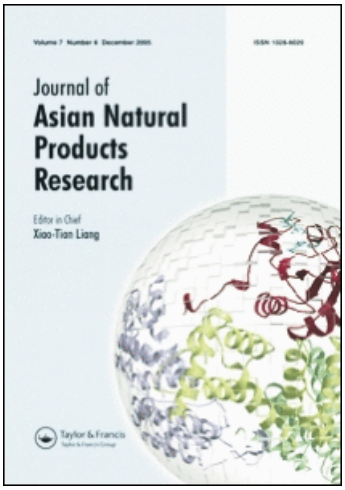

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Wang, Lei , Cui, Xi-Qiang , Gong, Ting , Yan, Ren-Yi , Tan, Yong-Xia and Chen, Ruo-Yun(2008) 'Three new compounds from the barks of *Morus nigra*', Journal of Asian Natural Products Research, 10: 9, 897 — 902
To link to this Article: DOI: 10.1080/10286020802181117
URL: http://dx.doi.org/10.1080/10286020802181117

Journal of Asian Natural Products Research
Vol. 10, No. 9, September 2008, 897–902

Three new compounds from the barks of *Morus nigra*

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(*Received 16 November 2007; final version received 16 April 2008*)

Three new compounds including two flavonoids and a new 2-phenylbenzofuran, named morunigrols A–C (**1**–**3**), together with three known compounds albafuran A (**4**), albafuran B (**5**), and mulberrofuran L (**6**), have been isolated from the barks of *Morus nigra*. Their structures have been elucidated by spectroscopic methods.

Keywords: *Morus nigra*; Moraceae; morunigrols A-C; flavonoid

1. Introduction

The dried bark of the genus *Morus*, widely distributed in most provinces of China [1, 2], is a well-known traditional Chinese medicine used for the treatment of diabetes, arthritis, and rheumatism. The chemical components of this genus *Morus*, such as the Diels-Alder-type adducts and flavonoids [3–7], have been widely studied and many compounds were obtained. However, the plant *Morus nigra* has been rarely studied. In our previous screening, the crude extract of the stem and root bark of *M. nigra* demonstrated moderate anti-inflammatory and antioxidative activities. Here, we describe the isolation and structural elucidation of three new compounds, two prenylated flavonoids and a benzofuran, named as morunigrols A–C, together with three known compounds albafuran A, albafuran B [8], and mulberrofuran L [9]. All compounds were obtained from this plant for the first time.

2. Results and discussion

The EtOAc part of the 95% EtOH extract of *M. nigra* was repeatedly subjected to silica gel, Sephadex LH-20, RP-18 column chromatography, and preparative HPLC to afford three new compounds (**1**–**3**) and three known compounds (**4**–**6**) (Figure 1).

Morunigrol A (**1**) has been isolated as a yellow powder. Its HRFABMS gave a pseudomolecular ion peak $[M + H]^+$at *m/z* 419.1497, consistent with the molecular formula $C_{25}H_{22}O_6$. The IR spectrum for **1** displayed absorption bands of hydroxyl group (3336 cm^{-1}), carbonyl group (1653 cm^{-1}), and aromatic ring functionalities (1559 and 1447 cm^{-1}). The UV spectrum showed absorption maxima at 278 and 376 nm. The ^1H NMR spectrum exhibited the presence of proton signals of two 2,2-dimethyl pyran rings at δ 5.78 (1H, d, $J = 10.0$ Hz), 6.92 (1H, d, $J = 10.0$ Hz), 5.48 (1H, d, $J = 9.5$ Hz), 6.19 (1H, d, $J = 9.5$ Hz), 1.68 (3H, s), 1.96

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802181117
http://www.informaworld.com

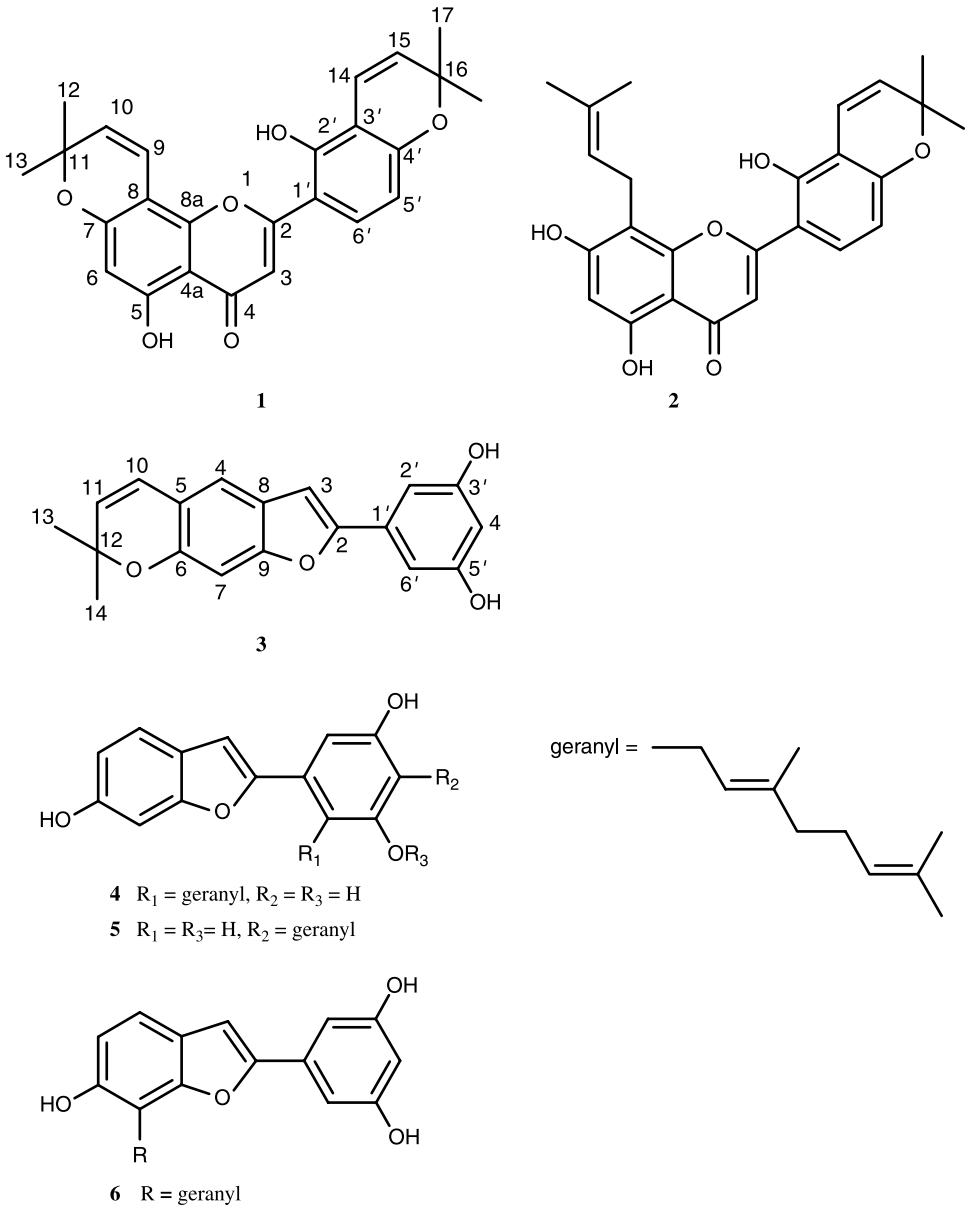

Figure 1. Structures of compounds **1**–**6**.

3H, s), and 1.46 (6H, s). The signal at δ 12.84 (1H, br s) was attributed to the hydroxyl group that was disappeared with D_2O exchange. Additionally, the ^{1}H NMR spectrum showed two singlets at δ 6.15 (1H, s) and 6.43 (1H, s) and one AB-type aromatic proton signals at δ 6.64 (1H, d, J = 8.0 Hz) and 7.80 (1H, d, J = 8.0 Hz). The ^{13}C NMR spectrum of **1** showed six oxygenated aromatic carbon signals at δ 164.1, 162.7, 159.9, 159.1, 156.5, and 152.0, one carbonyl resonance at δ 179.2, and a carbon resonance at δ 108.3, indicating that the skeleton of the compound is a flavonoid. In the HMBC experiment, H-9 at δ 6.92 showed correlations with C-11 (δ 70.8), C-7 (δ 159.9), C-8a (δ 152.0), and C-10

(δ 128.7), H-10 at δ 5.78 with C-12 (δ 28.3), C-13 (δ 28.3), and C-8 (δ 102.3), and H-6 at δ 6.15 with C-4a (δ 105.9) and C-8 (δ 102.3). These data suggested the presence of one 2,2-dimethyl pyran moiety formed by the isopentene group at C-8 with 7-OH in ring A. The following data also suggested the presence of the other 2,2-dimethyl pyran moiety in ring B. H-14 at δ 6.19 was coupled with C-5′ (δ 104.8), C-2′ (δ 159.1), C-4′ (δ 156.5), C-15 (δ 138.9), and C-16 (δ 70.3), and H-15 at δ 5.48 with C-17 (δ 18.6), C-18 (δ 26.8), C-16 (δ 70.3), and C-3′ (δ 109.9). To confirm whether the isopentene moiety formed the 2,2-dimethyl pyran group with 2′-OH or 4′-OH in ring B, compound **1** was dissolved in methanol and checked with the UV spectrum. Without adding any diagnose reagent, the UV spectrum showed the band I (300–400) at 376 nm and the band II (220–280 nm) at 278 nm. But after adding a diagnose reagent (unmelted NaOAc), bands I and II still did not show dramatically any red shift and the strength reduced. This phenomenon indicated that 4′-OH did not freely exist. So according to this result, it can be proved that the isopentene group formed the 2,2-dimethyl pyran group with 4′-OH. Thus, combined with the data of UV, ^{1}H NMR, ^{13}C NMR, and the HMBC experiment, the structure of compound **1** was elucidated as 7,8,3′,4′-bis-(2,2-dimethyl-chromano)-5,2′-dihydroxy flavonoid.

Morunigrol B (**2**) was isolated as a yellow powder. Its HRFABMS gave a pseudomolecular ion $[M + H]^{+}$at *m/z* 421.1651, consistent with the molecular formula $C_{25}H_{24}O_6$. The IR spectrum for **2** displayed absorption bands attributable to hydroxyl group (3345 cm^{-1}), carbonyl group (1651 cm^{-1}), and aromatic ring functionalities (1559 and 1444 cm^{-1}). The UV spectrum indicated its absorption maxima at 276 and 374 nm. Comparing the ^{1}H NMR spectral data of **2** with those of **1** revealed that there was only one 2,2-dimethyl pyran moiety with proton signals at δ 1.67 (3H, s), 1.92 (3H, s), 5.48 (1H, d, $J = 9.5$ Hz), and 6.19 (1H, d, $J = 9.5$ Hz), and an isopentene group with proton signals at δ 1.65 (3H, s), 1.83 (3H, s), 3.50 (2H, d, $J = 7.5$ Hz), and 5.32 (1H, t, $J = 7.5$ Hz) in **2**, instead of the other 2,2-dimethyl pyran in **1**. The ^{13}C NMR spectral data of **2** were also similar to those of **1**, except for the noticeable upshift of C-9 (Table 1). The HMBC correlations between H-9 at δ 3.50 and C-7 (δ 162.1), C-8a (δ 155.2), C-11 (δ 131.9), C-10 (δ 123.5), and C-8 (δ 107.5), H-10 at δ 5.32 and C-12 (δ 18.1), C-13 (δ 22.2), C-9 (δ 25.8), and C-8 (δ 107.5), H-6 at δ 6.32 and C-7 (δ 162.1), C-4a (δ 105.4), and C-8 (δ 107.5) suggested that the isopentene group was located at C-8 in ring A. Similar to **1**, it can be confirmed that the 2,2-dimethyl pyran moiety was attached to C-3′ in ring B by the HMBC experiment. Using the UV spectrum, the 2,2-dimethyl pyran moiety was elucidated with 4′-OH, not with 2′-OH. Thus, the structure of compound **2** was determined as 8-isopentene-3′,4′-(2,2-dimethyl-chromano)-5,7,2′-trihydroxy flavonoid.

Morunigrol C (**3**) had been isolated as a brown powder, a pseudomolecular ion $[M - H]^{-}$ at *m/z* 307.0948 by HRESIMS suggested the molecular formula $C_{19}H_{16}O_4$. The UV spectrum exhibited an absorption maximum at 204 nm. Its IR spectrum demonstrated that it contained hydroxyl groups (3287 cm^{-1}) and aromatic ring functionalities (1430, 1369, and 1336 cm^{-1}). The ^{1}H NMR spectrum of **3** exhibited the feature of the 2,2-dimethyl pyran moiety by showing proton signals at δ 1.42 (6H, s), 5.74 (1H, d, $J = 9.5$ Hz), and 6.49 (1H, d, $J = 9.5$ Hz), a set of AX_2-type aromatic protons at δ 6.36 (1H, d, $J = 2.0$ Hz) and 6.85 (2H, d, $J = 2.0$ Hz), and three aromatic proton singlets at δ 6.89 (1H, s), 7.02 (1H, s), and 7.24 (1H, s). The ^{13}C NMR spectrum of **3** contained 19 carbons signals, five of them are oxygenated aromatic carbons (δ 152.5, 156.1 × 2, and 159.8 × 2). From the ^{1}H NMR, ^{13}C NMR spectra, and the literature value [10,11], compound **3** can be speculated as a benzofuran with a 2,2-dimethyl pyran moiety and a phenyl group. In the HSQC and HMBC experiments, the 2,2-dimethyl pyran moiety can be confirmed to be located at C-5 with 6-OH and the phenyl group at C-2, because H-4 at

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compounds **1** and **2**.

	1		**2**	
Position	H[a]	C[b]	H[a]	C[b]
2		164.2		164.1
3	6.43	110.0	6.42, s	110.9
4		179.2		179.3
4a		105.4		105.9
5		162.7		160.9
6	6.15, s	100.4	6.32, s	99.4
7		159.9		162.1
8		102.3		107.5
8a		152.0		155.2
9	6.92, d (10.0)	115.1	3.50, d (7.5)	25.8
10	5.78, d (10.0)	128.7	5.32, t (7.5)	123.5
11		78.8		131.9
12	1.46, s	28.3	1.83, s	18.1
13	1.46, s	28.3	1.65, s	22.2
14	6.19, d (9.5)	122.0	6.19, d (9.5)	122.2
15	5.48, d (9.5)	138.9	5.48, d (9.5)	138.6
16		70.3		70.4
17	1.68, s	18.6	1.67, s	18.6
18	1.96, s	26.8	1.92, s	25.8
1′		108.3		108.6
2′		159.1		159.0
3′		109.9		109.6
4′		156.5		156.4
5′	6.64, d (8.0)	104.8	6.65 (1H, d, 8.0)	104.9
6′	7.80, d (8.0)	126.0	7.70 (1H, d, 8.0)	126.1
5-OH	12.84, s		12.80, s	

Chemical shift values are in ppm and *J* values in parentheses are in Hertz, assignments were confirmed by the experiment of HMBC and HSQC. [a] Recorded at 500 MHz in CD_3COCD_3. [b] Recorded at 125 MHz in CD_3COCD_3.

δ 7.24 demonstrated the HMBC correlations with C-8 (δ 156.1), C-6 (δ 152.5), C-10 (δ 123.4), and C-3 (δ 102.3), H-7 at δ 6.89 with C-5 (δ 119.3), C-8 (δ 152.5), and C-6 (δ 156.1), H-11 at δ 5.74 with C-5 (δ 119.3), H-10 at δ 6.49 with C-4 (δ 118.7) and C-6 (δ 152.5), H-2′, -6′ at δ 6.85 with C-2, C-4′, and C-6′, and H-4′ with C-2′, C-3′, C-5′, and C-6′. Thus, combined with the UV, ^{1}H NMR, ^{13}C NMR data, and the HMBC and HSQC experiments, this compound was characterized as 5,6-(2,2-dimethylchromano)-3′,5′-dihydroxyl benzofuran.

Compounds **4**–**6** were also isolated from the barks of *M. nigra.* By comparing those of spectroscopic data (UV, ^{1}H NMR, ^{13}C NMR, HMBC, and HSQC) with the literature value, they were identified as albafurans A and B [8] and mulberrofuran L [9], respectively.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT-100 X micromelting point apparatus and are uncorrected. The UV spectra were recorded on a Thermo Spectronic – Vision32 software V1.25. The IR spectra were taken on a NICOLET 5700 FT-IR spectrophotometer. The NMR spectra were run on INOVA-500 and MERCURY-400 with TMS as the internal standard. HRFABMS were performed on a VG-Autospec-300 mass spectrometer. ESIMS and HRESIMS were operated on the Agilent1100LC/MSD Trap SL mass spectrometer. Silica gel (200–300 mesh; Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia,

Table 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **3**.

No.	H[a]	C[b]	No.	H[a]	C[b]
2		156.1	12		76.9
3	7.02, s	102.1	13	1.42, s	28.0
4	7.24, s	118.7	14	1.42, s	28.0
5		119.3	1′		133.1
6		152.5	2′	6.85, d (2.0)	103.9
7	6.89, s	99.8	3′		159.8
8		156.1	4′	6.36, d (2.0)	103.7
9		123.7	5′		159.8
10	6.49, d (9.5)	123.4	6′	6.85, d (2.0)	103.9
11	5.74, d (9.5)	130.9			

Chemical shift values are in ppm and *J* values in parentheses are in Hertz, assignments were confirmed by the experiment of HMBC and HSQC. [a] Recorded at 500 MHz in CD_3COCD_3. [b] Recorded at 125 MHz in CD_3COCD_3.

Piscataway, NJ, USA), and RP-18 (40–60 μm; Merk, 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 province of China in July 2005, and identified as the bark of *M. nigra* L. by Professor 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

Pulverized barks of *M. nigra* (3.75 kg) were extracted 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 petroleum fraction (65 g) was chromatographed over a silica gel column (160–200 mesh, 10 × 80 cm, 2.6 kg) using petroleum ether–CH_3COCH_3 as gradient eluents [(95:5–9:1–8:2–1:1, v/v)–CH_3COCH_3)] to provide seven fractions P-1–P-7. Fraction P-4 (1.1 g) was separated by silica gel column chromatography (160–200 mesh, 2.5 × 50 cm, 100 g), eluted with petroleum ether–CH_3COCH_3 to give five fractions. Fraction P-4-2 was subjected to Sephadex LH-20 column (2 × 45 cm, MeOH) to give compound **1** (20 mg). 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-1 (3.24 g) was subjected to silica gel column chromatography (160–200 mesh, 4 × 44 cm, 240 g) and eluted by petroleum ether–CH_3COCH_3 [(9:1–8:2–7:3–6:4–5:5, v/v)–CH_3COCH_3], and then 10 fractions E-1-1–E-1-10 were obtained. Compound **2** (15 mg) was isolated by preparative HPLC (MeOH–H_2O 85:15) from fraction E-1-1 with two known compounds albafurans A and B, and from fraction E-1-2 compound **3** (5 mg) and mulberrofurans L were obtained by preparative HPLC (MeOH–H_2O, 8:2).

3.3.1 Morunigrol A

Obtained as a yellow powder; m.p. 134–136°C; UV (MeOH) λ_{max} (nm) (log ε) 203 (4.23), 219 (4.22), 255 (4.01), 278 (4.03), 295 sh (3.85), 376 (3.85); IR (KBr) ν_{max} (cm^{-1}) 3336, 2972, 2920, 2852, 1653, 1559, 1482, 1447, 1348, 1148, 1111, 1069, 806; ^{1}H NMR and ^{13}C NMR spectral data, see Table 1; ESIMS *m/z* 418 $[M]^+$(30), 403 (100), 363 (25), 203 (8); HRFABMS *m/z* 419.1497 $[M + H]^+$ (calcd for $C_{25}H_{23}O_6$, 419.1495).

3.3.2 Morunigrol B

Obtained as a yellow powder; m.p. 116–118°C; UV (MeOH) λ_{max} (nm) (log ε) 206 (4.66), 263 (4.22), 276 (4.29), 295 (4.03), 374 (4.20); IR (KBr) ν_{max} (cm^{-1}) 3345, 2914, 1651, 1620, 1559, 1444, 1372, 1158, 1060; ^{1}H NMR and ^{13}C NMR spectral data, see Table 1; ESIMS *m/z* 419.3 $[M - H]^-$, 421.2 $[M + H]^+$, 443.2 $[M + Na]^+$, 459.1 $[M + K]^+$; HRFABMS *m/z* 421.1656 $[M + H]^+$ (calcd for $C_{25}H_{25}O_6$, 421.1651).

3.3.3 Morunigrol C

Obtained as a brown powder; m.p. 231–234.5°C; UV (MeOH) λ_{max} (nm) (log ε) 230 (4.39), 262 (4.22), 309 (4.23), 337 (4.19); IR (KBr) ν_{max} (cm^{-1}) 3287, 2901, 1631, 1430, 1369, 1336, 1318, 1103, 1047; ^{1}H NMR and ^{13}C NMR spectral data, see Table 2; ESIMS *m/z* 307.7 $[M - H]^-$; HRESIMS *m/z* 307.0948 $[M - H]^-$ (calcd for $C_{19}H_{15}O_4$, 307.0970).

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Nos. 20432030 and 20572133). The authors acknowledge the Department of Instrumental Analysis, Institute of Materia Medica, Chinese Academy of Medical Sciences, and Peking Union Medical College for all the spectral analyses. We also thank Professor Lin Ma for the plant identification.

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