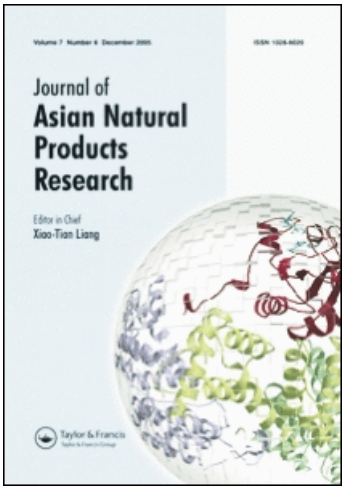

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Three new compounds from *Morus nigra* L.

Lei Wang[a]; Yan Yang[a]; Chao Liu[a]; Ruo-Yun Chen[a]
[a] The Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education & Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Online publication date: 15 June 2010

To cite this Article Wang, Lei , Yang, Yan , Liu, Chao and Chen, Ruo-Yun(2010) 'Three new compounds from *Morus nigra* L.', Journal of Asian Natural Products Research, 12: 6, 431 — 437
To link to this Article: DOI: 10.1080/10286020.2010.489824
URL: http://dx.doi.org/10.1080/10286020.2010.489824

Journal of Asian Natural Products Research
Vol. 12, No. 6, June 2010, 431–437

ORIGINAL ARTICLE

Three new compounds from *Morus nigra* L.

Lei Wang, Yan Yang, Chao Liu and Ruo-Yun Chen*

The Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education & Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

(*Received 23 February 2010; final version received 26 April 2010*)

A new 2-arylbenzofuran derivative, mornigrol D (**1**), along with two new flavones, mornigrol G (**2**) and mornigrol H (**3**), and six known compounds, norartocarpetin (**4**), dihydrokaempferol (**5**), albanin A (**6**), albanin E (**7**), moracin M (**8**), and albafuran C (**9**), were isolated from the barks of *Morus nigra*. Their structures were elucidated by spectroscopic analysis. Compounds **1** and **9** showed antioxidative activities *in vitro* with inhibition ratios of 98 and 99% at the concentration of 10^{-4} mol/l, and of 74 and 75% at the concentration of 10^{-5} mol/l. In addition, compounds **1** and **4** showed potent anti-inflammatory activities (inhibition of release of β-glucuronidase from rat polymorphonuclear leucocytes induced by platelet activating factor) with inhibitory ratios of 65.9% ($P < 0.01$) and 67.7% ($P < 0.01$) at a concentration of 10^{-5} mol/l.

Keywords: *Morus nigra*; Moraceae; 2-arylbenzofuran; isoprenyl flavonoid; anti-inflammation; antioxidation

1. Introduction

Many plants of genus *Morus*, such as *Morus nigra* L., distributed in Xin Jiang are well-known in traditional Chinese medicine, and have been used for the treatment of diabetes, arthritis, and rheumatism for thousands of years [1]. Previously, many flavones, stilbenes, and benzofuran derivatives [2–7] were isolated from the root barks or the stem barks of *M. alba*, *M. lhou*, *M. macroura*, and other related species. In our successive investigation of *Morus* species, it was found that the EtOAc extract of the stem bark of *M. nigra* exhibited anti-inflammatory and antioxidative activities. The consequent investigation resulted in the isolation of three new compounds, mornigrol D (**1**) and mornigrols G and H (**2** and **3**) (Figure 1), together with six known compounds, norartocarpetin (**4**) [8], dihydrokaempferol (**5**) [9], albanin A (**6**) [10], albanin E (**7**) [11], moracin M (**8**) [12], and albafuran C (**9**) [13]. The present paper deals with the isolation and structural elucidation of three new compounds, as well as the evaluation of their anti-inflammatory and antioxidative activities.

2. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. The molecular formula was determined to be $C_{24}H_{26}O_5$ by HR-ESI-MS, which showed a quasi-molecular ion peak at *m/z* 395.1853 $[M + H]^+$. The IR spectrum showed absorption bands ascribable to hydroxyl (3330 cm^{-1}) and aromatic (1620 and 1443 cm^{-1}) groups.

*Corresponding author. Email: rych@imm.ac.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.489824
http://www.informaworld.com

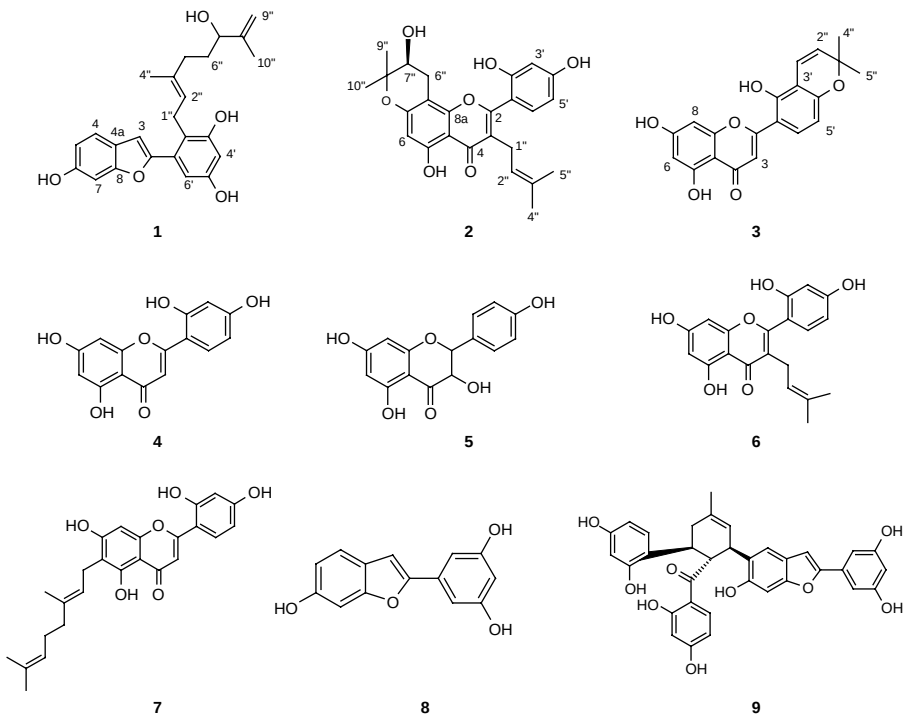

Figure 1. Structures of compounds **1**–**9** isolated from *M. nigra*.

The UV spectrum displayed absorption maxima at 212 and 311 nm, similar to those of 2-arylbenzofuran derivatives [3]. The ^{1}H NMR spectrum (Table 1) displayed the following proton signals: one proton of furan nucleus at δ_H 6.78 (1H, s, H-3); an ABX-type aromatic proton at δ_H 7.42 (1H, d, $J = 8.4$ Hz, H-4), 6.82 (1H, dd, $J = 2.1$, 8.4 Hz, H-5), 6.97 (1H, d, $J = 2.1$ Hz, H-7); AB-type aromatic protons at δ_H 6.74 (1H, d, $J = 1.8$ Hz, H-4′), 6.49 (1H, d, $J = 1.8$ Hz, H-6′); and a changed geranyl group, one of whose double bonds was hydrated, at δ_H 5.24 (1H, m, H-2″), 3.52 (2H, d, $J = 6.3$ Hz, H-1″), 4.84 (1H, s, H-9″$_a$), 4.69 (1H, s, H-9″$_b$), 3.96 (1H, t, $J = 6.3$ Hz, H-7″), 2.01 (2H, m, H-6″), 1.74 (3H, s, H-4″), 1.66 (3H, s, H-10″), 1.54 (2H, m, H-5″). The ^{13}C NMR signal at δ_C 75.2 further supported the presence of a hydroxy group. The complete structure was confirmed with the aid of HSQC and HMBC spectra. In the HMBC (Figure 2) spectrum, long-range correlations between H-9″ and C-7″, C-10″ and between H-7″ and C-5″, C-9″, and C-10″, demonstrated that the geranyl group was replaced by a 7″-hydroxy-3″, 8″-dimethylbut-2″, 8″-dioctenyl group. Furthermore, H-1″ showed long-range correlations with C-1′ and C-3′, supporting that the changed geranyl group is located at C-2′. Thus, the structure of **1** was elucidated as 2′-(7″-hydroxy-3″,8″-dimethylbut-2″,8″-dioctenyl)-3′,5′,7-trihydroxy-2-arylbenzofuran and was named as mornigrol D.

Compound **2** was obtained as a yellow amorphous powder. Its molecular formula of $C_{25}H_{26}O_7$ was determined based on the HR-ESI-MS spectrum at *m/z* 439.1761 $[M + H]^+$. The IR spectrum of **2** showed absorption bands of hydroxyl (3262 cm^{-1}), conjugated carbonyl (1656 cm^{-1}), and aromatic (1615, 1572, 1427 cm^{-1}) groups. The UV spectrum exhibited absorption maxima at 264 and 329 nm, similar to those of morusin [14]. The ^{1}H NMR spectrum (Table 1) showed the presence

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compounds **1**–**3** (in acetone-d_6).

	1		**2**		**3**	
No.	δ_H[a] (J, Hz)	δ_C[b]	δ_H[a] (J, Hz)	δ_C[b]	δ_H[a] (J, Hz)	δ_C[b]
2		155.6		161.9		164.3
3	6.78 (1H, s)	105.5		121.5	6.41 (1H, s)	104.8
4	7.42 (1H, d, $J = 8.4$)	121.9		182.9		178.9
4a		122.6		105.2		105.2
5	6.82 (1H, dd, $J = 8.4, 2.1$)	112.9		163.4		163.1
6		155.6	6.15 (1H, s)	93.7	6.23 (1H, d, $J = 1.5$)	95.2
7	6.97 (1H, d, $J = 2.1$)	98.3		167.3		165.5
8		156.4		104.2	6.51 (1H, d, $J = 1.5$)	99.9
8a				153.8		158.2
1′		132.8		112.8		110.9
2′		130.9		161.5		159.1
3′		157.4	6.57 (1H, d, $J = 8.4$)	103.8		108.3
4′	6.49 (1H, d, $J = 1.8$)	103.7		157.3		156.4
5′		155.6	6.51 (1H, dd, $J = 8.4, 2.1$)	108.0	6.62 (1H, d, $J = 7.5$)	109.9
6′	6.74 (1H, d, $J = 1.8$)	118.6	7.23 (1H, d, $J = 2.1$)	130.1	7.67 (1H, d, $J = 7.5$)	126.2
1″	3.52 (2H, d, $J = 6.3$)	26.2	3.12 (1H, d, $J = 6.3$)	24.6	6.18 (1H, d, $J = 9.6$)	138.6
2″	5.24 (1H, m)	125.2	5.13 (1H, t, $J = 6.3$)	132.3	5.46 (1H, d, $J = 9.6$)	122.3
3″		134.9		122.6		70.3
4″	1.74 (3H, s)	16.5	1.53 (3H, s)	25.5	1.66 (3H, s)	18.6
5″	1.54 (2H, m)	35.1	1.41 (3H, s)	17.6	1.92 (3H, s)	25.8
6″	2.01 (2H, m)	36.5	3.16 (1H, dd, $J = 17.1, 5.7$); 2.85 (1H, dd, $J = 17.1, 5.7$)	25.5		
7″	3.96 (1H, t, $J = 6.3$)	75.2	4.78 (1H, m)	92.5		
8″		149.3		71.3		
9″	4.84 (1H, s); 4.69 (1H, s)	110.3	1.26 (3H, s)	25.8		
10″	1.66 (3H, s)	25.8	1.20 (3H, s)	25.5		

Note: [a]300 MHz.
[b]125 MHz.

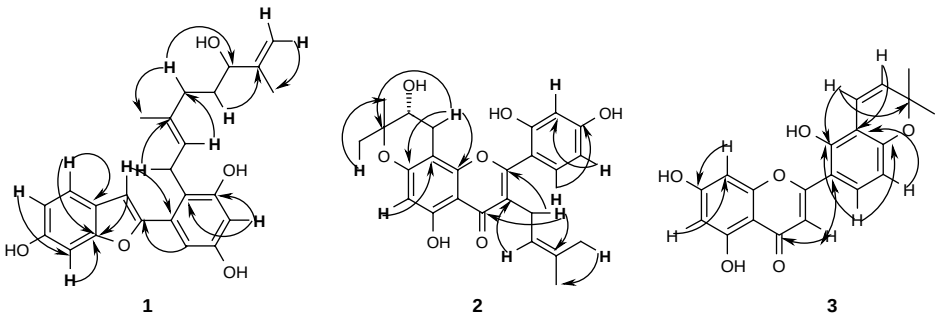

Figure 2. Key HMBC correlations of compounds **1**–**3**.

of one prenyl group; a 2,2-dimethyl-3-hydroxy-pyran ring moiety at δ_H 1.20 (3H, s), 1.26 (3H, s), 3.16 (1H, dd, $J = 17.1$, 5.7 Hz), 2.85 (1H, dd, $J = 17.1$, 5.7 Hz), and 4.78 (1H, m); ABX-type aromatic protons at δ_H 6.57 (1H, d, $J = 8.4$ Hz), 6.51 (1H, dd, $J = 8.4$, 2.1 Hz), and 7.23 (1H, d, $J = 2.1$ Hz) as well as one isolated aromatic proton at δ_H 6.15 (1H, s). Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of morusin suggested that compound **2** has the same framework as morusin and the structural difference probably is the 2,2-dimethylpyran ring in morusin replaced by a 2,2-dimethyl-3-hydroxy-pyran ring in **2** [14]. In the HMBC (Figure 2) spectrum, long-range correlations are between H-6″ and C-7, C-8a as well as between H-7″ and C-8, whereas H-1″ showed cross-peaks with C-2 and C-4. These data supported the location of the prenyl group at C-3 and the 2,2-dimethyl-3-hydroxy-pyran ring moiety.

The absolute configuration of the 2,2-dimethyl-3-hydroxy-pyran was solved by CD methods. A method involving *in situ* complexes of dirhodium tetrakis (trifluoroacate) [$Rh_2(OCOCF_3)_4$], which was developed by Gerards and Snatzke [15] and extended by Frelek and Szczepek [16], can be applied for the determination of the absolute configuration of chiral secondary alcohols. According to this method, it is only necessary to mix the chiral secondary alcohol and $Rh_2(OCOCF_3)_4$ at a molar ratio of 1:0.3–1:0.7 in methylene chloride and record the CD spectra in the 230–700-nm spectral range. From the sign of the CD bands occurring at 350 nm, it is possible to establish the chirality of the secondary alcohol expressed by the bulkiness rule for correlation of the alcohol geometry with the sign of the CD band E [15]. The positive sign was observed in the CD spectrum. Therefore, the absolute configuration of C-7″ was *S* and the structure of **2** was elucidated as 2′,4′,5-trihydroxy-3-(3″-methyl-2″-butenyl)-(8″,8″-dimethyl-7″-hydroxy-pyran)-(12″,13″;7,8)-flavone and was named as mornigrol G.

Compound **3** was obtained as a yellow amorphous powder. It exhibited a quasi-molecular ion peak at *m/z* 353.1026 $[M + H]^+$ in HR-ESI-MS, suggesting a molecular formula of $C_{20}H_{16}O_6$. The UV spectrum showed absorption maxima at 264 and 316 nm. The IR spectrum of **3** displayed absorption bands of hydroxyl group (3203 cm^{-1}), carbonyl group (1651 cm^{-1}), and aromatic ring functionalities (1562 and 1445 cm^{-1}). The ^{1}H NMR spectrum (Table 1) displayed the following proton signals: a pair of *meta*-coupled aromatic protons (A-ring), one proton singlet at δ_H 6.41(H-3), a pair of *ortho*-coupled aromatic protons (C-ring), and a 2,2-dimethylpyran ring unit. On the basis of the observation from the ^{1}H, ^{13}C NMR, and HMBC spectra, compound **3** can be confirmed as a flavonoid skeleton with a prenyl group located at C-3′ that formed the 2,2-dimethylpyran group

with 2′-OH or 4′-OH (H-1″, H-2″, H-4″, H-5″). By using the HMBC experiment we could not confirm that the isoprenyl group formed the 2,2-dimethylpyran group with 2′-OH or 4′-OH, so compound **3** was dissolved in methanol and checked with a UV spectrum. Without adding any diagnosing reagent, the UV spectrum showed the band I (300–400 nm) at 316 nm and band II (220–280 nm) at 264 nm. But after the addition of the diagnostic reagent (unmelted and melted NaOAc), bands I and II still did not show dramatically any red shift and the strength was reduced. This phenomenon indicated that 4′-OH did not freely exist. According to this result, it can be proved that the isoprenyl group formed the 2,2-dimethylpyran group with 4′-OH. Thus, compound **3** was confirmed as 2′,5′,7-trihydroxy-(2″,2″-dimethylpyrano)-[5″,6″; 3′,4′]-flavone and was named as mornigrol H.

The structure of the remaining six compounds **4**–**9** (Figure 1) was identified as norartocarpetin (**4**) [8] dihydrokaempferol (**5**) [9], albanin A (**6**) [10], albanin E (**7**) [11], moracin M (**8**) [12], and albafuran C (**9**) [13], respectively, by comparing their spectroscopic (MS and NMR) data with those reported in the literature. All these compounds were assayed for their anti-inflammatory and antioxidative activities. Compounds **1** and **9** showed antioxidative activities and **1** and **4** showed potent anti-inflammatory activities.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT-100× micro-melting point apparatus and are uncorrected. UV spectra were recorded on a Thermo Spectronic ~ Vision32 software V1.25. IR spectra were taken on a NICOLET 5700 FT-IR spectrophotometer. NMR spectra were run on INOVA-500 and MERCURY-300 with TMS as internal standard. HR-ESI-MS were recorded on the Agilent1100LC/MSD Trap SL mass spectrometer. Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and RP-18 (40–60 μm, Merck, Darmstadt, Germany) were used for column chromatography, and silica gel GF-254 (Qingdao Marine Chemical Factory) was used for TLC. Spots on the plate were observed under UV light and visualized with 10% H_2SO_4 followed by heating.

3.2 Plant material

Plant material was gathered from Kashi (Xinjiang, China) in July 2005, and identified as the bark of *M. nigra* L. by Prof. Lin Ma. A voucher specimen (No. 21738) has been deposited in the Herbarium of Materia Medica, Department of Phytochemistry, Institute of Materia Medica.

3.3 Extraction and isolation

Extraction of the pulverized barks of *M. nigra* (3.75 kg) was performed with 95% EtOH under reflux. After evaporation of the solvents under vacuum, the residue (650 g) was dissolved in hot water and then extracted with petroleum ether, chloroform, ethyl acetate, and *n*-BuOH successively. The EtOAc fraction (107 g) was chromatographed over a silica gel column (160–200 mesh, 9 × 80 cm, 3.0 kg) eluted by $CHCl_3$–CH_3COCH_3 [(9:1–8:2–7:3–6:4–5:5, V/V)–CH_3COCH_3] to give eight fractions E-1–E-8. Fraction E-4 (10.2 g) was subjected to silica gel column chromatography (160–200 mesh, 5.5 × 35 cm, 250 g) and eluted by petroleum ether–CH_3COCH_3 [(9:1–8:2–7:3–6:4–5:5, V/V)–CH_3COCH_3], then six fractions E-1-1–E-1-6 were obtained. Compound **1** (10 mg) was isolated by preparative HPLC (MeOH–H_2O 75:25) from fraction E-1-3 (0.56 g) with one known compound albafuran C and in the fraction E-1-5 (0.91 g), compounds **2** (7 mg) and **3** (5 mg) with norartocarpetin (**4**), albanin A (**6**), albanin

E (**7**) were obtained by preparative HPLC (MeOH–H_2O 7:3) (flow rate 4 ml/min, UV detection at 254 nm). Fraction E-1-6 (0.8 g) was subjected to Sephadex LH-20 column chromatography using MeOH (1:1) to obtain a target portion, which was further purified on RP-HPLC with an ODS column with MeOH–H_2O (7:3) to yield compounds **5** and **8**.

*3.3.1 Mornigrol D (**1**)*

Yellow amorphous powder, mp 92–94°C; UV (MeOH) λ_{max}: (log ε) 212 (4.59), 311 (4.39) nm; IR (KBr) ν_{max} 3330, 1620, 1443, and 1363 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS *m/z* 395.1853 $[M + H]^+$ (calcd for $C_{24}H_{27}O_5$, 395.1858).

*3.3.2 Mornigrol G (**2**)*

Yellow amorphous powder, mp 145–147°C; UV (MeOH) λ_{max} (log ε) 205 (4.52), 264 (4.29), 329 (3.82) nm; IR (KBr) ν_{max}: 3262, 2974, 1656, 1615, 1572, 1427, 1355, 1132, and 1066 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS *m/z* 439.1761 $[M + H]^+$ (calcd for $C_{25}H_{27}O_7$, 439.1756).

*3.3.3 Mornigrol H (**3**)*

Yellow amorphous powder, mp 148–150°C; UV (MeOH) λ_{max} (log ε) 209 (4.56), 264 (4.18), and 316 (3.58) nm; UV (MeOH + NaOAc) λ_{max} (log ε) 210 (4.48), 266 (4.12), and 317 (3.53) nm; IR (KBr) ν_{max}: 3203, 2968, 1651, 1619, 1562, 1445, 1365, 1176, 1079 $cm^{-1;}$ ^1H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS *m/z* 353.1026 $[M + H]^+$ (calcd for $C_{20}H_{17}O_6$, 353.1025).

3.4 Determination of absolute configuration of the 2,2-dimethyl-3-hydroxy-pyran moiety using the CD method

Dirhodium tetrakis (trifluoroacate) was purchased from Acros (Geel, Belgium). CH_2Cl_2 was obtained from Beijing Chemical Works (Beijing, China), and dried according to the common procedures. According to the published procedure [16], about 1:2 adducts of the general formula of $[Rh_2(OCOCF_3)_4(alcohol)_2]$ were prepared using 0.65 mg/ml of compound **2**. Soon after mixing, the first CD spectrum was recorded and its evolution monitored until it remained stable (30–60 min). The sign of the diagnostic band at 350 nm (band E) is correlated to the absolute configuration of the 2,2-dimethyl-3-hydroxy-pyran. The inherent CD of the secondary alcohol was subtracted. The value of the diagnostic band at 350 nm of compound **2** was +0.06.

3.5 Anti-inflammation and antioxidation bioassays

The anti-inflammatory activity was assayed by releasing β-glucuronidase from rat polymorphonuclear leucocytes (PMNs) induced by platelet activating-factor (PAF). The suspension of rat PMNs (245 μl) at a density of 2.5×10^6 cells/ml and test samples were incubated at 37°C for 20 min and 2.5 μl of 1 mM cytochalasin B was added for a further 5 min, followed by 0.2 μM PAF (2.5 μl). Then, following the steps as previously described [7], the inhibitory rate was calculated. The antioxidative assay was operated as reported previously [6]. In short, from checking the percentage of inhibition of malondialdehyde formation for the absorption at 532 nm, the inhibitory ratio can be calculated.

Acknowledgements

This research program was financially supported by the Special Foundation for Basic Research of the Ministry of Science and Technology, China (No. 2007FY130100). The authors acknowledge the Department of Instrumental Analysis, Institute of Materia Medica, Chinese Academy of Medical Sciences, and Peking Union Medical College for all spectral analysis. We especially thank Prof. Yi-Kang Si for his help in the determination of absolute configuration and Prof. Lin Ma for the plant identification.

References

[1] T. Nikaido, T. Ohmoto, and T. Nomura, *Chem. Pharm. Bull.* **32**, 4929 (1984).

[2] T. Nomura and T. Fukai, *Heterocycles* **15**, 1531 (1981).

[3] T. Nomura, in *Progress in the Chemistry of Organic Natural Products*, edited by W. Herz, H. Grisebach, and C.W. Kirby (Springer, Vienna, New York, 1988), Vol. 53, p. 87.

[4] Y. Yang, T. Gong, C. Liu, and R.Y. Chen, *Chem. Pharm. Bull.* **58**, 257 (2010).

[5] S.J. Dai, Y. Wu, Y.H. Wang, W.Y. He, R.Y. Chen, and D.Q. Yu, *Chem. Pharm. Bull.* **52**, 1190 (2004).

[6] S.J. Dai, R.Y. Chen, and D.Q. Yu, *Phytochemistry* **3**, 3135 (2004).

[7] J. Kang, R.Y. Chen, and D.Q. Yu, *Planta Med.* **72**, 52 (2006).

[8] C.N. Lin, C.M. Lu, and P.L. Huang, *Phytochemistry* **39**, 1447 (1995).

[9] Y.B. Yang, N.H. Tan, L. Wang, S.M. Wang, R.R. Jia, L.H. Jiang, and X. Fu, *Nat. Prod. Res. Dev.* **15**, 203 (2003).

[10] F. Franco, M. Irene, and C.M. Maria, *Planta Med.* **55**, 70 (1989).

[11] T. Fukai and T. Nomura, *Heterocycles* **32**, 499 (1991).

[12] G.D.W.F. Kapche, C.D. Fozing, J.H. Donfack, G.W. Fotso, D. Amadou, A.N. Tchana, M. Bezabih, P.F. Moundipa, B.T. Ngadjui, and B.M. Abegaz, *Phytochemistry* **70**, 216 (2009).

[13] Y. Hano, H. Tsubura, and T. Nomura, *Heterocycles* **24**, 1807 (1986).

[14] T. Nomura, T. Fukai, and K. Masa, *Chem. Pharm. Bull.* **26**, 1453 (1978).

[15] M. Gerards and G. Snatzke, *Tetrahedron: Asymm.* **1**, 221 (1990).

[16] J. Frelek and W.J. Szczepek, *Tetrahedron: Asymm.* **10**, 1507 (1999).