A Novel 3-Arylcoumarin and Three New 2-Arylbenzofurans from *Mucuna birdwoodiana*

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A novel coumarin (1, mucodianin A), three new 2-arylbenzofurans (2—4, mucodianins B—D) along with four known ones were isolated from the vine stems of *Mucuna birdwoodiana*. Their structures were elucidated on basis of spectral analysis. This is the first report of 7-quinonylcoumarin (1) as stable form in natural products.

Key words *Mucuna birdwoodiana*; Leguminosae; 3-arylcoumarin; 2-arylbenzofuran

Mucuna birdwoodiana TUTCH. (Leguminosae), distributed in southern China, has long been used as a traditional Chinese medicine named "ji-xue-teng." The vine stems of this plant are effective in promoting blood circulation or relieving stasis and have been used to treat pain or numbness of the wrists, knees or other joints and irregular menstruation.[1)] Our continuing study on searching for the bioactive constituents from the plant "ji-xue-teng" led to the isolation of a novel coumarin (**1**, mucodianin A), three new 2-arylbenzofurans (**2**—**4**, mucodianins B—D) and four known ones, 6-demethylvignafuran (**5**), eryvarin L (**6**), isopterofuran (**7**), lespeflorin F_1 (**8**) from the 50% EtOH extract of *M. birdwoodiana*. We herein describe the isolation and structural elucidation of these compounds.

Results and Discussion

Compound **1** was obtained as a fluorescein solid. The negative HR-electrospray ionization (ESI)-MS exhibited the quasi-molecular ion peak $[M-H]^-$ at *m/z* 343.0827 (Calcd for $C_{18}H_{15}O_7$, 343.0823), suggesting the molecular formula $C_{18}H_{16}O_7$ of compound **1**. The UV spectrum showed the absorption maxima at 338, 260, 203 nm and the fluorescent nature suggested that it might be a 3-arylcoumarin derivative.[2,3)] The ^{1}H-NMR spectral data (Table 1) of **1** indicated the presence of three methoxyl signals at δ_H 3.67, 3.72, 3.75 and five aromatic protons, appearing as one singlet at δ_H 7.43; two pairs of doublets at δ_H 6.84 and 6.23 (each 1H, J=8.8 Hz), δ_H 6.78 and 6.59 (each 1H, J=8.4 Hz). The ^{13}C-NMR spectrum (Table 1) displayed three methoxyl signals and fifteen nucleus signals. In the heteronuclear multiple bond correlations (HMBC) experiment (Table 1), the proton signal δ_H 6.84 (1H, d, J=8.8 Hz) coupling to the typical carbon signal for coumarin (C-4) at δ_C 143.2 must be assigned as H-5. Based on the correlations of H-5 to δ_C 168.7 and H-4 to δ_C 161.4, the signals δ_C 168.7 and δ_C 161.4 could be assigned as C-7 and C-2 respectively, which were opposite to the data of hydranget,[4)] implying that **1** should be in the form of 7-quinonylcoumarin rather than in its normal form (**1a**). The methoxy at δ_H 3.72 was located at C-8 on the basis of the HMBC spectrum (δ_H 3.72 to C-8 at δ_C 168.7). HMBC correlations of H-5′ to C-1′ and C-3′; H-6′ to C-2′ and C-3; methoxy at δ_H 3.67 to C-2′; methoxy at δ_H 3.75 to C-3′, suggested 2′,3′-dimethoxyl, 4′-hydroxyl groups substitued in ring C. Based on the above spectral evidence, the structure of **1** was determined as shown in Fig. 1. **1** and **1a** which have never been discovered before are two tautomeric forms of the compound, named mucodianin A. Theoretically, structure **1a** is more stable than **1**. However, **1** was isolated in our experi-

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data and ^2J and $^3J_{H-C}$ Correlations of Compound **1** (in DMSO)

No.	δ_H	δ_C	^2J	^3J
2		161.4		
3		110.3		
4	7.43 (s)	143.2	104.7 (C-4a)	161.4 (C-2), 148.8 (C-8a), 123.7 (C-5), 121.6 (C-1′)
4a		104.7		
5	6.84 (d, 8.8)	123.7	104.7 (C-4a)	168.7 (C-7), 148.8 (C-8a), 143.2 (C-4)
6	6.23 (d, 8.8)	118.9		135.6 (C-8), 104.7 (C-4a)
7		168.7		
8		135.6		
8a		148.8		
1′		121.6		
2′		151.6		
3′		140.7		
4′		150.9		
5′	6.59 (d, 8.4)	111.2	150.9 (C-4′)	121.6 (C-1′), 140.7 (C-3′)
6′	6.78 (d, 8.4)	125.3		110.3 (C-3), 151.6 (C-2′)
8-OMe	3.72 (s)	58.7		135.6 (C-8)
2′-OMe	3.67 (s)	60.2		151.6 (C-2′)
3′-OMe	3.75 (s)	59.9		140.7 (C-3′)

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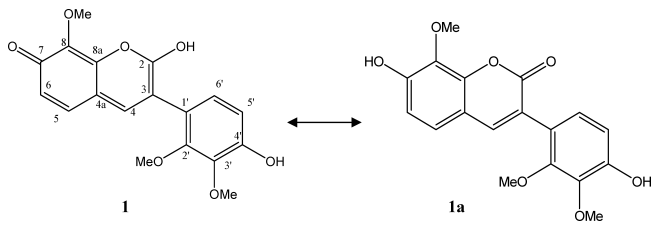
ment and was stable in dimethyl sulfoxide (DMSO). It was reported that 7-hydroxycoumarin could be converted to 7-quinonylcoumarin in aquenous solutions, which mainly was caused by the transfer of a proton between two sites in the molecule by the participation of water moleculues.[5] The reason of why **1** was stable in DMSO may be the structure itself or external factors.

The UV spectra with maxima at 324 and 338 nm and the characteristic aromatic proton signal (δ_H 6.98—7.04, br s) in the ^{1}H-NMR spectra indicated that compounds **2**—**8** all were 2-arylbenzofuran derivatives.[6]

Compound **2** was obtained as a yellow solid with the molecular formula $C_{16}H_{14}O_5$, deduced from the HR-ESI-MS ($C_{16}H_{14}O_5Na$ *m/z* 309.0739 $[M+Na]^+$, Calcd for 309.0733). The ^{1}H-NMR spectrum (Table 2) displayed two broad proton singlets at δ_H 6.98 and 6.94, assignable to the two aromatic protons H-3 and H-7 of 2-arylbenzofuran. One ABX coupling system at δ_H 6.53 (d, J=2.4 Hz), 6.46 (dd, J=8.7, 2.4 Hz), and 7.63 (d, J=8.7 Hz) similar to the ^{1}H-NMR data of **5**,[7] was assigned to the 2′-methoxyl, 4′-hydroxyl-2-phenyl moiety (ring C). This was also confirmed by the HMBC spectrum (Fig. 2). Another methoxy at δ_H 3.79 must be positioned at C-5 and one aromatic proton singlet at δ_H 7.07 assigned as H-4 determined by the key nuclear Overhauser effect (NOE) correlations between H-4 at δ_H 7.07 and 5-OMe at δ_H 3.79, H-3 at δ_H 6.98. The ^{13}C-NMR (Table 2) and HMBC (Fig. 2) experiments confirmed this structure of **2** and assigned all carbon resonances. Thus, it was determined to be 2-(4-hydroxy-2-methoxylphenyl)-5-methoxyl-benzofuran-6-ol, named mucodianin B.

Compound **3** was isolated as a pale yellow solid. The molecular formula was established as $C_{17}H_{16}O_6$ by HR-ESI-MS, which displayed a quasi-molecular ion peak at *m/z* 339.0843 $[M+Na]^+$, Calcd for 339.0839. The ^{1}H-NMR spectrum (Table 2) showed the presence of one proton singlet at δ_H 7.10 (H-4), two broad proton singlets at δ_H 6.96 (H-7) and 7.03 (H-3), one methoxyl group at δ_H 3.79. Its similatity to that of **2** indicated that 5-methoxyl, 6-hydroxyl groups substituted in ring A. The AB coupling system at δ_H 6.71 (d, J=8.7 Hz) and 7.38 (d, J=8.7 Hz) in ^{1}H-NMR spectrum (Table 2) indicated that there were three substituents in ring C. Except two methoxys at δ_H 3.79 and 3.85, it was deduced that there should be a hydroxyl group according to the molecular formula. In the NOE spectrum, the methoxy at δ_H 3.85 correlating to H-3 meant it should be at C-2′. Due to one methoxy resonate in the range 60—63 ppm in the ^{13}C-NMR spectrum, which required the presence of both *ortho*-positions substituted,[8] the methoxy at δ_H 3.79 was determined to be at C-3′ and the hydroxy was assigned to be at C-4′. The structure was further confiremed by the HMBC spectrum (Fig. 3) and also by comparison with the data of ring C of **7**.[7] Therefore, the structure of **3** was determined as 2-(4-hydroxy-2,3-dimethoxylphenyl)-5-methoxylbenzofuran-6-ol and named mucodianin C.

Compound **4**, a brown solid, had the molecular formula of $C_{16}H_{14}O_6$ established by HR-ESI-MS (*m/z* 303.0877 $[M+H]^+$, Calcd for 303.0863). Its ^{1}H-NMR spectral data (Table 2) were reminiscent of those of **3**, only missing the signal of 3′-methoxy at δ_H 3.79 and the hydroxyl group substituted, so the AB type proton signals upfield shifted to δ_H



1 1a

Fig. 1. Chemical Structures of Compounds **1** and **1a**

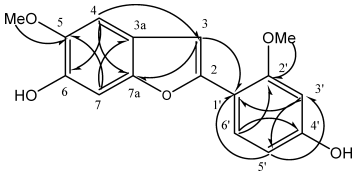
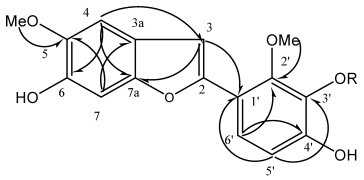


Fig. 2. Chemical Structure and Key HMBC Correlations of Compound **2**

Table 2. ^{1}H-NMR (300 MHz) and ^{13}C-NMR Spectral Data of Compounds **2**—**4** in DMSO

No.	**2**		**3**		**4**	
	δ_H	δ_C [a)]	δ_H	δ_C [b)]	δ_H	δ_C [a)]
2		150.8		150.3		145.4
3	6.98 (br s)	103.6	7.03 (br s)	103.5	7.02 (br s)	103.1
3a		120.5		120.4		120.5
4	7.07 (s)	103.0	7.10 (s)	102.9	7.10 (s)	103.0
5		145.3		145.4		145.4
6		144.9		145.2		145.0
7	6.94 (br s)	97.8	6.96 (br s)	97.8	6.95 (br s)	97.8
7a		147.8		148.0		148.0
1′		110.4		115.5		115.4
2′		157.0		150.4		150.9
3′	6.53 (d, 2.4)	99.4		141.3		138.9
4′		158.6		151.3		147.0
5′	6.46 (dd, 8.7, 2.4)	107.6	6.71 (d, 8.7)	112.3	6.64 (d, 8.7)	111.3
6′	7.63 (d, 8.7)	126.6	7.38 (d, 8.7)	120.6	7.12 (d, 8.7)	115.8
5-OMe	3.79 (s)	56.1	3.79 (s)	56.1	3.79 (s)	56.1
2′-OMe	3.88 (s)	55.4	3.85 (s)	59.9	3.76 (s)	59.0
3′-OMe			3.79 (s)	60.2		

a) Recorded in 100 MHz, *b*) recorded in 125 MHz.



3 R = Me, **4** R = H

Fig. 3. Chemical Structures and Key HMBC Correlations of Compounds **3** and **4**

6.64 (d, J=8.7 Hz) and 7.12 (d, J=8.7 Hz). The key NOEs (H-3/2′-OMe), the molecular formula and the HMBC spectral data (Fig. 3) also supported the structure of **4**. Thus, it was characterized as 2-(3,4-dihydroxy-2-methoxylphenyl)-5-methoxylbenzofuran-6-ol and named mucodianin D.

Phytochemical study of this plant also resulted in the isolation of four known 2-arylbenzofurans: 6-demethylvignafuran (**5**),[7] eryvarin L (**6**),[9] isopterofuran (**7**),[7] and lespeflorin F_1 (**8**),[10] which were characterized by comparison of the respective spectral data (^{1}H-, ^{13}C-NMR, MS) with those found in the literatures.

2-Arylbenzofuran derivatives isolated from leguminous plants were considered to be of isoflavonoid origin,[2] the co-occurrence of compounds **1** and **8** also confirmed this hypothesis.

Experimental

General Experimental Procedures Melting points were determined by XT Digital Melting-Point Apparatus with Microscope and uncorrected. UV spectra were obtained by JASCO U-650 spectrophotometer. IR spectra were run on IMPACT 400 spectrometer. HR-ESI-MS were performed on Autospec Ultima-TOF mass spectrometer and ESI-MS on Agilent 1100 LC/MSD Trap-SL mass spectrometer. ^{1}H-NMR (500 MHz), ^{13}C-NMR (125 MHz), NOE difference, heteronuclear single quantum coherence (HSQC) and HMBC spectra were run on INOVA-500 spectrometer with TMS as internal standard, ^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz), NOE difference, HSQC and HMBC spectra were recorded on Mercury-400 spectrometer, and ^{1}H-NMR (300 MHz) spectrum on Mercury-300 spectrometer. Sephadex LH-20 (Pharmacia), Macroporous resin D101 (26—60 mesh, tianjin, China) and Polyamide resin (30—50 mesh, zhejiang, China) were used for Column Chromatography. HPLC separations were performed on preparation YMC-Pack ODS-A column (10 μm, 250×20 mm i.d.) equipped with Shimadzu SPD-6A UV spectrophotometric detector and Thermo constametric pumping system.

Plant Material The vine stem of *M. birdwoodiana* was collected in the County of Jin Xiu, Guangxi Province, China, in August 2006, and identified by Prof. Shou-yang Liu, Guangxi Traditional Chinese Medical College. A voucher specimen (No. S2263) has been deposited in the herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

Extraction and Isolation Crushed dry vine stem of *M. birdwoodiana* (10 kg) was extracted with 50% EtOH (3×10 l) under reflux to yield a crude extract (1600 g). The extract was passed over a D101 macroporous resin column chromatography (CC) (8 kg) eluting with a gradient of aqueous EtOH (0, 30, 60, 75, 100%, v/v) to yield five fractions (Fr. 1—5). Fr. 3 (145 g) was further fractionated on a Polyamide resin CC (2.5 kg) eluting with a gradient of aqueous EtOH (0, 15, 30, 50, 70, 100%, v/v) to give six fractions (Frs. 3.1—3.6). Fr. 3.4 (16.3 g) was separated by reversed-phase silica gel (300 g) CC and eluted with 30—70% aqueous MeOH to provide nine fractions (Frs. 3.4.1—3.4.9). Fr. 3.4.4 (624 mg) was applied to repeated ODS, Sephadex LH-20 CC, and preparative HPLC (43% aqueous MeOH, 4 ml/min) to give **4** (4.5 mg, t_R=45.3 min) and **1** (5.2 mg, t_R=87.0 min). Fr. 3.4.5 (390 mg) was purified by Sephadex LH-20 CC and preparative HPLC (48% aqueous MeOH, 4 ml/min) to yield compounds **2** (6.2 mg, t_R=75.2 min) and **7** (2.3 mg, t_R=94.0 min). Fr. 3.4.6 (1.0 g) was further purified by repeated ODS CC and preparative HPLC (50% aqueous MeOH, 4 ml/min) to give compounds **3** (3.6 mg, t_R=64.2 min) and **8** (5.3 mg, t_R=72.0 min). Fr. 4 (10.6 g) was subjected to silica gel CC (160—200 mesh, 400 g), eluted with $CHCl_3$–MeOH (100 : 0—50 : 1—20 : 1—10 : 1—8 : 1—4 : 1—1 : 1, v/v) to provide eight fractions. Fr. 4.2 (810 mg) was purified by Sephadex LH-20 CC to yield compound **6** (6.2 mg). Fr. 4.3 (391 mg) was applied to Sephadex LH-20 CC and preparative HPLC (47% aqueous MeOH, 4 ml/min) to yield compound **5** (7.2 mg, t_R=84.0 min).

Mucodianin A (**1**): Fluorescein solid (MeOH). mp 116.2—117.5 °C. UV (MeOH) λ_{max} (log ε): 338 (3.81), 260 (3.76), 203 (4.33) nm. IR ν_{max}: 3372, 2918, 2850, 1706, 1589, 1503, 1465, 1415, 1362, 1312, 1069, 983, 827 cm^{-1}. HR-ESI-MS m/z: 343.0827 $[M-H]^-$ (Calcd for $C_{18}H_{15}O_7$, 343.0823). ^{1}H-NMR (300 MHz) and ^{13}C-NMR (100 MHz) in DMSO: see Table 1.

Mucodianin B (**2**): Yellow solid (MeOH). mp 155.1—156.6 °C. UV (MeOH) λ_{max} (log ε): 342 (4.52), 327 (4.47), 282 (4.18), 208 (4.68) nm. IR ν_{max}: 3167, 2928, 2850, 1716 (w), 1612, 1488 1460, 1429, 1330, 1308, 1202, 1025, 960, 837 cm^{-1}. HR-ESI-MS m/z: 309.0739 $[M+Na]^+$ (Calcd for $C_{16}H_{14}O_5Na$, 309.0733). ^{1}H-NMR (300 MHz) and ^{13}C-NMR (100 MHz) in DMSO: see Table 2.

Mucodianin C (**3**): Pale yellow solid (MeOH). mp 125.4—126.5 °C. UV (MeOH) λ_{max} (log ε): 338 (4.33), 324 (4.34), 293 (4.08), 284 (4.07), 211 (4.41) nm. IR ν_{max}: 3394, 2917, 2850, 1715(w), 1605, 1468, 1420, 1329, 1203, 1030, 961 cm^{-1}. HR-ESI-MS m/z: 339.0843 $[M+Na]^+$ (Calcd for $C_{17}H_{16}O_6Na$, 339.0839). ^{1}H-NMR (300 MHz) and ^{13}C-NMR (100 MHz) in DMSO: see Table 2.

Mucodianin D (**4**): Brown solid (MeOH). mp 140.6—142.0 °C. UV (MeOH) λ_{max} (log ε): 338 (4.18), 324 (4.22), 293 (3.97), 285 (3.95), 212 (4.23) nm. IR ν_{max}: 3352, 2925, 2851, 1609, 1467, 1424, 1329, 1205, 1124, 1024, 961, 932 cm^{-1}. HR-ESI-MS m/z: 303.0877 $[M+H]^+$ (Calcd for $C_{16}H_{15}O_6$, 303.0863). ^{1}H-NMR (300 MHz) and ^{13}C-NMR (125 MHz) in DMSO: see Table 2.

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