)	1.82 (*t*, *J* = 8.0)	19.4 (*t*)
CH_2(12)	1.70–1.73, 2.83–2.86 (2*m*)	37.8 (*t*)	1.48 (overlapped), 2.18 (overlapped)	32.3 (*t*)
H–C(13)	2.61–2.69 (*m*)	37.5 (*d*)	2.92 (*dd*, *J* = 8.0, 4.0)	34.9 (*d*)
CH_2(14)	2.20, 1.81 (2*dd*, *J* = 12.0, 5.2)	32.0 (*t*)	2.48, 2.00 (2*dd*, *J* = 12.0, 4.0)	29.7 (*t*)
H–C(15) or C(15)	5.59 (*s*)	81.6 (*d*)		200.4 (*s*)
C(16)		158.0 (*s*)		151.2 (*s*)
CH_2(17)	5.43, 5.16 (2 br. *s*)	109.5 (*t*)	6.07, 5.41 (2 br. *s*)	117.6 (*t*)
Me(18)	1.63 (*s*)	21.8 (*q*)	1.30 (*s*)	27.8 (*q*)
Me(19)	1.28 (*s*)	32.7 (*q*)	0.97 (*s*)	22.6 (*q*)
CH_2(20)	4.45, 4.20 (*AB*, *J* = 10.4)	60.3 (*t*)	4.41, 4.34 (*AB*, *J* = 8.0)	75.3 (*t*)
C(21)				171.3 (*s*)
CH_2(22)			2.12 (*t*, *J* = 8.0)	36.2 (*t*)
CH_2(23)			1.42–1.49 (*m*)	18.1 (*t*)
Me(24)			0.76 (*t*, *J* = 8.0)	13.2 (*q*)



1 **2**

Fig. 2. *Key NOESY correlations of compounds* **1** *and* **2**

spectrum exhibited the presence of CO (1754, 1717) and OH groups (3525). In the ^{13}C-NMR and DEPT spectra of **2** (*Table*), besides four C-atom signals for a butyroxy residue, there were another 20 signals for the skeleton of a 6,7-seco-6,20-epoxy-ent-kaurane deduced from the characteristic signals of one lactonic CO (δ(C) 171.7, *s*) and one keto CO (δ(C) 200.4, *s*), four quanternary C-atoms (there of one olefinic), six CH groups, thereof three O-bearing, six CH_2 groups (including one olefinic, one O-

bearing), and two Me groups. Comparison of the NMR data of **2** with those of enmein [8], a similar diterpenoid isolated from this plant, indicated that the butyroxy group should be at C(6) in **2**. The chemical shift of H–C(6) changed from 5.90 ppm for enmein to 6.58 ppm for **2**. Moreover, the signal of C(21) (δ(C) 171.3) showed correlations with those of H–C(6) (δ(H) 6.58, *s*) and CH_2(22) (δ(H) 2.12, *t*) in the HMBC spectrum, which further confirmed the above assignment. On the other hand, the position of the OH group at C(3) was determined by the HMBCs between the signal of H–C(3) (δ(H) 3.75, br. *s*) and those of C(1) (δ(C) 73.3), C(5) (δ(C) 49.2), C(18) (δ(C) 27.8), C(19) (δ(C) 22.6). The relative configuration of **2** was confirmed by a NOESY experiment (*Fig. 2*). In the NOESY spectrum, there were correlations of the signal of H–C(3) (δ(H) 3.75, br. *s*) with the one of H–C(2) (δ(H) 2.27–2.30, *m*), but no correlation with those of H–C(1) (δ(H) 5.34, *dd*) and H–C(5) (δ(H) 2.62, *s*); the signal of H–C(6) (δ(H) 6.58, *s*) correlated with those of H–C(9) (δ(H) 2.64, *dd*) and Me(19) (δ(H) 0.97, *s*). Thus, H–C(3) and H–C(6) were both in α-orientation. Based on the above spectral analysis and on the comparison of the spectral data with those of enmein reported in the literature [8], the structure of compound **2** was established as 6β-butyroxy-3β-hydroxy-6,7-seco-6,20-epoxy-7,1α-olide-entkaur-16-en-15-one.

The known compounds **3**–**27** were identified by comparison of their NMR data with literature data. They were identified as rabdosichuanin C (**3**) [9], epinodosin (**4**) [10], lasiodonin (**5**) [11], oridonin (**6**) [12], enmenol (**7**) [13], rabdosinate (**8**) [14], rabdosin B (**9**) [15], isodonoiol (**10**) [16], lasiokaurin (**11**) [17], effusanin A (**12**) [18], acetonide derivative of lasiodonin (**13**) [19], shikokianal acetate (**14**) [20], parvifoline G (**15**) [21], epinodosinol (**16**) [22], isodonal (**17**) [23], wikstroemioidin B (**18**) [24], longirabdolide C (**19**) [25], maoyerabdosin (**20**) [26], maoecrystal E (**21**) [27], sodoponin (**22**) [28], 6α,15α-dihydroxy-20-aldehyde-6,7-seco-6,11α-epoxy-1,7-olide-entkaur-16-ene (**23**) [29], isojaponin A (**24**) [4], shikokianin (**25**) [30], 1α-*O*-β-D-glucopyranosylenmenol (**26**) [31], and eriocaside A (**27**) [32].

This work was supported by the *Natural Science Foundation of Henan Province* (No. 102102310011).

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Co.*, P. R. China). Thin layer chromatography (TLC): silica gel (GF_{254}, 10–40 μm; *Qingdao Marine Chemical Co.*, P. R. China); spots were detected on TLC under UV or by heating after spraying with 5% H_2SO_4 in EtOH. Optical rotations: *Perkin-Elmer 341* polarimeter. M.p.: *X-4 Digital Display* micro-melting point apparatus; uncorrected. IR Spectra: *Nicolet 170 SX* FT-IR spectrometer. ^{1}H-, ^{13}C- and 2D-NMR spectra: *Bruker AM-400* NMR spectrometer using TMS as the internal standard. HR-ESI-MS: *Waters HPLCQ-Tof* HR-MS spectrometer.

Plant Material. Isodon japonicus (BURM.f.) H.HARA was collected in Qinyuan County, Shanxi Province, P. R. China, in August 2010, and identified by Prof. *Changshan Zhu*, Henan Agriculture University, P. R. China. A voucher specimen (No. 201003) has been deposited with Pharmacy College, Xinxiang Medical University.

Extraction and Isolation. The air-dried leaves of *Isodon japonica* (BURM. f.) HARA (16 kg) were pulverized and extracted four times with Me_2CO/H_2O (7:3 *v*/*v*) at r.t. for 7 d and filtered. The filtrate was concentrated and partitioned successively between AcOEt and H_2O. The AcOEt extract (436 g) was subjected to a SiO_2 column (12 × 150 cm, 3000 g, 200–300 mesh), eluted with a gradient system of $CHCl_3$/MeOH (30:1, 20:1, 10:1, 5:1) to give four factions according to their TLC analysis. From *Fr. 1*

($CHCl_3$/MeOH 30:1), compounds **1** (78 mg), **2** (11 mg), **3** (22 mg), **4** (23 mg), **8** (92 mg), **9** (235 mg), **10** (112 mg), **11** (76 mg), **12** (38 mg), **13** (86 mg), **14** (38 mg), **15** (32 mg), **17** (22 mg), **18** (21 mg), **23** (22 mg), **24** (13 mg), and **25** (34 mg) were obtained by repeated CC (SiO_2; $CHCl_3$/Me_2CO 30:1, 20:1, 10:1; petroleum ether (PE)/Me_2CO 10:1, 8:1, 5:1), and subsequent recrystallization from Me_2CO. From *Fr. 2* ($CHCl_3$/MeOH 20:1), compounds **5** (125 mg), **6** (22 g), **16** (89 mg), **19** (46 mg), **20** (37 mg), **21** (28 mg), and **22** (67 mg) were isolated by further purification by CC (SiO_2; PE/Me_2CO 5:1, 3:1, 2:1, 1:1; $CHCl_3$/Me_2CO 20:1, 10:1), and subsequent recrystallization from Me_2CO or MeOH. From *Fr. 3* ($CHCl_3$/MeOH 10:1), compounds **7** (408 mg) and **27** (21 mg) were separated by repeated CC (SiO_2; $CHCl_3$/MeOH 15:1, 10:1). From *Fr. 4* ($CHCl_3$/MeOH 5:1), compound **26** (353 mg) was obtained by repeated CC (SiO_2; $CHCl_3$/MeOH 10:1, 5:1).

15α,20-Dihydroxy-6,7-seco-entkaur-16-en-7,1α(6,11α)-diolide (=*(3a*S*,5a*S*,6*R*,8*S*,9a*S*,11a*R*,11b*S*,11c*S*)-Decahydro-6-hydroxy-11b-(hydroxymethyl)-1,1-dimethyl-7-methylidene-1*H*-5a,8-methano-4,10-dioxacyclohepta[*cd*]phenalene-5,11(2*H*)-dione*; **1**). Colorless crystals from Me_2CO. M.p. 306–308°. $[\alpha]_D^{20} = -122.7$ ($c = 1.05$, pyridine). IR (KBr): 3525, 3441, 3007, 2983, 2954, 2917, 2898, 1759, 1701, 1664, 1317, 1262, 1078, 990, 898. ^{1}H- and ^{13}C-NMR: see *Table*. HR-ESI-MS: 363.1805 ($[M+H]^+$, $C_{20}H_{27}O_6^+$; calc. 363.1808).

6β-Butyroxy-3β-hydroxy-6,7-seco-6,20-epoxy-7,1α-olide-entkaur-16-en-15-one (=*(2*S*,3a*S*,5a*S*,8*R*,10a*S*,10b*S*,13a*R*)-Dodecahydro-2-hydroxy-1,1-dimethyl-7-methylidene-5,6-dioxo-5a,8-methanocyclohepta[*c*]furo[3,4-*e*]chromen-13(5*H*)-yl Butanoate*; **2**). Colorless needles from Me_2CO. M.p. 87–89°. $[\alpha]_D^{23} = -88.6$ ($c = 1.07$, Me_2CO). IR (KBr): 3525, 3089, 2964, 2876, 1754, 1717, 1643, 1259, 1075, 940, 916. ^{1}H- and ^{13}C-NMR: see *Table*. HR-ESI-MS: 433.2257 ($[M+H]^+$, $C_{24}H_{33}O_7^+$; calc. 433.2226).

REFERENCES

[1] B. S. Jung, M. K. Shin, 'Encyclopedia of Illustrated Korean Natural Drugs', Young Lim Sa, Seoul, 1990, p. 845.
[2] B. Li, X. Tian, *Phytochemistry* **2001**, *58*, 543.
[3] J.-X. Zhang, Q.-B. Han, A.-H. Zhao, H.-D. Sun, *Fitoterapia* **2003**, *74*, 435.
[4] L.-B. Yang, S.-X. Huang, L.-M. Li, Y. Zhao, C. Lei, W.-L. Xiao, J.-X. Pu, Q.-B. Han, H.-D. Sun, *Helv. Chim. Acta* **2007**, *90*, 2375.
[5] H. D. Sun, Y. L. Xu, B. Jiang, 'Diterpenoids from Isodon Species', Science Press, Beijing, 2001, pp. 93–106.
[6] Q. Han, J. Zhang, Y. Lu, Y. Wu, Q. Zheng, H. Sun, *Planta Med.* **2004**, *70*, 581.
[7] R.-J. Xie, F.-L. Yan, G.-F. Hai, R.-J. Hou, M.-M. Ding, Y.-X. Bai, *Fitoterapia* **2011**, *82*, 726.
[8] T. Ikeda, S. Kanatomo, *Yakugaku Zasshi* **1958**, *78*, 1128.
[9] H. Huang, H. Zhang, H. Sun, *Phytochemistry* **1990**, *29*, 2591.
[10] T. Kubota, I. Kubo, *Chem. Commun.* **1968**, *13*, 763.
[11] G. Y. Xu, *Acta Chim. Sin.* **1985**, *43*, 35.
[12] S. Mori, K. Shudo, T. Ageta, T. Koizumi, T. Okamoto, *Chem. Pharm. Bull.* **1970**, *18*, 871.
[13] X.-R. Wang, H.-P. Wang, H.-P. Hu, H.-D. Sun, S.-Q. Wang, S. Ueda, Y. Kuroda, T. Fujita, *Phytochemistry* **1995**, *38*, 921.
[14] M.-T. Wang, T.-Z. Zhao, J.-C. Li, C.-J. Liu, X.-Z. An, *Acta Chim. Sin*. **1987**, *45*, 871.
[15] X. Meng, Y. Chen, Y. Cui, J. Cheng, *J. Nat. Prod*. **1988**, *51*, 812.
[16] Q. B. Han, J. X. Zhang, Y. H. Shen, H. D. Sun, *Chin. J. Nat. Med*. **2003**, *1*, 16.
[17] Y. H. Gao, Z. X. Wan, X. W. Lai, Y. Zhu, G. Y. Li, S. H. Wu, *China J. Chin. Mat. Med.* **1994**, *19*, 295.
[18] T. Fujita, Y. Takeda, T. Shingu, A. Ueno, *Chem Lett.* **1980**, *9*, 1635.
[19] Q.-B. Han, R.-T. Li, M. Li, Y.-K. Mou, Q.-E Tian, S.-W. Li, H.-D. Sun, *J. Asian Nat. Prod. Res.* **2005**, *7*, 31.
[20] M. Ochi, M. Okamura, H. Kotsuki, I. Miura, I. Kubo, T. Kubota, *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2208.
[21] L.-M. Li, G.-Y. Li, S.-X. Huang, S.-H. Li, Y. Zhou, W.-L. Xiao, L.-G. Lou, L.-S. Ding, H.-D. Sun, *J. Nat. Prod.* **2006**, *69*, 645.
[22] Y. Takeda, T. Matsumoto, Y. Futatsuishi, H. Terada, H. Otsuka, *J. Nat. Prod.* **1995**, *58*, 794.

[23] I. Kubo, T. Kamikawa, T. Kubota, *Tetrahedron* **1974**, *30*, 615.
[24] S.-H. Wu, H.-J. Zhang, Y.-P. Chen, Z.-W. Lin, H.-D. Sun, *Phytochemistry* **1993**, *34*, 1099.
[25] Y. Takeda, T. Matsumoto, H. Otsuka, *J. Nat. Prod.* **1994**, *57*, 650.
[26] Q. Z. Zhao, J. H. Chao, H. Q. Wang, H. D. Sun, *Chin. Tradit. Herbal Drugs* **1984**, *15*, 49.
[27] C. Li, J. Zhou, H. Sun, *Acta Bot. Yunnan.* **1985**, *7*, 115.
[28] E. Fujita, T. Fujita, M. Taoka, H. Katayama, M. Shibuya, *Chem. Pharm. Bull.* **1973**, *21*, 1357.
[29] F. L. Yan, L. B. Zhang, J. X. Zhang, H. D. Sun, *J. Chem. Res.* **2007**, 362.
[30] B. Li, Y. Pan, W. Pan, *Helv. Chim. Acta* **2001**, *84*, 3418.
[31] H.-M. Liu, X. Yan, F. Kiuchi, Z. Liu, *Chem. Pharm. Bull.* **2000**, *48*, 148.
[32] X. Shen, A. Isogai, K. Furihata, H. Sun, A. Suzuki, *Phytochemistry* **1993**, *34*, 1595.

Received January 1, 2012