A NEW ANTITUMOR ANTIBIOTIC, FR-66979

Sir:

We have previously reported on a new antibiotic, FR-900482, possessing a hydroxylamine function whose hydroxyl group exists as a hemiketal moiety, an aziridine function and a carbamoyloxymethyl group in the structure[1,2]. In addition to producing FR-900482, *Streptomyces sandaensis* No. 6897 also biosynthesizes FR-66979 (Fig. 1), a dihydro derivative of FR-900482[3]. In this paper, the isolation, physicochemical properties and biological activities of FR-66979 are reported.

The strain *S. sandaensis* No. 6897 was cultured at 30°C for 72 hours in a 30-liter jar fermentor with aeration at 20 liters/minute and agitation at 200 rpm in a production medium containing soluble starch 8%, dried yeast 1%, peanut powder 3% and soybean meal 0.5% (pH 6.2). The cultured broth (18 liters) was filtered with the aid of diatomaseous earth. The filtrate was passed through a Diaion HP-20 column, which was washed with water and eluted with 50% aqueous methanol. The eluate was concentrated *in vacuo* to remove methanol and then loaded onto an ion exchange resin, Amberlite IRC-50 column (H^+ form). This column was washed with deionized water and eluted with 0.1 N HCl. The eluate was neutralized with 2 N NaOH and passed through a Diaion HP-20 column. After washing the column with deionized water it was eluted with 50% aqueous methanol, and the active eluate was concentrated *in vacuo* and lyophilized to give a crude powder. The crude powder mixed with Silicar CC-4, was suspended in chloroform and chromatographed on a column of Silicar CC-4. The column was eluted with a mixture of chloroform - methanol (5 : 1), and the active fraction was concentrated *in vacuo* to give a powder. The powder was dissolved into methanol and purified by HPLC on silica gel (YMC-Pack S-043, Shimakyu Co., Ltd., Japan) using a mixture of methanol - chloroform (5 : 1) as an eluent. The active fraction was concen-

Fig. 1. Structure of FR-66979.

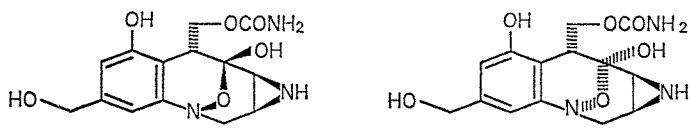


Table 1. Physico-chemical properties of FR-66979.

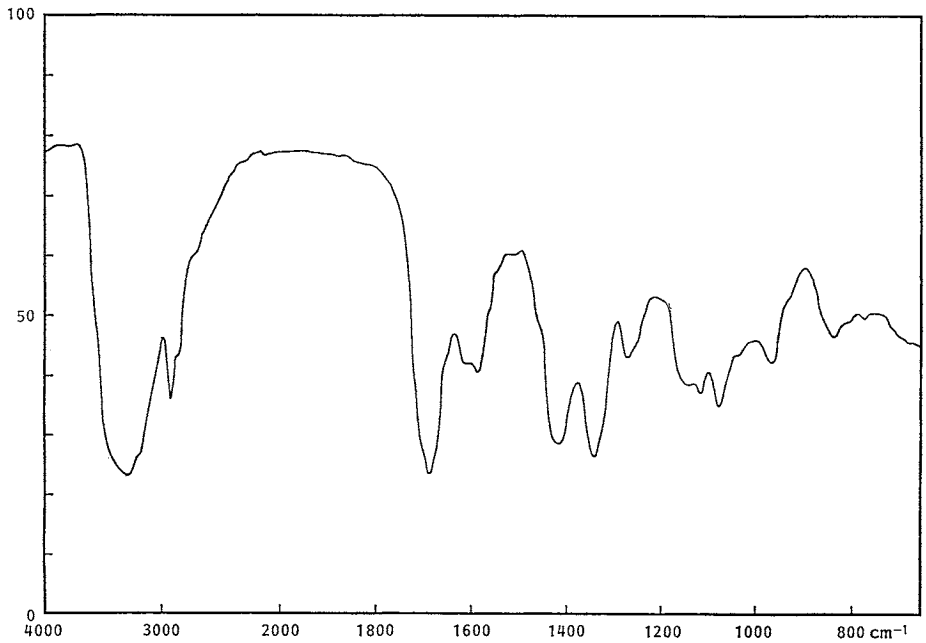
Appearance	White powder
Molecular formula	$C_{14}H_{17}N_3O_6$
Molecular weight (*m/z*)	323
Elementary analysis	
Calcd for $C_{14}H_{17}N_3O_6 \cdot \frac{1}{2}H_2O$:	C 50.60, H 5.46, N 12.65.
Found:	C 49.76, H 5.34, N 12.10.
SI-MS (*m/z*)	324 $(M+H)^+$
MP	165~170°C (dec)
$[\alpha]_D^{23}$	+12.5° (*c* 0.85, H_2O)
UV $\lambda_{max}^{H_2O}$ nm (ε)	215 (20,000), 240 (sh, 5,000), 280 (1,600)
$\lambda_{max}^{H_2O+HCl}$ nm	214, 240 (sh), 280
$\lambda_{max}^{H_2O+NaOH}$ nm	223, 250 (sh), 298
TLC (Silica gel plate)	
Rf[a]	0.28 and 0.52
Rf[b]	0.40 and 0.60

SI-MS: Secondary ion mass spectrum.

[a] Solvent system: Chloroform - methanol, 6 : 4.

[b] Solvent system: 2-Propanol - H_2O, 9 : 1.

Fig. 2. IR spectrum of FR-66979 in KBr.



trated *in vacuo* and a colorless powder (0.7 g) of FR-66979 was obtained.

The physico-chemical properties of FR-66979 are summarized in Table 1. The IR, ^{1}H and ^{13}C NMR spectra are represented in Figs. 2, 3 and 4, respectively. FR-66979 is soluble in water and methanol, and insoluble in acetone, ethyl acetate and chloroform. FR-66979 gave positive reactions to sulfuric acid 2,4-dinitrophenylhydrazine, iodine vapor and ferric chloride-potassium ferricyanide reagents, though negative to ferric chloride and Sakaguchi reactions. As can be seen in TLC behavior and ^{13}C NMR spectrum (Table 1 and Fig. 4), FR-66979 exists as an equilibrium mixture of two isomers, which was shown to be attributable to tautomerism of the anomeric hydroxyl group (Fig. 1, **A** and **B**).

FR-66979 is identical in physico-chemical analysis with synthetic FR-66979 derived from FR-900482 by catalytic hydrogenation[4].

The antimicrobial spectrum of FR-66979 is shown in Table 2. FR-66979 inhibited *Bacillus stearothermophilus* var. *calidolactis* C 953 at low concentrations. However, FR-66979 has weak antimicrobial activities against other bacteria, fungi and yeast.

Table 2. Antimicrobial activity of FR-66979.

Strains	MIC (μg/ml)
Escherichia coli NIHJ JC-2	50
Pseudomonas aeruginosa NCTC 10490	12.5
Staphylococcus aureus 209P JC-1	100
Bacillus subtilis ATCC 6633	100
B. stearothermophilus var. *calidolactis* C 953	0.8
Candida albicans	1,000
Aureobasidium pullulans IFO 4466	1,000

FR-66979 was effective against mouse leukemia P388 cells (IC_{50} 0.8 μg/ml), melanoma B16 cells (IC_{50} 0.8 μg/ml) and baby hamster kidney (BHK-21) cells (IC_{50} 1.6 μg/ml). The acute toxicity of FR-66979 was determined in *dd*Y mice (5 weeks old, female) by a single intravenous injection of graded doses of FR-66979 into 5 mice. The LD_{50} was approximately 40 mg/kg. Further studies on the antitumor activity *in vivo* are ongoing.

Fig. 3. 400 MHz ^{1}H NMR spectrum of FR-66979 in D_2O, pD=7.

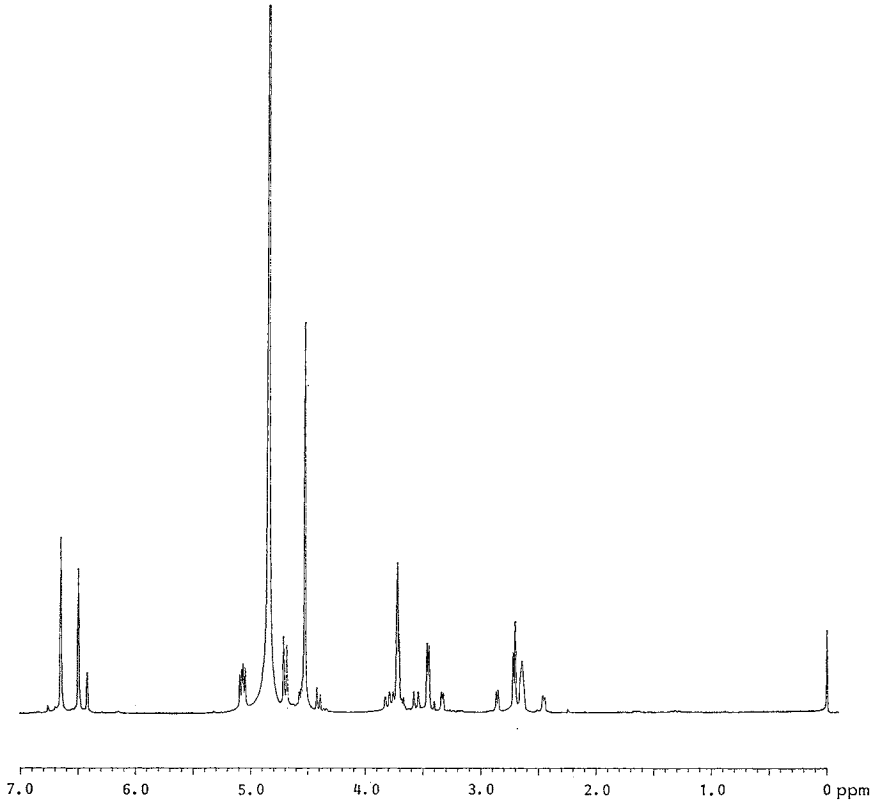
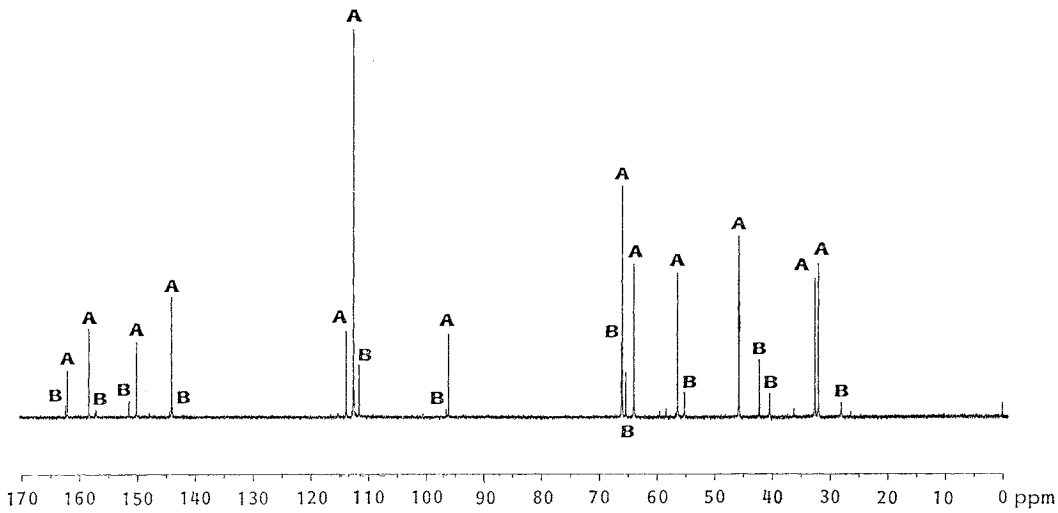


Fig. 4. 100 MHz ^{13}C NMR spectrum of FR-66979 in D_2O, pD=7.



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