Four New 2-Arylbenzofuran Derivatives from Leaves of *Morus alba* L.

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Four new 2-arylbenzofuran derivatives, moracins V—Y (1—4), together with two known compounds, moracin N (5) and moracin P (6), were isolated from the leaves of *Morus alba* L. Their structures were elucidated by spectroscopic analysis. Moracins X (3) and Y (4) represent unusual substituted group compared with 2-arylbenzofuran derivatives, which were isolated from the genus *Morus*. Compounds 2—5 were evaluated for cytotoxicity against several human cancer cell lines.

Key words *Morus alba* L.; benzofuran; moracin V; moracin W; moracin X; moracin Y

The mulberry tree has been widely cultivated in China; its leaves have been an indispensable food source for silkworms, and its root barks have been used to treat 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 *Morus alba*, *M. lhou*, *M. macroura*, and other related species. As part of our continuing study on the difference of the constituents between the root bark and the leaves, an investigation on the ethanol extract of the leaves of *M. alba* L. was carried out. Further detailed investigation yielded four new 2-arylbenzofuran derivatives moracins V—Y (**1**—**4**) (Fig. 1), along with two known compounds moracin N (**5**) and moracin P (**6**). This paper describes the isolation and structure elucidation as well as evaluation of the cytotoxic effects of these 2-arylbenzofuran derivatives.

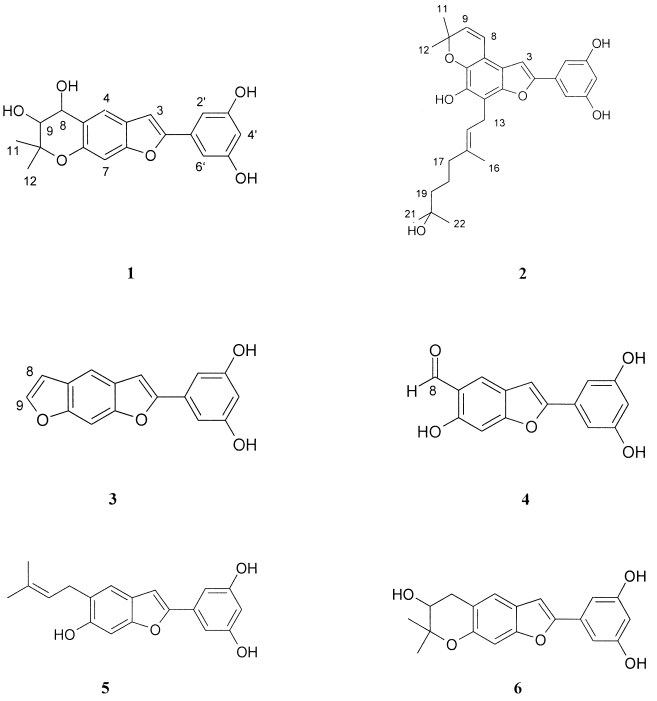

Fig. 1. Structures of Compounds **1**—**6**

Results and Discussion

Moracins V—Y (**1**—**4**) showed a dark blue fluorescence on TLC plates under UV light at 254 nm. The UV spectrum of these compounds resembled those of 2-arylbenzofuran derivatives.[8] The ^{1}H-NMR (Table 1) spectrum of these compounds showed signals of *meta*-coupled aromatic protons (A_2M-type, B-ring), and in the ^{13}C-NMR (Table 2) spectrum, oxygenated aromatic carbons at δ_C 159.8 (C-3′, 5′) and aromatic carbons at δ_C 103.8 (C-2′, 6′), δ_C 103.6 (C-4′), and δ_C

Table 1. ^{1}H-NMR Data of Compounds **1**—**4** (in Acetone-d_6, 300 MHz)

Position	1	2	3	4
H-3	7.05 (1H, s)	7.15 (1H, s)	7.22 (1H, s)	7.21 (1H, s)
4	7.67 (1H, s)		7.78 (1H, s)	8.03 (1H, s)
7	6.86 (1H, s)		7.71 (1H, s)	7.08 (1H, s)
8	4.60 (1H, d, 8.7)	6.70 (1H, d, 9.6)	6.93 (1H, d, 2.1)	10.1 (1H, s)
9	3.56 (1H, d, 8.7)	5.74 (1H, d, 9.6)	7.82 (1H, d, 2.1)	
11	1.44 (3H, s)	1.43 (3H, s)		
12	1.21 (3H, s)	1.43 (3H, s)		
13		3.62 (2H, d, 7.2)		
14		5.46 (1H, br t, 7.2)		
16		1.87 (3H, s)		
17		1.97 (2H, br t, 2.1)		
18		1.36 (2H, m)		
19		1.49 (2H, m)		
21		1.10 (3H, s)		
22		1.10 (3H, s)		
2′, 6′	6.85 (2H, d, 1.0)	6.87 (2H, d, 2.1)	6.93 (2H, d, 2.1)	6.89 (2H, d, 2.1)
4′	6.36 (1H, t, 1.0)	6.34 (1H, t, 2.1)	6.41 (1H, t, 2.1)	6.42 (1H, t, 2.1)

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Table 2. ^{13}C-NMR Data of Compounds **1**—**4** (in Acetone-d_6, 125 MHz)

Position	1	2	3	4
C-2	156.1	155.7	157.2	158.0
3	102.3	100.5	102.2	102.2
3a	123.0	118.1	127.1	123.7
4	120.7	111.1	112.3	128.4
5	123.6	137.6	125.5	119.4
6	151.8	142.5	154.3	160.9
7	98.8	112.3	94.7	99.4
7a	156.0	149.4	154.0	160.4
8	70.1	120.2	107.4	197.6
9	76.7	130.7	146.7	
10	79.6	77.2		
11	27.3	27.4		
12	19.5	27.4		
13		23.2		
14		122.6		
15		136.1		
16		16.3		
17		40.8		
18		23.6		
19		44.2		
20		70.3		
21		29.8		
22		29.8		
1′	133.2	133.6	133.1	132.2
2′, 6′	103.8	103.8	104.1	104.2
3′, 5′	159.8	159.8	159.8	159.9
4′	103.6	103.5	103.9	104.3

133.2 (C-1′), which resembled the B-ring carbons of moracin P,[9] suggesting that a symmetrical 1,3,5-trisubstituted phenyl ring (3,5-dihydroxyphenyl, B-ring) is present in the structure of **1**—**4**.

Compound **1** was obtained as a brown amorphous powder. The molecular formula was determined $C_{19}H_{18}O_6$ from the HR-ESI-MS, which showed a quasi-molecular ion peak at *m*/*z* 365.0997 $[M+Na]^+$. The IR spectrum of **1** showed absorptions of hydroxyl (3413 cm^{-1}), aliphatic bond (2914, 2879 cm^{-1}), and aromatic bond (1603, 1470 cm^{-1}). The UV spectrum showed maxima at 219, 320, and 334 nm. The ^{1}H-NMR (Table 1) of **1** showed signals of the following protons: *meta*-coupled aromatic protons (B-ring); one proton of furan nucleus signal at [δ_H 7.05 (1H, s, H-3)]; two aromatic proton signals at [δ_H 6.86 (1H, s, H-7), 7.67 (1H, s, H-4)]; two oxygenated methine protons [δ_H 4.60 (1H, d, J=8.7 Hz, H-8), 3.56 (1H, d, J=8.7 Hz, H-9)]; and two methyl groups [δ_H 1.44 (3H, s, H-11), 1.21 (3H, s, H-12)]. Furthermore, the ^{13}C-NMR (Table 2) showed the presence of two oxygenated methine carbon signals (δ_C 70.1, 76.7) and one oxygenated quaternary carbon (δ_C 79.6), which were characteristic of two hydroxy groups located on the 2,2-dimethylpyran ring. From the above information, it can be concluded that a 2,2-(3,4-dihydroxy)-dimethylpyran ring as well as a 3,5-dihydroxyphenyl ring were present in the structure of **1**. The complete structure of compound **1** was determined by the key heteronuclear multiple bond connectivity (HMBC) correlations (Fig. 2) as follows. H-4 showed correlations to C-6 (δ_C 151.8), C-7a (δ_C 156.0), and C-8 (δ_C 70.1); H-8 to C-4 (δ_C 120.7), and C-10 (δ_C 79.6); H-9 to C-11 (δ_C 27.3), and C-12 (δ_C 19.5). The relative configuration at H-8 and H-9 was determined to be *trans* from the coupling constant (J=8.7 Hz) between H-8 and H-9. Optical rotation of **1** gave a low value ($[\alpha]_D^{20}$ +2.0), indicating that compound **1** existed as a racemic mixture. Therefore the absolute configuration of **1** was not studied. As a result, the structure of **1** was assigned as 3′,5′-dihydroxy-[2″,2″-(3″,4″-dihydroxy)-dimethylpyrano]-(5″,6″:5,6)-2-arylbenzofuran and named moracin V.

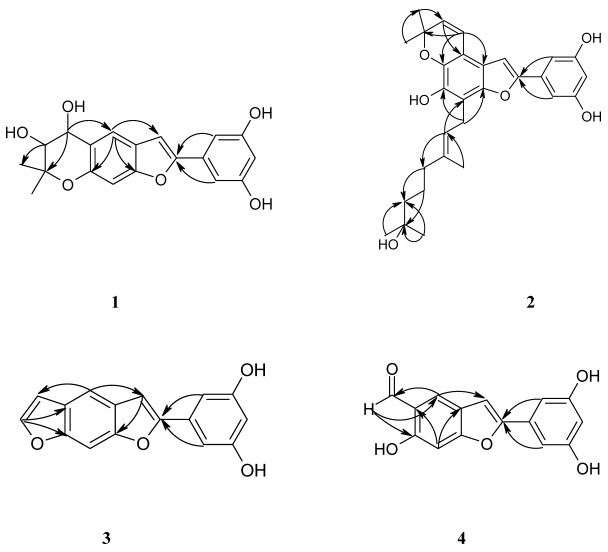

Fig. 2. Key HMBC Correlations of Compounds **1**—**4**

Compound **2** was obtained as a brown amorphous powder. The molecular formula was determined as $C_{29}H_{34}O_6$ by HR-electrospray ionization (ESI)-MS of its $[M+Na]^+$ ion peak at *m*/*z* 501.2275. The IR spectrum of **2** showed absorption bonds of hydroxyl (3388 cm^{-1}), aliphatic bond (2971, 2867 cm^{-1}), and aromatic groups (1603, 1442 cm^{-1}). The UV spectrum exhibited maxima at 208, 317, 331, and 352 nm. The ^{1}H-NMR (Table 1) spectrum of **2** exhibited the presence of one proton of furan nucleus signal at [δ_H 7.15 (1H, s, H-3)], *meta*-coupled aromatic protons (B-ring), a 2,2-dimethylpyran ring unit [δ_H 1.43 (6H, s, H-11, 12), 5.74 (1H, d, J=9.6 Hz, H-8), 6.70 (1H, d, J=9.6 Hz, H-9)], a changed geranyl group, one of whose double bonds was hydrated [δ_H 3.62 (2H, d, J=7.2 Hz, H-13), 5.46 (1H, br t, J=7.2 Hz, H-14), 1.87 (3H, s, H-16), 1.97 (2H, t, J=2.1 Hz, H-17), 1.36 (2H, m, H-18), 1.49 (2H, m, H-19), 1.10 (6H, s, H-21, 22)]. The ^{13}C-NMR signal at δ_C 70.3 further supported that compound **2** bore an aliphatic hydroxy group. The location of the hydroxy group was determined to be at C-20 based on the HMBC (Fig. 2) correlation between C-20 and H-18, H-21, H-22. Moreover, H-13 showed long-range correlations with C-6 (δ_C 142.4), C-7a (δ_C 149.4), and C-15 (δ_C 136.0), supporting that the changed geranyl group located at C-7. The position of the 2,2-dimethylpyran ring was confirmed by the long-range correlations between H-8 and C-3a (δ_C 118.1), C-5 (δ_C 137.6), and C-10 (δ_C 77.1) as well as H-9 and C-4 (δ_C 111.1). On the basis of these observations, the structure of **2** was elucidated as 3′,5′,6-trihydroxy-7-[(8″-hydroxy)-geranyl]-(2‴,2—dimethylpyrano)-(5‴,6‴:4,5)-2-arylbenzofuran and named moracin W.

Compound **3**, obtained as a yellow amorphous powder, was assigned the molecular formula $C_{16}H_{10}O_4$ by HR-ESI-MS *m*/*z* 265.0513 $[M-H]^-$. The IR spectrum of **3** showed absorption at 3493, 1612, 1581, and 1548 cm^{-1}. The UV spectrum showed absorption at 227, 259, 268, 318, and

333 nm. The B-ring protons and carbons resembled those of moracin N.[10] However, the protons in the benzofuran nucleus [δ_H 7.22 (1H, s, H-3), 7.78 (1H, s, H-4) and 7.71 (1H, s, H-7)] are more deshielded (0.5—0.7 ppm) than that of moracin N.[10] The ^{1}H-NMR (Table 1) spectrum showed AX-type aromatic protons at [δ_H 6.93 (1H, d, J=2.1 Hz, H-8) and 7.82 (1H, d, J=2.1 Hz, H-9)] from a 1,2-disubstituted olefin. The ^{13}C-NMR (Table 2) spectrum showed the signals of 14 carbon atoms from 2-arylbenzofuran nucleus and two more olefinic methenyl carbon atoms. Since a 2-arylbenzofuran nucleus accounted for ten of twelve unsaturations, it was concluded that **3** contains one more ring. From the HMBC (Fig. 2) spectrum, H-4 showed long-range correlations with C-3 (δ_C 102.2) and C-8 (δ_C 107.4); H-8 with C-6 (δ_C 154.3); H-9 with C-5 (δ_C 125.5) and C-6 (δ_C 154.3). These data support that **3** is a 2-arylbenzofuran fused with a new furan ring at C-5 and C-6, which resulted in the downfield shift of the protons in the A-ring and C-ring. Therefore the structure was determined 3′,5′-dihydroxy-furan-(4″,5″:5,6)-2-arylbenzofuran and named moracin X.

Compound **4** was obtained as a yellow powder. HR-ESI-MS of **4** showed a quasi-molecular ion peak at *m/z* 269.0459 $[M-H]^-$, which accorded to the molecular formula $C_{15}H_{10}O_5$. The IR spectrum of **4** showed absorption at 3390, 1636, 1581, and 1457 cm^{-1}. The UV spectrum showed absorption at 215, 235, 268, 300, 332, and 353 nm. The ^{1}H-NMR (Table 1) spectrum showed signals of one proton of furan nucleus signal at [δ_H 7.21 (1H, s, H-3)], one aromatic proton signal at [δ_H 7.08 (1H, s, H-7)], and *meta*-coupled aromatic protons (A_2M-type, B-ring). Furthermore, the existence of C-8 formoyl [δ_H 10.1 (1H, s, H-8); δ_C 197.6; IR: 1636 cm^{-1}], one hydroxyl proton [δ_H 13.01 (1H, s)], and the chemical shift of H-4 downfield shifted to 8.01 were observed. In the HMBC spectrum (Fig. 2), H-4 showed long-range correlations with C-3 (δ_C 102.2), C-8 (δ_C 197.6); H-7 with C-3a (δ_C 123.7), C-5 (δ_C 119.4); H-8 with C-4 (δ_C 128.4) and C-6 (δ_C 160.9). These results allowed that the formoyl located at the C-5 and the signal at δ_H 8.01 would be assigned to the proton at the C-4. Thus compound **4** was assigned as 4-formoyl-3′,5′,6-trihydroxy-2-arylbenzofuran and named moracin Y.

Two known compounds moracin N (**5**)[10] and moracin P (**6**)[9] were identified by comparison of their physical and spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with reported values.

Compounds **2**—**5** were evaluated for cytotoxicity against five tumor cell lines A549 (lung cancer), BEL7402 (liver cancer), BGC823 (gastric cancer), HCT8 (colon cancer), and A2780 (oophoroma) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method,[11] but were found to exhibit only moderate cytotoxicity as shown in Table 3. In the present study, compound **3**, with a new furan ring at C-5 and C-6, demonstrated the strongest cytotoxic activity against A549, BEL7402, BGC823, and A2780 cell lines. Compound **4**, which has a formoyl at the C-5 position of A-ring, exhibited the most potent inhibitory activities with an IC_{50} value of 6.3 μg/ml for HCT8 and close to compound **3**. From the above results, it could be tentatively concluded that the cytotoxicity of more aromatic 2-arylbenzofurans may be stronger than that with long aliphatic chains.

Table 3. Cytotoxicity of Compounds **2**—**5** against A549, BEL7402, BGC823, HCT8, and A2780 Human Cancer Cell Lines

Compounds	IC_{50} (μg/ml)				
	A549	BEL7402	BGC823	HCT8	A2780
2	>10	>10	8.3	9.2	>10
3	7.3	5.9	3.8	6.4	5.9
4	>10	6.8	7.6	6.3	6.9
5	>10	>10	>10	>10	>10
5-FU[a)]	0.18	0.54	0.70	0.54	0.65

a) 5-FU: reference compound.

Experimental

General Experimental Procedures Melting points were determined by XT digital melting-point apparatus with a microscope and are uncorrected. Optical rotations were measured by Jasco P2000 polarimeter. IR spectra were carried out on a Nicolet IMPACT 400 spectrophotometer with KBr disks. UV spectra were determined by Jasco V650 spectrophotometer. ^{1}H-NMR (300 MHz) specta were recorded by Mercury-300 spectrophotometer. ^{13}C-NMR (125 MHz), HMBC spectra were run on an INOVA-500 with TMS as internal standard. HR-ESI-MS and ESI-MS were performed on an Agilent 1100 LC/MSD Trap-SL mass spectrometer. Silica gel (Qingdao Marine Chemical Factory, 160—200 mesh), Sephadex LH-20 (Pharmacia), and RP-18 (Merck, 40—60 μm) were used for column chromatography and silica gel GF-254 (QingDao Marine Chemical Factory) was used for TLC. HPLC experiments were performed on a preparation YMC-Pack ODS-A column (10 μm, 250×20 mm) equipped with Shimadzu SPD-6A UV spectrophotometric detector at 230 nm and a Thermo Consta Metric pumping system running with flow rate of 4 ml/min.

Plant Material The leaves of *M. alba* L. were collected in the County of Anding, Beijing, China, in October 2007, and identified by Prof. Lin Ma, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. A voucher specimen (No. 21742) has been deposited at the Herbarium of Institude of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

Extraction and Isolation The air-dried leaves (26 kg) of *M. alba* L. were extracted with 95% EtOH (3×10 l; 3 h) under reflux condition. After evaporation of the solvents under reduced pressure, the residue (2500 g) was submitted to chromatography over a silica gel column (160—200 mesh; 15×140 cm; 4.0 kg) and eluted with petroleum ether (60—90 °C), $CHCl_3$, EtOAc, CH_3COCH_3, and CH_3OH, successively. The EtOAc fraction (160 g) was chromatographed over a silica gel column (160—200 mesh; 10×110 cm; 3.0 kg) using $CHCl_3$–CH_3OH as gradient eluent (50 : 1—25 : 1—15 : 1—10 : 1—5 : 1—2 : 1—0 : 1, v/v) to provide 4 fractions. Fraction 1 (118 g) was purified by silica gel column chromatography (160—200 mesh; 5×100 cm; 2.0 kg), eluted with petroleum ether–CH_3COCH_3 (95 : 5—9 : 1—8 : 2—7 : 3—1 : 1—0 : 1, v/v) to give 8 fractions. Fraction 1.3 (6.8 g) was subjected to a Sephadex LH-20 column chromatography using $CHCl_3$–MeOH (1 : 1) as eluent to give 5 fractions. Fraction 1.3.2 (300 mg) was submitted to RP-18 column chromatography then purified on RP-HPLC with an ODS column with MeOH/H_2O (5 : 5) to yield compound **1** (5 mg). Fraction 1.3.4 (100 mg) was purified on RP-HPLC with an ODS column with MeOH/H_2O (7 : 3) to give compounds **3** (8 mg), **4** (6 mg), and **6** (14 mg). Fraction 1.4 (6.0 g) was subjected to silica gel CC (160—200 mesh; 4×85 cm) eluted with $CHCl_3$–CH_3COCH_3 (99 : 1—98 : 2—95 : 5—9 : 1—8 : 2—7 : 3—1 : 1—0 : 1, v/v) and fraction 1.4.3 was purified first by Sephadex LH-20 column and again by preparative HPLC (MeOH/H_2O 7 : 3) to yield compounds **2** (17 mg) and **5** (20 mg).

Moracin V (**1**): Brown amorphous powder, mp >250 °C, $[\alpha]_D^{20}$ +2.0 (c=0.09, MeOH). UV (MeOH) λ_{max} (log ε): 219 (4.23), 320 (4.22), 334 (4.18). IR (KBr) ν_{max}: 3413, 2914, 2879, 1603, and 1470 cm^{-1}. ESI-MS *m/z*: 341.6 $[M-H]^-$. HR-ESI-MS *m/z*: 365.0997 $[M+Na]^+$ (Calcd for $C_{19}H_{18}O_6$, 365.0996). ^{1}H-NMR data see Table 1 and ^{13}C-NMR data see Table 2.

Moracin W (**2**): Brown amorphous powder, mp 216—217 °C, UV (MeOH) λ_{max} (log ε): 208 (4.37), 317 (4.20), 331 (4.19), 352 (4.06). IR (KBr) ν_{max}: 3388, 2971, 2867, 1603, 1573, and 1442 cm^{-1}. ESI-MS *m/z*: 501.3 $[M+Na]^+$. HR-ESI-MS *m/z*: 501.2275 $[M+Na]^+$ (Calcd for $C_{29}H_{34}O_6$, 501.2306). ^{1}H-NMR data see Table 1 and ^{13}C-NMR data see Table 2.

Moracin X (**3**): Yellow amorphous powder, mp 201—203 °C, UV

(MeOH) λ_{max} (log ε): 227 (4.15), 259 (3.89), 268 (3.91), 318 (4.17), 333 (4.16). IR (KBr) ν_{max}: 3493, 1612, 1581, and 1548 cm^{-1}. ESI-MS *m/z*: 265.2 $[M-H]^-$. HR-ESI-MS *m/z*: 265.0513 $[M-H]^-$ (Calcd for $C_{16}H_{10}O_4$, 265.0506). ^{1}H-NMR data see Table 1 and ^{13}C-NMR data see Table 2.

Moracin Y (**4**): Yellow powder, mp 236—238 °C, UV (MeOH) λ_{max} (log ε): 215 (4.02), 235 (4.10), 268 (4.18), 300 (4.11), 332 (3.69), 353 (3.62). IR (KBr) ν_{max}: 3390, 1636, 1581, and 1457 cm^{-1}. ESI-MS *m/z*: 269.1 $[M-H]^-$. HR-ESI-MS *m/z*: 269.0459 $[M-H]^-$ (Calcd for $C_{15}H_{10}O_5$, 269.0455). ^{1}H-NMR data see Table 1 and ^{13}C-NMR data see Table 2.

Cytotoxicity Bioassays Cytotoxicity was determined by MTT method[11] using human cell lines A549 (lung cancer), BEL7402 (liver cancer), BGC823 (gastric cancer), HCT8 (colon cancer) and A2780 (oophoroma) grown in RPMI-1640 medium supplied with 10% fetal bovine serum. Cells in logarithmic phase were cultured at a density of 10000 cells/ml per well in a 96-well microtiter plate. Then different concentrations of test compounds dissolved in dimethyl sulfoxide (DMSO) were added to each well. Each concentration was tested in triplicate. After incubation at 37 °C in 5% CO_2 for 96 h, 100 μl of MTT (0.4 mg/ml) was added to each well and incubated for another 4 h then liquid in the wells was removed. DMSO (150 μl) was added to each well. The absorbance was recorded on a microplate reader (Bio-Rad model 550) at a wavelength of 540 nm. The IC_{50} value resulted from 50% reduction of absorbance in the control assay, which was treated with 2.5% DMSO alone.

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