Cyathane Diterpenoids and Nitrogenous Terphenyl Derivative from the Fruiting Bodies of Basidiomycete *Phellodon niger*

Sheng-Tao FANG,[a,b] Ling ZHANG,[a,b] Zheng-Hui LI,[a] Bo LI,[a] and Ji-Kai LIU*,[a]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences; Kunming 650204, P.R. China: and* [b] *Graduate School of the Chinese Academy of Sciences; Beijing 100039, P.R. China.* Received April 20, 2010; accepted June 18, 2010; published online June 28, 2010

Two new cyathane-type diterpenoids, nigernin A and B (1, 2), one new nitrogenous terphenyl derivative, phellodonin (3), together with three known compounds, 2′,3′-diacetoxy-3,4,5′,6′,4″-pentahydroxy-*p*-terphenyl, grifolin, and 4-*O*-methylgrifolic acid, were isolated from the fruiting bodies of basidiomycete *Phellodon niger*. The structures of these new compounds were elucidated by spectroscopic methods and comparison with the data of known compounds in the literature. All these compounds were isolated from this fungus for the first time.

Key words *Phellodon niger*; basidiomycete; nigernin A; phellodonin; diterpenoid; terphenyl

The fungus *Phellodon niger* (Fr.) KARST. belongs to the family Hydnaceae, is an edible mushroom.[1)] There is only one literature about the report of chemical constituents of the genus *Phellodon* in which phellodonic acid was isolated from *Phellodon melaleucus*.[2)] Previous chemical investigation of the related genus *Sarcodon*, which also belongs to Hydnaceae family, a number of cyathane diterpenoids have been isolated from the fruiting bodies of basidiomycetes (for instance *Sarcodon scabrosus*,[3—9)] *S. cyrneus*[10)] and *S. glaucopus*[11)]), including scabronines A—G, which are stimulators of nerve growth factor (NGF) synthesis. All these cyathane-type diterpenoids consisting of angularly condensed five-, six-, and seven-membered rings, and were mainly isolated from a diverse variety of fungi and few sponges.[12)] Recently, a series of unusual nitrogenous terphenyl derivatives were isolated from the fruiting bodies of *Sarcodon leucopus*,[13,14)] *S. scabrosus*,[15)] *Hydnellum suaveolens*, and *H. geogerirum*.[16)] These compounds were found to have cytotoxity in tumor cell lines, and antioxidant activity. As our continuing studies on search for novel and secondary metabolites of higher fungi of Yunnan Province in China,[17—19)] we now report the isolation and elucidation of two new cyathane-type diterpenoids, nigernin A and B (**1**, **2**), one new nitrogenous terphenyl derivative, phellodonin (**3**), and three known compounds, 2′,3′-diacetoxy-3,4,5′,6′,4″-pentahydroxy-*p*-terphenyl,[20)] grifolin,[21)] and 4-*O*-methylgrifolic acid[22)] from the fruiting bodies of basidimycete *P. niger*. Their structures were identified on the basis of spectroscopic analysis and by comparison with previously reported physical and spectral data of the known compounds. All these six compounds were isolated from this fungus for the first time.

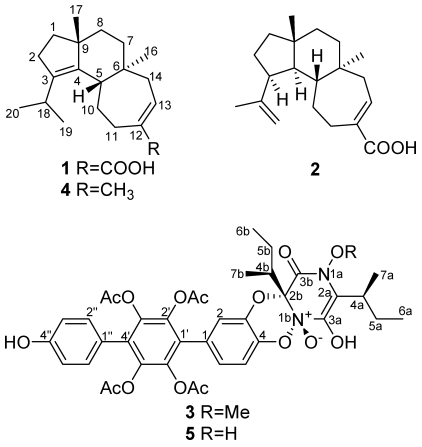


Fig. 1. Structures of Compounds **1**—**5**

Results and Discussion

Compound **1** was obtained as white amorphous solid, giving the molecular formula $C_{20}H_{30}O_2$ by positive high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) (*m*/*z* 303.2316 $[M+1]^+$, Calcd for 303.2324). Its IR spectrum showed the presence of a hydroxyl (3423 cm^{-1}), a carbonyl (1681 cm^{-1}), and a double bond (1640 cm^{-1}) group, respectively. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** (Table 1) showed the presence of 20 carbons consisting of one carbonyl (δ_C 172.7), two double bonds (δ_C 145.1, 136.3; 139.5, 138.7), two sp^3 quaternary carbons (δ_C 49.3, 38.5), two sp^3 methines (δ_C 52.1, 26.7), seven methylenes and four methyls. The ^{1}H- and ^{13}C-NMR spectral data of **1** were very similar to those of cyatha-3,12-diene (**4**), a known diterpenoid with cyathane skeleton which was isolated from the basidiomycete *Hericium erimaceum*,[23)] indicated that **1** also possessed the same cyathane skeleton. The only difference between these two compounds was a carboxyl group in **1** instead of a methyl group in **4**. The heteronuclear multiple bond connectivity (HMBC) spectrum of **1** (Fig. 2) displayed correlations from H-11 and H-13 to C-15 (δ_C 172.7), revealed that the carboxyl group was located at C-15. The relative configuration of **1** was assigned on the basis of the rotating-frame Overhauser enhancement spectroscopy (ROESY) correlations (Fig. 3). Correlations of H-5 with H-17 and H-10β, of H-17 with H-8β, and of H-16 with H-10α and H-8α, indicated that **1** has the same configuration as that of **4**. Thus, the structure of **1** was determined to be cyatha-3,12-diene-15-oic acid, and named nigernin A.

Compound **2** was obtained as colorless needle crystals, and had the molecular formula $C_{20}H_{30}O_2$, as established from HR-ESI-MS (*m*/*z* 325.2135 $[M+Na]^+$, Calcd for 325.2143), the same as **1**. Analysis of the ^{1}H- and ^{13}C-NMR spectra (Table 1) revealed that the structure of **2** was similar to that of **1**, except for the position of one double bond. In the NMR spectra, the olefinic carbons δ_C 109.3 (t), 150.6 (s) and corre-

* To whom correspondence should be addressed. e-mail: jkliu@mail.kib.ac.cn

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1** and **2**

Position	**1**[a]		**2**[b]	
	δ_H (mult, *J*, Hz)	δ_C	δ_H (mult, *J*, Hz)	δ_C
1	1.57 (m), 1.48 (m)	38.1 t	1.46 (m), 1.10 (m)	41.5 t
2	2.25 (td, 8.0, 1.2)	28.6 t	2.00 (dd, 12.5, 9.5)	30.6 t
3		139.5 s	2.39 (m)	44.6 d
4		138.7 s	1.39 (m, overlap)	51.8 d
5	2.40 (br d, 12.5)	52.1 d	1.94 (m)	48.8 d
6		38.5 s		37.0 s
7	1.64 (td, 13.5, 4.0)	39.9 t	1.56 (td, 14.0, 4.0)	39.1 t
	1.21 (dt, 13.5, 3.0)		1.09 (m)	
8	1.53 (m)	37.1 t	1.39 (m, overlap)	37.0 t
	1.38 (dt, 12.5, 2.4)		1.31 (td, 13.5, 3.5)	
9		49.3 s		42.2 s
10	1.92 (br dd, 12.5, 7.5)	26.5 t	1.90 (dd, 14.0, 6.0)	24.6 t
	1.73 (m)		1.18 (m)	
11	2.97 (m, overlap)	26.9 t	4.43 (dd, 15.0, 6.0)	28.1 t
	2.09 (m, overlap)		2.23 (m)	
12		136.3 s		137.5 s
13	7.17 (m)	145.1 d	7.42 (m)	141.3 d
14	2.32 (dd, 14.0, 5.5)	43.4 t	2.25 (m)	44.4 t
	2.09 (m, overlap)		2.06 (dd, 14.0, 9.5)	
15		172.7 s		170.4 s
16	0.75 (s)	17.1 q	0.81 (s)	17.3 q
17	1.07 (s)	24.1 q	0.96 (s)	20.8 q
18	2.97 (m, overlap)	26.7 d		150.6 s
19	0.92 (d, 7.0)	21.7 q[c]	4.83 (s), 4.73 (s)	109.3 t
20	0.92 (d, 7.0)	21.8 q[c]	1.65 (s)	25.2 q

a) Measured in $CDCl_3$ at 400/100 MHz. *b*) Measured in C_5D_5N at 500/125 MHz. *c*) Values may be interchanged.

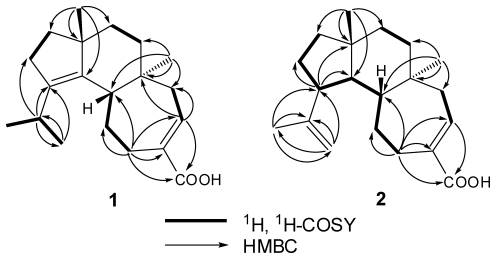


Fig. 2. Key ^{1}H, ^{1}H-COSY and HMBC Correlations of Compounds **1** and **2**

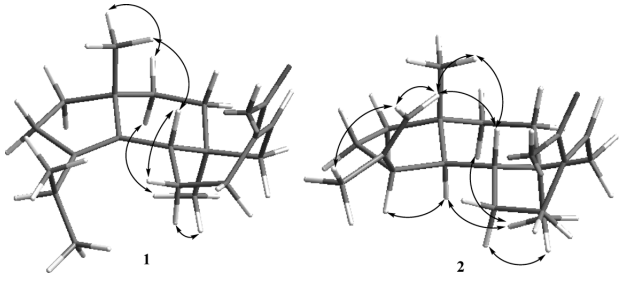


Fig. 3. Key ROESY Correlations of Compounds **1** and **2**

sponding protons δ_H 4.83, 4.73 (each 1H, s) suggested an olefinic linkage between C-18 and C-19. This was further confirmed by the HMBC spectrum (Fig. 2), which showed correlations between H-19 with C-18, C-20 and C-3. The stereochemistry of **1** was elucidated by the ROESY correlations (Fig. 3). The ROESY correlations of H-5 with Me-17 and H_a-19, and of Me-17 with H_a-19, as well as of H-4 with H-3 and Me-16, suggested H-3 and H-4 were both α-orientation. Therefore, compound **2** was determined as (3*S**,4*R**)-cyatha-12,18-diene-15-oic acid, named nigernin B.

Compound **3** was obtained as colorless amorphous solid, and has a pseudomolecular ion peak at *m/z* 801 $[M+Na]^+$ in its positive ESI-MS, corresponding to the molecular formula $C_{39}H_{42}O_{15}N_2$ as determined by HR-ESI-MS (*m/z* 801.2461 $[M+Na]^+$, Calcd for 801.2482), with 20 degrees of unsaturation. Careful analysis of the ^{1}H- and ^{13}C-NMR spectral data of **3** (Table 2), as well as IR and UV data, showed features very similar to those of *p*-terphenyl derivatives sarcodonin β (**5**) and the other related compounds sarcodonin α, γ and δ,[14,15] suggesting that they were analogues. Particularly, in the NMR spectrum of **3**, the typical low-field signals of the *p*-terphenyl core and the majority of the signals of the side chain in the aliphatic moiety closely resemble the related compounds.

From comparison of the ^{1}H- and ^{13}C-NMR spectral data of **3** with those of sarcodonin β (**5**),[14] (Table 2) it was apparent that **3** contained a *p*-terphenyl core bearing oxygenated functions at the same positions. In addition to the *p*-terphenyl resonances, it also revealed the presence of two aliphatic side chains, four acetoxyl groups, and a methoxyl group in compound **3** on the basis of the DEPT and 2D NMR experiments. Two aliphatic side chains were elucidated as the *sec*-butyl partial structure by ^{1}H–^{1}H correlated spectroscopy (^{1}H–^{1}H COSY) and HMBC spectra (Fig. 4), which were located at C-2a and C-2b by the HMBC correlations between H-4a and C-2a, H-4b and C-2b. Four acetoxyl groups were located at C-2′, C-3′, C-5′ and C-6′, respectively. This was confirmed by the observed HMBC correlations of the oxygen-bearing quaternary carbons with the methyls in acetyls. The NMR spectral data were assigned mainly by comparison with those of sarcodonin β (**5**), except for the presence of one more methoxyl group in **3**. The HMBC correlations of δ_H 6.37 (br s) with C-3″, 5″ and C-4″, and of δ_H 8.22 (br s) with C-3a were observed (Fig. 4), indicated that two hydroxyl groups located at C-4″ and C-3a, respectively. In addition, no HMBC correlations of aromatic quaternary carbons with the protons of methoxy were observed, so the methoxy was connected directly with N-1a. The relative stereochemistry of four chiral centers at N-1b, C-2b, C-4a and C-4b of **3** was the same as that of sarcodonin β (**5**)[14] and sarcodonin[13] by comparison of their ^{1}H- and ^{13}C-NMR spectral data. (Table 2). Thus, the structure of **3** was elucidated as shown in Fig. 1, and named phellodonin.

Experimental

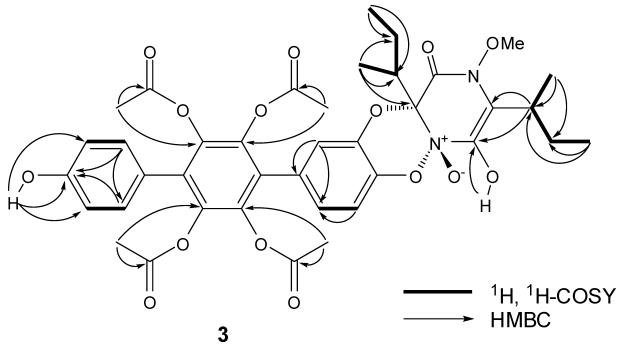
General Experimental Procedures Melting point was measured on an XRC-1 apparatus (Sichuan University, Sichuan, People's Republic of China). Optical rotation was measured with a Horiba SEPA-300 polarimeter. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS (tetramethylsilane) as an internal standard. UV spectra were recorded on Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained on Bruker Tensor 27 spectrometer with KBr pellets. ESI-MS and HR-ESI-MS were recorded on a VG Autospec-3000 and API Qstar-Pulsar LC/time-of-flight (TOF) mass spectrometers. Silica gel (200—300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. TLC was performed on silica gel plates (GF254, Qingdao Marine Chemical Inc., China). The spots on TLC were visualized by UV light (254/365 nm) and sprayed with 10%H_2SO_4 in ethanol, followed by heating.

Fungal Material The basidiomycete *P. niger* was collected at Wuding of Yunnan Province, China, in August 2009 and identified by Prof. Zhu-Liang Yang, Kunming Institute of Botany. The voucher specimen was de-

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data for **3** and **5**

Position	**3**[e]		**5**[f]	
	δ_H (mult, J, Hz)	δ_C	δ_H (mult, J, Hz)	δ_C
1		128.5 s		127.9 s
2	7.13 (d, 1.6)	118.4 d	7.07 (d, 2.0)	118.8 d
3		141.0 s[a]		141.1 s[a]
4		140.8 s[a]		140.8 s[a]
5	7.01 (d, 8.4)	117.0 d	7.13 (d, 8.5)	116.8 d
6	7.05 (dd, 8.4, 1.6)	126.4 d	7.06 (dd, 8.0, 2.0)	124.9 d
1′		122.8 s		123.1 s
2′		138.9 s[b]		139.0 s[b]
3′		139.3 s[b]		139.5 s[b]
4′		122.8 s		121.1 s
5′		139.3 s[b]		139.5 s[b]
6′		138.9 s[b]		139.0 s[b]
1″		128.7 s		128.6 s
2″, 6″	7.18 (d, 8.4)	130.8 d	7.18 (d, 8.5)	131.0 d
3″, 5″	6.85 (d, 8.4)	115.3 d	6.86 (d, 8.5)	115.3 d
4″		156.2 s		155.9 s
2′-$COCH_3$	1.99 (s)[c]	167.9 s, 20.1 q	1.99 (s)[c]	167.7 s, 20.0 q
3′-$COCH_3$	2.03 (s)[c]	167.9 s, 20.1 q	2.01 (s)[c]	167.7 s, 20.0 q
5′-$COCH_3$	2.03 (s)[c]	167.9 s, 20.1 q	2.01 (s)[c]	167.7 s, 20.0 q
6′-$COCH_3$	1.99 (s)[c]	167.9 s, 20.1 q	1.99 (s)[c]	167.7 s, 20.0 q
2a		166.6 s[d]		166.2 s
3a		159.0 s		158.7 s
4a	3.02 (m)	33.7 d	3.07 (m)	33.4 d
5a	1.63 (m), 1.39 (m)	26.0 t	1.62 (m), 1.42 (m)	25.7 t
6a	0.90 (t, 7.4)	12.1 q	0.89 (t, 7.5)	11.5 q
7a	1.09 (d, 7.2)	16.1 q	1.10 (d, 7.0)	16.5 q
2b		93.3 s		91.0 s
3b		159.3 s		159.4 s
4b	2.55 (m)	42.6 d	2.51 (m)	41.7 d
5b	1.90 (m), 1.41 (m)	23.7 t	1.82 (m), 1.33 (m)	23.1 t
6b	1.05 (t, 7.4)	12.4 q	1.04 (t, 7.5)	12.3 q
7b	1.33 (d, 6.8)	14.2 q	1.29 (d, 7.5)	13.8 q
1a-OCH_3	3.93 (s)	66.6 q		
4″-OH	6.37 (br s)			
3a-OH	8.22 (br s)			

a—*c*) Values with identical superscripts within each column may be interchanged. *d*) Data was not detected in ^{13}C-NMR, and obtained from HMBC experiment. *e*) Measured in $CDCl_3$ at 400/100 MHz. *f*) Measured in $CDCl_3$ at 500/125 MHz in ref. 14.



3

Fig. 4. Key ^{1}H, ^{1}H-COSY and HMBC Correlations of Compound **3**

posited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation The air-dried fruiting bodies (950 g) were extracted three times with $CHCl_3$/MeOH (1 : 1, v/v) at r.t. After removal of the solvent by evaporation, the residue (98.0 g) was subjected to silica gel column chromatography, eluting with a petroleum ether–acetone gradient system (100 : 1—1 : 1) to give fractions A—I. Fraction C was subjected to Sephadex LH-20 using $CHCl_3$–MeOH (1 : 1, v/v) to give 5 subfractions: C1—C5. Subfraction C4 was chromatographed on silica gel (petroleum ether–Me_2CO, 4 : 1) to afford compound **1** (35 mg) and 4-*O*-methylgrifolic acid (16 mg). Subfraction C5 was subjected to silica gel chromatography column ($CHCl_3$–Me_2CO, 30 : 1) followed by recrystallization to obtain compound **2** (7 mg). Fraction F was passed through Sephadex LH-20 using $CHCl_3$–MeOH (1 : 1, v/v) and repeated column chromatography over silica gel to afford compound **3** (263 mg). Grifolin (45 mg) was purified from fraction G by column chromatography (silica gel, $CHCl_3$–MeOH, 20 : 1—10 : 1). Fraction D was purified on a Sephadex LH-20 ($CHCl_3$–MeOH, 1 : 1) and a silica gel column (petroleum ether–Me_2CO, 3 : 1) to give 2′,3′-diacetoxy-3,4,5′,6′,4″-pentahydroxy-*p*-terphenyl (31 mg).

Nigernin A (**1**): White amorphous solid. $[\alpha]_D^{16.5}$ −176.3° (c=0.4, $CHCl_3$). UV λ_{max} ($CHCl_3$) nm (log ε): 241 (3.58). IR (KBr) cm^{-1}: 3423, 2956, 2931, 1681, 1641, 1280, 933. ^{1}H- and ^{13}C-NMR data, see Table 1. EI-MS *m*/*z*: 302 $[M]^+$. Positive HR-ESI-MS *m*/*z*: 303.2316 $[M+1]^+$ (Calcd for $C_{20}H_{31}O_2$, 303.2324).

Nigernin B (**2**): Colorless needles. mp 177—178 °C. $[\alpha]_D^{16.9}$ +91.4° (c=0.2, MeOH). UV λ_{max} (MeOH) nm (log ε): 225 (3.87). IR (KBr) cm^{-1}: 3427, 2945, 2933, 1668, 1641, 1289, 1265, 886. ^{1}H- and ^{13}C-NMR data, see Table 1. EI-MS *m*/*z*: 302 $[M]^+$. Positive HR-ESI-MS *m*/*z*: 325.2135 $[M+Na]^+$ (Calcd for $C_{20}H_{30}O_2Na$, 325.2143).

Phellodonin (**3**): Colorless amorphous solid. $[\alpha]_D^{16.7}$ −105.9° (c=0.4, $CHCl_3$), UV λ_{max} ($CHCl_3$) nm (log ε): 266 (3.97). IR (KBr) cm^{-1}: 3503, 3443, 2971, 2941, 1784, 1676, 1524, 1458, 1371, 1191, 1027. ^{1}H- and ^{13}C-NMR data, see Table 2. Positive ESI-MS *m*/*z*: 801 $[M+Na]^+$, 1580 $[2M+Na+1]^+$. Positive HR-ESI-MS *m*/*z*: 801.2461 $[M+Na]^+$ (Calcd for $C_{39}H_{42}O_{15}N_2Na^+$, 801.2482).

Acknowledgments This project was supported by the National Basic Research Program of China (973 Program, 2009CB522300), the National Natural Sciences Foundation of China (30830113), and MOST

(2009ZX09501-029; 2009ZX09501-013).

References

1) Mao X.-L., "The Macrofungi in China," Henan Science and Technology Press, Zhengzhou, 2000, p. 418.
2) Stadler M., Anke T., Dasenbrock J., Steglich W., *Z. Naturforsch.*, **48c**, 545—549 (1993).
3) Shibata H., Tokunaga T., Karasawa D., Hirota A., Nakayama M., Nozaki H., Tada T., *Agric. Biol. Chem.*, **53**, 3373—3375 (1989).
4) Kita T., Takaya Y., Oshima Y., Ohta T., Aizawa K., Hirano T., Inakuma T., *Tetrahedron*, **54**, 11877—11886 (1998).
5) Shibata H., Irie A., Morita Y., *Biosci. Biotechnol. Biochem.*, **62**, 2450—2452 (1998).
6) Hirota M., Moritura K., Shibata H., *Biosci. Biotechnol. Biochem.*, **66**, 179—184 (2002).
7) Kamo T., Imura Y., Hagio T., Makabe H., Shibata H., Hirota M., *Biosci. Biotechnol. Biochem.*, **68**, 1362—1365 (2004).
8) Ma B.-J., Zhu H.-J., Liu J.-K., *Helv. Chim. Acta*, **87**, 2877—2881 (2004).
9) Ma B.-J., Liu J.-K., *J. Basic Microbiol.*, **45**, 328—330 (2005).
10) Marcotullio M. C., Pagiotti R., Maltese F., Mwankie G. N. O.-M., Hoshino T., Obara Y., Nakahata N., *Bioorg. Med. Chem.*, **15**, 2878—2882 (2007).
11) Curini M., Maltese F., Marcotullio M. C., Menghini L., Pagiotti R., Rosati O., Altinier G., Tubaro A., *Planta Med.*, **71**, 194—196 (2005).
12) Cassidy M. P., Ghisalberti E. L., *J. Nat. Prod.*, **56**, 1190—1193 (1993).
13) Geraci C., Neri P., Paternò C., Rocco C., Tringali C., *J. Nat. Prod.*, **63**, 347—351 (2000).
14) Calì V., Spatafora C., Tringali C., *Eur. J. Org. Chem.*, **2004**, 592—599 (2004).
15) Ma B.-J., Liu J.-K., *Z. Naturforsch.*, **60b**, 565—568 (2005).
16) Hashimoto T., Quang D. N., Kuratsune M., Asakawa Y., *Chem. Pharm. Bull.*, **54**, 912—914 (2006).
17) Liu J.-K., *Chem. Rev.*, **106**, 2209—2223 (2006).
18) Liu J.-K., *Chem. Rev.*, **105**, 2723—2744 (2005).
19) Liu J.-K., *Heterocycles*, **57**, 157—167 (2002).
20) Tringali C., Piattelli M., Geraci C., Nicolosi G., Rocco C., *Can. J. Chem.*, **65**, 2369—2372 (1987).
21) Luo D.-Q., Shao H.-J., Zhu H.-J., Liu J.-K., *Z. Naturforsch.*, **60c**, 50—56 (2005).
22) Ishii N., Takahashi A., Kusano G., Nozoe S., *Chem. Pharm. Bull.*, **36**, 2918—2924 (1988).
23) Kenmoku H., Kato N., Shimada M., Omoto M., Mori A., Mitsuhashi W., Sassa T., *Tetrahedron Lett.*, **42**, 7439—7442 (2001).