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Citridones, New Potentiators of Antifungal Miconazole Activity, Produced by *Penicillium* sp. FKI-1938

II. Structure Elucidation

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Abstract The structures of citridones A, B, B′ and C, new potentiators of miconazole activity against *Candida albicans* produced by *Penicillium* sp. FKI-1938, were elucidated by various spectroscopic analyses including UV, NMR, and MS and degradation experiments. Although citridones B and B′ were isolated as a mixture, each structure was also elucidated, indicating that they exist in equilibrium of hemiacetal epimerization. Citridones A, B and B′ have a similar phenylfuropyridone moiety.

Keywords citridones, phenylfuropyridone, *Penicillium*, anti-infective, azole potentiator

Introduction

During the course of screening for potentiators of antifungal miconazole activity, new citridones A, B, B′ and C (Fig. 1) were isolated from the culture broth of *Penicillium* sp. FKI-1938. The fermentation, isolation and their biological properties are described in the preceding paper [1]. We report herein the structure elucidation of citridones A, B, B′ and C.

Materials and Methods

Materials

Citridones A and C were purified from the culture broth of *Penicillium* sp. FKI-1938, but citridones B and B′ were isolated as a mixture as described in the preceding paper [1].

General Experimental Procedures

Optical rotations were recorded with a DIP-370 digital polarimeter (JASCO, Tokyo, Japan). Melting points were measured with a micro melting apparatus (Yanaco, Kyoto, 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, USA) 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, USA).

Results

Physico-chemical Properties of Citridones A, B, B′ and C

Physico-chemical properties of citridone A, a mixture of

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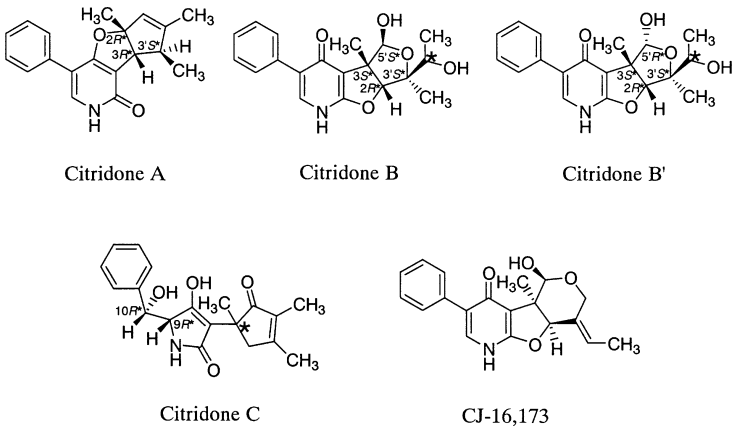

Fig. 1 Structures of citridones, A, B, B′, C and CJ-16,173.

Table 1 Physico-chemical properties of citridones A, B+B′ and C

	Citridone A	Citridones B+B′	Citridone C
Appearance	white needle	white needle	pale yellow amorphous
Melting point	172～175°C	168～170°C	—
$[\alpha]_D^{25}$	−1.6 (*c* 0.1, CH_3OH)	+102.4 (*c* 0.1, CH_3OH)	−74.4 (*c* 0.1, CH_3OH)
Molecular formula	$C_{19}H_{19}NO_2$	$C_{19}H_{21}NO_5$	$C_{19}H_{21}NO_4$
Molecular weight	293	343	327
HR-FAB-MS *m/z* $(M+H)^+$			
Calcd	294.1493 (for $C_{19}H_{20}NO_2$)	344.1497 (for $C_{19}H_{22}NO_5$)	328.1549 (for $C_{19}H_{22}NO_4$)
Found	294.1493	344.1506	328.1546
UV $\lambda_{max}^{CH_3OH}$ nm (ε)	205 (9,800), 246 (10,700)	207 (21,000), 233 (18,900)	203 (16,900), 233 (11,800)
IR ν_{max}^{KBr} cm^{-1}	2964, 2859, 1654, 1604	3390, 2981, 2346, 1646	3355, 2923, 1670, 1639
	1498, 1430	1596, 1475,1455	1452, 1392
Solubility			
Soluble	DMSO, CH_3OH	DMSO, CH_3OH	DMSO, CH_3OH
	$CHCl_3$, EtOAc	$CHCl_3$, EtOAc	$CHCl_3$, EtOAc
Insoluble	*n*-Hexane, H_2O	*n*-Hexane, H_2O	*n*-Hexane, H_2O

citridones B and B′, and citridon C are summarized in Table 1. All citridones showed very similar UV spectra with absorption maxima at 203～207 nm and 233～246 nm, suggesting the presence of phenylfuropyridone as reported by Sakemi *et al.* [2]. Absorptions at about 1670～1639 cm^{-1} in IR spectra suggested the presence of carbonyl groups. Thus similarity in their data indicated that they are structurally related.

Structure of Citridone A

The molecular formula of citridone A was determined to be $C_{19}H_{19}NO_2$ on the basis of HRFAB-MS measurement (Table 1). The ^{13}C NMR spectrum (in $CDCl_3$) showed 19 resolved signals, which were classified into three methyl carbons, two methine carbons, seven sp^2 methine carbons, six (five sp^2) quaternary carbons and one carbonyl carbon by analysis of DEPT spectra. The ^1H NMR spectrum (in $CDCl_3$) showed three methyl signals, four methine signals, five aromatic signals and one nitrogen proton signal. The connectivity of proton and carbon atoms was established by the ^{13}C-^1H HMQC spectrum as shown in Table 2. Analysis of the ^1H-^1H COSY and ^{13}C-^1H HMBC spectra revealed the three partial structures I, II and III (Fig. 2).

The ^{13}C-^1H long range couplings of ^2J and ^3J observed in the ^{13}C-^1H HMBC experiments (Fig. 3) gave the following evidence. 1) The cross peaks from 3-H (δ 3.26) to C-3a (δ 113.5), C-7a (δ 165.2) and C-6′ (δ 26.2), from 6-H (δ 7.45) to C-4 (δ 162.4) and C-7a and from 6′-H_3 (δ 1.65) to C-2 (δ 104.1) and C-3 (δ 56.4) supported the partial structure I. 2) The cross peaks from 9-H (δ 7.52) to

Table 2 ^{1}H and ^{13}C NMR chemical shifts of citridones A, B and B′

	Citridone A		Citridone B		Citridone B′	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]
C-2	104.1		92.2	4.87 (1H, s)	90.2	4.83 (1H, s)
C-3	56.4	3.26 (1H, d, *J*=2.0 Hz)	58.8		58.1	
C-3a	113.5		110.3		108.4	
C-4	162.4[c]		165.0		162.0	
C-5		124.1			122.9	
C-6	133.8	7.45 (1H, s)	141.0	7.49 (1H, s)	146.0	7.74 (1H, s)
C-7	111.7					
C-7a	165.2[c]		164.0		166.5	
C-8	133.2		134.0		134.4	
C-9	127.5	7.52 (2H, m)	129.2	7.36 (2H, m)	129.1	7.43 (2H, m)
C-10	128.5	7.38 (2H, m)	128.6	7.35 (2H, m)	128.4	7.35 (2H, m)
C-11	127.3	7.29 (1H, m)	127.7	7.27 (1H, m)	127.2	7.22 (1H, m)
C-1′	126.2	5.38 (1H, t, *J*=1.5 Hz)	18.1	1.17 (3H, d, *J*=6.5 Hz)	17.6	1.16 (3H, d, *J*=6.5 Hz)
C-2′	150.5		72.4	3.75 (1H, q, *J*=6.5 Hz)	72.6	3.72 (1H, q, *J*=6.5 Hz)
C-3′	49.0	2.90 (1H, dq, *J*=2.0, 7.0 Hz)	91.4		88.4	
C-4′	20.2	1.29 (3H, d, *J*=7.0 Hz)	19.6	1.08 (3H, s)	18.9	1.08 (3H, s)
C-5′	14.7	1.72 (3H, br.s)	99.5	5.59 (1H, s)	103.4	5.54 (1H, s)
C-6′	26.2	1.65 (3H, s)	17.3	1.47 (3H, s)	19.8	1.46 (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. c) The assigments may be exchangeable.

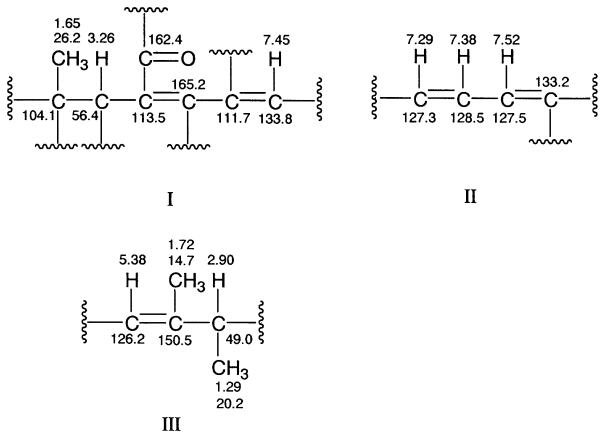

Fig. 2 Partial structures I, II and III of citridone A.

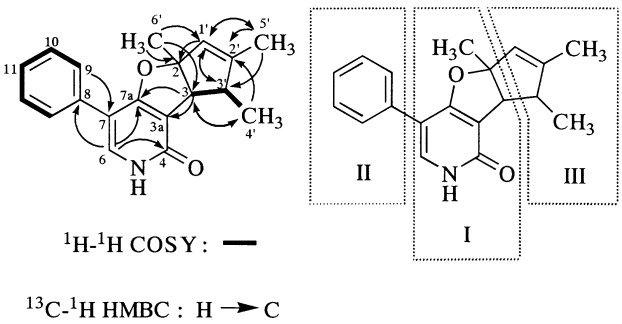

Fig. 3 Key cross peaks observed in ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC experiments of citridone A.

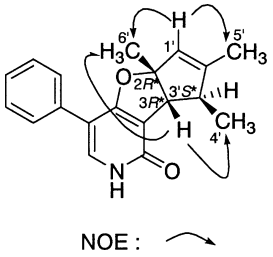

Fig. 4 NOE experiments of citridone A.

C-11 (δ 127.3), from 10-H (δ 7.38) to C-8 (δ 133.2) and C-9 (δ 127.5) and from 11-H (δ 7.29) to C-9 supported the partial structure II. 3) The cross peaks from 1′-H (δ 5.38) to C-2′ (δ 150.5), C-3′ (δ 49.0) and C-5′ (δ 14.7), from 3′-H (δ 2.90) to C-4′ (δ 20.2), from 4′-H_3 (δ 1.29) to C-2′ and C-3′ and from 5′-H_3 (δ 1.72) to C-1′ (δ 126.2), C-2′

and C-3′ supported the partial structure III. 4) The cross peaks from 6-H to C-8 and from 9-H to C-7 (δ 111.7) indicated that the partial structures I and II are linked as shown in Fig. 3. 5) The cross peaks from 3-H to C-1′, C-2′ and C-4′, from 6′-H_3 to C-1′, from 1′-H to C-2 and C-3, from 3′-H to C-3 and from 4′-H_3 to C-3 indicated that the partial structures I and III are joined at C-2 and C-3 as shown in Fig. 3. Thus, the planar structure of citridone A is shown in Fig. 3. This is reasonable in the molecular formula ($C_{19}H_{19}NO_2$) and the UV spectra at 205 and 246 nm, which indicated the presence of 4-hydroxy-7-phenylfuropyridine as previously reported for CJ-15,696 derivative [2].

The relative configurations of C-2, C-3 and C-3′ were determined by NOE experiments. Observation of NOEs from 3-H to 4′-H_3 and 6′-H_3 (Fig. 4) indicated that it forms a *cis*-geometry. Accordingly, the relative configurations are 2*R**, 3*R** and 3′*S**. Taken together, the structure of citridone A was elucidated as shown in Fig. 1.

Structure of Citridones B and B′

Citridones B and B′ were isolated as a mixture, and existed in an equilibrium of 3 : 2 in a solution from the HPLC analysis.

The molecular formula of citridone B was determined to be $C_{19}H_{21}NO_5$ on the basis of HRFAB-MS measurement (Table 1). The ^{13}C NMR spectrum (in $CDCl_3$) showed 19 resolved signals, which were classified into three methyl carbons, three methine carbons, six sp^2 methine carbons, six (four sp^2) quaternary carbons and one carbonyl carbon by analysis of DEPT spectra. The ^{1}H NMR spectrum (in $CDCl_3$) showed three methyl signals, four methine signals and five aromatic signals, but nitrogen and hydroxy protons were not detected. The connectivity of proton and carbon atoms was established by the ^{13}C-^{1}H HMQC spectrum as shown in Table 2. Analysis of the ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC spectra revealed the two partial structures IV and V (Fig. 5). For citridone B, the cross peaks from 2-H (δ 4.87) to C-3 (δ 58.8), C-3a (δ 110.3), C-7a (δ 164.0), C-5′ (δ 99.5), and C-6′ (δ 17.3), from 6-H (δ 7.49) to C-4 (δ 165.0), C-5 (δ 124.1), C-7a and C-8 (δ 124.0), from 9-H (δ 7.36) to C-5, C-8 and C-11 (δ 127.7), from 10-H (δ 7.35) to C-9 (δ 129.2) and C-11, from 5′-H (δ 5.59) to C-2, C-3 and C-3a and from 6′-H_3 (δ 1.47) to C-2, C-3, C-3a and C-5′ were observed in the ^{13}C-^{1}H HMBC experiments to give the partial structure IV (Fig. 5). The cross peaks from 1′-H_3 (δ 1.17) to C-2′ (δ 72.4) and C-3′ (δ 91.4), from 2′-H (δ 3.75) to C-1′ (δ 18.1) and C-3′ and from 4′-H_3 (δ 1.08) to C-2′ and C-3′ were observed in the ^{13}C-^{1}H HMBC experiments to give the partial structure V (Fig. 5). The cross peaks from 2-H to C-2′ and C-3′, from 2′-H to C-2

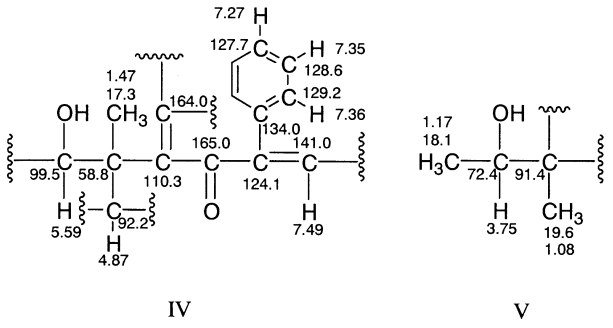

Fig. 5 Partial structures IV and V of citridine B.

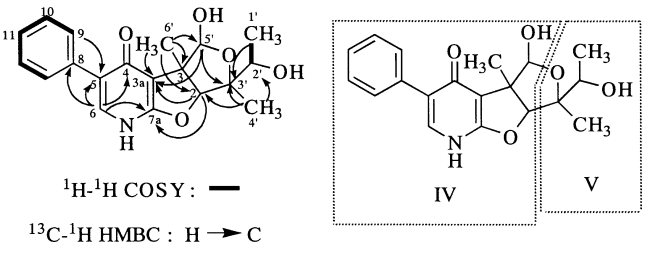

Fig. 6 Key cross peaks observed in ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC experiments of citridones B and B′.

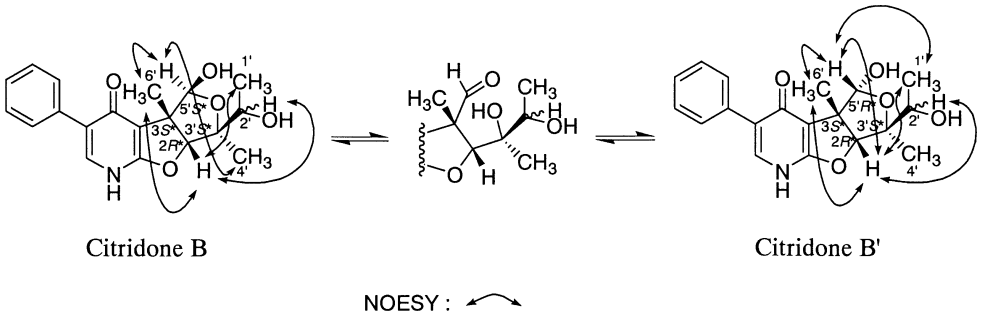

Fig. 7 NOESY experiments of citridones B and B′, and the possible intermediate structure in epimerization between citridones B and B′.

Table 3 ^{1}H and ^{13}C NMR chemical shifts of citridone C

	Citridone C	
	^{13}C chemical shifts (ppm)[a]	^{1}H chemical shifts (ppm)[b]
C-1	216.9	
C-2	132.2	
C-3	175.3	
C-4	45.9	2.62 (1H, d, *J*=20.0 Hz) 3.18 (1H, d, *J*=20.0 Hz)
C-5	47.3	
C-6	174.2	
C-7	104.3	
C-8	169.4	
C-9	60.5	4.11 (1H, d, *J*=7.0 Hz)
C-10	75.7	4.74 (1H, d, *J*=7.0 Hz)
C-11	138.9	
C-12	126.7	7.32 (2H, m)
C-13	128.1	7.25 (2H, m)
C-14	128.2	7.27 (1H, m)
C-15	7.7	1.72 (3H, s)
C-16	17.4	2.04 (3H, s)
C-17	27.0	1.32 (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.

and 4′-H to C-2 indicated that the partial structures IV and V are linked at C-2 as shown in Fig. 6. The cross peak from 5′-H to C-3′ indicated that the partial structure IV is cycled between C-3′ and C-5′ beyond an oxygen. The number and position of the hydroxyl groups were confirmed by the acetylation and the methylation. Accordingly, the planar structure of citridone B is shown in Fig. 6. This is reasonable in the molecular formula ($C_{19}H_{21}NO_5$) and the UV spectra at 207 and 233 nm, which indicated the presence of 4-hydroxy-5-phenylfuropyridine as previously reported for CJ-15,696 derivative [2]. The relative configurations of C-2, C-3, C-3′ and C-5′ were determined by the NOESY experiments (Fig. 7). The cross peaks from 2-H to 1′-H_3, 2′-H, and 6′-H_3 and from 5′-H to 4′-H_3 indicated that it forms a *cis*-geometry. Thus the relative configurations were 2*R**, 3*S**, 3′*S** and 5′*S**. However, the relative configuration of C-2′ was not defined. Taken together, the structure of citridone B was elucidated as shown in Fig. 1.

Citridone B′ was an epimer at C-5′ of citridone B. The cross peaks from 2-H to 1′-H_3, 2′-H and 6′-H_3 and from 5′-H to 2-H, 2′-H and 6′-H_3 in the NOESY experiments (Fig. 7) showed that it forms a *cis*-geometry. Thus the relative configuration is 5′*R**. Finally, the structure of citridone B′ was elucidated as shown in Fig. 1.

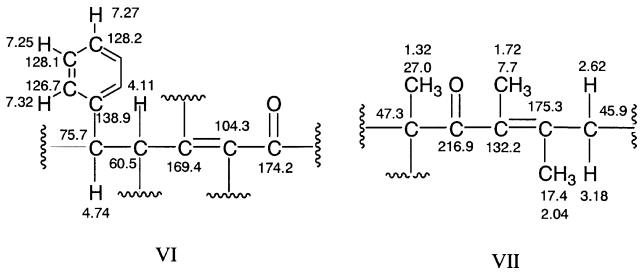

Fig. 8 Partial structures VI and VII of citridone C.

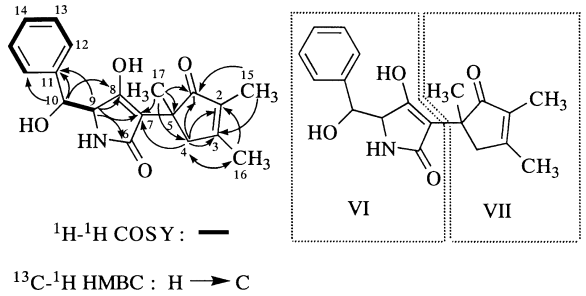

Fig. 9 Key cross peaks observed in ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC experiments of citridone C.

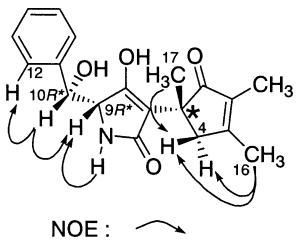

Fig. 10 NOE experiments of citridone C.

Thus, it is plausible that citridones B and B′ exist in an equilibrium of hemiacetal epimerization *via* the aldehyde as the intermediate (Fig. 7).

Structure of Citridone C

The molecular formula of citridone C was determined to be $C_{19}H_{19}NO_4$ on the basis of HRFAB-MS measurement (Table 1). The ^{13}C NMR spectrum (in $CDCl_3$) showed 19 resolved signals, which were classified into three methyl carbons, one methylene carbons, two methine carbons, five sp^2 methine carbons, six (five sp^2) quaternary carbons and two carbonyl carbons by analysis of DEPT spectra. The ^{1}H NMR spectrum (in $CDCl_3$) showed three methyl signals, one methylene signal, two methine signals and five aromatic signals. The connectivity of proton and carbon atoms was established by the ^{13}C-^{1}H HMQC spectrum as

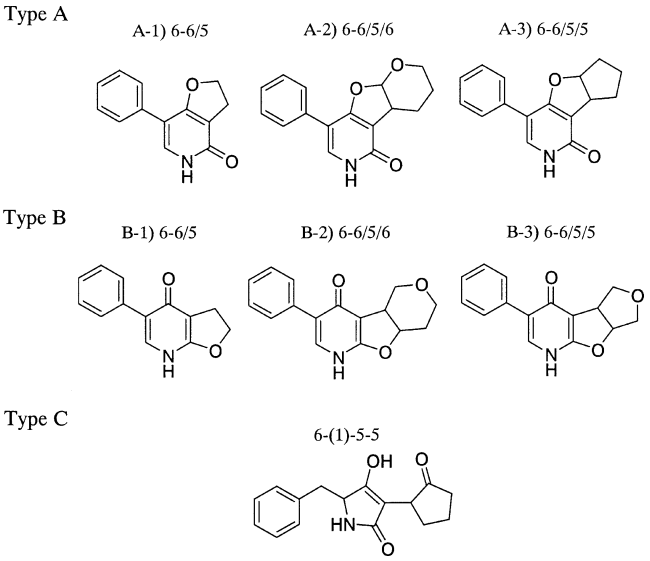

Fig. 11 Structural classification of phenylfuropyridones and related compounds.

shown in Table 3. Analysis of the ^{1}H-^{1}H COSY and ^{13}C-^{1}H HMBC spectra revealed the two partial structures VI and VII (Fig. 8).

The ^{13}C-^{1}H long range couplings of ^{2}J and ^{3}J observed in the ^{13}C-^{1}H HMBC experiments (Fig. 9) gave the following evidence. 1) The cross peaks from 9-H (δ 4.11) to C-6 (δ 174.2), C-7 (δ 104.3), C-8 (δ 169.4), C-10 (δ 75.7) and C-11 (δ 138.9), from 10-H (δ 4.74) to C-9 (δ 60.5), C-10, C-11 and C-12 (δ 126.7) and from 12-H (δ 7.32) to C-10, C-11, C-13 (δ 128.1) and C-14 (δ 128.2) supported the partial structure VI. 2) The cross peaks from 15-H_3 (δ 1.72) to C-1 (δ 216.9), C-2 (δ 132.2) and C-3 (δ 175.3), from 16-H_3 (δ 2.04) to C-2, C-3 and C-4 (δ 45.9), from 4-H_2 (δ 2.62 and 3.18) to C-1, C-2, C-3, C-5 (δ 47.3), C-16 (δ 17.4) and C-17 (δ 27.0), and from 17-H_3 (δ 1.32) to C-1, C-4 and C-5 supported the partial structure VII. 3) The cross peaks from 4-H_2 to C-7 and from 17-H_3 to C-7 indicated that the partial structures VI and VII are joined at C-5 and C-7. 4) The cross peak from 9-H to C-6 indicated that the partial structure VII is linked to give a cyclic structure beyond nitrogen as shown in Fig. 9. 5) Taking the chemical shifts and molecular formula (Table 1) into consideration, C-8 and C-10 should be hydroxy carbons as shown in Fig. 9.

The relative configurations of C-9 and C-10 were determined by the NOE experiments (Fig. 10). Observation of NOEs from NH (δ 5.78) to 9-H and from 10-H to 9-H and 12-H indicated that it forms a *cis*-geometry. Thus, the relative configurations of C-9 and C-10 were 9*R** and 10*R**. The relative configuration of C-5 was not determined by the NOE experiments because it was far from C-9. However, NOEs from 16-H_3 to 4-H_2 and from 17-H_3 to only 4-H (δ 2.62) were observed as shown in Fig. 10. Thus, the structure of citridone C was elucidated as shown in Fig. 1.

Discussion

Eight phenylfuropyridones (or hydroxy-phenylfuropyridines) were reported as antibacterial or antifungal antibiotics from *Cladobotryum varium* [2∼4]. Based on the ring system of the furopyridone moiety, they are classified into the two types as summarized in Fig. 11. Type A is the phenyl-α-furopyridone family containing the A-1 group (6-6/5 ring system) such as CJ-16,170, CJ-16,196 and CJ-16,197 [2], and the A-2 group (6-6/5/6 ring system) such as CJ-16,171 [2]. Citridone A is a member of Type A having a new 6-6/5/5 ring system (A-3 group). Type B is the phenyl-γ-furopyridone family containing the B-1 group (6-6/5 ring system) such as CJ-15,696, CJ-16,169, CJ-16,174 [2], and cladobotryal [5], and the B-2 group (6-6/5/6 ring system) such as CJ-16,173 [2]. Citridones B and B′ are members of Type B having a new 6-6/5/5 ring system (B-3 group). Citridone C has an isolated ring system (Type C).

Regarding the chemical shift of the C-2 quaternary carbon for citridone A (A-3 group in Fig. 11), the value (104.1 ppm) seemed very lower than the expected one. However, it was reported that the analogous chemical shift (95.1 ppm) of the C-2 methine carbon for CJ-16,170 (A-1 group in Fig. 11) also showed a lower value [2]. It might be that the C-2 carbons in Type A compounds show lower chemical shifts due to the unexpected reasons. But further experiments such as X-ray crystallography or synthetic approaches are necessary to define this point. Citridones B and B′ are illustrated as a pyridone-type structure in Figs. 1, 6 and 7, because *N*-methyl derivative was obtained by methylation of citridone B (data not shown). However, they might co-exist with a pyridinol-type structure because tri-*O*-acetyl derivative was obtained by acetylation of citridone B (data not shown).

Thus, citriodnes are found to be a new type of phenylfuropyridones possessing unique ring systems. Their biosynthesis appeared to be related to that of fungal tenellin case [6].

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