Anti-inflammatory Biphenyls and Dibenzofurans from *Rhaphiolepis indica*

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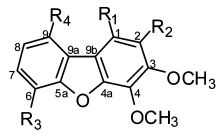
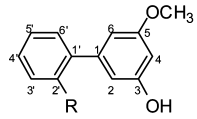
Bioassay-guided fractionation of the methanolic extract of the roots of *Rhaphiolepis indica* var. *tashiroi* afforded four new dibenzofurans, 2-hydroxy-3,4,6-trimethoxydibenzofuran (**1**), 2-hydroxy-3,4,9-trimethoxydibenzofuran (**2**), 2-hydroxy-3,4,6,9-tetramethoxydibenzofuran (**3**), and 1,2-methylenedioxy-3,4,6-trimethoxydibenzofuran (**4**), two new biphenyls, 3-hydroxy-2′,5-dimethoxybiphenyl (**5**) and 2′,3-dihydroxy-5-methoxybiphenyl (**6**), and 3-hydroxy-5-methoxybiphenyl (**7**). Among the isolates, **3**, **5**, and **6** exhibited inhibitory effects on *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced superoxide production, with in vitro IC_{50} values < 8.36 μM.

Rhaphiolepis indica (L.) Lindl. *ex* Ker var. *tashiroi* Hayata *ex* Matsum. & Hayata (Rosaceae) is one of three varieties of an evergreen shrub or small tree in Taiwan. *R. indica* is also found in countries throughout Asia, including India, southern China, the Ryukyus in Japan, Korea, and low-altitude areas of Taiwan.[1] Phytochemical studies of *R. umbellata* have revealed the presence of dibenzofurans,[2] biphenyls,[3] flavanol glycosides,[4] and procyanidins.[5] Eucidafuran and aucuparin are known to have anti-inflammatory activity.[6] However, the chemical constituents and biological activities of *R. indica* var. *tashiroi* have not been investigated. A root extract tested positive for anti-inflammatory activity in an in vitro screening of Formosan plants. Bioassay-guided fractionation of the EtOAc extract of the roots of this plant afforded six new compounds, including four dibenzofurans, **1**−**4**, two biphenyls, **5** and **6**, and one known biphenyl, **7**. This study communicates the structures and anti-inflammatory activities of these isolates.



1 R_1= H, R_2= OH, R_3= OCH_3, R_4= H
2 R_1= H, R_2= OH, R_3= H, R_4= OCH_3
3 R_1= H, R_2= OH, R_3= OCH_3, R_4= OCH_3
4 R_1 + R_2= OCH_2O, R_3= OCH_3, R_4= H



5 R= OCH_3
6 R= OH
7 R= H

Results and Discussion

Compound **1** was isolated as a colorless oil. The HRESIMS analysis of **1** revealed an $[M + Na]^+$ ion peak at *m/z* 297.0741 (calcd 297.0739), which corresponds to the molecular formula $C_{15}H_{14}O_5$. The UV absorption bands were at 219, 258, 293, 300, and 315 (sh) nm; a bathochromic shift was observed with the addition of alkali, indicative of the presence of a phenolic dibenzofuran moiety.[7] The IR spectrum revealed a hydroxy group at 3402 cm^{-1}, which was further confirmed by a ^1H NMR hydroxy signal at δ 7.90 (1H, br s, D_2O exchangeable). The ^1H NMR spectrum of **1** (Table 1) resembles that of α-cotonefuran,[7] except that H-7 occurs in **1** in place of OH-7 in α-cotonefuran. Three mutually coupled aromatic proton signals were observed at δ 7.07 (1H, dd, J = 7.8, 0.6 Hz, H-7), 7.25 (1H, t, J = 7.8 Hz, H-8), and 7.52 (1H, dd, J = 7.8, 0.6 Hz, H-9). The aromatic proton at δ 7.20 (singlet), which shows long-range HMBC correlations (Figure 1) to C-2 (δ 149.1), C-3 (δ 141.2), C-4a (δ 143.4), C-9a (δ 127.5), and C-9b (δ 122.1), was designated as H-1. Similarly, H-9 showed long-range HMBC correlations to C-9a, C-9b, C-5a (δ 147.0), C-7 (δ 110.8), and C-8 (δ 125.1). The long-range HMBC correlations from OH-2 (δ 7.90) to C-1 (δ 101.3) and C-2 (δ 149.1), from OMe-3 (δ 3.93) to C-3 (δ 141.2), from OMe-4 (δ 4.20) to C-4 (δ 140.4), and from OMe-6 (δ 4.03) to C-6 (δ 147.3) established the 2-hydroxy-3,4,6-trimethoxy substitution pattern of the dibenzofuran. On the basis of these data, **1** was identified as 2-hydroxy-3,4,6-trimethoxydibenzofuran. The ^{13}C NMR (Table 1), HSQC, HMBC, and NOESY (Figure 2) analyses provided additional structural confirmation.

Compound **2** was obtained as a colorless oil. The molecular formula was established as $C_{15}H_{14}O_5$ by ESIMS (*m/z* 297 $[M + Na]^+$) and HRESIMS (*m/z* 297.0740 $[M + Na]^+$). The UV (220, 260, 295, 299, and 317 [sh] nm), IR (OH: 3417 cm^{-1}), and ^1H and ^{13}C NMR (Table 1) data were similar to those of **1**. The ^1H NMR spectrum showed three coupled aromatic proton signals at δ 7.18 (1H, dd, J = 8.4, 0.6 Hz, H-6), 7.39 (1H, t, J = 8.4 Hz, H-7), and 6.89 (1H, dd, J = 8.4, 0.6 Hz, H-8). The aromatic singlet at δ 7.30, which shows long-range HMBC correlations to C-2 (δ 148.9), C-3 (δ 140.5), C-4a (δ 142.5), C-9a (δ 115.0), and C-9b (δ 121.2), was designated as H-1. The long-range HMBC experiments showed correlations between H-6 and C-9a, C-5a (δ 159.1), C-7 (δ 129.1), and C-8 (δ 105.5). The location of the methoxy (δ 4.06, 3H, s) group of **2** at C-9 (δ 157.3) was further confirmed by an HMBC experiment. The NMR data indicated that **2** (OMe-9) and **1** (OMe-6) are regioisomers. The data indicate that the structure of **2** is 2-hydroxy-3,4,9-trimethoxydibenzofuran, the structure of which was further confirmed by HSQC, NOESY (Figure 2), and HMBC (Figure 1) experiments.

Compound **3** was isolated as a colorless oil. ESIMS (*m/z* 327 $[M + Na]^+$) and HRESIMS (*m/z* 327.0844 $[M + Na]^+$) analysis indicated that the molecular formula of **3** was $C_{16}H_{16}O_6$. Compound **3** might have an additional methoxy group compared to **1** or **2**, as the ESIMS of **3** was 30 amu $[CH_2O]$ greater than **1** and **2**. The UV absorption bands and a bathochromic shift in alkaline solution

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Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Data for Compounds **1**−**4** (in Acetone-d_6)

	1		**2**		**3**		**4**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	7.20 s	101.3	7.30 s	103.5	7.29 s	103.5		140.7
2		149.1		148.9		149.0		134.9
3		141.2		140.5		140.6		127.1
4		140.4		139.9		140.1		134.4
4a		143.4		142.5		142.7		144.8
5								
5a		147.0		159.1		147.8		146.4
6		147.3	7.18 dd (8.4, 0.6)	105.6		141.6		147.0
7	7.07 dd (7.8, 0.6)	110.8	7.39 t (8.4)	129.1	6.98 d (8.4)	111.5	7.03 dd (7.8, 1.2)	109.9
8	7.25 t (7.8)	125.1	6.89 dd (8.4, 0.6)	105.5	6.76 d (8.4)	105.0	7.24 t (7.8)	125.3
9	7.52 dd (7.8, 0.6)	114.0		157.3		151.0	7.58 dd (7.8, 1.2)	115.5
9a		127.5		115.0		116.6		127.0
9b		122.1		121.2		121.4		112.5
OMe-3	3.93 s	62.3	3.92 s	62.3	3.92 s	62.3	4.09 s	61.7
OMe-4	4.20 s	61.8	4.18 s	61.8	4.19 s	61.8	4.15 s	61.0
OMe-6	4.03 s	57.1			3.97 s	57.6	4.02 s	57.2
OMe-9			4.06 s	56.8	3.99 s	56.9		
OH-2	7.90 br s		7.81 br s		7.90 br s			
OCH_2O							6.08 s	103.5

indicated that **3** also had a phenolic dibenzofuran moiety. The ^{1}H NMR spectrum of compound **3** indicated the presence of four methoxy groups, at δ 3.92 (3H, s, OMe-3), 4.19 (3H, s, OMe-4), 3.97 (3H, s, OMe-6), and 3.99 (3H, s, OMe-9), and two *ortho*-coupled aromatic proton doublets at δ 6.98 (1H, d, J = 8.4 Hz, H-7) and 6.76 (1H, d, J = 8.4 Hz, H-8). The locations of two methoxy groups at C-6 and C-9 were identified by the long-range HMBC correlations from H-7 (δ 6.98) to C-5a (δ 147.8), C-6 (δ 141.6), and C-9 (δ 151.0) and from H-8 (δ 6.76) to C-6 (δ 141.6), C-9 (δ 151.0), and C-9a (δ 116.6). Furthermore, the long-range HMBC correlations of the aromatic singlet at δ 7.29 with C-2 (δ 149.0), C-3 (δ 140.6), C-4a (δ 142.7), C-9a, and C-9b (δ 121.4) led to the designation of this proton as H-1. Thus, the structure of **3** was identified as 2-hydroxy-3,4,6,9-tetramethoxydibenzofuran, which was further confirmed by HSQC, NOESY (Figure 2), and HMBC (Figure 1) experiments.

Compound **4** was isolated as colorless needles. HRESIMS of **4** exhibited an $[M + Na]^+$ ion peak at m/z 325.0687 (calcd 325.0688). The UV spectrum of **4** had a similar skeleton to those of dibenzofurans **1**−**3**. The ^{1}H NMR spectrum of compound **4** was similar to that of **1**, except that the methylenedioxy group (δ 6.08, 2H, s) in **4** replaces H-1 and OH-2 in **1**. The ^{1}H NMR data (Table 1) of **4** indicated three mutually coupled aromatic protons at δ 7.03 (1H, dd, J = 7.8, 1.2 Hz, H-7), 7.24 (1H, t, J = 7.8 Hz, H-8), and 7.58 (1H, dd, J = 7.8, 1.2 Hz, H-9). The long-range HMBC correlations from OMe-6 (δ 4.02) to C-6 (δ 147.0), from OMe-4 (δ 4.15) to C-4 (δ 134.4), from OMe-3 (δ 4.09) to C-3 (δ 127.1), and from OCH_2O (δ 6.08) to C-1 (δ 140.7) and C-2 (δ 134.9) determined the positions of the three methoxy groups and the methylenedioxy group of dibenzofuran **4**. Similarly, long-range HMBC correlations were observed between H-9 and C-9a, C-9b, C-5a, C-7, and C-8; between H-8 and C-9, C-9a, and C-6; and between H-7 and C-9, C-6, and C-5a. NOESY correlations (Figure 2) between OMe-3 and OMe-4, between H-7 and OMe-6, and between H-7 and H-8 were observed. According to a computer-assisted 3D structure developed using the molecular modeling program CS CHEM 3D Ultra 10.0, with MM2 force-field calculations for energy minimization, the distance between OMe-1 and H-9 was 2.1 Å. However, H-9 showed no NOESY correlations with any methoxy groups, suggesting that C-1 lacked a methoxy group. Therefore, the location of the methylenedioxy group is reasonably at C-1 and C-2. These data indicate that the structure of **4** is 1,2-methylenedioxy-3,4,6-trimethoxydibenzofuran, which was further

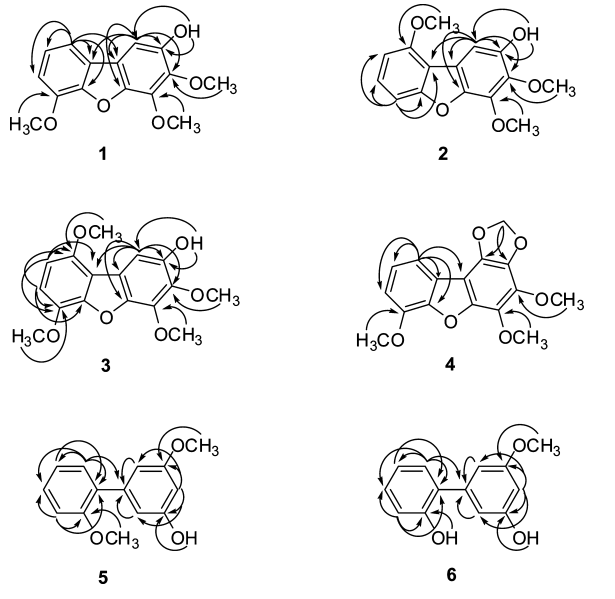

Figure 1. HMBC correlations for compounds **1**−**6**.

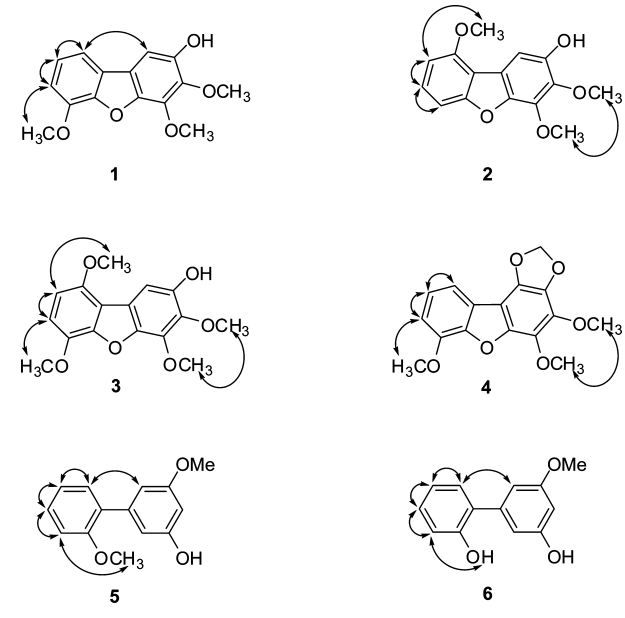

Figure 2. NOESY correlations for compounds **1**−**6**.

Table 2. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data for Compounds **5** and **6** (in Acetone-d_6)

	5		**6**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		132.2		130.1
2	6.61 dd (2.4, 1.2)	110.7	6.66 dd (1.8, 1.2)	110.5
3		159.5		159.7
4	6.39 dd (2.4, 2.0)	101.4	6.37 dd (2.4, 1.8)	101.5
5		162.1		162.3
6	6.56 dd (2.0, 1.2)	108.2	6.61 dd (2.4, 1.2)	107.9
1′		142.2		142.2
2′		158.2		155.6
3′	7.07 dd (7.6, 1.2)	113.1	6.95 dd (7.8, 1.2)	117.7
4′	7.30 td (7.6, 1.2)	130.2	7.15 td (7.8, 1.2)	130.0
5′	6.99 td (7.6, 1.2)	122.1	6.89 td (7.8, 1.2)	121.3
6′	7.32 dd (7.6, 1.2)	131.9	7.26 dd (7.8, 1.2)	131.9
OMe-5	3.79 s	56.5	3.78 s	56.1
OMe-2′	3.77 s	56.1		
OH-2′			8.12 br s	
OH-3	8.35 br s		8.34 br s	

confirmed by HSQC, COSY, NOESY (Figure 2), and HMBC (Figure 1) experiments.

Compound **5** was isolated as a colorless oil. The molecular formula of **5** was established as $C_{14}H_{14}O_3$ by ESIMS (m/z 253 [M + Na]$^+$) and HRESIMS (m/z 253.0842 [M + Na]$^+$). The UV absorptions at 226 and 295 nm were similar to those of 3-hydroxy-5-methoxybiphenyl (**7**) and showed a bathochromic shift after alkali was added, which indicated the presence of a phenolic biphenyl skeleton.[8] The IR spectrum showed a hydroxy group at 3400 cm^{-1}. In the ^{1}H NMR spectrum of **5** (Table 2), a 1,3,5-trisubstituted benzene ring was established by the presence of three aromatic protons at δ 6.61 (1H, dd, J = 2.4, 1.2 Hz, H-2), 6.39 (1H, dd, J = 2.4, 2.0 Hz, H-4), and 6.56 (1H, dd, J = 2.0, 1.2 Hz, H-6), together with a hydroxy group at δ 8.35 (1H, br s, D_2O exchangeable) and a methoxy group at δ 3.79 (3H, s, OMe-5). A 1,2-disubstituted benzene ring was indicated by the presence of four aromatic proton signals at δ 7.07 (1H, dd, J = 7.6, 1.2 Hz, H-3′), 7.30 (1H, td, J = 7.6, 1.2 Hz, H-4′), 6.99 (1H, td, J = 7.6, 1.2 Hz, H-5′), and 7.32 (1H, dd, J = 7.6, 1.2 Hz, H-6′) and a methoxy group at δ 3.77 (3H, s, OMe-2′). The positions of the OH-3 and OMe-5 substitutions were further confirmed by long-range HMBC correlations between OH-3 (δ 8.35) and C-3 (δ 159.5) and between OMe-5 (δ 3.79) and C-5 (δ 162.1), respectively. Similarly, the location of the OMe-2′ group was confirmed by the long-range HMBC correlations between OMe-2′ (δ 3.77) and C-2′ (δ 158.2). The quaternary carbon signal at δ 142.2 was attributed to C-1′ by HMBC correlations with H-3′, H-5′, and H-6′, whereas the quaternary carbon signal at δ 132.2 was attributed to C-1 by HMBC correlations with H-2 and H-6. Thus, **5** was determined to be 3-hydroxy-2′,5-dimethoxybiphenyl, which was further confirmed by HSQC, COSY, NOESY (Figure 2), and HMBC techniques.

Compound **6** was isolated as a colorless oil. ESIMS (m/z 239 [M + Na]$^+$) and HRESIMS (m/z 239.0686 [M + Na]$^+$) established that the molecular formula of **6** was $C_{13}H_{12}O_3$. The UV, IR, ^{1}H NMR, and ^{13}C NMR spectroscopic data were similar to those of **5**, except that **6** had an OH-2′ moiety instead of an OMe-2′ moiety (Table 2). From these data, the structure of **6** was determined to be 2′,3-dihydroxy-5-methoxybiphenyl, which was further confirmed by HSQC, COSY, NOESY (Figure 2), and HMBC (Figure 1) experiments.

3-Hydroxy-5-methoxybiphenyl (**7**) was identified by comparing its spectroscopic data (UV, IR, ^{1}H NMR, ^{13}C NMR, and MS) with literature data.[8]

The anti-inflammatory effects of the isolates (Table 3) were evaluated by measuring their suppression of the *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced generation of the superoxide anion, an inflammatory mediator produced by neutrophils. The clinical anti-inflammatory agent ibuprofen was used as the positive control. The effects of compounds **3** (IC_{50} 7.61 ± 2.31 μM), **5** (IC_{50} 8.36 ± 0.89 μM), and **6** (IC_{50} 2.04 ± 0.57 μM) on fMLP-induced superoxide generation were more potent than that of ibuprofen (IC_{50} 27.53 ± 3.58 μM). A literature review revealed only one study of the anti-inflammatory effects of biphenyls and dibenzofurans.[6] In our study, the anti-inflammatory effects of compounds **3**, **5**, and **6** were more potent than those of eucidafuran and aucuparin.[6] The new biphenyl **6** was the most effective of the tested isolates. Of the biphenyl analogues, **5** (with OMe-2′) showed stronger inhibition than **7** (without the 2′ substituent). Moreover, the inhibitory effects of **6** (with OH-2′) were stronger than those of its analogue **5**. Of the dibenzofuran analogues, **3** (with OMe-6,9) was more effective than **1** (with OMe-6), **2** (with OMe-9), and **4** (with OCH_2O-1,2 and OMe-6). Isomers **1** (OMe-6) and **2** (OMe-9) showed marginal activities, with the same IC_{50} values of 27.42 ± 3.98, which were weaker than that of **3** but still better than that of ibuprofen.

Table 3. IC_{50} Values for **1**−**7** in the Inhibition of fMLP-Induced Superoxide Generation in Human Neutrophils

compound	IC_{50} (μM)[a]
2-hydroxy-3,4,6-trimethoxydibenzofuran (**1**)	27.42 ± 3.98
2-hydroxy-3,4,9-trimethoxydibenzofuran (**2**)	27.42 ± 3.98
2-hydroxy-3,4,6,9-tetramethoxydibenzofuran (**3**)	7.61 ± 2.31
1,2-methylenedioxy-3,4,6-trimethoxydibenzofuran (**4**)	>100
3-hydroxy-2′,5-dimethoxybiphenyl (**5**)	8.36 ± 0.89
2′,3-dihydroxy-5-methoxybiphenyl (**6**)	2.04 ± 0.57
3-hydroxy-5-methoxybiphenyl (**7**)	34.07 ± 4.24
ibuprofen[b]	27.53 ± 3.58

[a] IC_{50} values were calculated from the slopes of the dose−response curves. The values are expressed as means ± standard errors of the means (SEM) of three independent experiments. [b] Ibuprofen was used as the positive control.

Experimental Section

General Experimental Procedures. Melting points were determined with a Yanaco micro melting apparatus and are uncorrected. The UV spectra were obtained with a Jasco V-530 UV/vis spectrophotometer, and the IR spectra (KBr or neat) were acquired with a Genesis II FTIR spectrophotometer. The 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, NOESY, HMQC, HMBC) NMR spectra, detected using acetone-d_6 (^{1}H, δ 2.05; ^{13}C, δ 30.5) solvent, were recorded on a Varian Unity Plus 400 spectrometer (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) and a Varian Unity Inova 600 spectrometer (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR). Chemical shifts are given as δ (ppm) using TMS as the internal standard. Low-resolution MS spectra were obtained with Micromass Trio-2000 GC/MS, VG Biotech Quattro 5022, and JEOL-JMS-HX 100 mass spectrometers. The HRMS spectra were recorded on JEOL JMS-SX102A GC/LC/MS and Finnigan MAT-95XL high-resolution mass spectrometers. Silica gel (70−230 and 230−400 mesh; Merck) and Spherical C18 100 Å reversed-phase silica gel (RP-18; particle size 20−40 μm; Silicycle) were used for column chromatography, and silica gel 60 F254 (Merck) and RP-18 F254S (Merck) were used for TLC and preparative TLC. Further purification was performed with HPLC (Shimadzu; pump, LCC-6AD; UV/vis detector, SPD-10A; integrator, C-R7A Plus).

Plant Material. The roots of *R. indica* var. *tashiroi* were collected on September 2007 in Wutai, Pingtung County, Taiwan, and identified by one of the authors (I.-S.C.). A voucher specimen (no. Chen 6060) was deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University.

Extraction and Isolation. Dried roots (32.8 kg) of *R. indica* var. *tashiroi* were extracted three times with cold MeOH (40 L) to yield a MeOH extract (1.9 kg), which was partitioned in EtOAc−H_2O (1:1; 2 L × 3) to produce an EtOAc-soluble fraction (600 g) and an H_2O-soluble fraction. The H_2O-soluble fraction was partitioned in *n*-BuOH−H_2O (1:1; 3 L × 3) to obtain an *n*-BuOH-soluble fraction (700 g) and an H_2O-soluble fraction (400 g). The active EtOAc-soluble fraction (100 g) was subjected to silica gel column chromatography (CC) using *n*-hexane as the primary eluent and gradually increasing the eluent polarity with EtOAc and MeOH to produce 12 fractions (A-1−A-12). Fractions A-5 and A-7 showed anti-inflammatory activity.

Fraction A-5 (490 mg) was subjected to silica gel CC using *n*-hexane−EtOAc (15:1) as the eluent to produce 12 fractions (A-5-1−A-5-12). Fraction A-5-4 (94.5 mg) was subjected to silica gel CC using *n*-hexane−CH_2Cl_2 (1:1) as the eluent to yield **4** (2.1 mg). Fraction A-6 (1.46 g) was subjected to silica gel CC using *n*-hexane−acetone (7:1) as the eluent to yield eight fractions (A-6-1−A-6-8). Fraction A-6-5 (268 mg) was subjected to silica gel CC using *n*-hexane−acetone (5:1) as the eluent to yield nine fractions (A-6-5-1−A-6-5-9). Fraction A-6-5-7 (98.6 mg) was subjected to silica gel CC using CH_2Cl_2−acetone (30:1) as the eluent to yield six fractions (A-6-5-7-1−A-6-5-7-6). Fraction A-6-5-7-2 (56.2 mg) was subjected to silica gel CC using CH_2Cl_2−acetone (20:1) as the eluent to yield **7** (24.5 mg). Fraction A-6-5-7-6 (9.6 mg) was purified by preparative reversed-phase HPLC (RP-18 column 250 × 10 mm, 5 μm, Merck) using MeOH−H_2O (2:1) as the eluent to yield **1** (2.4 mg, t_R 18 min, 2 mL/min) and **2** (3.1 mg, t_R 24 min, 2 mL/min). Fraction A-6-8 (142.6 mg) was subjected to silica gel CC using CH_2Cl_2−EtOAc (20:1) as the eluent to yield nine fractions (A-6-8-1−A-6-8-9). Fraction A-6-8-1 (10.1 mg) was purified by preparative normal-phase TLC developed with *n*-hexane−EtOAc (5:1) to yield **3** (4.5 mg). Fraction A-6-8-9 (89.3 mg) was subjected to silica gel CC using CH_2Cl_2−EtOAc (10:1) as the eluent to produce **5** (67.7 mg). Fraction A-7 (685 mg) was subjected to silica gel CC using *n*-hexane−acetone (8:1) as the eluent to produce seven fractions (A-7-1−A-7-7). Fraction A-7-6 (54.8 mg) was subjected to silica gel CC using *n*-hexane−acetone (3:1) as the eluent to produce nine fractions (A-7-6-1−A-7-6-9). Fraction A-7-6-8 (12.8 mg) was further purified by preparative reversed-phase TLC developed with MeOH−H_2O (6:1) to produce **6** (2.7 mg).

2-Hydroxy-3,4,6-trimethoxydibenzofuran (1): colorless oil; UV (MeOH) λ_{max} (log ε) 219 (4.35), 258 (4.05), 293 (4.00), 300 (4.04), 315 (sh) (3.71) nm; UV (MeOH + KOH) λ_{max} (log ε) 210 (4.37), 227 (sh) (4.33), 330 (4.03) nm; IR (neat) ν_{max} 3402, 1600, 1587, 1465 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 297 $[M + Na]^+$; HRESIMS *m*/*z* 297.0741 $[M + Na]^+$ (calcd for $C_{15}H_{14}O_5Na$, 297.0739).

2-Hydroxy-3,4,9-trimethoxydibenzofuran (2): colorless oil; UV (MeOH) λ_{max} (log ε) 220 (4.31), 260 (4.08), 295 (4.09), 299 (4.01), 317 (sh) (3.67) nm; UV (MeOH + KOH) λ_{max} (log ε) 209 (4.35), 230 (sh) (4.28), 325 (4.01) nm; IR (neat) ν_{max} 3417, 1603, 1589, 1459 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 297 $[M + Na]^+$; HRESIMS *m*/*z* 297.0740 $[M + Na]^+$ (calcd for $C_{15}H_{14}O_5Na$, 297.0739).

2-Hydroxy-3,4,6,9-tetramethoxydibenzofuran (3): colorless oil; UV (MeOH) λ_{max} (log ε) 218 (4.28), 257 (4.04), 299 (4.12), 301 (4.06), 320 (sh) (3.58) nm; UV (MeOH + KOH) λ_{max} (log ε) 208 (4.33), 230 (sh) (4.28), 326 (4.04) nm; IR (neat) λ_{max} 3403, 1601, 1590, 1466 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 327 $[M + Na]^+$; HRESIMS *m*/*z* 327.0844 $[M + Na]^+$ (calcd for $C_{16}H_{16}O_6Na$, 327.0845).

1,2-Methylenedioxy-3,4,6-trimethoxydibenzofuran (4): colorless needles (MeOH); mp 127−128 °C; UV (MeOH) λ_{max} (log ε) 220 (4.23), 256 (4.01), 292 (3.98), 303 (4.07), 313 (sh) (3.69) nm; IR (KBr) ν_{max} 1605, 1589, 1460, 1040, 920 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 325 $[M + Na]^+$; HRESIMS *m*/*z* 325.0687 $[M + Na]^+$ (calcd for $C_{16}H_{14}O_6Na$, 325.0688).

3-Hydroxy-2′,5-dimethoxybiphenyl (5): colorless oil; UV (MeOH) λ_{max} (log ε) 226 (4.19), 295 (4.38) nm; UV (MeOH + KOH) λ_{max} (log ε) 209 (4.23), 229 (sh) (4.13), 335 (4.07) nm; IR (neat) ν_{max} 3400, 1599, 1581, 1463 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 253 $[M + Na]^+$; HRESIMS *m*/*z* 253.0842 $[M + Na]^+$ (calcd for $C_{14}H_{14}O_3Na$, 253.0941).

2′,3-Dihydroxy-5-methoxybiphenyl (6): colorless oil; UV (MeOH) λ_{max} (log ε) 219 (4.35), 293 (4.00) nm; UV (MeOH + KOH) λ_{max} (log ε) 210 (4.37), 227 (sh) (4.33), 330 (4.03) nm; IR (neat) ν_{max} 3402, 1600, 1587, 1465 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 239 $[M + Na]^+$; HRESIMS *m*/*z* 239.0686 $[M + Na]^+$ (calcd for $C_{13}H_{12}O_3Na$, 239.0684).

Anti-inflammatory Activity Assay: Evaluation of $O_2^{\bullet-}$ Release by Human Neutrophils. The anti-inflammatory effects of the compounds isolated from the roots of *R. indica* were evaluated by measuring the inhibition of superoxide anion production, which was tested with a continuous spectrophotometric assay of ferricytochrome *c* reduction by an isolated preparation of human neutrophils.

Preparation of Human Neutrophils. Human neutrophils from the venous blood of healthy[9] adult volunteers (20−28 years old) were isolated using a standard method of dextran sedimentation followed by centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of the erythrocytes.[10] The purified neutrophils, containing >98% viable cells as determined by the Trypan blue exclusion method, were resuspended in a Ca^{2+} (1 mM) Hank's balanced salt solution (pH 7.4) and maintained at 4 °C until use.

Measurement of $O_2^{\bullet-}$ Generation. The assay for measuring $O_2^{\bullet-}$ generation was based on the superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome *c*.[11] Briefly, neutrophils (1×10^6 cells/mL), pretreated with various concentrations of the test compounds for 5 min at 37 °C, were stimulated with fMLP (1 μmol/L) in the presence of ferricytochrome *c* (0.5 mg/mL). Extracellular $O_2^{\bullet-}$ production was assessed with a UV spectrophotometer at 550 nm (Hitachi, UV-3010). The percentage of superoxide inhibition by the test compound was calculated as {[(control − resting) − (compound − resting)]/(control − resting)} × 100. SigmaPlot software was used to determine the IC_{50} values.

Statistical Analysis. The results are expressed as means ± SEM, and comparisons were made with Student's *t* test. A probability of 0.05 or less was considered significant.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **1**−**6** are available free of charge via the Internet at http://pubs.acs.org.

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