Fischerisin A and B, Cytotoxic Sesquiterpenoid-Geranylhydroquinones from *Ligularia fischeri*

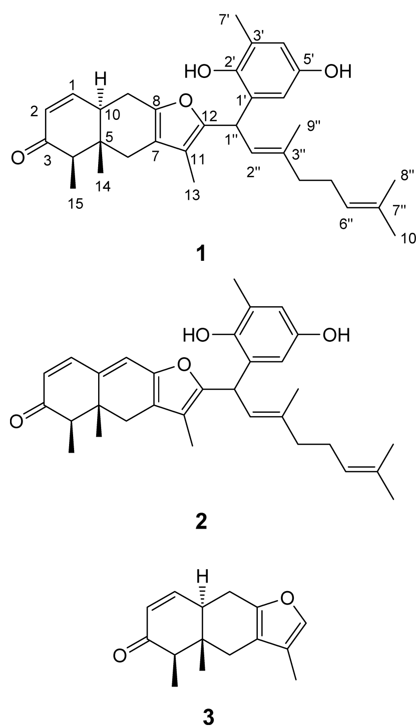
Wei-Dong XIE,*,[a] Xia LI,[a] Cheng-Wu WENG,[a] Shan-Shan LIU,[a] and Kyung Ho ROW[b]

[a] *Marine College, Shandong University at Weihai; Weihai 264209, P. R. China: and* [b] *Department of Chemical Engineering, Inha University; Incheon 402751, Korea.*
Received December 11, 2010; accepted January 26, 2011; published online January 27, 2011

During the course of screening natural sesquiterpenoids for new antitumor agents, two novel compounds, fischerisin A (1) and fischerisin B (2), were isolated from the roots of *Ligularia fischeri*. Their structures were elucidated by interpretation of their IR, high resolution-mass spectrometry (HR-MS), 1D- and 2D-NMR data. Fischerisin A and B are the first representatives of a novel sesquiterpenoid-geranylhydroquinone hybrid, and both compounds exhibited *in vitro* cytotoxicity against cultured human oral epidermoid carcinoma (KB) and human breast cancer (MCF-7) cell lines with IC_{50} values of 9.7 and 10.2 μM, and 9.8 and 17.8 μM, respectively.

Key words *Ligularia fischeri*; sesquiterpenoid; geranylhydroquinone; cytotoxicity; fischerisin A; fischerisin B

Sesquiterpenoids are the characteristic secondary metabolites of the Asteraceae family.[1] Some of them showed a wide range of biological and pharmacological properties including insect antifeedant,[2] anti-inflammatory activity,[3] antibacterial activity,[4] antimalarial activity,[5] cytotoxicity,[6] and nuclear factor κB (NF-κB) inhibition activity[7] *etc.* The investigation of the structure diversities, structure–activity relationships and biosynthetic approaches of the sesquiterpenoid constitutes in traditional Chinese medicines to screen for cytotoxic leads to drug has been the principal subject of our researchs.[8,9] *Ligularia fischeri* (LEDEB.) TURCZ. (Asteraceae) has long been used as a folk medicine in China for the treatment of coughs, inflammations, jaundice, scarlet fever, rheumatoidal arthritis, and hepatic diseases.[10] In the mid-1970s, Japanese researchers initially isolated furanoeremophilane-type sesquiterpenoids from this plant for the first time.[11] Subsequent phytochemical researches of this plant collected in different region have resulted in the isolation of many eremophilane-type lactones and norsesquiterpene derivatives.[12—14] Recently, we reported the structures of eremophilane dimers and an eremophilane lactam from this plant.[15,16] This paper describes the isolation and structure elucidation of two sesquiterpenoid-geranylhydroquinone hybrids, namely fischerisin A (**1**) and fischerisin B (**2**), isolated from the roots of *L. fischeri*. To the best of our knowledge, these two compounds represent a kind of unprecedented sesquiterpenoid derivative. In addition, the *in vitro* cytotoxicity of both compounds against cultured human oral epidermoid carcinoma (KB) and human breast cancer (MCF-7) cell lines was also evaluated.



Results and Discussion

The petroleum ether (60—90 °C)–Et_2O–CH_3OH (1 : 1 : 1) extract of the root of *L. fischeri* was chromatographed on a silica gel column with a gradient of hexane–acetone as eluent. After the sequential purification, fischerisin A (**1**) and fischerisin B (**2**) were finally obtained from the part of hexane–acetone (10 : 1).

Fischerisin A (**1**) was obtained as pale amorphous powder with a molecular formula of $C_{32}H_{40}O_4$ determined by the molecular ion peak at *m/z* 488.2890 ($[M]^+$, $C_{32}H_{40}O_4^+$; Calcd 488.2916) in high resolution electronic ionization mass spectrometry (HR-EI-MS). Its IR spectrum showed the absorption bands of hydroxy group (3423 cm^{-1}) and α,β-unsaturated ketone moiety (1677 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table 1) exhibited signals for a tetrasubstituted aromatic ring [δ_H 6.38 (1H, d, J=1.8 Hz, H-4′) and 6.31 (1H, d, J=1.8 Hz, H-6′)], four olefinic protons [δ_H 6.39 (1H, dd, J=10.0, 1.8 Hz, H-1), 5.94 (1H, dd, J=10.0, 3.2 Hz, H-2), 5.23 (1H, d, J=9.7 Hz, H-2″) and 5.02 (1H, m, H-6″)], seven methyl groups [δ_H 2.08 (3H, s, Me-7′), 1.74 (3H, d, J=0.9 Hz, Me-13), 1.61 (3H, br s, Me-10″), 1.58 (3H, d, J=1.1 Hz, Me-9″), 1.49 (3H, br s, Me-8″), 1.04 (3H, d, J=6.8 Hz, Me-15) and 0.45 (3H, s, Me-14)], and several signals attributed to aliphatic protons. The ^{13}C-NMR and the distortionless enhancement by polarization transfer (DEPT) spectra displayed 32 carbon signals assigned to seven methyls, four methylenes, nine methines and twelve quaternary carbons. The carbon signal at δ_C 200.1 indicated the presence of a ketone

* To whom correspondence should be addressed. e-mail: wdxie@sdu.edu.cn © 2011 Pharmaceutical Society of Japan

group. Sixteen carbon resonances between δ_C 105 and δ_C 150 could be allocated to an aromatic ring and five double bonds. The remaining carbon resonances were upfield of δ_C 53. Detailed examination of the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) (Fig. 1), ^{1}H-detected heteronuclear multiple quantum coherence spectrum (HMQC) and heteronuclear multiple bond coherence spectroscopy (HMBC) (Table 1) completed the establishment of three substructures of **1a**, **1b** and **1c** (Fig. 1), and allowed the unambiguous assignment of all signals (Table 1). Among the ^{1}H-NMR signals, the chemical shifts and coupling patterns of methyl signals [δ_H 1.74 (3H, d, J=0.9 Hz), 1.04 (3H, d, J=6.8 Hz) and 0.45 (3H, s)], and olefinic proton signals [δ_H 6.39 (1H, dd, J=10.0, 1.8 Hz) and 5.94 (1H, dd, J=10.0, 3.2 Hz)] were very similar to that of methyls and α,β-unsaturated ketone moiety in furanoligularenine (**3**) obtained from the same species.[16,17] Hence, compound **1** was supposed to be a derivative of furanoligularenine. The HMBC correlations of H_3-15 (δ_H 1.04) to C-3 (δ_C 200.1), C-4 (δ_C 52.7) and C-5 (δ_C 42.3), H_3-14 (δ_H 0.45) to C-4 (δ_C 52.7), C-5 (δ_C 42.3), C-6 (δ_C 34.1) and C-10 (δ_C 42.6), and H_3-13 (δ_H 1.74) to C-7 (δ_C 105.2), C-11 (δ_C 120.2) and C-12 (δ_C 128.8) further confirmed the presence of a furanoeremophilene moiety with a ketone group at C-3 (Table 1). The ^{1}H–^{1}H COSY spectrum resolved the spin system of (H-2)–(H-1)–(H-10)–(H_2-9) (Fig. 1, substructure **1a**). The HMBC correlations of H-1 (δ_H 6.39) and H-2 (δ_H 5.94) to C-3 (δ_C 200.1) suggested the double bond location between C-1 and C-2, thus completing substructure **1a**. In the HMBC spectrum (Table 1), the correlations of H-4′ (δ_H 6.38) and H-6′ (δ_H 6.31) to C-2′ (δ_C 149.0), and the correlations of H_3-7′ (δ_H 2.08) to C-2′ (δ_C 149.0), C-3′ (δ_C 119.0) and C-4′ (δ_C 114.9) were observed. The observed ^3J HMBC correlations of H-4′ (δ_H 6.38) and H-6′ (δ_H 6.31) to C-2′ (δ_C 149.0) and ^2J HMBC correlations of H-4′ (δ_H 6.38) and H-6′ (δ_H 6.31) to C-5′ (δ_C 149.0) suggested that two hydroxy groups located at C-2′ and C-5′

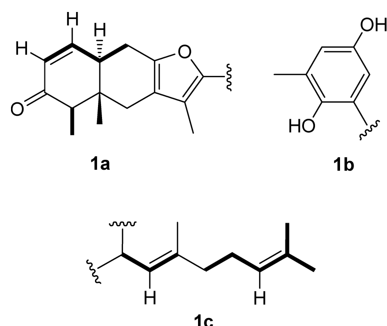


Fig. 1. Substructures of **1a**, **1b** and **1c**, and the Partial Structures (in Bold) Resolved by ^{1}H–^{1}H COSY Spectrum

Table 1. ^{1}H- (500 MHz), ^{13}C- (125 MHz) NMR (DEPT) and HMBC Data for Fischerisin A (**1**) and Fischerisin B (**2**) ($CDCl_3$, δ in ppm, J in Hz)[a]

No.	Fischerisin A (**1**)			Fischerisin B (**2**)		
	δ_C	δ_H mult. (J in Hz)	HMBC	δ_C	δ_H mult. (J in Hz)	HMBC
1	150.1 CH	6.39 dd (10.0, 1.8)	3, 9, 10	144.2 CH	7.02 d (9.7)	3, 5, 9, 10
2	128.0 CH	5.94 dd (10.0, 3.2)	4, 10	124.1 CH	5.99 d (9.7)	4, 10
3	200.1 C	—	—	200.7 C	—	—
4	52.7 CH	2.36 q (6.8)	2, 5, 6, 14	51.9 CH	2.49 q (6.9)	3, 5, 6, 14, 15
5	42.3 C	—	37.7 C	—	—	
6a	34.1 CH_2	2.32 d (13.3)	5, 7, 8	33.6 CH_2	2.68 d (16.4)	5, 7, 8, 10, 11, 14
6b		2.09 br d (13.3)	5, 7, 8		2.42 d (16.4)	4, 5, 7, 8, 10, 11, 14
7	105.2 C	—	—	123.3 C	—	—
8	136.7 C	—	—	148.2 C	—	—
9a	37.5 CH_2	2.06 dd (13.5, 3.3)	1, 5, 7, 8	120.3 CH	6.60 s	1, 5, 7, 8, 10
9b		1.56 dd (13.5, 13.3)	1, 5, 7, 8			
10	42.6 CH	2.86 dddd (13.3, 3.3, 3.2, 1.8)	1, 2, 5, 14	137.0 C	—	—
11	120.2 C	—	—	116.3 C	—	—
12	128.8 C	—	—	153.0 C	—	—
13	8.5 CH_3	1.74 d (0.9)	7, 11, 12	8.0 CH_3	1.96 s	7, 11, 12
14	9.1 CH_3	0.45 s	3, 4, 6, 10	17.4 CH_3	0.77 s	3, 4, 6, 10
15	6.2 CH_3	1.04 d (6.8)	3, 4, 5	7.2 CH_3	1.18 d (6.9)	3, 4, 5
1′	129.1 C	—	—	129.1 C	—	—
2′	149.0 C	—	—	145.5 C	—	—
3′	119.0 C	—	—	126.2 C	—	—
4′	114.9 CH	6.38 d (1.8)	2′, 6′, 7′	116.0 CH	6.53 d (2.9)	2′, 5′, 6′, 7′
5′	149.5 C	—	—	149.1 C	—	—
6′	108.0 CH	6.31 d (1.8)	2′, 4′, 1″	112.7 CH	6.39 d (2.9)	2′, 4′, 5′, 1″
7′	14.3 CH	2.08 s	2′, 3′, 4′	16.2 CH_3	2.19 s	2′, 3′, 4′
1″	44.9 CH	4.25 d (9.7)	11, 12, 2′, 6′, 2″, 3″	37.3 CH	5.11 d (9.3)	11, 12, 1′, 2′, 6″, 2″, 3″
2″	118.2 CH	5.23 d (9.7)	1′, 1″, 4″, 9″	122.4 CH	5.63 br d (9.3)	12, 1′, 4″, 9″
3″	139.1 C	—	—	139.0 C	—	—
4″	38.8 CH_2	1.97 m	2″, 3″, 5″, 9″	39.5 CH_2	2.10 m	2″, 5″, 9″
5″	25.6 CH_2	1.99 m	3″, 4″, 6″, 7″	26.1 CH_2	2.12 m	3″, 4″, 6″
6″	122.8 CH	5.02 m	4″, 8″, 10″	123.6 CH	5.04 m	8″, 10″
7″	130.8 C	—	—	132.1 CH_3	—	—
8″	16.7 CH_3	1.49 br s	6″, 7″, 10″	17.1 CH_3	1.57 br s	6″, 7″, 10″
9″	15.5 CH_3	1.58 d (1.1)	2″, 3″, 4″	16.4 CH_3	1.73 d (1.3)	2″, 3″, 4″
10″	24.6 CH_3	1.61 br s	6″, 7″, 8″	25.7 CH_3	1.66 br s	6″, 7″, 8″

a) Assignments were based on ^{1}H–^{1}H COSY, gHMQC and HMBC experiments.

respectively. Thus, the tetrasubstituted phenyl group was assigned to 2,5-dihydroxy-3-methylbenzoyl group (Fig. 1, substructure **1b**). The ^{1}H-^{1}H COSY spectrum also established the spin systems from H-1″ through H-2″ and from H_2-4″ through H-6″ (Fig. 1, substructure **1c**). In addition, the long rang coupling correlations of H_3-9″ to H-2″, and H_3-8″ and H_3-10″ to H-6″ were also observed. The HMBC correlations of H_3-9″ (δ_H 1.58) to C-2″ (δ_C 118.2) and C-4″ (δ_C 38.8), and H_3-8″ (δ_H 1.49) and H_3-10″ (δ_H 1.61) to C-6″ (δ_C 122.8) connected these two spin systems to a geranyl group which connected to two substitutes at C-1″ simultaneously (Fig. 1, substructure **1c**). Finally, HMBC correlations of H-1″ (δ_H 4.25) to aromatic carbon of C-1′ (δ_C 129.1), C-2′ (δ_C 149.0) and C-6′ (δ_C 108.0), and H-6′ (δ_H 6.31) to C-1″ (δ_C 44.9) connected the geranyl group to aromatic ring. The connections of C-12 to C-1″ could be deduced by the HMBC correlations of H-1″ (δ_H 4.25) to C-11 (δ_C 120.2) and C-12 (δ_C 128.8), and H-2″ (δ_H 5.23) to C-12 (δ_C 128.8). As for relative configuration in **1**, CH_3-14 and CH_3-15 were assigned as β orientation on the basis of biogenetic considerations.[18] Furthermore, the nuclear Overhauser effect correlation spectroscopy (NOESY) also showed the correlations between H_3-14 and H_3-15. The absence of NOESY correlation between H_3-14 and H-10 implied the *trans*-fused rings system in substructure **1a**. The *E* configuration of the double bond between C-2″ and C-3″ in substructure **1c** was assigned according to the correlation between H-2″ and H_2-4″ in the NOESY spectrum. The absolute configuration of C-1″ remains to be defined.

Fischerisin B (**2**) is an analog of **1**. The high resolution electrospray ionization mass spectrometry (HR-ESI-MS) give a quasi molecular ion peak at *m/z* 487.2841 ($[M+H]^+$, $C_{32}H_{39}O_4^+$; Calcd 487.2843) in accordance with the formula of $C_{32}H_{38}O_4$. The ^{1}H-, ^{13}C-NMR and DEPT spectra of **2** (Table 1) were very similar to that of **1**, except for the presence of an additional double bond group [δ_H 6.60 (1H, s, H-9); δ_C 120.3 (C-9) and 137.0 (C-10)] and the obvious downfield shift of H-1 (from δ_H 6.39 in **1** to δ_H 7.02 in **2**) (Table 1). The long rang ^{1}H–^{1}H COSY cross-peak of H-1 (δ_H 7.02) to H-9 (δ_H 6.60) and the HMBC correlation of H_3-14 (δ_H 0.77) to the olefinic carbon C-10 (δ_C 137.0) supported the location of a double bond between C-9 and C-10. Analysis of ^{1}H–^{1}H COSY, gradient heteronuclear multiple quantum coherence spectrum (gHMQC), HMBC and NOESY spectroscopic data indicated that the rest of structure of **2**, including the relative configuration, was same as that of **1**.

Several investigations indicated that sesquiterpenoids could be converted to radicals under the condition of oxidation to form a dimmer in plants.[19,20] Up to now, a few of sesquiterpenoid dimmers have been isolated from Asteraceae plants.[16,19—21] Though, fischerisin A and B are the first representatives of a hybrid consisted of a sesquiterpenoid and a geranylhydroquinone moiety, to our knowledge.

Fischerisin A (**1**) was found to exhibit *in vitro* cancer cell cytotoxicity with IC_{50} of 9.7 and 10.2 μM toward human oral epidermoid carcinoma (KB) and human breast cancer (MCF-7) cell lines respectively. Interestingly, furanoligularenine (**3**) isolated at the same time, possess no cytotoxicity ($IC_{50}>100\,\mu$M), suggesting that the presence of geranylhydroquinone moiety is a crucial structural requirement for the cytotoxic effect of fischerisin A on the cancer cell lines tested. Fischerisin B (**2**) also showed significant cytotoxicity against KB and MCF-7 with IC_{50} of 9.8 and 17.8 μM. The IC_{50} of control drug Doxorubicin were 1.65 and 0.82 μM for KB and MCF-7 cell lines.

Experimental

General Procedures Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were measured on Varian Cary 100 Scan UV–Visible spectrometer. IR spectra were obtained from a Nicolet NEXUS 470 Fourier transform (FT)-IR spectrometer in KBr plate. ^{1}H-, ^{13}C-NMR (DEPT) and 2D-NMR spectra were recorded on Bruker AVANCE 500 spectrometer. Chemical shifts are given on the δ (ppm) scale with tetramethylsilane (TMS) as internal reference. HR-EI-MS and HR-ESI-MS spectra were acquired on a Thermo Fisher LTQ-Orbitrap XL spectrometer. Column chromatography (CC) was carried out on silica gel (200—300 and 300—400 mesh) and TLC on silica gel (GF_{254} 10—40 μm), with both materials supplied by Qingdao Marine Chemical Factory in China. Silica gel C-18 (25—50 μm) used for low pressure CC was purchased from Merck in Germany. The purity of the samples were checked on TLC (silica gel, GF_{254} and C-18) under UV light at 254 nm or by heating after spraying with 5% H_2SO_4 in C_2H_5OH.

Plant Material The roots of *L. fischeri* were collected from Changbai Mountain, Tonghua, Jilin Province, P. R. China, in September 2008, and identified by Associate Prof. Hong Zhao, Marine College, Shandong University at Weihai. A voucher specimen (No. CB200809) was deposited at the herbarium in the Laboratory of Botany, Marine College, Shandong University at Weihai.

Extraction and Isolation The air-dried roots of *L. fischeri* (8.2 kg) were powdered and extracted with mixed solvent of petroleum ether/Et_2O/MeOH (1 : 1 : 1) three times (7 d each time) at room temperature. The extracts were concentrated under reduced pressure to afford a residue (712 g). This residue was subjected to a silica gel column chromatography (CC) (200—300 mesh; 2500 g) with a gradient of hexane/acetone (1 : 0, 20 : 1, 10 : 1, 5 : 1, v/v) as eluent. Four fractions (Fr. A—Fr. D) were collected according to TLC analysis. Fr. A and Fr. B (with hexane/acetone 1 : 0, 20 : 1; 422 g) were combined together, because they exhibited similar constituents on the basis of TLC analysis. The main components of Fr. A and Fr. B are essential oil and a colorless crystal. After filtration and recrystallization in acetone, furanoligularenine (126 g) was obtained. Fr. C (with hexane/acetone 10 : 1; 48 g) was separated on a silica gel CC (200—300 mesh; 800 g) with a hexane/acetone (20 : 1, 10 : 1, 5 : 1, 3 : 1, v/v) gradient to obtained four subfractions (Fr. C1—Fr. C4). Subfraction Fr. C4 (with hexane/acetone 3 : 1; 7.6 g) was isolated by a silica gel CC (300—400 mesh; 80 g) with a hexane/EtOAc (10 : 1, 5 : 1, 3 : 1, v/v) gradient to give three subfraction (Fr. C1a—Fr. C1c). Fr. C1c (with hexane/EtOAc, 3 : 1; 1.5 g) was purified was subjected to silica gel CC (300—400 mesh; 20 g) with a hexane/EtOAc (5 : 1, v/v) elution to give a mixture. This mixture (62 mg) was further purified by a low pressure silica gel C-18 CC eluting with H_2O/MeOH (3 : 2, v/v) to yield **1** (12 mg) and **2** (16 mg).

Fischerisin A (**1**): Pale amorphous powder; $[\alpha]_D^{20}$ -300 (c=0.02, $CHCl_3$); IR (KBr) ν_{max} 3423, 3029, 2967, 2922, 2854, 1677, 1474, 1334, 1261, 1209, 1143, 1067, 1018, 925, 819 cm^{-1}; UV ($CHCl_3$): λ_{max} (log ε) 267 (3.27), 296 (3.56), 327 (2.84) nm; HR-EI-MS *m/z* 488.2890 ($[M]^+$, Calcd for $C_{32}H_{40}O_4$: 488.2916); ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR (DEPT) (125 MHz, $CDCl_3$): see Table 1.

Fischerisin B (**2**): Pale amorphous powder; $[\alpha]_D^{20}$ -106 (c=0.12, $CHCl_3$); IR (KBr) ν_{max} 3430, 3029, 2969, 2924, 2854, 1678, 1474, 1384, 1209, 1144, 1079, 1017, 925, 820, 773 cm^{-1}; UV ($CHCl_3$): λ_{max} (log ε) 265 (3.01), 296 (3.38), 335 (2.49) nm; HR-ESI-MS *m/z* 487.2841 ($[M+H]^+$, Calcd for $C_{32}H_{39}O_4$: 487.2843). ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR (DEPT) (125 MHz, $CDCl_3$): see Table 1.

Cytotoxicity Assay The cytotoxic activity of fischerisin A and fischerisin B was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the purified tested compounds were initially dissolved in dimethyl sulfoxide (DMSO) to adjust its concentration for use in experiments. Human oral epidermoid carcinoma (KB) cells and human breast cancer (MCF-7) cells were seeded into 96-well culture plates (4—5×10^3 per well) respectively. After incubation for 24 h, the cells were treated with various concentrations of tested compounds and incubated for pointed time. Then, MTT (5 mg/ml) was added to each well for 4 h, and the resulting crystals were dissolved in DMSO. Parallel controls consisted of deionized H_2O with the same DMSO concentration. Optical density was measured by MTT assay. Triplicate experiments were conducted

for each test.

Acknowledgment This work was supported by the Independent Innovation Foundation of Shandong University, P. R. China (No. 2010ZRYB005).

References

1) Zdero C., Bohlmann F., *Plant Syst. Evol.*, **171**, 1—14 (1990).
2) Kopczacki P., Gumulka M., Masnyk M., Grabarczyk H., Nowak G., Daniewski W. M., *Phytochemistry*, **58**, 775—787 (2001).
3) Hwangbo C., Lee H. S., Park J., Choe J., Lee J. H., *Int. Immunol.*, **9**, 1578—1584 (2009).
4) Roll D. M., Manning J. K., Carter G. T., *J. Antibiot.*, **51**, 635—639 (1998).
5) Okasaka M., Takaishi Y.,Kashiwada Y., Kodzhimatov O. K., Ashurmetov O., Lin A. J., Consentino L. M., Lee K. H., *Phytochemistry*, **67**, 2635—2640 (2006).
6) Oh H., Jensen P. R., Murphy B. T., Fiorilla C., Sullivan J. F., Ramsey T., Fenical W., *J. Nat. Prod.*, **73**, 998—1001 (2010).
7) Hehner S. P., Hofmann T. G., Droge W., Schmitz M. L., *J. Immunol.*, **163**, 5617—5623 (1999).
8) Xie W. D., Weng C. W., Niu Y. F., Lai P. X., Row K. H., *Chem. Biodivers.*, **7**, 221—224 (2010).
9) Dong X. W., Li R. J., Gao X., Row K. H., *Fitoterapia*, **81**, 153—156 (2010).
10) Li L. B., *Chin. Wild Plant Resour.*, **20**, 8—13 (2001).
11) Sato T., Moriyama Y., Nagano H., Tanahashi Y., Takahashi T., *B. Chem. Soc. Jnp.*, **48**, 112—114 (1975).
12) Wang W. S., Gao K., Yang L., Jia Z. J., *Planta Med.*, **66**, 189—191 (2000).
13) Deng M. C., Dong W. W., Jiao W., Lu R. H., *Helv. Chim. Acta*, **92**, 495—501 (2009).
14) Zhang W. J., Qi H. Y., Shi Y. P., *Planta Med.*, **76**, 159—164 (2010).
15) Zhao H., Weng C. W., Tian X., Lai P. X., Huang J. J, Xie W. D., *Chin. Chem. Lett.*, **21**, 613—615 (2010).
16) Xie W. D., Weng C. W., Li X., Row K. H., *Helv. Chim. Acta*, **93**, 1983—1989 (2010).
17) Torres P., Ayala J., Grande C., Anaya J., Granda M., *Phytochemistry*, **52**, 1507—1513 (1999).
18) Moriyama Y., Takahashi T., *Bull. Chem. Soc. Jpn.*, **49**, 3196—3199 (1976).
19) Wu Q. H., Wang C. M., Cheng S. G., Gao K., *Tetrahedron Lett.*, **45**, 8855—8858 (2004).
20) Wu Q. X., Yang A. M., Shi Y. P., *Tetrahedron*, **61**, 10529—10535 (2005).
21) Zhao Y., Jiang H., MacLeod M., Parsons S., Rankin D. W. H., Wang P., Cheng C. H. K., Shi H., Hao X. J., Gueritte F., *Chem. Biodivers.*, **1**, 1546—1559 (2004).