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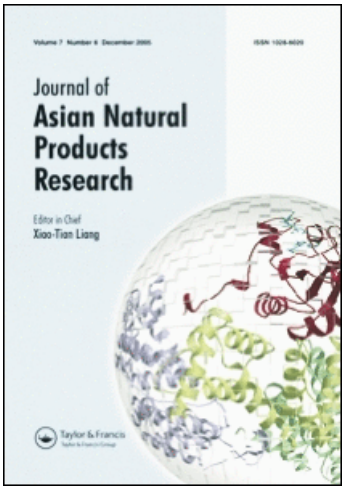

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Two novel flavanes from the leaves of *Morus alba* L.

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ORIGINAL ARTICLE

Two novel flavanes from the leaves of *Morus alba* L.

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Two new flavanes, (2*R*,4*S*)-2′,4′-dihydroxy-2*H*-furan-(3″,4″:8,7)-flavan-4-ol (**1**) and (2*S*)-2′,4′-dihydroxy-7-methoxyl-8-butyricflavane (**2**), together with four known flavonoids, were isolated from the leaves of *Morus alba* L. Their structures were determined on the basis of spectroscopic analysis.

Keywords: *Morus alba* L.; flavane; (2*R*,4*S*)-2′,4′-dihydroxy-2*H*-furan-(3″,4″:8,7)-flavan-4-ol; (2*S*)-2′,4′-dihydroxy-7-methoxyl-8-butyricflavane

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1. Introduction

The root bark of *Morus alba* L. has been commonly used as a traditional medicine to treat diabetes, arthritis, and rheumatism for thousands of years [1]. *M. alba* was investigated in the 1970s. Since then, many flavones, stilbene, and benzofuran derivatives [2–4] have been isolated. In recent years, the root barks or the stem barks of seven species of the genus *Morus* have been investigated systematically by our group [5–7]. As part of the ongoing study on the difference in the constituents between the root bark and the leaves, an investigation on the ethanol extracts of the leaves of *M. alba* L. was carried out. Further detailed investigation yielded one novel flavan-4-ol (**1**) and one novel flavane (**2**), together with four known flavonoids (Figure 1). This paper describes the isolation and structural elucidation of these compounds.

2. Results and discussion

Compound **1** was obtained as a brown amorphous powder, $[\alpha]_D^{20}$ −38.01 ($c = 0.142$, CH_3OH). The molecular formula was determined to be $C_{17}H_{16}O_5$ from the HR-ESI-MS, which showed a quasi-molecular ion peak at *m/z* 323.0895 $[M+Na]^+$. The IR spectrum of **1** showed absorption bands ascribed to hydroxyl (3345 cm^{-1}) and aromatic groups (1611, 1505, and 1453 cm^{-1}). The UV spectrum showed absorption maxima at 286, 277, 235, and 211 nm. The ^1H NMR spectrum of **1** displayed the following proton signals: ABX-type aromatic protons at δ 7.15 (1H, d, $J = 8.1$ Hz), 6.35 (1H, dd, $J = 8.1$, 2.4 Hz), and 6.19 (1H, d, $J = 2.4$ Hz); *ortho*-coupled aromatic protons at δ 7.03 (1H, d, $J = 8.1$ Hz) and 6.40 (1H, d, $J = 8.1$ Hz); one hydroxylated methene protons at δ 3.59 (2H, m); and two aliphatic protons at δ 2.80 (2H, m).

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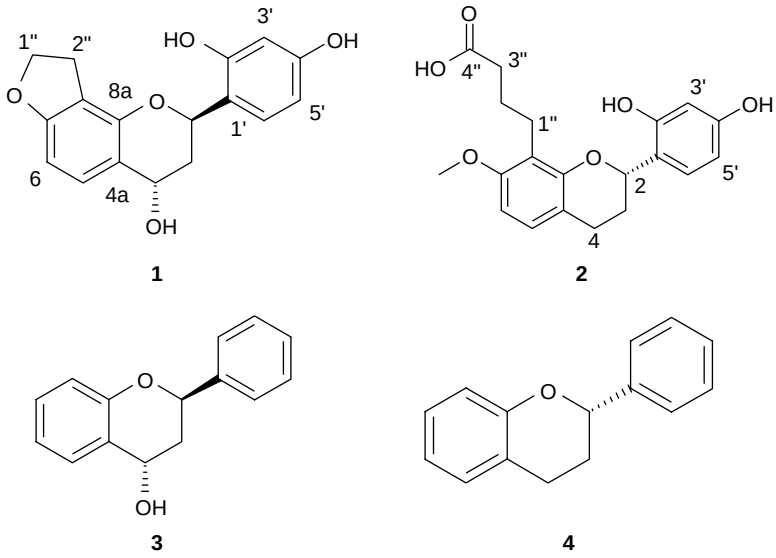

Figure 1. Structure of compounds **1**–**4**.

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Furthermore, the lower field shifted signals at δ 5.31 (1H, br s, H-2), 5.24 (1H, br s, H-4), and 2.18 (2H, m, H-3), which suggested the presence of a flavan-4-ol moiety. The ^{13}C NMR chemical shifts for C-2 (δ 68.1) and C-4 (δ 68.5) were in agreement with the flavan-4-ol moiety. From the above information, it was concluded that a flavan-4-ol moiety as well as a dihydrofuran ring and a 1,2,4-trisubstituted aromatic ring were present in the structure of **1**. The location of the hydrofuran ring was supported by the HMBC correlations (Figure 2) as follows: the methylene proton signals at δ 2.80 (H-2″) exhibited long-range correlations with C-8 (δ 114.4), C-7 (δ 158.2), and C-8a (δ 152.9), while H-1″ showed a long-range correlation with C-8 (δ 114.4).

In the nuclear Overhauser effect (NOE) experiment, irradiation at δ 2.18 due to H-3 produced a significant enhancement of the proton signal at δ 5.31 (H-2) and δ 5.24 (H-4). However, irradiation at H-2 or H-4 only produced significant enhancement at δ 2.18 (H-3), suggesting that the flavan-4-ol moiety has a 2,4-*trans*-type stereochemistry. On the basis of the 2*R*, 4*S* absolute configuration of compound **3** [8] and the fact that the CD spectrum of **1** showed a negative Cotton effect in the 280 nm region (^1L_b transition), similar to

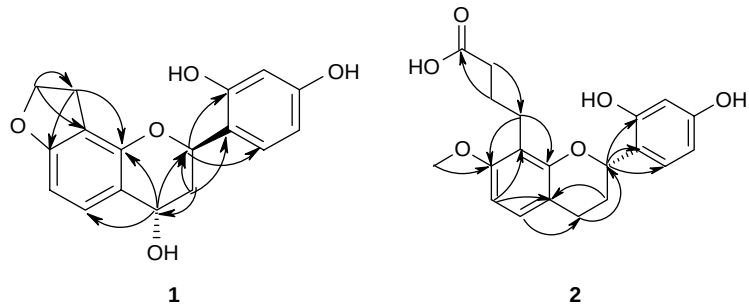

Figure 2. Key HMBC correlations of compounds **1** and **2**.

compound **3**, the absolute configuration of C-2 in compound **1** was determined as *R* [8]. Together with the NOE and the optical rotation ($[\alpha]_D^{20}$ −38.01), the absolute configuration was determined to be 2*R*, 4*S*. Thus, compound **1** was assigned as (2*R*,4*S*)-2′,4′-dihydroxy-2*H*-furan-(3″,4″:8,7)-flavan-4-ol.

Compound **2** was obtained as a brown amorphous powder, $[\alpha]_D^{20}$ + 2.69 ($c = 0.100$, CH_3OH). The molecular formula was determined as $C_{20}H_{22}O_6$ by its $[M+Na]^+$ ion peak at *m/z* 381.1320 in HR-ESI-MS. The IR spectrum of **2** showed absorption bands of hydroxyl (3383 cm^{-1}) and aromatic groups (1613 and 1519 cm^{-1}). The UV spectrum showed absorption maxima at 286, 279, 226, and 206 nm. Comparison of its ^1H and ^{13}C NMR spectral data with those of 1,3-benzenediol,4-[3,4-dihydro-7-methoxy-8-(3-methyl-2-butenyl)-2*H*-1-benzopyran-2-yl] [9] suggested that they have the same framework and the structural difference might be the substituent group at the C-8 position. The existence of the carboxylic group (δ_C 170.8, IR 1710 cm^{-1}), six aliphatic protons, as well as three aliphatic carbons indicated that a butanoic acid existed in compound **2**. The complete structure was revealed with the aid of HMQC and HMBC spectra. As shown in Figure 2, H-1″ showed long-range correlations with C-7 (δ 157.9), C-8a (δ 154.9); H-2″ with C-4″ (δ 170.8); H-3″ with C-1″ (δ 23.3); H-2 with C-4 (δ 25.5), C-2′ (δ 155.7), C-6′ (δ 128.1); H-3 with C-4a (δ 115.6); and H-4 with C-2 (δ 74.1). Based on the known 2*S* absolute configuration of compound **4** [8,10], and the same fact that a negative Cotton effect ascribed to the ^1L_b transition at 280 nm was observed in the CD spectrum of **2**, the absolute configuration at C-2 was assigned as *S*. Thus, the structure of **2** was elucidated as (2*S*)-2′,4′-dihydroxy-7-methoxyl-8-butyricflavane.

Four known compounds morusin (**5**) [11], monogolin B (**6**) [6], 4*H*,8*H*-benzo[1,2-*b*:3,4-*b*]dipyran-4-one,2-(2,4-dihydroxyphenyl)-5-hydroxy-3-(2-hydroxy-3-methyl-3-butenyl)-8,8-dimethyl (**7**) [12], and kaempferol-3-*O*-β-D-glucoside (**8**) [13] were identified by the comparison of their physical and spectral data (^1H NMR, ^{13}C NMR, MS) with the reported values in the literature.

3. Experimental

3.1 General experimental procedures

The optical rotations were measured on a JASCO P2000 polarimeter. Melting points were determined on an XT digital melting-point apparatus with a microscope and are uncorrected. IR spectra were carried out on a Nicolet IMPACT 400 spectrophotometer as KBr disks. UV spectra were determined with a JASCO V650 spectrophotometer. NMR and HMBC spectra were recorded on Mercury-400 and Mercury-300 spectrophotometers with TMS as an internal standard. HR-ESI-MS and ESI-MS were performed on an Agilent 1100 LC/MSD Trap-SL mass spectrometer. Silica gel (160–200 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. HPLC experiments were carried out on a preparative YMC-Pack ODS-A column (10 μm, 250 × 20 mm i.d.; YMC, Kyoto, Japan) equipped with a Shimadzu SPD-6A UV spectrophotometric detector at 230 nm and a Thermo Constametric pumping system.

3.2 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 Science and Peking Union Medical College. A voucher specimen (No. 21742) has been deposited

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at the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3 Extraction and isolation

The air-dried leaves (26 kg) of *M. alba* were finely cut and extracted with 95% EtOH (3× 10 liters, 3 h) under reflux condition. After evaporation of the solvent under reduced pressure, the residue (2500 g) was subjected 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 MeOH, successively. The EtOAc fraction (160 g) was chromatographed over a silica gel column (160–200 mesh, 10×110 cm, 3.0 kg) using $CHCl_3$–MeOH as the gradient eluent [(50:1–25:1–15:1–10:1–5:1–2:1–0:1, v/v)] to provide four 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 eight fractions (1-1–1-8). Fraction 1-4 (10.1 g) was subjected to silica gel column chromatography (160–200 mesh, 5×60 cm, 350 g), eluted with $CHCl_3$–CH_3COCH_3 (9:1–8:2–7:3–1:1, v/v) to give 15 fractions. Fraction 1-4-6 (150 mg) was subjected to RP-18 column chromatography, and then purified on RP-HPLC with an ODS column (flow rate 4 ml/min) with MeOH–H_2O (5:5) to yield compounds **1** (10 mg) and **6** (9 mg).

Fraction 1-2 (6.7 g) was subjected to Sephadex LH-20 column chromatography using $CHCl_3$–MeOH (1:1) as the eluent to give five fractions. Fraction 1-2-3 (100 mg) was purified on RP-HPLC with an ODS column (flow rate 4 ml/min) with MeOH–H_2O (6:4) to yield compound **2** (6 mg). Fraction 1-2-4 (1.2 g) was

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** in acetone-d_6 (300 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR).

	1		**2**	
Position	H (*J*, Hz)	C	H (*J*, Hz)	C
2	5.31 (1H, br s)	68.1	5.29 (1H, dd, $J = 2.1, 9.9$)	74.1
3	2.18 (2H, m)	27.7	2.20 (1H, m), 1.94 (1H, m)	30.0 (overlapped)
4	5.24 (1H, br s)	68.5	2.92 (1H, m), 2.70 (1H, dt, $J = 16.2, 4.5$)	25.5
4a		109.0		115.6
5	7.03 (1H, d, $J = 8.1$)	129.9	6.93 (1H, d, $J = 8.7$)	128.6
6	6.40 (1H, d, $J = 8.1$)	114.1	6.52 (1H, d, $J = 8.7$)	103.8
7		158.2		157.9
8		114.4		113.6
8a		152.9		154.9
1′		114.5		120.5
2′		155.4		155.7
3′	6.19 (1H, d, $J = 2.4$)	103.5	6.42 (1H, d, $J = 2.4$)	103.4
4′		160.0		158.6
5′	6.35 (1H, dd, $J = 8.1, 2.4$)	109.4	6.37 (1H, dd, $J = 2.4, 8.1$)	107.6
6′	7.15 (1H, d, $J = 8.1$)	132.3	7.20 (1H, d, $J = 8.1$)	128.1
1″	3.59 (2H, m)	62.6	2.94 (2H, m)	23.3
2″	2.80 (2H, m)	27.4	1.90 (2H, m)	20.8
3″			4.14 (1H, m), 4.06 (1H, m)	63.7
4″				170.8
—OCH_3			3.78 (3H, s)	56.0

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subjected to Sephadex LH-20 column chromatography using $CHCl_3$–MeOH (1:1) as the eluent to give six fractions. Fraction 1-2-4-3 was purified on RP-HPLC with an ODS column (flow rate 4 ml/min) with MeOH–H_2O (8:2) to yield compounds **3** (75 mg), **4** (32 mg), and **5** (30 mg).

*3.3.1 (2R,4S)-2′,4′-dihydroxy-2H-furan-(3″,4″:8,7)-flavan-4-ol (**1**)*

A brown amorphous powder, mp 106–108°C, $[\alpha]_D^{20}$ −38.01 ($c = 0.142$, CH_3OH). UV (MeOH) λ_{max} (log ε): 210 (4.69), 235 (3.93), 277 (3.56), 286 (3.58) nm. IR (KBr) ν_{max}: 3345, 2958, 2920, 1611, 1505, 1453 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS *m/z*: 323.1 $[M+Na]^+$. HR-ESI-MS *m/z*: 323.0895 $[M+Na]^+$ (calcd for $C_{17}H_{16}O_5Na$, 323.0890).

*3.3.2 (2S)-2′,4′-dihydroxy-7-methoxyl-8-butyricflavane (**2**)*

A brown amorphous powder, mp 148–151°C, $[\alpha]_D^{20}$ +2.69 ($c = 0.100$, CH_3OH). UV (MeOH) λ_{max} (log ε): 206 (4.62), 226 (4.06), 279 (3.54), 286 (3.52) nm. IR (KBr) ν_{max}: 3388, 2926, 2850, 1710, 1613, 1519 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS *m/z*: 381.2 $[M+Na]^+$. HR-ESI-MS *m/z*: 381.1320 $[M+Na]^+$ (calcd for $C_{20}H_{22}O_6Na$, 381.1309).

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