Five New 2-Arylbenzofuran Derivatives from *Morus wittiorum*

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Five new 2-arylbenzofuran derivatives wittifurans A—C, F and G (1—5) have been isolated from the stem bark of *Morus wittiorum*. Their structures were determined on the basis of spectroscopic analysis. Compounds 1, 3—5 were evaluated for their antioxidant and anti-inflammatory activities respectively.

Key words *Morus wittiorum*; Moraceae; 2-arylbenzofuran; antioxidation; anti-inflammation

From the plants of *Morus* were involved in investigations in 1970s to the latest days, hundreds of compounds have been isolated and identified. Among which flavanoids and Diels–Alder type adducts were mostly reported.[1—5] These phenolic compounds mostly occurred in the EtOAc extract, which exhibited significant antioxidant activity. In our ongoing investigation on the bioactive constituents of *Morus* species, *Morus wittiorum* was taken into investigation for its roots, twigs, leaves and fruits as well are used as folk medicine.[6] Furthermore, there are no reports on chemical constituents of *M. wittiorum* before our investigation. Seven 2-arylbenzofuran derivatives obtained from the stem bark of *M. wittiorum* in our previous work have been reported.[7] And the successive investigation resulted in the isolation of five new 2-arylbenzofuran derivatives wittifurans A—C, F and G (**1**—**5**). We herein describe the isolation and structure elucidation of those new derivatives as well as the assays of antioxidant and anti-inflammatory activities of compounds **1**, **3**—**5**.

Results and Discussion

The stem bark of *M. wittiorum* Hand.-Mazz. was collected in Guangxi Province, China and identified by Professor Shou-Yang Liu, Guangxi College of Traditional Chinese Medicine. The 95% EtOH extract of the stem bark of *M. wittiorum* was fractioned by different solvents, among which EtOAc fraction displayed significant antioxidant activity in a Fe^{2+}/cysteine system induced lipid peroxidation in the liver microsomal with an inhibitory ratio of 88.0% at a concentration of 10 μg/ml. The successive fractionation on the EtOAc fraction led to the isolation of five new 2-arylbenzofuans as wittifurans A—C, F and G (**1**—**5**).

Wittifuran A (**1**) was obtained as a white crystalline powder. Its molecular formula of $C_{24}H_{26}O_6$ was established by HR-FAB-MS data (*m*/*z* 410.1730 $[M]^+$, Calcd for 410.1729). The UV spectrum of **1** showed absorption maximum at 214, 300 and 344 nm, which were similar to that of a 2-arylbenzofuran derivative sorocenol A.[8] The ^{1}H-NMR spectrum exhibited an AX_2 coupling system at δ 6.70 (2H, d, J=2.0 Hz), 6.20 (1H, t, J=2.0 Hz), which indicated the presence of a 3,5-dihydroxyphenyl. Two *cis*-coupled olefinic protons at δ 6.72 (1H, d, J=10.0 Hz), 5.80 (1H, d, J=10.0 Hz) respectively, alongside the signals of two equivalent methyls at δ 1.42 (6H, s) implicated the existence of a 2,2-dimethylpyran ring. The signals at δ 2.77 (2H, m), 1.59 (2H, m), along with 1.18 (6H, s) in high field suggested the presence of a saturated isoprenoid group. The ^{13}C-NMR (Table 1) spectrum displayed 16 aromatic carbons, excluding two carbons on the pyran ring, there were 14 carbons left, which provided a further proof that compound **1** possessed the skeleton of 2-arylbenzofuran. The structure was further confirmed by the heteronuclear multiple bond correlations (HMBC) spectrum. The correlations of H-13 with C-6, C-7a and H-14 with C-7 suggested that the pyran ring was connected at C-6, C-7. The correlations of H-8 with C-3a, C-5, and H-9 with C-4 as well revealed the position of isoprenol group was at C-4. Therefore, compound **1** was elucidated as wittifuran A.

Wittifuran B (**2**) was isolated as a brown amorphous powder. The FAB-MS data showed its molecular weight was 393 $[M+H]^+$, which lost 18 compared to compound **1**. Its molecular formula of $C_{24}H_{24}O_5$ was determined by HR-FAB-MS spectrum (*m*/*z* 393.1694 $[M+H]^+$, Calcd for 393.1702). The ^{1}H-NMR spectrum was similar to that of wittifuran A, except to the two groups of coupling signals at δ 5.33 (1H, t, J=7.2 Hz) and 3.54 (2H, d, J=7.2 Hz) along with the shifting of two methyls at δ 1.82 (3H, s) and 1.66 (3H, s). The assignment was finally confirmed by the ^{13}C-NMR (Table 1) spectrum.

Wittifuran C (**3**) was isolated as a white crystalline powder. HR-FAB-MS spectrum exhibited its molecular formula

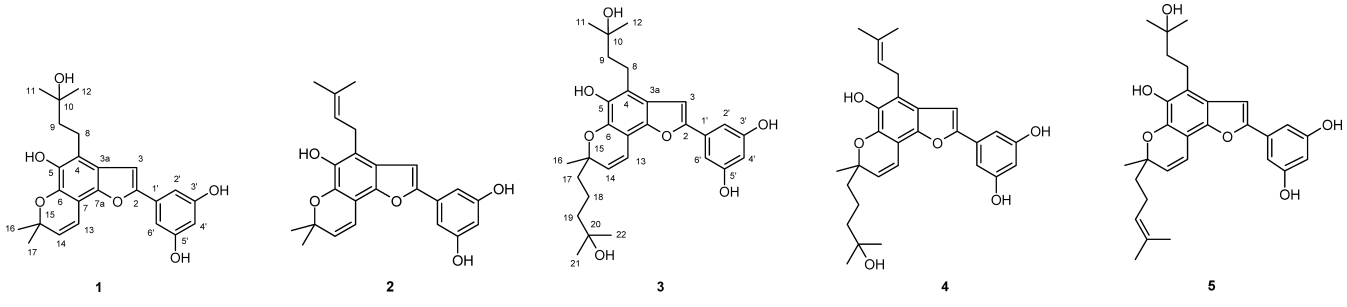

Fig. 1. Structures of Compounds **1**—**5**

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of $C_{29}H_{36}O_7$ (*m/z* 496.2460 $[M]^+$, Calcd for 496.2461), which had a group of $C_5H_{10}O$ more than compound **1**. Moreover, there appeared another two equivalent methyls at δ 1.27 (6H, s) besides the signal at δ 1.13 (6H, s) in the ^{1}H-NMR spectrum, which suggested it should exist another isoprenol. The two signals at δ 70.4, 70.2 in the ^{13}C-NMR (Table 1) spectrum which were characteristic signals for the carbons with oxygen of isoprenols, further confirmed the presence of two isoprenols. Due to the integration area of methyls on 2,2-dimethylpyran ring at δ 1.42 (3H, s) reduced, one of the isoprenols was inferred to be connected to one of the methyls on 2,2-dimethylpyran ring. The situation of the substituents was finally confirmed by the HMBC spectrum as shown in Fig. 2, in which H-17 correlated with C-14, C-15, C-18 and H-18 correlated with C-17.

Wittifuran F (**4**) and wittifuran G (**5**) were isomers, sharing the same molecular weight of 478. The lost of 18 comparing with compound **3** implied the two isomers may derive from compound **3** by losing H_2O between C-9 and C-10, or C-19 and C-20.

Wittifuran F (**4**) was obtained as a brown amorphous powder. Its molecular formula of $C_{29}H_{34}O_6$ was established by HR-FAB-MS data (*m/z* 479.2431 $[M+H]^+$, Calcd for 479.2434). The ^{1}H-NMR spectrum displayed two groups of coupling signals at δ 5.33 (1H, t, J=7.2 Hz) and 3.54 (2H, d, J=7.2 Hz) together with two methyls at δ 1.82 (3H, s) and 1.66 (3H, s) respectively, which were characteristic for a γ,γ-dimethyl allyl group. Therefore, compound **4** was postulated to be derived from compound **3** by losing H_2O between C-9 and C-10. And the structure of compound **4** was consequently confirmed by the HMBC spectrum as the correlations of H-8 with C-3a, C-5, C-10 and H-9 with C-8, C-11, C-12 as well.

Wittifuran G (**5**) was obtained as a brown amorphous powder. HR-FAB-MS spectrum determined its molecular formula of $C_{29}H_{34}O_6$ (*m/z* 479.2409 $[M+H]^+$, Calcd for 479.2434). Because the signal around δ 3.54 (2H, d, J=7.2 Hz), the most characteristic signal for α-H of a γ,γ-dimethyl allyl group, did not show in the ^{1}H-NMR spectrum. But the signal specific for α-H of an isoprenol group, appeared at δ 2.95 (2H, m) instead, which suggest the substituent at C-4 should be isoprenol group. Therefore, compound **5** was inferred to be derived from compound **3** by losing H_2O between C-19 and C-20. The connections of the substituents were eventually confirmed by the HMBC spectrum as displayed in Fig. 2.

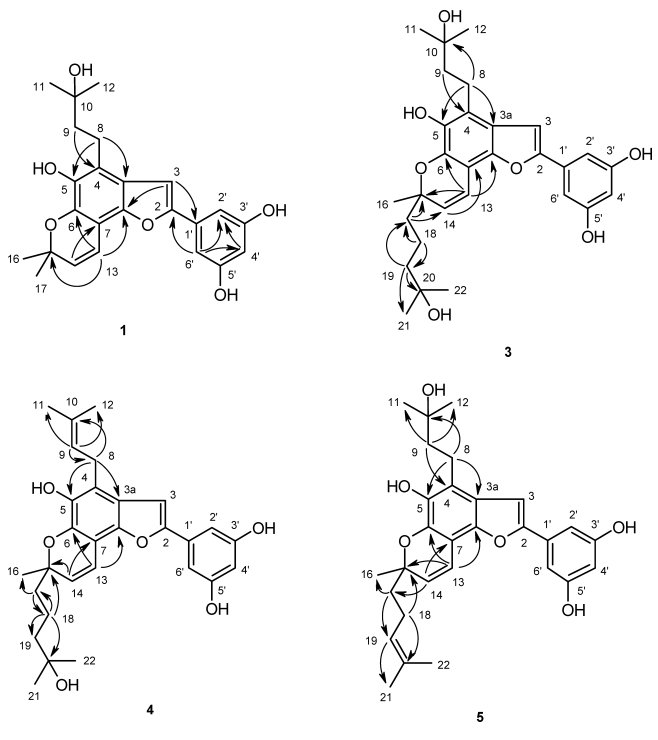

Fig. 2. Key HMBC Correlations of Compounds **1**, **3**—**5**

Due to the optical rotations of compounds **3**—**5** were measured to be 0, and there was only one chiral center in each structure, they were supposed to be racemic mixtures respectively. So chiral separations were conducted, as the results showed that there were two peaks with equal peak area in each chromatogragh of compounds **3**—**5**. Therefore, compounds **3**—**5** were determined to be racemic mixtures individualy.

All the four bioassayed compounds showed significant antioxidant activity in a Fe^{2+}-Cys system induced lipid peroxidation in the liver microsomal. The inhibitory ratios of compounds **1**, **3**—**5** along with vitamin E (positive control) against the production of malondialdehyde (MDA) were 88.4%, 87.3%, 100.1%, 99.8% and 67.2% respectively at a concentration of 10^{-5} M. Compounds **3**—**5** also exhibited strong inhibition to the release of β-glucuronidase from rat polymorphonuclear leukocytes (PMNs) induced by platelet activating factors (PAF) with the inhibitory ratios of 87.4%, 70.9%, 64.2% and 58.4% as well for ginkgolide B (positive control) respectively at a concentration of 10^{-5} M.

Experimental

General Procedures Melting points were measured on an uncorrected Boetius micro-melting point apparatus. Optical rotations were measured on a Perkin-Elmer 241 digital polarimeter. UV and IR spectra were recorded with a Jasco V-650 spectrophotometer and a Thermo Nicolet 5700 infrared spectrometer with KBr pellets. FAB-MS and HR-FAB-MS were determined by a VG-Autospec-300 mass spectrometer. NMR spectra were recorded on a Varian Mercury-400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C), with trimethylsilyl as internal standard. Silica gel (Qingdao Marine Chemical Factory, 160—200 and 200—300 mesh), Sephadex LH-20 (Pharmacia) and RP-C_{18} (Merk, 40—60 μm) were used for column chromatography (CC). Precoated plates of silica gel GF254 and silica gel RP-C_{18} F254s (Merk) were used for TLC, and detected under UV light or by heating after spraying with 98% H_2SO_4–EtOH (10 : 90, v/v). Reversed-phase HPLC separations were performed on a Shimadzu 6A system, detected by a UV detector at 254 nm and equipped with a YMC semipreparative C_{18} column (10 μm, 10×250 mm) running with flow rate of 4 ml/min. Chiral separations were performed on a Chiralcell OD column (4.6×250 mm, Daicel Chemical Industries, Ltd.), with *n*-hexane–isopropanol (70 : 30) as eluent at 0.5 ml/min and detected at 300 nm.

Plant Material The stem bark of *M. wittiorum* Hand.-Mazz. was collected in Guangxi Province, People's Republic of China, in May 2006, and identified by Professor Shou-Yang Liu, Guangxi College of Traditional Chinese Medicine. A voucher specimen (No. 21181) has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

Extraction and Isolation The air-dried stem bark (11.5 kg) of *M. wittiorum* were crushed properly and extracted with 95% EtOH (3×15 l, 2 h for each) under reflux. After removal of the solvent under reduced pressure, the EtOH extract (618 g) was chromatographed over a silica gel column (160—200 mesh, 10×60 cm, 1.0 kg), eluted with petroleum ether, $CHCl_3$, and EtOAc successively, 15 l for each solvent. The EtOAc fraction (198.8 g) was subjected to a silica gel column (160—200 mesh, 2.6 kg, 10×130 cm), with gradient $CHCl_3$–MeOH (98 : 2, 95 : 5, 90 : 10, 85 : 15, 80 : 20, 70 : 30, 1 : 1, v/v) as eluent and finally gave 7 crude fractions. Fraction 2 (52.1 g) was fur-

Table 1. ^{13}C-NMR (100 MHz, Acetone-d_6) Data of Compounds **1**—**5**

No.	**1**[a)]	**2**	**3**	**4**	**5**
2	153.8	155.3	155.2	155.2	155.2
3	100.7	101.7	101.5	101.7	101.4
3a	121.0	122.4	122.3	122.3	122.3
4	120.7	119.4	121.0	119.3	121.0
5	139.5	140.6	140.5	140.5	140.5
6	138.4	139.1	139.3	139.3	139.2
7	103.5	104.8	104.6	104.7	104.5
7a	143.3	144.9	144.9	144.9	144.9
8	21.9	26.7	22.8	26.7	22.8
9	43.4	123.6	44.4	123.7	44.4
10	69.0	131.7	70.4	131.7	70.4
11	29.2	18.0	29.7	18.0	29.6
12	29.2	25.8	29.7	25.8	29.6
13	115.6	116.7	117.2	117.1	117.3
14	130.2	130.8	129.9	130.0	129.7
15	76.4	77.8	80.4	80.5	80.2
16	27.0	27.6	26.1	26.2	26.1
17	27.0	27.6	42.1	42.1	41.3
18			19.7	19.7	25.7
19			45.0	45.0	125.0
20			70.2	70.1	132.0
21			29.7	29.7	23.5
22			29.7	29.7	17.6
1′	131.8	133.5	133.6	133.5	133.5
2′	102.2	103.7	103.7	103.7	103.7
3′	158.7	159.8	159.7	159.8	159.7
4′	102.5	103.4	103.3	103.4	103.3
5′	158.7	159.8	159.7	159.8	159.7
6′	102.2	103.7	103.7	103.7	103.7

a) Measured in DMSO-d_6.

ther fractioned on CC over silica gel (160—200 mesh, 6×110 cm, 1.5 kg) with $CHCl_3$–MeOH (95 : 5, v/v) as the eluent and resulted in 4 sub-fractions according to the result of TLC. Fr. 2-3 (14.8 g) was separated on a silica gel column (200—300 mesh, 4.5×60 cm, 250 g) eluted with petroleum ether–Me_2CO (5 : 3, v/v), and afforded seven fractions monitored by TLC. Fr. 2-3-3 (2.9 g) was subjected to a Sephadex LH-20 column (2.5×125 cm, 130 g) eluting with MeOH and afforded **1** (6 mg). Fr. 2-3-6 (1.6 g) was purified according the same procedure as Fr. 2-3-3 and resulted in **3** (62 mg). Fr. 2-2 (20.5 g) was further fractioned on CC over silica gel (160—200 mesh, 6×80 cm, 500 g) with petroleum ether–Me_2CO (7 : 3, 6 : 4, 1 : 1, v/v) as the eluent and gave 10 sub-fractions according to the result of TLC. Fr. 2-2-6 (3.1 g) was separated on a RP-C_{18} column (4.5×60 cm, 250 g) eluted with gradient MeOH–H_2O (60 : 40, 65 : 35, 70 : 30, 80 : 20, v/v), and correspondingly afforded 4 fractions. The second fraction Fr. 2-2-6-2 (470 mg) was purified on RP-C_{18} HPLC eluting isocratically with MeOH–H_2O (70 : 30) to give **4** (110 mg, t_R 55 min), **2** (3 mg, t_R 70 min) and **5** (12 mg, t_R 90 min).

Wittifuran A (**1**): A white crystalline powder (Me_2CO), decomposed over 250 °C; UV (MeOH) λ_{max} (log ε): 214 (4.41), 300 (4.30), 344 (4.09) nm; IR (KBr) ν: 3426, 2970, 1615, 1596, 1496, 1462, 1367 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.06 (1H, s, H-3), 6.72 (1H, d, J=10.0 Hz, H-13), 6.70 (2H, d, J=2.0 Hz, H-2′, 6′), 6.20 (1H, t, J=2.0 Hz, H-4′), 5.80 (1H, d, J=10.0 Hz, H-14), 2.77 (2H, m, H-8), 1.59 (1H, m, H-9), 1.42 (6H, s, H-16, 17), 1.18 (6H, s, H-11, 12); ^{13}C-NMR data are displayed in Table 1; HR-FAB-MS m/z: 410.1730 [M]$^+$ (Calcd for $C_{24}H_{26}O_6$, 410.1729).

Wittifuran B (**2**): A brown amorphous powder; UV (MeOH) λ_{max} (log ε): 213 (4.43), 298 (4.29), 343 (4.11) nm; IR (KBr) ν: 3387, 2973, 2920, 2851, 1606, 1577, 1424, 1373 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ: 7.05 (1H, s, H-3), 6.86 (2H, d, J=2.0 Hz, H-2′, 6′), 6.81 (1H, d, J=10.0 Hz, H-13), 6.35 (1H, t, J=2.0 Hz, H-4′), 5.80 (1H, d, J=10.0 Hz, H-14), 5.33 (1H, t, J=7.2 Hz, H-9), 3.54 (2H, d, J=7.2 Hz, H-8), 1.82 (3H, s, H-11), 1.66 (3H, s, H-12), 1.46 (6H, s, H-16, 17); ^{13}C-NMR data are displayed in Table 1; HR-FAB-MS m/z: 393.1694 [M+H]$^+$ (Calcd for $C_{24}H_{24}O_5$, 393.1702).

Wittifuran C (**3**): A white crystalline powder (Me_2CO), mp 158—160 °C; $[\alpha]_D^{20}$ 0 (c=0.19, MeOH); UV (MeOH) λ_{max} (log ε): 214 (4.50), 300 (4.41), 346 (4.20) nm; IR (KBr) ν: 3385, 2969, 1606, 1575, 1424, 1369 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ: 7.09 (1H, s, H-3), 6.86 (2H, d, J=2.0 Hz, H-2′, 6′), 6.84 (1H, d, J=10.0 Hz, H-13), 6.35 (1H, t, J=2.0 Hz, H-4′), 5.78 (1H, d, J=10.0 Hz, H-14), 2.94 (2H, m, H-8), 1.71—1.80 (4H, m, H-9, 17), 1.50—1.64 (2H, m, H-18), 1.42 (3H, s, H-16), 1.40—1.45 (2H, m, H-19), 1.27 (6H, s, H-11, 12), 1.13 (6H, s, H-21, 22); ^{13}C-NMR data are displayed in Table 1; HR-FAB-MS m/z: 496.2460 [M]$^+$ (Calcd for $C_{29}H_{36}O_7$, 496.2461).

Wittifuran F (**4**): A brown amorphous powder; $[\alpha]_D^{20}$ 0 (c=0.19, MeOH); UV (MeOH) λ_{max} (log ε): 214 (4.47), 300 (4.30), 345 (4.05) nm; IR (KBr) ν: 3397, 2972, 1605, 1575, 1424, 1369 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ: 7.05 (1H, s, H-3), 6.86 (2H, d, J=2.0 Hz, H-2′, 6′), 6.84 (1H, d, J=10.0 Hz, H-13), 6.35 (1H, t, J=2.0 Hz, H-4′), 5.79 (1H, d, J=10.0 Hz, H-14), 5.33 (1H, t, J=7.2 Hz, H-9), 3.54 (2H, d, J=7.2 Hz, H-8), 1.82 (3H, s, H-11), 1.69—1.78 (2H, m, H-17), 1.66 (3H, s, H-12), 1.54—1.62 (2H, m, H-18), 1.45—1.46 (2H, m, H-19), 1.42 (3H, s, H-16), 1.13 (6H, s, H-21, 22); ^{13}C-NMR data are displayed in Table 1; HR-FAB-MS m/z: 479.2431 [M+H]$^+$ (Calcd for $C_{29}H_{34}O_6$, 479.2434).

Wittifuran G (**5**): A brown amorphous powder; $[\alpha]_D^{20}$ 0 (c=0.20, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.48), 297 (4.32), 342 (4.10) nm; IR (KBr) ν: 3374, 2971, 2929, 2870, 1606, 1576, 1446, 1424, 1373 cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ: 7.09 (1H, s, H-3), 6.87 (2H, d, J=2.0 Hz, H-2′, 6′), 6.85 (1H, d, J=10.0 Hz, H-13), 6.35 (1H, t, J=2.0 Hz, H-4′), 5.78 (1H, d, J=10.0 Hz, H-14), 5.11 (1H, t, J=7.2 Hz, H-19), 2.95 (2H, m, H-8), 2.16 (2H, m, H-18), 1.74—1.83 (4H, m, H-9, 17), 1.62 (3H, s, H-21), 1.55 (3H, s, H-22), 1.43 (3H, s, H-16), 1.28 (6H, s, H-11, 12); ^{13}C-NMR data are displayed in Table 1; HR-FAB-MS m/z: 479.2409 [M+H]$^+$ (Calcd for $C_{29}H_{34}O_6$, 479.2434).

Bioassays Antioxidant activity of compounds **1**, **3**—**5** with vitamin E as the positive control were assayed in a Fe^{2+}/cysteine system induced lipid peroxidation in the liver microsomal according to established procedures.[3] Compounds **1**, **3**—**5** along with the positive control ginkgolide B were assayed for their anti-inflammatory activity against the release of β-glucuronidase from rat PMNs induced by PAF as described in the literature.[3] The purities of test compounds **1**, **3**—**5** were all >90% by the detection of HPLC. The positive controls vitamin E and ginkgolide B were purchased from Sigma Co. and their purities were all >98%.

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References

1) Nomura T., Fukai T., *Heterocycles*, **15**, 1531—1567 (1981).
2) Dai S. J., Ma Z. B., Wu Y., Chen R. Y., Yu, D. Q., *Phytochemistry*, **65**, 3135—3141 (2004).
3) Dai S. J., Mi Z. M., Ma Z. B., Li S., Chen R. Y., Yu D. Q., *Planta Med.*, **70**, 758—763 (2004).
4) Dai S. J., Wu Y., Wang Y. H., He W. Y., Chen R. Y., Yu D. Q., *Chem. Pharm. Bull.*, **52**, 1190—1193 (2004).
5) Kang J., Chen R. Y., Yu D. Q., *Planta Med.*, **72**, 52—59 (2006).
6) Chen Z. Y., *Chin. Wild Plant Res.*, **17**, 44—45 (1998).
7) Tan Y. X., Liu C., Chen R. Y., *Acta Pharm. Sin.*, **43**, 1119—1122 (2008).
8) Hano Y., Yamanaka J., Nomura T., Momose Y., *Heterocycles*, **41**, 1035—1043 (1995).