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Anti-inflammatory Benzenoids from *Antrodia camphorata*

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Four new compounds, including three new benzenoids, antrocamphin A (**1**), antrocamphin B (**2**), and 2,3,4,5-tetramethoxybenzoyl chloride (**3**), and a new 1,3-dioxolan-2-one derivative, antrodioxolanone (**4**), together with 13 known compounds have been isolated from the fruiting body of *Antrodia camphorata*. The structures of these new compounds were determined through spectral analyses including extensive 2D-NMR data. Among the isolates, antrocamphin A (**1**), antcin A (**10**), and antcin B (**11**) exhibited potent inhibition against fMLP-induced superoxide production with IC_{50} values less than 10 μM.

Antrodia camphorata Wu, Ryvarden & Chang (Polyporaceae, Aphyllophorales)[1] is a parasitic fungus on the inner heartwood wall of the endemic species *Cinnamomum kanehirai* Hay (Lauraceae) in Taiwan. The fruiting body of *A. camphorata* is well known in Taiwan by the name niu-chang-chih or jang-jy and is also popular and very expensive as a medicinal material. It has been used for the treatment of food, alcohol, and drug intoxication, diarrhea, abdominal pain, hypertension, itching, and liver cancer in Chinese folk medicine.[2] Previous chemical studies of the fruiting body of this fungus have reported the isolation of several components including phenyl derivatives, triterpenoids, diterpenes, sesquiterpenoids, steroids, lignans, fatty acids, and maleic and succinic acid derivatives.[3−13] Cytotoxic,[11] anti-inflammatory,[12] and neuroprotective[13] activities have been demonstrated for some of these compounds. In a screening program searching for anti-inflammatory compounds in Formosan fungi, *A. camphorata* was shown to be one of the active species. Investigation on the *n*-hexane-soluble fraction of the fruiting body of *A. camphorata* has led to the isolation and characterization of three new benzenoids, antrocamphin A (**1**), antrocamphin B (**2**), and 2,3,4,5-tetramethoxybenzoyl chloride (**3**), and a new 1,3-dioxolan-2-one derivative, antrodioxolanone (**4**), and 13 known compounds. This paper describes the structural elucidation of **1**−**4** and the anti-inflammatory activities of the isolates.

Extensive chromatographic purification of the *n*-hexane-soluble fraction of the fruiting body of *A. camphorata* on a silica gel column and preparative TLC afforded four new (**1**−**4**) and 13 known compounds (**5**−**17**).

Results and Discussion

Antrocamphin A (**1**) was isolated as a yellowish oil. The HRESIMS gave an $[M + H]^+$ ion at *m*/*z* 247.1337, consistent with the molecular formula $C_{15}H_{19}O_3$. The IR spectrum showed a C≡C stretch at 2196 cm^{-1} and an aromatic ring C═C stretch at 1593, 1490, and 1462 cm^{-1}. The ^1H NMR spectrum of **1** showed the presence of three methoxy groups, an aromatic methyl group, an aromatic proton, and a 3-methylbut-3-en-1-ynyl group. On the basis of the NOESY correlations (Figure 1) between H-6 and OMe-1 and OMe-5 and between OMe-5 and Me-3′ of 3-methylbut-3-en-1-ynyl group, the 3-methylbut-3-en-1-ynyl group was assigned to reside at C-4. The assignments of OMe-2 and Me-3 were confirmed by HMBC correlations (Figure 1) between H-6 and OMe-2 and C-2 and between Me-3 and C-2 and C-4. Thus, the structure of **1** was elucidated as 1,2,5-trimethoxy-3-methyl-4-(3-methylbut-3-en-1-ynyl)benzene, named antrocamphin A. This was further confirmed by $^1H-^1H$ COSY and NOESY experiments (Figure 1). The assignment of ^{13}C NMR resonances was confirmed by HSQC and HMBC techniques (Figure 1).

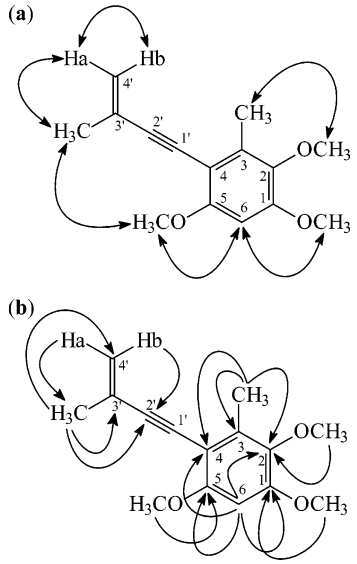
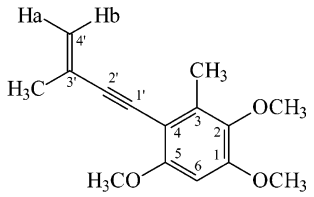


Figure 1. NOESY (a) and HMBC (b) correlations of **1**.



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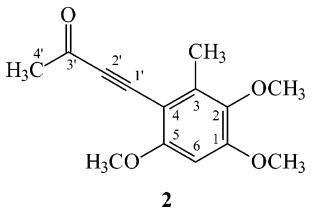

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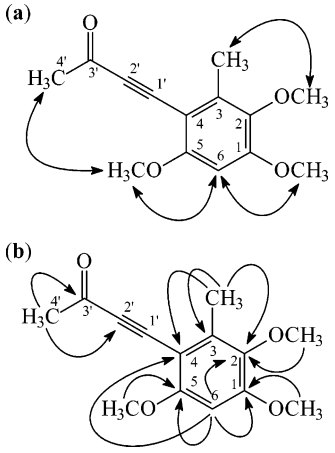


Figure 2. NOESY (a) and HMBC (b) correlations of **2**.

Antrocamphin B (**2**) was isolated as a yellowish powder. The EIMS afforded the molecular ion $[M]^+$ at *m/z* 248, implying a molecular formula of $C_{14}H_{16}O_4$, which was confirmed by the HRESIMS. The IR spectrum showed a C≡C stretch at 2182 cm^{-1} and a carbonyl absorption at 1661 cm^{-1}. The ^1H NMR spectrum of **2** was similar to that of antrocamphin A (**1**), except that a 3-oxobut-1-ynyl group of **2** replaced the 3-methylbut-3-en-1-ynyl group of **1**. Analysis of the ^1H NMR spectrum of **2** showed resonances for an aromatic proton, three aromatic methoxy groups, an aromatic methyl group, and a 3-oxobut-1-ynyl group. In the NOESY spectrum of **2**, the proton at δ 6.32 (H-6) showed correlations with the methoxy resonances at δ 3.89 (OMe-1) and 3.92 (OMe-5), and the methoxy at δ 3.92 (OMe-5) with Me-3′ (δ 2.46) of the 3-oxobut-1-ynyl group. Thus, the 3-oxobut-1-ynyl group was assigned to reside at C-4. Furthermore, the assignments of OMe-2 and Me-3 were confirmed by HMBC correlations (Figure 2) between H-6, OMe-2, and C-2 and between Me-3 and C-2 and C-4. On the basis of the above data, the structure of **2** was elucidated as 4-(3,4,6-trimethoxy-2-methylphenyl)but-3-yn-2-one, named antrocamphin B. This was further confirmed by $^1H-^1H$ COSY and NOESY (Figure 2) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 2) techniques.

2,3,4,5-Tetramethoxybenzoyl chloride (**3**) was isolated as an amorphous powder with a molecular formula of $C_{11}H_{13}ClO_5$ as determined by positive-ion HRESIMS and ^{13}C NMR data. The presence of a carbonyl group was revealed by a band at 1768 cm^{-1} in the IR spectrum, which was confirmed by the resonances at δ 168.1 in the ^{13}C NMR spectrum. Comparison of the IR and ^1H and ^{13}C NMR data of **3** with those of 2,3,4,5-tetramethoxybenzoic acid[14] suggested that their structures are closely related except that the chlorocarbonyl group of **3** replaced the carboxy group of 2,3,4,5-tetramethoxybenzoic acid. Analysis of the ^1H NMR spectrum of **3** showed resonances for an aromatic proton and four aromatic methoxy groups. A lower field aromatic proton at δ 7.13 suggested that it was adjacent to the chlorocarbonyl group. In the NOESY spectrum of **3**, the proton at δ 7.13 (H-6) showed correlations with the methoxy resonance at δ 3.91 (OMe-5). Thus, the other three methoxy groups were assigned to C-2, C-3, and C-4. On the basis of the above data, the structure of **3** was elucidated as 2,3,4,5-tetramethoxybenzoyl chloride. This was further confirmed by $^1H-^1H$ COSY and NOESY (Figure 4) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 4) techniques. This is the first report of the occurrence of **3** in a natural source, although it has been used as a reagent.[15] Further evidence to support the structure of **3** was also sought in a

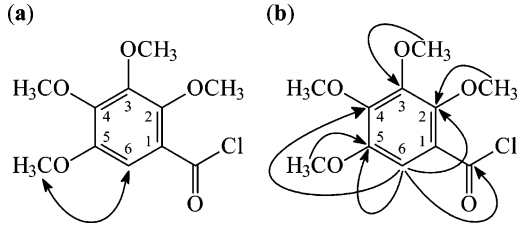


Figure 3. NOESY (a) and HMBC (b) correlations of **3**.

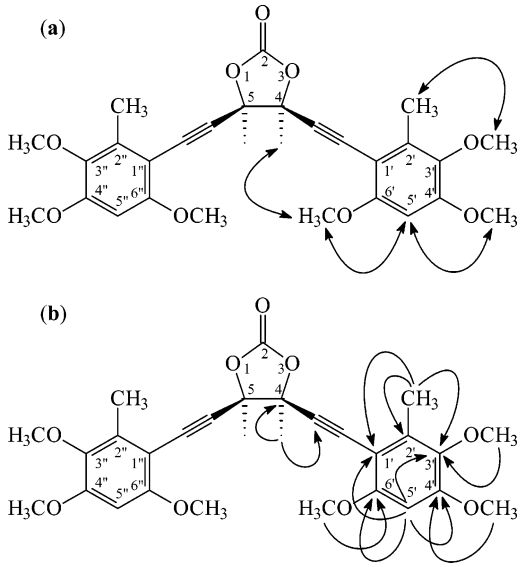
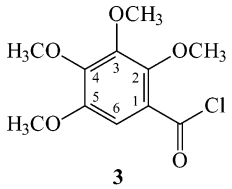


Figure 4. NOESY (a) and HMBC (b) correlations of **4**.

methanolysis experiment of **3** to give methyl 2,3,4,5-tetramethoxybenzoate (**3a**), which was confirmed by ^1H NMR and HREIMS data.



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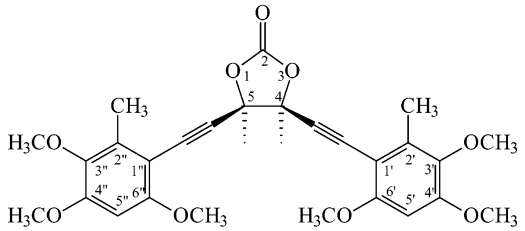

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Antrodioxolanone (**4**) was obtained as a yellowish, amorphous solid, and the molecular formula was confirmed to be $C_{29}H_{32}O_9$ from the pseudomolecular ion peak at $m/z = 547.1946$ $[M + Na]^+$ obtained by HRESIMS. In the IR spectrum, absorptions for alkyne (2186 cm^{-1}), 1,3-dioxolan-2-one carbonyl (1765 cm^{-1}), and aromatic olefinic functions (1598, 1497, and 1455 cm^{-1}) were observed. In the ^1H and ^{13}C NMR spectra, the number of resonances observed was half that expected, suggesting that **4** had a symmetrical structure. The ^1H NMR spectrum of **4** was similar to that of **2** except that the C-2′ acetyl group of **2** was replaced by a 4,5-dimethyl-2-oxo-5-((3,4,6-trimethoxy-2-methylphenyl)ethynyl)-1,3-dioxolan-4-yl group of **4**. The formation of **4** may be the result of intermolecular cyclization[16] at the acetyl group of **2** to give a cyclic

Table 1. IC_{50} Values of Compounds Isolated from the Fruiting Body of *A. camphorata* in the Inhibition on fMLP-Induced Superoxide Generation in Human Neutrophils

compound	IC_{50} (μM)[a]
antrocamphin A (**1**)	9.33 ± 3.31
antrocamphin B (**2**)	>100
2,3,4,5-tetramethoxybenzoyl chloride (**3**)	>100
antrodioxolanone (**4**)	>100
4,7-dimethoxy-5-methyl-1,3-benzodioxole (**5**)	26.09 ± 3.34
2,2′,5,5′-tetramethoxy-3,4,3′,4′-bimethylene-dioxy-6,6′-dimethylbiphenyl (**6**)	>100
(−)-sesamin (**7**)	90.23 ± 8.11
α-tocospiro B (**8**)	>100
epi-friedelinol (**9**)	>100
antcin A (**10**)	8.55 ± 1.04
antcin B (**11**)	9.82 ± 4.40
dehydroburicoic acid (**12**)	77.50 ± 7.43
ergosta-4,6,8(14),22-tetraen-3-one (**13**)	>100
eburicol (**14**)	50.47 ± 2.84
β-sitostenone (**15**)	>100
mixture of β-sitosterol (**16**) and stigmasterol (**17**)	>100
ibuprofen[b]	27.5 ± 3.45

[a] The IC_{50} values were calculated from the slope of the dose−response curves. Values are expressed as the SEM of three independent experiments. [b] Ibuprofen was used as a positive control.

carbonate in the biogenetic process. Compound **4** is a meso compound, $[\alpha]_D^{25} = \pm 0$; thus **4** possessed a 4*S*,5*R*-configuration. According to the above data, the structure of **4** was elucidated as (4*S*,5*R*)-4,5-dimethyl-4,5-bis[(3,4,6-trimethoxy-2-methylphenyl)-ethynyl]-1,3-dioxolan-2-one, named antrodioxolanone. This was confirmed by $^1H-^1H$ COSY and NOESY (Figure 4) experiments. The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC (Figure 4) techniques.

The known isolates were readily identified by comparison of physical and spectroscopic data (UV, IR, ^1H NMR, $[\alpha]_D$, and MS) with corresponding authentic samples or literature values, and this included a benzenoid, 4,7-dimethoxy-5-methyl-1,3-benzodioxole (**5**),[5] a biphenyl, 2,2′,5,5′-tetramethoxy-3,4,3′,4′-bimethylenedioxy-6,6′-dimethylbiphenyl (**6**),[5] a lignan, (−)-sesamin (**7**),[17] an α-tocopheroid, α-tocospiro B (**8**),[18] three triterpenoids, *epi*-friedelinol (**9**),[19] antcin A (**10**),[3] antcin B (**11**),[3] a steroidal acid, dehydroburicoic acid (**12**),[7] and five steroids, ergosta-4,6,8(14),22-tetraen-3-one (**13**),[20] eburicol (**14**),[21] β-sitostenone (**15**),[22] and a mixture of β-sitosterol (**16**)[23] and stigmasterol (**17**).[23]

The anti-inflammatory effects of the isolates from the fruiting body of *A. camphorata* were evaluated by suppressing *N*-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced production of superoxide anion, an inflammatory mediator produced by neutrophils. The anti-inflammatory activity data are shown in Table 1. The clinically used anti-inflammatory agent ibuprofen was used as the positive control. From the results of our anti-inflammatory tests, the following conclusions can be drawn: (a) Antrocamphin A (**1**), 4,7-dimethoxy-5-methyl-1,3-benzodioxole (**5**), antcin A (**10**), and antcin B (**11**) exhibited more potent inhibition ($IC_{50} \leq 26.09$ μM) than ibuprofen ($IC_{50} = 27.52$ μM) against fMLP-induced superoxide production. (b) Antrocamphin A (**1**), with a 3-methylbut-3-en-1-ynyl group, showed strong inhibition against fMLP-induced superoxide production, but its analogue, antrocamphin B (**2**), with a 3-oxobut-1-ynyl group, was inactive. (c) 4,7-Dimethoxy-5-methyl-1,3-benzodioxole (**5**) exhibited more effective inhibition than its dimer, 2,2′,5,5′-tetramethoxy-3,4,3′,4′-bimethylenedioxy-6,6′-dimethylbiphenyl (**6**), against fMLP-induced superoxide production. (d) Antcin A (**10**) is the most effective among the isolates, with an IC_{50} value of 8.55 ± 1.04 μM against fMLP-induced production of superoxide anion by neutrophils. (e) Compounds **1**, **5**, **10**, and **11** may exert their anti-inflammatory action through inhibiting superoxide generation.

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. IR spectra (KBr or neat) were recorded on a Perkin-Elmer system 2000 FT-IR spectrometer. Optical rotations were measured using a Jasco DIP-370 polarimeter in $CHCl_3$. UV spectra were obtained on a Jasco UV-240 spectrophotometer. EI, ESI, and HRESI mass spectra were recorded on a Bruker APEX II mass spectrometer. HREI, FAB, and HRFAB mass spectra were recorded on a JEOL JMX-HX 110 mass spectrometer. NMR spectra, including COSY, NOESY, HMBC, and HSQC experiments, were recorded on a Varian Unity 400 or a Varian Inova 500 spectrometer operating at 400 and 500 MHz (^1H) and 100 and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using TMS as an internal standard. Silica gel (70−230, 230−400 mesh) (Merck) was used for CC. Silica gel 60 F-254 (Merck) was used for TLC and preparative TLC.

Plant Material. The fruiting body of *A. camphorata* was collected from Nantou County, Taiwan, in December 2004. A voucher specimen (Chen 6001) was deposited in the herbarium of the Department of Pharmacy, Tajen University, Pingtung, Taiwan.

Extraction and Separation. The air-dried fruiting bodies of *A. camphorata* (200 g) were extracted with *n*-hexane (3 × 2 L, each 24 h), EtOAc (3 × 2 L, each 24 h), and MeOH (3 × 2 L, each 24 h) successively, and the extracts concentrated under reduced pressure. The *n*-hexane extract (fraction A, 15 g) was chromatographed on silica gel (70−230 mesh, 1.8 kg), eluting with *n*-hexane, gradually increasing the polarity with EtOAc and MeOH to give 17 fractions: A1 (2.5 L, *n*-hexane), A2 (1.5 L, *n*-hexane−EtOAc, 100:1), A3/A4 (each 2 L, *n*-hexane−EtOAc, 80:1), A5 (1.5 L, *n*-hexane−EtOAc, 60:1), A6/A7 (each 1.5 L, *n*-hexane−EtOAc, 40:1), A8/A9 (each 2 L, *n*-hexane−EtOAc, 30:1), A10/A11 (each 2.5 L, *n*-hexane−EtOAc, 20:1), A12 (1.5 L, *n*-hexane−EtOAc, 10:1), A13 (2.5 L, *n*-hexane−EtOAc, 5:1), A14 (2 L, *n*-hexane−EtOAc, 3:1), A15 (2.5 L, *n*-hexane−EtOAc, 1:1), A16 (3 L, EtOAc), A17 (4 L, MeOH). Fraction A2 (0.6 g) was chromatographed further on silica gel (230−400 mesh, 90 g) eluting with *n*-hexane−EtOAc (20:1) to give five fractions (each 1 L, A2-1−A2-5). Fraction A2-3 (155 mg) was purified further by preparative TLC (*n*-hexane−acetone, 30:1) to obtain **5** (13.8 mg) ($R_f = 0.35$). Fraction A5 (0.9 g) was chromatographed further on silica gel (230−400 mesh, 100 g) eluting with *n*-hexane−acetone (10:1) to give six fractions (each 1 L, A5-1−A5-6). Fraction A5-2 (115 mg) was purified further by preparative TLC (*n*-hexane−acetone, 3:1) to obtain **15** (6.8 mg) ($R_f = 0.67$). Fraction A5-3 (196 mg) was purified further by preparative TLC (*n*-hexane−acetone, 10:1) to obtain **16** and **17** (15.5 mg) ($R_f = 0.40$). Fraction A5-5 (127 mg) was purified further by preparative TLC (*n*-hexane−acetone, 3:1) to obtain **7** (2.1 mg) ($R_f = 0.27$). Fractions A6 and A7 (1.5 g) were chromatographed further on silica gel (230−400 mesh, 110 g) eluting with *n*-hexane−acetone (5:1) to give seven fractions (each 1.5 L, A6-1−A6-7). Fraction A6-2 (75 mg) was purified further by preparative TLC ($CHCl_3$−EtOAc, 60:1) to obtain **8** (2.8 mg) ($R_f = 0.68$) and **14** (8.5 mg) ($R_f = 0.38$). Fraction A6-3 (50 mg) was purified further by preparative TLC (*n*-hexane−acetone, 3:1) to obtain **1** (6.8 mg) ($R_f = 0.58$). Fraction A6-4 (40 mg) was purified further by preparative TLC (CH_2Cl_2−EtOAc, 60:1) to obtain **3** (2.0 mg) ($R_f = 0.78$) and **4** (2.7 mg) ($R_f = 0.65$). Fraction A6-5 (55 mg) was purified further by preparative TLC ($CHCl_3$−EtOAc, 10:1) to obtain **2** (4.7 mg) ($R_f = 0.72$). Fractions A8 and A9 (1.2 g) were chromatographed further on silica gel (230−400 mesh, 100 g) eluting with *n*-hexane−acetone (3:1) to give seven fractions (each 1.2 L, A8-1−A8-7). Fraction A8-3 (145 mg) was purified further by preparative TLC (*n*-hexane−EtOAc, 5:1) to obtain **13** (15.8 mg) ($R_f = 0.36$). Fraction A8-5 (125 mg) was purified further by preparative TLC (*n*-hexane−EtOAc, 3:1) to obtain **6** (12.4 mg) ($R_f = 0.55$). Fraction A13 (0.9 g) was chromatographed further on silica gel (230−400 mesh, 100 g) eluting with $CHCl_3$−acetone (10:1) to give eight fractions (each 1.2 L, A13-1−A13-8). Fraction A13-5 (185 mg) was purified further by preparative TLC (*n*-hexane−acetone, 5:1) to obtain **9** (11.4 mg) ($R_f = 0.58$). Fraction A14 (0.8 g) was chromatographed further on silica gel (230−400 mesh, 90 g) eluting with CH_2Cl_2−acetone (5:1) to give seven fractions (each 1 L, A14-1− A14-7). Fraction A14-4 (135 mg) was purified further by preparative TLC (*n*-hexane−EtOAc, 1:1) to obtain **12** (7.4 mg) ($R_f = 0.60$). Fraction A14-6 (128 mg) was purified further by preparative TLC ($CHCl_3$−acetone, 2:1) to obtain **11** (6.7 mg) ($R_f = 0.72$). Fraction A15 (0.75 g) was chromatographed further on silica gel (230−400

mesh, 90 g) eluting with CH_2Cl_2−acetone (3:1) to give six fractions (each 1 L, A15-1−A15-6). Fraction A15-5 (128 mg) was purified further by preparative TLC (CH_2Cl_2−MeOH, 20:1) to obtain **10** (6.9 mg) ($R_f = 0.55$).

Anti-inflammatory Activity Assay: Evaluation of $O_2^{\bullet-}$ Release by Human Neutrophils. Superoxide anion production was tested by using a continuous spectrophotometric assay of ferricytochrome *c* reduction by isolated neutrophils. Briefly, neutrophils were isolated from the venous blood[24] of consenting healthy volunteers (20−35 years old) by double-gradient Ficoll−Hypaque centrifugation and hypotonic lysis of contaminating red blood cells as previously described.[25] Neutrophils (1×10^6 cells/mL) pretreated with the various test agents (100 μmol/L) at 37 °C for 5 min 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 of the test compound was calculated as the percentage of inhibition = {(control − resting) − (compound − resting)}/(control − resting) × 100.

Antrocamphin A (1): yellowish oil; UV (MeOH) λ_{max} (log ϵ) 268 (4.12), 284 (4.23), 308 (4.13) nm; IR (KBr) ν_{max} 2196 (C≡C), 1593, 1490, 1462 (aromatic ring C═C stretch) cm^{-1}; ^1H NMR (400 MHz) δ 2.02 (3H, s, H-3′), 2.36 (3H, s, Me-3), 3.72 (3H, s, OMe-2), 3.87 (3H, s, OMe-1), 3.88 (3H, s, OMe-5), 5.25 (1H, br s, Ha-4′), 5.37 (1H, br s, Hb-4′), 6.33 (1H, s, H-6); ^{13}C NMR (100 MHz) δ 14.1 (Me-3), 23.7 (Me-3′), 55.8 (OMe-5), 56.3 (OMe-1), 60.4 (OMe-2), 83.6 (C-1′), 94.3 (C-6), 97.5 (C-2′), 104.8 (C-4), 120.7 (C-4′), 127.3 (C-3′), 135.3 (C-3), 141.1 (C-2), 153.5 (C-5), 157.2 (C-1); EIMS *m*/*z* (rel int) 246 ([M]$^+$, 73), 231 (50), 203 (72), 188 (40), 173 (27), 160 (28), 145 (34), 129 (33), 128 (47), 127 (23), 117 (55), 115 (82), 91 (32), 89 (20), 77 (43), 73 (32), 69 (100), 65 (25), 63 (44), 57 (23), 55 (37), 51 (43); HRESIMS *m*/*z* 247.1337 [M + H]$^+$ (calcd for $C_{15}H_{19}O_3$, 247.1334).

Antrocamphin B (2): yellowish powder; UV (MeOH) λ_{max} (log ϵ) 295 (3.98), 337 (4.07) nm; IR (KBr) ν_{max} 2182 (C≡C), 1661 (C═O), 1591, 1488, 1462 (aromatic ring C═C stretch) cm^{-1}; ^1H NMR (400 MHz) δ 2.46 (3H, s, H-4′), 2.39 (3H, s, Me-3), 3.73 (3H, s, OMe-2), 3.89 (3H, s, OMe-1), 3.92 (3H, s, OMe-5), 6.32 (1H, s, H-6); ^{13}C NMR (100 MHz) δ 14.3 (Me-3), 33.0 (C-4′), 56.1 (OMe-5), 56.4 (OMe-1), 60.7 (OMe-2), 88.1 (C-1′), 94.0 (C-6), 96.5 (C-2′), 101.5 (C-4), 137.6 (C-3), 141.4 (C-2), 156.4 (C-5), 160.0 (C-1), 184.8 (C-3′); EIMS *m*/*z* (rel int) 248 ([M]$^+$, 98), 233 (77), 175 (31), 161 (29), 153 (31), 147 (23), 136 (26), 107 (34), 105 (20), 91 (24), 89 (48), 78 (35), 77 (100), 76 (28), 74 (23), 69 (62), 65 (22), 63 (58); HRESIMS *m*/*z* 249.1128 [M + H]$^+$ (calcd for $C_{14}H_{17}O_4$, 249.1127).

2,3,4,5-Tetramethoxybenzoyl chloride (3): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 210 (4.59), 245 (4.08), 300 (3.69) nm; IR (KBr) ν_{max} 1768 (C═O) cm^{-1}; ^1H NMR (400 MHz) δ 3.89 (3H, s, OMe-4), 3.91 (3H, s, OMe-5), 3.92 (3H, s, OMe-3), 3.93 (3H, s, OMe-2), 7.13 (1H, s, H-6); ^{13}C NMR (100 MHz) δ 56.3 (OMe-5), 61.1 (OMe-4), 61.2 (OMe-3), 62.4 (OMe-2), 109.7 (C-6), 116.5 (C-1), 151.0 (C-4), 146.5 (C-5), 147.9 (C-2), 148.2 (C-3), 168.1 (C═O); EIMS *m*/*z* (rel int) 260 ([M]$^+$, 100), 245 (64), 231 (16), 229 (54), 227 (39), 217 (56), 219 (19), 213 (32), 202 (25), 173 (21), 171 (54), 143 (21), 131 (25), 129 (32), 105 (32), 103 (35), 84 (48), 77 (81); HRESIMS *m*/*z* 283.0350 [M + Na]$^+$ (calcd for $C_{11}H_{13}ClO_5Na$, 283.0349).

Methyl 2,3,4,5-tetramethoxybenzoate (3a). Methanol (0.3 mL) was added to a solution of **3** (1.9 mg) in pyridine (0.3 mL), and the mixture was stirred for 10 h at room temperature. The excess pyridine was removed with saturated aqueous $CuSO_4$ solution to give a residue (1.8 mg). The residue was chromatographed on silica gel to give **3a** (1.5 mg), colorless oil: UV (MeOH) λ_{max} (log ϵ) 209 (4.50), 243 (4.04), 306 (3.61) nm; IR (KBr) ν_{max} 1714 (C═O) cm^{-1}; ^1H NMR (400 MHz) δ 3.89 (3H, s, OMe-4), 3.90 (3H, s, COOMe), 3.90 (3H, s, OMe-5), 3.91 (3H, s, OMe-3), 3.91 (3H, s, OMe-2), 7.03 (1H, s, H-6); EIMS *m*/*z* (rel int) 256 ([M]$^+$, 100), 241 (54), 225 (51), 210 (57), 194 (14), 171 (55), 131 (27), 129 (29), 103 (31), 84 (42), 77 (78); HREIMS *m*/*z* 256.0942 [M]$^+$ (calcd for $C_{12}H_{16}O_6$, 256.0947).

Antrodioxolanone (4): yellowish, amorphous solid; $[\alpha]^{25}_D$ ±0 (*c* 0.10, $CDCl_3$); UV (MeOH) λ_{max} (log ϵ) 285 (4.15), 313 (4.08) nm; IR (KBr) ν_{max} 2186 (C≡C), 1765 (C═O), 1598, 1497, 1455 (aromatic ring C═C stretch) cm^{-1}; ^1H NMR (400 MHz) δ 1.70 (6H, s, Me-4 and Me-5), 2.36 (6H, s, Me-2′ and Me-2″), 3.72 (6H, s, OMe-3′ and Me-3″), 3.85 (6H, s, OMe-4′ and OMe-4″), 3.87 (6H, s, OMe-6′ and OMe-6″), 6.33 (2H, s, H-5′ and H-5″); ^{13}C NMR (100 MHz) δ 14.2 (Me-2′ and Me-2″), 20.1 (Me-4 and Me-5), 55.8 (OMe-6′ and OMe-6″), 56.4 (OMe-4′ and OMe-4″), 60.5 (OMe-3′ and OMe-3″), 81.9 (C-4 and C-5), 82.8 (Ph-*C*≡C-4 and Ph-C≡*C*-5), 94.2 (C-5′ and C-5″), 92.6 (Ph−C≡*C*-4 and Ph−C≡*C*-5), 102.2 (C-1′ and C-1″), 135.8 (C-2′ and C-2″), 141.3 (C-3′ and C-3″), 152.5 (C-2), 154.0 (C-6′ and C-6″), 158.5 (C-4′ and C-4″); EIMS *m*/*z* (rel int) 524 (M$^+$, 8), 509 (4), 369 (9), 355 (16), 295 (11), 281 (18), 246 (63), 231 (16), 221 (40), 209 (100), 207 (21), 203 (8), 165 (7), 129 (10), 111 (13), 98 (15); HRESIMS *m*/*z* 547.1946 [M + Na]$^+$ (calcd for $C_{29}H_{32}O_9Na$, 547.1944).

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References and Notes

(1) Wu, S. H.; Ryvarden, L.; Chang, T. T. *Bot. Bull. Acad. Sin.* **1997**, *38*, 273−275.
(2) Tsai, Z. T.; Liaw, S. L. *The Use and the Effect of Ganoderma*; Sang-Yun Press: Taichung, Taiwan, 1982; p 116.
(3) Cherng, I. H.; Chiang, H. C. *J. Nat. Prod.* **1995**, *58*, 365−371.
(4) Chen, C. H.; Yang, S. W. *J. Nat. Prod.* **1995**, *58*, 1655−1661.
(5) Chiang, H. C; Wu, D. P.; Cherng, I. W.; Ueng, C. H. *Phytochemistry* **1995**, *39*, 613−616.
(6) Wu, D. P.; Chiang, H. C. *J. Chin. Chem. Soc.* **1995**, *42*, 797−800.
(7) Yang, S. W; Shen, Y. C.; Chen, C. H. *Phytochemistry* **1996**, *41*, 1389−1392.
(8) Cherng, I. H.; Wu, D. P.; Chiang, H. C. *Phytochemistry* **1996**, *41*, 263−267.
(9) Shen, Y. C.; Yang, S. W.; Lin, C. S.; Chen, C. H.; Kuo, Y. H.; Chen, C. F. *Planta Med.* **1997**, *63*, 86−88.
(10) Shen, C. C.; Kuo, Y. C.; Huang, R. L.; Lin, L. C.; Don, M. J.; Chang, T. T.; Chou, C. J. *J. Chin. Med.* **2003**, *14*, 247−258.
(11) Nakamura, N.; Hirakawa, A.; Gao, J. J.; Kakuda, H.; Shiro, M.; Komatsu, Y.; Sheu, C. C.; Hattori, M. *J. Nat. Prod.* **2004**, *67*, 46−48.
(12) Shen, Y. C.; Wang, Y. H.; Chou, Y. C.; Chen, C. F.; Lin, L. C.; Chang, T. T.; Tien, J. H.; Chou, C. J. *Planta Med.* **2004**, *70*, 310−314.
(13) Chen, C. C.; Shiao, Y. J.; Lin, R. D.; Shao, Y. Y.; Lai, M. N.; Lin, C. C.; Ng, L. T.; Kuo, Y. H. *J. Nat. Prod.* **2006**, *69*, 689−691.
(14) Meyers, A. I.; Flisak, J. R.; Aitken, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 5446−5452.
(15) Ghosal, S.; Chaudhuri, R. K. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2538−2541.
(16) Lu, L.; Fang, J. M.; Lee, G. H.; Wang, Y. *J. Chin. Chem. Soc.* **1997**, *44*, 279−289.
(17) Yu, H. J.; Chen, C. C.; Shieh, B. J. *J Chin. Chem. Soc.* **1998**, *45*, 773−778.
(18) Chiang, Y. M.; Kuo, Y. H. *Tetrahedron Lett.* **2003**, *44*, 5125−5128.
(19) Salazer, G. C. M.; Silva, G. D. F.; Duarte, L. P.; Vieira, F. S. A.; Lula, I. S. *Magn. Reson. Chem.* **2000**, *38*, 977−980
(20) Kobayashi, M.; Krishna, M. M.; Ishida, K.; Anjanetulu, V. *Chem. Pharm. Bull.* **1992**, *40*, 72−74.
(21) Shirane, N.; Takenaka, H.; Ueda, K.; Hashimoto, Y.; Katoh, K.; Ishii, H. *Phytochemistry* **1996**, *41*, 1301−1308.
(22) Betancor, C.; Freire, R.; Gonzalez, A. G.; Salazar, J. A.; Pascard, C.; Prange, T. *Phytochemistry* **1980**, *19*, 1989−1993.
(23) Chen, J. J.; Huang, Y. C.; Chen, Y. C.; Huang, Y. T.; Wang, S. W.; Peng, C. Y.; Teng, C. M, Chen, I. S. *Planta Med.* **2002**, *68*, 980−985.
(24) Boyum, A. *Scand. J. Lab. Invest.* **1968**, *21*, 77−97.
(25) English, D.; Andersen, B. R. *J. Immunol. Methods* **1974**, *5*, 249−252.

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