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2-Arylbenzofuran, Flavonoid, and Tyrosinase Inhibitory Constituents of *Morus yunnanensis*

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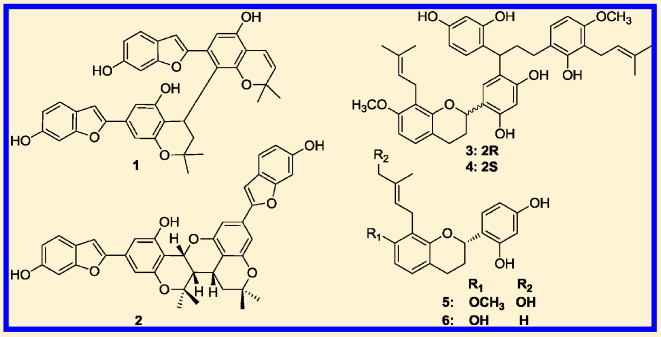
Supporting Information

ABSTRACT: Two novel 2-arylbenzofuran dimers, morusyunnansins A and B (**1** and **2**), two new biflavonoids, morusyunnansins C and D (**3** and **4**), two new flavans, morusyunnansins E and F (**5** and **6**), and four known flavans (**7**−**10**) were isolated from the leaves of *Morus yunnanensis*. Compounds **5**−**8** showed potent inhibitory effects on mushroom tyrosinase with IC_{50} values ranging from 0.12 ± 0.02 to 1.43 ± 0.43 μM.



Tyrosinase, also known as polyphenoloxidase, plays a key role in melanin synthesis in mammals.[1] Overproduction of melanin results in skin hyperpigmentations, such as age spots, melasma, and chloasma.[2] Using tyrosinase inhibitors to prevent pigmentation disorders and whiten skin by suppressing melanin formation has become increasingly important in medicinal and cosmetic products. However, many of the current popular skin-whitening agents exhibit toxicity and adverse side-effects.[3−5] In addition, tyrosinase is responsible for enzymatic browning of many plant-derived foods.[6] Tyrosinase inhibitors are applicable to food preservation by preventing the undesirable browning reactions and should also be effective and safe. Hence, the need for new potent tyrosinase inhibitors for use in medicinal, cosmetic, and food products has been widely recognized. Plants are one of the main sources of natural tyrosinase inhibitors.

The genus *Morus* (Moraceae) comprises about 16 species, and some of them have been used as traditional Chinese medicines. In recent years, extracts from some *Morus* plants have been used as popular skin-lightening agents in cosmetics. Some flavonoids are regarded as active constituents for their inhibitory effects on tyrosinase and melanin biosynthesis.[7−9]

Morus yunnanensis Koidz. (Moraceae) is an arbor tree that grows in Yunnan Province of China. Diels−Alder adducts, 2-arylbenzofurans, and stilbenes were isolated from the bark of this plant previously, and some of these showed antioxidant and anti-inflammatory avtivities.[10,11] As part of our program on the discovery of tyrosinase inhibitory agents from natural products, we investigated the chemical constituents of *M. yunnanensis*. An ethanol extract of leaves of *M. yunnanensis* afforded two novel 2-arylbenzofuran dimers, morusyunnansins A and B (**1** and **2**), two new biflavonoids, morusyunnansins C and D (**3** and **4**), two new flavans, morusyunnansins E and F (**5** and **6**), and four known flavans, (2*R*)-2′,4′-dihydroxy-7-methoxy-8-hydroxyethylflavan (**7**), (2*S*)-2′,4′-dihydroxy-7-methoxy-8-prenylflavan (**8**), (2*S*)-7,2′-dihydroxy-4′-methoxy-8-prenylflavan (**9**), and (2*S*)-7,4′-dihydroxy-8-prenylflavan (**10**). We herein present the structure elucidation of compounds **1**−**6** and their tyrosinase inhibitory activities.

RESULTS AND DISCUSSION

Compound **1**, a yellowish oil, was assigned the molecular formula $C_{38}H_{32}O_8$ by HRESIMS. The UV absorption maxima at λ_{max} 221, 320, and 333 nm were similar to those of 2-arylbenzofurans.[12] The IR spectrum showed absorptions for OH (3430 cm^{-1}) and aromatic (1625 and 1488 cm^{-1}) moieties. The ^1H NMR spectrum showed four hydroxy signals at δ_H 8.47 (3H, s, OH-6, OH-6′, OH-12′) and 7.63 (1H, s, OH-12), two 1,2,4-trisubstituted aromatic systems (A and A′ rings) at δ_H 7.38 (1H, d, J = 8.2 Hz, H-4), 6.99 (1H, br s, H-7), and 6.79 (1H, dd, J = 2.0, 8.2 Hz, H-5) and at δ_H 7.44 (1H, d, J = 8.2 Hz, H-4′), 6.96 (1H, br s, H-7′), and 6.82 (1H, dd, J = 2.0, 8.2 Hz, H-5′), two *meta*-coupled aromatic protons (B ring) at δ_H 6.74 (1H, d, J = 1.6 Hz, H-9) and 6.83 (1H, d, J = 1.6 Hz, H-13), and an aromatic singlet (B′ ring) at δ_H 6.72 (1H, s, H-13′) (Table 1).

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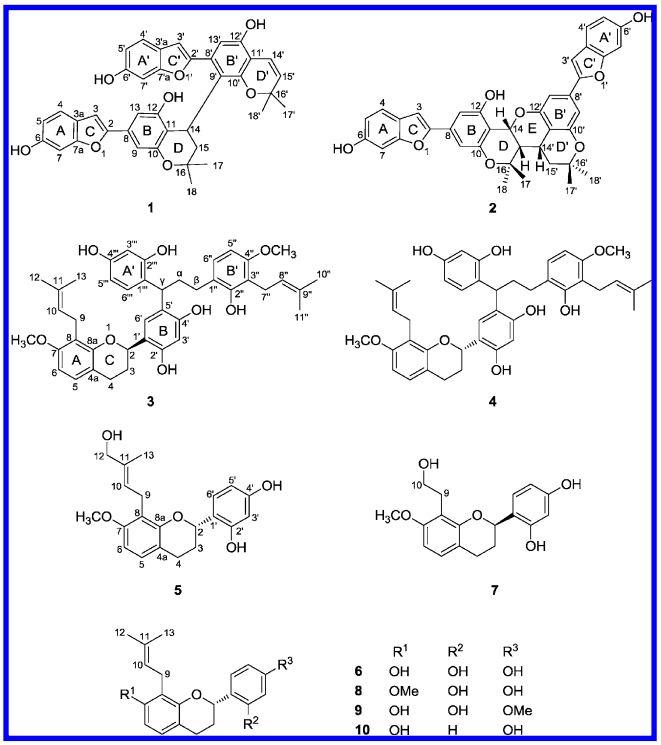

Two broad olefinic singlets at δ_H 6.97 (1H, br s, H-3) and 6.94 (1H, br s, H-3′) suggested the presence of two 2-arylbenzofuran units. The ^{1}H NMR spectrum also displayed signals of two isoprenoid moieties, a 2,2-dimethyldihydropyran ring at δ_H 4.63 (1H, dd, J = 7.4, 11.3 Hz, H-14), 2.39 (1H, t, J = 12.1 Hz, H-15a), 2.11 (1H, dd, J = 7.4, 13.3 Hz, H-15b), 1.45 (3H, s, H_3-18), and 1.24 (3H, s, H_3-17), and a 2,2-dimethylpyran ring at δ_H 6.61 (1H, d, J = 10.2 Hz, H-14′), 5.53 (1H, d, J = 10.2 Hz, H-15′), 1.26 (3H, s, H_3-17′), and 0.70 (3H, s, H_3-18′). The ^{13}C NMR spectrum exhibited 17 quaternary sp^2, 13 methine sp^2, two quaternary sp^3, one methine sp^3, one methylene sp^3, and four methyl carbon signals (Table 1). The aforementioned spectral data indicated that **1** was a diprenylated 2-arylbenzofuran dimer. The ^{1}H and ^{13}C NMR signals of the two 2-arylbenzofuran skeletons were assigned by the HSQC and HMBC spectra. The signal at δ_H 7.63 was assigned to OH-12, and the three overlapped OH signals at δ_H 8.47 were attributed to OH-6, OH-6′, and OH-12′ by the HMBC correlations (Figure 1a in the Supporting Information). The 2,2-dimethyldihydropyran ring was fused at C-10/11 by the HMBC correlations from H-14 to C-10, C-11, and C-12, while the 2,2-dimethylpyran moiety was attached at C-10′/11′ according to the HMBC correlations from H-14′ to C-10′, C-11′, and C-12′ and from H-15′ to C-11′. The two monomeric units were linked through a C-14−C-9′ bond, as determined by the key HMBC correlations from H-14 to C-8′, C-9′, and C-10′. In the ROESY experiment, the correlations of H-14/H_3-17 and H-14/H-15b suggested that they are cofacial, which was supported by the correlation of H_3-18/H-15a (Figure 1b in the Supporting Information). Thus, the structure of **1** (morusyunnansin A) was elucidated as 3,4-dihydro-2,2-dimethyl-4-[2,2-dimethyl-5-hydroxy-7-(6-hydroxybenzofuran-2-yl)-2*H*-1-benzopyran-8-yl]-7-(6-hydroxybenzofuran-2-yl)-2*H*-1-benzopyran-5-ol.

Compound **2** was assigned the molecular formula $C_{38}H_{32}O_8$ by HRESIMS, indicating 23 degrees of unsaturation. The ^{1}H NMR spectrum of **2** showed signals similar to those of **1**, including two broad olefinic singlets (H-3 and H-3′) and two

Table 1. NMR Data of Compounds 1 and 2 (400 MHz for ^{1}H, 100 MHz for ^{13}C, acetone-d_6, δ in ppm, J in Hz)

	1		2	
position	δ_H	δ_C	δ_H	δ_C
2		155.8		154.9
3	6.97 br s	101.3	7.16 br s	103.0
3a		122.7		122.5
4	7.38 d (8.2)	121.7	7.43 d (8.2)	122.0
5	6.79 dd (2.0, 8.2)	113.0	6.82 dd (2.0, 8.2)	113.4
6	8.47 s (OH)	156.5		156.8
7	6.99 br s	98.3	7.01 br s	98.4
7a		157.0		156.9
8		129.7		133.7
9	6.74 d (1.6)	104.1	6.89 br s	105.1
10		156.8		156.0
11		115.6		108.6
12	7.63 s (OH)	156.7		158.9
13	6.83 d (1.6)	105.5	7.03 br s	103.8
14	4.63 dd (7.4, 11.3)	31.0	4.84 d (4.4)	69.5
15	2.39 t (12.1, H-15a)	40.2	2.27 br t (4.4)	48.3
	2.11 dd (7.4 13.3, H-15b)			
16		75.2		78.3
17	1.24 s	23.2	1.59 s	28.7
18	1.45 s	30.2	1.47 s	21.2
2′		156.0		155.3
3′	6.94 br s	105.8	7.13 br s	102.2
3′a		122.5		122.6
4′	7.44 d (8.2)	121.9	7.45 d (8.2)	122.1
5′	6.82 dd (2.0, 8.2)	113.0	6.84 dd (2.0, 8.2)	113.2
6′	8.47 s (OH)	156.5		156.7
7′	6.96 br s	98.4	7.01 br s	98.4
7′a		156.6		156.8
8′		132.1		130.6
9′		123.2	6.93 br s	106.5
10′		152.5		155.6
11′		111.0		112.7
12′	8.47 s (OH)	151.7		158.4
13′	6.72 s	108.8	7.01 br s	105.4
14′	6.61 d (10.2)	117.4	2.80 overlap	26.3
15′	5.53 d (10.2)	129.8	2.19 dd (4.7, 13.0, H-15′β)	42.4
			1.97 t (13.0, H-15′α)	
16′		76.0		77.1
17′	1.26 s[a]	28.2[a]	1.49 s	26.8
18′	0.70 s[a]	26.1[a]	1.48 s	30.5

[a]The assignments are exchangeable.

1,2,4-trisubstituted aromatic systems (A and A′ rings) (Table 1). It also exhibited two sets of *meta*-coupled aromatic protons (B and B′ rings) at δ_H 6.89 (1H, br s, H-9) and 7.03 (1H, br s, H-13) and at δ_H 6.93 (1H, br s, H-9′) and 7.01 (1H, br s, H-13′). The ^{1}H NMR signals of two isoprenoid groups both in the form of 2,2-dimethyldihydropyran rings were observed at δ_H 4.84 (1H, d, J = 4.4 Hz, H-14), 2.27 (1H, br t, J = 4.4 Hz, H-15), 1.59 (3H, s, H_3-17), and 1.47 (3H, s, H_3-18) and at δ_H 2.80 (1H, overlap, H-14′), 2.19 (1H, dd, J = 4.7, 13.0 Hz, H-15′β), 1.97 (1H, br t, J = 13.0 Hz, H-15′α), 1.49 (3H, s, H_3-17′), and 1.48 (3H, s, H_3-18′). The ^{13}C NMR spectrum showed 38 carbon signals, corresponding to a diprenylated 2-arylbenzofuran dimer (Table 1). The ^{1}H and ^{13}C NMR signals of the two 2-arylbenzofuran units were assigned by the HSQC and HMBC spectra. The two 2,2-dimethyldihydropyran

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moieties were respectively fused at C-10/11 and C-10′/11′ by the HMBC correlations from H-14 to C-10, C-11, and C-12 and from H-15′ to C-11′ (Figure 2a in the Supporting Information). The ^{1}H−^{1}H COSY and HMBC correlations identified the sequence of the two isoprenoid groups as −CH(14)−CH(15)[C(CH_3)$_2$−O−]−CH(14′)−CH_2(15′)−C(CH_3)$_2$−O− (Figure 2a in the Supporting Information). The unsaturation degree and the downfield shift of C-14 (δ_C 69.5) suggested an additional ring (E ring) formed by an ether bridge between C-14 and C-12′. Thus, the connectivity of the two 2-arylbenzofuran monomers was established. The coupling constant of H-15 with H-14 and H-14′ (J = 4.4 Hz) indicated their *cis* relationship, shown in β-orientation. The ROESY correlations of H-15/H-14, H-15/H_3-17, H-15/H-15′β, H-14′/H_3-17, and H-14′/H_3-17′ confirmed their β-orientation (Figure 2b in the Supporting Information). H_3-18 and H_3-18′ were then assigned as α-orientation. Thus, the structure of **2** (morusyunnansin B) was elucidated as 2,2′-{(7a*R**,13a*S**,13b*R**)-8-hydroxy-2,2,13,13-tetramethyl-1,2,7a,13,13a,13b-hexahydropyrano[3,2-*c*:4,5,6-*d*′*e*′]di(3,4-dihydro-2*H*-1-benzofuran)-5,10-diyl}bis(benzofuran-6-ol).

Compounds **3** and **4** were assigned identical molecular formulas ($C_{42}H_{48}O_8$) by HRMALDIMS. The IR spectrum of **3** showed absorptions for OH and aromatic moieties. The UV absorption maxima at λ_{max} 225 and 282 nm were similar to those of flavans.[13] The ^{1}H NMR spectrum of **3** displayed five hydroxy signals at δ_H 8.43, 8.30 (each 1H, br s, OH-4′, 2‴), 8.21 (1H, s, OH-2′), 8.01 (1H, s, OH-4‴), and 6.92 (1H, s, OH-2″), two sets of *ortho*-coupled aromatic protons (A and B′ rings) at δ_H 6.88 (1H, d, J = 8.6 Hz, H-5) and 6.50 (1H, d, J = 8.6 Hz, H-6) and at δ_H 6.81 (1H, d, J = 8.2 Hz, H-6″) and 6.38 (1H, d, J = 8.2 Hz, H-5″), a 1,2,4-trisubstituted aromatic system (A′ ring) at δ_H 7.08 (1H, d, J = 8.6 Hz, H-6‴), 6.37 (1H, d, J = 2.0 Hz, H-3‴), and 6.31 (1H, dd, J = 2.0, 8.6 Hz, H-5‴), two aromatic singlets (B ring) at δ_H 7.46 (1H, s, H-6′) and 6.45 (1H, s, H-3′), two sets of 3-methyl-2-butenyl (prenyl) signals at δ_H 5.26 (1H, br t, J = 7.0 Hz, H-10), 3.39 (1H, br dd, J = 7.8, 13.3 Hz, H-9a), 3.26 (1H, br dd, J = 6.0, 13.3 Hz, H-9b), and 1.65, 1.59 (each 3H, br s, H_3-13, H_3-12) and at δ_H 5.14 (1H, br t, J = 7.0 Hz, H-8″), 3.33 (2H, br d, J = 7.0 Hz, H_2-7″), and 1.71, 1.58 (each 3H, br s, H_3-11″, H_3-10″), and two methoxy groups at δ_H 3.78 (3H, s, OMe-7) and 3.73 (3H, s, OMe-4″) (Table 2). One set of aliphatic proton signals at δ_H 5.27 (1H, overlap, H-2), 2.89 (1H, m, H-4a), 2.71 (1H, br d, J = 14.9 Hz, H-4b), 2.27 (1H, m, H-3a), and 1.92 (1H, m, H-3b) and another set at δ_H 4.49 (1H, br t, J = 7.5 Hz, H-γ), 2.53 (2H, m, H_2-β), and 2.28 (2H, m, H_2-α) were characteristic of a flavan and a deoxotetrahydrochalcone moiety.[14] The ^{13}C NMR spectrum showed 17 quaternary sp^2, 11 methine sp^2, two methine sp^3, six methylene sp^3, and six methyl carbon signals (Table 2). These NMR signals indicated that **3** was a biflavonoid consisting of the flavan-deoxotetrahydrochalcone monomers with five hydroxy, two methoxy, and two prenyl groups. Analysis of the HSQC and HMBC spectra identified the structures of the two monomers as 2′,4′-dihydroxy-7-methoxy-8-prenylflavan and 2″,2‴,4‴-trihydroxy-4″-methoxy-3″-prenyldeoxotetrahydrochalcone, which were linked through a C-γ−C-5′ bond according to the HMBC correlations from H-γ to C-4′, C-5′, and C-6′ and from H_2-α to C-5′ (Figure 3 in the Supporting Information). The absolute configuration at C-2 was determined as *R* by the CD spectrum of **3**, which exhibited a positive Cotton effect at λ_{max} 291 nm.[13,15] The absolute configuration at C-γ remains unknown. As for **4**, its UV, IR, ^{1}H NMR, ^{13}C NMR, and MS data were similar to those of **3**. It was inferred that both compounds had the same planar structure. Opposite of **3**, the CD spectrum of **4** showed a negative Cotton effect at λ_{max} 288 nm, assigning the absolute configuration at C-2 as *S*.[15] Thus, the structure of **3** (morusyunnansin C) was elucidated as 4-{1-(2,4-dihydroxyphenyl)-3-[2-hydroxy-4-methoxy-3-(3-methyl-2-buten-1-yl)phenyl]propyl}-6-[(2*R*)-3,4-dihydro-7-methoxy-8-(3-methyl-2-buten-1-yl)-2*H*-1-benzopyran-2-yl]-1,3-benzenediol, and **4** (morusyunnansin D) was elucidated as its 2*S*-diastereomer.

Compound **5** had the molecular formula $C_{21}H_{24}O_5$, as deduced from HREIMS. The ^{1}H NMR spectrum showed hydroxy signals at δ_H 8.33 (1H, s, OH-2′) and 8.19 (1H, s, OH-4′), *ortho*-coupled aromatic protons (A ring) at δ_H 6.88 (1H, d, J = 8.2 Hz, H-5) and 6.50 (1H, d, J = 8.2 Hz, H-6), a 1,2,4-trisubstituted aromatic system (B ring) at δ_H 7.20 (1H, d, J = 8.3 Hz, H-6′), 6.43 (1H, d, J = 2.0 Hz, H-3′), and 6.37 (1H, dd, J = 2.0, 8.3 Hz, H-5′), and a methoxy group at δ_H 3.79 (3H, s, OMe-7) (Table 2). In addition, a set of aliphatic proton signals at δ_H 5.29 (1H, dd, J = 2.0, 10.0 Hz, H-2), 2.89 (1H, m, H-4a), 2.70 (1H, dt, J = 4.0, 16.0 Hz, H-4b), and 2.18, 1.89 (each 1H, m, H-3a, H-3b) indicated a flavan skeleton for **5**. The presence of a 4-hydroxy-3-methyl-2-butenyl group was inferred from the following ^{1}H and ^{13}C NMR data: δ_H 5.45 (1H, br t, J = 7.0 Hz, H-10), 3.85 (2H, br d, J = 5.9 Hz, H_2-12), 3.49 (1H, t, J = 5.9 Hz, OH-12), 3.37 (1H, br dd, J = 6.3, 13.7 Hz, H-9a), 3.32 (1H, br dd, J = 7.3, 13.7 Hz, H-9b), and 1.66 (3H, br s, H_3-13); δ_C 135.4 (C-11), 123.9 (C-10), 68.6 (C-12), 22.4 (C-9), and 13.8 (C-13) (Table 2). These NMR data suggest that **5** is a prenylated flavan containing a methoxy and two hydroxy groups. The 4-hydroxy-3-methyl-2-butenyl group was located at C-8 according to the HMBC correlations from H_2-9 to C-7, C-8, and C-8a (Figure 4 in the Supporting Information). The HMBC correlation from the protons at δ_H 3.79 to C-7 assigned the methoxy group to be at C-7. The 2′,4′-dihydroxylated B ring was deduced by the HMBC correlations from OH-2′ to C-1′, C-2′, and C-3′ and from OH-4′ to C-3′, C-4′, and C-5′. The upfield shift of C-13 caused by the *γ-gauche* effect determined the *E*-configuration of the double bond at C-10/11.[16] The CD spectrum showed a negative Cotton effect at 284 nm, assigning the absolute configuration at C-2 as *S*.[15] Thus, the structure of **5** (morusyunnansin E) was elucidated as 4-{(2*S*)-3,4-dihydro-7-methoxy-8-[(2*E*)-3-methyl-2-buten-4-ol-1-yl]-2*H*-1-benzopyran-2-yl}-1,3-benzenediol.

Compound **6** was assigned the molecular formula $C_{20}H_{22}O_4$ by HREIMS. It was also regarded as a prenylated flavan by the UV, IR, and NMR spectroscopic data. Similar to **5**, the ^{1}H NMR spectrum of **6** showed two *ortho*-coupled aromatic protons (A ring), a 1,2,4-trisubstituted aromatic system (B ring), and a set of aliphatic protons (C ring) (Table 2). Different from **5**, the A ring of **6** contained a hydroxy and a prenyl group. The prenyl group was located at C-8 by the HMBC correlations from H_2-9 to C-7, C-8, and C-8a. The absolute configuration at C-2 was determined as *S* by the CD spectrum.[15] Thus, the structure of **6** (morusyunnansin F) was elucidated as 4-[(2*S*)-3,4-dihydro-7-hydroxy-8-(3-methyl-2-buten-1-yl)-2*H*-1-benzopyran-2-yl]-1,3-benzenediol.

Four known compounds were identified as (2*R*)-2′,4′-dihydroxy-7-methoxy-8-hydroxyethylflavan (**7**),[17] (2*S*)-2′,4′-dihydroxy-7-methoxy-8-prenylflavan (**8**),[18] (2*S*)-7,2′-dihydroxy-4′-methoxy-8-prenylflavan (**9**),[19] and (2*S*)-7,4′-dihydroxy-8-prenylflavan (**10**)[20] by comparison of their spectroscopic data with those reported.

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Table 2. NMR Data of Compounds 3−6 (400 MHz for ^{1}H, 100 MHz for ^{13}C, acetone-d_6, δ in ppm, J in Hz)

	3		4		5		6	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.27 overlap	74.3	5.27 overlap	74.3	5.29 dd (2.0, 10.0)	74.0	5.27 dd (2.0, 10.0)	74.0
3	2.27 m, 1.92 m	29.5	2.16 m, 1.84 m	29.5	2.18 m, 1.89 m	29.4	2.17 m, 1.87 m	29.5
4	2.71 br d (14.9)	25.7	2.67 br d (15.3)	25.7	2.70 dt (4.0, 16.0)	25.6	2.64 dt (4.0, 16.0)	25.6
	2.89 m		2.84 overlap		2.89 m		2.86 m	
4a		115.5		115.5		115.5		113.9
5	6.88 d (8.6)	127.6	6.87 d (8.5)	127.6	6.88 d (8.2)	127.7	6.72 d (8.2)	127.4
6	6.50 d (8.6)	104.0	6.51 d (8.5)	104.0	6.50 d (8.2)	103.9	6.39 d (8.2)	108.2
7		157.2		157.2		157.3	7.97 br s (OH)	154.6
8		117.8		117.8		117.3		116.1
8a		154.3		154.3		154.4		154.6
9	3.39 br dd (7.8, 13.3)	22.9	3.40 br dd (7.5, 13.0)	23.0	3.37 br dd (6.3, 13.7)	22.4	3.36 br dd (7.0, 13.7)	23.0
	3.26 br dd (6.0, 13.3)		3.31 br dd (6.6, 13.0)		3.32 br dd (7.3, 13.7)		3.30 br dd (7.6, 13.7)	
10	5.26 br t (7.0)	124.3	5.25 br t (7.0)	124.1	5.45 br t (7.0)	123.9	5.26 br t (7.0)	124.4
11		130.8		130.9		135.4		130.5
12	1.59 br s	26.0	1.54 br s	26.0	3.85 d (5.9)	68.6	1.61 br s	25.9
					3.49 t (5.9, OH)			
13	1.65 br s	18.0	1.61 br s	18.0	1.66 br s	13.8	1.65 br s	17.9
1′		121.2		121.2		120.6		120.7
2′	8.21 s (OH)	153.3	8.17 s (OH)	153.4	8.33 s (OH)	155.7	8.29 br s (OH)	155.7
3′	6.45 s	103.6	6.45 s	103.5	6.43 d (2.0)	103.3	6.43 d (2.0)	103.3
4′		154.9		154.8	8.19 s (OH)	158.6	8.29 br s (OH)	158.5
5′		123.4		123.7	6.37 dd (2.0, 8.3)	107.6	6.38 dd (2.0, 8.2)	107.5
6′	7.46 s	126.1	7.41 s	126.1	7.20 d (8.3)	128.2	7.22 d (8.2)	128.2
α	2.28 m	35.7	2.31 m	35.5				
β	2.53 m	29.4	2.55 m	29.4				
γ	4.49 t (7.5)	35.3	4.49 t (7.5)	35.3				
1″		122.4		122.3				
2″	6.92 s (OH)	153.8	6.96 s (OH)	153.8				
3″		117.1		117.1				
4″		157.3		157.3				
5″	6.38 d (8.2)	103.5	6.40 d (8.6)	103.3				
6″	6.81 d (8.2)	127.8	6.85 d (8.6)	127.8				
7″	3.33 br d (7.0)	23.1	3.35 br d (7.0)	23.1				
8″	5.14 br t (7.0)	124.1	5.17 br t (7.0)	124.1				
9″		131.2		131.1				
10″	1.58 br s	25.8	1.61 br s	25.8				
11″	1.71 br s	17.8	1.73 br s	17.8				
1‴		123.7		123.6				
2‴		155.5		155.7				
3‴	6.37 d (2.0)	103.3	6.36 d (2.2)	103.3				
4‴	8.01 s (OH)	157.1	8.00 s (OH)	157.1				
5‴	6.31 dd (2.0, 8.6)	108.3	6.31 dd (2.2, 8.2)	108.3				
6‴	7.08 d (8.6)	128.8	7.08 d (8.2)	128.8				
OMe-7	3.78 s	56.0	3.79 s	56.0	3.79 s	56.0		
OMe-4″	3.73 s	55.8	3.75 s	55.8				
OH-4′, 2‴	8.43 br s, 8.30 br s		8.36 br s					

For the reported 2-arylbenzofuran dimers, two monomers are connected by the carbon atoms of two 2-arylbenzofuran skeletons.[21,22] It is found for the first time that two monomers in **1** and **2** were linked through the isoprenoid groups. As for the reported bioflavonoids consisting of a flavan and a deoxotetrahydrochalcone moiety, the linkage of two monomers is at the A ring of flavan.[14,23,24] The linkage position at the B ring as shown in **3** and **4** in this class of compounds is reported for the first time.

Compounds **3**−**10** were screened for their inhibitory effects on mushroom tyrosinase. Kojic acid was used as positive control in this test. Compounds **5**−**8** showed potent inhibitory activities, with IC_{50} values ranging from 0.12 ± 0.02 to 1.43 ± 0.43 μM (Table 3), whereas compounds **3**, **4**, **9**, and **10**

Table 3. Inhibitory Effects of Compounds 5−8 on Mushroom Tyrosinase

compound	IC_{50} ± SD (μM)
5	1.43 ± 0.43
6	0.15 ± 0.04
7	0.12 ± 0.02
8	0.81 ± 0.17
kojic acid	32.62 ± 1.24

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did not exhibit significant effects (less than 50% inhibition at 25 μM). It was noteworthy that the IC_{50} values of **6** and **7** were 217-fold and 271-fold lower than that of kojic acid, respectively, indicating their potential as effective natural tyrosinase inhibitors.

The bioassay results and the structure characteristics of compounds **3**–**10** implied the following structure–activity relationships: (1) The 2′,4′-dihydroxylated B ring of the flavan skeleton is crucial to the tyrosinase inhibitory activity. Substitution of a methyl group at the OH-4′ (**9**) or the absence of the OH-2′ (**10**) would greatly compromise the inhibitory effect. (2) The presence of a bulky group at the 2′,4′-dihydroxylated B ring (**3** and **4**) negatively influences the tyrosinase inhibitory effect.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on a SGW X-4 melting point apparatus. Optical rotations were measured on a Jasco P-1020 polarimeter. UV spectra were recorded on a Hitachi U-2900 spectrophotometer. CD spectra were obtained on a JASCO J-715 spectropolarimeter. IR spectra were measured on a Nicolet Avatar-360 spectrometer with KBr pellets. NMR spectra were obtained on Varian Mercury Plus 400 and Bruker Avance 600 instruments using acetone-d_6 as solvent. Chemical shifts were reported with TMS as internal standard or with respect to acetone-d_6 (δ_H 2.04, δ_C 206.0 ppm). EIMS (70 eV) and ESIMS were recorded on Waters Micromass GCT, Agilent LC/MSD, and API Qstar Pulsar mass spectrometers, respectively. HREIMS, HRMALDIMS, and HRESIMS were carried out on Waters Micromass GCT, IonSpec 4.7 T FTMS, Bruker Daltonics ApexIII, and API Qstar Pulsar mass spectrometers, respectively. Semipreparative HPLC was performed on an Agilent 1200 (Agilent Technologies, USA) and a Sepax Amethyst C_{18} column (150 × 10 mm, 5 μm, Sepax Techologies, Inc., USA). Column chromatography (CC) was performed on silica gel (200–300 mesh, Yantai Institute of Chemical Technology, Yantai, P. R. China), ODS (50 μm, YMC Co., Kyoto, Japan), Sephadex LH-20 (GE Healthcare Amersham Biosciences, Uppsala, Sweden), and Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan). TLC analysis was run on precoated silica gel GF254 plates (10–40 μm, Yantai Institute of Chemical Technology, Yantai, P. R. China).

Plant Material. Leaves of *M. yunnanensis* were collected in Mengzi County of Yunnan Province, P. R. China, in July 2008. The plant material was identified by Dr. Yun Kang, Fudan University, and a voucher specimen (TCM 08-07-08 Hou) has been deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Fudan University.

Extraction and Isolation. The milled, air-dried leaves of *M. yunnanensis* (5.0 kg) were percolated with 95% EtOH (60 L) at room temperature. The filtrate was evaporated in vacuo to give a residue (500 g), which was suspended in H_2O and extracted successively with petroleum ether, $CHCl_3$, and EtOAc. The petroleum ether and $CHCl_3$ extracts were combined (350 g) on the basis of TLC analysis and subjected to CC on Diaion HP-20 eluted with 90% EtOH and EtOH. The 90% EtOH fraction (75 g) was separated by CC on silica gel eluted with a gradient of petroleum ether–EtOAc (8:1, 5:1, 3:1, 1:1) to yield fractions A–I. Fraction D was separated by CC on silica gel eluted with a gradient of petroleum ether–Me_2CO (7:1, 4:1) to yield fractions D1–D4. Fraction D3 was chromatographed over ODS eluted with a gradient of CH_3OH–H_2O (7:3, 8.5:1.5) to give fractions D3a–D3d. Fraction D3b was separated by semipreparative HPLC (CH_3OH–H_2O, 7.3:2.7, flow rate 1 mL/min, UV detector 210 nm) to provide **6** (13 mg) and **8** (20 mg). Fraction E was subjected to CC on silica gel eluted with a gradient of $CHCl_3$–MeOH (150:1, 80:1) to afford fractions E1–E5. Fraction E3 was chromatographed over ODS eluted by a gradient of CH_3OH–H_2O (7:3, 9:1) and CH_3OH to give fractions E3a–E3d. Fraction E3b was separated by semipreparative HPLC (CH_3OH–H_2O, 7:3, flow rate 1 mL/min, UV detector 210 nm) to provide **10** (6 mg) and **9** (4 mg). Fraction E3d was purified by semipreparative HPLC (CH_3OH–H_2O, 8.5:2.5, flow rate 1 mL/min, UV detector 210 nm) to yield **4** (4 mg) and **3** (7 mg). Fraction E4 was chromatographed over Sephadex LH-20 eluted with CH_3OH to afford fractions E4a–E4c. Fraction E4b was separated by semipreparative HPLC (CH_3CN–H_2O, 6.8:3.2, flow rate 1 mL/min, UV detector 210 nm) to yield **7** (6 mg) and **5** (4 mg). Fraction G was subjected to CC on silica gel eluted with a gradient of $CHCl_3$–EtOAc (40:1, 20:1, 10:1) to afford fractions G1–G5. Fraction G4 was purified by CC on Sephadex LH-20 eluted with CH_3OH to give fractions G4a–G4e. Fraction G4e was separated by semipreparative HPLC (CH_3CN–H_2O, 7.5:2.5, flow rate 1 mL/min, UV detector 210 nm) to afford **1** (4 mg) and **2** (4 mg).

*Morusyunnansin A (**1**):* yellowish oil; $[\alpha]^{25}_D$ −2.0 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} (log ε) 221 (4.37), 320 (4.45), 333 (4.40) nm; IR (KBr) ν_{max} 3430, 2926, 1625, 1488, 1455, 1361, 1244, 1115, 1068, 1033 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 639 $[M + Na]^+$; HRESIMS *m/z* 639.2004 $[M + Na]^+$ (calcd for $C_{38}H_{32}O_8Na$, 639.1994).

*Morusyunnansin B (**2**):* yellowish oil; $[\alpha]^{25}_D$ −11.0 (*c* 0.1, CH_3OH); UV (MeOH) λ_{max} (log ε) 221 (4.30), 318 (4.42), 333 (4.38) nm; IR (KBr) ν_{max} 3391, 2927, 1603, 1490, 1443, 1380, 1211, 1187, 1073, 1032 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 615 $[M - H]^-$; HRESIMS *m/z* 615.2050 $[M - H]^-$ (calcd for $C_{38}H_{31}O_8$, 615.2024).

*Morusyunnansin C (**3**):* yellowish powder; mp 118–120 °C; $[\alpha]^{25}_D$ +7.2 (*c* 0.6, acetone); UV (MeOH) λ_{max} (log ε) 225 (4.28), 282 (3.69) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 240 (−0.80), 291 (+0.36); IR (KBr) ν_{max} 3423, 2924, 1701, 1613, 1491, 1442, 1269, 1170, 1091, 973 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 2; ESIMS *m/z* 679.2 $[M - H]^-$; HRMALDIMS *m/z* 703.3264 $[M + Na]^+$ (calcd for $C_{42}H_{48}O_8Na$, 703.3241).

*Morusyunnansin D (**4**):* yellowish powder; mp 120–122 °C; $[\alpha]^{25}_D$ −6.7 (*c* 0.3, acetone); UV (MeOH) λ_{max} (log ε) 225 (4.23), 282 (3.22) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 242 (+0.28), 288 (−0.60); IR (KBr) ν_{max} 3419, 2922, 1699, 1614, 1489, 1441, 1268, 1169, 1091, 973 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 2; ESIMS *m/z* 679.2 $[M - H]^-$; HRMALDIMS *m/z* 703.3259 $[M + Na]^+$ (calcd for $C_{42}H_{48}O_8Na$, 703.3241).

*Morusyunnansin E (**5**):* yellowish powder; mp 110–111 °C; $[\alpha]^{25}_D$ −6.5 (*c* 0.6, acetone); UV (MeOH) λ_{max} (log ε) 224 (4.12), 280 (3.55), 285 (sh) (3.54) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 253 (+0.43), 284 (−0.26); IR (KBr) ν_{max} 3410, 2918, 1611, 1489, 1381, 1269, 1168, 1098, 975 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 2; EIMS *m/z* 356 $[M]^+$ (8), 338 (100), 323 (42), 293 (66), 283 (29), 203 (72), 187 (27), 149 (40), 123 (35); HREIMS *m/z* 356.1628 $[M]^+$ (calcd for $C_{21}H_{24}O_5$, 356.1624).

*Morusyunnansin F (**6**):* yellowish powder; mp 115–116 °C; $[\alpha]^{25}_D$ −6.8 (*c* 0.5, acetone); UV (MeOH) λ_{max} (log ε) 225 (4.02), 280 (3.45), 285 (sh) (3.43) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 253 (+0.31), 283 (−0.21); IR (KBr) ν_{max} 3399, 2924, 1605, 1514, 1453, 1374, 1283, 1164, 1057, 975 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 2; EIMS *m/z* 326 $[M]^+$ (81), 270 (18), 204 (25), 191 (58), 175 (63), 147 (94), 136 (100), 123 (62); HREIMS *m/z* 326.1522 $[M]^+$ (calcd for $C_{20}H_{22}O_4$, 326.1518).

Assay of Tyrosinase Activity. L-Dopa oxidase activity of mushroom tyrosinase was determined by the spectrophotometric method described in our previous study.[7] The extent of tyrosinase inhibition is expressed as the concentration necessary for 50% inhibition (IC_{50}).

ASSOCIATED CONTENT

Supporting Information

NMR and MS spectra of compounds **1**–**7** and Figures 1–4 are available free of charge via the Internet at http://pubs.acs.org.

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