CATIONOMYCIN, A NEW POLYETHER IONOPHORE ANTIBIOTIC PRODUCED BY *ACTINOMADURA* NOV. SP.

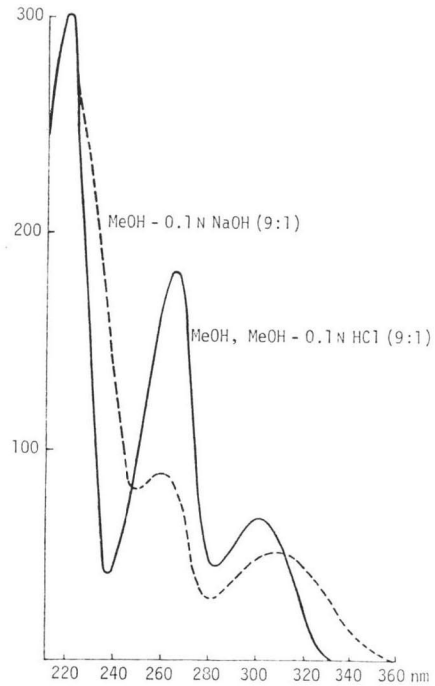
Sir:

We wish to report here the isolation and characterization of a new polyether ionophore antibiotic named cationomycin from a fermentation broth of a rare actinomycete, strain No. 76-11, which was isolated from a soil sample collected in Masuda-shi, Shimane-ken, Japan. Taxonomic study showed that the strain's cell wall is of type IIIb and the mycelium shows a characteristic dark blue color on oat meal agar and often breaks up into coccoid forms in submerged culture. The strain was defined as a new species belonging to the *Actinomadura* and named *Actinomadura azurea* nov. sp. NAKAMURA et ISONO. Details of taxonomy will be reported separately. The absolute structure of cationomycin was determined by X-ray analysis of the thallium salt (Scheme 1), the details of which will be reported elsewhere.

The strain was fermented at 28°C for 210 hours in a jar fermenter containing 18 liters of a medium consisted of oat meal (3 %), glycerol (1.5 %), dry yeast (0.5 %), KH_2PO_4 (0.5 %), $Na_2HPO_4 \cdot 12H_2O$ (0.5 %), $MgCl_2 \cdot 6H_2O$ (0.1 %). The fermentation broth was filtered with the aid of Celite and the filtrate was extracted with ethyl acetate. The mycelium was extracted with acetone and the extract was concentrated *in vacuo* to give an aqueous solution, which was then extracted with ethyl acetate. Both ethyl acetate extracts were combined and concentrated *in vacuo* to dryness. The residue was purified by silicic acid chromatography using the solvent system, benzene - ethyl acetate, (5: 1)~(1: 1) (v/v). The active fractions were combined, concentrated, and the residue was recrystallized several times from methanol to give pure crystals of a sodium salt (yield 150 mg).

Cationomycin sodium salt melted at 184~188°C with decomposition. It is optically active, $[\alpha]_D^{25}$ +36.6° (*c* 0.38, chloroform). Elementary analysis and FD mass spectrum [$(M+1)^+$ 874, $(M+Na)^+$ 896] indicated the molecular formula, $C_{45}H_{69}O_{15}Na$: *Anal.* Calcd. for $C_{45}H_{69}O_{15}Na$: C 61.91, H 7.97, Na 2.63. Found:

Fig. 1. UV absorption spectra of cationomycin.



Scheme 1. The structure of cationomycin.

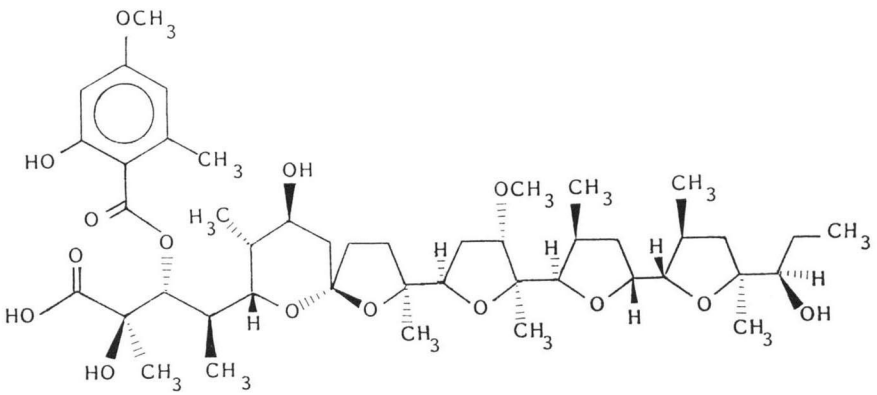


Fig. 2. IR absorption spectrum of cationomycin sodium salt in KBr.

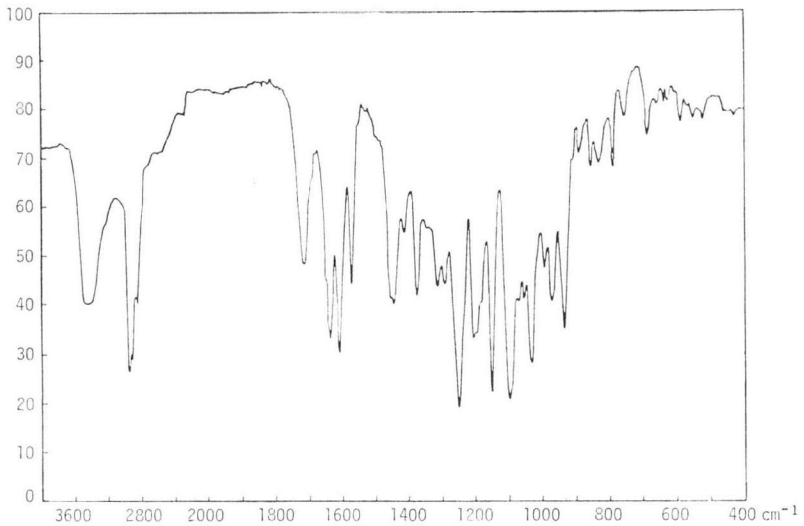


Table 1. Antibacterial spectrum of cationomycin.

Microorganism	Minimal inhibitory concentration (mcg/ml)
Staphylococcus aureus 209P	0.4
Staphylococcus aureus (multi resistant)	0.4
Bacillus subtilis PCI 219	0.4
Bacillus subtilis (rec$^+$)	0.4
Bacillus subtilis (rec$^-$)	0.4
Mycobacterium sp. 607	0.4
Mycobacterium phlei	0.4
Mycobacterium avium	0.4
Escherichia coli	>100
Salmonella typhimurium	>100

Conventional agar dilution method was employed using bouillon agar.

C 61.09, H 8.13, Na 2.38. The free acid was obtained as a white powder with m.p. 108~112°C. *Anal.* Calcd. for $C_{45}H_{70}O_{15}$: C 63.58, H 8.18. Found: C 62.61, H 8.27. It is a dibasic acid with *pKa*'s of 4.6 and 11.1 (66.7 % dioxane). The thallium salt was obtained as crystals with m.p. 205~208°C. *Anal.* Calcd. for $C_{45}H_{69}O_{15}T1$: C 51.25, H 6.59, T1 19.38. Found: C 51.08, H 6.80, T1 18.59. The antibiotic has a characteristic UV absorption spectrum: λ_{max}^{MeOH} nm (ε); 217 (27,300), 262 (16,400), 301 (6100). The spectrum did not change in acidic methanol but shifted to 260 (7800) and 308 (4480) in alkaline methanol (Fig. 1.). The antibiotic is soluble in most organic solvents but barely soluble in water and hexane. It reduced a permanganate solution, but gave a negative reaction to ferric chloride. The IR spectrum of the sodium salt indicated the presence of carboxylate (1610 and 1380 cm^{-1}) and ester (1718 cm^{-1}) groups.

Cationomycin inhibited Gram-positive bacteria including mycobacteria (Table 1). The Gram-negative bacteria, yeast, and fungi tested were not sensitive. It showed coccidiostat activity *in vivo* when mixed in feed at the level of 50~100 ppm for chickens infected with *Eimeria tenella*. The acute toxicity was relatively low for this class of compound. Mice tolerated an intraperitoneal dose of 200 mg per kg of body weight.

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