Fungal Citridone D Having a Novel Phenylfuropyridine Skeleton

Takashi FUKUDA,[a] Yasunari SAKABE,[b] Hiroshi TOMODA,*[,b] and Satoshi ŌMURA[a,c]

[a] *Kitasato Institute for Life Sciences & Graduate School of Infection Control Sciences, Kitasato University;* [b] *School of Pharmacy, Kitasato University; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan: and* [c] *Kitasato Institute; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8642, Japan.*

Received July 31, 2006; accepted October 2, 2006; published online October 5, 2006

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

Opportunistic 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 actofunicone,[3)] beauvericins[4)] and phenatic acids[5)] of microbial origin as potentiators of miconazole activity against *C. albicans*. Recently, citridones A, B, B' and C[6)] 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 on 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-AX505H 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.).

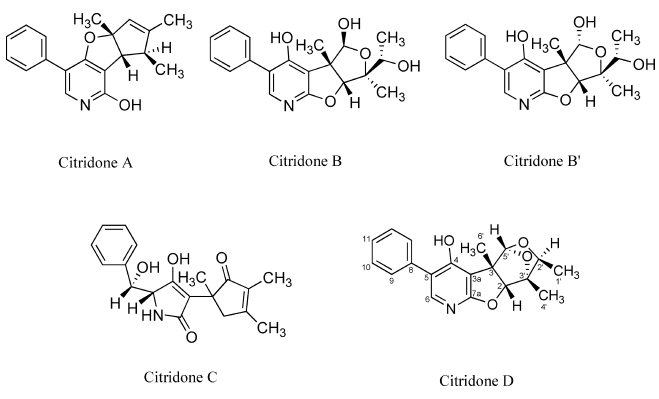

Fig. 1. Structures of Citridones

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, GY agar (glucose 1%, yeast extract 0.5% and agar 0.8%) (Plate A) and GY 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 (20 l) of *Penicillium* sp. FKI-1938, acetone (20 l) was added. After the acetone extracts were filtered and concentrated, the aqueous solution (pH 7.1) was extracted with ethyl acetate (5 l) 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 Na_2SO_4 and concentrated *in vacuo* to dryness to yield an oily material (1.4 g). The material was dissolved in a small volume of $CHCl_3$, applied to a silica gel column (80 g, 4.0×18 cm, 70—230 mesh, Merck), and eluted with $CHCl_3$–CH_3OH solutions. Citridone D was recovered in the 25 : 1 fraction, which was concentrated to give a brown material (233.8 mg). The material was purified by HPLC; ODS column (4.6×250 mm, Pegasil, Senshu Sci. Co. Tokyo, Japan), a 30-min linear gradient from 20 to 40% CH_3CN in 0.05% CF_3COOH, 1.0 ml/min, and UV at 240 nm. Under these conditions, citridone D was eluted as a peak with a retention time of 16.0 min, and the peak was pooled and concentrated to yield pure citridone D (16.4 mg) as pale yellow amorphous solid.

Physico-chemical Properties The physico-chemical properties of citridone D are summarized in Table 1. The HR-FAB-MS spectrum indicated a molecular formula of $C_{19}H_{20}NO_4$ ($[M+H]^+$ 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 pres-

* To whom correspondence should be addressed. e-mail: tomodah@pharm.kitasato-u.ac.jp

Table 1. Physico-chemical Properties of Citridone D

	Citridone D
Appearance	Pale yellow amorphous
Melting point	—
$[\alpha]_D^{25}$	+73.5 (c=0.1, CH_3OH)
Molecular formula	$C_{19}H_{19}NO_4$
Molecular weight	325
HR-FAB-MS m/z $(M+H)^+$	
Calcd	326.1392 (for $C_{19}N_{20}NO_4$)
Found	326.1381
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	203 (24000), 232 (17000)
IR ν_{max}^{KBr} cm^{-1}	2983, 1671, 1646, 1600, 1444, 1386
Solubility	
Soluble	DMSO, CH_3OH, $CHCl_3$, EtOAc
Insoluble	n-Hexane, H_2O

Table 2. ^{1}H- and ^{13}C-NMR Chemical Shifts of Citridone D

Position	Citridone D δ_C (mult.)	δ_H (mult. J)
2	91.1 (CH)	4.75 (1H, s)
3	59.6 (C)	
3a	110.8 (C)	
4	163.4 (C)	
5	123.5 (C)	
6	138.6 (CH)	7.52 (1H, s)
7a	165.5 (C)	
8	131.4 (C)	
9	129.3 (CH)	7.30 (2H, d, J=7.5 Hz)
10	129.3 (CH)	7.40 (2H, m)
11	129.0 (CH)	7.40 (1H, m)
1′	13.7 (CH_3)	1.32 (3H, d, J=7.0 Hz)
2′	78.2 (CH)	3.79 (1H, q, J=7.0 Hz)
3′	89.6 (C)	
4′	12.2 (CH_3)	1.50 (3H, s)
5′	102.2 (CH)	5.52 (1H, s)
6′	15.6 (CH_3)	1.68 (3H, s)

a) Chemical shifts are shown with reference to $CDCl_3$ as 77.0 ppm. *b*) Chemical shifts are shown with reference to $CDCl_3$ as 7.26 ppm.

ence of a phenylfuropyridine moiety, as reported by Sakemi *et al.*[7]

Structural Elucidation The ^{13}C-NMR spectrum (in $CDCl_3$) showed 19 resolved signals, which were classified as three methyl carbons, three methine carbons, six sp^2 methine carbons and seven (five sp^2) quaternary carbons by analysis of the DEPT spectra (Table 2). The ^{1}H-NMR spectrum (in $CDCl_3$) showed three methyl signals, four methine signals and five aromatic signals. The connectivity of proton and carbon atoms was established by the HMQC spectrum, as shown in Table 2. Analysis of the ^{1}H–^{1}H COSY and HMBC spectra revealed two partial structures, I and II (Fig. 2). As shown in the bold line in the partial structure I, the presence of a mono-substituted benzene ring was suggested from the chemical shifts of C-9 (δ 129.3), C-10 (δ 129.3) and C-11 (δ 129.0) in ^{13}C-NMR and 9-H (δ 7.30), 10-H (δ 7.40) and 11-H (δ 7.40) in the ^{1}H-NMR and H–H COSY spectrum. Cross peaks from 6-H (δ 7.52) to C-4 (δ 163.4), C-5 (δ 123.5), C-7a (δ 165.5) and C-8 (δ 131.4) and from 9-H to C-5 were observed in the HMBC experiments to give the partial structure I. These data showed good agreement with those of citridone B[6]. Regarding the partial structure II, extraordinary chemical shifts were observed for the methane carbon (C-5′, δ_C 102.2; δ_H 5.52), suggesting that it is bound to two oxygens. Additionally, the chemical shifts of the two methine carbons (C-2, δ_C 91.1; δ_H 4.75 and C-2′, δ_C 78.2; δ_H 3.79) and the quaternary carbon (C-3′, δ_C 89.6) suggested that they are bound to an oxygen. 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′-H_3 (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′-H_3 (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′-H_3 (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′-H_3 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.

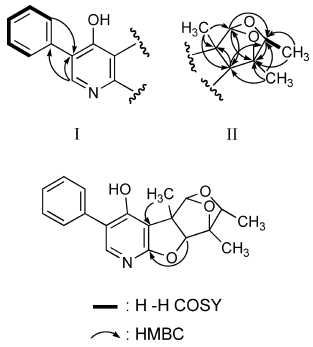

Fig. 2. Partial Structures I and II and Key Cross Peaks Observed in ^{1}H–^{1}H COSY and HMBC Experiments for Citridone D

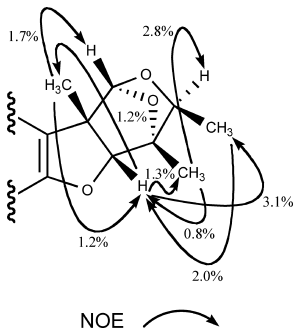

Fig. 3. NOE Experiments for Citridone D

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′-H_3, 4′-H_3 and 6′-H_3, from 1′-H_3 to 2′-H and from 6′-H_3 to 2-H and 5′-H indicated that 2-H, 1′-H_3, 4′-H_3 and 6′-H_3 were located with *cis*-geometry. Accordingly, the relative configurations are 2*R**, 3*S**, 2′*S**, 3′*S**, 5′*S**. Taking these findings together, the structure of citridone D was elucidated to be as shown in Fig. 1.

Citridones A, B and B′ have a phenyltricyclic structure but only citridone D has a very unique phenyltetracyclic 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).

Acknowledgments We wish to thank Ms. Noriko Sato, Ms. Akiko Nakagawa and Ms. Chikako Sakabe, School of Pharmaceutical Sciences, Kitasato University, for measurements of NMR and mass spectra. This study was supported in part by the 21st Century COE Program and Kakenhi 16073215, Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1) Nishiyama Y., Yamaguchi H., *Antibiot. Chemother.*, **16**, 19—26 (2000).
2) Ōmura S., *Nippon Saikingaku Zasshi*, **54**, 795—813 (1999).
3) Arai M., Tomoda H., Okuda T., Wang H., Tabata N., Masuma R., Yamaguchi Y.,Ōmura S., *J. Antibiot.*, **55**, 172—180 (2002).
4) Fukuda T., Arai M., Yamaguchi Y., Masuma R., Tomoda H., Ōmura S., *J. Antibiot.*, **57**, 110—116 (2004).
5) Fukuda T., Matsumoto A., Takahashi Y., Tomoda H., Ōmura S., *J. Antibiot.*, **58**, 252—259 (2005).
6) Fukuda T., Yamaguchi Y., Masuma R., Tomoda H., Ōmura S., *J. Antibiot.*, **58**, 309—314 (2005).
7) Sakemi S., Border J., Decosta D. L., Dekker K. A., Hirai H., Inagaki T., Kim Y. J., Kozima N., Sims J. C., Sugie Y., Sugiura A., Sutcliffe J. A., Tachikawa K., Truesdell S. J., Wong J. W., Toshikawa N., Kozima Y., *J. Antibiot.*, **55**, 6—18 (2002).