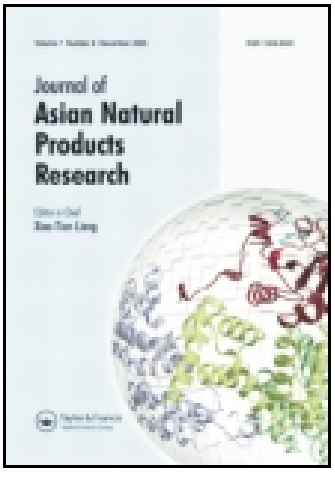


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Compounds from the Chinese black ant (Polyrhachis dives) and NMR behavior of the isomers with formamide group

Jian-Jun Tang[ab], Qi Luo[b], Lei Di[b], Li Zhang[c], Qing Lu[b], Bo Hou[b], Zhi-Li Zuo[b], Hou-Lin Xia[a], Xiu-Jing Ma[d] & Yong-Xian Cheng[ab]

[a] College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China

[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[c] Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China

[d] Center for Drug Evaluation, State Food and Drug Administration, Beijing 100038, China

Published online: 31 Oct 2014.

To cite this article: Jian-Jun Tang, Qi Luo, Lei Di, Li Zhang, Qing Lu, Bo Hou, Zhi-Li Zuo, Hou-Lin Xia, Xiu-Jing Ma & Yong-Xian Cheng (2015) Compounds from the Chinese black ant (Polyrhachis dives) and NMR behavior of the isomers with formamide group, Journal of Asian Natural Products Research, 17:1, 20-26, DOI: 10.1080/10286020.2014.966698

To link to this article: http://dx.doi.org/10.1080/10286020.2014.966698

Journal of Asian Natural Products Research, 2015
Vol. 17, No. 1, 20–26, http://dx.doi.org/10.1080/10286020.2014.966698

Compounds from the Chinese black ant (*Polyrhachis dives*) and NMR behavior of the isomers with formamide group

Jian-Jun Tang[a,b1], Qi Luo[b1], Lei Di[b1], Li Zhang[c], Qing Lu[b], Bo Hou[b], Zhi-Li Zuo[b], Hou-Lin Xia[a]*, Xiu-Jing Ma[d]* and Yong-Xian Cheng[a,b]*

[a]*College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China;* [b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;* [c]*Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China;* [d]*Center for Drug Evaluation, State Food and Drug Administration, Beijing 100038, China*

(*Received 25 August 2014; final version received 14 September 2014*)

Two new dopamine derivatives divesamides A (**1**) and B (**2**), along with six known *N*-containing compounds were isolated from the Chinese black ant (*Polyrhachis dives*). Their structures were determined on the basis of spectroscopic methods. Compound **1** is a racemate, and chiral HPLC separation yielded a pair of antipodes. The absolute configuration of (+)-**1** was assigned by a computational method. The double signals in the ^{1}H and ^{13}C NMR spectra of **2** that resulted from the presence of a formamide group were discussed. The T- and B-lymphocytes proliferation assay showed that **2** has moderate immunosuppressive activity toward T- and B-lymphocytes proliferation at a concentration of 20 μM.

Keywords: *Polyrhachis dives*; black ant; *N*-containing compound; amide bond; immunosuppressive activity

1. Introduction

The Chinese black ant (*Polyrhachis dives*) is mainly distributed in Southeast Asia and Australia [1]. It has been used as a traditional medicine and food ingredient in China for centuries. As a traditional medicine, it has been used to treat rheumatoid arthritis, hepatic disorders, and diabetes [2,3]. Pharmacological studies showed that *P. dives* possesses immune-regulatory, antiinflammatory, hepatoprotective, and analgesic activities [4–6]. However, the small molecules in the title species are largely unknown. In the course of searching for active compounds from the insects, eight *N*-containing compounds were isolated (Figure 1), two of which are new dopamine derivatives. In this contribution, we describe the isolation, structure elucidation, and biological evaluation of these compounds.

2. Results and discussion

Compound **1** had the molecular formula $C_{12}H_{17}NO_4$ derived from its HR-EI-MS at *m/z* 239.1153 $[M]^+$, ^{13}C NMR, and DEPT spectra, indicating 5 degrees of unsaturation. The ^{13}C NMR and DEPT spectra showed 12 carbons attributed to 2 methyl, 2 methylene, 4 methine including 3 aromatic ones and an oxygenated one, and 4 quaternary carbons including a carbonyl group (δ_C 173.4). The ^{1}H NMR spectrum (Table 1) revealed an ABX spin system (δ_H 6.75, d, $J = 1.9$ Hz, H-2; δ_H 6.63, dd, $J = 8.1$, 1.9 Hz, H-6; δ_H 6.74, d, $J = 8.1$ Hz, H-5). These data resembled those of **6**, suggesting that **1** is a dopamine

*Corresponding authors. Email: 18980555665@163.com; maxiujing@sina.com; yxcheng@mail.kib.ac.cn

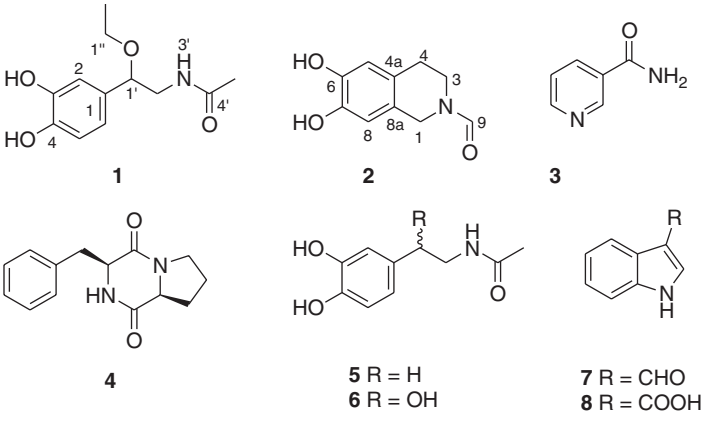


Figure 1. The structures of compounds **1**–**8**.

derivative. Compared to **6**, an additional ethoxy group occurred in the structure of **1**, the HMBC correlation of H-1″/C-1′ indicated that this group was located at C-1′ (Figure 2). The racemic nature of **1** was indicative of its optical rotation. Chiral HPLC separation afforded (+)- and (−)-antipodes of **1**. Computational methods were used to assign the absolute configuration. A theoretical calculation of electronic circular dichroism (ECD) spectra was performed by TDDFT/B3LYP/6-311 + + G(2d,2p) level with three different solvent models (the polarizable continuum model (PCM), the conductor-like screening model (COSMO), and the gas phase). Because the CD spectrum of the compound is strongly dependent upon the dihedral angle formed around the axis of chromophore, the Boltzmann distribution of the different conformers has to be taken into account. A good agreement was found between the experimental and calculated ECD spectra. TD/B3LYP/6-311++(2d, dp) with COSMO model was proved to be able to predicate the strength of the experimental peak at 203 nm (Figure 3). All ECD spectra led to unambiguous assignment of *S*-configuration to (+)-**1**. Thus, the absolute configuration of the antipodes of **1** was determined as shown.

Table 1. ^{1}H and ^{13}C NMR spectral data (400 MHz for ^{1}H and 100 MHz for ^{13}C, methanol-d_4) of **1** (δ in ppm, J in Hz).

	1	
No.	δ_H	δ_C
1		132.9 (s)
2	6.75 (d, 1.9)	114.6 (d)
3		146.2 (s)
4		146.5 (s)
5	6.74 (d, 8.1)	116.2 (d)
6	6.63 (dd, 8.1, 1.9)	119.5 (d)
1′	4.22 (dd, 8.3, 4.7)	81.3 (d)
2′	2.33–2.35 (m)	47.1 (t)
4′		173.4 (s)
5′	1.92 (s)	22.5 (q)
1″	3.38–3.42 (m)	65.0 (t)
2″	1.16 (t-like, 7.0)	15.6 (q)

The molecular formula of compound **2** was established as $C_{10}H_{11}NO_3$ from its HR-EI-MS at m/z 193.0733 $[M]^+$, ^{13}C NMR, and DEPT spectra, suggesting six degrees of unsaturation. The ^{13}C NMR and DEPT spectra of **2** showed 10 carbons including 3 methylene, 2 aromatic methine, an aldehyde and 4 aromatic quaternary carbons (2 are oxygenated). The ^{1}H NMR spectrum (Table 2) showed two singlets in the aromatic region, suggesting a 1,2,4,5-tetrasubstituted benzene. The ^{1}H–^{1}H COSY correlations of H-3/H-4 and HMBC correlations of H-3/C-4a, H-9/C-3, in consideration of the molecular formula, revealed the structure fragment of C-4–C-3–*N*–C-9. In addition to a benzene group

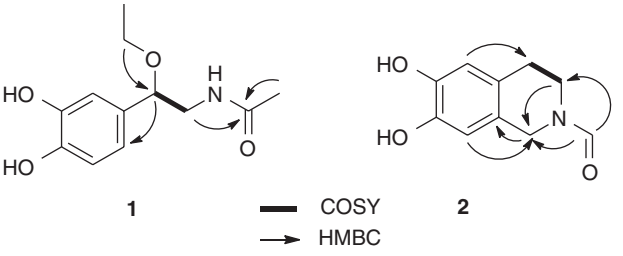


Figure 2. The key COSY and HMBC correlations of **1** and **2**.

and an aldehyde, the remaining 1 degree of unsaturation required the formation of one ring. The HMBC correlations of H-1/C-8a, H-3, and H-9/C-1 indicated that the *N*-atom was connected to C-8a via C-1. Interestingly, the double signals were observed in both the ^{1}H and ^{13}C NMR spectra of **2** (Figure S1 in the Supplementary material), although it is of HPLC grade purity. This may result from conformational isomerization due to restricted C–*N*-amide bond rotation. Actually, the structures with formamide group are readily isomerized in solution [7–11]. Usually, conformational isomerization varies according to the temperature; however, we tried to record the NMR spectra of **2** even at 333 K, the ratio of two isomers (approximately 3:2) remained almost unchanged. Furthermore, ROESY experiments showed correlations of H-9/H-3 (major peaks), and of H-9/H-1 (minor peaks) (Figure S2 in the Supplementary material), suggesting that the *cis*-isomer predominates in the equilibrium mixture (Figure 4).

The known compounds were identified as niacinamide (**3**) [12], cyclo-(L-Pro–L-Tyr) (**4**) [13], *N*-acetyldopamine (**5**) [14], (±)-*N*-(2-(3,4-dihydroxyphenyl)-2-hydroxyethyl)acetamide (**6**) [15], 1*H*-indole-3-carboxaldehyde (**7**) [16], and 1*H*-indole-3-carboxylic acid (**8**) [17] by comparison of

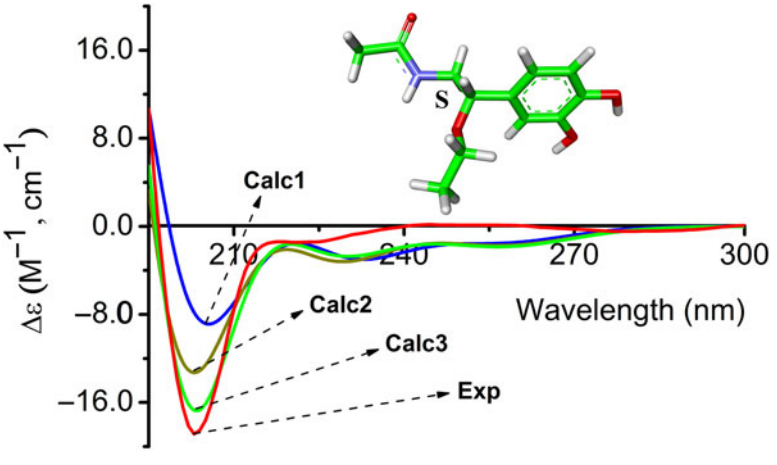


Figure 3. Key structure and relative energy of the 10 lowest-energy conformers used for ECD calculations at the TDDFT/B3LYP/6-311 + + G(2d,2p) level, 50 first excited state. All calculated spectra were wavelength corrected to match the experimental UV spectra (bandwidth: 0.4 eV). (Calc1 in the gas phase; Calc2 with the PCM in MeOH; Calc3 with the COSMO model in MeOH; Exp experimental in MeOH).

Table 2. ^{1}H and ^{13}C NMR spectral data (600 MHz for ^{1}H and 150 MHz for ^{13}C, DMSO-d_6) of **2** (δ in ppm, J in Hz).

	2-*cis* (major)		**2**-*trans* (minor)	
No.	δ_H	δ_C	δ_H	δ_C
1	4.34 (s)	41.1 (t)	4.36 (s)	37.5 (t)
3	3.54 (t, 5.8)	42.8 (t)	3.55 (t, 5.8)	46.0 (t)
4	2.62 (t, 5.8)	28.5 (t)	2.56 (t, 5.8)	26.8 (t)
4a		124.3 (s)		124.5 (s)
5	6.49 (s)	115.6 (d)	6.49 (s)	115.6 (d)
6		144.1[a](s)		144.2 (s)
7		144.0[a] (s)		143.9 (s)
8	6.52 (s)	113.2 (d)	6.49 (s)	112.8 (d)
8a		122.3 (s)		123.4 (s)
9	8.10 (s)	161.7 (d)	8.16 (s)	161.2 (d)

[a] The signals may be interchanged.

their spectroscopic data with literature data.

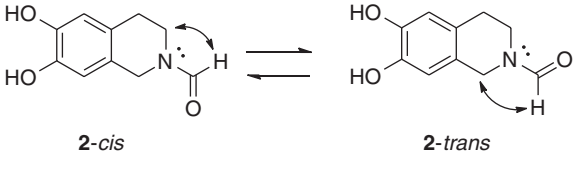
Considering that *P. dives* has been used for the treatment of rheumatoid and osteoarthritis, the antiproliferation action of compounds **2** and **3** was tested against T- and B-lymphocytes of Kunming mice. Compound **2** exhibited immunosuppressive effects on the proliferation of T- and B-lymphocytes at 20 μM by 31.27% and 24.18%, respectively. In contrast, compound **3** was inactive on either.

Mediators including TNF-α are involved in inflammation. We investigated the effects of compounds **2** and **3** on the production of TNF-α from lipopolysaccharides (LPS)-activated RAW264.7 macrophages cultures in the presence and absence of the compounds. The results showed that compound **2** could inhibit the production at 20 and 4 μM by 31.63% and 20.9%, respectively. But compound **3** has no inhibitive effect on the production of TNF-α.

3. Experimental

3.1 General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter (Horiba, Kyoto, Japan). CD spectra were measured on a Chirascan instrument (Applied Photophysics, Leatherhead, UK). UV spectra were recorded on a Shimadzu UV-2401PC spectrometer (Shimadzu, Kyoto, Japan). IR spectra were recorded on a Tensor 27 spectrometer (Bruker Optics, Ettlingen, Germany) with KBr pellets. NMR spectra were recorded on a Bruker AV-600 spectrometer (Bruker, Rheinstetten, Germany), with TMS as an internal standard. EI-MS and HR-EI-MS were collected by an AutoSpec Premier P776 spectrometer (Waters, Milford, MA, USA). ESI-MS were collected by an API QSTAR Pulsar 1 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA). Semi-preparative HPLC was carried out using an Agilent 1200 liquid



2-*cis* **2**-*trans*

Figure 4. The equilibrium of **2** in solution.

chromatograph (Agilent, Santa Clara, CA, USA), the column used was a 250 mm × 9.4 mm, i.d., 5 μm, Zorbax SB-C_{18} and a 250 mm × 4.6 mm, i.d., 5 μm, Daicel Chiralpak IC. Column chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China), C-18 silica gel (40–60 μm; Daiso Co., Osaka, Japan), MCI gel CHP 20P (75–150 μm; Mitsubishi Chemical Industries, Tokyo, Japan), and Sephadex LH-20 (Amersham Pharmacia, Biosciences, Uppsala, Sweden).

3.2 Insect material

The whole bodies of *P. dives* were collected in September 2012 from Wenshan, Yunnan Province, China, and were identified by Prof. Zheng-Hui Xu at Southwest Forestry University. A voucher specimen (CHYX-0588) is deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

Dried powders of *P. dives* (50 kg) were extracted thrice with 70% EtOH (each 25 l, 72 h) at room temperature followed by concentration under reduced pressure to give an extract (2 kg), which was suspended in H_2O and partitioned by EtOAc (each 5 l × 15 l) to afford an EtOAc extract. The EtOAc extract (500 g) was separated into fractions A–H by MCI gel CHP 20P with gradient aqueous MeOH (10–100%) as the eluent. Fraction B (15 g) was gel filtered over Sephadex LH-20 (MeOH), followed by RP-18 column (aqueous MeOH, 5–35%,) to give B3.4 (150 mg), which was further purified by using preparative TLC developed with $CHCl_3$/MeOH (7:1) and semi-preparative HPLC (aqueous acetonitrile, 25%) to give compounds **1** (t_r = 14.4 min, 10 mg), **5** (t_r = 16.2 min, 12 mg), and **6** (t_r = 20.9 min, 13 mg). Fraction C (10 g) was gel filtered over Sephadex LH-20 (MeOH) followed by RP-18 column (aqueous MeOH, 10–40%) to afford fr. C3.6 (130 mg), which was submitted to preparative TLC ($CHCl_3$/MeOH, 9:1) followed by semi-preparative HPLC (MeOH/H_2O, 25%) to give compounds **2** (t_r = 12.4 min, 13 mg), **3** (t_r = 14.4 min, 3 mg), and **4** (t_r = 17.4 min, 2 mg). Fraction E (12 g) was subjected to Sephadex LH-20 (MeOH) followed by RP-18 column (aqueous MeOH, 30–70%) to yield fr.E4.5 (80 mg), which was separated by preparative TLC ($CHCl_3$/MeOH, 9:1) and finally purified via semi-preparative HPLC (aqueous MeOH, 25%) to produce compounds **7** (t_r = 11.5 min, 2 mg) and **8** (t_r = 13.4 min, 2.2 mg). In addition, racemic **1** was separated by chiral phase HPLC (*n*-hexane/iPrOH, 77:23, flow rate: 1 ml/min) to afford (+)-**1** (t_r = 8.5 min, 3 mg) and (−)-**1** (t_r = 12.0 min, 4.3 mg).

3.3.1 Divesamide A (*1*)

Yellowish gum; {$[\alpha]_D^{23}$ + 23.0 (*c* 0.30, MeOH); CD (MeOH) $\Delta\varepsilon_{204\,nm}$ − 4.75, $\Delta\varepsilon_{230\,nm}$ − 3.16, $\Delta\varepsilon_{280\,nm}$ − 3.12; (+)-divesamide A}; {$[\alpha]_D^{23}$ − 22.0 (*c* 0.44, MeOH); CD (MeOH) $\Delta\varepsilon_{204\,nm}$ + 4.67, $\Delta\varepsilon_{230\,nm}$ + 3.59, $\Delta\varepsilon_{280\,nm}$ + 3.25; (−)-divesamide A}; IR (KBr) ν_{max}: 3439, 3427, 2975, 2931, 2879, 1635, 1524, 1445, 1376, 1284, 1097, 599 cm^{-1}; UV (MeOH) λ_{max} (log ε): 284 (3.42), 203 (4.45) nm. For ^1H (400 MHz) and ^{13}C NMR (100 MHz) data, see Table 1. EI-MS: *m/z* 239 $[M]^+$. HR-EI-MS: *m/z* 239.1153 $[M]^+$ (calcd for $C_{12}H_{17}NO_4$, 239.1158).

3.3.2 Divesamide B (*2*)

Yellowish gum; IR (KBr) ν_{max}: 3440, 3424, 2957, 2925, 2854, 1649, 1527, 1453, 1396, 1359, 1335, 1277, 1188, 1098, 872 cm^{-1}; UV (MeOH) λ_{max} (log ε): 290 (3.40), 204 (4.41) nm. For ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data, see

Table 2. ESI-MS: *m/z* 192 $[M - H]^-$. HR-EI-MS: *m/z* 193.0733 $[M]^+$ (calcd for $C_{10}H_{11}NO_3$, 193.0739).

3.4 Activities assay

3.4.1 Antiproliferative assay

Lymphocytes were isolated from the spleen of Kunming mice. Proliferation of lymphocytes was analyzed *in vitro* using the CCK-8 colorimetric assay. Lymphocytes were seeded into 96-well plates at a cell density of 1×10^5/well. Activation of T- and B-lymphocytes was induced by ConA and LPS (final concentration, 5 μg/ml). The final concentration of the test compounds in the assay was 20 μM. Wells containing PRMI 1640 was used as a negative control. All assays were carried out in duplicate. After 72 h of incubation, CCK-8 was added and the cells was cultured for 4 h, and then spectrophotometric measurement were carried out at 450 nm using a microplate reader. Inhibition (%) was calculated using the following formula:

$$\text{inhibition}(\%) = \frac{A - B}{A - C} \times 100.$$

$A - C$: *A*: ConA or LPS (+), sample (−); *B*: ConA or LPS (+), sample (+); *C*: ConA and LPS (−), sample (−).

3.4.2 Assay for TNF-α production inhibition

Inhibitory effects of compounds **2** and **3** on the TNF-α production in LPS-induced RAW264.7 were evaluated. RAW264.7 cells were seeded into 96-well plates at a cell density of 5×10^3/well and precultured at 37°C, 5% CO_2 in air for 4 h. Nonadherent cells were removed by washing the cells with phosphate-buffered saline. The adherent cells were cultured in a fresh medium (200 μl) containing 10 μg/ml LPS and various concentrations of compounds **2** and **3** for 24 h. Production of TNF-α was assessed by the ELISA kit. Inhibition (%) was calculated using the following formula:

$$\text{inhibition}(\%) = \frac{A - B}{A - C} \times 100.$$

$A - C$: *A*: LPS (+), sample (−); *B*: LPS (+), sample (+); *C*: LPS (−), sample (−).

Acknowledgments

This work was financially supported by the Open Research Fund of State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources.

Note

1. These authors contributed equally to this paper.

References

[1] J. Wang and T.X. Liu, *Mod. Food Sci. Technol.* **26**, 1092 (2010).
[2] N.J. Huang and S.X. Xiao, *Res. Pract. Chin. Med.* **17**, 60 (2003).
[3] J. Li and H. Gang, *Chin. Pharm. 21*, 75 (2012).
[4] Y.X. Li and X.G. He, *Food Res. Dev.* **33**, 11 (2012).
[5] J. Chen, J.S. Liu, C.X. Fan, F.L. Wu, M. Z. Zhang, L. Tan, J.Q. Zhu, and W. Q. Sun, *J. Jilin Univ.* (Medicine Edition) **30**, 543 (2004).
[6] C.H. Pu, X.X. Shao, and W.J. Guo, *Chin. Tradit. Patent Med.* **29**, 1150 (2007).
[7] R. Quintanilla-Licea, J.F. Colunga-Valladares, A. Caballero-Quintero, C. Rodríguez-Padilla, R. Tamez-Guerra, R. Gómez-Flores, and N. Waksman, *Molecules* **7**, 662 (2002).
[8] W. Kim, H.J. Lee, Y.S. Choi, J.H. Choi, and J. Yoon, *J. Chem. Soc. Faraday Trans.* **94**, 2663 (1998).
[9] S. Ullrich, G. Tarczay, X. Tong, C.E. H. Dessent, and K. Müller-Dethlsfs, *Phys. Chem. Chem. Phys.* **3**, 5450 (2001).
[10] J. Vaara, J. Kaski, J. Jokisaari, and P. Diehl, *J. Phys. Chem. A* **101**, 5069 (1997).
[11] I.P. Gerothanassis, C. Vakka, and A. Troganis, *J. Magn. Reson. Ser. B* **111**, 220 (1996).

[12] B.J. Shi, G.X. Chou, and Z.T. Wang, *J. Chin. Pharm. Univ.* **41**, 26 (2010).
[13] H.C. E, H. Tang, B.S. Liu, and L. Ling, *Chin. J. Mar. Drugs* **32**, 31 (2010).
[14] N. Naoki, K. Shinichi, and M. Yoko, *Chem. Pharm. Bull.* **48**, 1749 (2000).
[15] J.S. Steven and S. Manickam, *J. Biol. Chem.* **265**, 16992 (1990).
[16] Y.M. Yan, X.P. Dong, Y. Li, and Y. X. Cheng, *Chin. Tradit. Herb. Drugs* **44**, 269 (2013).
[17] B.Q. Bao, P. Zhang, and Y.M. Lee, *Mar. Drugs* **5**, 31 (2007).