Fungal Citridone D Having a Novel Phenylfuropyridine Skeleton

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Citridone D was isolated from the culture broth of Penicillium sp. FKI-1938 by solvent extraction, silica gel column chromatography and HPLC. The structure of citridone D was elucidated by spectroscopic analysis including NMR analysis. Citridone D was found to have a novel phenylfuropyridine skeleton different from those of other citridones. Citridone D potentiated miconazole activity against Candida albicans.

Key words citridone; miconazole potentiator; Penicillium sp.; phenylfuropyridine; fungal metabolite

Oppportunistic infections caused by certain fungi, in particular problematic Candida albicans, have increased and the therapy of these infections is clinically important.1) Patients with compromised immune systems, e.g., patients receiving organ transplants and cancer chemotherapy or those infected by human immunodeficiency virus (HIV) are particularly prone to such infections.1) Azole derivatives, which inhibit fungal ergosterol biosynthesis by blockade of the cytochrome P-450 reaction involved in 14-alpha demethylation, are the most commonly used antifungal agents.

Employing the new concept of “anti-infective drugs,”2) including classical antibiotics, vaccines and agents which control microbial adaptation/survival or pathogenesis, potentiate the activity of known antibiotics or enhance the host immune system against microbial infection, we discovered actofunicine,3) beauvericins5) and phenatic acids5) of microbial origin as potentiators of miconazole activity against C. albicans. Recently, citridones A, B, B’ and C6) were also discovered from the culture broth of Penicillium sp. FKI-1938 during a screening program (Fig. 1). Further investigation of the culture broth lead to discovery of a new citridone (designated citridone D). In this study, we describe the isolation, structural elucidation and miconazole-potentiating activity of citridone D. Citridone D was found to have a novel phenylfuropyridine skeleton different from those of other citridones.

Experimental

General Experimental Procedures The strain FKI-1938 was isolated from soil collected at Ishigaki Island, Okinawa, Japan and was used for production of citridone D. C. albicans ATCC64548 was purchased from ATCC (Virginia, U.S.A.). Optical rotations were recorded with a DIP-370 digital polarimeter (Jasco, Tokyo, Japan). FAB-MS spectrometry was conducted on a JMS-AXS05H spectrometer (Jeol, Tokyo, Japan). UV and IR spectra were measured with a DU640 spectrophotometer (Beckman, California, U.S.A.) and an FT-210 Fourier transform infrared spectrometer (Horiba, Kyoto, Japan), respectively. The various NMR spectra were measured with a MERCURY plus 300 MHz spectrometer (Varian, California, U.S.A.).

Assay for Miconazole-Potentiating Activity C. albicans was inoculated into a 50-ml test tube containing 10 ml of seed medium (potato extract containing peptone 0.5% and glucose 1%), and was grown for 24 h on a rotary shaker. In this method,3) the seed culture of C. albicans (0.1%, v/v) was transferred to the two different agar plates, CY agar (glucose 1%, yeast extract 0.5% and agar 0.8%) (Plate A) and CY agar plus miconazole (0.06 mm) (Plate B). The concentration (0.06 mm) of miconazole used was one-fourth the MIC value against C. albicans, and showed no effect on the growth of C. albicans. Paper disks (8 mm, Toyo Roshi Kaisya, Ltd., Tokyo, Japan) containing a sample were put on Plates A and B, which were incubated at 27 °C for 24 h.

Results and Discussion

Isolation of Citridone D from the Culture Broth of Penicillium sp. FKI-1938 To 7-d old culture broth (201) of Penicillium sp. FKI-1938, acetone (201) was added. After the acetone extracts were filtered and concentrated, the aqueous solution (pH 7.1) was extracted with ethyl acetate (51) to remove citridones A to C. The pH of the resulting aqueous solution was adjusted to 3.0 with HCl and extracted with ethyl acetate again. The organic layer was dried over Na2SO4 and concentrated in vacuo to dryness to yield an oily material (1.4 g). The material was dissolved in a small volume of CHCl3, applied to a silica gel column (80 g, 4.0×18 cm, 70–230 mesh, Merck), and eluted with CHCl3–CH3OH solutions. Citridone D was recovered in the 25:1 fraction, which was concentrated to give a brown material (233.8 mg).

The physico-chemical properties of citridone D are summarized in Table 1. The HR-FAB-MS spectrum indicated a molecular formula of C19H20NO4 ([M+H]+ Found m/z 326.1381; Calcd 326.1392), implying eleven degrees of unsaturation for the compound. The IR spectrum indicated the presence of a hydroxyl group (3500–3100 cm−1) and the UV spectrum (MeOH) displayed two peaks of absorption (203, 232 nm), suggesting the presence of hydroxy compounds.
of the DEPT spectra (Table 2). The 1H-NMR spectrum (in CDCl₃) showed 19 resolved signals, which were classified as four methyl carbons, three methine carbons, six sp² methine carbons and seven (five sp²) quaternary carbons by analysis of the DEPT spectra (Table 2). The 1H-NMR spectrum (in CDCl₃) showed three methyl signals, four methine signals and five aromatic signals. The connectivity of proton and carbon atoms was established by the HMOC spectrum, as shown in Table 2. Analysis of the 1H-1H COSY and HMBC spectra revealed two partial structures, I and II (Fig. 2). As done B6). Regarding the partial structure II, extraordinary cross peaks from 2-H to C-7a yielded a connection between C-3 and C-3a, and the quaternary carbon (C-3', δc 89.6) suggested that it is bound to two oxygens. Cross peaks were observed from 2-H (δ 4.75) to C-7a (δ 165.5), C-2' (δ 78.2), C-3' (δ 89.6), C-5' (δ 102.2), and C-6' (δ 15.6), from 1'-H₃ (δ 1.32) to C-2' and C-3', from 2'-H (δ 3.79) to C-2 (δ 91.1), C-3' and C-4' (δ 12.2), from 4'-H₁ (δ 1.50) to C-2, C-2' and C-3', from 5'-H (δ 5.52) to C-2, C-3 (δ 59.6), C-2' and C-3' and from 6'-H₁ (δ 1.68) to C-2, C-3, C-3a and C-5' in the HMBC experiments to give the partial structure II (Fig. 2). Finally, the partial structures I and II were connected as shown in Fig. 2 for the following reasons; the cross peaks from 6'-H₁ to C-3a yielded a connection between C-3 and C-3a, and the cross peaks from 2-H to C-7a yielded a connection between C-2 and C-7a beyond an oxygen. The structure satisfied the molecular formula and the degree of unsaturation.

The relative configuration of C-2, C-3, C-2', C-3' and C-5' was studied by NOE experiments (Fig. 3). The cross peaks from 2-H to 1'-H₃, 4'-H₁ and 6'-H₁, from 1'-H₃ to 2'-H and from 6'-H₁ to 2-H and 5'-H indicated that 2-H, 1'-H₃, 4'-H₁ and 6'-H₁ were located with cis-geometry. Accordingly, the relative configurations are 2R*, 3S*, 2'S*, 3'S*, 5'S*. Taking these findings together, the structure of citridone D was elucidated to be as shown in Fig. 1.

Citrifolones A, B and B' have a phenyltricyclic structure but only citridone D has a very unique phenyltetrayclic structure. To our knowledge, this is the first report of the finding of this skeleton.

**Biological Properties** Citridone D showed inhibition zones only on Plate B (20 mm at 10 µg/disk) indicated that citridone D potentiates the miconazole activity against C. al-
bicans, but the activity is less potent than that of citridone A (23 mm at 10 μg/disk on Plate B). Citridone D showed no antimicrobial activity against several microorganisms at 10 μg/6 mm disk (data not shown).

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