Notes

FR900403, A NEW ANTIFUNGAL ANTIBIOTIC PRODUCED BY A *KERNIA* SP.

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(Received for publication February 27, 1990)

In the course of our screening for new antibiotics, a novel anti-*Candida* substance, FR900403, was isolated from the culture broth of *Kernia* sp. F-19849. This paper describes the fermentation, isolation, structural elucidation and biological properties of FR900403.

A seed medium (160 ml) consisting of sucrose 4%, cotton seed flour 2%, dried yeast 1%, peptone 1%, KH_2PO_4 0.2%, $CaCO_3$ 0.2% and Tween 80 0.1% was poured into each of two 500-ml Erlenmeyer flasks and sterilized. A loopful of slant culture of *Kernia* sp. F-19849 was inoculated into each flask and cultured under shaking at 25°C for 4 days. A production medium (20 liters) consisting of soluble starch 3%, sucrose 1%, corn meal 1%, dried yeast 1%, $NaNO_3$ 0.2%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.05% and $CaCO_3$ 0.2% was poured into a 30-liter jar fermenter and sterilized. The seed culture broth (320 ml) was transferred to the production medium and cultured at 25°C for 5 days with agitation at 200 rpm and aeration at 20 liters per minute.

The cultured broth (50 liters) was filtered with the aid of diatomaceous earth. The filtrate was passed through a Dowex 1X2 column (OH^- type), which was washed with water and eluted with 0.5 M NaCl.

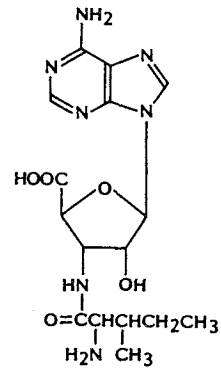

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Fig. 1. ^1H NMR spectrum of FR900403 in D_2O (400 MHz).

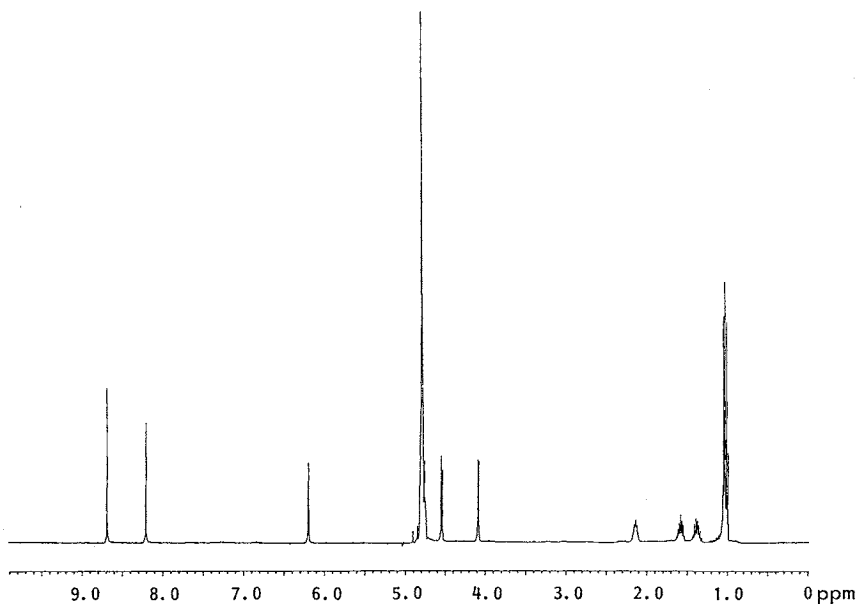
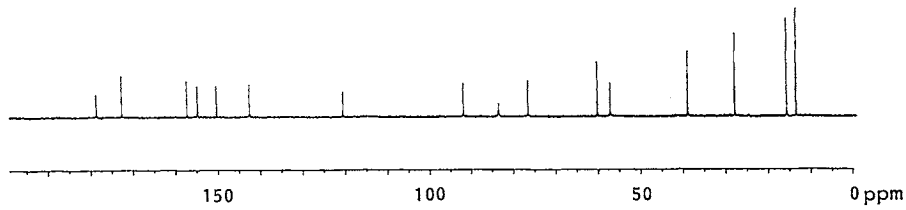


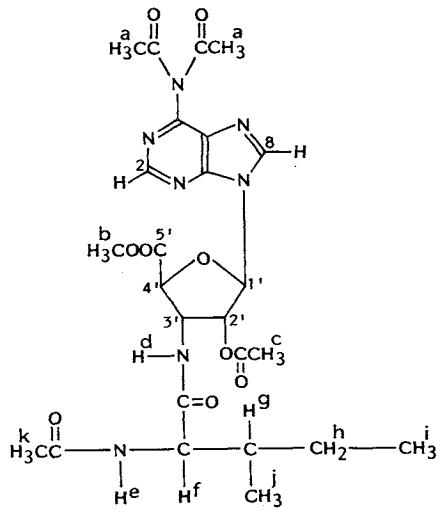
Fig. 2. ^{13}C NMR spectrum of FR900403 in D_2O (100 MHz).



The eluate was passed through an active carbon column. The column was washed with water and eluted with 50% aqueous acetone. The eluate was concentrated *in vacuo* to remove acetone and passed through a Diaion SP-207 column, which was washed with water and eluted with 50% aqueous methanol. The eluate was concentrated *in vacuo* to remove methanol and applied on a CM Sephadex C-25 column. The column was washed with water and eluted with 0.08 M NaCl. The active fractions were pooled and adsorbed on a SP-207 column. After washing with water, the column was eluted with 50% aqueous methanol. The eluate was concentrated *in vacuo* to remove methanol and freeze-dried to give 620 mg of FR900403.

FR900403 (**1**) is soluble in water, slightly in methanol and ethanol, but insoluble in chloroform and ethyl acetate. It is amphoteric white powder, mp 218~229°C (dec), $[\alpha]_D^{20}+54.1°$ (*c* 1.0, H_2O). The molecular formula $C_{16}H_{23}N_7O_5$ of **1** was established by elementary analysis, FAB mass spectrum and ^{13}C NMR spectrum; elementary analysis, calcd for $C_{16}H_{23}N_7O_5 \cdot 2H_2O$: C 44.75, H 6.34, N 22.83, found: C 45.95, H 6.52, N 22.08; FAB-MS, *m/z* 394 (M + H). The ^{13}C NMR spectrum showed 16 carbon resonances. FR900403 gave positive color reactions with ninhydrin and $CeSO_4$-H_2SO_4. The ^1H and ^{13}C NMR spectra are shown in Figs. 1 and 2, respectively. The UV absorption at 260 nm in water and the two proton signals at δ 8.67 (s) and 8.19 (s) in the ^1H NMR spectrum, together with five carbon signals at δ 157.9, 155.1, 150.9, 142.7 and 121.1 in the ^{13}C NMR spectrum suggested the presence of the adenine nucleus in FR900403 molecule. In addition, the presence of carboxylic acid and/or amide function was indicated from the two carbon signals at δ 172.6 and 178.6 in the ^{13}C NMR spectrum. Acid hydrolysis of **1** with 6 N HCl at 110°C for 12 hours yielded adenine and one amino acid which was determined to be alloisoleucine by the amino acid analyzer. The signals attributed to an anomeric proton[1] was found to have a coupling to the multiplet signal at δ 4.75. This result suggested the presence of a sugar moiety. Acetylation of **1** with Ac_2O in pyridine and successive treatment of the solution with methanol gave the tetraacetyl methyl ester derivative (**2**). Analysis of the ^1H NMR spectrum of **2** using a double resonance technique

Table 1. ^1H NMR spectral data of **2** in $CDCl_3$ (400 MHz).



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Proton	δ (ppm)	m	*J* (Hz)
2-H	8.73	s	
8-H	8.97	s	
1'-H	6.34	d	2.0
2'-H	5.83	dd	2.0, 5.5
3'-H	5.21	m	
4'-H	4.60	d	8.3
H^a	2.37	s	
H^b	3.80	s	
H^c	2.25	s	
H^d	7.05	d	8.2
H^e	6.01	d	8.8
H^f	4.28	t	8.0
H^g	1.91	m	
H^h	1.46, 1.18	m, m	
H^i	0.94	t	7.3
H^j	0.95	d	5.9
H^k	2.02	s	

m: Multiplicity.

(Table 1) enabled us to assign all the proton signals as shown in Table 1 and to reveal the structure (**2**). Thus, the coupling constants $J_{1',2'}=2.0$ Hz, $J_{2',3'}=5.5$ Hz, and $J_{3',4'}=8.3$ Hz are determined. Analogy of these values with those of puromycin[2] and chryscandin[3] assisted assignment of the relative stereochemistry of the aminosugar moiety as shown in the structure of **2**. Based on the chemical structure of **2**, the structure of FR900403 was deduced to be **1**.

FR900403 exhibits antimicrobial activity against *Candida albicans*, but not against filamentous fungi. The MIC value of FR900403 against *C. albicans* FP633 was 0.4 μg/ml in EAGLE minimum essential medium at 37°C under 5% CO_2 atmosphere. FR900403 was effective against a generalized infection of *C. albicans* FP633 in mice at a dose of 10 mg/kg. It was not lethal to mice at a dose of 320 mg/kg when intravenously administered.

Acknowledgments

The authors are grateful to Drs. N. SHIGEMATSU, S. TAKASE and H. TANAKA for their help in structural elucidation.

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