Sesquiterpenoids and Diarylheptanoids from *Nidus Vespae* and Their Inhibitory Effects on Nitric Oxide Production

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Two coriamyrtin-type sesquiterpenes, fengfangin A (**1**) and tutin (**2**), and six diarylheptanoids, namely alnusone (**3**), centrolobol (**4**), muricarpone B (**5**), 1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-one (**6**), (3*S*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-ol (**7**), and (3*S*)-1-(4-hydroxyphenyl)-7-(3,4-dihydroxyphenyl)heptan-3-ol (**8**), were isolated from the 95% EtOH extract of *nidus vespae*, the nest of *Polistes* species. Their structures were identified by spectroscopic methods. Compounds **1** and **8** are new products. The absolute configuration of **1** was determined by single-crystal X-ray diffraction analysis using *Flack* parameter. The biological tests showed that compounds **5**, **6**, and **8** could inhibit nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells with IC_{50} values in the range of 13–17 μM, whereas the sesquiterpenes were inactive in this assay (>25 μM). In addition, the ecological significance of the presence of neurotoxic sesquiterpene lactones in *nidus vespae* is briefly discussed.

Introduction. – For millennia, animals or their products have been used in medicine as remedies in various cultures [1–3]. Even in modern societies, animal-based remedies are still essential alternatives among many known therapies practiced around the world. For example, in India, *ca.* 15–20 percent of the Ayurvedic medicine is derived from animal substances [4], and, in traditional Chinese medicine (TCM), over 1500 animal species have been recorded to be of medicinal significance [5]. It is estimated that *ca.* 13% of the medicines used in TCM today are animal-based products. Obviously, medicinal animal ingredients have become a feature of traditional Chinese culture. However and unfortunately, systematic investigation on medicinal animals has often been neglected in the past when compared with plant species. In particular, compounds used by animals as chemical defense might be an intriguing source of active compounds for pharmaceutical uses. *Nidus vespae*, the nest of *Polistes* species, is a commonly used traditional Chinese medicine for the treatment of cancers, rheumatoid arthritis, skin tinea, and teeth pain [6]. Recently, a neuroactive compound, polybioside, was characterized from the venom of the social wasp *Polybia paulista* [7], whereas

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small molecules present in *nidus vespae* responsible for its therapeutic effects are still not well-understood. The aim of this study is to unveil the chemical profile of *nidus vespae*, which led to the isolation of two coriamyrtin-type sesquiterpene lactones and six diarylheptanoids (*Fig. 1*). In correlation with its traditional use, anti-inflammatory potential of these isolates were evaluated by measuring NO release against lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells.

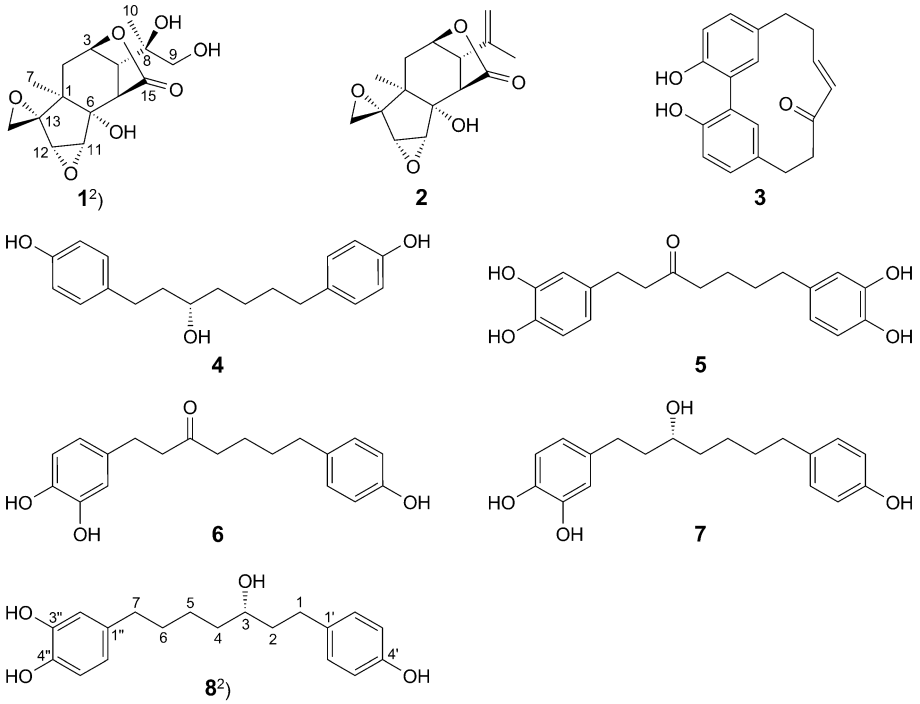

Fig. 1. *The structures of compounds* **1**–**8**

Results and Discussion. – *Nidus vespae* collected from Deqin County of Yunnan Province, P. R. China, was soaked with 95% EtOH. The EtOH extract was submitted to a combination of chromatographic methods including column chromatography (CC) on silica gel, C_{18} reversed-phase silica gel, *MCI* gel *CHP 20P*, and *Sephadex LH-20*, and semi-preparative HPLC on C_{18} to afford compounds **1**–**8** (see *Exper. Part*).

Compound **1** was obtained as colorless crystals. The molecular formula $C_{15}H_{20}O_7$ was established on the basis of HR-ESI-MS (*m*/*z* 311.1128 ($[M-H]^-$, $C_{15}H_{19}O_7^-$; calc. 311.1130)), indicating six degrees of unsaturation The IR spectrum showed an absorption band at 1751 cm^{-1} assignable to the lactone group. The ^{1}H-NMR spectrum exhibited signals of two tertiary Me groups at δ(H) 0.90 (*s*, H–C(7)) and 1.13 (*s*, H–C(10)) (*Table 1*). Two diagnostic vicinal O-bearing CH-group signals at δ(H) 3.65

[2]) Arbitrary numbering. For systematic names, see *Exper. Part*.

Table 1. *NMR Data for Compound* **1**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Position	δ(H)	δ(C)
C(1)		39.4
CH_2(2)	1.51 (*dd*, J=15.6, 4.4, H_α), 1.71 (*d*, J=15.6, H_β)	31.8
H–C(3)	4.75 (br. *t*, J=3.6)	78.6
H–C(4)	2.49 (*dd*, J=4.4, 4.0)	47.0
H–C(5)	2.89 (*d*, J = 4.0)	48.9
C(6)		74.5
Me(7)	0.90 (*s*)	23.0
C(8)		71.4
CH_2(9)	3.40 (*dd*, J=10.8, 5.2, H_α), 3.16 (*dd*, J=10.8, 5.2, H_β)	68.5
Me(10)	1.13 (*s*)	23.4
H–C(11)	3.65 (*d*, J=3.2)	60.9
H–C(12)	3.19 (*d*, J=3.2)	58.0
C(13)		67.2
CH_2(14)	2.78 (*d*, J=4.0, H_α), 2.96 (*d*, J=4.0, H_β)	51.9
C(15)		175.5
HO–C(6)	7.15 (*s*)	
HO–C(8)	6.36 (*s*)	
HO–C(9)	5.23 (br. *t*, J=5.1)	

(*d*, J=3.2, H–C(11)) and 3.19 (*d*, J=3.2, H–C(12)) suggested the presence of an epoxide moiety. Two resonances of geminal H-atoms at δ(H) 2.96 (*d*, J=4.0, H_α–C(14)) and 2.78 (*d*, J=4.0, H_β–C(14)) indicated another epoxide group in the molecule. The ^{13}C-NMR spectrum exhibited signals for two Me, three CH_2, and five CH groups, and five quarternary C-atoms. These data were similar to those of tutin (**2**) [8], differing only in that a C=C bond in **2** was replaced by two O-bearing C-atoms of **1**. This was confirmed by the HMBCs H–C(4)/C(8), H–C(9)/C(4), and H–C(10)/C(4), C(9) (*Fig. 2*). The relative configuration of **1** was assigned by ROESY experiment. The ROESY correlations H_α–C(14)/H_β–C(12), H_β–C(11), HO–C(6); HO–C(8)/H_α–C(5), H–C(7); and H–C(10)/ H_α–C(2), H_α–C(3) provided the relative configurations of the stereogenic centers of **1**. The absolute configuration of **1** was established as (1*S*,3*S*,4*S*,5*S*,6*R*,8*R*,11*S*,12*R*,13*S*) by X-ray diffraction analysis according to *Flack* parameter of 0.0 (3) [9], using anomalous dispersion with copper radiation (*Fig. 3*). Taken together, the structure of **1** was elucidated as depicted in *Fig. 1* and named fengfangin A[2]).

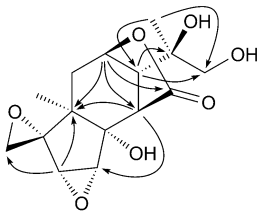

Fig. 2. *Key HMBCs for compound* **1**

The molecular formula of compound **8** was determined as $C_{19}H_{24}O_4$ by the HR-ESI-MS (*m*/*z* 315.1601 ($[M-H]^-$, $C_{19}H_{23}O_4^-$; calc. 315.1596). The ^{1}H- and ^{13}C-NMR

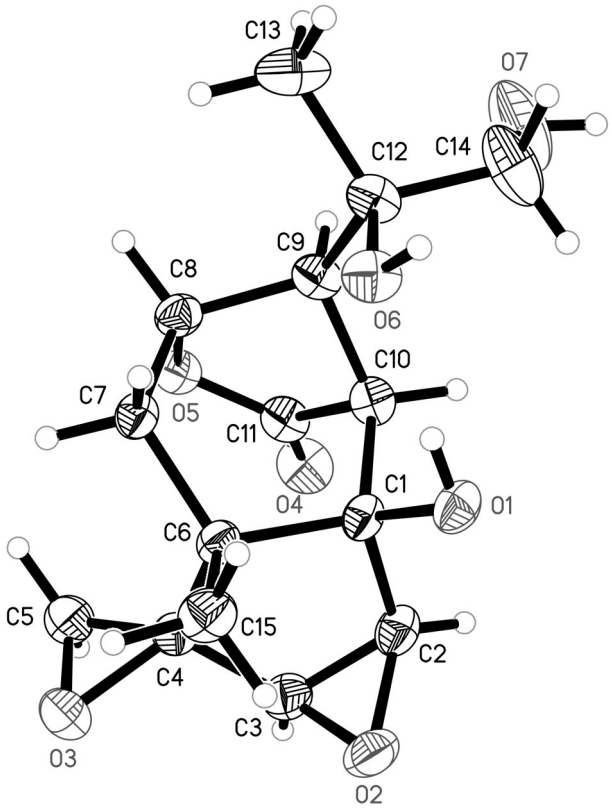

Fig. 3. *ORTEP Plot of* **1**. Displacement ellipsoids are drawn at the 30% probability level.

spectra indicated the presence of a 3-hydroxyphenyl group, a 3,4-dihydroxyphenyl group, six CH_2 C-atoms, and an O-bearing CH group. These signals were similar to those of **4**, differing in that a 4-hydroxyphenyl group in **4** was replaced by a 3,4-dihydroxyphenyl group in **8**. The OH group was positioned at C(3) by the HMBCs of H–C(1)/C(3), C(1′), and C(2′). The absolute configuration at C(3) of **8** was assigned as (*S*) by comparison of its specific optical rotation with those of a series of similar diarylheptanoids isolated from *Curcuma kwangsiensis* [10]. Thus, compound **8** was deduced as (3*S*)-1-(4-hydroxyphenyl)-7-(3,4-dihydroxyphenyl)heptan-3-ol[2]).

The known compounds were identified as tutin (**2**) [8], alnusone (**3**) [11], centrolobol (**4**) [12], muricarpone B (**5**) [13], and (3*S*)-1-(3,4-dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-ol (**7**) [10] by comparison of their spectroscopic data with those reported in the literature. The NMR data of compound **6** are reported for the first time. Compound **7** and its enantiomer have been recently isolated from the rhizomes of *Curcuma kwangsiensis* [10]. We assigned the absolute configuration of **7** by comparison of its specific rotation ($[\alpha]_D^{17} = -6.7$ ($c = 0.30$, MeOH) for **7**) [10].

Considering the medical applications of *nidus vespae*, the selected isolates from this animal material were assessed for their anti-inflammatory potentials by determining

NO release against LPS-stimulated RAW 264.7 macrophage cells with MG132 as a positive control, as MG132 is an inhibitor of the proteasome which could prevent LPS-stimulated NF-κB signaling activation [14]. It was found that diarylheptanoids **5**, **6**, and **8** exhibited inhibitory effects in this assay with IC_{50} values of 16.8, 13.2, and 13.8 μM, respectively (*Table 2*), whereas the sesquiterpene lactones were inactive (>25 μM). To exclude the effects of **5**, **6**, and **8** related with their cellular toxicity, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) control assay was performed in every experiment. The results showed that these compounds exhibit no cytotoxicity in RAW 264.7 macrophage cells at the concentrations of 0.04, 0.2, 1, 5, and 25 μM.

Table 2. *Inhibitory Effects of Compounds* **1**–**8** *on NO Release* (IC_{50} values ± SD, $n=3$)

Compound	IC_{50} [μM]
1	>25
2	>25
4	>25
5	16.8 ± 0.8
6	13.2 ± 2.1
8	13.8 ± 1.9
MG132[a])	0.1 ± 0.0

[a]) Positive control.

Compounds **1** and **2** are typical neurotoxins mainly found in the family Coriaceae. It is interesting to discover these plant-derived substances in *nidus vespae*, moreover, The analogs of **1** or **2** were also found in the honeydew produced in some regions of New Zealand [15–17]. We, therefore, hypothesize that insects of the genus *Polistes* may be able to utilize these toxins as deterrent against their predators.

This work was financially supported by the following grants: *Sino-German International Collaboration Project from Yunnan Province* (No. 2009AC011); *Talent Scholarship of Yunnan Youth* (No. 2007PY01-48); *The Open Research Fund of State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources*; and *Key Project for Drug Innovation* (2008ZX09401-004) from the *Ministry of Science and Technology of China*.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical Inc.*, P. R. China), on C_{18} reversed-phase silica gel (40–60 μm; *Daiso Co.*, Japan), *MCI gel CHP 20P* (75–150 μm, Tokyo, Japan), and *Sephadex LH-20* (*Amersham Pharmacia*, Sweden). Semi-prep. HPLC: *Agilent 1200* liquid chromatograph with a *Zorbax SB-C_{18}* column (5 μm; 9.4 × 250 mm, i.d.). Optical rotations: *Horiba SEPA*-300 polarimeter. UV Spectra: *Shimadzu double-beam 210A* spectrometer, λ_{max} in nm. IR Spectra: *Tensor 27* spectrometer, with KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AV-400* or *DRX-500* or *DRX-600* spectrometer, with TMS as an internal standard. ESI-MS and HR-ESI-MS: *API QSTAR Pulsar 1* spectrometer.

Material. Nidus vespae was collected from Deqin County of Yunnan Province, P. R. China, in August 2008. A voucher specimen (CHYX-0571) was deposited with the State Key Laboratory of Photochemistry and Plant Resources in West China of our institution.

Extraction and Isolation. The dried and powdered *nidus vespae* (20 kg) was soaked with 95% EtOH (3×50 l) at r.t. The extracts were combined and concentrated under reduced pressure to yield a dark residue, which was suspended in H_2O (5 l), followed by successive partition with petroleum ether (PE; 3×5 l), AcOEt (3×5 l), and BuOH (3×5 l). The AcOEt extract (150 g) was separated by CC SiO_2 (10×120 cm, 200–300 mesh, 2.0 kg); gradient of $CHCl_3$/MeOH) to afford *Frs. A–D. Fr. A* (10.2 g) was subjected to CC (*MCI gel CHP 20P*; aq. MeOH 50–90%) to yield *Frs. AI–AIII. Fr. AII* (2.3 g) was loaded by CC (*Sephadex LH-20* MeOH) to obtain **3** (8.2 mg). *Fr. AIII* (1.1 g) was purified by CC (*Sephadex LH-20*; MeOH) to yield **2** (24 mg) and **6** (6.1 mg). *Fr. B* (16.7 g) was subjected to CC (*MCI gel CHP 20P*; aq. MeOH (40–90%) to afford *Frs. BI* and *BII. Fr. BI* (3.1 g) was submitted to CC (*Sephadex LH-20*; MeOH) to yield **4** (40 mg). *Fr. BII* (7.2 g) was purified by CC (*Sephadex LH-20*; MeOH) to afford **5** (120 mg). *Fr. C* (8.6 g) was fractionated by CC (*MCI gel CHP 20P*; aq. MeOH 20–90%) to afford *Frs. CI* and *CII. Fr. CI* (1.0 g) was further separated by CC (*Sephadex LH-20*; MeOH) to give *Fr. CI–I* (50 mg), and *CI–II* (300 mg), and *CI–III* (500 mg). *Fr. CI–II* was purified by semi-prep. HPLC (MeOH/H_2O 45 :55) to yield **7** (8.5 mg, flow rate 2 ml/min; t_R 32.5 min) and **8** (0.6 mg; flow rate 2 ml/min; t_R 30.4 min). *Fr. D* (6.1 g) was divided into three *Fr. DI–DIII* by CC (*MCI gel CHP 20P*; aq. MeOH 20–70%). *Fr. DII* (1.1 g) was subjected to CC (*Sephadex LH-20*; MeOH) to give *Fr. DII–I* (200 mg), which was further purified by CC RP C_{18} silica gel; MeOH/H_2O 70 :30) to afford **1** (28 mg).

*(1a*R*,1b*S*,2*R*,5*R*,6a*S*,7*S*,7a*S*)-8-[(2*R*)-1,2-Dihydroxy-1-methylethyl]hexahydro-1b-hydroxy-6a-methylspiro[2,5-methanooxireno[3,4]cyclopenta[1,2-*d*]oxepine-7,2′-oxiran]-3(2*H*)-one* (**1**). Colorless prism. M.p. 155–158°. $[\alpha]_D^{24} = -6.0$ ($c = 0.45$, MeOH). UV (MeOH): 219.0 (2.39). IR (KBr): 3455, 3229, 2938, 1751, 1070, 924. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz): see *Table 1*. ESI-MS (neg.): 311 ($[M-H]^-$). HR-ESI-MS (neg.): 311.1128 ($[M-H]^-$, $C_{15}H_{19}O_7^-$; calc. 311.1130).

1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-one (**6**). White oil. UV (MeOH): 282 (3.62), 203 (4.40). ^{1}H-NMR (500 MHz, (D_6)acetone)^2): 1.50–1.52 (*m*, H–C(5), H–C(6)); 2.42 (*t*, $J =$ 6.8, H–C(4)); 2.48 (*t*, $J = 6.8$, H–C(7)); 2.65–2.68 (*m*, H–C(2)), H–C(1)); 6.50 (*dd*, $J = 7.8, 2.0$, H–C(6′)); 6.66 (*d*, $J = 2.0$, H–C(2′)); 6.69 (*d*, $J = 7.8$, H–C(5′)); 6.72 (*dd*, $J = 8.8, 2.0$, H–C(3″), H–C(5″)); 6.98 (*d*, $J =$ 8.3, H–C(2″)); 7.00 (*d*, $J = 8.3$, H–C(6″)). ^{13}C-NMR (125 MHz, (D_6)acetone)^2): 23.9 (C(5)); 29.2 (C(1)); 32.0 (C(6)); 35.4 (C(7)); 42.9 (C(4)); 44.9 (C(2)); 115.8 (C(3″)); 115.9 (C(5′)); (C(5″)); 116.1 (C(2′)); 120.2 (C(6′)); 130.0 (C(2″), C(6″)); 133.8 (C(1″)); 134.0 (C(1′)); 143.9 (C(4′)); 145.6 (C(3′)); 156.1 (C(4″)); 209.8 (C(3)). ESI-MS: 313 ($[M-H]^-$).

*4-[(5*S*)-5-Hydroxy-7-(4-hydroxyphenyl)heptyl]benzene-1,2-diol* (**8**). White oil. $[\alpha]_D^{16} = -13.8$ ($c =$ 0.13, MeOH). UV (MeOH): 281 (3.53), 222 (4.06). ^{1}H-NMR (600 MHz, CD_3OD)^2): 1.31–1.35 (*m*, H–C(5a)); 1.41–1.45 (*m*, H–C(5b)); 1.44–1.47 (*m*, H–C(4)); 1.52–1.58 (*m*, H–C(6)); 1.60–1.69 (*m*, H–C(2)); 2.45 (*t*, $J = 7.5$, H–C(7)); 2.49–2.54 (*m*, H–C(1b)); 2.62–2.66 (*m*, H–C(1a)); 3.48–3.50 (*m*, H–C(3)); 6.47 (*dd*, $J = 8.0, 1.6$, H–C(6″)); 6.65 (*d*, $J = 8.0$, H–C(5″)); 6.60 (*d*, $J = 1.6$, H–C(2″)); 6.69 (*d*, $J = 8.3$, H–C(3′), H–C(5′)); 6.99 (*d*, $J = 8.3$, H–C(2′), H–C(6′)). ^{13}C-NMR (150 MHz, CD_3OD)^2): 26.4 (C(5)); 32.3 (C(1)); 33.1 (C(6)); 36.3 (C(7)); 38.3 (C(4)); 40.8 (C(2)); 71.8 (C(3)); 116.2 (C(3′), C(5′)); 116.3 (C(5″)); 116.7 (C(2″)); 120.8 (C(6″)); 130.5 (C(2′), C(6′)); 134.7 (C(1′)); 135.8 (C(1″)); 144.1 (C(4″)); 146.1 (C(3″)); 156.3 (C(4′)). ESI-MS (neg.): 315 ($[M-H]^-$). HR-ESI-MS (neg.): 315.1601 ($[M-H]^-$, $C_{19}H_{23}O_4^-$; calc. 315.1596).

The colorless crystal of **1** was obtained from a mixture of MeOH/DMSO. The X-ray data were collected on a *Bruker AXS SMART APEX II CCD* diffractometer; formula, $C_{15}H_{19}O_7^-$; space group, $P2_12_12_1$, with unit cell parameters $a = 6.9558$ (5) Å, $b = 13.6655(10)$ Å, $c = 14.9197(10)$ Å, $\alpha/\beta/\gamma = 90$, temp.: 296 K, $V = 1418.18(17)$ Å^3, $Z = 4$, $D_c = 1.463$ g/cm^3, $F(000) = 664.0$, the final indices were $R_1 =$ 0.0678, wR_2 0.1623. Crystallographic data (excluding structure factors) of the crystal structure have been deposited as supplementary publication No. CCDC-798735. Copies of the data can be obtained free of charge from the CCDC *via* www.ccdc.cam.ac.uk.

Inhibition of NO production and cell viability in LPS-stimulated RAW 264.7 macrophage cell line. The NO production assay was carried out according to the in method described [18]. Briefly, the murine monocytic RAW 264.7 macrophages were seeded into 96-well plates (2×10^5 cells/well) containing *RPMI 1640* medium (*Hyclone*, USA) with 10% FBS under a humidified atmosphere of 5% CO_2 at 37°. After 24 h pre-incubation, cells were treated with the compounds with the maximum concentration of 25 μM in the presence of 1 μg/ml LPS for 18 h. Each compound was dissolved in DMSO and further diluted in

medium to obtain different concentrations. NO Production was assessed by adding 100 µl of *Griess* reagents *A* and *B* to 100 µl of each supernatant from LPS or the compound-treated cells in triplicate. After 5 min incubation, the absorbance was measured at 570 nm with a *2104 Envision Multilabel Plate Reader* (*Perkin-Elmer Life Sciences, Inc.*, Boston, Ma, USA). *MG132* (*Sigma–Aldrich*, USA) was included as a positive control. Cytotoxicity was determined by the MTT assay as described in [19].

REFERENCES

[1] E. Lev, *J. Ethnopharmacol.* **2003**, *85*, 107.
[2] M. O. Adeola, *Environ. Conserv.* **1992**, *19*, 125.
[3] L. R. Angeletti, U. Agrimi, C. Curia, D. French, R. Mariani-Costantini, *Lancet* **1992**, *340*, 223.
[4] M. M. Mahawar, D. P. Jaroli, *J. Ethnobiol. Ethnomed.* **2007**, *3*, 25.
[5] China National Corporation of Traditional and Herbal Medicine, 'Materia Medica Commonly Used in China', Science Press, Beijing, 1995.
[6] Z. Q. Li, Y. F. Wang, *Shizhen Guoyi Guoyao* **2005**, *16*, 1801.
[7] D. M. Saidemberg, L. C. da Silva-Filho, L. M. M. Cesar Tognoli, C. F. Tormena, M. S. Palma, *J. Nat. Prod.* **2010**, *73*, 527.
[8] N. B. Perry, M. Aiyaz, D. S. Kerr, R. J. Lake, M. T. Leach, *Phytochem. Anal.* **2001**, *12*, 69.
[9] H. D. Flack, *Acta Crystallogr., Sect. A* **1983**, *39*, 876; H. D. Flack, G. Bernardinelli, *J. Appl. Crystallogr.* **2000**, *33*, 1143.
[10] J. Li, F. Zhao, M. Z. Li, L. X. Chen, F. Qiu, *J. Nat. Prod.* **2010**, *73*, 1667.
[11] M. Nomura, T. Tokoroyama, T. Kubota, *Phytochemistry* **1981**, *20*, 1097.
[12] L. V. Alegrio, R. Braz-Filho, O. R. Gottlieb, *Phytochemistry* **1989**, *28*, 2359.
[13] P. M. Giang, P. T. Son, K. Matsunami, H. Otsuka, *Chem. Pharm. Bull.* **2006**, *54*, 139.
[14] A. Trauzold, H. Wermann, A. Arlt, S. Schütze, H. Schäfer, S. Oestern, C. Röder, H. Ungefroren, E. Lampe, M. Heinrich, H. Walczak, H. Kalthoff, *Oncogene* **2001**, *20*, 4258.
[15] M. D. Sutherland, *Anal. Proc.* **1992**, *29*, 112.
[16] T. Palmer-Jones, *New Zeal. Med. J.* **1965**, *64*, 631.
[17] J. W. Blunt, M. G. Munro, W. Swallow, *Aust. J. Chem.* **1979**, *32*, 1339.
[18] X.-J. Zhou, X.-L. Chen, X.-S. Li, J. Su, J.-B. He, Y.-H. Wang, Y. Li, Y.-X. Cheng, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 373.
[19] D. A. Israf, T. A. Khaizurin, A. Syahida, N. H. Lajis, S. Khozirah, *Mol. Immunol.* **2007**, *44*, 673.

Received December 20, 2010