Antifungal Agents from the Roots of Cudrania cochinchinensis against Candida, Cryptococcus, and Aspergillus Species

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Bioassay-guided fractionation resulted in the isolation of four antifungal agents from the roots of Cudrania cochinchinensis. Two of these were new compounds, cudraxanthone S and cudraflavanone B, and the remaining two compounds were known compounds, toxylxanthone C and wighteone. Among these compounds, 1 and 4 exhibited antifungal activities against Cryptococcus neoformans, Aspergillus fumigatus, and A. nidulans (MICs = 2–8 μg/mL). Compounds 1 and 3 also showed antifungal activity against Candida glabrata (MICs = 4–8 μg/mL).

The incidences of infections by opportunistic fungi are increasing, especially in patients whose immune systems are compromised by AIDS, cancer, diabetes, age, and other causes. Many antifungal compounds have been identified, but safe and effective antifungal drugs have not yet been developed because of the high degree of similarity between fungi and mammalian cells. Therefore, amphotericin B (AMPH), which was developed many years ago, is still widely used in treatment for deep-seated mycoses despite its serious side effects. Azole group antifungal agents, miconazole (MCZ), ketoconazole, fluconazole, and itraconazole, are also used clinically. The side effects of these antibiotics are relatively weak, but these medicines have nephrotoxicity and hepatotoxicity and cause vomiting and impotence. This has consequently resulted in a strong demand for drugs that have much weaker side effects. Medicinal plants that have been used for a long time may be good sources of safe antifungal agents. The water or traditional Chinese medicine. There is no report on side effects of this liquid medicine, but warnings are given for the treatment of gonorrhea, rheumatism, jaundice, hepatitis, boils, scabies, and bruising in C. cochinchinensis Lour. (Moraceae) is used for the treatment of gonorrhea, rheumatism, jaundice, hepatitis, boils, scabies, and bruising in traditional Chinese medicine. There is no report on side effects of this liquid medicine, but warnings are given for the treatment of gonorrhea, rheumatism, jaundice, hepatitis, boils, scabies, and bruising.

Cudraxanthone S decomposed at 162 °C and gave a positive reaction with FeCl₃ reagent by TLC. The compound was assigned a molecular formula of C₁₃H₁₆O₆ as inferred from its HR-MALDI-TOF-MS and NMR data. UV data suggested that it should have a 1,3,5,6-tetraoxygenated xanthone skeleton. The ¹H NMR spectrum of 1 contained signals of four hydroxyl groups in which one was hydrogen-bonded (δ 14.23), ortho-coupled aromatic signals (B ring), an aromatic singlet signal (A ring), and a set of signals due to a 1,1-dimethyl-2-propenyl group. The chemical shift of the hydrogen-bonded OH indicated the isoprenoid group occurred at the C-2 position. The ¹³C NMR signals of 1 were assigned with its HMQC and HMBC spectra (data not shown). The chemical shifts of the A ring carbons (C-1′-C-4, C-4a, and C-9a) of 1 resembled those of cudraxanthone P, having the same A ring, and those of the B ring (C-5′-C-8, C-4b, and C-8a) resembled those of macluraxanthone, with the same B ring (see Supporting Information). Furthermore, in the HMBC spectrum, the olefinic proton signal at δ 6.36 (H-2′) showed a cross-peak with C-2, also indicating that the group was located at C-2. Thus, cudraxanthone S was concluded to be 1,3,5,6-tetrahydroxy-2-(1,1-dimethyl-2-propenyl)xanthone (1). Cudraflavanone B (2), C₂₀H₂₀O₆, δ [α]₀° gave a positive reaction with FeCl₃ reagent by TLC. Its UV spectrum indicated that 2 was a flavanone or an isoflavanone. The NMR spectra showed characteristic signals of a flavanone together with a signal of an aromatic proton.
Trichophyton mentagrophytes and Microsporum gypseum (MICs = 12.5–25 μg/mL).  

**Experimental Section**

**General Experimental Procedures.** AMPH and MCLZ were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma (St. Louis, MO), respectively. α-Cyano-4-hydroxycinnamic acid (Aldrich, Milwaukee, WI) was used as the matrix of HR-MALDI-TOF-MS, and calibration was performed with two peaks of the matrix, m/z 190.05042 [M + H]+ and m/z 379.09301 [2M + H]+. The other general procedures, instruments, and chemicals were as described in our previous paper.  

**Plant Material and Extraction.** The roots of *C. cochinchinesis* Lour. (Moraceae) were collected in Xishuangbanna, Yunnan, PRC, in July 1998, and air-dried. The identity of plant material was verified by Prof. Zhong-Wen Lin (Kunning Institute of Botany), and a voucher specimen (KIB 98-720 Lin) was deposited in the Herbarium of the Department of Taxonomy, Kunning Institute of Botany, Academia Sinica. The dried and powdered roots (8.7 kg) were extracted with EtOH under reflux three times and filtered. The filtrate was evaporated to dryness to give a residue (1 kg), which was suspended in water and partitioned successively with n-hexane, EtOAc, and n-BuOH. The EtOAc-soluble portion investigated here was the same as that described previously.  

**Isolation.** The EtOAc-soluble portion (182 g) of the EtOH extract (DIZ = 13 mm) was subjected to column chromatography (CC) over silica gel eluted with a mixture of n-hexane and acetone to yield fractions 1–5. The following isolation procedure was guided by antifungal activity against *C. albicans* with a disk diffusion method (DDM), in which a paper disk was dipped directly into the eluted solution of each fraction of CC without concentration. Fraction 2 (n-hexane–acetone, 3:1; 93.7 g) was rechromatographed over silica gel (benzene–EtOAc, column 2) to yield fractions 2.1–2.20. Fraction 2.4 (benzene–EtOAc, 5:1; 8.3 g) was subjected to CC over silica gel (first column, benzene–chloroform (CHCl3); second column, n-hexane–EtOAc) to yield cudraxanthone S (1, 48 mg), toxylaxanthone C (3, 34 mg), and wighteone (4, 3 mg). From fraction 2.5 (15.5 g), 76 mg of (−)-aromadendrin (6, a) and 10.7% (c.p., MeOH), was obtained with crystallization from CHCl3–EtOAc. The mother liquor was chromatographed over silica gel (first column, benzene–acetone; second column, n-hexane–CHCl3; third column, benzene (saturated with H2O)–MeOH; fourth column, CHCl3 only), followed by ODS CC (MeOH–H2O, 4:1) to give cudraflavanone B (2, 11 mg). The known compounds were identified by comparison of their spectral data with those reported previously (see Supporting Information).  

**Cudraxanthone S (1):** granule (acetone); mp 162 °C (dec); FeCl3 reaction on TLC plates, blue-green; UV (MeOH) λmax (log ε) 202 (4.24), 252 (4.53), 284 (3.88), 327 (4.24) nm; H NMR (acetone-d6, 400 MHz) δ 14.23 (1H, s, OH-1), 8.8–9.5 (3H, br, OH), 7.61 (1H, d, J = 9 Hz, H-8), 6.93 (1H, d, J = 9 Hz, H-7), 6.43 (1H, s, H-4), 6.36 (1H, dd, J = 10, 17 Hz, H-2), 6.94 (1H, dd, J = 1, 17 Hz, H-3), 4.84 (1H, dd, J = 1, 12 Hz, H-3), 1.61 (6H, s, H-1′′ and H-5′′); 13C NMR (acetone-d6, 100 MHz) δ 181.4 (C-8), 168.6 (C-3), 163.9 (C-5), 156.4 (C-4a), 151.9 (C-6), 150.8 (C-2), 146.5 (C-6), 135.1 (C-5), 117.5 (C-8), 115.6 (C-2), 114.7 (C-8a), 113.5 (C-7), 108.6 (CH2, C-3′), 103.1 (C-9a), 95.2 (CH-4), 41.6 (C-1′), 29.2 (CH3, C-4′ and C-5′); HRMALDITOF MS m/z 329.1063 [M + H]+ (calcld for C18H17O6, 329.1025).  

**Cudraflavanone B (2):** resin (acetone–n-hexane); αP20° = 0° (c 0.200, MeOH); UV (MeOH) λmax (log ε) 202 (4.58), 225 (3.44), 291 (4.19), 335 (3.44) nm; H NMR (acetone-d4, 400 MHz) δ 12.49 (1H, s, OH-5), 9.4–8.3 (3H, br, OH), 7.29 (1H, d, J = 8 Hz, H-6), 6.46 (1H, d, J = 2 Hz, H-3), 6.31 (1H, dd, J = 2, 8 Hz, H-5′), 5.76 (1H, dd, J = 3, 13 Hz, H-2), 5.67 (1H, s, H-8), 5.23 (1H, m, H-2′′), 3.24 (2H, br, d, J = 7 Hz, H-2′), 3.13 (3H, dd, J = 3, 17 Hz, H-3), 2.68 (1H, dd, J = 13, 17 Hz, H-3), 1.73 (3H, br, s, H-5′′), 1.63 (3H, br, s, H-4′′); 13C NMR (acetone-d6, 100 MHz) δ 197.8 (C-1′), 164.8 (C-7), 162.5

(A ring), AXY type aromatic protons (B ring), a set of signals indicating a 3-methyl-2-buteryl (prenyl) group, and protons of a hydrogen-bonded OH and three normal OH groups. The chemical shift of 5-OH (δ 12.49) indicated that C-6 of 2 was substituted with the prenyl group. In the 1H NMR spectrum signals were assigned with HMOC and HMB spectra (data not shown), the signals of the ring A (C-4a, C-5–C-8, and C-8a) resembled those of 6-prenylidiotyl (3,4,5,7-tetrahydroxy-6-prenylflavanone), and those of the B ring (C-1′–C-6′) were similar to those of leachianone G (2,4,5,7-tetrahydroxy-8-prenylflavanone) (see Supporting Information). In the HMB spectrum, the methylene signal of the prenyl group (H-2′) showed cross-peaks with C-6 and C-5. Consequently, cudraflavanone B was elucidated as 2,4,5,7-tetrahydroxy-6-prenylflavanone (2).

Previously, we isolated 27 phenolic compounds from the benzene-soluble portion of an EtOH extract of *C. cochinchinesis.* This portion also showed antifungal activity using a disk diffusion method in the present investigation (diameter of inhibition zone (DIZ) = 9 mm). On the prescreening of antifungal compounds obtained from these active fractions (benzene- and EtOAc-soluble portions), eight compounds, 1, 2, 3, 4, 1.3, 7-trihydroxy-2-prenylxanthone (5), 12 cudraphenones C and D (6), and cudraphenone,11 were active compounds (DIZs = 9–11 mm). Minimum inhibitory concentrations (MICs) of 12 phenolic compounds (the eight active compounds and some related compounds) from the plant against human pathogenic fungi, Candida, Cryptococcus, and Aspergillus species, were determined (see Supporting Information).

Compound 1 exhibited antifungal activities against Candida glabrata, Cryptococcus neoformans, Aspergillus fumigatus, and *A. nidulans* (MICs = 2–4 μg/mL). The activities of 3, a cyclic derivative of 1, against these strains were slightly weaker than those of 1 (MICs = 8 μg/mL). The 1,3,7-trihydroxyxanthone with a hydrophobic group but without OHs at C-5 and C-6 (5) exhibited no activity against these microorganisms. The 1,5,6-trihydroxyxanthone with a hydrophobic group (6-deoxyacetereubin) also showed no activity against these strains (MICs > 128 μg/mL) with the exception of *C. neoformans* (MIC = 32 μg/mL). Recently, antifungal xanthones were isolated from Calophyllum caledonicum and *Tovomita krukovii.13* These compounds exhibit antifungal activities against *A. fumigatus* and/or *A. albicans* and have a hydrophobic group on the A ring and a hydroxyl group at C-5 or C-6. It is likely that antifungal xanthones from plants require a hydrophobic group on ring A and three or four OHs in which one or two OHs must be at C-5 and/or C-6. Benzophenones are considered to be biosynthetic precursors of xanthones. The benzophenones with two hydrophobic groups and three or four OHs (6 and cudraphenone B) exhibited antifungal activities against *C. neoformans,* *A. fumigatus,* and *A. nidulans* (MICs = 2–16 μg/mL).

Flavanone 2 showed only weak antifungal activity against some species (see Supporting Information). The isoflavone with a hydrophobic group on ring A (4) was the most potent of the 12 compounds, with MICs for two Aspergillus species (MICs = 2–4 μg/L) and *C. neoformans* (MIC = 4 μg/mL). On the other hand, the isoflavone without a hydrophobic group (3-O-methylorobol) showed no anti- fungal effect against all fungi investigated here. Previously, it was reported that flavones with two hydrophobic groups (morusin and kuwanon C, which are more hydrophobic than 4) exhibit no antifungal activity except against...
solution (12.8 mg/mL) was diluted with RPMI-1640 medium.

All compounds were dissolved in dimethyl sulfoxide, and the standards with a little modification as described previously.17

(C, C-5), 162.3 (C, C-8a), 156.3 (C, C-2′), 131.1 (C, C-3′), 129.0 (CH, C-6′), 123.7 (CH, C-2′′), 117.6 (C, C-1′), 108.9 (C, C-6), 107.9 (CH, C-5′), 103.5 (C and CH, C-4a and C-3′), 95.3 (CH, C-8), 75.3 (CH, C-2′′), 42.8 (CH2, C-3), 25.8 (CH3, C-4′′), 21.5 (CH2, C-1′′), 17.8 (CH3, C-5′′); HRMALDITOFMS m/z 357.1336 [M + H]+ (calcd for C20H21O6, 357.1338).

Test Microorganisms. Strains of IFM (the collection of the former Institute of Food Microbiology, present RCPFMT) were derived from the collection of RCPPM (Aspergillus fumigatus IFM 41374, A. fumigatus IFM 41236, A. nidulans IFM 5369), and Candida albicans ATCC 90028, C. parapsilosis ATCC 22019, C. glabrata ATCC 90030, C. krusei ATCC 6258, and Cryptococcus neoformans ATCC 90112 were from American Type Culture Collection (Rockville, MD). Yeasts were maintained on modified Sabouraud’s dextrose agar slant (2% glucose, 1% peptone, and 1.5% agar) (SDA) or potato dextrose agar (Difco Laboratories, Detroit, MI) slant (PDA). Filamentous fungi were also maintained on PDA.

Disk Diffusion Method (DDM). Standard samples were dissolved in MeOH at 100 μg/mL (AMPH) and 10 μg/mL (MCZ). A paper disk (1.5 mm thick, 8 mm diameter, Tokyo Roshi, Tokyo, Japan) was dipped into the solution, and then the disk was air-dried on a filter paper on a clean bench. The inoculum amount of the stock culture of C. albicans ATCC 90028, preincubated in YPD-Broth (Difco), used for the DDM was adjusted by measuring the diameter of inhibition zone (DIZ) of the standard samples as follow: DIZs of AMPH and MCZ were 22 and 14 mm, respectively, when incubated on (DIZ) of the standard samples as follow: DIZs of AMPH and MCZ were 22 and 14 mm, respectively, when incubated on agar (Difco Laboratories, Detroit, MI) slant (PDA). Filamentous fungi were also maintained on PDA.

MIC Measurement. Broth microdilution was according to the method of the National Committee for Clinical Laboratory Standards with a little modification as described previously.17 All compounds were dissolved in dimethyl sulfoxide, and the final concentration of the solvents was less than 1%. The stock solution (12.8 mg/mL) was diluted with RPMI-1640 medium with a serial 2-fold dilution to concentrations from 0.128 to 128.0 μg/mL. Details of the procedure are given in the Supporting Information.

Supporting Information Available: Table of antifungal activity (MICs). 13C NMR data of compounds related to 1 and 2, data of known compounds, structures of tested compounds, and details of the MIC measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes


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