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COMMUNICATIONS TO THE EDITOR

Caprazamycin B, a Novel Anti-tuberculosis Antibiotic, from *Streptomyces* sp.

Sir:

Tubelculosis is still the greatest single infectious cause of mortality in the world. Moreover, the spread of the HIV promoted to increase the number of tuberculosis patients[1]. However, powerful new anti-TB drugs with new mechanism of action have not been developed in last over thirty years, only 5 antituberculous first-line drugs can be used still now. We have screened for new anti-mycobacterial antibiotics from microbial products having effective and a specific narrow-range spectrum. As a part of this program, we discovered a novel anti-tuberculosis antibiotic named caprazamycin B (Fig. 1, CPZ-B) from the culture broth of the Actinomycete strain *Streptomyces* sp. MK730-62F2.

A slant culture of the caprazamycin-producing organism was inoculated into a 500-ml baffled Erlenmeyer flask containing 110 ml of the seed medium consisting of galactose 2%, dextrin 2%, Bacto-soytone (Difco) 1.0%, corn steep liquor (Iwaki Co.) 0.5%, glycerol 1.0%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% in deionized water (pH 7.4 before sterilization). The culture was incubated on a rotary shaker (180 rpm) at 30°C for 2 days. The seed culture (330 ml) of the strain were transferred into 30-liter fermentors containing 15 liters of a producing medium which was consisting of Tomato-paste (Kagome Co.) 2.4%, dextrin 2.4%, yeast extract 1.2%, and $CoCl_2 \cdot 6H_2O$ 0.0006% in deionized water (pH 7.0) before sterilization. The fermentation was carried out at 27°C for 6 days under agitation of 200 rpm. The fermentation broth (12 liters) was centrifuged for separating the mycelial cake and supernatant.

The mycelial cake was extracted with MeOH (6 liters) and filtered. The MeOH extract without evaporation of the solvent was added to the supernatant. The combined solution applied to a 750 ml of Diaion HP20 (Mitsubishi Chemical Co.) column. The active substance was eluted with 80% aq acetone (2.25 liters) after washing with water (2.25 liters) and 80 % MeOH (2.25 liters). The eluate was concentrated under reduced pressure to give brownish oil (10.1 g).

The crude oily material was charged on a 500 ml of silica gel column and developed with $CHCl_3$-MeOH-H_2O (4 : 1 : 0.1, 2 : 1 : 0.2 and 1 : 1 : 0.2, each of 1350 ml). The active fractions (Fr. Nos. 66~83, 20 g/Fr.) were collected and concentrated *in vacuo* to give a brownish residue (625 mg). The residue was dissolved with MeOH and left overnight at 5~6°C when the active material deposited as

Fig. 1. Caprazamycin B.

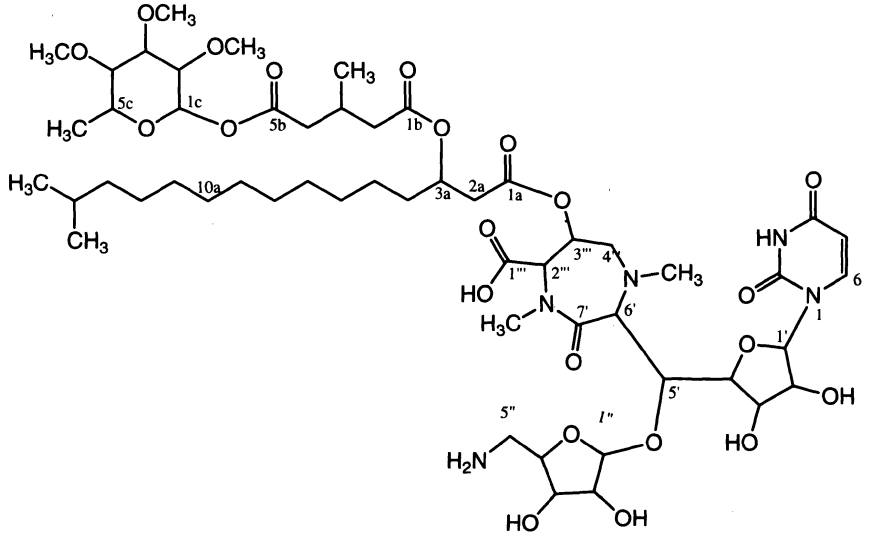


Fig. 2. ^{1}H NMR spectrum of caprazamycin B (DMSO-d_6 : D_2O=10 : 1, 500 MHz).

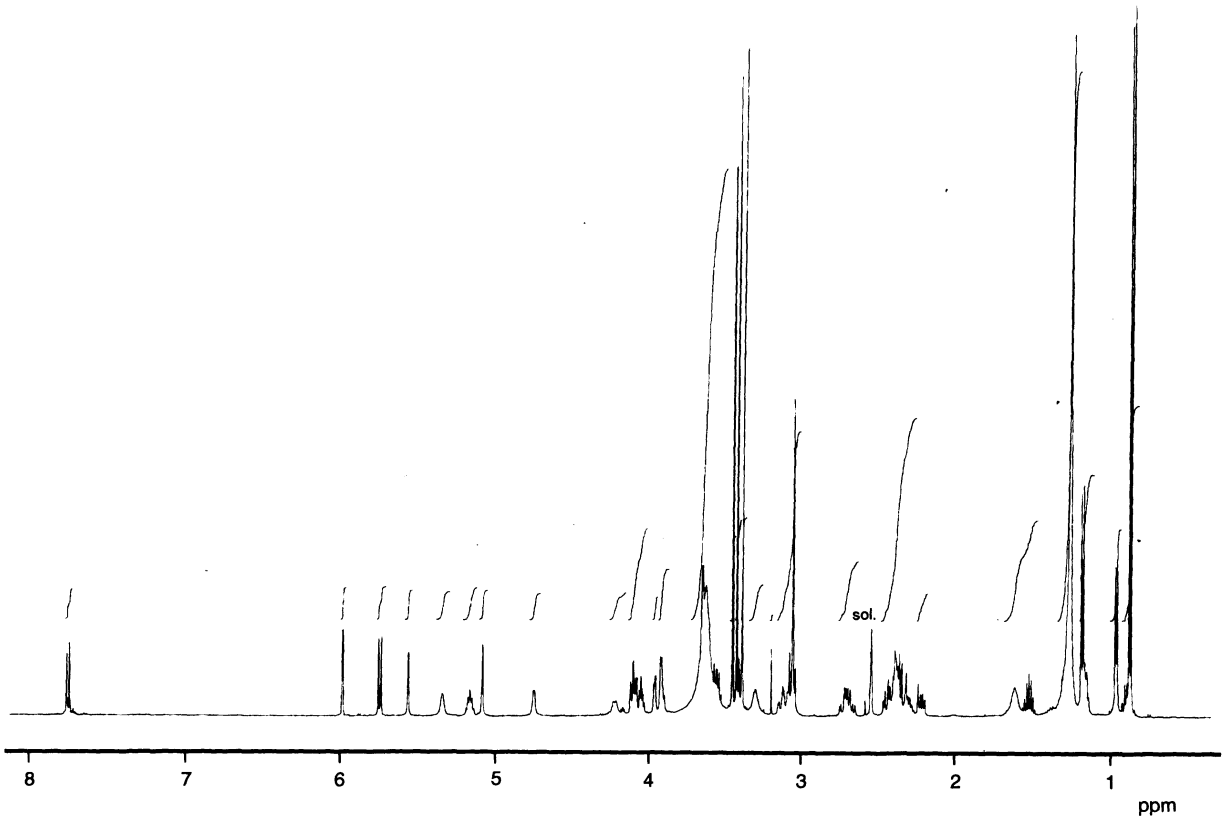
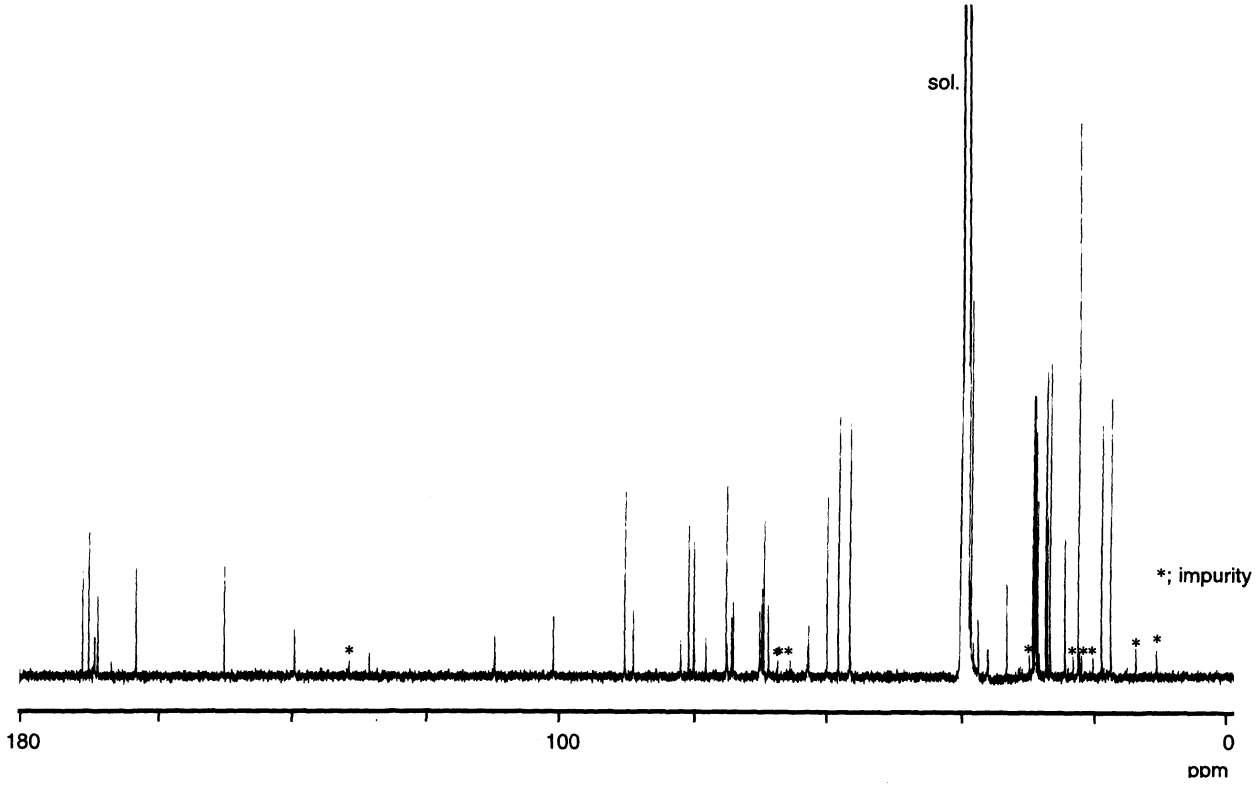


Fig. 3. ^{13}H NMR spectrum of caprazamycin B (DMSO-d_6 : D_2O=10 : 1, 125 MHz).



light brownish powder (537 mg). The powder was further purified by using reversed phase HPLC, CAPCELL PACK-C18 column (20×250 mm, Shiseido Co., Ltd., Japan; mobile phase, 50% aq acetonitrile in 0.05% formic acid; flow rate, 12.0 ml/minute; detection, UV; 258 nm). CPZ-B was eluted at 52~60 minutes as isolated peak. The eluate was collected and concentrated *in vacuo* to give a pure CPZ-B (90.3 mg).

The antibiotic is soluble in DMSO, pyridine, slightly soluble in MeOH and insoluble in water. CPZ-B was detected by molybdophosphoric acid-sulfuric acid positive spot (Rf 0.44) on a silica gel TLC (Kieselgel 60 F254, art 5715, Merck) developing with butanol - MeOH - H_2O (4 : 1 : 2) as a solvent system. The molecular formula of CPZ-B was established as $C_{53}H_{86}N_5O_{22}$ on the basis of HRFAB-MS [*m/z* 1144.5750 $(M-H)^-$, error −1.5 mmu]. The physico-chemical properties of CPZ-B were as follows, $[\alpha]_D^{23}$ −2.6° (*c* 0.91, DMSO), UV λ_{max} in MeOH (ε), 262 (8,000) nm, IR ν_{max} (KBr) 3400, 2925, 2854, 1739, 1701 (sh), 1674, 1630 (sh), 1467, 1386, 1193, 1126, and 1001 cm^{-1}. The planar structure of CPZ-B was determined from NMR experiments including DEPT experiment, $^1H^1H$ COSY, HMQC, HMBC, and NOESY. The ^1H and ^{13}C NMR spectra of CPZ-B show in Figs. 2 and 3. The ^1H and ^{13}C NMR assignments (in DMSO : D_2O=10 : 1) for CPZ-B are indicated Table 1. These data revealed that CPZ-B was new lipo-nucleoside antibiotics, which is related to liposidomycins[2~7] and capuramycin[8]. The structure elucidation of CPZ-B including stereo-chemistry will be detailed elsewhere.

CPZ-B showed excellent anti-mycobacterial activity *in vitro* against drug-susceptible and multidrug-resistant *M. tuberculosis* strains. The MICs of CPZ-B were 3.13 μg/ml for *M. tuberculosis* H37Rv and Kurono strains, 3.13 μg/ml for and *M. bovis* Ravenel strain, 6.25~12.5 μg/ml for drug-susceptible *M. tuberculosis* (n=22), 6.25~12.5 μg/ml for multi drug-resistant *M. tuberculosis* (n=12). Furthermore, CPZ-B was exhibited equivalents of *in vitro* activities with clarithromycin for MIC values and MIC distribution curves in anti-MAC activity. The MIC of CPZ-B were 6.25~50 μg/ml for *M. avium* (n=33) and 1.56~25 μg/ml for *M. intracellulare* (n=17). CPZ-B did not demonstrate any significant toxicity in mice received a single dose (>200 mg/kg, i.v.), repeated dose (100 mg/kg/14 days), as well as genotoxicity and cytotoxicity tests (5000 μg/ml). In

Table 1. NMR data of caprazamycin B (DMSO-d_6 : D_2O=10 : 1).

No.	δ_C		δ_H	No.	δ_C		δ_H
1	150.3	*s*		6a*	28.7	*t*	1.28
2	163.5	*s*		7a*	28.9	*t*	1.28
3	101.3	*d*	5.77	8a*	29.0	*t*	1.28
4	139.9	*d*	7.78	9a*	29.1	*t*	1.28
1'	89.3	*d*	5.58	10a*	29.1	*t*	1.28
2'	74.5	*d*	3.98	11a	29.4	*t*	1.28
3'	68.9	*d*	3.93	12a	26.9	*t*	1.28
4'	82.2	*d*	4.13	12a	38.6	*t*	1.18
5'	75.1	*d*	4.24	14a	27.5	*d*	1.54
6'	62.9	*d*	3.63	14a-Me	22.6	*q*	0.89
6'-*N* -Me	36.1	*q*	2.41	15a	22.6	*q*	0.89
7'	169.6	*s*		1b	171.4	*s*	
7'-*N*- Me	37.5	*q*	3.07	2b	40.2	*t*	2.38, 22.3
1''	110.1	*d*	5.10	3b	27.2	*d*	2.32
2''	74.2	*d*	3.94	3b-Me	19.2	*q*	0.98
3''	70.3	*d*	4.10	4b	39.5	*t*	2.47, 23.2
4''	78.3	*d*	4.07	5b	170.5	*s*	
5''	40.4	*t*	3.13	1c	90.6	*d*	6.01
1'''	170.0	*s*		2c	75.3	*d*	3.67
2'''	63.0	*d*	4.76	2c-OMe	56.8	*q*	3.41
3'''	70.0	*d*	5.36	3c	80.2	*d*	3.42
4'''	56.7	*t*	3.32	3c-OMe	58.5	*q*	3.44
1a	169.1	*s*		4c	81.0	*d*	3.08
2a	38.7	*t*	2.75, 2.69	4c-OMe	60.2	*q*	3.47
3a	69.9	*d*	5.18	5c	69.6	*d*	3.58
4a	33.3	*d*	1.63	6c	17.8	*q*	1.20
5a	24.6	*t*	1.30				

consequence of the following biological experimental results, CPZ-B was considered to be the promising candidate as an anti-TB drug in future. Detailed biological study of CPZ-B is now in progress.

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