SF-701, A NEW STREPTOTHRICIN-LIKE ANTIBIOTIC

Sir:

A new antibiotic, SF-701 was isolated from the culture broth of *Streptomyces griseochromogenes* obtained from a soil sample collected in Java, Indonesia. General properties of this antibiotic resemble those of the known streptothricins, but obvious difference from them was found by chromatography of the antibiotic and its acid hydrolysate.

For antibiotic production, the following medium was used: starch 2.0 %, soybean meal 2.5 %, wheat-embryo 1.0 % and sodium chloride 0.5 %. Antimicrobial activity attained a maximum after cultivation for 50 hours in a jar fermentor.

The antibiotic in the culture filtrate was adsorbed on a column of Amberlite IRC 50 (Na^+). After washing the column with water, active principle was eluted by 0.5 N hydrochloric acid. The active eluate was neutralized with Amberlite IR-45 (OH^-) and concentrated under reduced pressure to a solution containing 10~20 mg/ml of the active principle. To the concentrate, saturated aqueous ammonium reineckate was added to precipitate crystalline reineckate of the antibiotic. It was converted to hydrochloride by using pyridine hydrochloride.

Further purification was accomplished by carbon chromatography using distilled water as developing solvent. Active fractions were concentrated, freeze-dried and hydrochloride of SF-701 substance was obtained as white crystalline powder.

Table 1. Thin-layer chromatography (cellulose)

Solvent systems	Rf values		
	SF-701 HCl	Racemomycin A HCl	A-249 HCl
n-Propanol·pyridine·acetic acid·water (15 : 10 : 3 : 12)	0.66	0.53	0.48
n-Butanol·acetic acid·water (2 : 1 : 1)	0.40	0.28	0.23
Wet *n*-butanol containing 2% of *p*-toluene sulfonic acid	0.23	0.20	0.21
Phenol·water (6 : 4)	0.42	0.28	0.23

Table 2. Thin-layer chromatography (silica gel)

Solvent systems	Rf values		
	SF-701 HCl	Racemomycin A HCl	A-249 HCl
Chloroform·methanol·17% NH_4OH (2 : 1 : 1) upper layer	0.72	0.36	0.31
n-Butanol·acetic acid·water (2 : 1 : 1)	0.15	0.12	0.10

Physical and chemical properties of the antibiotic hydrochloride were as follows: It melted at 210~213°C under decomposition. It was optically active, $[\alpha]_D^{24}$ −68° (c 1,H_2O). Molecular weight determination by titration method gave a value of 570 (pKa′ 7.2 and 9.3). Elemental analysis indicated a molecular formula of $C_{18}H_{36}N_7O_{11}\cdot 2HCl$.

Found: C 36.12, H 6.23, N 16.31, Cl 12.18%
Calcd: C 36.06, H 6.01, N 16.36, Cl 11.89%
(M.W. 599).

Fig. 1. Infrared absorption spectrum of SF-701 substance HCl-salt

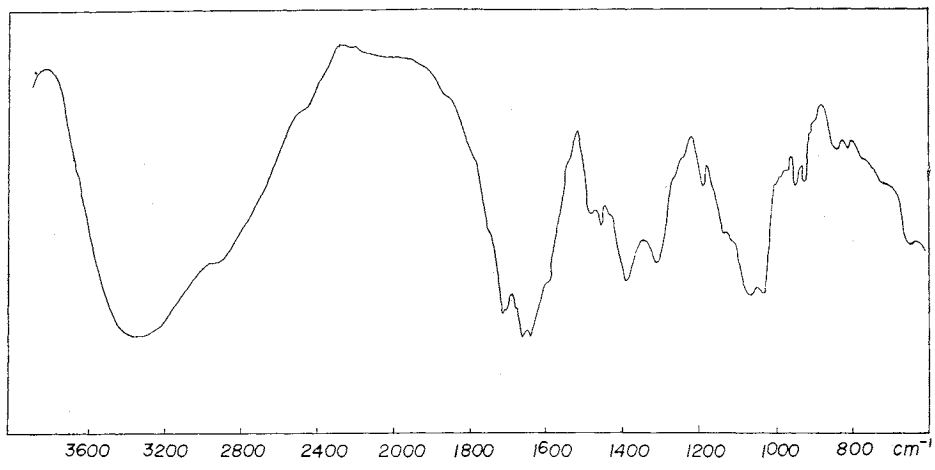
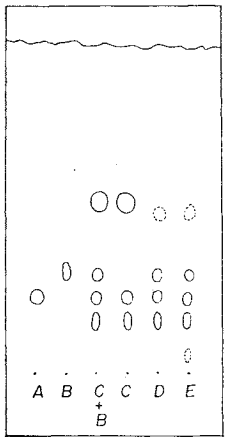


Fig. 2. Paper chromatography of acid hydrolysates of SF-701 substance and known streptothricins



A; streptolidine HCl
B; β-lysine HCl
C; hydrolysate of SF-701 substance
D; hydrolysate of A-249
E; hydrolysate of racemomycin A

Solvent system : *n*-butanol·acetic acid·water (2 : 1 : 1)
Detection : ninhyrin

The antibiotic was soluble in water and methanol, slightly soluble in ethanol, and insoluble in acetone, ethyl acetate, chloroform and ether. Ultraviolet absorption spectrum in water exhibited end absorption. Infrared absorption spectrum is shown in Fig. 1. It gave positive ninhydrin, FEHLING, BENEDICT, ELSON-MORGAN and PAULY, but negative ferric chloride, biuret, SAKAGUCHI and MOLISCH reactions. Reineckate of this antibiotic was plate crystals which decomposed at 155~160°C. On circular paper chromatography, with the solvent system of *n*-butanol·pyridine·acetic acid·water (15 : 10 : 3 : 12), this antibiotic showed a single spot (Rf=0.39). SF-701 was distinguished from racemomycin A and A-249 by thin-layer chromatography (Tables 1 and 2).

The antimicrobial activity is shown in Table 3.

The antibiotic was injected intravenously into mice weighing 20 g. All the mice injected with 6 mg died after 2 days and those with 4 mg died after 3 days. Such a delayed toxicity indicates a characteristic streptothricin-like nature of this substance.

Paper chromatographic patterns of acid hydrolysates of SF-701 and the known streptothricins (in 6 N HCl at 100°C for 10 hours) are shown in Fig. 2. The hydrolysate of SF-701 shows a spot which has very similar Rf value to streptolidine but lacks β-lysine moiety.

Table 3. Antimicrobial activity of SF-701 substance

Test organisms*	M.I.C (mcg/ml)
Bacillus subtilis ATCC 6633	1.56
Bacillus cereus IAM 1072	>100
Staphylococcus aureus 209P	6.25
〃 (A-249·SM-R)	>100
〃 (KM-R)	>100
〃 (SM·TC·EM·PC-R)	25.0
Sarcina lutea	3.12
Escherichia coli K-12	3.12
〃 IAM 1253	6.25
Shigella dysenteriae	1.56
Salmonella typhi	1.56
Klebsiella pneumoniae	3.12
Pseudomonas aeruginosa	25.0
Xanthomonas oryzae	3.12
Mycobacterium smegmatis 607	6.25
〃 〃 (KM-R)	6.25
〃 〃 (SM-R)	6.25
Candida albicans	6.25
Cryptococcus neoformans	0.8

* -R : resistant, SM : streptomycin, KM : kanamycin, EM : erythromycin, PC : penicillin G.

Furthermore, on acid hydrolysis in 6 N HCl at 110°C for 15 hours, this antibiotic yielded a crystalline product which was identified as N-methylglycine (sarcosine) by n.m.r. spectrum ($\delta=3.58$, singlet ($-\underline{CH_2}$), $\delta=2.70$, singlet ($\underline{H_3}NCNH-$) in D_2O, 100 MC) and infrared absorption spectrum.

Thus, SF-701 was concluded to be a new member of the streptothricin group. The β-lysine moiety of the known streptothricins seems to be substituted by another component which may be N-methylglycine.

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