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CHEMICAL CONSTITUENTS FROM THE ROOTS OF *Cinnamomum subavenium*

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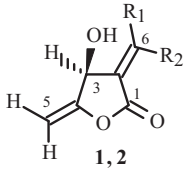
Cinnamomum subavenium Miq. (Lauraceae) is a medium-sized evergreen tree found in central to southern mainland China, Burma, Cambodia, Taiwan, Malaysia, and Indonesia [1]. *C. subavenium* was chosen for further phytochemical investigation. In previous studies, we have investigated the chemical constituents of the Formosan Lauraceous plants and have identified a novel cytotoxic monoterpenoid, subamone, five new butanolides, subamolide A–E, two new secobutanolides, secosubamolide and secosubamolide A, along with 27 known compounds from the stems and leaves of *C. subavenium* [1–3]. The MeOH extract of its roots was subjected to solvent partitioning and chromatographic separation to afford five pure substances. The chemical constituents in the roots of *C. subavenium* were separated with column chromatography. Five compounds, including isoobtusilactone A (**1**) [4], obtusilactone A (**2**) [4], eugenol (**3**) [5], myristicin (**4**) [6], and 3,4-methylenedioxy-5-methoxy cinnamyl alcohol (**5**) [7], were isolated from the roots of *C. subavenium*. All of these compounds were found for the first time from this plant.

The specimen of *C. subavenium* was collected from Wulai Hsiang, Taipei County, Taiwan in May, 2005. A voucher specimen (Cinnamo. 5) was identified by Dr. Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University) and was deposited in the School of Medical and Heath Science, The Fooyin University, Kaohsiung County, Taiwan. The air-dried roots of *C. subavenium* Miq. (1.1 kg) were extracted with MeOH (50 L × 6) at room temperature, and a MeOH extract (74.3 g) was obtained upon concentration under reduced pressure. The MeOH extract, suspended in H_2O (1 L), was partitioned with $CHCl_3$ (2 L × 5) to give fractions soluble in $CHCl_3$ (42.8 g) and H_2O (21.5 g). The $CHCl_3$-soluble fraction (42.8 g) was chromatographed over silica gel (800 g, 70–230 mesh) using *n*-hexane–EtOAc–MeOH mixtures as eluents to produce five fractions. Part of fraction 1 (7.22 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (1:5), enriched with EtOAc to furnish five further fractions (1-1–1-5). Fraction 1-2 (2.33 g) was subjected to silica gel chromatography, eluting with $CHCl_3$–MeOH (100:1), and enriched gradually with MeOH, to obtain five fractions (1-2-1–1-2-5). Fractions 1-2-1 (0.62 g) and 1-2-2 (0.32 g) were subjected to further silica gel column chromatography and purified by preparative TLC (thin layer chromatography) to yield isoobtusilactone A (**1**) (54 mg) and obtusilactone A (**2**) (21 mg). Part of fraction 2 (10.58 g) was subjected to silica gel chromatography by eluting with *n*-hexane–EtOAc (50:1), enriched with EtOAc to furnish two further fractions (2-1−2-5). Fraction 2-1 (2.24 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain eugenol (**3**) (183 mg). Part of fraction 2-3 (3.23 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain myristicin (**4**) (12 mg). Fraction 2-4 (1.98 g) was further purified on a silica gel column using *n*-hexane–EtOAc mixtures to obtain 3,4-methylenedioxy-5-methoxycinnamyl alcohol (**5**) (26 mg).



1, 2

1: $R_1 = (CH_2)_{12}CH_3$, $R_2 = H$
2: $R_1 = H$, $R_2 = (CH_2)_{12}CH_3$

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TABLE 1. Anti-oxidation, Reducing Power, and Metal Chelating Inhibition Effects of Compounds **1–5** at 100 μM

Compound	DPPH inhibition	Reducing power	Metal chelating inhibition
1	–	12	10
2	–	–	–
3	46	31	–
4	19	10	10
5	13	43	–

Vitamin C was used as a positive control on DPPH reactions. BHA (3-*tert*-butyl-4-hydroxyanisole) was used as a positive control on reducing power. EDTA was used as a positive control on chelating ability. (–): no significance inhibition ability; Results are mean values (n = 3).

Isoobtusilactone A (1) as in [4], colorless oil, $[\alpha]_D^{25}$ –32.3° (*c* 0.55, $CHCl_3$), UV (λ_{max}, nm): 220. IR (ν_{max}, cm^{-1}): 3400 (OH), 1745, 1680 (*α,β*-unsaturated *γ*-lactone). ^{1}H NMR (500 MHz, acetone-d_6, δ, ppm, J/Hz): 6.94 (1H, td, J = 8.0, 2.0, H-6), 5.32 (1H, br.s, H-3), 4.78 (1H, dd, J = 2.5, 1.5, H-5b), 4.63 (1H, dd, J = 2.5, 1.5, H-5a), 2.49 (2H, m, H-7), 1.54 (2H, m, H-8), 1.29 (20H, br.s, H-9–H-18), 0.88 (3H, t, J = 6.5, H-19), ^{13}C NMR (125 MHz, acetone-d_6, δ): 167.3 (C-1), 129.3 (C-2), 66.6 (C-3), 159.9 (C-4), 90.0 (C-5), 148.5 (C-6), 30.4–29.0 (C-7~C-16), 32.7 (C-17), 23.4 (C-18), 14.4 (C-19), FAB-MS *m/z:* 309 $[M + H]^+$.

Obtusilactone A (2) as in [4], colorless oil, $[\alpha]_D^{25}$ –28.1° (*c* 0.55, $CHCl_3$), UV (λ_{max}, nm): 220. IR (ν_{max}, cm^{-1}): 3400 (OH), 1745, 1680 (*α,β*-unsaturated *γ*-lactone). ^{1}H NMR (500 MHz, acetone-d_6, δ, ppm, J/Hz): 6.64 (1H, t, J = 7.5, H-6), 5.16 (1H, br.s, H-3), 4.71 (1H, s, H-5b), 4.57 (1H, s, H-5a), 2.73 (2H, m, H-7), 1.49 (2H, m, H-8), 1.29 (20H, br.s, H-9–H-18), 0.88 (3H, t, J = 7.0, H-19). ^{13}C NMR (125 MHz, acetone-d_6, δ): 166.1 (C-1), 128.7 (C-2), 69.1 (C-3), 159.7 (C-4), 89.0 (C-5), 149.6 (C-6), 28.6–30.7 (C-7–C-16), 32.7 (C-17), 23.4 (C-18), 14.4 (C-19), FAB-MS *m/z*: 309 $[M + H]^+$.

Eugenol (3) as in [5], colorless oil, UV (λ_{max}, nm): 230, 282. IR (ν_{max}, cm^{-1}): 3500, 1630, 1500, 1430. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.93 (1H, d, J = 8.8, H-6), 6.76 (1H, d, J = 2.0, H-3), 6.75 (1H, dd, J = 8.8, 2.0, H-5), 6.03 (1H, m, H-2′), 5.86 (1H, s, OH), 5.13 (2H, m, H-3′), 3.89 (3H, s, OCH_3), 3.38 (2H, d, J = 6.8, H-1′), ^{13}C NMR (100 MHz, $CDCl_3$, δ): 143.8 (C-1), 146.4 (C-2), 111.0 (C-3), 131.8 (C-4), 121.1 (C-5), 114.2 (C-6), 39.8 (C-1′), 137.8 (C-2′), 115.4 (C-3′), 55.8 (OCH_3), EI-MS *m/z*: 164 $[M]^+$.

Myristicin (4) as in [6], colorless oil, UV (λ_{max}, nm): 225, 292. IR (ν_{max}, cm^{-1}): 1655, 1040, 936 (-OCH_2O-). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.38 (1H, d, J = 1.2, H-4), 6.35 (1H, d, J = 1.2, H-6), 5.93 (2H, s, -OCH_2O-), 5.92 (1H, m, H-2′), 5.07 (2H, m, H-3′), 3.88 (3H, s, OCH_3), 3.29 (2H, d, J = 6.4, H-1′). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 143.4 (C-1), 148.8 (C-2), 134.5 (C-3), 102.6 (C-4), 133.4 (C-5), 107.6 (C-6), 40.2 (C-1′), 137.3 (C-2′), 115.8 (C-3′), 101.2 (-OCH_2O-), 56.4 (OCH_3), EI-MS *m/z*: 192 $[M]^+$.

3,4-Methylenedioxy-5-methoxy cinnamyl alcohol (5) as in [7], colorless needles, UV (λ_{max}, nm): 232, 272. IR (ν_{max}, cm^{-1}): 3420, 1045, 942. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.61 (1H, d, J = 1.2, H-4), 6.53 (1H, d, J = 1.2, H-6), 6.49 (1H, d, J = 15.6, H-1′), 6.21 (1H, dt, J = 15.6, 5.6, H-2′), 5.96 (2H, s, -OCH_2O-), 4.29 (2H, dd, J = 6.0, 1.6, H-3′), 3.90 (3H, s, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 143.5 (C-1), 149.1 (C-2), 135.0 (C-3), 100.0 (C-4), 131.6 (C-5), 106.7 (C-6), 130.9 (C-1′), 127.2 (C-2′), 63.6 (C-3′), 101.5 (-OCH_2O-), 56.5 (OCH_3), EI-MS *m/z*: 208 $[M]^+$.

The importance of diet for the prevention of some diseases is well recognized [8]. Antioxidant properties elicited by plant species have a full range of perspective applications in human health care. In recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables, or teas rich in natural antioxidants [9]. Antioxidant activities of plants have been claimed to have beneficial health functions for retarding aging and preventing cancer and cardiovascular diseases. The interest in antioxidants has increased remarkably over the last decade because of their protective effects against different diseases, including cardiovascular, inflammatory, and neurological diseases, as well as cancers. Antioxidant components are most important in foods because of their ability to reduce free radical-mediated degradation of cells and tissues in an organism [8–10].

The antioxidant properties of natural plant compounds should be evaluated in a variety of model systems using various different indices to ensure the effectiveness of such antioxidant materials.

In order to investigate the antioxidant activity of the roots of *C. subavenium*, free radical scavenging tests were conducted. The inhibition values for five pure compounds from the roots of *C. subavenium* in DPPH are given in Table 1. Eugenol (**3**) of *C. subavenium* showed a higher inhibitory effect for DPPH radical. Myristicin (**4**) and 3,4-methylenedioxy-5-methoxy cinnamyl alcohol (**5**) demonstrated minor inhibition results. However, isoobtusilactone A (**1**) and obtusilactone A (**2**) did not display antioxidation effects in DPPH. The antioxidant properties of *C. subavenium* compounds were due to the supply of hydrogen by the samples, which combined with radicals and formed a stable radical to terminate the radical chain reaction by acting as a chain break antioxidant. The other possibility was that the samples could combine with the radical ions that were necessary for the radical chain reaction, thus terminating the reaction.

In this assay, the yellow color of the test solution changed to various shades of green and blue depending upon the reducing power of each antioxidant sample. Table 1 depicts the reducing power of five pure compounds. The reducing power of *C. subavenium* compounds exhibited the following order: 3,4-methylenedioxy-5-methoxycinnamyl alcohol (**5**) > eugenol (**3**) > isoobtusilactone A (**1**) > myristicin (**4**) > obtusilactone A (**2**).

The ferrous ion chelating activities of *C. subavenium* compounds are shown in Table 1. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents such as antioxidant compounds, the complex formation is disrupted, resulting in a decrease in the red color of the complex. Isoobtusilactone A (**1**) and myristicin (**4**) showed minor metal scavenging effect among the samples.

The results obtained in this study clearly showed that *C. subavenium* compounds have mid-powerful antioxidant activity against various antioxidant systems *in vitro*; moreover, these pure compounds can be used as easily accessible sources of natural antioxidants and as a possible food supplement, and in cosmetics products and pharmaceutical applications. It can also be used in stabilizing food and cosmetics against oxidative deterioration.

Determination of DPPH Radical Scavenging Capacity. The antioxidant activity of the tested compounds was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH· method as modified by Katalinic et al. [11]. Briefly, various concentrations of the sample were added to 0.2 mL of DPPH· (60 μM) solution. When DPPH· reacts with an antioxidant compound donating hydrogen, it is reduced, resulting in a decrease in absorbance at 517 nm. The absorbance was recorded at 10 min intervals up to 30 min using a UV-vis spectrophotometer. The percentages of the remaining DPPH were plotted against the sample to obtain the amount of antioxidant necessary to reduce the initial concentration of DPPH. Scavenging activity (%) was determined as

$$100 \times (A_{\text{control}} - A_{\text{sample}})/\text{b}A_{\text{control}}$$

The preliminary data are listed in Table 1.

Reducing Power. The reducing powers of our natural pure compounds were determined according to the method of Gulcin et al. [12]. Briefly, various concentrations of sample (10^{-2} mol/L scale) in 63 μL of methyl alcohol were mixed with sodium phosphate buffer (0.1 mL 0.2 M, pH 6.8) and 2.5 μL of 20% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50° for 20 min, then 160 μL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 g. The upper layer of solution (75 μL) was mixed with distilled water (25 μL) and $FeCl_3$ (25 μL, 2%), and the absorbance was measured with a 96-well plate spectrophotometer at 650 nm (Table 1). A higher absorbance indicates a higher reductive capability.

Metal Chelating Inhibition Activity. The ferrous ion-chelating potential of chlorophyll was investigated according to the method of Decker and Welch [13]. Briefly, test samples at 100 μM final concentrations dissolving in DMSO were added to a solution of 2 mM $FeCl_2 \cdot 4H_2O$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL), and the mixture was vigorously shaken and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the mixture was read at 650 nm against a blank. EDTA was used as a positive control (Table 1). Data are expressed as means of three experiments.

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