A New Meroterpenoid, Chrodrimanin C, from YO-2 of Talaromyces sp.

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Received November 10, 2011; Accepted December 15, 2011; Online Publication, April 7, 2012

The new meroterpenoid, chrodrimanin C (3), together with chrodrimamins A (2) and B (1) were isolated from okara (the insoluble residue of whole soybean) that had been fermented with strain YO-2 of Talaromyces sp. Their structures were elucidated by spectroscopic methods. The partial structures of 1 essential for exhibiting insecticidal activity were investigated by using a silkworm assay. The absolute configuration of 1 was also determined.

Key words: insecticidal compound; chrodrimamin; meroterpen; Talaromyces

Fungal metabolites have long been known to show a wide variety of bioactivities;¹ for example, azaphilones have been reported to exhibit a broad range of interesting biological effects such as antimicrobial, antifungal, antiviral, cytotoxic, nematicidal, and anti-inflammatory activities.² In addition, torrubliellones isolated from the spider pathogenic fungus of Torrubiella sp. have been shown to exhibit antimalarial activity.³ Omura has recently published a fascinating review of microbial metabolites with biological activities.⁴

In our continuing studies on bioactive fungal metabolites using a bioassay with silkworms (Bombyx mori), we have so far isolated some strains from soil samples collected in Osaka. Penicillium simplicissimum ATCC 90288 produced the insecticidal indole alkaldoids, okaramines A,⁵ B,⁵ K,⁵ L,⁶ Q⁶ and R.⁷ A soil isolate of Penicillium expansum MY-57 produced the new conmenisin congeners, conmenisins D, E and F, as insecticidal compounds.⁸

We also screened microbes for insecticidal activity, resulting in the isolation of strain YO-2 of Talaromyces sp. This strain produced the known metabolites chrodrimamins A (2) and B (1), together with the new derivative chrodrimanin C (3). We report here the isolation and identification of insecticidal compound 1, the structural determination of 3, and their biological activities. The absolute configuration of chrodrimanin B (1) is also described.

Materials and Methods

Melting point (mp) data are uncorrected, and optical rotation was measured with a Horiba model SEPA-300 polarimeter. IR spectra were recorded with a Jasco FT/IR-460 spectrophotometer, mass spectra were recorded with a Jeol JMS-700 instrument, and ¹H- and ¹³C-NMR spectra were obtained with a Jeol JNM AL-400 spectrometer. Chemical shifts are referenced to residual CHCl₃ at 7.26 ppm for the ¹H spectra, to CDCl₃ at 77.0 ppm for the ¹³C spectra, to DMSO at 2.50 ppm for the ¹H spectra, and to DMSO-d₆ at 39.5 ppm for the ¹³C spectra, each being reported relative to TMS. Column chromatography was performed with Wakogel C-200 (Wako Pure Chemical Industries) and Inertsil ODS-3 (GL Sciences). The okara used as a medium in this experiment was kindly supplied by the Shirayukihime tofu (bean-curd) shop (Sakai, Osaka, Japan).

Bioassay. Larvae of B. mori were used for the bioassay and were cultured on an artificial diet purchased from Nippon Nossan Kogyo Co.

To one gram of the diet, 100 μL of an acetone extract or a certain amount of the sample to be tested was added in a Petri dish. After removing the solvent, ten larvae at the third instar stage were introduced into the Petri dish. Thirty larvae (three Petri dishes) were treated at each dosage, and the insecticidal activity of the silkworms was observed 24 h after initiating the administration. The survival rates were measured and the LD₅₀ value was determined from the survival curve.

Fermentation. The YO-2 strain of Talaromyces sp. was isolated from a soil sample collected in Izumi (Osaka, Japan) in the usual manner. This strain was identified at Centraalbureau voor Schimmelcultures (the Netherlands).

A loopful of spores from a slant culture of the Talaromyces strain was inoculated into 30 g of okara in a Petri dish of 9 cm in diameter, and cultivation was carried out at 25°C for 14 d.

Extraction and preliminary separation. The okara (10 kg) that had been fermented with strain YO-2 was soaked in acetone. Evaporation of the acetone gave an aqueous concentrate which was extracted three times with EtOAc. The resulting EtOAc extract (13.3 g) was chromatographed on Wakogel C-200 by eluting with MeOH:CHCl₃ (M : C) 28:54 (M⁺); mp 276–291°C (dec.); EIMS m/z 484 (M⁺)

Chrodrimanin B (1). Colorless needles; [α]D₃⁰ = –41.1° (c 0.111, MeOH-CHCl₃ 1:1); [α]D₃⁰ = –54.5° (c 0.055, CHCl₃); mp 276–291°C (dec.); EIMS m/z 484 (M⁺). CHrodrimanin C (2). Colorless needles; [α]D₃⁰ = +8.2° (c 0.098, MeOH-CHCl₃ 1:1); mp 279–288°C (dec.); FAB-MS m/z 443 (M + H⁺).

Chrodrimanin C (3). Colorless needles; [α]D₃⁰ = –49° (c 0.021, MeOH-CHCl₃ 1:1); mp 254–256°C. HR-FAB-MS m/z 413.2336 (M + H⁺) (calcd. for C₂₅H₃₆O₃, 413.2328). EI-MS m/z (%): 412 (M⁺, 54), 245 (16), 207 (100), 195 (24), 194 (49), 189 (13). UV max (MeOH) nm (ε): 220 (19,200), 272 (7600), 312 (3600). IR νmax (KBr) cm⁻¹: 3395, 1668, 1476, 1384, 1131, 1254, 1172, 1111. ¹H- and ¹³C-NMR: see Table 1.

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Preparation of the (S)- and (R)-MTPA ester derivatives of I. (R)- (−)-MTPA chloride (15.1 mg), 4-dimethylaminopyridine (6.0 mg) and triethylamine (10 μL) were added to a solution of chrodrimanin B (1; 5.0 mg) in CHCl₃ (500 μL). The reaction mixture was stirred at room temperature. After 10 h, the reaction mixture was diluted with 1.0 N HCl and the aqueous phase was extracted with EtOAc. The organic layer was recovered, dried over anhydrous Na₂SO₄, filtered and concentrated to dryness. The product was purified by HPLC, employing isocratic elution with MeCN-water (80:20, v/v) at a flow rate of 5.0 mL/min to yield 4 (7.0 mg). Similarly, 5 (5.5 mg) was obtained by using (S)-(+)-MTPA chloride.

7.4′-(S)-α-Methoxy-α-(trifluoromethyl)phenylacetoxy chrodrimanin B (4). FAB-MS m/z: 917 (M + H⁺). ¹H-NMR (400 MHz, CDCl₃) δ: 0.90 (3H, s), 1.02 (3H, s), 1.15 (3H, s), 1.39 (3H, s), 1.44 (3H, d, 6.6), 1.73 (1H, ddd, 15.0, 14.2, 1.4), 2.09 (1H, dd, 14.0, 5.0), 2.20 (3H, s), 2.24 (1H, dd, 15.0, 8.8, 5.6), 2.64 (1H, dd, 16.0, 14.0), 3.00 (1H, dd, 16.0, 5.0), 3.54 (3H, s), 3.77 (3H, s), 4.69 (1H, qd, 6.6, 1.6), 5.38 (1H, dd, 8.6, 3.8), 6.01 (1H, d, 10.2), 6.14 (1H, d, 1.6), 6.69 (1H, dd, 14.0, 5.4), 7.16 (1H, d, 10.2), 11.02 (1H, s).

Chrodrimanin B 7-acetate (7). EI-MS (m/z): 526 (M⁺), 486 (10), 451 (20), 406 (54), 391 (100), 270 (11), 205 (12). ¹H-NMR (400 MHz, CDCl₃) δ: 1.14 (3H, s), 1.16 (3H, s), 1.34 (6H, s), 1.47 (3H, d, 6.6), 1.80 (1H, td, 14.2, 3.8), 2.11 (3H, s), 2.16 (3H, s), 2.15–2.35 (3H, m), 2.60 (1H, ddd, 15.0, 14.0), 2.97 (1H, dd, 15.0, 5.0), 4.71 (1H, qd, 6.6, 1.6), 5.38 (1H, dd, 8.6, 3.8), 6.01 (1H, dd, 10.2), 6.14 (1H, d, 1.6), 6.45 (1H, s), 7.16 (1H, d, 10.2), 11.02 (1H, s).

Chrodrimanin C 7,4-diacetate (8). EI-MS m/z (%): 568 (M⁺), 7, 526 (15), 508 (12), 466 (23), 406 (80), 391 (100), 270 (14), 205 (13). ¹H-NMR (400 MHz, CDCl₃) δ: 1.14 (3H, s), 1.16 (3H, s), 1.35 (3H, s), 1.35 (3H, s), 1.44 (3H, d, 6.6), 1.86 (1H, td, 14.4, 2.8), 2.16 (3H, s), 2.15–2.35 (3H, m), 2.37 (3H, s), 2.63 (1H, d, 15.8, 14.0), 3.02 (1H, dd, 15.8, 5.0), 4.66 (1H, qd, 6.6, 1.6), 6.01 (1H, d, 10.2), 6.15 (1H, d, 1.6), 6.69 (1H, s), 7.16 (1H, d, 10.2).

Chrodrimanin B 7,4-di trifluoromethylacetate (9). Chrodrimanin B (20 mg) was dissolved in CHCl₃ (1.0 mL). Palladium-activated carbon (0.5 mg) was added to this solution. The solution was stirred at room temperature for 24 h and then poured into ice-cooled water. The mixture was extracted with EtOAc, and the extract (110 mg) was loaded into an Inertsil ODS-3 column (250 × 10.0 mm) equipped with a photodiode array detector. Separation by employing isocratic elution with MeCN-water (80:20, v/v) at a flow rate of 2.36 mL/min gave 7 (14.6 mg, tR 16.2 min), 8 (18.0 mg, tR 23.3 min), and 6 (5.7 mg, tR 33.3 min).

Chrodrimanin B 7-acetate. EI-MS m/z (%): 526 (M⁺), 326 (55), 393 (71), 270 (100), 255 (21), 205 (58). ¹H-NMR (400 MHz, CDCl₃) δ: 0.99 (3H, s), 1.09 (3H, s), 1.11 (3H, s), 1.38 (3H, s), 1.46 (3H, d, 6.4), 1.58 (1H, m), 1.70 (1H, m), 1.90–2.05 (3H, m), 2.13 (3H, s), 2.16 (1H, m), 2.4–2.7 (4H, m), 3.00 (1H, brs, 7.0-H), 4.03 (1H, t, 7.6), 4.69 (1H, dd, 6.4, 1.6), 6.09 (1H, d, 1.6), 6.49 (1H, s), 11.03 (1H, s).

Results and Discussion

Strain YO-2 obtained as a soil isolate of Talaromyces sp. showed insecticidal activity against the silkworm. An acetone extract of okara that had been fermented with this strain was extracted with EtOAc after evaporating the acetone. The ethyl acetate fraction was chromatographed on Wakogel with a hexane-ethyl acetate mixture, using the insecticidal activity to monitor the separation. The 60–100% EtOAc eluates were crystallized to yield chrodrimanin B (1), and the filtrate was rechromatographed on Wakogel with a hexane-acetone mixture to obtain chrodrimanins A (2) and C (3).

Chrodrimanins B (1) and A (2) were isolated as colorless needles. Their structures were established on the basis of EIMS, IR, UV, and NMR data. The spectral data for 1 and 2 were identical to those previously reported in the literature.⁹ Chrodrimanin C (3) was found to have the molecular formula of C₂₅H₂₉O₈ by the HR-FABMS and NMR data shown in Table 1, indicative of ten degrees of unsaturation. The ¹⁳C-NMR spectrum in DMSO-d₆ showed 35 resolved signals, these being classified by an analysis of DEPT and HMQC spectra into five methyl carbons, six methylene carbons, two sp² methine carbons, one oxygenated sp³ methine carbon, two sp² quaternary carbons, one oxygenated sp³ quaternary carbon, one sp² methine carbon, three sp² quaternary carbons, two oxygenated sp² quaternary carbons, and two carbonyl carbons. The ¹H-NMR spectrum in DMSO-d₆ exhibited 32 proton signals, one of which was assigned to a hydroxyl proton (δ₁H 11.02). The connectivity of the proton and carbon atoms was established by the HMQC spectrum (Table 1). These spectral data from the NMR data and physicochemical properties indicated that chrodrimanin C (3) had the same carbon skeleton as that of chrodrimanin A (2). A comparison of the ¹H-NMR spectra between 2 and 3 indicated that two hydroxy groups at C-7 and C-7′ in 2 were diminished in 3. In addition, a double bond at C-1=C-2 in 2 was saturated in 3. The COSY spectrum of 3 showed correlations of H-1/H-2, H-5/H-6/H-7, H-9/H-11, and H₂-7'/H₂-8'/H₃-9', indicating four partial units of a sesquiterpene. The HMBC spectrum shown in Table 1 permitted completion of the decalin ring system (A and B), a dihydropyran (C), an aromatic ring (D), and a 4-lactone (E). These spectral characteristics, shown in Fig. 2, led to the conclusion that chrodrimanin C (3) was 7',7'-dihydroxy-1,2-dihydrochrodrimanin A. The structure satisfied the degree of unsaturation and the molecular formula. The relative stereochemistry of chrodrimanin C was established by the NOESY spectrum, indicating trans-fused A/B and B/C ring systems as shown in Fig. 1.

To elucidate the absolute configuration of the chrodrimanins, the (S)- and (R)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) esters (5) were prepared by treating chrodrimanin B with (R)-(−)- and (S)-(+) MTPA chloride in the presence of 4-dimethylaminopyridine and triethylamine. The ¹H-NMR spectra of 4 and 5 were completely assigned. As shown in Fig. 3, the
calculated Δδ values (Δδ = δS - δR) for H-9, H2-11, H3-12, H-5', H-7' and H-8' were all positive, whereas those for H-5, H2-6, H3-13, H1-14 and H3-15 were all negative, indicating the absolute stereochemistry of the seven chiral centers in chrodrimanin B to be 5R,7SR,9S,10S7'R8'R. It is plausible that all chrodrimanins were biosynthesized by the same pathway, enabling the absolute stereochemistry of chrodrimanins A and C to be deduced to be the same as that of chrodrimanin B.

The insecticidal activity of chrodrimanins 1, 2 and 3 against the third instar larva of the silkworm is listed in Table 2. Chrodrimanin B exhibited insecticidal activity with an LD50 value of 10μg/g of diet, while chrodrimanins A (2) and C (3) were inactive. To evaluate the partial structures responsible for exhibiting insecticidal activity, we made acetates at C-7 and C-4, and a dihydroderivative of 1, 7-Acetate (6), 4'-acetate (7), and 7,4'-diacetate (8) showed almost the same activity as that of 1, strongly indicating that the hydroxy groups at C-7 and C-4' did not play an important role in exhibiting insecticidal activity. Hydrogenation of the double bond at C1 = C2 in 1 diminished this activity, indicative of the importance of this double bond. These findings strongly suggested that the co-existence of an acetoxy group at C-7 and a double bond at C1 = C2 was essential to exhibit strong insecticidal activity.

Chrodrimanins A and B were first reported as metabolites produced by Penicillium variabils, with chrodrimanin B being insecticidal and having insect-repelling effects on Lepidoptera.20

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<th>Compounds</th>
<th>Activity (LD50 μg/g diet)</th>
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Apart from chrodrimanins, kampanols, \textsuperscript{10} thailandolides, \textsuperscript{11} and pentacecilides\textsuperscript{12,13} have been reported as merodrimanes. Kampanols isolated from a fungal culture of \textit{Stachybotrys kampalensis} have inhibited Ras farnesyl-protein transferase. \textsuperscript{10} Thailandolides have been discovered as metabolites of \textit{Talaromyces hacilliporus}. \textsuperscript{11} Pentacecilides produced by \textit{Penicillium cecidica} has inhibited lipid droplet formation in mouse macrophages. \textsuperscript{12} Thailandolides, pentacecilides, and chrodrimanins have a common carbon skeleton, in which the decalin ring system is trans-fused in all three groups. Ring fusion of the B ring of a decalin unit and a dihydropyran ring (C) is of cis-type in thailandolides and pentacecilides, and of trans-type in chrodrimanins. Thailandolide B is a C-9 epimer of chrodrimanin B. Although the structure of thailandolide B was confirmed by X-ray crystallography, observation of the ORTEP drawing of thailandolide A\textsuperscript{11} strongly suggested the fusion of the B and C rings to not be cis, but trans fusion. Moreover, thailandolide B has given proton and carbon spectra nearly identical to those observed in this study, and previously reported for chrodrimanin B.\textsuperscript{10} We conclude from all these findings that thailandolide B was the same compound as chrodrimanin B.

\textbf{References}