Pyrenolide D, a New Cytotoxic Fungal Metabolite from Pyrenophora teres

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A new tricyclic spiro-γ-lactone with cytotoxic activity, named as pyrenolide D (I), was isolated from the phytopathogenic fungus Pyrenophora teres (Diedicke) Drechsler (IFO 7508), from which pyrenolides A, B, and C had previously been isolated. In this paper, we describe the structural determination of I by spectroscopic methods. Similar tricyclic fungal metabolites, cephalosporolide E and F, have been reported from Cephalosporium aphidicolare.

During an investigation on fungal morphogenic metabolites, a new crystalline substance was isolated from the ethyl acetate extract of a culture broth of the fungus. The fungus was surface-cultured in a malt-dextrose medium (350 ml in a 1 liter flat-bottomed flask) for a month. The filtered culture broth (pH 6.5, 85 liters) was passed through a column of XAD-2, and the column eluted with methanol. The methanol eluate was concentrated to an aqueous suspension and extracted by ethyl acetate. The extract (16 g) was subjected to silica gel (Fujigel, BW-6:3:5-MH) column chromatography in mixed solvent systems of ethyl acetate-n-hexane (stepwise gradients of the following compositions: EtOAc:n-hexane = 1:5:85, 2:20:80, 3:30:70, 4:50:50, 5:70:30, and 6:100:0 (v/v)). The fractions containing I revealed by TLC monitoring (silica gel 60 F254 aluminium sheet (Merck), Rf = 0.75, CHCl3:MeOH = 5:1 (v/v)) were combined and applied to a column of Sephadex LH-20 (MeOH). The pyrenolide D fractions were combined and evaporated to afford a crude crystalline residue, which was recrystallized from ethyl acetate-n-hexane to give colorless needles (800 mg), mp 158°C, [α]D 102.5 + 79.5 (c 0.9, CHCl3).

The molecular formula of I was determined to be C16H18O3 (M+, m/z 212.0679; caled. 212.0684) through high-resolution mass spectrometry, the mass spectrum showing the following prominent ions: m/z 194 (65%, C14H11O2), 116 (99%, C9H11O4), and 97 (100%, C5H10O4). The latter two fragment ions are thought to have been formed by fission of a hydroxyl group into two parts, one forming the ion m/z 116 and the other producing the ion m/z 97 accompanied by a hydrogen shift. The UV spectrum of I showed an end absorption in methanol. This absorption can be assigned to the γ-butenolide moiety in I, the presence of which was deduced from the following IR, 1H, and 13C-NMR data. The IR spectrum of I showed hydroxyl (3450 cm⁻¹) and α,β-unsaturated γ-lactone (1775 and 1760 (sh) cm⁻¹) groups. The 1H- and 13C-NMR (δ4-acetone) data of I were as follows: δH 7.47 (1H, d, J = 5.5 Hz, H-3), 6.21 (1H, d, J = 5.5 Hz, H-2), 5.05 (1H, dd, J = 7.4, 4.9 and 3.4 Hz, H-6), 4.67 (1H, d, J = 4.9 Hz, H-7), 4.57 (1H, d, J = 5.6 Hz, O=CH), 4.10 (1H, dq, J = 6.3 and 3.0 Hz, H-9), 3.99 (1H, dd, J = 5.6 and 3.0 Hz, H-8), 2.54 (1H, dd, J = 14.8 and 7.4 Hz, H-5), 2.36 (1H, dd, J = 14.8 and 3.4 Hz, H-5a), and 1.20 (3H, d, J = 6.3 Hz, H-10); δC 170.19 (C-1), 153.07 (C-3), 124.30 (C-2), 115.89 (C-4), 92.26 (C-7), 81.17 (C-6), 76.71 (C-9), 76.53 (C-8), 42.86 (C-5), and 13.45 (C-10). The assignments of their chemical shifts were based on 1H-1H and 1H-13C COSY spectra. The magnitude of the vicinal coupling constant, JHH = 5.5 Hz, is characteristic of Z-olefinic protons on a five-membered ring. In the long-range 1H-13C COSY spectra, each olefinic proton of H-2 and H-3, and each carbon of the quaternary acetel at δc 115.89 (C-4) and of the ester carbonyl at δc 170.19 (C-1) showed correlation peaks to each other. To the β-carbon of the butenolate at δc 153.07 (C-3), the proton at δh 2.36 (H-5a) was cross-correlated, showing connectivity of the methylene group to the next acetal carbon. The remaining part of the middle five-membered ring in I was deduced from the 1H-1H COSY spectrum. Since no proton–proton coupling between H-7 and H-8 was observed, the dihedral angle between these protons should be nearly 90 degree. The connectivity of this part was deduced from the long-range 1H-13C COSY spectrum. The proton appearing at δh 3.99 (H-8) was cross-correlated to δc 92.26 (C-7) and to δc 81.17 (C-6). The remaining part was deduced from the 1H-1H COSY spectrum. Based on these spectral data, the unique tricyclic structure shown in I was deduced. This structural assignment is also supported by the HMBC spectrum of I. The relative stereochemistry of the tricyclic system was assigned from the phase-sensitive NOESY spectrum of I. The β-olefinic proton of H-3 showed correlation peaks to the protons at δh 2.36 (H-5a) and at δc 4.10 (H-9). Correlation peaks were observed between three of the four protons, leaving the one appearing at δh 2.36 (H-5a), on the middle five-membered ring. Therefore, the one appearing at δh 2.36 (H-5a) was located opposite to the remaining three protons on the ring. The correlation peak observed between H-7 and the hydroxyl protons indicated a β orientation of the hydroxyl group. The H-9 proton showed correlation to the protons of H-8, H-5a, and H-3, indicating the relative stereochemistry of the methyl, the hydroxyl and the γ-butenolide ring groups as shown in I (see Fig. 2). Therefore, the structure of pyrenolide D was established to be I, although its absolute stereochemistry remains to be determined.

Biogenetically, pyrenolide D may arise from hydration of the epoxide group in pyrenolide A, followed by reacionization and acetal formation, an analogous sequence to that postulated for the cephalosporolides. Attempts to transform pyrenolide A to pyrenolide D in the laboratory were unsuccessful. Moreover, we could not isolate other possible structural isomers like the cephalosporolides. We consider that the formation of pyrenolide D only occurred by fungal metabolism.

Although I was not active to fungi like other pyrenolides, I was found to be cytotoxic to HL-60 cells at IC50 4 μg/ml.

Fig. 1. Structure of I.

Fig. 2. The Arrows Indicate the Pairs of Protons among Which Correlation Peaks Were Observed in the Phase-sensitive NOESY Spectrum of I.

Not all of the pairs are shown. This model was created by the Chem3D program and may not represent the precise conformation of I.

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References and Notes
1) One of the authors (M.N.) presented the plain structure of 1 at the 30th Symposium on The Chemistry of Natural Products held at Fukuoka in 1988. Although at that time the name pyrenolidol I was used, we prefer to use pyrenolidol D instead of pyrenolidol I to avoid confusing 1 and I.


