

A NEW ANTIBIOTIC LIBRAMYCIN A

Sir:

A new antibiotic libramycin A has been isolated from the culture filtrate of a *Streptomyces* sp. No. 72-192 which was isolated from a soil sample collected at Sendai-shi, Miyagi Prefecture, Japan. Libramycin A, $C_{11}H_{20}N_2O_8$, is a fat-soluble, weakly acidic substance, effective against some bacteria and fungi, especially against *Mycobacterium phlei* and *Xanthomonas oryzae*.

The composition of the culture medium used to produce libramycin A was 2% glucose, 1% starch, 2.5% soybean meal, 0.4% dry yeast, 0.1% beef extract, 0.2% sodium chloride and 0.005% dipotassium phosphate. The fermentation was carried out in a 600-liter tank for 96 hours at 27°C. The active substance was adsorbed on active carbon (1% w/v) from the culture filtrate (400 liters) at pH 6 and eluted with 70% aqueous acetone. The active eluate was concentrated, acidified to pH 2, and extracted with *n*-butanol. The antibiotic was re-extracted into water adjusted to pH 9 with

sodium hydroxide. The aqueous phase was neutralized to pH 6 and the antibiotic in it was adsorbed on an active carbon column. The column was washed with water, and the antibiotic was eluted with 70% aqueous acetone. The active eluate was concentrated and applied to a Sephadex G-10 column. The column was developed with distilled water and the active fractions were combined and concentrated *in vacuo* to yield 1.2g of crude

Fig. 1. Ultraviolet spectrum of libramycin A.

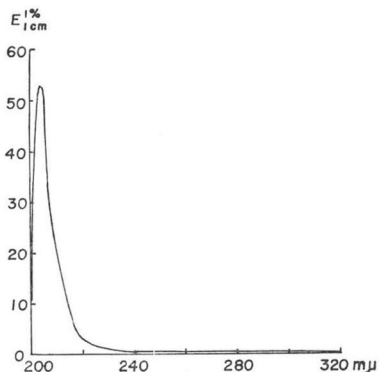


Fig. 2. Infrared spectrum of libramycin A (KBr).

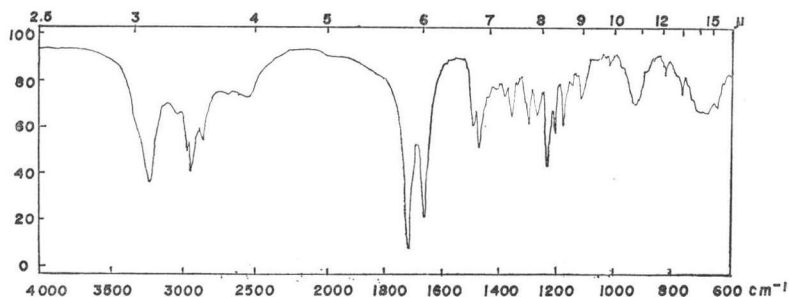
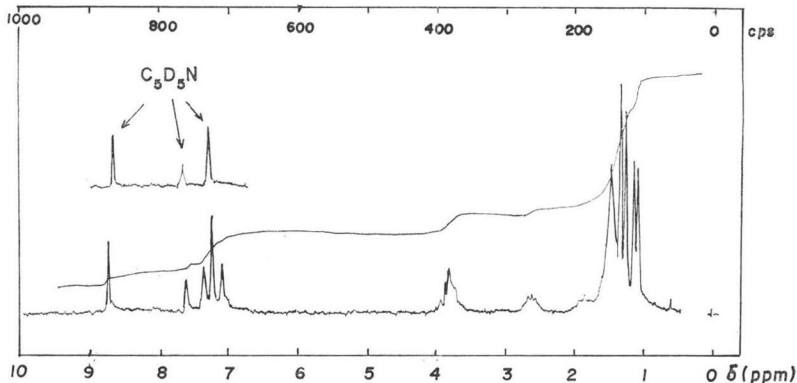


Fig. 3. NMR spectrum of libramycin A (100 MHz C_5D_5N).



crystals. Recrystallization from hot water gave 0.98 g of libramycin A as colorless needles, m.p. 161 ~ 162°C. It is an acidic substance with a pK_a of 6.60, and is optically active, $[\alpha]_D^{25} - 6.3^\circ$ (c 1, methanol). It is easily soluble in methanol, ethanol, pyridine and alkali, soluble in acetone, ethyl acetate, chloroform and water, slightly soluble in ether and benzene, and insoluble in *n*-hexane.

Elementary analysis gave:

Calcd. for $C_{11}H_{20}N_2O_3$ (M.W. 228):

C 57.89, H 8.77, N 12.28, O 21.05

Found: C 57.75, H 8.55, N 12.31, O 21.75

The molecular ion was observed at m/e 228.14266 (calculated m/e 228.14737 for $C_{11}H_{20}N_2O_3$) by high resolution mass spectrometry, thus establishing the molecular formula. Libramycin A gives positive color reactions with anisaldehyde, $KMnO_4$, ferric chloride, DRAGENDORFF, MOLISCH and EHRlich reagents; negative reactions with ninhydrin, FEHLING, SAKAGUCHI, BIURET, TOLLENS and anthrone reagents. The ultraviolet absorption spectrum, Fig. 1, shows end absorption at 204 nm ($E_{1\%}^{1\text{cm}}$ 53). The infrared spectrum is shown in Fig. 2. A 100 MHz NMR spectrum of libramycin A dissolved in C_5D_8N with TMS as an internal standard is shown in Fig. 3. Two signals, at δ 7.09 and δ 7.36, disappeared on the addition of a few drops of D_2O .

R_f values by paper chromatography were as follows: 0.80 (*n*-butanol saturated with water), 0.97 (3% ammonium chloride), 0.90 (75% phenol), 0.96 (50% acetone), 0.83 (*n*-butanol-methanol-water, 4:1:2), and 0.66 (benzene-methanol, 4:1). Thin-layer chromatography with silica gel (Kieselgel-G, Merck) gave R_f values: 0.53 (*n*-butanol saturated with water), 0.56 (*n*-butanol-methanol-water, 4:1:2), 0.88 (propanol-pyridine-acetic acid-water, 15:10:3:10), 0.87 (*n*-butanol-acetic acid-water, 3:1:1) and 0.32 (methanol-ethyl acetate, 15:100).

The antimicrobial spectrum of libramycin A by the agar dilution method is shown in Table 1. The intraperitoneal injection of 400 mg/kg in mice did not elicit any toxic response. Two known antibiotics having N_2O_3 in their molecular formulae, nocardamin^{1,2)} and elaiomycin³⁾,

Table 1. Antimicrobial spectrum of libramycin A

Microorganisms	M.I.C.(mcg/ml)
<i>Staphylococcus aureus</i> FDA 209P	>100
<i>Pseudomonas aeruginosa</i> IFO 3445	20
<i>Mycobacterium phlei</i> LEHMAN et NEUMANN	1
<i>Mycobacterium smegmatis</i> ATCC 607	2
<i>Escherichia coli</i> NIHJ	>100
<i>Klebsiella dysenteriae</i>	>100
<i>Xanthomonas oryzae</i> ATCC 10031	2
<i>Trichophyton mentagrophytes</i>	>100
<i>Trichophyton rubrum</i>	>100
<i>Cryptococcus neoformans</i>	>100
<i>Candida albicans</i> Tokyo Univ.	>100
<i>Candida albicans</i> 57	>100
<i>Colletotrichum lagenarium</i>	2
<i>Aspergillus fumigatus</i>	>100
<i>Alternaria kikuchiana</i>	>100
<i>Fusarium oxysporum</i>	>100
<i>Pyricularia oryzae</i> cavara	>100

are differentiated from libramycin A on the basis of their physico-chemical properties.

These physico-chemical and biological properties indicate that libramycin A is a new antibiotic.

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